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CONTENTS / CUPRINS

I. GEOLOGY / GEOLOGIE

BORDEIANU Marian, GRĂDIANU Ionuț, TRIF Nicolae, CODREA Vlad - Commented list of the lower oligocene fish fauna from the Coza Valley (Marginal Folds Nappe, Eastern Carpathians, Romania) / Lista comentată a faunei de pești oligoceni inferiori din Valea Coza (Pânza Cutelor Marginale, Carpații Orientali, România) 7

GRIGORE Dan - Oppelid and haploceratid ammonites from the upper jurassic deposits of Hăghimaș MTS. (The Eastern Carpathians – Romania) / Oppelide și haploceratide din depozitele jurasic superioare din Munții Hăghimaș (Carpații Orientali – România) 15

MUÇI Redi, FOCIRO Oltion, SKRAME Klodian - The effect of lime as a stabilizing agent in plastic clayey soils in Vila Hill, Durres, Albania / Efectul varului ca agent de stabilizare în pământurile plastice din Vila Hill, Durres, Albania 23

SEITAJ Bardhosh, PRIFTI Irakli - Identification of the gude oil level in the geological section of Marinza-3111 well / Identificarea nivelului petrolului brut în secțiunea forajului Marinza-3111..... 29

ASIMOPOLOS Natalia-Silvia, ASIMOPOLOS Laurențiu - Evaluation of electromagnetic field variations from monitored data in planetary observatories / Evaluarea variațiilor câmpului electromagnetic din datele monitorizate în observatoarele planetare 37

KARRIQI Altin, PEKMEZI Jeton - Qualitative and quantitative resistivity (VES) study in bedrock mapping. Case study Moglica / Studiul calitativ și cantitativ al rezistivității (VES) în cartarea rocilor. Studiu de caz Moglica 45

II. VEGETAL BIOLOGY / BIOLOGIE VEGETALĂ

IVANOVA Raisa, SMEREA Svetlana - Effects of seeds irradiation with gamma-ray on plant growth and yield attributing characters of safflower / Efectele iradierii seminţelor cu raze gamma asupra creşterii plantelor şi a caracterelor atribuite productivităţii şofrănelului 51

YAVUZ Mustafa, ÇOBANOĞLU Gülşah - Lichen diversity of Gölcük Nature Park (Isparta), including new records for Turkey / Diversitatea lichenilor din Parcul Natural Gölcük (Isparta), inclusiv înregistrări noi pentru Turcia 57

SMEREA Svetlana, ANDRONIC Larisa, SCHIN Victoria - Morphogenetic potential of calluses derived from gamma irradiated safflower seeds / Potențialul morfogenetic al calusului obținut din semințe iradiate de șofrănel 67

VICOL Ioana - Distribution of the *Leptorhaphis* Körb. 1855 genus in Romania / Distribuția genului *Leptorhaphis* Körb. 1855 în România 72

NICULAE Marilena - Phenological, autecological and phytocoenological characteristics of a *Sedum caespitosum* Cav (Crassulaceae) population from Central Dobrogea (SE Romania), lessons for broader, more effective conservation / Caracteristici fenologice, autecologice şi fitocenologice ale unei populaţii de *Sedum caespitosum* Cav (Crassulaceae) din Dobrogea Centrală (SE România), învăţăminte pentru o mai largă şi mai eficientă conservare 79

III. ANIMAL BIOLOGY / BIOLOGIE ANIMALĂ

III.a. INVERTEBRATES VARIOUS / NEVERTEBRATE DIVERSE

MARNICHE Faiza, MILLA Amel, TIMTAOUCINE Khaoula, BACHA Assia - Coproscopy of wild mammals: the case of red fox *Vulpes vulpes* (Thomas Say, 1823), common jackal *Canis aureus* (Linné, 1758) and wild boar *Sus scrofa* (Linné, 1758) in marsh of Réghaia (Algiers) / Coproscopia mamiferelor sălbatice: cazul vulpii roșii *Vulpes vulpes* (Thomas Say, 1823), șacalului comun *Canis aureus* (Linnaeus 1758) și mistrețului *Sus scrofa* (Linné, 1758) în mlaștina din Réghaia (Alger) 89

BODOG Denisa-Emilia, POPOVICI Paula-Vanda, MOLNÁR Krisztina, SAS-KOVÁCS István, FERENŢI Sára - Terrestrial isopods (Isopoda, Onsicidea) in Sebiș town, Arad county (Romania) / Izopode terestre (Isopoda, Oniscidea) în orașul Sebiș, județul Arad (România) 97

POPOVICI Paula-Vanda, BONDAR Alexandra, BODOG Denisa-Emilia, VICAŞ Patricia-Teodora, CUPŞA Diana - Road mortality on two secondary roads near Abrămuț locality, western Romania: effects of year period and road surrounding habitats / Mortalitatea rutieră pe două drumuri secundare lângă localitatea Abrămuț, vestul României: efectele perioadei din an și al habitatelor înconjurătoare 103

LILA Gima - Preliminary data regarding interspecific relationships beetle species collected from different ecosystems met in Dolj county in 2017. Note 2 / Date preliminare privind relații interspecifice la coleoptere din diferite ecosisteme din județul Dolj colectate în 2017. Nota 2 111

PETRESCU Ana-Maria, PETRESCU Iorgu - The catalogue of the collection bought by Grigore Antipa from Václav Frič (Czech Republic) / Catalogul colecției cumpărate de Grigore Antipa de la Václav Frič (Republica Cehă) 114

BÂRCĂ Valentin, NICULAE Marilena - Life history and plurivoltinism of *Scolitantides orion* (Pallas 1771), (Lepidoptera, Lycaenidae) from Romanian Southern Dobrogea, in captive breeding on *Hylotelephium telephium*, sozological implications / Ciclul de viață și plurivoltinismul la *Scolitantides orion* (Lepidoptera, Lycaenidae) din Dobrogea Sudică Română, crescut în captivitate pe *Hylotelephium telephium*, implicații sozologice 123

STANCĂ-MOISE Cristina - The species of macrolepidoptera collected from the Gușterița Hill, Sibiu, existing within the collection of dr. Viktor Weindel / Specii de macrolepidoptere colectate din Dealul Gușteriței-Sibiu, existente în cadrul colecției dr. Viktor Weindel 131

III.b. VERTEBRATES / VERTEBRATE

PETRESCU Angela, PETRESCU Ana-Maria, CHIȘAMERA Gabriel Bogdan, RIDICHE Mirela Sabina - The expedition of Humberto dos Passos-Freitas in the Danube Delta (Romania) - 1922 and the "Grigore Antipa" National Museum of Natural History from Bucharest / Expediția lui Humberto dos Passos Freitas în Delta Dunării (România) - 1922 și Muzeul Național de Istorie Naturală ,,Grigore Antipa" din București 137

BĂLESCU Carmen Daniela, MURARIU Dumitru - Preliminary data on small and medium-sized mammals from Craiova municipality (Romania) / Date preliminare despre fauna de mamifere mici și mijlocii din municipiul Craiova (România) 149

TAMBA-BEREHOIU Radiana, POPA Nicolae-Ciprian, VIȘAN Luminița - Statistical evaluation of seminal plasma vs. blood serum biochemical parameters in Bruna and Holstein Friesian breeding bulls / Evaluarea statistică comparativă a parametrilor biochimici ai plasmei seminale și ai serului sangvin la taurii de reproducţie din rasele Brună și Holstein Friză 157

IV. ECOLOGY - THE ENVIRONMENT PROTECTION / ECOLOGIE - PROTECŢIA MEDIULUI

VIȘAN Luminița, TAMBA-BEREHOIU Radiana Maria, POPA Ciprian Nicolae, DĂNĂILĂ-GUIDEA Silvana Mihaela - Aspects on the impact of climate change on viticulture / Aspecte privind impactul schimbărilor climatice asupra viticulturii 165

BOIU-SICUIA Oana-Alina, CONSTANTINESCU Florica, DIACONU Aurelia, DRĂGHICI Reta - Current status and future perspectives in Romania on biological control of sweet potato fungal pathogen / Stadiul actual şi perspective privind controlul biologic al cartofului dulce cultivat în România 172

M'BARKI Lhassan, ABIOUI Mohamed, BENSSAOU Mohammed, MSANDA Fouad, AXINI Monica - The role of the flora in agricultural system development in mountain areas - case study: Tafraoute Ida Ouzdoute, Morocco / Rolul florei în dezvoltarea sistemului agricol din zonele montane – studiu de caz: Tafraoute Ida Ouzdoute, Maroc 180

CORNEANU C. Gabriel, CORNEANU Mihaela, CRĂCIUN Constantin, TRIPON Septimiu, LĂCĂTUȘU Anca Rovena, COJOCARU Luminița - Ultrastructural aspects of cellular resistance to pollution. Case study - *Phragmites australis* (Cav.) Trin. ex Steud. / Aspecte ultrastructurale ale rezistenței celulare la poluare. Studiu de caz - *Phragmites australis* (Cav.) Trin. ex Steud. 188

NEAGU Simona, COJOC Roxana, GOMOIU Ioana, TUDORACHE Mădălina, ENACHE Mădălin - Purification and characterization of decarboxylase from a halophilic microorganism with potential in biotransformation of waste glycerol from biodiesel industry / Purificarea și caracterizarea decarboxilazei de la un microorganism halofil cu potențial în biotransformarea glicerolului rezidual din industria biodiesel 199

GAVRILESCU Elena, CIOBOIU Olivia, TOMUȘ Nicolae, DEAK Ștefania Elena, CISMAȘIU Carmen-Mădălina - Studies on environmental pollution in large installation of combustion in regard with international standards / Studiul privind poluarea mediului de către instalaţiile mari de ardere în acord cu standardele internaționale 204

CÎRSTEA Doina Maria, ȘTEFĂNESCU Mugur Cristian - A review of general aspects of microorganisms involved in the corrosion process / O revizuire a aspectelor generale ale microorganismelor implicate în procesul de coroziune 212

LEBEDENCO Liubovi - Studies on the distribution of zooplanktonic communities in the Prut - Danube ecosystems / Studii privind distribuția comunităților zooplanctonice în ecosistemele Prut – Dunăre 216

ILIE Aurelian Leonardo, MARINESCU Mariana, ILIE Lorena Cosma - Observations regarding the entry in the hiemal diapause of *Chrysolina fastuosa* (Scopoli, 1763) (Choleoptera, Chrysomelidae) in the north-western part of Romania (Tinca area, Bihor county) / Observații privind intrarea în diapauza hiemală (iernare) la *Chrysolina fastuosa* (Scopoli 1763) (Coleoptera, Chrysomelidae) în partea nord-vestică a României (zona Tinca, județul Bihor) 220

YAPANTO Lis Melissa, MODJO LESTARI Meilinda - Assessing the public awareness level on the preservation of coral reefs (The case study in Biak Numfor, Papua, Indonesia) / Evaluarea nivelului de conştientizare a publicului privind conservarea recifelor de corali (Studiul de caz în Biak Numfor, Papua, Indonezia) 224

V. SCIENTIFIC ESSAYS / REFERATE ŞTIINŢIFICE

ANTONIE Iuliana - August von Spiess - under the sign of the destiny / Sub semnul destinului- August von Spiess 233

CORNEANU C. Gabriel, CORNEANU Mihaela - From idea to achievement - From the experience of a biologist / De la idee la realizare - Din experiența unui biolog 239

Recommendations regarding the elaboration of the papers for the scientific journal *"Oltenia. Studii şi comunicări. Ştiinţele Naturii"/* Recomandări privind elaborarea lucrărilor științifice pentru revista *"Oltenia. Studii şi comunicări. Ştiinţele Naturii"*..... 245

COMMENTED LIST OF THE LOWER OLIGOCENE FISH FAUNA FROM THE COZA VALLEY (MARGINAL FOLDS NAPPE, EASTERN CARPATHIANS, ROMANIA)

BORDEIANU Marian, GRĂDIANU Ionuţ, TRIF Nicolae, CODREA Vlad

Abstract. The study of Oligocene fishes from the external sector of the Eastern Carpathians Flysch of Romania (Marginal Folds Nappe or Vrancea Nappe) started more than a century ago. During all this time the research was focused mainly on the Northern exposures of these deposits, while the southern areas remained almost neglected. The aim of this research is to report the occurrence of several Oligocene fishes discovered from a new outcrop. This new outcrop which brings to the surface a part of the Bituminous Marls Formation is found on Coza Valley (Vrancea District). This site could be considered an important landmark in the study of the fish fossils, due to the relative richness of the specimens. Seven genera and five related species were collected and assigned to the following taxa: *Keasius parvus* Leriche, 1908, *Sardinella sardinites* Heckel, 1850, *Glossanodon musceli* Paucă, 1929, *Palaeogadus* sp., *Aeoliscus* sp., *Oliganodon budensis* Heckel, 1856, *Anenchelum glarisianum* Blainville, 1818.

Keywords: fossil fishes, Oligocene, Marginal Folds Nappe, Vrancea half-window, Eastern Carpathians, Romania.

Rezumat. Lista comentată a faunei de pești oligoceni inferiori din Valea Coza (Pânza Cutelor Marginale, Carpații Orientali, România). Studiul peştilor oligoceni identificaţi în flişul extern al Carpaţilor Orientali din România (Pânza Cutelor Marginale = Pânza de Vrancea) are începuturi care depășesc un secol. În tot acest timp studiile s-au focalizat asupra sectoarelor nordice în care aceste depozite aflorează, cele sudice fiind în mare parte neglijate. Scopul acestui studiu este de a semnala descoperirea unor pești oligoceni într-un afloriment identificat recent. Aflorimentul permite studiul unei părți a Formațiunii Marnelor Bituminoase în Valea Coza (Județul Vrancea) și poate fi considerat drept un reper important în studiul peștilor fosili datorită numeroaselor specimene identificate. Șapte genuri și cinci specii de pești au fost colectate și determinate: *Keasius parvus* Leriche, 1908, *Sardinella sardinites* Heckel, 1850, *Glossanodon musceli* Paucă, 1929, *Palaeogadus* sp., *Aeoliscus* sp., *Oliganodon budensis* Heckel, 1856, *Anenchelum glarisianum* Blainville, 1818.

Cuvinte cheie: pești fosili, Oligocen, Pânza Cutelor Marginale, Semifereastra Vrancei, Carpații Orientali, România.

INTRODUCTION

The presence of fish remains in the Oligocene formations of Romania has been known since 1883, when Leon C. Cosmovici collected the first fossils from the Cozla Mountain, Piatra Neamţ area. In the following years he completed the systematic description of the discovered specimens. The results were published in a paper issued in Paris (COSMOVICI, 1886). In the next century, the research of the Oligocene-lowermost Miocene fish fauna from Eastern Carpathians thrived, as new outcrops were found (for details see CONSTANTIN, 1999).

In the following decades, other researchers added new and important contributions to this topic (e.g. SIMIONESCU, 1904; PAUCĂ, 1934; JONET, 1949; CIOBANU, 1977). CONSTANTIN (1999) published the first inventory of the Oligocene-lowermost Miocene fish fauna reported in Romania. BACIU (2001), in his PhD studies realized a detailed research on the fossil fish fauna from Piatra Neamţ area, adding a new perspective on the studied exposures and to the importance of the fish fossils. GRĂDIANU (2010) studied the fish fauna from the Cenozoic flysch deposits (Tarcău and Marginal Folds nappes) exposed between Moldova and Suceava rivers (Suceava District).

ŞTEFAN (1988) was the first to attempt a study on fossil fishes from the Vrancea half-window (Marginal Folds Nappe). He described six species collected from the Lower Dysodilic Shales Formation from the Coza Valley. The specimens herein reported were collected from a new outcrop located on the same valley, more specifically on the southwestern side of the Coza Village, upstream on the river (Fig. 1). It is placed on a slope of the Coza Mountain, in the Eastern Carpathians (Romania, Vrancea District).

Figure 1. Satellite view of the Coza Valley; the outcrop is marked by a white rectangle (after maps.google.com, modified).

GEOLOGICAL SETTING

The Carpathian Orogen is the result of the gradual collision between the African and Eurasian plates. As a consequence, the Neo-Tethys Ocean vanished and the Paratethys Sea to the north and the Mediterranean Sea to the south begun their evolutions (DERCOURT et al., 2000; BERRA & ANGIOLINI, 2014). According to SĂNDULESCU (1984) and BĂDESCU (2005) the eastern Moldavids, i.e. Tarcău, Marginal Folds [= External Unit – BĂNCILĂ (1958); Marginal Unit – DUMITRESCU (1952); Submarginal Unit – JOJA (1952); Marginal Folds Nappe – SĂNDULESCU (1984)] and Peri-Carpathian nappes were erected in Miocene. As the overlying Tarcău Nappe covers completely the Marginal Folds Nappe, in many sectors, this one can be observed in detail only in half-windows which occurred due to the river erosion (SĂNDULESCU, 1984). The studied fish-bearing outcrop belongs to this thrusting nappe, located in the half-window named Vrancea (Fig. 2).

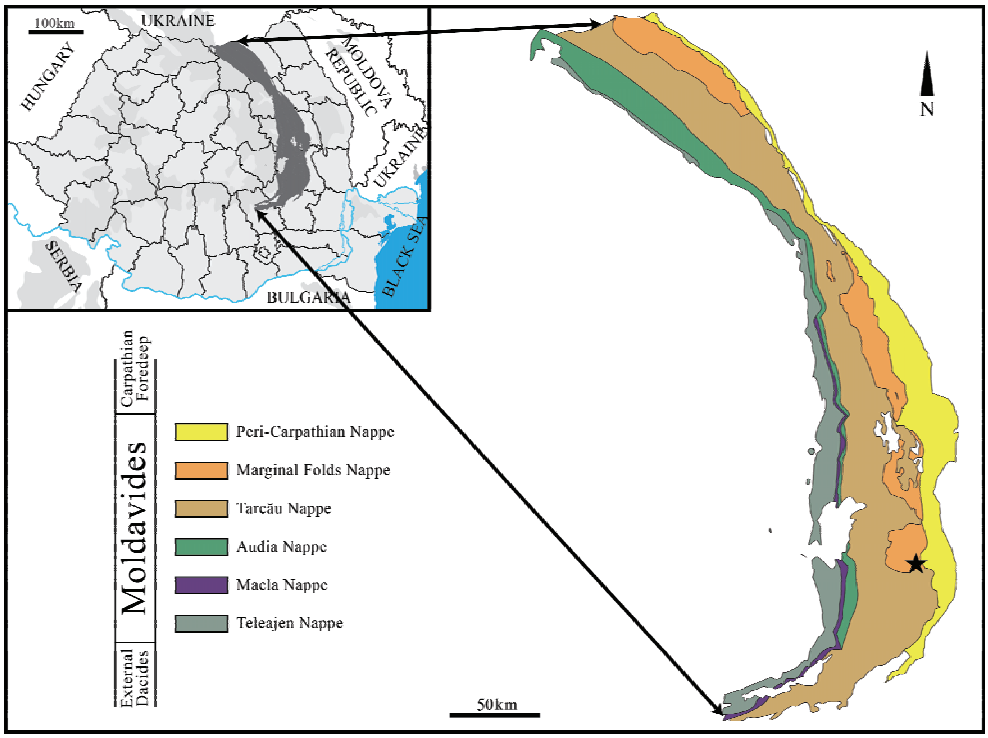

Figure 2. The Moldavides and the related nappes; location of the outcrop in the Vrancea half-window (based on SĂNDULESCU, 1984; BĂDESCU, 2005).

The Marginal Folds Nappe includes mainly deep sea sediments with anoxic tendencies, ideal for specific fossilizations (PAUCĂ, 1979). In this nappe, sedimentation started in the Early Cretaceous with the black schist, followed by Upper Cretaceous deposits. At a small scale in the Vrancea half-window, the Eocene sediments are part of the Coza anticlinoria, situated in the southern area of the Putna Valley. DUMITRESCU (1948) divided this anticlinorium into six distinct anticlines. The Oligocene sedimentary series (Fig. 3) starts with the menilitic lithofacies divided by ATANASIU (1943) in two "terms" (members): a lower one that is now named Lingurești Member (GRASU, 1988) and the upper one, the Compact Menilitic Member. The succession continues with the Bituminous Marls Formation, which DUMITRESCU (1952) and GRASU et al. (1988) described as an excellent cartographic marker for the Oligocene in the Tarcău and Vrancea nappes. It is well known that in several studies (CIOBANU 1977, GRASU et al. 1988, 2004), this unit is also called the 'Bituminous Brown Marls Formation', associated mostly with the marls from the northern region of the Tarcău and Vrancea nappes. The Lower Dysodilic Formation marks a series of episodic alternating regressions and transgressions, followed by a subsidence of the basin bottom that allowed a high stand sedimentation represented by the Kliwa lithofacies (ȘTEFĂNESCU et al., 2006). The Oligocene sedimentation is ending with the Upper Menilitic and Dysodilic Formation. BĂNCILĂ (1958) considered that the whole Oligocene comprises bituminous facies with rare flysch interbeddings, mostly to the top of this succession. Due to the erosion, the Miocene sedimentation is represented in the first stage particularly by clays associated with arenites, silts and rudites, and with olistoliths (IONESI & BOGATU, 1986).

The fossil fish specimens were collected between 2012 and 2014 by the senior author during several field campaigns. The outcrop is situated on the left bank of the Coza River, almost 3.5 km upstream from the Coza Village limits. Starting from this village towards the outcrop, Miocene and Oligocene deposits are visible. Even though there are complex folds and faults, Oligocene sediments can be easily distinguished due to their mineralization, composition and emplacement. Unlike the other Oligocene formations in the area, the Bituminous Marls Formation outcrop is not crossed by the stream as a transversal section, but is parallel with the stream, which is following the beds direction.

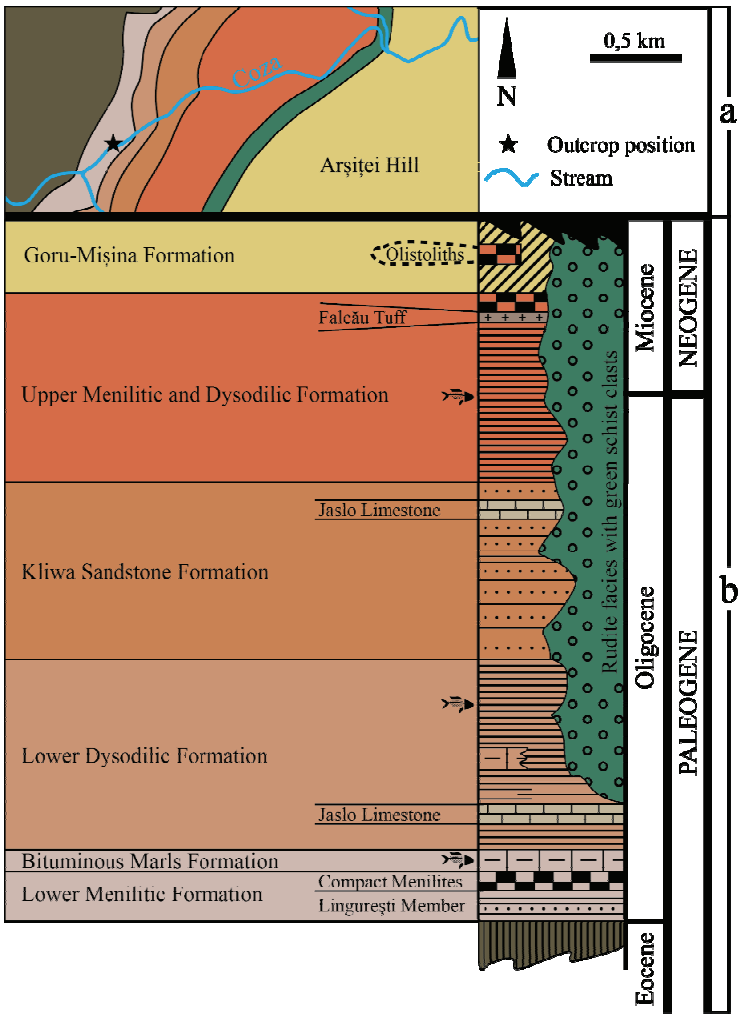

Figure 3. a. Simplified geological map of the studied area (based on DUMITRESCU, 1952),
b. lithostratigraphic column of the Oligocene deposits from the Marginal Folds Nappe (based on GRASU et al., 1988).

MATERIAL AND METHODS

Preparation: Most of the fishes were found articulated and isolated on the bedding surfaces, devoid of other associated fossils. Some of the specimens were found without excess of covering matrix. However, the specimens that needed sediment removal were prepared using various tools, such as needles and scalpels, a rotary rubbing tool was used in some cases (commercial Dremel^{TM}). The preparation was done under the magnification of a binocular stereo microscope (Carl Zeiss Jena). The fish fossils presented herein are inventoried in the collection of the Paleontology-Stratigraphy Museum from the "Babeş-Bolyai" University of Cluj Napoca (V547-V560, 23936).

SYSTEMATIC PALEONTOLOGY

Class **Chondrichthyes** Goodrich, 1909
Subclass **Elasmobranchii** Bonaparte, 1838
Order **Lamniformes** Berg, 1940
Family **Cetorhinidae** Gill, 1862
Genus ***Keasius*** Welton, 2013
Keasius parvus **(**Leriche, 1908)
Plate I - 1
1977 *Cetorhinus parvus* Leriche; CIOBANU, p. 43, pl. VI, fig. 1, 2, 3;
1991 *Cetorhinus parvus* LerichE; PHARISAT, p. 24, fig. 9;
2013 *Keasius parvus* (Leriche); WELTON, p. 39.
Material: one gill raker (V547).
Diagnosis: according to HOVESTADT & HOVESTADT EULER (2012) and WELTON (2013).
Description: The specimen is represented by a complete imprint that preserves two parts of the gill raker, the base and a medial part of the filament. The length of the filament starting from curvature of the base to its apex is 6.3 cm. The

filament base is strongly curved and has well-formed distal protuberance. Bight shape is rounded and has an intermediate width. Medial process length is longer than basal length. Mesial edge of medial process is rounded. Basal edge of the base is rounded and it forms with the mesial edge a sub-angular basal shape. The vascular foramens of attachment surface are present on the entire length of the mesial curvature of the gill raker base. Starting from these foramens, several fine parallel edges are visible on the entire length of the specimen.

Subdivision **Teleostei** *sensu* Patterson & Rosen, 1977
Order **Clupeiformes** *sensu* Grande, 1985
Family **Clupeidae** Cuvier, 1817
Genus ***Sardinella*** Valenciennes, 1847
Sardinella sardinites (Heckel, 1850)
Plate I – 2
1850 *Meletta sardinites* HECKEL, p. 227, pls 23, 24;
1934 *Clupea longimana* (HECKEL); PAUCĂ, p. 601, pl. I, figs 3a, 3b, pl. V, figs 2, 5;
1934 *Clupea sardinites* (HECKEL); PAUCĂ, p. 603, pl. I, fig. 1;
1980 *Sardinella sardinites* (HECKEL); DANIL'CHENKO, p. 9;
1985 *Sardinella sardinites* (HECKEL); GRANDE, P. 322;
1991 *Clupea sardinites* (HECKEL); PHARISAT, p. 27, figs 13, 14, 15;
2006 *Sardinella sardinites* (HECKEL); CARNEVALE et al., p. 686, figs 4.1, 4.2.
Material: three incomplete specimens (V551, V552, V553).
Diagnosis: according to DANIL'CHENKO (1980) and GRANDE (1985).
Description: Vertebrae: 43-46; D: 17-19; A: 17; P: 15-17; V: 9.
Body it is elongated and laterally compressed. The head is relatively large. The diameter of the orbit it is smaller than the preorbital distance. The vertebral column consists of 43-46 vertebrae. The dorsal fin it is inserted approximately in the middle of the body. Anal fin is located posteriorly on the body. Cycloid scales are visible.

Order **Argentiniformes** Johnson & Patterson, 1996
Family **Argentinidae** Bonaparte, 1846
Genus ***Glossanodon*** Guichenot, 1867
Glossanodon musceli (Paucă, 1929)
Plate I – 3, 6
1934 *Nemachilus musceli* Paucă, p. 598, pl. II, figs 1, 2;
1967 *Glossanodon musceli* (Paucă); JERZMAŃSKA, p. 200, text-figs 2, 4, 6, 8, 10, pl.I, figs 1, 2;
2011 *Glossanodon musceli* (Paucă); GREGOROVÁ, p. 8, pl. III, fig. 1.
Material: six incomplete specimens (V548, V549, V550, V553, V555, V559 and three unnumbered specimens).
Diagnosis: according to JERZMAŃSKA (1967).
Description: Vertebrae: 45-46; D: 11-12; A: 16-18; P: 17-18; V: 10-12.
Small sized fishes. The body is elongated and slender. The head is almost triangular in shape. The diameter of the orbit is relatively big. The premaxilla is elongated. No teeth on the premaxilla and dentary. The dorsal fin it is inserted approximately in the middle of the body. The anal fin it is placed posteriorly to the last ray of the dorsal fin.

Order **Gadiformes** *sensu* Endo, 2003
Family **Merlucciidae** Adams, 1864
Genus ***Palaeogadus*** von Rath, 1859
Palaeogadus sp.
Plate I – 4
Material: one specimen poorly preserved (V554).
Diagnosis*:* according to DANIL'CHENKO (1960) and JERZMAŃSKA (1968).
Description: Vetebrae: 42; DI: 9, DII: 31-32.
Body it is elongated and laterally compressed. The head it is large; morphology of the cranial region it is not visible because of the preservation state. The vertebral column contains 42 vertebrae; relatively long, robust and aciculated parapophyses are visible on the abdominal vertebrae but the preservation state does not allow us to establish the precise number. Two dorsal fins. The anal fin it is poorly preserved. The caudal peduncle is moderately deep.

Order **Gasterosteiformes** Gill, 1872
Family **Centriscidae** Rafinesque, 1826
Genus ***Aeoliscus*** Jordan & Starks, 1902
Aeoliscus sp.
Plate I – 5
Material: one poorly preserved specimen (V560).
Diagnosis: according to DANIL'CHENKO (1960).

Description: Wedge-shaped small fishes with elongated body. The length of the head is bigger than the body height. The snout is long and tubular. The body is almost totally covered with thin bony shields. Dorsal shields are extended down to the middle line of the body. There are 15 visible vertebrae. First dorsal spine it is long and pointed. Soft dorsal and anal fins are poorly preserved. Caudal fin it is small.

Order **Perciformes** Bleeker, 1859
Percoidei *incertae familiae*
Genus ***Oliganodon*** Bannikov, 2010
Oliganodon budensis (Heckel, 1856)
Plate I – 6
1856 *Smerdis budensis*, HECKEL, p. 264, pl. XI, fig. 16;
1960 *Serranus budensis (*HECKEL); DANIL'CHENKO, p. 101, fig. 21, pl. XXV, fig. 4;
1991 *Serranus budensis* (HECKEL); PHARISAT, p. 44, figs 32, 33;
2010 *Oliganodon budensis* (HECKEL); BANNIKOV, p. 86, pl. VII, figs 2, 3.
Material: one incomplete specimen (V555).
Diagnosis: according to BANNIKOV (2010).
Description: Vertebrae: 24; D: X+10; A: III+9; P: 16; V: I+5.
Body elongated, laterally compressed. Head length is bigger than body height. The orbits are large. The preopercle is serrated on the ventral and posterior margins. Two small spines are visible on the opercle. The vertebral column is composed from 24 vertebrae from which ten are abdominal. Dorsal fin is continuous. The anal fin insertion is located below the first dorsal soft rays. The caudal fin contains 17 principal rays.

Suborder **Scombroidei** Bleeker, 1859
Family **Trichiuridae** Rafinesque 1810
Genus ***Anenchelum*** Blainville, 1818
Anenchelum glarisianum Blainville, 1818
Plate I – 7, 8
1818 *Anenchelum glarisianum*, Blainville, p. 314;
1901 *Lepidopus glarisianus* (Blainville); WOODWARD, p. 477;
1977 *Lepidopus glarisianus* (Blainville); CIOBANU, p. 119, pl. XL, fig. 1;
1977 *Anenchelum glarisianum* Blainville; CIOBANU, p. 120, pl. XLI, figs 1, 2;
2011 *Anenchelum glarisianum* Blainville; GREGOROVÁ, p. 17, pl. VI, figs 1, 2.
Material: three incomplete specimens and one almost complete (V557, V558, V559, 23936).
Diagnosis: according to JERZMAŃSKA (1968).
Description: Body elongated and laterally compressed. Head is triangularly shaped; the length of the head it is bigger than the body depth. Two canine-like teeth followed by small conical teeth are present on the upper maxilla. The vertebrae are elongated; 116 vertebrae are visible on the specimen no. 23936. The dorsal fin is continuous almost on the total length of the body; 33-35 spines and are visible. The anal fin is poorly preserved. The pectoral fins are composed from about 12-13 soft rays each.

CONCLUDING REMARKS

In 2012, a new outcrop exposing the Bituminous Marls Formation of Early Oligocene (Rupelian) age, belonging to the Vrancea Nappe (Eastern Carpathians of Romania) was identified nearby to the Coza locality.

The fish fossil specimens discovered from this outcrop are referred to known taxa in this region, already reported from the same formation of the Piatra Neamț area as well as from several Early Oligocene deposits from the Central and Eastern Paratethys and include the following species: *Keasius parvus* (Leriche, 1908), *Sardinella sardinites* (Heckel, 1850), *Glossanodon musceli* (Paucă, 1929), *Palaeogadus* sp., *Aeoliscus* sp., *Oliganodon budensis* (Heckel, 1856), *Anenchelum glarisianum* Blainville, 1818.

Based on the interpolation method proposed by GAUDANT (1979) with the distribution of the actual taxa and the data indicated by COMPAGNO (2002) and NELSON (2006), the identified fish fossil assemblage probably indicate the following bathymetric distribution: *Keasius* and *Sardinella* – shallow waters, *Glossanodon*, *Anenchelum* and *Paleogadus* – benthopelagic waters, *Oliganodon* and *Aeoliscus* – continental shelf and coastal waters. Presently, no typical meso- and bathypelagic fish species were discovered in the Coza outcrop, although they are common in the Bituminous Marls Formation from the northern part of the Marginal Folds Nappe (Piatra Neamț area).

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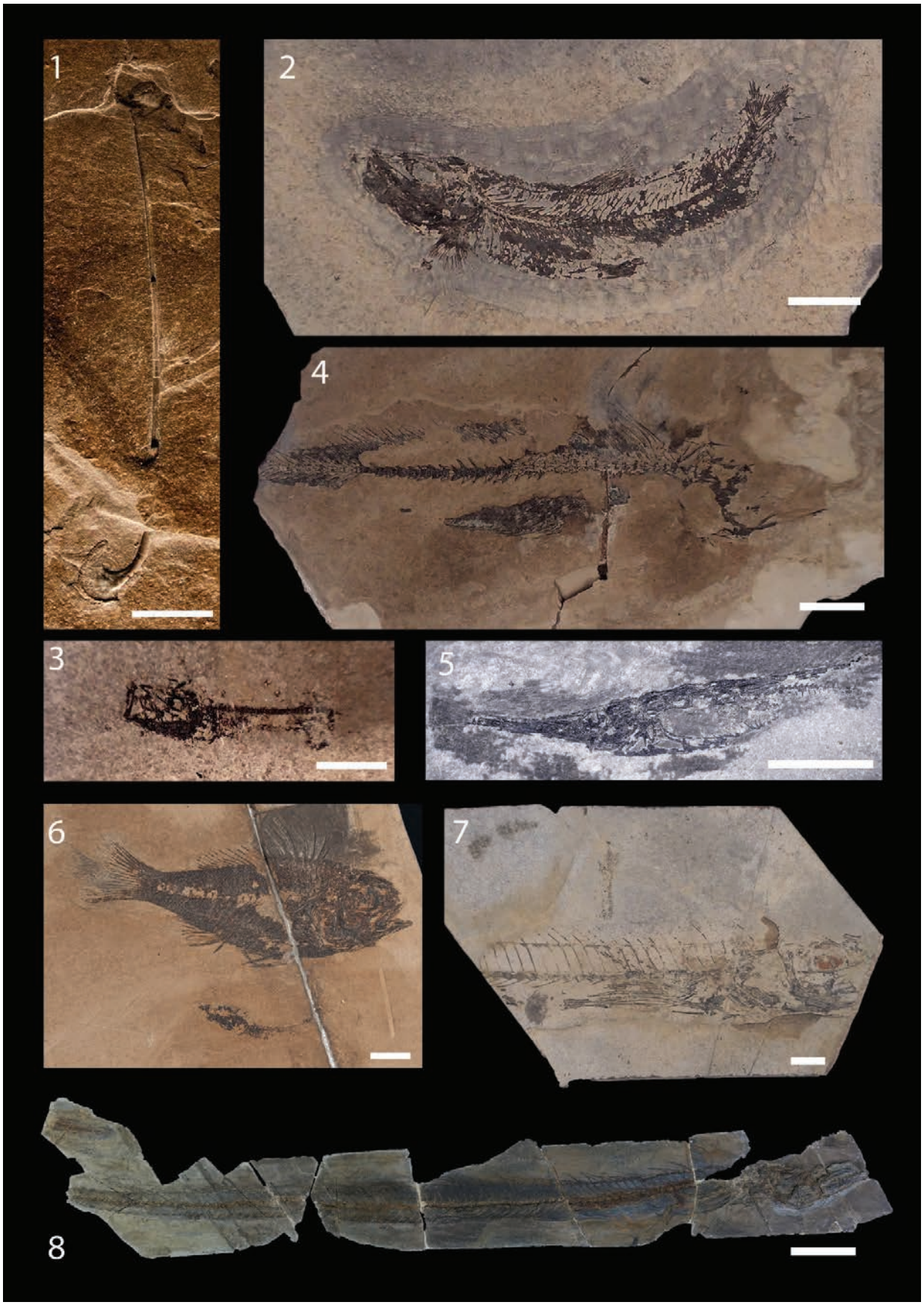

Plate I: 1 - *Keasius parvus* (V547), **2** - *Sardinella sardinites* (V551), **3** - *Glossanodon musceli* (V550), **4** - *Paleogadus* sp. (V554), **5** - *Aeoliscus* sp. (V560), **6** - *Oliganodon budensis* and *Glossanodon musceli* (V555), **7** - *Anenchelum glarisianum* and *Glossanodon musceli* (V559). Scale bar: 1 cm. **8** - *Anenchelum glarisianum* (23936). Scale bar: 10 cm.

OPPELID AND HAPLOCERATID AMMONITES FROM THE UPPER JURASSIC DEPOSITS OF HĂGHIMAŞ MTS. (THE EASTERN CARPATHIANS – ROMANIA)

GRIGORE Dan

Abstract. This paper deals with the taxonomic study of the oppeliidas (Streblitinae) and haploceratidas from the Kimmeridgian-Tithonian deposits from Ghilcoș and Ciofronca (Hăghimaș Mts). Fore species of Streblitinae are now described in addition to those known to date from these localities and haploceratidas previously described by PREDA (1973) are reviewed. For the first time species belonging to *Semiformiceras* Spath, 1925 are described from the studied region.

Keywords: Oppeliidae**,** Haploceratidae, paleontology, Hăghimaș, Carpathians, Romania.

Rezumat. Oppelide și haploceratide din depozitele jurasic superioare din Munții Hăghimaș (Carpații Orientali – România). În lucrare este prezentat studiul taxonomic al speciilor din Familia Oppeliidae – Subfamilia Streblitinae și Familia Haploceratidae găsite în depozitele kimmeridgian-tithoniene din Ghilcoș și Ciofronca (Munții Hăghimaș). Sunt descrise acum alte patru specii de Streblitinae pe lângă cele cunoscute până în prezent din aceste aflorimente și, revizuite haploceratidele prezentate anterior de PREDA (1973). Pentru prima dată sunt descrise din regiune specii de *Semiformiceras* Spath, 1925.

Cuvinte cheie: Oppeliidae**,** Haploceratidae, paleontologie, Hăghimaș, Carpați, România.

INTRODUCTION

The described ammonite fauna comes from the "Acanthicum Beds" of Lacu Roșu (Ghilcoș/Ucigașu and Ciofronca) area, Haghimaș Massif - Eastern Carpathians (Fig. 1). Outcrops and Upper Jurassic litho- and bio-stratigraphy of this region have been previously described (GRIGORE et al., 2009 and GRIGORE, 2011) in detail.

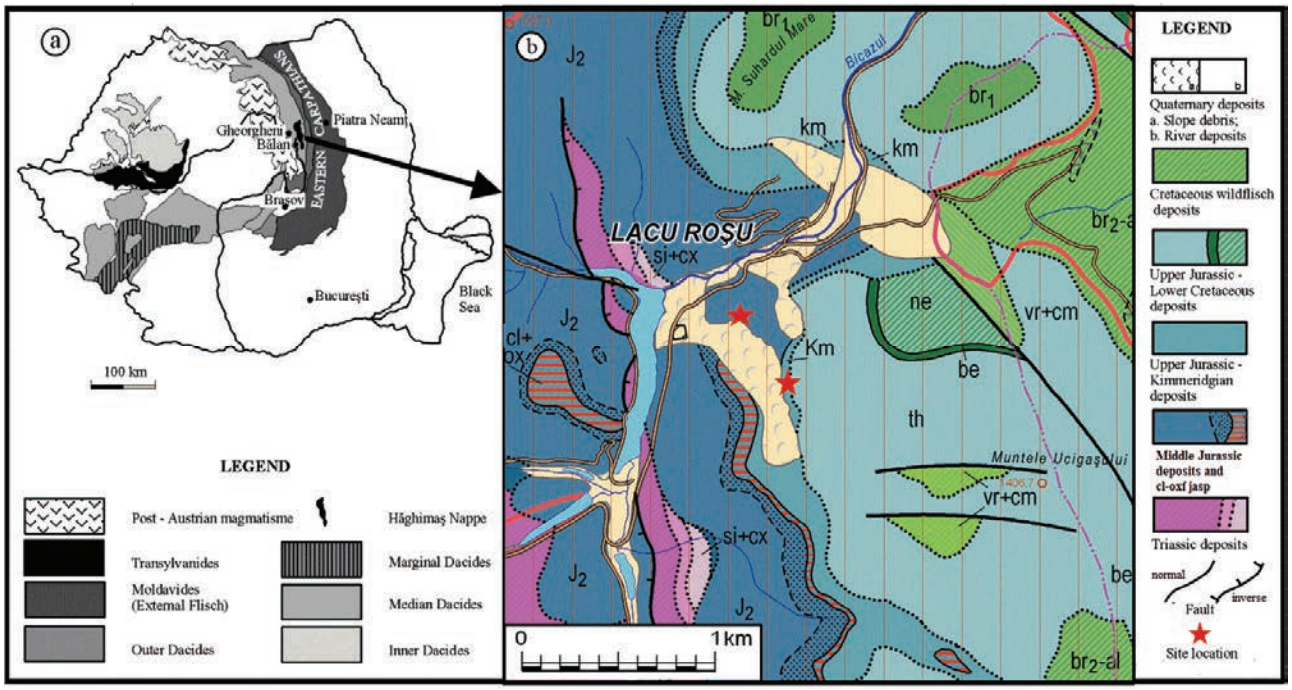

Figure 1. Localization of sites: a) in Romania - geotectonic sketch (after SĂNDULESCU, 1984); b) on the geological map scale 1: 50,000 (after SĂNDULESCU al. 1975, revised GHEUCA & GRIGORE, 2010).

The species described here belong to the families Oppeliidae Douvillé (Subfamily Streblitinae Spath) and Haploceratidae Zittel. The Oppeliidae family is well represented in the region by number of species and specimens, on the entire interval Kimmeridgian - Early Tithonian; the representatives of Taramelliceratinae are the most abundant, while those of Streblitinae were less known until now. From the previous papers of NEUMAYR (1873), HERBICH (1878) and PREDA (1973) the species *Streblites tenuilobatus* (Oppel, 1863) and *Creniceras dentatum* (Reinecke, 1818) only were reported from the Kimmeridgian of this area, while Tithonian streblitid occurrences remain unknown yet (Fig. 2).

Discussions over the last 20 years on the sexual dimorphism of ammonites have led to the attempt to join under the same species name dimorphs previously ascribed to as different genera or even subfamilies. For example, among the

Kimmeridgian oppeliids the following taxa were synonimized in such a manner: *Taramelliceras compsum* (Oppel) with *Glochiceras crenosum* (Quenstedt) and *Streblites weinlandi* (Oppel) with *Creniceras dentatum* (Reinecke) (BAUDOUIN & AL, 2011).

As Wierzbowski shows (WIERZBOWSKI et al., 2010), in some cases, this review is premature, with no detailed ontogenetic and extensive biostratigraphic analysis; synonimization of morphospecies in some unreal biological species can also cause biostratigraphic problems. For this reason I used the old classification in some cases, but with the mention in the chapter of synonymy the modification brought by newer authors.

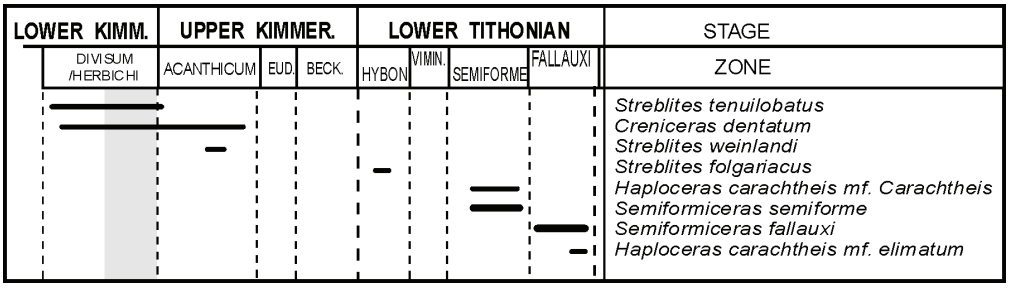

Figure 2. Stratigraphic distribution of studied taxa through these sections.

MATERIAL AND METHOD

The analysed material belongs to the author's collection, deposited in the National Geological Museum (MNG - GIR, Geological Institute of Romania) - Bucharest. Also, the opelids and haploceratides specimens from the collections of the University of Bucharest and the Museum of Natural Sciences in Piatra Neamț belonging to Professor Ion PREDA were analyzed. All specimens described by previous authors (NEUMAYR, 1873, HERBICH, 1878, PREDA, 1973) are reviewed here to complete the picture of the association existing in this region.

The determination of the species used the method of comparison with the specimens known in the literature, mentioned in synonymy. The specific determination was made especially related to the holotype (or other type specimens), comparing the morphometric parameters and the morphology.

SYSTEMATICS

Abbreviations for the measurements, collections and outcrops:

Dmax = maximal diameter
Dph = phragmocone diameter
D = measured diameter
U = diameter of umbilicus
H = whorl height
W = whorl width

GIR = Geological Institute of Romania
GBA = Geological Institute of Austria (Bundesanstalt)
UBB = "Babeș Bolyai" University from Cluj Napoca
LGB = Geology Laboratory of Bucharest University
MPN = Museum of Natural Sciences - Piatra Neamț

F1 = Outcrop from western Ghilcoș walls
F2 = Outcrop from north-western Ghilcoș slope
F17 = Outcrop from "Ciofronca"; all in GRIGORE et al., 2009
A, D… K = studied sections (GRIGORE, 2002, 2011)

Family Oppeliidae Douvillé 1890
Subfamily Streblitinae Spath 1925
Genus *Streblites* Hyatt 1900
Streblites tenuilobatus (Oppel 1857)
Pl. 1, Figs. 8, 11, 12

1857 *Ammonites tenuilobatus* - OPPEL; p. 388.
1863 *Ammonites tenuilobatus* Oppel - OPPEL; p. 160; Pl. 50, Fig. 1.
1876 *Oppelia tenuilobata* Oppel - LORIOL; p. 29; Pl. 2, Fig. 5.
1877 *Ammonites* (*Oppelia*) *tenuilobatus* Oppel - FAVRE; p. 26, Pl. 2, Fig. 5.
1879 *Oppelia tenuilobata* Oppel - FONTANNES; p. 22; Pl. 3, Fig. 5.
1888 *Ammonites pictus* - QUENSTEDT; Pl. 119, Figs. 12, 13, 15, 17.
1929 *Streblites tenuilobatus* Oppel - WEGELE; p. 11; Pl. 1, Figs. 8, 9.
1973 *Oppelia* (*Streblites*) *tenuilobatus* Oppel - PREDA; Pl. 10, Fig. 5.
1973 *Oppelia* sp. - PREDA; Pl. 7, Fig. 3.
1978 *Streblites tenuilobatus* (Oppel) - OLORIZ; p. 44; Pl. 4, Figs. 2, 3.
1979 *Streblites tenuilobatus* (Oppel) - SAPUNOV; p. 62; Pl. 14, Figs. 1 a, b.

Material: LRp212A8, LRp210A2, LRp76K6, LRp213A3, LR81A, LRp80A2; *S.* cf. *tenuilobatus*: LRp356T Grigore Collection in GIR. Neumayr's specimen (1973): Collection of GBA – collected by Herbich from red nodular limestones – Ghilcoș outcrop (F1). Herbich's specimen (1878): Collection of UBB; originates from red nodular limestones – Ciofronca outcrop (F17). Preda's specimens (1973): Collection of MPN inv. 5MPN (Pl. 10, Fig. 5); originates from grey nodular limestones – Ghilcoș outcrop (F2); *Oppelia* sp. (Pl. 7, Fig. 3) originates from red nodular limestones of Ghilcoș outcrop (lost).

Table 1. Measurements of *Streblites tenuilobatus* (Oppel 1863) specimens.

Specimen	Dmax	Dph	D	U	H	W	U/D	H/D	W/D	W/H
Lectotype	80	70	80	6	44	20	0.07	0.55	0.25	0.45
Preda 5MPN specimen	95	95	95	6	56	17	0.06	0.59	0.18	0.30
LRp212A8	42	38	42	3	24	9?	0.07	0.57	0.21	0.37

Remarks: The specimen of the Preda collection (5MPN) is a large phragmocone, undeformed and well-preserved, showing all morphological and morphometric characters comparable to the holotype (Table 1). My specimens are fragments of different sizes (LRp212A2, LRp210A2 are two juveniles) and in various conservation states; thus most of the specimens conserve partially the characteristic ornamentation, the internal ribs and the medio-lateral tubercles being hardly observable. The specimens could be determined mainly on the morphological characteristics of the outer half of the flanks, and on the whorl section and the morphology of the ventral side.

Occurrence: Early Kimmeridgian – Divisum Zone in Ghilcoș outcrops (A, B, D profiles); Early Kimmeridgian in Europe (Italy, Spain, Switzerland, Bulgaria, France and Germany).

Streblites weinlandi (Oppel 1863)
Pl. 1, Fig. 10

1863 *Ammonites weinlandi* nov.sp. - OPPEL; p. 198; Pl. 53, Fig. 1.
1879 *Oppelia levipicta* (Fontannes) - FONTANNES; p. 22; Pl. 3, Figs. 3, 4.
1929 *Streblites levipictus* Fontannes - WEGELE; p. 13; Pl. 25, Fig. 13.
?1978 *Streblites weinlandi* (Oppel) - *levipictus* (Fontannes) - OLORIZ; p. 47; Pl. 4, Figs. 1 a, b.
1983 *Streblites weinlandi* (Oppel) - SANTANTONIO; p. 152; Pl. 1, Fig. 3.
2011 *Streblites weinlandi* (Oppel) Morph *weinlandi* [M] (= Macroconch) - BAUDOUIN et al.; p. 634; Pl. 1, Fig. 8; Pl. 2, Fig. 1; Pl. 9, Figs. 1-8; Pl. 10, Figs. 1-5.

Material: LRp82D2 Grigore Collection in GIR.

Table 2. Measurements of *Streblites weinlandi* (Oppel 1863) specimens.

Specimen	Dmax	Dph	D	U	H	W	U/D	H/D	W/D	W/H
Lectotype	74	-	60	4	35	12	0.07	0.58	0.20	0.34
LRp82D2	51	51	51	3	30	13	0.06	0.59	0.25	0.43

Remarks: my specimen is a poorly preserved fragmocon, but with the distinctive characters of the species (Table 2).

Occurrence: Late Kimmeridgian-Acanthicum Zone in Ghilcoș outcrops (A, B, D profiles); Kimmeridgian - Strombecki - Compsum interval in Spain; Early Kimmeridgian in Italy, Germany, France.

Streblites folgariacus (Oppel 1863)
Pl. 1, Fig. 9

1863 *Ammonites Folgariacus* - OPPEL; p. 199; Pl. 54, Figs. 6 a, b.
1959 *Streblites folgariacus* (Oppel) - HOLDER & ZIEGLER; p. 202; Pl. 22, Fig. 4.
1978 *Streblites folgariacus* (Oppel) - OLORIZ; p. 52; Pl. 4, Fig. 6.
1993 *Streblites folgariacus* (Oppel) - SARTI; p. 69; Pl. 4, Figs. 6 a, b.
1994 *Streblites folgaricus* (Oppel) - FOZY et al.; Pl. 1, Figs. 2, 6, 7, 8.

Material: LRp222G12 Grigore Collection in GIR.

Remarks: my specimen (LRp222G12) is a small phragmocone, deformed in silty deposits. It preserves very well the specific ornamentation (ribs, tubers and keel), as well as the whorl section shape.

Occurrence: Early Tithonian – Hybonotum Zone in Ghilcoș outcrops (G profile); Late Kimmeridgian - Early Tithonian – Beckeri – Hybonotum interval in Europe (Switzerland, Germany, Italy, Spain, Hungary, Poland).

Genus *Creniceras* Munier Chalmas 1892
Creniceras dentatum (Reinecke 1818)
Pl. 1, Figs. 1, 2, 3.

1818 *Ammonites dentatus* - REINECKE; p. 73; Pl. 4, Figs. 43, 44.
1876 *Ammonites* (*Oppelia*) *dentatus* Reinecke - LORIOL; p. 46; Pl. 5, Figs. 4, 5.
1877 *Ammonites* (*Oppelia*) *dentatus* Reinecke - FAVRE; p. 57; Pl. 2, Fig. 4.
1879 *Oppelia dentata* Reinecke - FONTANNES; p. 52; Pl. 7, Fig. 10.
1958 *Creniceras dentatum* (Reinecke) - ZIEGLER; Pl. 11, Fig. 28.
1973 *Oppelia* (*Taramelliceras*) *dentata* Reinecke - PREDA; Pl. 7, Fig. 5.
1978 *Creniceras dentatum* (Reinecke) - OLORIZ; p. 119.
2011 *Streblites weinlandi* (Oppel) Morph *dentatum* [m] (= microconch) - BAUDOUIN et al.; p. 638; Pl. 1, Figs. 5, 6; Pl. 10, Figs. 6-14.

Material: LRp190R1, LRp184A2, LRp188A, LRp186D3, LRp504X5, LRp353T3,0, LRp448A2, LRp214A Grigore Collection in GIR. Preda's specimen (1973): was in the inventory of MPN Collection (lost or removed) – originates from red nodular limestones of Ghilcoș outcrop.

Table 3. Measurements of *Creniceras dentatum* (Reinecke, 1818) specimens.

Specimen	**Dmax**	**Dph**	**D**	**U**	**H**	**W**	**U/D**	**H/D**	**W/D**	**W/H**
Loriol (1876; fig.4) specimen	22	-	22	5	9	6	0.23	0.41	0.27	0.67
LRp190R1	16.5	15.5	16.5	3	8	4.5	0.18	0.48	0.27	0.56
LRp184A2	22	-	22	6	9	5	0.27	0.41	0.23	0.55
LRp188A	21	-	21	5	9	5.5	0.24	0.43	0.26	0.61
LRp214A	25,2	13.3	20	4.4	9.2	6	0.22	0.46	0.30	0.65

Remarks: the LRp190R1 specimen only preserves the phragmocone, the other specimens have and the living chamber, only one preserves the apophyses (LRp214A); all exhibit specific morphological and morphometric parameters (Table 3).

Occurrence: Late Kimmeridgian-Acanthicum Zone in Ghilcoș outcrops (R, B, D profiles); Acanthicum /Compsum Zone in Spain, France, Switzerland and Germany.

Genus *Semiformiceras* Spath, 1925 emended Oloriz 1978
Semiformiceras semiforme (Oppel 1865)
Pl. 1, Fig. 5

1865 *Ammonites semiformis* - OPPEL; p. 547.
1870 *Oppelia semiformis* Oppel - ZITTEL; p. 59; Pl. 4, Figs. 7, 8.
1973 *Semiformiceras semiforme* (Oppel) - ENAY & GEYSSANT; p. 43.
1978 *Semiformiceras semiforme semiforme* (Oppel) - OLORIZ; p. 67; Pl. 3, Figs. 5, 6.
1984 *Semiformiceras semiforme* (Oppel) - SARTI; p. 494; Pl. 1, Fig. 7.
1988 *Semiformiceras semiforme* (Oppel) - FOZY; p. 46; Pl. 1, Figs. 1 – 4.
1991 *Semiformiceras semiforme* (Oppel) - ENAY & CECCA; p. 56; Pl. 2, Figs. 18, 19.
1994 *Semiformiceras semiforme* (Oppel) - FOZY et al.; Pl. 1, Figs. 19-21.
1994 *Semiformiceras semiforme* (Oppel) - ZEISS et al.; Pl. 1, Fig. 3.

Material: LRp512aK42, LRp512bK42, LRp484K41 Grigore Collection in GIR.

Table 4. Measurements of *Semiformiceras semiforme* (OPPEL, 1865) specimens.

Specimen	**Dmax**	**Dph**	**D**	**U**	**H**	**W**	**U/D**	**H/D**	**W/D**	**W/H**
Lectotype	73	52	73	5	36	-	0.06	0.49	-	-
Paratype	67	-	67	4	32	18	0.06	0.48	0.27	0.56
LRp512aK42	40	~29	31	3	16	~9	0.09	0.51	0.30	0.56

Remarks: the LRp512aK42 specimen is small and has an elliptical shape; preserves a part of the living chamber, which shows the beginning of the specific ventral groove. The specimens LRp484K41 and LRp512bK42 are conch halves that partially preserves the specific ornamentation (Table 4).

Occurrence: Early Tithonian-Semiforme /Verruciferum Zone in Ghilcoș outcrops (K profile); Early Tithonian - Semiforme /Verruciferum Zone in Europe (Italy, Spain, Germany, France) and Russian Far East (SEY & KALACHEVA, 1997).

Semiformiceras fallauxi (Oppel 1865)
Pl. 1, Fig. 4

1865 *Ammonites fallauxi* - OPPEL; p. 547.
1870 *Oppelia fallauxi* Oppel - ZITTEL; p. 61; Pl. 4, Figs. 4 – 6.
1973 *Semiformiceras falllauxi* (Oppel) - ENAY & GEYSSANT; p. 44.
1978 *Semiformiceras falllauxi* (Oppel) - OLORIZ; p. 74; pl.
1991 *Semiformiceras fallauxi* (Oppel) - ENAY & CECCA; p. 54; Pl. 2, Figs. 11 – 17.

Material: LRp483K44, LRp526K43 Grigore Collection in GIR.

Table 5. Measurements of *Semiformiceras fallauxi* (Oppel 1865) specimens.

Specimen	Dmax	Dph	D	U	H	W	U/D	H/D	W/D	W/H
Lectotype	32	23	32	9.5	12.5	9	0.30	0.39	0.28	0.72
LRp483K44	33	25	33	9	14	›7	0.27	0.42	›0.21	›0.50
LRp526K43	25	23	23	5	11	7	0.22	0.48	0.30	0.64

Remarks: the LRp483K44 specimen is medium-sized and elliptically coiled; it preserves a part of the living chamber, which shows the beginning of the ventral groove and the specific ornamentation. The LRp526K43 specimen is smaller in size and preserves the beginning of the living chamber with the specific ornamentation (Table 5).

Occurrence: Early Tithonian-Fallauxi Zone in Ghilcoş outcrops (K profile); Early Tithonian-Fallauxi Zone in Europe (Italy, Spain, Germany, France and Crimea).

Family HAPLOCERATIDAE Zittel (1884) emended Callomon 1981
Genus *Haploceras* Zittel (1870) emended Enayi & Cecca 1986
Haploceras (*Haploceras*) *carachtheis* (Zeuschner 1846)
morphotype *carachtheis* (Zeuschner 1846)
Pl. 1, Fig. 6

1846 *Ammonites carachtheis* - ZEUSCHNER; Pl. 4, Fig. 1; refigured in ZITTEL (1868) Pl. 15, Figs. 1-3.
1962 *Glochiceras carachtheis* (Zeuschner) - BARTHEL; p. 17; Pl. 2, Figs. 1-4, Pl. 3, Figs. 1-7.
*non*1973 *Haploceras carachteis* Zeuschner - PREDA; Pl. 17, Fig. 13 (= *Aspidoceratidae*).
1976 *Haploceras* (*Neoglochiceras*) *carachtheis* (Zeuschner) - AVRAM; p. 168; Pl. 3, Fig. 8.
1986 *Haploceras* (*Haploceras*) *carachtheis* (Zeuschner, 1846)(m) - ENAY & CECCA; p. 49; Pl. 3, Fig. 1.
1991 *Haploceras* (*Haploceras*) *carachtheis* (Zeuschner, 1846) - CECCA & ENAY; p. 43; Pl. 1, Figs. 8 a, b.
1994 *Haploceras carachtheis* (Zeuschner) - FOZY et al.; Pl. 1, Figs. 16, 22.
1994 *Haploceras* (*Hypoglochiceras*) *carachtheis* (Zeuschner) - ZEISS et al.; p. 370; Pl. 2, Fig. 3.
1995 *Haploceras carachtheis* (Zeuschner, 1846) - FOZY; p. 136; Pl. 20, Fig. 12.

Material: LRp507K42, LRp482K41 Grigore Collection in GIR.

Remarks: the LRp507K42 is a whorl fragment from the aperture of a large individual: H> 23. The whorl section is oval, compressed, the ventral side is provided with wrinkles on the last portion of the living chamber. The LRp482K41 specimen is also a smaller fragment with the same characteristics.

Occurrence: Early Tithonian - Semiforme Zone in Ghilcoş outcrops (K profile); Early/Middle Tithonian in Europe.

morphotype *elimatum* (Oppel 1865) in Zittel 1868
Pl. 1, Fig. 7

1865 *Ammonites elimatus* - OPPEL; p. 549.
1868 *Ammonites elimatus* Oppel - ZITTEL; p. 79; Pl. 13, Figs. 1 – 7.
1962 *Haploceras elimatum* (Oppel) - BARTHEL; p. 11; Pl. 1, Figs. 12-17.
1973 *Haploceras subelimatum* Fontannes - PREDA; Pl. 9, Fig. 2.
1986 *Haploceras elimatum* (Oppel)(M) - ENAY & CECCA; p. 50; Pl. 4, Fig. 1.
1994 *Haploceras elimatum* (Oppel) - FOZY et al.; Pl. 1, Fig. 10.
1994 *Haploceras elimatum* Oppel-ZEISS et al.; p. 370; Pl. 1, Fig. 4.
1995 *Haploceras elimatum* (Oppel, 1865) - FOZY; p. 136; Pl. 20, Fig. 9.

Material: LRp525K44 Grigore Collection in GIR. Preda's specimens (1973): Collection of MPN inv. 21aMPN (Pl.10, Fig.5); originates from greenish sandstones (F1) – Ghilcoş outcrop.

Remarks: the specimen of Preda (*Haploceras subelimatum*: Pl. 9, Fig. 2) is of medium size and keeps the living chamber almost undeformed, but with aperture not well preserved; it shows the morphometric parameters almost identical to those of the Oppel species holotype. Morphologically, the species *H. subelimatum* Fontannes, differs from

H. elimatum Oppel by the presence of periombilical ribs (in the periombilical depressed area), which brings him closer to the *Glochiceras* group taxa.

The LRp525K44 specimen is a half-phragmocone of a small individual with morphometric parameters close to those of Oppel's holotype (Table 6).

Table 6. Measurements of *Haploceras* (*Haploceras*) *carachtheis* mph. *elimatum* (Oppel, 1865) specimens.

Specimen	**Dmax**	**Dph**	**D**	**U**	**H**	**W**	**U/D**	**H/D**	**W/D**	**W/H**
Lectotype	145	82	145	35	67	49	0.24	0.46	0.34	0.73
Preda 21aMPN specimen	49	-	43	11	20	14.5	0.25	0.46	0.34	0.72
LRp525K44	26	-	26	6	13	›8	0.23	0.50	0.31	›0.62

Occurrence: Early Tithonian - Fallauxi Zone in Ghilcoș outcrops (K profile); Early and Middle Tithonian in Europe, Russia, Madagaskar, Mexic, etc.

CONCLUSIONS

In conclusion, as mentioned in the introduction, only two species of the Streblitinae subfamily have been known in the region so now there are four other species: *Streblites weinlandi*, *S. folgariacus*, *Semiformiceras semiforme* and *S. fallauxi*. Thus the association and species of other genres, some of the Early Tithonian, have been completed. At the same time, the presence of haploceratids species (both morphotypes of *H. carachtheis carachtheis* and *elimatum*) from Early Tithonian was confirmed in the region, and the specimens mentioned by PREDA (1973) were also reviewed.

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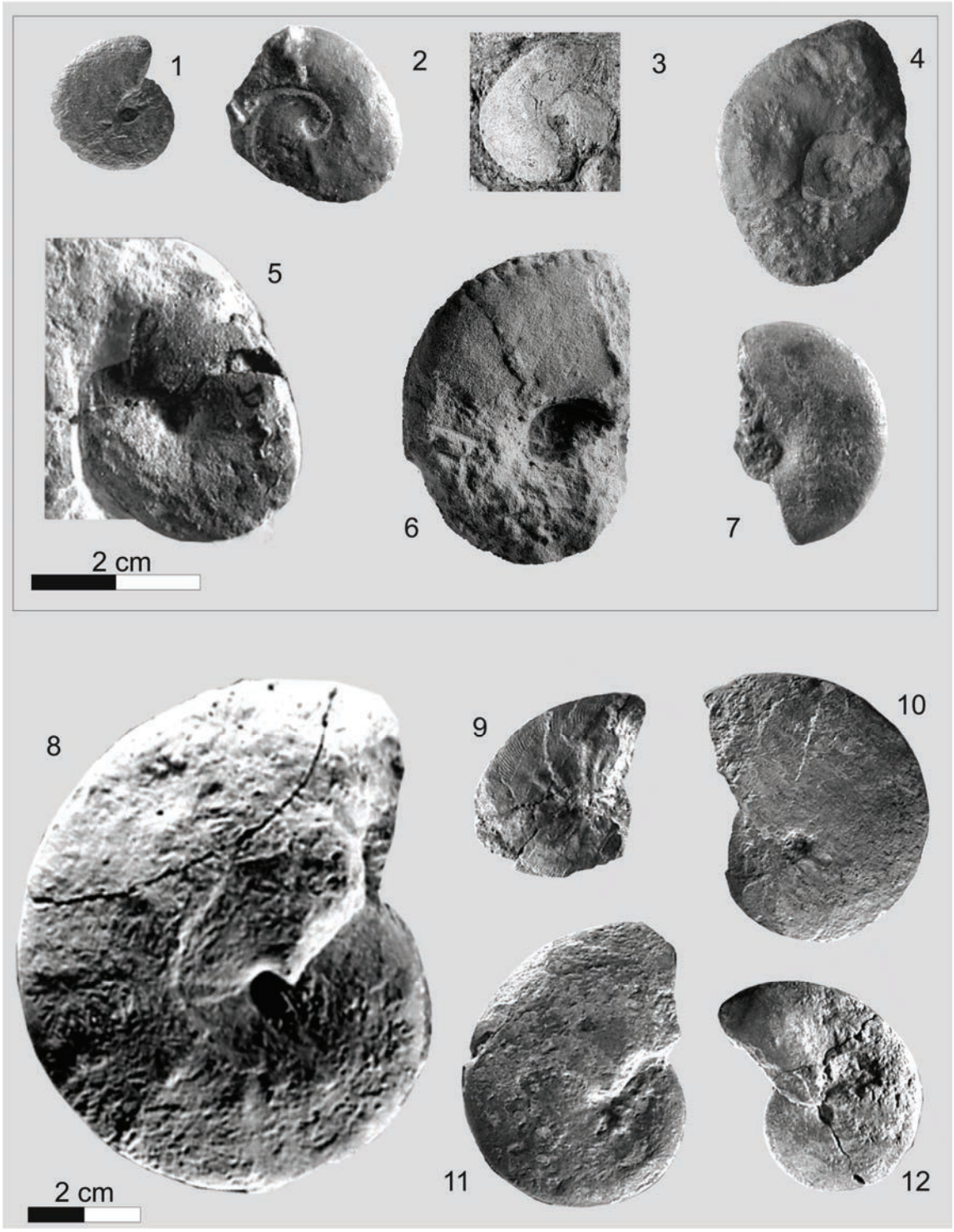
PLATE I



1. *Creniceras dentatum* (Reinecke) (LRp190R1); reddish nodular limestone, Early Kimmeridgian-Divisum Zone, Ghilcoş; 2. *Creniceras dentatum* (Reinecke) (LRp214A), green nodular limestone, Kimmeridgian, Ghilcoş; 3. *Creniceras dentatum* (Reinecke) (LRp504X5); green nodular limestone, Early Kimmeridgian-Divisum Zone, Ghilcoş; 4. *Semiformiceras fallauxi* (Oppel) (LRp483K44); grey-yellowish silts, Early Tithonian-Fallauxi Zone, Ghilcoş; 5. *Semiformiceras semiforme* (Oppel) (LRp512K42); grey-yellowish silts, Early Tithonian-Semiforme Zone, Ghilcoş; 6. *Haploceras carachtheis* (Zeuschner) morphotyp *carachtheis* (Zeuschner) (LRp507K42); yellowish silts, Early Tithonian-Semiforme Zone, Ghilcoş; 7. *Haploceras carachtheis* (Zeuschner) morphotyp *elimatum* (Oppel) (LRp525K44); yellowish silty marls, Early Tithonian-Fallauxi Zone, Ghilcoş; 8. *Streblites tenuilobatus* (Oppel) Preda's specimen-phragmocone; green nodular limestone, Early Kimmeridgian-Strombecki(?) Zone, Ghilcoş; 9. *Streblites folgariacus* (Oppel) (LRp222G12); yellowish silts, Early Tithonian-Semiforme(?) Zone, Ghilcoş; 10. *Streblites weinlandi* (Oppel) (LRp82D2); green nodular limestone, Late Kimmeridgian-Acanthicum Zone, Ghilcoş; 11. *Streblites tenuilobatus* (Oppel) (LRp80A2); green nodular limestone, Early Kimmeridgian-Divisum Zone, Ghilcoş; 12. *Streblites tenuilobatus* (Oppel) (LRp212A8); green nodular limestone, Early Kimmeridgian-Divisum Zone, Ghilcoş.

THE EFFECT OF LIME AS A STABILIZING AGENT IN PLASTIC CLAYEY SOILS IN VILA HILL, DURRES, ALBANIA

MUÇI Redi, FOCIRO Oltion, SKRAME Klodian

Abstract. Soil stabilization through the addition of chemical agents such as lime, Portland cement, kiln dust, fly-ash etc., is dependent on the reaction of various oxides such Al_2O_3, SiO_2, with CaO, in the presence of water, to form chemical compounds such as $3CaO\text{-}2SiO_2\text{-}4H_2O$ (CSH), as well as CAH, CS'H and CAFS'H. These reactions increase the overall strength parameters of the soil. The purpose of this paper is to analyse the effect of lime in stabilizing clayey soils by influencing the plastic behaviour and thus reducing its swell potential, as well as altering its compaction characteristics. The samples were collected along the steep hill of Kodër Vilë in Durrës, Albania, a coastal area known for its problematic and recurring slope failures. Soil properties were determined along with Atterberg limits, maximum dry density and optimum moisture content. An X-ray diffraction (XRD) analysis was performed on the clay samples in order to determine the mineralogical composition of the soil, as well as an X-fluorescence (XRF) analysis on the soil and the lime for the purpose of identifying the main oxides present in them. The optimum lime content was determined to be 1.75% according to the pH-test. The mixing of this amount of lime with clay resulted in a flattened compaction curve where maximum dry density was reduced from 1.79 g/cm^3 to 1.69 g/cm^3, whereas optimum moisture content increased from 17.1% to 20%. The overall Plasticity Index of the soil decreased from 31.7 to 25.9 after 28 days of curing.

Keywords: soil-mixing, lime, expansive soil, slope stability.

Rezumat. Efectul varului ca agent de stabilizare în pământurile plastice din Vila Hill, Durres, Albania. Adaosul de agenți chimici în soluri, cum ar fi varul hidratat, cimentul Portland, praful de cuptor, cenușă etc., în scopul stabilizării, se bazează pe reacția diferiților oxizi prezenți în acești agenți, cum ar fi Al2O3, Si02 și CaO, în prezența apei, pentru a forma compuși chimici cum ar fi 3CaO-2Si02-4H20 (CSH), precum și CAH, CS'H și CAFS'H. Aceste produse măresc parametrii de rezistență ai solului. Scopul acestei lucrări este de a analiza efectul varului hidratat în stabilizarea solurilor argiloase prin influențarea comportamentului plastic și reducând astfel potențialul de umflare, precum și modificarea caracteristicilor de compactare. Mostrele au fost colectate de-a lungul dealului abrupt al Kodër Vilë din Durrös, Albania, o zonă de coastă cunoscută pentru rupturile sale problematice și recurente. Proprietățile solului au fost determinate împreună cu limitele Atterberg, densitatea maximă uscată și conținutul optim de umiditate. A fost efectuată o analiză a difracției cu raze X (XRD) pe probele de argilă pentru a determina compoziția mineralogică a solului, precum și o analiză X-fluorescentă (XRF) asupra solului și a varului hidratat în scopul identificării principalilor oxizi prezenți în ele. Conținutul optim de var a fost determinat la 1,75% în funcție de testul de pH. Amestecarea acestei cantități de var de hidrat cu argilă a condus la o curbă de compactare aplatizată, unde densitatea maximă uscată a fost redusă de la 1,79 g / cm3 la 1,69 g / cm3, în timp ce conținutul optim de umiditate a crescut de la 17,1% la 20%. Indicele general de plasticitate a solului a crescut de la 27,3 la 40,5. Aceste rezultate indică faptul că sunt necesare mai multe teste pentru a determina cantitatea potrivită de var hidratat pentru reducerea PI.

Cuvinte cheie: amestecarea solului, var, sol expansiv, stabilitate în pantă.

INTRODUCTION

The city of Durrës is located at the Western part of the Albanian lowlands, along the coastline of the Adriatic Sea (Fig. 1). The area under consideration, Kodër Vilë (or Currila), is a hilly section stretching along the coast as a segment of Durrës Mountain. It is composed of soft clays of Pliocene marine deposits, aligned at 45 degrees slope-angle and lacking any significant presence of vegetation. Heavy rainfall during the winter season – reaching a maximum precipitation of 120mm in December (PUMO E. et al., 1990) – and surface-water streams have caused slope failures along the years due to saturation of the clayey soil and loss of strength parameters. The recurrent slope failures have caused considerable damage to the university building and local business located in the area. Furthermore, erosion is a visible phenomenon in this area. Various attempts have been made by the central and local governments for rehabilitating the area, with a major project undertaken by the Ministry of Environment. Two years later, however, the project appears to have failed its objective in stabilising the hill and providing safety for buildings in the area. This paper aims at introducing soil mixing with lime as a possible solution for enhancing the

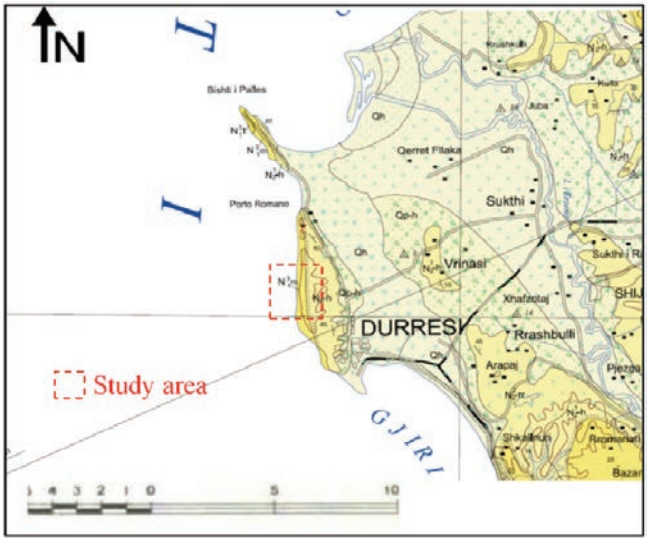


Figure 1. Location of the study area in geological map 1: 200.000 SHALLO et al., 2002.

strength parameters of the soil. The addition of lime for stabilization purposes has recently found a widespread application in various construction projects in Sweden as well as around the world (ÅHNBERG, 2006). Furthermore, lime has been used in soil mixing for the purpose of controlling the volume changes in expansive soils (ZHANG & CAO 2002). The clayey soil from the area was mixed with lime in order to observe the changes in plasticity and compaction characteristics. This data will be used in the future for an evaluation of slope stability.

MATERIALS AND METHODS

The collected samples represent the debris flow material that was deposited near the surface due to slope failure. Initially, the physical properties of the soil, including bulk density, moisture content and specific gravity were determined based on ASTM standards. The soil classification was based on grain size analysis and plasticity of the clay. An X-ray diffraction analysis was conducted in order to identify the main mineralogical components in the clay. Moreover, the chemical composition of the clayey soil and lime were determined using the X-fluorescence by fusion bead method.

Several authors have suggested various amounts of lime to be used in soil mixing as appropriate. For the majority of soil types, 5-10% of lime is suggested (DAS 1990). OLA (1978) proposes 10% of lime for expansive soils, whereas AKAWWI & AL-KHARABSHEH (2002) 3.5-5%. In order to determine the optimal percentage of lime for stabilization, the pH method was used according to ASTM D6276-99. This procedure, based on EADES & GRIM (1966), determines the optimum content of lime by looking at the level of pH that would create the appropriate environment for the pozzolanic reactions to take place. During the reaction of quicklime with water for obtaining hydrated lime, the pH value increases, thus stimulating cation exchange and as well as pozzolanic reactions. The former process, in which calcium and magnesium ions are exchanged for potassium and sodium ions, significantly reduces the plasticity index (ZHANG, 2002). This is followed by flocculation which allows the water to flow through the particles instead of being trapped in them. Furthermore, the increase in pH value causes the dissolution of silica and alumina present in the soil, which initiates the pozzolanic reactions responsible for the long-term strength-gain of the soil.

The pH method examines the bare minimum amount of lime that would potentially be required to mix with the soil for improving its properties, and more specifically reduce the plasticity index. The procedure consists of eleven 150 ml plastic bottles filled with different percentages of lime-soil mixture in water, where pH level is measured for the smallest amount of lime necessary to reach a value of 12.4.

The proportions of lime and soil content in the lime-soil mixture were defined as the ratio of their dry weights to the total mixture. Four curing periods were considered for the mixture: 48 hours, 7 days, 14 days, 28 days. The cation exchange process takes place relatively rapidly, whereas flocculation and the pozzolanic reactions are considered as secondary processes due to the fact that they continue even after a few years (HOPKINS, 2008). The properties of the mixture, such as liquid limit, plastic limit, plasticity index and compaction characteristics, were later considered.

For the purpose of determining the Liquid Limit of the soil, two methods were used, according to ASTM D4318 and BS EN ISO 17892-6:2017, and the results were compared. The first method consists of the Casagrande apparatus, where the liquid limit corresponds to that moisture content for which the groove in the soil inside the brass plate closes for 13mm after 25 blows. The second method used, known as the fall cone test, determines the liquid limit to be the value of the moisture content for which the standard cone will penetrate by 20mm the soil in the cup for five seconds. The two apparatuses are shown in Fig. 2. The plastic limit was determined based on the ASTM procedure.

Furthermore, the Proctor compaction test was performed based on ASTM D558, in order to determine the maximum dry density and optimal moisture content. According to MALLELA et al. (2004), the treatment of soil with lime causes an increase of the optimal moisture content value and decrease of the maximum dry density. The latter is a result of the flocculation process during which the particles occupy a wider space than previously, thus decreasing the density of the mixture, whereas the former stems from the fact that the addition of quicklime increases the percentage of the fine particles present in the soil, thus requiring a larger quantity of water for lubrication during the compaction process. The Atterberg limits as well as compaction properties were compared before and after the treatment of the soil with lime.

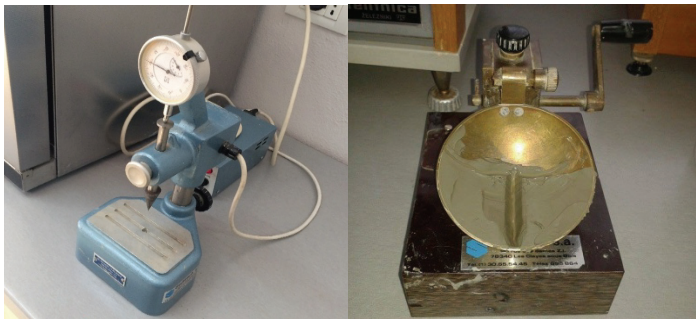


Figure 2. Casagrande and Fall-cone test apparatuses (original).

RESULTS

Soil properties

The Pliocene marine deposits composing the hill at Kodër Vilë in Durrës, have a grey-bluish colour and sparse organic content. The main physical properties are given in Table 1. More than 95% of the material is fine-grained and it has a natural moisture content of 20%. Based on a grain size analysis and the plastic properties, the soil was categorized according to the Unified Soil Classification System (USCS) as Fat Clay (CH), as shown in Fig. 3. This is clay of high plasticity that is associated with a high swell potential.

Table 1. Soil properties.

% passing 75 micron sieve	> 95
% clay	35
USCS classification	CH
Liquid Limit (%)	56.7
Plastic Limit (%)	25.0
Plasticity Index (%)	31.7
Bulk density (g/cm^3)	1.97
Moisture content (%)	20
Specific gravity	2.74

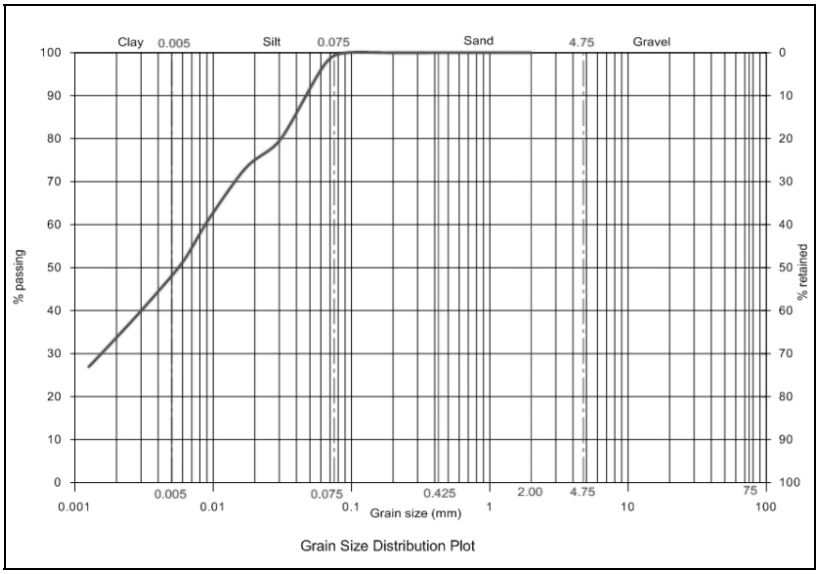


Figure 3. Particle size distribution.

X-ray diffraction analysis

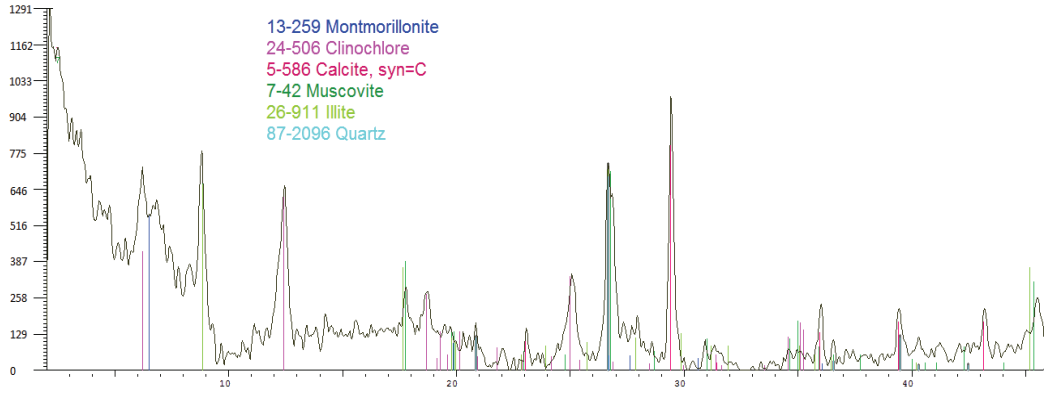


Figure 4. XRD results for the soil.

The x-ray diffraction analysis, as shown in Fig. 4, demonstrates an abundance of quartz and calcite present in the clay. In terms of clay minerals, the presence of illite (or mallachite) and chlinoclore seems significantly larger than that of montmorillonite, which is considered to be a mineral with high swelling potential (CERATO, 2001).

Composition of clay and quicklime

The chemical oxides present in the soil and in the quicklime used as stabilising agent were determined via x-fluorescence by fusion bead method. The main oxides are shown in Table 2:

Table 2. Chemical composition of clay and lime.

	Clay soil	Quicklime
CaO	10.56%	74.45%
SiO_2	46.93%	0.73%
Al_2O_3	12.26%	0.34%
Fe_2O_3	7.01%	0.12%
MgO	5.60%	0.67%
SO_3	0.51%	0.12%
K_2O	2.40%	0.04%
Na_2O	1.51%	0.02%
LOI	12.43	22.4

The optimum lime content was determined based on the minimum amount of lime that would yield a 12.4 value of pH for the lime-soil mixture. For this experiment, eleven different percentages of limes were mixed with the soil. Due to the rather high natural pH value of the clay, namely 9.53, the 12.4 value of pH was obtained for 1.75% of lime added in the mixture, as shown in Fig. 5. This percentage of lime was used for treating the soil when performing the compaction test and in determining the Atterberg limits of the soil.

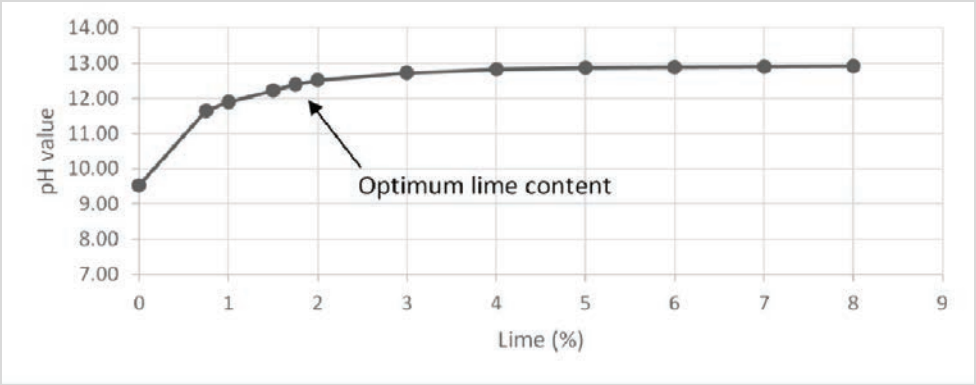


Figure 5. pH test method for determining the optimum lime content.

Compaction test

The results obtained from the Proctor test for compaction are shown in Fig. 6. For the untreated soil sample, the maximum dry density and optimum moisture content were 1.79 g/cm^3 and 17.1%, respectively. The curve is typically bell-shaped. After 1.75% of lime was added to the soil, the compaction curve changed into a flattened-shape one, where the maximum dry density decreased to 1.69 g/cm^3 and the optimum moisture content increased to 20%. The flat shape of the curve indicates that the dry density of the soil varies less with the fluctuation of the moisture content.

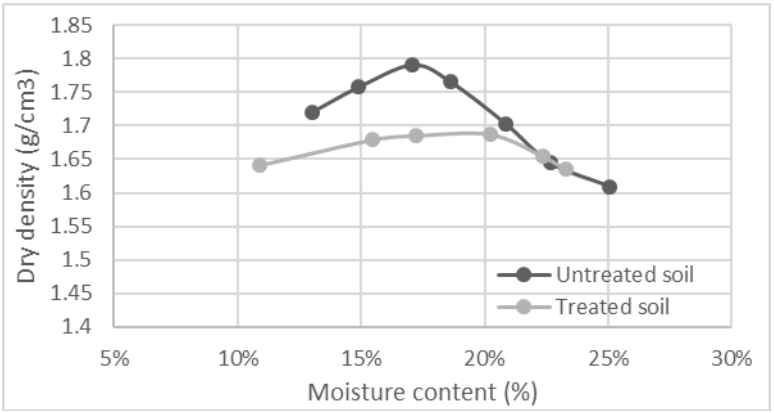


Figure 6. Proctor compaction test for untreated and treated soil.

Atterberg Limits

The Liquid Limit, Plastic Limit and Plasticity Index were determined for both untreated soil and treated soil with 1.75% lime. The determination of liquid limit was conducted with Casagrande method and Fall cone test. It can be observed in Fig. 7 that with the addition of lime the liquid limit decreases and the plastic limit increases, thus decreasing the overall plasticity of the soil. Furthermore, the delay period plays a significant part in continuing this trend, with the final values after 28 days of liquid limit decreasing from 56.7% to 54.2%, the plastic limit increasing from 25% to 28.3% and the plasticity index overall reducing from 31.7% to 25.9% (the results are shown in Table 3). According to MALLELA et al. (2004), a variation of the Plasticity Index from 50% to 40% relates to a decrease in swelling from 45% to less than 20%.

Table 3. Atterberg Limit test results for soil treated with 1.75% lime with Casagrande and Fall-cone methods.

		Casagrande test			Fall-cone test			Average		
Lime %	Delay	LL	PL	PI	LL	PL	PI	LL	PL	PI
0	Natural soil	56.7%	25.0%	31.7%	56.7%	25.0%	31.7%	56.7%	25.0%	31.7%
1.75	2 days	56.4%	26.2%	30.2%	55.3%	27.1%	28.2%	55.8%	26.6%	29.2%
	7 days	55.9%	27.1%	28.8%	55.5%	26.2%	29.3%	55.7%	26.7%	29.0%
	14 days	55.0%	27.5%	27.5%	55.3%	27.6%	27.6%	55.1%	27.6%	27.6%
	28 days	54.2%	28.4%	25.8%	54.2%	28.1%	26.1%	54.2%	28.3%	25.9%

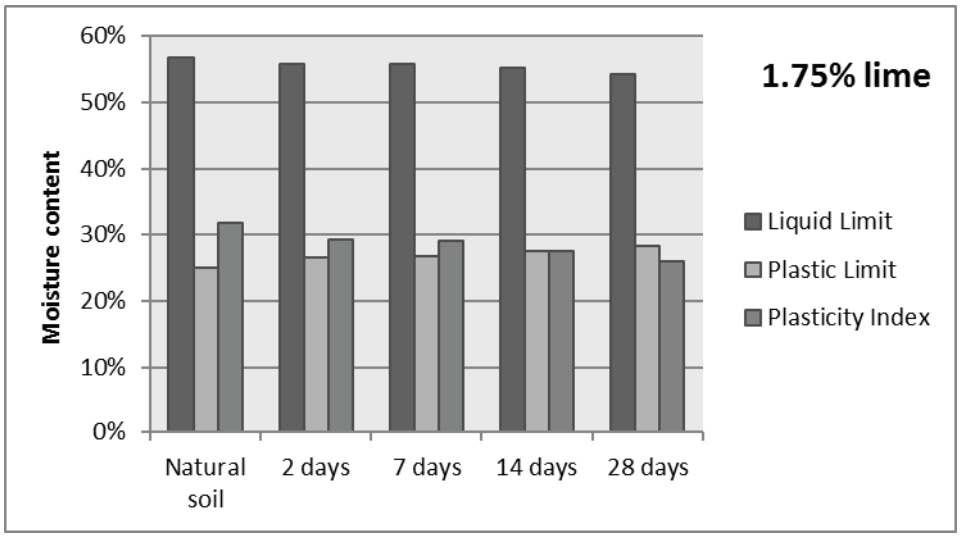


Figure 7. Natural soil vs treated soil with 1.75% lime for 2, 7, 14, and 28 days.

DISCUSSION & CONCLUSIONS

The XRD results showed that montmorillonite was not the predominant clay mineral in the soil. Nonetheless, illite and chlinoclore also have significant expansive potential, based on their specific surface value.

The pH test method determined the optimum value of lime – to be used as a stabilizing agent – to be 1.75%. With the addition of this percentage of lime, the mixture reached the appropriate pH level of 12.4, necessary for the stabilizing chemical reactions to take place.

With the addition of lime, the compaction curve obtained from the Proctor test flattened from a bell-shaped curve and shifted towards the right. A flat curve shows that the dry density (and therefore the volume) does not fluctuate as much with the change in water content.

The value of PI decreased with the addition of 1.75% lime. This effect amplified with the curing time. A higher amount of lime should be used for the value of PI to decrease significantly, since it is considered to be an indicator of swelling potential and thus directly affecting the stability of the slope.

Overall, the addition of 1.75% of lime in the soil, as a minimum amount indicated by the pH method, marginally improved the compaction parameters of the soil and decreased the PI value. Future research will consider the addition of higher percentages of lime in order to assess the increase in strength parameters and soil stability.

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IDENTIFICATION OF THE GUDE OIL LEVEL IN THE GEOLOGICAL SECTION OF MARINZA-3111 WELL

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Abstract. Accumulations in the Marinza complex oilfield especially within Miocene deposits are related to the Patos – Verbas anticline. This structure is included in the Kurveleshi Belt of theJonian zone. The Patos – Verbas anticline represents the west – northern and deeper part of Ionian orogene. The oil accumulations occur in limestone reservoirs and sandstone reservoirs within the flysch deposits, while the accumulations in Miocene deposits are dispersed in conformity with five formations such as "Bubullima", "Marinza", "Kucova", "Gorani", and "Driza", where accumulations are within the classic reservoirs. Two oil levels were identified in two formations: the "Kuçova" formation and the deepest formation of "Marinza".All types of oil field deposits encountered in Marinza oil field include oils of a broad spectrum. Both levels of natural reservoirs have been exploited at the same time. The maximum crude oil production was in September 1996. Two main migration phases of HC are determined, which are in conformity with tectonic phases. The earliest massive oil migration has occurred during the tectonic phase Middle Miocene and the second during Post – Pliocene time. During exploration process around the Patos – Verbas anticline, gas samples from oil accumulations of Miocene deposits were taken where methane was rich in isotopic carbon (C^{13} of CH_4 up to – 26%). Values of stable carbon isotope of methane are characteristics for the thermogenous gas. There is not a good correction between C^{13} of methane and with others geochemical parameters. We have concluded that the youngest migration phase of thermogenic gas happened during Post – Pliocene time through a heterogeneous medium. This process has contaminated oil accumulations and deposits around the oil fields mentioned above. The Marinza-3111 oil trap is small after reducing the natural reservoirs, which is located on the contour of the oiltrap.The data were taken from the Marinza-3111 well file.

Keywords: Crude oil, reservoirs, formation, geochemical parameters.

Rezumat. Identificarea nivelului petrolului brut în secțiunea forajului Marinza-3111. Acumularea în câmpul petrolifer complex Marinza, în special în depozitele miocenice, este legată de anticlinele Patos - Verbas. Această structură este inclusă în zona BeltKurveleshi din zona Jonian. Anticlinalul Patos - Verbas reprezintă partea de nord-vest, mai adâncă, a orogenului ionian. Acumularea de petrol se produce în rezervoarele de calcar și în rezervoarele de gresie din depozitele de fliș, în timp ce acumulările din depozitele miocene sunt dispersate în conformitate cu cinci formații precum ,,Bubulima", ,,Marinza", ,,Kucova", ,,Gorani" și ,,Driza" , unde acumulările se află în rezervoarele clasice. Două niveluri de petrol au fost identificate în două formațiuni: formațiunea ,,Kuçova" și cea mai profundă formare a ,,Marinza". Toate tipurile de depuneri de câmp petrolifer întâlnite în câmpul petrolifer Marinza includ petrol de un spectru larg. Ambele nivele de rezervoare naturale au fost exploatate în același timp. Producția maximă de țiței a fost în septembrie 1996. Se determină două faze principale de migrație ale HC, care sunt în conformitate cu fazele tectonice. Cea mai veche migrație masivă a petrolului a avut loc în timpul fazei tectonice Miocen mediu,iar cea de-a doua în timpul post Pliocen. În timpul procesului de explorare din jurul anticlinalului Patos - Verbas, au fost prelevate probe de gaze din acumularea de petrol din depozite miocenice, unde metanul era bogat în carbonizotopic (C13 de CH4 până la 26%). Valorile izotopului de carbon stabil al metanului sunt caracteristice pentru gazul termogen. Nu există o corelație bună între C13 al metanului și alți parametri geochimici. Am concluzionat că cea mai tânără fază de migrare a gazului termogen sa produs în timpul post-Pliocen printr-un mediu eterogen. Acest proces a contaminat acumulările de petrol și depozitele în jurul câmpurilor petroliere menționate mai sus. Capcana de petrol Marinza-3111 este mică după reducerea rezervoarelor naturale, care se află pe conturul capcanei de petrol. Datele au fost preluate din fișierul Marinza-3111.

Cuvinte cheie: Petrol brut, rezervor, formare, parametrii geochimici.

GEOLOGICAL SECTION PENETRATED BY MARINZA-3111 WELL

The Marinza oilfield is among the largest in Europe in sandstone reservoirs. Its orientation is southeast and is located in the geological section of the Adriatic Depression.

The Marinza-3111 well was drilled on the northeastern contour of the Marinza oilfield. It can be called the contour well (Fig. 1). It has passed a reduced geological section.

The lithological description of the geological section of the well will be treated according to previously conducted studies (PRIFTI & DORRE, 2015).

Marinez Formation. This formation lies discordantly on the buried erosion surface (Fig. 2).

The lithofacies is represented by massive beds of loose sandstones containing carbonate sandstone concretions. The lithofacies is represented by massive beds of loose sandstones containing carbonate sandstone concretions (PRIFTI & DORRE, 2015). Accumulations of crude oils can be found on the upper part of this formation, at a depth of 1506-1516 m and 1520-1529.2 m. Sandstones alternate with clays containing carbonate concretions. The thickness is 150 m.

Driza and Gorani Formations. In Marinez, the section is mainly argillaceous alternating with sandstones. It is represented by an alternation of loose sandstones up to gravel with clays and carbonate aleurites. During the drilling and the testing process, no oil traps have been identified. Their thickness is 220 m.

Kuçova Formation is propagated all over the region and lies normally on Gorani Formation. It is represented by an alternation between clays and loose sandstones. In the depth of 1168.8-1179.6 m there are identified oil traps. The thickness is 230m.

Polovina Formationis eroded by Pliocene transgression. It is characterized by the alternation between clays and loose sandstones. The thickness is 150m.

Pliocene deposits. They include two lithological formations: Helmes and Rrogozhina.

Helmes Formation lies by transgression on the Messinian deposits, represented by clays and aleuritic clays withsandstones and aleurites intercalations. While Rrogozhina formation there is no interest in oil production.

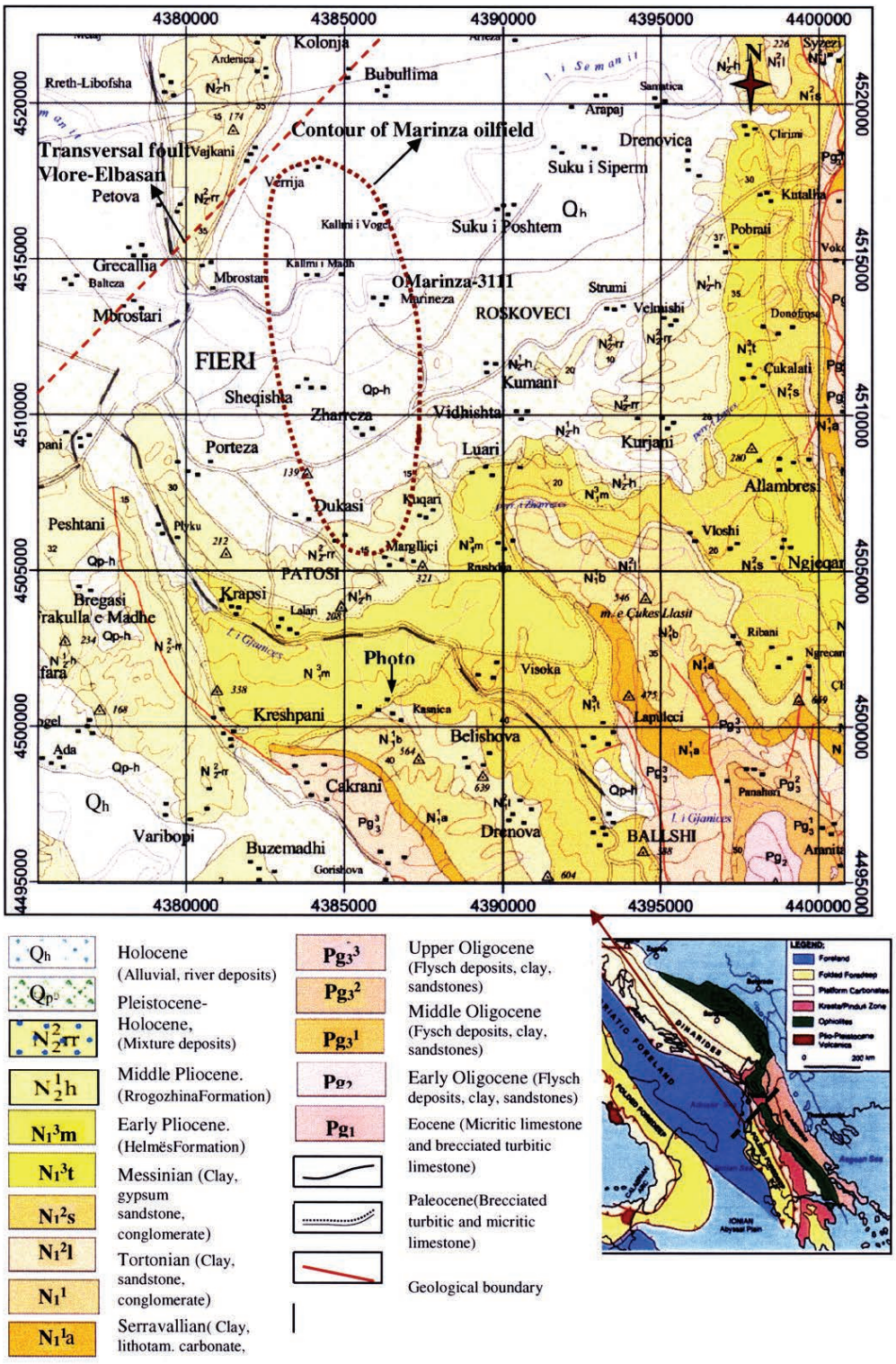

Figure 1. Position of Marinza oilfield and Marinza-3111 well on geological map (scale:1/200 000, based on PRIFTI & DORRE, 2015, modified by Seitaj).

PROSPECTING OF OIL ACCUMULATIONS BY GEOCHEMICAL PARAMETERS OF HC GASSES

Thermogenic gases have been met for the first time in the Kolonja-8 well. This well is drilled to the north of the Marinza oilfield complex and exploits the oil from the carbonate reservoirs (eroded Patos-Verbas anticline). Precisely this was the reason we carried out the study during the drilling of the Marinza-3111 well to verify the presence of thermogene gasses in the northern part of the Marinza oilfield complex.

We have analyzed thermovacuum gasses derived from mud in interval 1060 (m) – 1660 (m) during drilling by two methods, gaschromotography (content of hydrocarbon gasses from methane to hexane) and methane stable carbon isotope ratio (Table 1).

Table 1. Hydrocarbon composition of gaseous samples from mud during drilling of Marinza-3111 well.

Depth (m)	Content (ml/l)		Hydrocarbon composition (%)									
	CH4	C2H6	CH4	C2H6	C3H8	iC4H10	nC4H10	C4H8	iC5H12	nC5H12	C6H14	C7H16
1120	2.7675	0.0646	99.558	0.24	0.027	0.005	0.006	0.059	0.01	0.001	0.002	0.022
1150	2.3394	0.0204	96.119	0.838	0.445	0.664	0.317	0.044	0.548	0.042	0.493	0.451
1190	5.2762	0.0539	90.975	1.01	0.029	1.537	0.472	0.138	2.36	4.379	1.976	1.219
1210	0.95553	0.00884	86.676	0.804	0.507	1.471	0.696	0.041	2.973	0.378	3.409	2.893
1240	1.12462	0.00906	89.231	0.719	0.098	1.053	0.409	0.179	2.634	0.373	2.881	2.217
1270	1.09299	0.00645	91.318	0.542	0.303	0.746	0.365	0.157	2.147	0.338	2.383	1.341
1300	0.8445	0.0038	90.54	0.489	0.293	0.371	0.303	0.139	1.929	0.377	2.467	2.681
1330	2.15354	0.00694	97.207	0.314	0.113	0.123	0.094	0.052	0.41	0.071	0.721	0.76
1360	2.1297	0.0037	90.649	0.321	0.072	0.101	0.061	0.059	0.406	0.287	0.952	1.181
1390	5.92327	0.02398	97.453	0.395	0.209	0.216	0.132	0.014	0.217	0.184	0.638	0.504
1420	5.4955	0.0251	97.238	0.444	0.301	0.216	0.246	0.023	0.297	0.232	0.581	0.347
1460	2.1735	0.0327	91.348	1.379	1.286	0.538	1.049	0.035	0.78	0.718	1.688	1.147
1480	5.8068	0.0188	94.269	1.003	0.942	0.355	0.833	0.021	0.493	0.493	0.946	0.6
1500	4.8755	0.0655	94.495	1.273	1.182	0.228	0.453	0.024	0.509	0.466	0.914	0.411
1540	6.52573	0.09994	89.472	1.371	1.243	0.538	1.382	0.043	1.192	1.263	2.404	1.07
1560	2.426	0.0706	83.618	2.178	1.537	0.214	0.897	0.083	2.08	2.376	4.872	2.072
1600	3.5775	0.2867	77.808	6.247	2.172	0.796	1.793	0.036	1.666	1.821	4.826	2.928

Geochemical properties of HC gasses samples are determined by parameters such as; C1/ C2, C1/ C2+, C2/C3 and C^{13} of methane (Table 2). Methane stable carbon isotope has a different behavior compared to others parameters and is not a good correction between them (PRIFTI & BITRI, 1992).

Based on the hydrocarbon composition of gasses the following indicators are calculated:

1. C1/C2 = Methane (CH4)/Ethane (C2H6),
2. C1/C2+ = Methane /(Ethane+Propane + Butane+ Pentane+Hexane+Heptane),
3. C2/C3 = Ethane/ Propa

In conformity with C1/C2 and C1/C2+ (Table 1, Fig. 2), all types of HC gasses are present (very dry, dry, wet and very wet gasses). The first level (1115 m – 1180 m, including the first production interval) is reflected very weak because the geological section is argillised.

In the interval 1300 (m) - 1390 (m) the values of C1/C2 and C1/C2+, increase immediately and HC gasses are included in dry to very dry gasses. This phenomenon is conditioned by two factors:

- HC gasses are generated from Miocene deposits.
- Or a new migration phase of methane gas from deeper part has occurred during Post – Pliocene time.

Table 2. Parameters of hydrocarbon gasses and Carbon isotopic ratio of methane (C^{13}).

Depth (m)	C1/C3	C1/C2+	C2/C3	Carbon isotopic ratio of methane (C^{13}- ‰)
1120	42.84056	267.629	8.888889	-32,8
1150	114.6765	25.01796	1.883146	
1190	97.88868	6.93407	34.82759	-35,25
1210	108.0916	6.580322	1.585799	
1240	124.1302	8.447505	7.336735	-34,5
1270	169.4558	10.97308	1.788779	-31,1
1300	222.2368	10.00553	1.668942	
1330	310.3084	36.57148	2.778761	-30,9
1360	575.5946	26.35145	4.458333	
1390	247.0088	38.84137	1.889952	-31,11
1420	218.9442	36.18831	1.475083	-30,85
1460	66.46789	10.59722	1.072317	-28,45
1480	308.8723	16.57914	1.064756	
1500	74.43511	17.30678	1.076988	-30,2
1540	65.29648	8.516276	1.102977	
1560	34.36261	5.127108	1.417046	-27,2
1600	12.4782	3.491497	2.876151	-29,1
1660	13.29994	3.653106	2.039411	-26,4

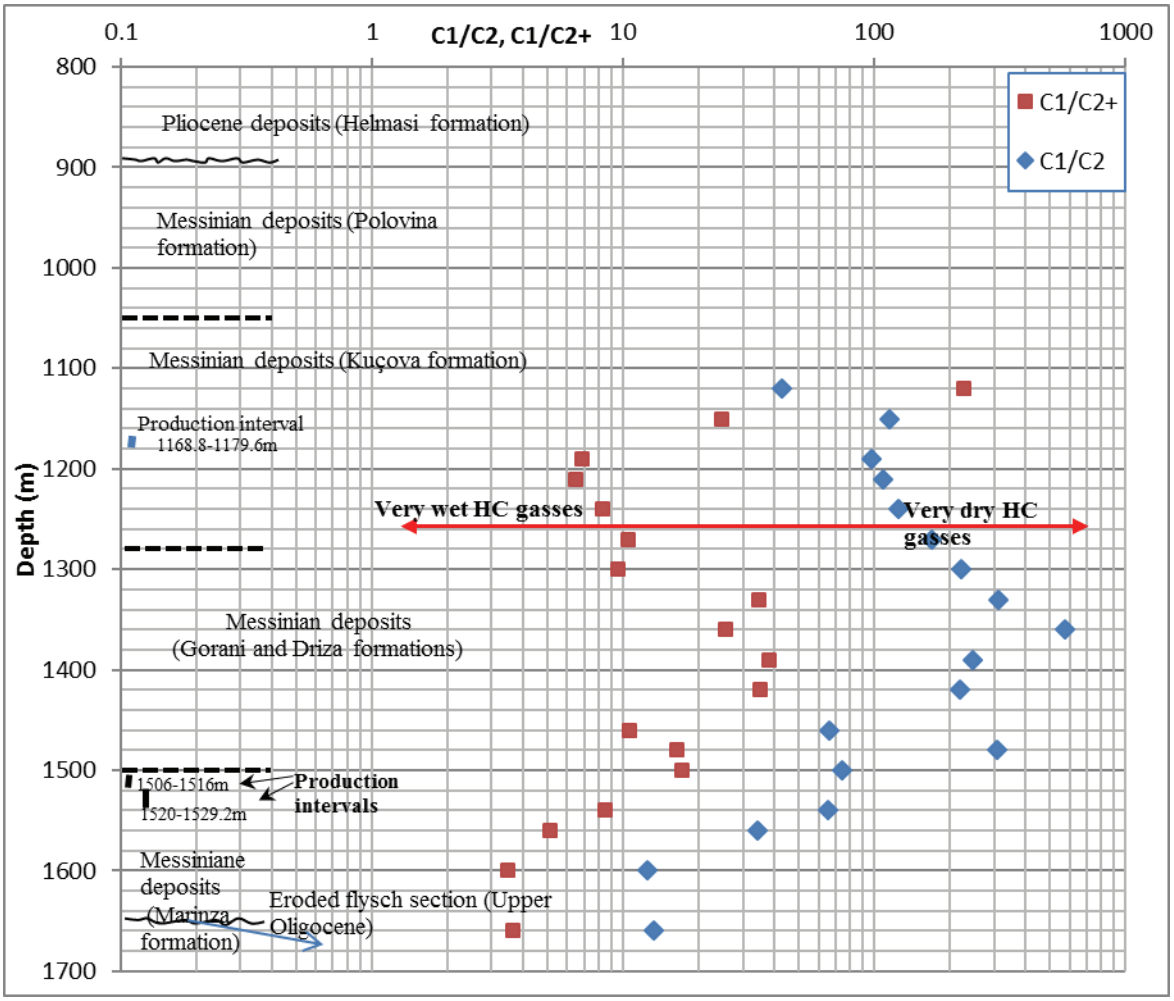

Figure 2. Type of geochemical HC gasses on geological section of Marinza-3111 well.

In deeper section more than 1390 (m) the values C1/C2 and C1/C2+, decrease and HC gasses become wet and very wet one, which express e gaseous anomaly caused by oil accumulation in 1506 (m) – 1529 (m) interval (Fig. 2). Oil accumulation is expressed more clearby C2/C3 ration, because lower are typical for oil associate gas (Fig. 3).

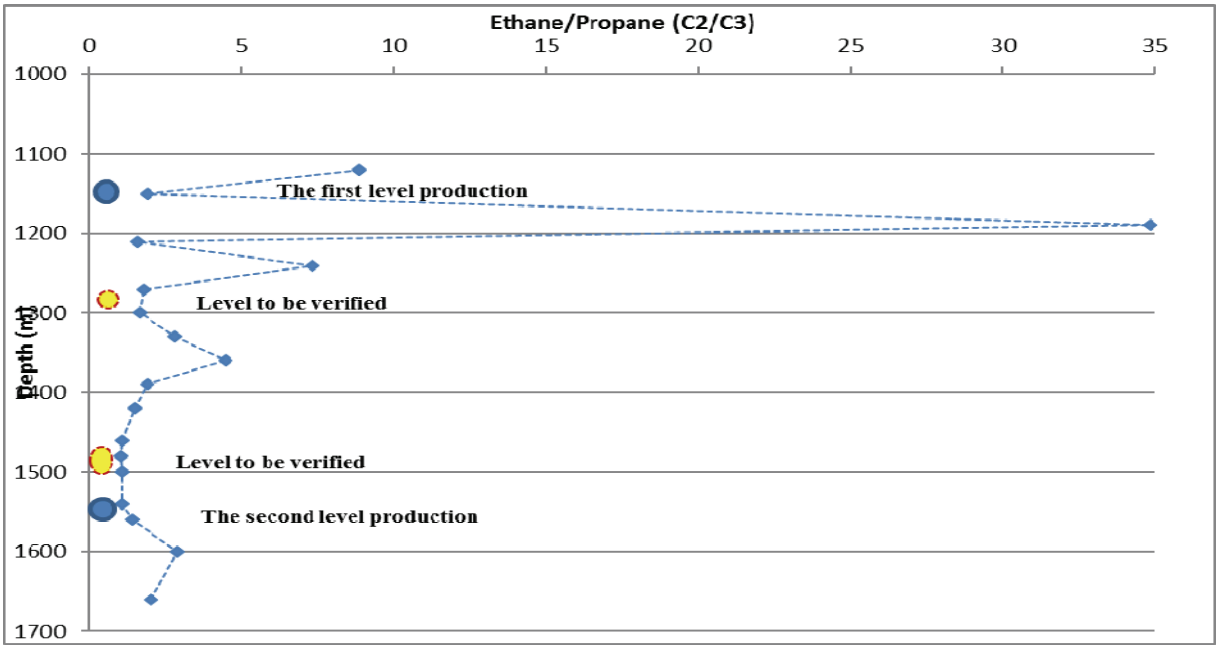

Figure 3. Determination of oil accumulations in the geological section of Marinza-3111 well by C2 / C3 ratio.

Different values of these parameters depend on some conditions:

- Gas samples where C2/C3>5 are generated from Middle Miocene deposits and are not influenced by oil accumulation.
- HC gasses were migrated.
- Decreasing of C2/C3 ratio in deeper section is caused by oil accumulation on "Marinza" formation.

The non-compliantsurface has contributed to the decrease of the C2/C3 ratio because matured hydrocarbon gaseshad migrated from this surface d (PRIFTI & BITRI, 1998).

The carbon isotopic ratio of methane (C^{13}) values ranges from -35.28 % to -26.84 % which express high level of maturity. Based on this parameter, a separation in matured HC gases is also allowed (C^{13} of CH4 = -31.65% + -26.84%) (Fig. 4).

The first group is taken from the shallow part of the geological well (up to 1390(m) and the second from deeper part (under 1390 m), therefore methane stable carbon isotope values increase with depth. High values of this parameter exclude the possibility of generation from Miocene deposits (PRIFTI & MUSKA, 1994).

There is no proper connection between C^{13} of methane and other geochemical parameters of HC gasses. On the top of geological section (1060(m) – 1390(m)) there isn't any connection with gaseous parameters. It does not exist in nature matured HC gases with high content of iso-butane and iso-pentane (Table 1).

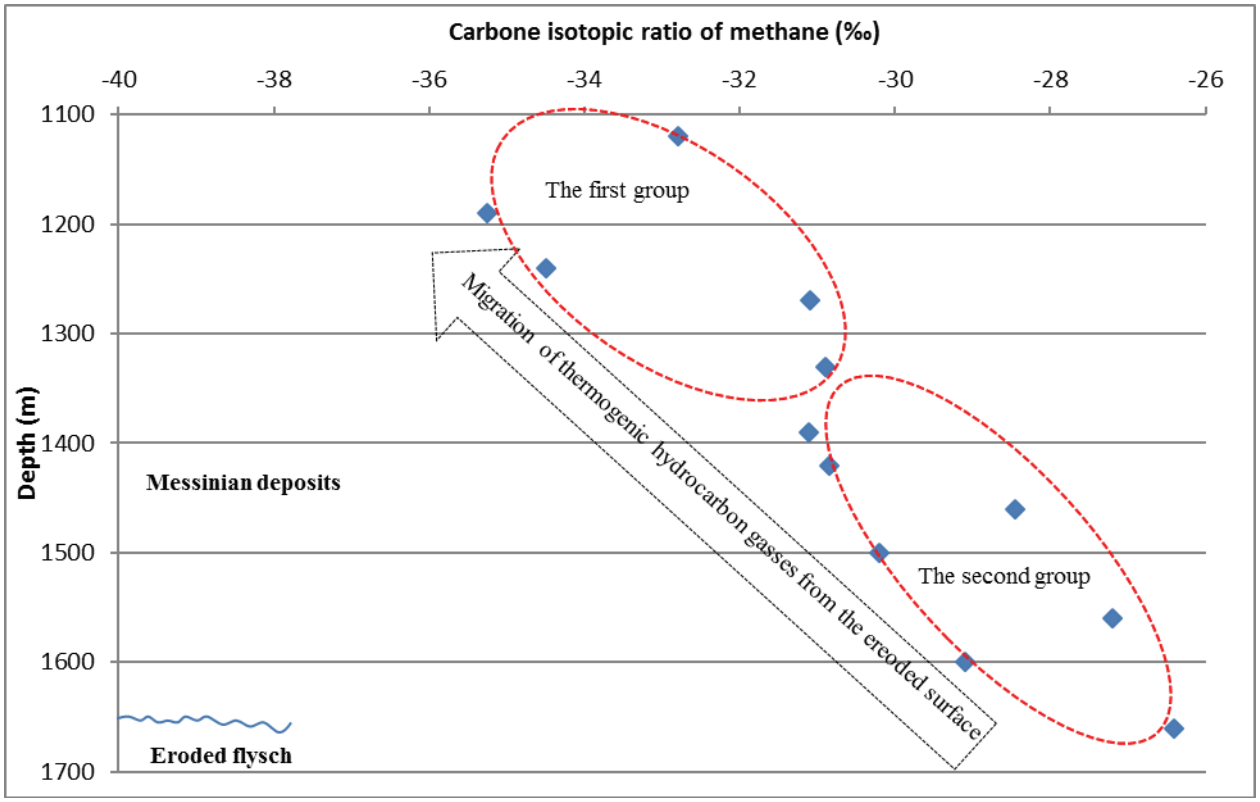

Figure 4. Variations of the carbon isotopic ratio of methane to the geological section of the Marinza-3111 well.

HC gasses which are taken from interval 1390(m) – 1660(m) are overmatured (C^{13} of CH4) and include in thermogene gasses, while according to other geochemical parameters (C1/C2 and C1/C2+), become wet and very wet.

There is no connection between them because thermogenic gasses are dry and very dry. The non-compliance of C^{13} of CH4 with other parameters results from a general contamination in the Marinza oilfield during the youngest migration phase of thermogene HC gasses.

Source rocks of Miocene deposits are immature, generate only biological gas (Carbon isotopic ratio of methane-C^{13}< -55‰) (Fig. 5).

Source rocks of the Ionian zone are within the "oil window",while the deeper blocks content over matured source rocks, which generate mature hydrocarbon gas (SHKURTAJ et al., 2002)

The first group of hydrocarbon gasses isgenerated by source rocks which are in stage of "**Condensates and wet gas generation** (δC_1^{3}= -37°/oo ÷ -31°/oo)". This group of hydrocarbon gases correlates with those of the Kolonja-8 well.

The second group of hydrocarbon gasses are generated by source rocks which are in stage of "**Stage of thermogene gas generation** (δC_1^{13}>-32°/oo)".

Hydrocarbon gases with high of carbon isotopic ratio of methane (C^{13}) values are generated from over matured source rocks.

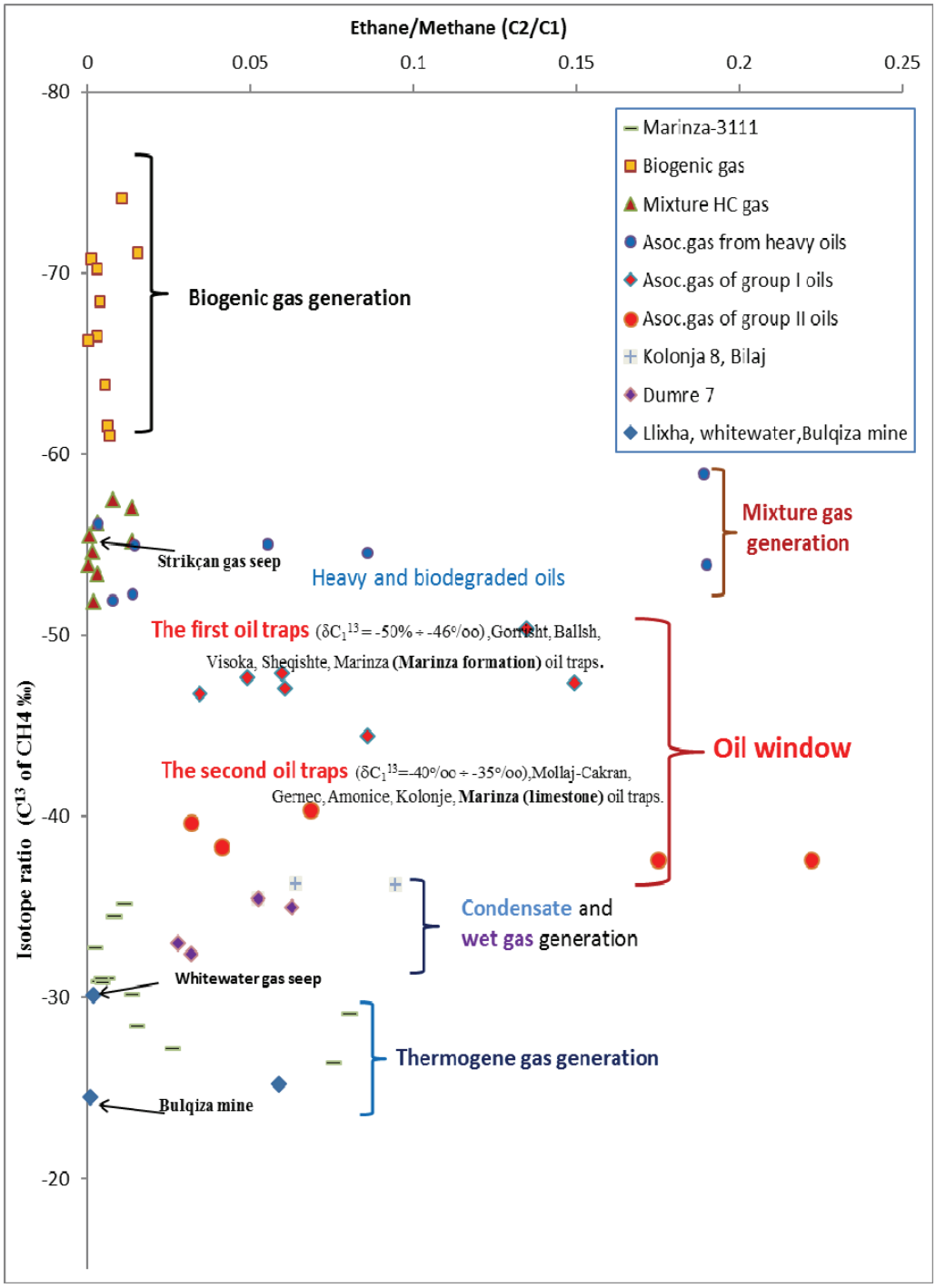

Figure 5. The position of hydrocarbon gasses from the Marinza-3111 well on the geochemical model of petroleum generation (based on SHKURTAJ et al., 2002, modification by Prifti and Seitaj).

THE PRODUCTION RHYTHMS OF THE MARINZA-3111 WELL

The oil production process of a well is carried out according to several cycles:
The first cycle utilizes the internal energy of the oil trap,
The second cycle continues with the application of the second methods,
In the third cycle begins the drilling of horizontal wells,
The fourth cycle is the repetition of the second cycle.

Once the oil extraction reserves have been extracted from the well, then technological water and CO2 injections start. The Marinza-3111 well was drilled into the northeastern contour of the Marinzaoilfield. It can be called the contour well. After the drilling, the well started producing crude oil in June 1996 (File of Marinza-3111 well).

We have studied the first cycle of oil production in the Marinza-3111 well. The duration of the first cycle is dependent on several factors: the physical-chemical characteristics of the crude oil (light petroleum tend to be the

shortest time), the elasticity of the reservoir and the fluids, the energy of the hydrocarbon gas dissolved in the crude oil (the gas factor is high rhythm of energy), the energy of the capillary, the energy of the bottom water, the dimensions of the oiltrap. The Marinza-3111 oil trap is small after reducing the natural reservoirs. This is related to the well position, which is located on the contour of the oiltrap, where the lithological section of the Micene deposits is clayed.

The ones we discussed above have affected the fact that the first production cycle has short time.

There are cases when the first cycle has not been completedsince 1935 (wells of heavy crude oil in Kuçova oilfield) (GJOKA et al., 2002).

The communication of the natural crude oil reservoir with the column of the well was achieved after three months of work. This was reflected in the increased quantity of crude oil produced. The production rhythm was preserved the same until February 1997 (Table 3).

At this stage, the communication paths between the reservoir and the wells columns began to get blocked. This is the result of the arrival of the sand in the well column. Communication routes are unblocked with the upgrade process. This process is related to cleaning the sand from the well's forehead. The same working method was carried out in the month of August 1997. Oil production from the well continued until the end of 1997. Subsequently, the well was transferred to another company's property and second methods were applied for the production of crude oil. After this time we are not authorized to publish the data. But other levels have been tested, as we have recommended. New secondary methods have been applied. The results were very good and more crude oil was produced (Fig. 6).

Table 3. Crude oil production by Marinza-3111 well.

Nr	year	Month	Grude oil (ton)	Gas (000/m^3)
1	1996	June	6.6	7.2
2	1996	July	8.7	12.6
3	1996	August	60.8	4.32
4	1996	September	197.8	5.58
5	1996	October	125.6	5.27
6	1996	November	116.4	5.51
7	1996	December	121.9	3.72
8	1997	January	98.8	3.36
9	1997	February	79.6	3.72
10	1997	March	102.7	3.48
11	1997	April	111.1	3.72
12	1997	May	112.6	3.6
13	1997	June	96.7	3.72
14	1997	July	57.5	2.76
15	1997	August	98.4	3.36
16	1997	September	95.4	3.6
17	1997	October	39.7	3.48
18	1997	November	7.2	0.96

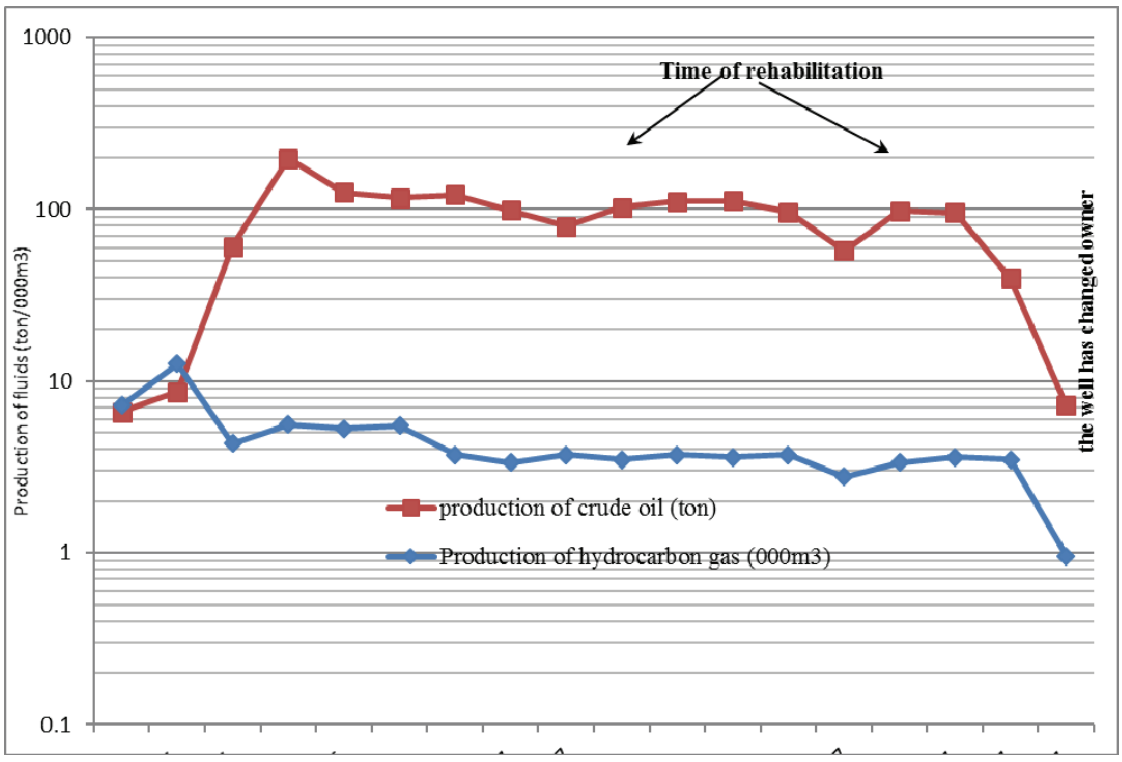

Figure 6. Crude oil production by Marinza-3111 well.

CONCLUSIONS

The geological section reached by the Marinza – 3111 well is reduced.Reducing the geological section is the result of the well position on the eastern side ofMarinza oilfield.

From geochemical investigations on the geological section reached by the Marinza – 3111 we shall draw some important conclusions regarding the interpretations of geochemical parameters for putting in evidence the oil accumulations and the prospecting for gas accumulations in deeper part of limestone section.

According to geochemical parameters (C1/ C2, C1/ C2+, C2/ C3), oil accumulations are identified in two levels of Miocene deposits, while for the two other levels they should be verified by the testing process.

Values of methane stable carbon isotope in oilfield complex in Marinza range from -47.9% to -26.84%.

A general contamination of oil accumulations and geological section from thermogene HC gasses occurred during the post-Pliocene migration phase.

Thermogene HC gases was mixedwith oil associate gases in deeper (under 1390 m) of Miocene deposits in Marinza 3111 well, so that the values of $C^{13}$of CH4 are higher.

Oil production from the well has been at acceptable rates since cutting is generally argillised.

Rehabilitation processeswere related to cleaning the sand from the well's forehead. These processes were carried out when the oil production was blocked.

During the interpretation of geochemical indicators two levels were identified in the depth of 1290 m and 1490 m. These levels should be subjected to the testing process.

Based on the above discussion, we conclude thatthermogenic gas traps must exist in the north of the Marinza oilfield.

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EVALUATION OF ELECTROMAGNETIC FIELD VARIATIONS FROM MONITORED DATA IN PLANETARY OBSERVATORIES

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Abstract. The Earth's electromagnetic field varies over time. Its changes have a large variety of both morphology and generation mechanisms, at the same time showing parallels with various geophysical and helio-physical phenomena. The correlation of these phenomena based on their physical substrate proved very useful to clarify several issues concerning field morphology. The geomagnetic field, as part of the Earth's electromagnetic field, is monitored in planetary observatories, from the INTERMAGNET network, with high-resolution and accurate triaxial magnetometers. In this paper we present the main types of variations exemplified through the data analysis from Surlari Geomagnetic Observatory (located at about 30 km North of Bucharest) for different time periods and acquisition rates. Geomagnetic data were analysed for the whole period of activity of the Observatory Surlari from 1943 to present. Thus, the data used for the study of the large periodicities of the geomagnetic field are presented as a series of hourly averages covering the period of analogic measured. We have highlighted, for long series of recordings for several solar cycles, 22-years and 11-years periodicities. Following the same procedures for the medium (multiannual) series, the annual, seasonal and monthly periodicities were highlighted. For shorter data series, we highlighted the diurnal, semi-diurnal (12-hour), 8-hour and even lower periodicities. For very short series with a high sampling rate and for some magnetotellurics records, we have highlighted various types of continuous pulsations (Pc1 - Pc5) and irregular pulsations (Pi1 - Pi2). Time - frequency analyses allow identify the frequency characteristics of the signal at a time. For this we chose a mobile window, moving along the signal from time t_0 to any position t_i, on temporal axis. The frequency content of each window was analysed, finally obtaining the frequency spectrum well localized in time.

Keywords: Earth's electromagnetic field, geomagnetic pulsations, geomagnetic storms, planetary observatories, INTERMAGNET network.

Rezumat. Evaluarea variațiilor câmpului electromagnetic din datele monitorizate în observatoarele planetare. Câmpul electromagnetic al Pământului variază de-a lungul timpului. Schimbările sale au o mare varietate morfologică și mecanisme de generare si prezintă paralelizari cu diverse fenomene geofizice și heliofizice. Corelarea acestor fenomene pe baza substratului lor fizic s-a dovedit foarte utilă pentru a clarifica mai multe aspecte legate de morfologia câmpului. Câmpul geomagnetic, ca parte a câmpului electromagnetic al Pământului, este monitorizat în observatoarele planetare, din rețeaua INTERMAGNET, cu magnetometre triaxiale de înaltă rezoluție și de precizie. În această lucrare prezentăm principalele tipuri de variații exemplificate prin analiza datelor de la Observatorul Geomagnetic Surlari (situat la cca 30km la nord de București), pentru diferite perioade de timp și rate de achiziție. Datele geomagnetice au fost analizate pentru întreaga perioadă de activitate a Observatorului Surlari din 1943 până în prezent. Astfel, datele utilizate pentru studiul periodicităților mari ale câmpului geomagnetic sunt prezentate ca o serie de medii orare care acoperă perioada măsurată analogic. Am evidențiat, pentru serii lungi de înregistrări pentru mai multe cicluri solare, periodicități de 22 ani și 11 ani. Urmând aceleași proceduri pentru seriile de înregistrări medii (multianuale), au fost evidențiate periodicitățile anuale, sezoniere și lunare. Pentru serii de date mai scurte, am evidențiat periodicitățile diurne, semidiurne, de 8 ore și chiar mai mici. Pentru serii foarte scurte cu o rată de eșantionare ridicată și pentru câteva înregistrări magnetotelurice, am evidențiat diferite tipuri de pulsații continue (Pc1 - Pc5) și pulsații neregulate (Pi1-Pi2). Analizele de timp - frecvență permit identificarea caracteristicilor frecvenței semnalului la un moment dat. Pentru aceasta am ales o fereastră mobilă, care se deplasează de-a lungul semnalului de la momentul t_0 la orice poziție t_i, pe axa temporală. Am analizat conținutul de frecvență al fiecărei ferestre, obținând în final spectrul de frecvențe bine localizat în timp.

Cuvinte cheie: câmpul electromagnetic al Pământului, pulsații geomagnetice, furtuni geomagnetice, observatoare planetare, rețeaua INTERMAGNET.

INTRODUCTION

The part of the geomagnetic field which has a simpler structure and determines its spatial distribution is mainly due to causes located inside the Earth, while that who diversify his morphology has external causes. The variation of the manifestations and of the space and time distribution of geomagnetic field have external causes.

Mechanisms from inside and outside the Earth are particularly important, from the core processes, which are responsible for producing the main field to the ionosphere and magnetosphere phenomena whose effects are the variations in geomagnetic disturbances.

The physical generation mechanism of the geomagnetic field of internal origin has been incompletely explained until now. Instead, the external field, although it has a higher morphological and phenomenological complexity, occurs as a result of a chain of physical processes framed in coherent explanatory concepts, largely verified by direct confrontation with reality.

Terrestrial magnetism has a determined spatial distribution and a characteristic temporal evolution. The dual variability of the geomagnetic field, with the place and time at which the observations are made, makes its space-time structure very complex.

The persistent part of the field, with a spatial distribution, corresponding in a first approximation to the field of a uniform magnetized sphere, has a slowly evolution in time, called secular variation. The transient geomagnetic field is represented by the calm and regular variations and by sudden and sporadic changes.

The two constituent parts of the geomagnetic field (the persistent emphasized by mediation for extended time and, respectively, transitional by momentary deviations from the mean) have different weights: the persistent, called the main geomagnetic field is over 90% of the total geomagnetic field, while the transient does not exceed 10% only during periods of extreme agitation of the field (geomagnetic storms).

The knowledge of the structure of the magnetosphere is based on the abundant data provided by satellite and land observations. The complicated problems of the structure of the magnetosphere and the nature of the geomagnetic variations have been largely dealt with in many publications and many authors: AKASOFU (1977), AKASOFU & CHAPMAN (1972), LYATSKY (1978), MATSUHITA (1975), MISHIN (1978), NISHIDA (1978), PUDOVKIN (1975, 1976) all in GEBBINS & HERRERO-BERVERA, 2007. Also, crustal magnetism is in relation with magnetics properties of geological formations (NICULESCU et al., 2012).

THE EARTH'S ELECTROMAGNETIC FIELD VARIATIONS

A simplified model for the magnetosphere and sources of the main variations is outlined in Fig. 1.

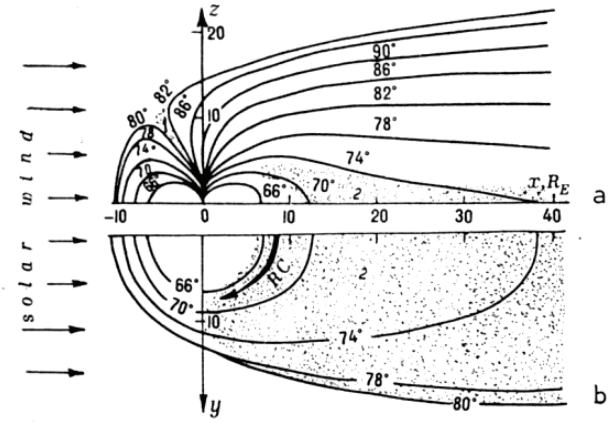


Figure 1. Structure of the magnetosphere (TRYGANENKO & PUDOVKIN, 1975 in GEBBINS & HERRERO-BERVERA, 2007).
a - meridian section; b - equatorial section.

The outer boundary of the magnetosphere, called magnetopause, is formed in the region where the geomagnetic field can stop the compressive forces exerted by the solar wind (solar plasma flow).

Moving around the magnetosphere, the solar wind gathers plasma and deforms the geomagnetic field lines from the periphery of the magnetosphere. Plasma moves back to Earth and to Sun along the peripheral area of the magnetosphere, this movement being called magnetospheric convection.

Due to high plasma conductivity, the electric field is closed at zero in the solar wind coordinate system. The solar wind has a Vsw speed relative to the Earth-linked coordinate system and thus induces the electric field in the latter.

The characteristic wind speed is about 300-400 km/s, and the Interplanetary Magnetic Field (IMF) increases with 3-5 nT, which gives the magnitude of Em of the order of 0.5-1 mV/m. Incorporating the southern face of the magnetosphere, the potential difference of 70 kV (Mishin 1978 in CONSTABLE CATHERINE – 2005) or even 300 kV (Nishidda 1978 in CONSTABLE CATHERINE – 2005) is reached. The effect of this electric field is an important cause of magnetospheric convection.

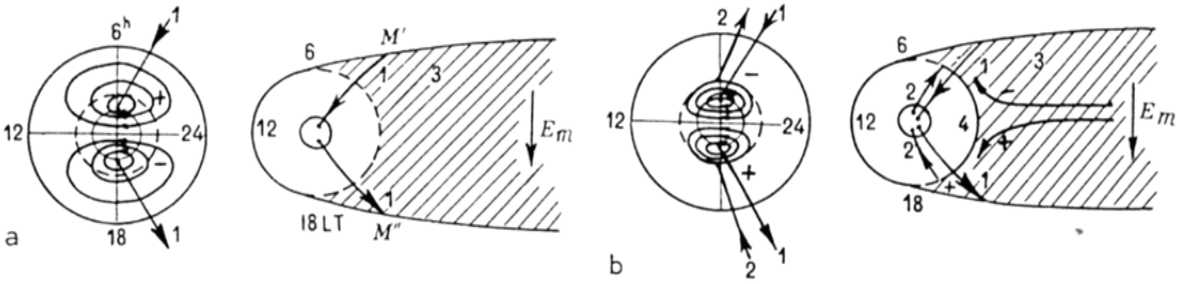


Figure 2. Diagram of electric particle circulation over a day
(TRYGANENKO & PUDOVKIN, 1975 in GEBBINS & HERRERO-BERVERA, 2007); a - ecuatorial section, b - polar section.

Figure 2 shows the electric field Em resulting from the southern orientation of the Bsw (South-West magnetic induction orientation). In the magnetosphere queue, the combined effect of the Earth and the geomagnetic field directed from the Earth to the southern part of the equatorial plane and towards the Earth in the north part results in the compaction of charged particles from the equatorial plane and their displacement to Earth. An increase in the Bz (vertically magnetic induction orientation) component when approaching the Earth favours the acceleration of the particles, the positive tasks - the protons being rejected towards the meridian of the evening and the negative charges towards the morning. Thus, the electrical polarization of the inner portion of the plasma jet known as the Alfven layer occurs. Figures 3 and 4 show two representative

schemes for the main source areas of the magnetosphere for different types of observed phenomena as well as the main components of the magnetosphere current systems.

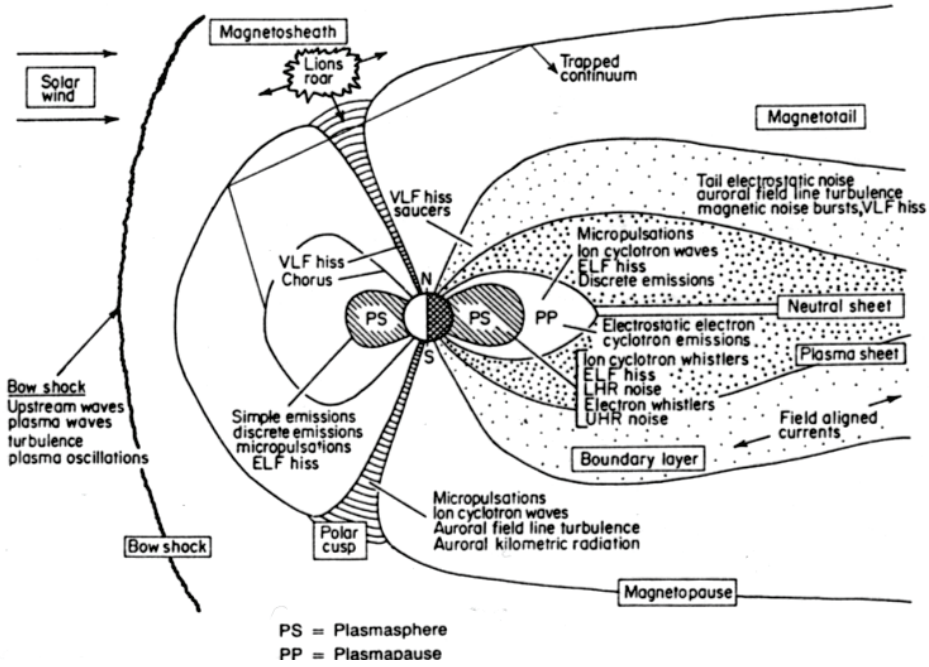


Figure 3. The main source areas in the magnetosphere for different types of observed phenomena (SHAWAN, 1979 in GEBBINS & HERRERO-BERVERA, 2007).

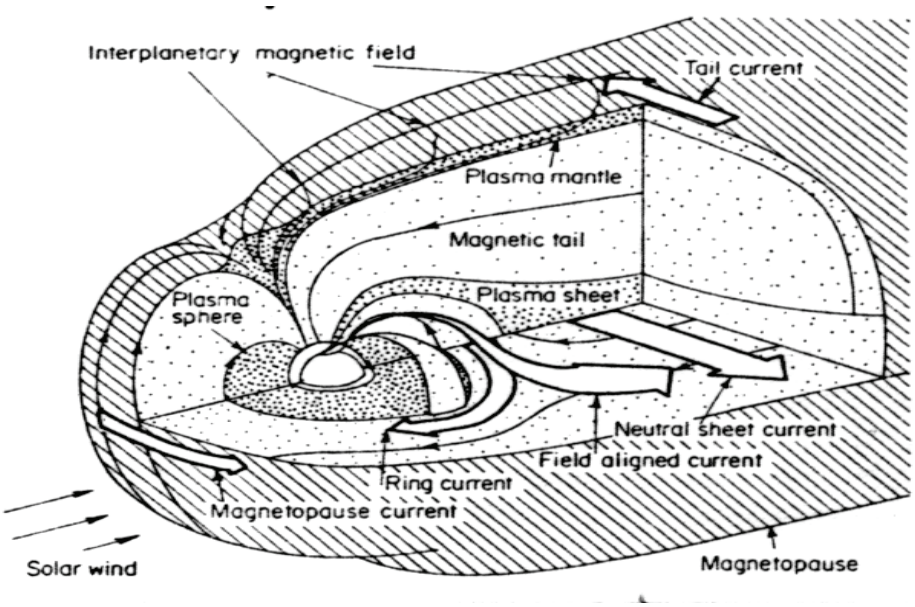


Figure 4. The main components of magnetospheric current systems. The earth and the atmosphere are represented by the two small spheres in the center of the figure. (POTEMRA, 1984 in GEBBINS & HERRERO-BERVERA, 2007).

The main geomagnetic field and the transient geomagnetic field can be evaluated by spherical harmonic analysis. Spherical functions are characterized by two remarkable properties:

- possibility to represent a certain distribution of values, no matter how complicated, on a sphere;
- their character of harmonic functions, satisfying the Laplace equation, which must undergo a field values derived from a potential.

Transitory part of geomagnetic field, represented by variation phenomena in a large range of periods, offers information both on external causes of the field with extensions in ionosphere and magnetosphere, as well as on internal ones, tightly connected to globe inner structure, anomalies in the distribution of electric properties in the crust, geodynamic processes in the mantle or convection phenomena in outer core.

The transient changes in the geomagnetic field are highlighted by continuous records of items made in magnetic observatories. On the one hand, periods of "quiet magnetic", characterized by slow variations, regular and predictable of

the geomagnetic components recorded, quasi-sinusoidal looking can be distinguished on the obtained magnetograms, and on the other hand, periods of "magnetic agitation", when the field presents deviations with an irregular distribution in time, with unequal amplitudes of the elements in relation to periods of magnetic quiet.

The external component of the geomagnetic field may present periodic variations (dependent on the position of the Sun and Moon relative to the meridian of the place) and aperiodic variations associated with increasing or reducing of solar activity at a certain moment.

In terms of how vary daily records of geomagnetic field elements to an observatory, the days can be classified into two categories:

- quiet magnetic days;
- disturbed magnetic days.

An extreme case of perturbation is a magnetic storm. Except the storm days in the records of the magnetic observatories can observe certain regularities of the daily variations.

The main part is the solar daily variation (S), with a 24-hour period and a lunar variation (L) with small amplitude (lunar daily variation) with a period of about 25 hours. Disturbance variation (D) is additional field which appears in disturbed or stormy days. S and D are relatively easy to recognize in the record, but to determine the variation produced by moon requires processing of long data sets.

Beside the statistical and spectral analyses, an important role at the beginning of a morphological study of the electromagnetic field is played by the visual analysis of analog magnetograms, which can easily highlight aperiodic magnetic events with very different morphology. For this reason it is useful to preserve the observatories' magnetograms. Several amplitude features allow the individualization of these events in several characteristic and standardized types (sudden impulses - and sudden storms commenced - Ssc, chromosphere eruptions - SFE, bays - b and pulsations) reflecting external causes related to different areas of the magnetosphere.

Thus, sudden jumps (SI), with a low nT amplitude, of the geomagnetic components are produced by a sudden change of solar wind pressure on the magnetosphere boundary (at a distance of about 10 Earth radii). When there is a rapid change of the magnetic field, with a few tens of nT or nT, precedes a geomagnetic storm (Ssc impulses), the magnetosphere is compressed and pushed to the Earth. Chromosphere eruptions (SFE) are caused by a sudden increase in solar UV and X radiation that ionizes the upper atmosphere and intensify the electrical currents. Magnetic bays (b) are a consequence of increasing of the polar electro-jets at night.

Continuous pulsations (Pc) of low-frequency are generated mainly by magneto-hydrodynamic instabilities of the contact between the solar wind and magneto - break. Many pulsations from the medium frequency band are caused by the instability proton - cyclotron in the solar wind. The continued pulsation of high frequency is mainly due to the ion - cyclotron instability in the magnetosphere where the energy of instability comes from anisotropic perturbations of the energetic protons.

Irregular pulsations (Pi) are generated by transient phenomena such as sudden impulses from the solar wind. The study of continuous pulsations Pc provide information about the near-Earth plasma, and the investigation of long-term changes of these pulses, in observatories is important for the study of plasma regions.

From the trend of the diurnal averages of geomagnetic components within a longer time (multiannual) overlapping oscillations representing large and small deviations from the monthly mean can be seen over a slow annual variation (secular variation). These deviations are more pronounced during equinox, because of the particular position of the Earth's magnetic dipole axis relative to the main direction of corpuscular solar radiation flow, their pressure on the magnetosphere is stronger, and the particle density penetrating the magneto-break (and concentrated in belts van Allen radiation) is higher.

GEOMAGNETIC DATA FROM SURLARI OBSERVATORY

Thus, the data used for the study of large periodicities of the geomagnetic field are presented as a series of hourly averages covering the period 1.01.1958-31.12.2006. These data were downloaded in the WDC format from the ftp site of the World Data Center for Geomagnetism from Kyoto. These were calculated annually based on final data both from analogue magnetograms and digital recordings (ASIMOPOLOS et al., 2010, 2012a, 2012b).

To study the diurnal variations, non-periodic variations (geomagnetic storms, phenomena SSC or SFE) were used as several minute averages for the entire period in which digital recordings were made in Surlari Observatory.

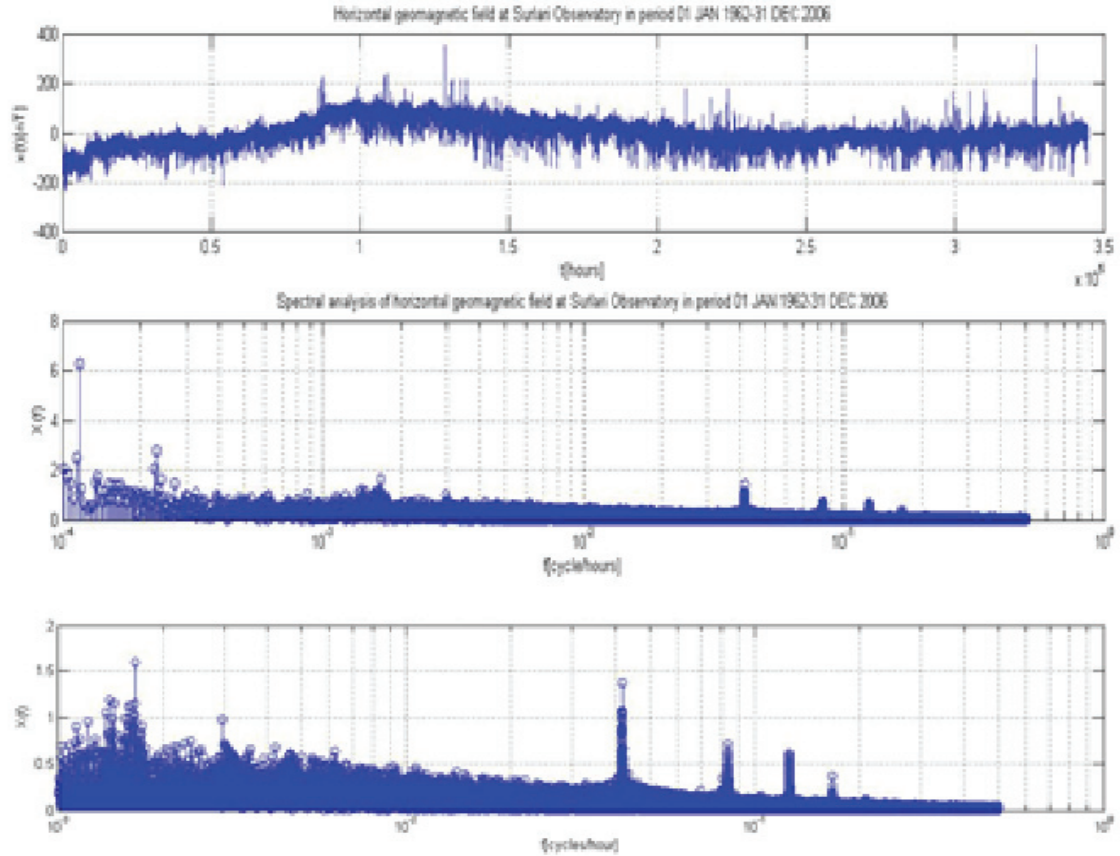
In order to study periodic phenomena with higher frequencies, we used gross values of the records of geomagnetic field components made with a sampling rate of 2 Hz, generally covering periods up to 24 hours.

The first processing was performed on a series of hourly values, based on final data and recorded during 1.01.1958-31.12.2006 in Șurlari geomagnetic observatory, with a major disruption in data acquisition in both years 1960 and 1961. Because of the existence of the disruption, the recorded values were treated separately for the intervals 1958-1959 and respectively 1962-2006.

We will illustrate, in the following, the spectral processing of the data acquired between 01.01.1962 and 31.12.2006, providing a long information series, almost continuous (about four solar cycles) as a series of values hourly average calculated annually based on final data.

At the latitude of the Surlari Observatory the horizontal component H has the highest degree of disturbance, reaching its variation amplitude of about 400 nT as shown in Fig. 5.

The first stage of the data processing (using WON et. al., 2004, www.matworks.com) is the linearization process. After applying linearization characteristic equations for each component is obtained extracting of the secular variation of the geomagnetic field component and is preserved the effect of external geomagnetic field.



A)

B)

C)

Figure 5. A) Diference between horizontal geomagnetic component and the linear trend, for Surlari Observatory for the period 1.01. 1962 - 31.12. 2006, based on hourly averages read from magnetograms; B) Spectral analysis for frequency range between 10^{-4} and 0.5 cycles / hour; C) Spectral analysis for frequency range between 10^{-3} and 0.5 cycles / hour.

For all three recorded components an upward trend is observed for the whole period. This trend represents the secular variation of the analysed components. Declination D and vertical component Z have variations with smaller amplitudes at latitude of Surlari Observatory. These variations are generally masked by the strong tendency of increasing values of these two components. Increasing trend is the dominant feature of these components.

Comparing sunspot number variation with linearized graphics components H, D and Z of the geomagnetic field can be seen overlapping periods of enhanced solar activity with periods of increasing amplitude variations of the geomagnetic field components.

The following figures exemplify the evolution graphs for the horizontal component, declination and vertical component for both the original data and after the removal of the linear trend.

A Gaussian distribution can be observed for the horizontal component H, whose upward trend is more pronounced (Fig. 6).

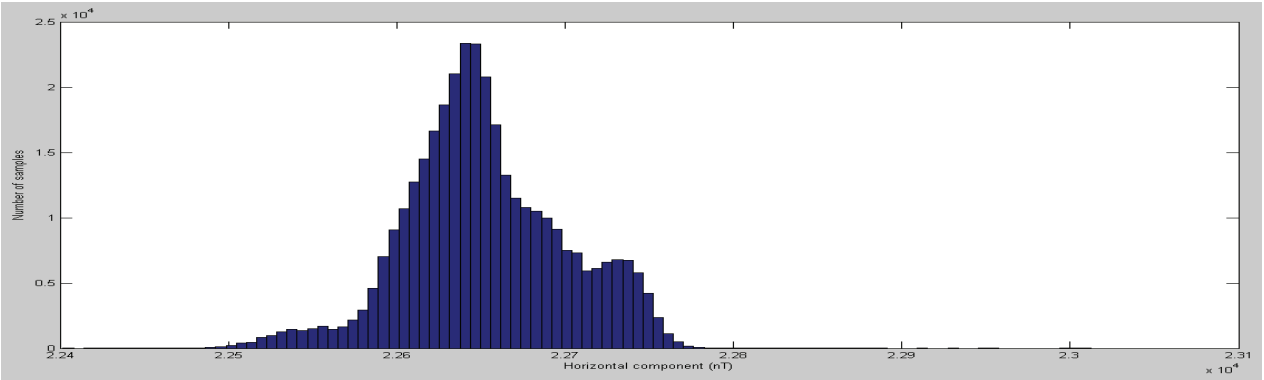


Figure 6. Histogram of horizontal component for year period 1962-2006.

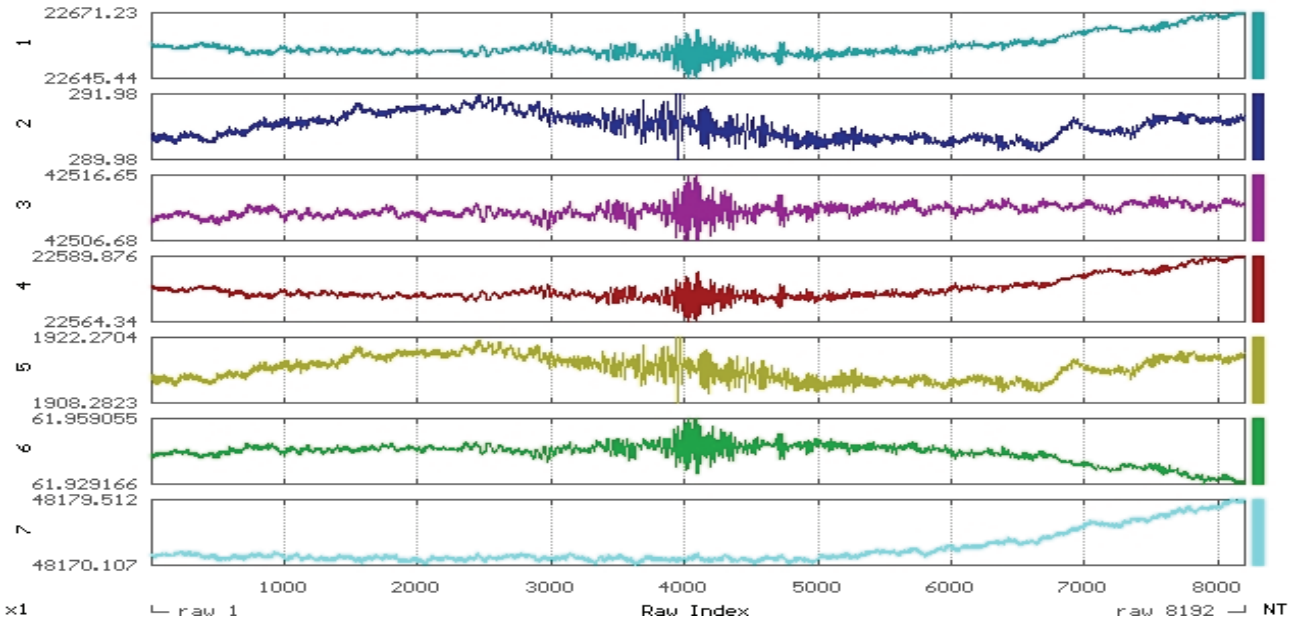


Figure 7. Graphical representation of the representative pulsations on 11.03.2011, in time interval 06:05:04 to 07:13:20 recorded at Șurlari Observatory. In this figure: 1 - horizontal geomagnetic component H, 2 – declination D, 3- vertical geomagnetic component Hz, 4-geomagnetic component Hx on the North direction, 5 – geomagnetic component Hy on the East direction, 6 – inclination I, 7 - total geomagnetic field F. The abscissa shows the number of samples with sampling rate of 0.5 seconds. In the y-axis components 1, 3, 4, 5 and 7 are given in nT, component 2 is given in minutes and component 6 is given in degrees.

We note the existence of the pulsations with different amplitudes and frequencies for the first six components. On the seventh component (total field F), these pulsations do not occur because they have a phase shift and through synthesis of the three perpendicular components (north, east and vertical) these pulsations disappear.

To explain better we selected a relevant detail for morphology pulsations.

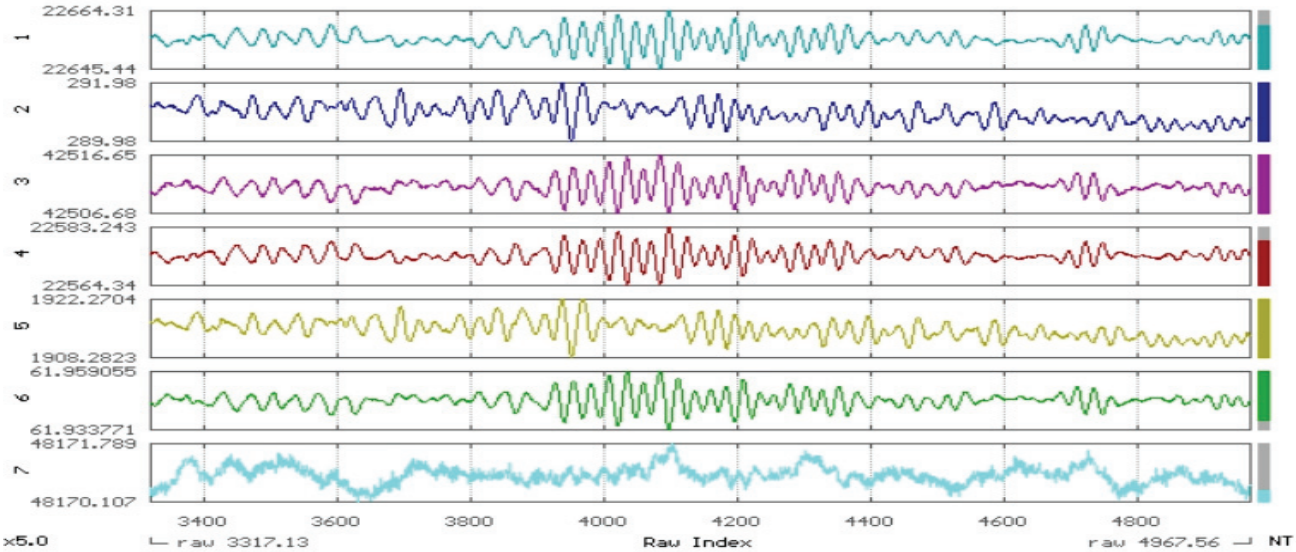


Figure 8. An detail of 1600 samples (800 seconds). Curves (1-7) have the same physical significance as in Fig. 7.

We performed a Fourier analysis to view the predominant frequencies for each geomagnetic component and can be distinguished frequencies in the range 0.02 Hz - 0.1 Hz, so in this example pulsations have periods between 10 and 50 seconds, fits in Pc3 and Pc4 categories.

We can show a variation of amplitudes for different geomagnetic components. Thus, the horizontal components were recorded amplitudes up to 20-22 nT, while the vertical component amplitudes of 10-12 nT occurred. Total geomagnetic field amplitudes were observed only 1-2 nT.

Predominant frequencies of these pulsations were different depending on the analyzed component.

We selected a sequence of 8192 samples (2^{13}), the horizontal and vertical components, which conducted to parametric spectrum (Figs. 9 and 10).

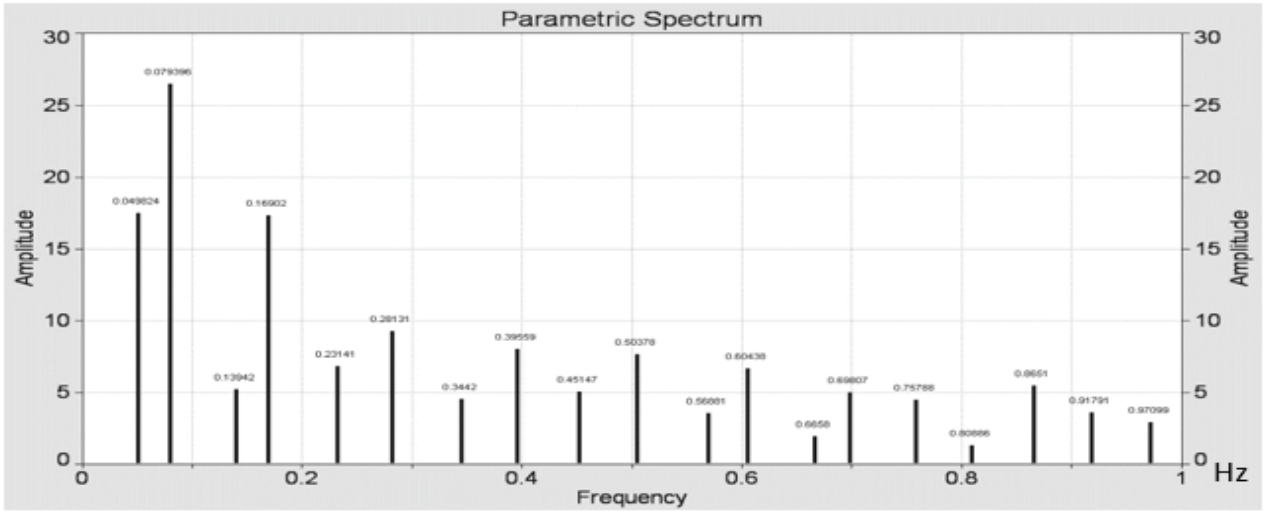


Figure 9. Parametric spectrum for horizontal geomagnetic field (H).

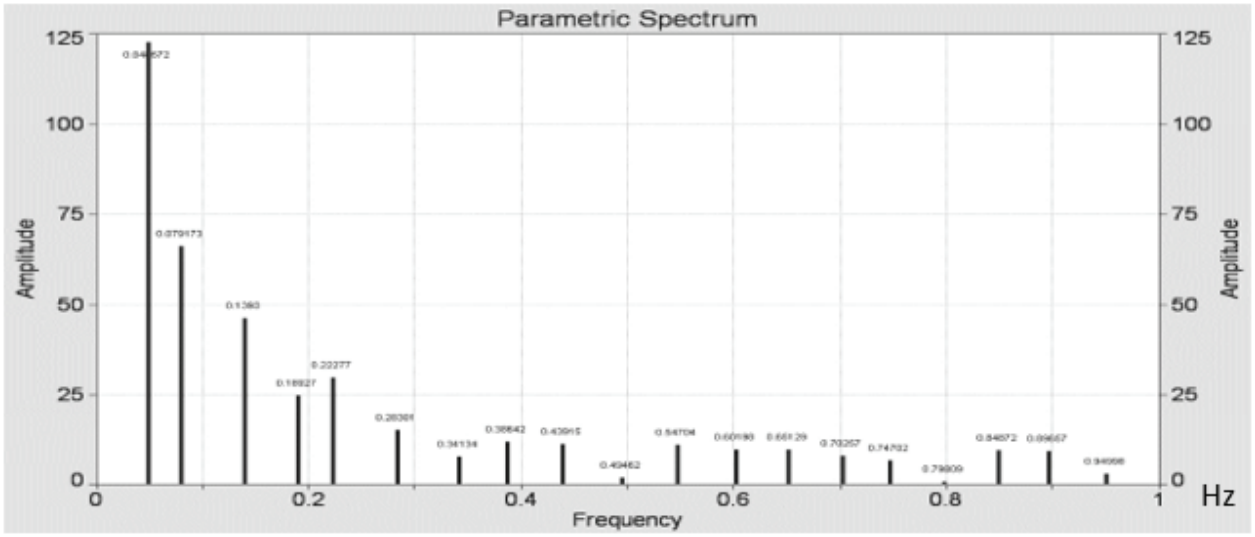


Figure 10. Parametric spectrum for vertical geomagnetic field (Hz).

CONCLUSIONS

Were reviewed the scanned analogic magnetograms recorded in the Surlary Observatory, identifying two types of even\: calm and disturbed variation.

From the whole set of data resulting from the processing of the records geomagnetic field components, several time series were selected with different sampling rates, to be subject to statistical and spectral studies.

Sampling rates were chosen based on the maximum resolution of the acquisition systems, data processing mode and the length of the analysed time series.

For the magnetograms recorded during the period 1943-1999 the maximum temporary resolution that can be achieved is 3 minutes, but because standardized processing requires processing of minute averages or hourly averages of these records, we used hourly average of the values of components recorded.

These data were downloaded in the WDC format from the ftp site of the World Data Centre for Geomagnetism from Kyoto. These were calculated annually based on final data both from analogy between magnetograms and digital recordings.

Digital recordings made between 1999 and February 2009, with a sampling rate of 0.2 Hz, and between March 2009 and to date, the sampling rate was 2 Hz for components D, H and Z and 0.2 Hz for scalar value of the total field F.

To study the diurnal variations, non-periodic variations (geomagnetic storms, phenomena SSC or SFE) were used as several minute averages for the entire period of digital recordings.

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QUALITATIVE AND QUANTITATIVE RESISTIVITY (VES) STUDY IN BEDROCK MAPPING. CASE STUDY MOGLICA

KARRIQI Altin, PEKMEZI Jeton

Abstract. A resistivity survey has been carried out in order to determine the subsurface structure of a site near Moglica village in Korça district. The survey line is located on the Devolli river valley, oriented perpendicular to the river bed. The scope of the survey was the delineation and mapping of bedrock, in help of geotechnical study of the site. Four Vertical Electrical Soundings (VES) have been conducted with maximum current electrodes separations of 130 m. In performing resistivity surveys, Schlumberger array has been used. Using those array parameters make possible a maximum survey of 26 m in depth. VES centers (stations) have been located 15 m apart, making possible a survey line length of 45 m. Both qualitative and quantitative interpretation of electrical resistivity data were carried out, giving information in terms of depth to bedrock, nature of superficial materials and geological features characterizing the subsurface basement rocks in the area.

Keywords: Vertical Electrical Soundings, bedrock, qualitative resistivity section, quantitative section.

Abstract. Studiul calitativ și cantitativ al rezistivității (VES) în cartarea rocilor. Studiu de caz Moglica. A fost efectuat un sondaj de rezistivitate pentru a determina structura de cartografiere stratigrafică subterană a unui sit în apropierea satului Moglica din cartierul Korça, în sprijinul studiilor geotehnice. Linia de sondaj este situată pe valea râului Devolli, orientată perpendicular pe versantul albiei. Scopul sondajului a fost delimitarea și cartografierea rocii de bază, în sprijinul studiului geotehnic al sitului. Au fost realizate patru sonde electrice verticale (VES) cu separări maxime de electrozi de 130 m. În efectuarea anchetelor de rezistivitate, a fost utilizată matricea Schlumberger. Utilizarea acestor parametri de matrice face posibilă o analiză maximă de 26 m în profunzime. Centrele VES (stații) au fost amplasate la o distanță de 15 m, ceea ce face posibilă o lungime a liniei de anchetă de 45 m. Au fost realizate atât interpretarea calitativă, cât și cantitativă a datelor de rezistivitate electrică, oferind informații în termeni de adâncime la roca de bază, natura materialelor superficiale și caracteristicile geologice care caracterizează rocile subterane de subsol din zonă.

Cuvinte cheie: Sunete electrice verticale, rocă de bază, secțiunea rezistivitate calitativă, secțiune cantitativă.

INTRODUCTION

Resistivity Survey method using Vertical Electrical Soundings (VES) is one of most used method in bedrock mapping and determining the subsurface structure. This method is easily applied in field, with no need of a large crew and depending of the depth of investigation needed, one can use easily transportable portable equipment. The accuracy of the method is very good when soil layers are horizontal or dipping with a smooth angle. These facts make this survey method a low cost one. VES data interpretation provides 1D results of rocks apparent resistivity at depth. Combining several VES surveys along a line, we can use the data to have a 2D image of rocks resistivity at depth (qualitative 2D section). On the other hand, using appropriate inversion software, we can interpret every VES in terms of number of layers, their true resistivity values and their thickness. After doing that we can interpolate several VES data, obtaining a 2D geophysical section. By knowing average resistivity values of different type of rocks, we can transform this in a geological – geophysical 2D section (quantitative section).

This is the case in our study, where 4 VES surveys are done along a line. VES centers are 15 m apart, resulting in a 45 m surveyed line length. The vertical electric soundings (VES) were conducted by using the symmetrical Schlumberger array with a maximum current electrode spacing (C1C2) of 130 m. This arrays parameters give us a maximal depth study of 26 m.

SITE DESCRIPTION AND GEOLOGICAL SETTINGS

The study site is located in SE part of Albania, near Moglica village, part of Korça district (Fig. 1). This area is under development because of HydroPower plants that are in construction in Devolli river cascade. The rural roads network is reconstructed and several bridges also. The surveyed line is located near Devolli river, on the side of a concrete bridge. The line is oriented perpendicular to the river bed and is surveyed in help of geotechnical studies for a new bridge and road construction.

The region is characterized by a complex geology combined with a very developed tectonic setting. In our site of study are represented these type of rocks:

Middle Eocene and Upper Eocene Flysch deposits (Pg_2^2 - Pg_2^3). The section is represented in lower part by combined clay – alevrolitic packs flysch deposits with sandstones and sporadic limestones. This pack underlays a sandstone – alevrolitic – clay pack with limestones and marl. In the upper part the section is represented by a clay – sandstone alevrolitic pack.

In Moglica village region this flysch is represented by alternation of clay – alevrolitic – sandstones packs with sandstone – clay packs. In between are encountered layers of micritic limestones, gray to beige in color, 10 – 15 cm thick, rich of planktonic foraminifers. Also conglomeratic turbidites with variable thickness are found.

In younger levels is noted flysch with layered conglomerates and turbidites with limestone olistolites of different sizes. This is covered by thin clay – marl flysch with sandstone (several cm thick) dated Upper Eocene (Pg_2^3). Those deposits have a high presence of folds and micro folds.

Alluvial deposits are represented in the site by sand and gravel sediments of actual river bed and old terraces of Devolli river.

Figure 1. Survey site and VES centers location (schematic presentation from Google Earth).

MATERIALS AND METHOD OF STUDY

Electrical resistivity techniques measure earth resistivity by passing an electrical current into the ground and measuring the resulting potentials created at the surface. This method involves the supply of direct current or low-frequency alternating current into the ground through a pair of current electrodes and the measurement of the resulting potential through another pair of electrodes called potential electrodes. As the distance between the current electrodes is increased, so the depth to which the current penetrates is increased. In the case of the Schlumberger array (Fig. 2), the potential electrodes (P1, P2) are placed at a fixed spacing (b) which is no more than a fifth of the current-electrode half-spacing (a) (REYNOLDS, 2011).

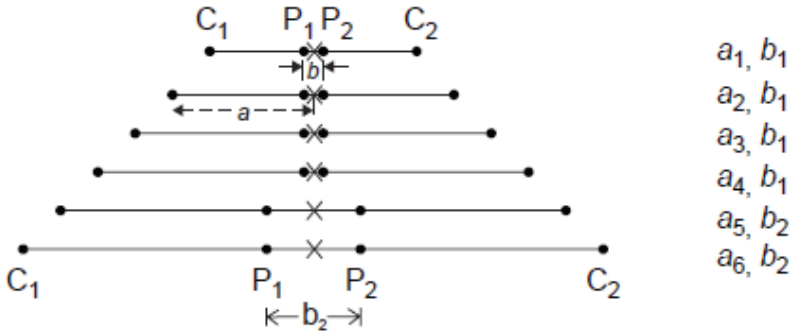

Figure 2. Expanded arrays with successive positions displaced at VES survey using Schlumberger array (REYNOLDS, 2011).

The current electrodes are placed at progressively larger distances. When the measured voltage between P1 and P2 falls to very low values (owing to the progressively decreasing potential gradient with increasing current electrode separation), the potential electrodes are spaced more widely apart (spacing b2). The measurements are continued and the potential electrode separation increased again as necessary until the VES is completed. The position of measurement is taken as the midpoint of the electrode array. For a depth sounding, measurements of the resistance ($\Delta V/I$) are made at the shortest electrode separation and then at progressively larger spacing (REYNOLDS, 2011).

At each electrode separation a value of apparent resistivity (ρa) is calculated using the measured resistance in conjunction with the appropriate geometric factor for the electrode configuration and separation being used.

The values of apparent resistivity are plotted on a graph ("field curve"), the *x*- and *y*-axes of which represent the logarithmic values of the current electrode half-separation (AB/2) and the apparent resistivity (ρa), respectively.

A total number of four VES surveys were conducted. The VES centers were projected in a line perpendicular to the river bed, starting from 2 m far from the riverbed with a step of 15 m along the line, making possible about 45 m of total length surveyed line. All the arrays are spread parallel with the riverbed. VES 4 center is displaced from the line to avoid the concrete bridge effects upon the current lines spread. The measurements were carried out with a Time Domain transmitter IPC- 8. As receiver it was used a Syscal (*Iris Instruments, France*). A transmitter time (*pulse*) of T = 2 sec and receiving time t = 2 sec were used for these measurements. The field data are interpreted using Geosoft Oasis Montaj for a qualitative section and IX1D software for VES data interpretation in terms of a quantitative section.

Potential electrodes (P1, P2) are placed in the center of survey array and served to measure the potential difference between them. The P1P2 electrodes distance was changed progressively from (P1P2)/2 = 1.5 m up to over 65 m. Tmaaking into consideration that survey depth is approximately 0.2 · P1P2, we have obtained 26 m maximum investigation depth. All resistivity surveys are high quality surveys. Several repeated surveys were done to assure the surveyed data quality. The standard deviation between repeated surveys was less than 5%. This accuracy is helpful in the processing and interpreting phase of field data.

The apparent resistivity (ρa) was calculated using the expression:

$$\rho_a = k \cdot \frac{\Delta V}{I}$$

where:

k – Geometrical factor of the array (in meter units), calculated as:

$$k = 2\pi/(1/C1P1 - 1/C1P2 - 1/C2P1 + 1/C2P2)$$

ΔV – Difference of potential between P1 and P2 electrodes (in Volts)

I – Current injected using the C1 and C2 electrodes (in Amps).

RESULTS AND DISCUSSION

After field data quality control and processing, the results are presented as both qualitative and quantitative interpretations. In the qualitative interpretation it is observed the shape of the VES curve and qualitative information about the number of layers and the resistivity of layers is obtained. The results of this method of interpretation involved geoelectrical sections using resistivity "Real Section" methodology of data presentation (KARRIQI & ALIKAJ, 2011; ALIKAJ et al., 2012*)*. All the apparent resistivity surveyed data are plotted for each VES and a 2D section is obtained.

In the quantitative method, true resistivity and layer thicknesses are obtained. The main objective of the quantitative interpretation of VES curves is to obtain the geoelectrical parameters and geoelectric section. A geoelectric layer is called by its fundamental characters, resistivity "ρ" and thickness "h". After obtaining geoelectrical parameters for each VES surveyed, we can correlate all VES data, obtaining a 2D quantitative section. By knowing the average resistivity of different lithologies, we may obtain a geoelectrical – geological 2D section.

Fig. 3 presents a 2D apparent resistivity section. In this 2D section we can notice the presence of high apparent resistivity values at VES 1, VES 2 and VES 3 in the upper part. This is an indicator of the gravel presence at the section. In the upper part (first 4 – 6 meters of the section) gravels are not mixed with clay material and this causes high values of apparent resistivity. At the upper part of VES 2 (about 1 m) we can note relatively lower apparent resistivity values, indicating the presence of mixed clay material in gravels in this part of the section. From 6 m to about 13 m of depth, the apparent resistivity values are lower, indicating a higher presence of clay material mixed with gravels. VES 4 is projected on the section for interpretation purposes. Normally this VES center is located about 30 m perpendicular to the line where other VES surveys are performed.

At the upper part of VES 4 (first 4 meters) can be noted low apparent resistivity values (in the range of clay and silt values). This is explained by deposition of clay and silt material eroded by flysch which is outcropping near the road. Also, river meanders may have deposited in this part clay and silt material.

Deeper, the apparent resistivity values are higher at VES 4 (at the level of gravel mixed with clay values). In all four VES surveys, at depth (below 12 – 13 meters), can be noted an immediate decrease of the apparent resistivity values, at the range of 40 – 100 ohm·m, indicating the presence of flysch deposits. The upper part of the flysch deposits is undulated and can be surveyed at different depths on VES surveys, but the average depth is on the range of 10 – 13 meters.

In quantitative interpretation, the geoelectrical parameters (true resistivity and thickness) are obtained for each geoelectrical layer through data inversion. In Fig. 4 are represented the quantitative interpretations of VES curves and true resistivity values and thicknesses for each interpreted layer.

From the quantitative sections it can be noticed that the flysch basement at the bottom part of the section is characterized by true resistivity values on the range of 40 -50 ohm·m. The depth of the flysch basement varies from 8 m to around 13 m.

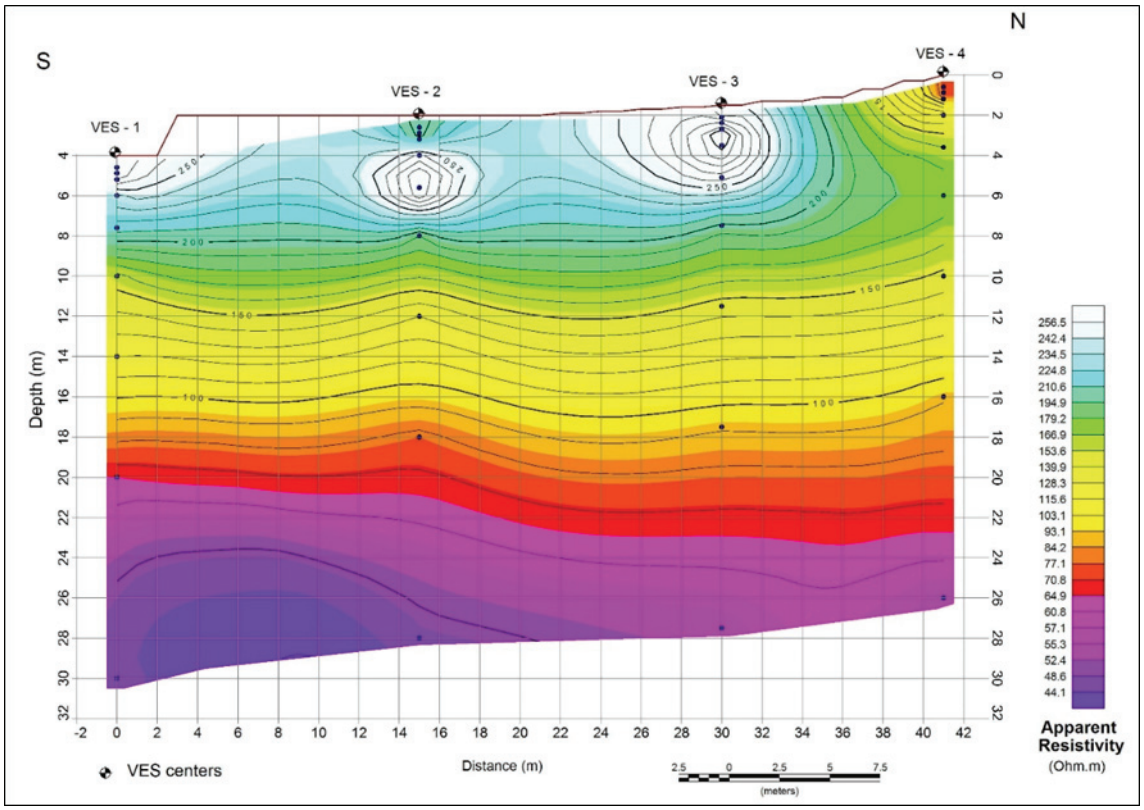

Figure 3. Qualitative 2D interpretation of apparent resistivity resulted from the VES surveys.

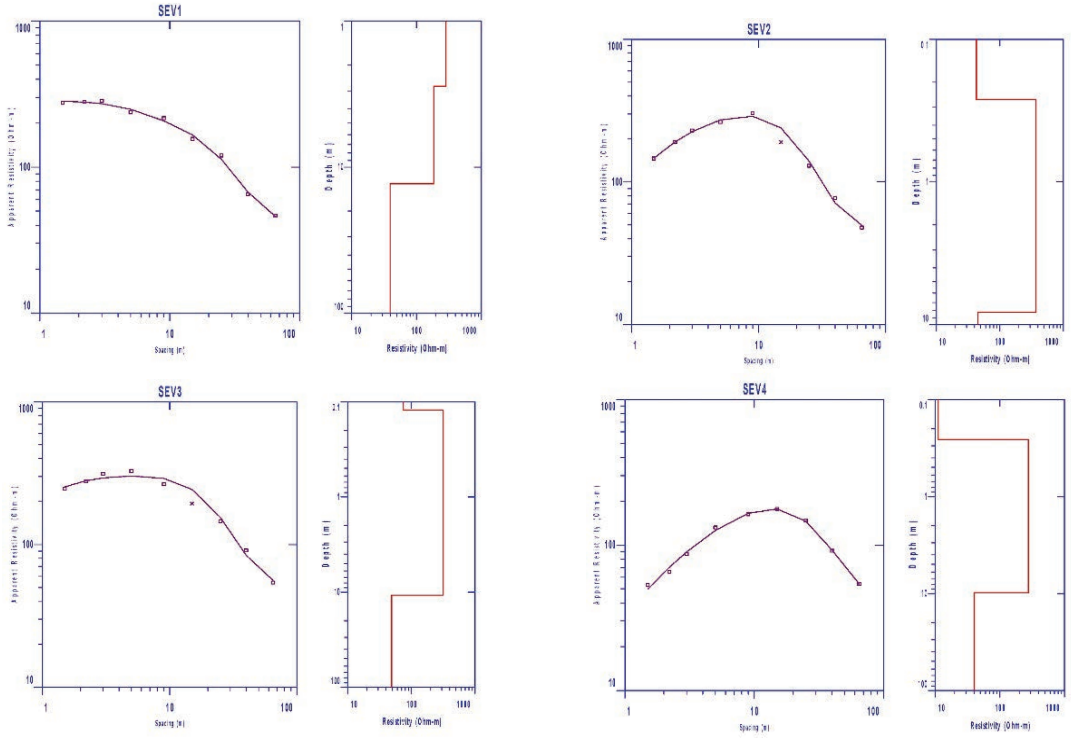

Figure 4. Interpretation curves of VES data defining true resistivity and thickness for each layer.

Above the flysch basement it is interpreted a gravel layer mostly heterogeneous, with some parts where clay and silt material is mixed with gravel. Because the upper part of the gravel is dry or with moderate moisture, the resistivity values are slightly higher (VES 1 first 2 meters). The gravel layer thickness is interpreted as 8 to 13.5 m. The level of underground water in gravel layers is below 3 m of depth, in the river bed quote. In the upper part of the sections is present a thin layer where predominant are silts and clays mixed with sand and gravel. The thickness of this layer varies from 0.2 to 0.4 m. At VES 1, which is about 3 m from the river bed, this thin layer is not present because of river erosion effect.

Qualitative 2D section served as a basis for an accurate quantitative interpretation, based on the correlation of geoelectrical section with the lithology, by plotting a 2D geoelectrical – geological section (Fig. 5).

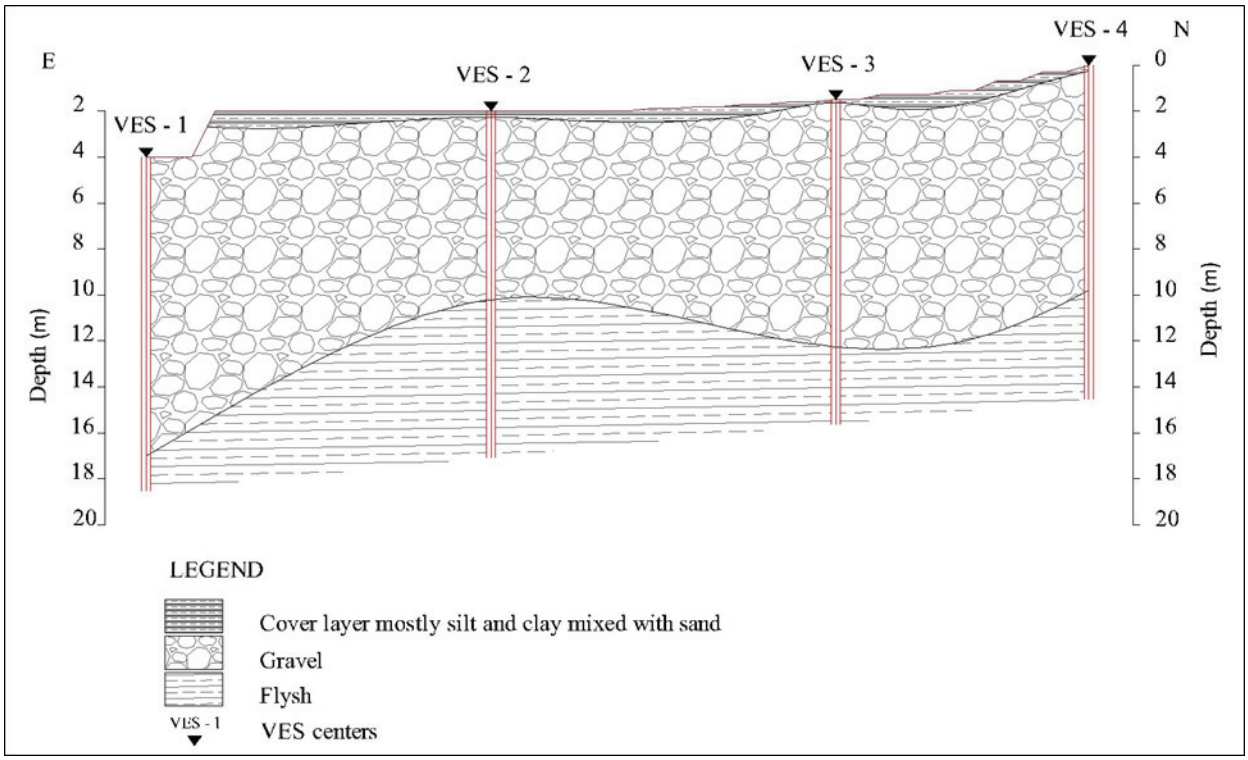

Figure 5. Quantitative geoelectrical - geological section derived from VES data interpretation.

CONCLUSIONS

Geoelectrical surveys can be very helpful in mapping bedrock. This surveys are easy to apply, they don't need a large crew, for shallow depth studies can be used portable, light weight equipments and VES surveys provide accurate results. VES surveys are giving information about depth from a point in surface which is the center of the survey array. In a certain way, a VES survey we may be called "electrical drilling". By surveying several VES along a line, it is possible to correlate all VES surveys and plot a 2D section.

Qualitative sections give information on how the apparent resistivity parameter varies in the section, where are the high and low resistivity areas located. In our study we have plotted a qualitative section using VES field data and apparent resistivity "Real Section" technique in data presentation. Qualitative 2D section served like a basis for an accurate quantitative interpretation, based on the correlation of geoelectrical section with the lithology

Quantitative sections give information about true resistivity and layer thickness (geoelectrical parameters). The flysch basement at the bottom part of the section is characterized by true resistivity values on the range of 40 -50 ohm·m. Depth of the flysch basement varies from 8 m to around 13 m. The flysch surface is undulated, so the gravel layer thickness varies along the line. Finding the flysch boundary pattern, helps geotechnical works in future.

A gravel layer mostly heterogeneous with some parts where clay and silt material is mixed with gravel is interpreted above the flysch basement. The gravel layer thickness is interpreted from 8 to 13.5 m. In the upper part of the sections is present a thin layer (0.2 – 0.4 m thickness), where the predominant lithology consists of silts and clays mixed with sand and gravel. At VES 1, this thin layer is not present because of the river erosion effect.

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EFFECTS OF SEEDS IRRADIATION WITH GAMMA-RAY ON PLANT GROWTH AND YIELD ATTRIBUTING CHARACTERS OF SAFFLOWER

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Abstract. The purpose of this study was to evaluate the impact of pre-sowing gamma irradiation with various doses of safflower seeds and time of seeds sowing on bio-morphological characters of plants. Based on ANOVA analysis there was showed that the time of seeds sowing statistically significantly influenced the height of safflower, number of developed and undeveloped inflorescences per plants more than the radiation. However, the contribution of radiation to the number of undeveloped inflorescences per plant was approximately seven times greater than the contribution of sowing time. The interaction between radiation and sowing time factors contributed to changes in number of secondary branches and developed inflorescences per plant. The correlations between the bio-morphological characters of plants growing under the same conditions demonstrated the strong direct proportional dependences of developed inflorescences on number of secondary branches per plant as well as the direct correlation between developed inflorescence and number of seeds per plant in all experimental variants. Thus, the safflower plants obtained from irradiated seeds which were sown in fall showed a more significant response to the abiotic factor (gamma radiation) by modifying the bio-morphological characters in comparison with the spring sown safflower.

Keywords: safflower, seed, gamma radiation, bio-morphological characters, yield.

Rezumat. **Efectele iradierii semințelor cu raze gamma asupra creşterii plantelor şi a caracterelor atribuite productivităţii şofrănelului.** Scopul acestui studiu a fost de a evalua impactul iradierii gamma cu diferite doze a semințelor de șofrănel înainte de semănat și epocii de semănat asupra caracterelor bio-morfologice ale plantelor. În baza analizei ANOVA s-a constatat că, epoca de semănat a influențat statistic semnificativ asupra înălțimii șofrănelului, numărului de inflorescențe dezvoltate și nedezvoltate per plantă mai mult decât radiația. Cu toate acestea, contribuția radiației la numărul de inflorescențe nedezvoltate per plantă a fost aproximativ de șapte ori mai mare decât contribuția epocii de semănat. Interacțiunea dintre factorii de iradiere și epoca de semănat a contribuit la modificarea numărului de lăstari secundari și a inflorescențelor dezvoltate per plantă. Corelațiile dintre caracterele bio-morfologice ale plantelor cultivate în aceleași condiții au demonstrat dependența puternică direct proporțională a inflorescențelor dezvoltate de numărul de lăstari secundari per plantă, precum și corelația directă dintre numărul de inflorescențe dezvoltate și numărul de semințe per plantă în toate variantele experimentale. Astfel, plantele de șofrănel obținute din semințe iradiate care au fost semănate în toamnă au demonstrat un răspuns mai semnificativ la factorul abiotic (radiația gamma) prin modificarea caracterelor bio-morfologice în comparație cu șofrănelul semănat primăvara.

Cuvinte cheie: şofrănel, semințe, gamma radiație, caractere bio-morfologice, productivitate.

INTRODUCTION

Safflower (*Carthamus tinctorius*) plants are valuable agronomic species for different practical purposes: seeds – bird and animal feed; seeds oil – food, containing alphatocopherol; bee plants – flower for honey production; red and yellow pigments of petals - food colorants; ornamental plants; post-harvesting silage for livestock feed; source of biologically active substances - folk medicine. The pre-sowing treatments of seed with physical methods are used in order to stimulate the physiological processes in plant metabolism: germination energy, photosynthetic and enzymes activity, redox processes, secondary metabolites accumulation (MOGHADDAM et al, 2011; AKSYONOV, 2013). The gamma rays are the most efficient ionizing radiation and often applied on plants for developing varieties which are agriculturally and economically important as well as comprise high productivity and efficiency potential (KIM et al., 2004; HEGAZI & HAMIDELDIN, 2010; JAN et al, 2012). It was reported (PATIL et al., 2001; KAYA et al., 2009) that the biological and genetic characters of safflower modified by gamma irradiation of seeds resulted in increasing of plants yield. Expression of various characters is often changed as results of changing breeding materials and environment. Therefore, the information about inter relationships between plant bio-morphological characters and yield is important for determination the component character on which selection can be based for improvement in safflower yield (ARSLAN, 2007). The present work was intended to investigate the impact of pre-sowing gamma irradiation of safflower seeds with various doses and effects of seed sowing time on bio-morphological characters of plants in term of growth and seed yield.

MATERIAL AND METHODS

Plant materials were obtained from field experiments which were carried out at the research field station of Institute of Genetics and Plant Protection in Chisinau area of Republic of Moldova (lat. $47^o01'$, long. $28^o75'$, alt. 85 m above sea level), in the season of 2016-2017. Safflower seeds were treated with three doses of γ-radiation (50, 100, 150Gy) using gamma RXM-V-20 system, the radiation source - ^{60}Co. Irradiated seeds were sown in experimental field in fall on November 07, 2016 and in spring on April 05, 2017. The row spacing of plantation was 50cm and intra-row

spacing - 15cm. Each row consisted of 50 seeds, in triplicate. Plants were grown in poor, dry soil, without irrigation. The plants grown from intact (untreated with gamma radiation) seeds served as control.

T**he bio-morphological characters of safflower plants** were studied according AHMADZADEH (2013), namely plant height (cm), number of secondary branches, number of inflorescences per plant, number of seeds per plant, number of seeds per capitulum, 1000-seed weight (g), seed yield (g).

The software package Statgraphics Plus 2.1 was used for statistical analysis. The ANOVA test was applied for variance analysis of bio-morphological characters, and Student test in assessment of statistically significant differences between treatments (RAUDONIUS, 2017).

RESULTS AND DISCUSSIONS

The morphological characters of safflower plants grown in experimental field showed that the plants height varied from 41 to 111cm (Table 1). The number of secondary branches per plant was minimum 2 and maximum 18, having up to 31 developed inflorescences and 38 undeveloped inflorescences. However, the average of developed inflorescences per plant was 2-3 times more than undeveloped inflorescences. It is important to note that the values of bio-morphological characters of safflower plants were modified depending on weather conditions of vegetation season. Our experiences of spring sown safflower in 2015 showed that the height of plant varied from 33 to 78cm (IVANOVA, 2016); and in 2016 it was 61-110cm (IVANOVA et al., 2017). In different seasons the average of safflower height was 59.25cm (2015) and 86.66cm (2016). Data obtained in 2017 (Table 1) statistically confirmed the growth potential of safflower in our experimental field and pedoclimatical conditions of the Republic of Moldova. The number of secondary branches, developed and undeveloped inflorescences per plant also differed from year to year. According to bio-morphological characters of safflower the best season was in 2016, the worst - in 2015.

Table 1. Bio-morphological characters of safflower sown in different time.

Time of sowing	Index	Characters of plants from seeds irradiated by different dose			
		Control	50Gy	100Gy	150Gy
Height					
Fall	average ±SE	76.51±2.56	77.38±1.83	75.39±2.39	71.65±2.29
	min ÷ max	47.0÷111.0	55.0÷98.0	56.0÷92.0	58.0÷100.0
	coefficient of variation,%	19.19	13.81	13.47	13.16
Spring	average ±SE	66.86±1.47	66.60±1.26	67.92±2.04	67.60±1.70
	min ÷ max	41.0÷95.0	46.0÷88.0	46.0÷81.0	54.0÷88.0
	coefficient of variation,%	16.47	14.44	14.69	12.57
Secondary branch					
Fall	average ±SE	6.48±0.37	7.24±0.53	8.67±0.92*	5.82±0.50
	min ÷ max	3÷12	3÷14	4÷16	2÷9
	coefficient of variation,%	32.96	43.05	45.12	35.52
Spring	average ±SE	7.38±0.42	7.31±0.29	7.21±0.41	8.32±0.49
	min ÷ max	2÷18	2÷14	3÷11	5÷14
	coefficient of variation,%	42.38	30.23	27.74	29.59
Developed inflorescence					
Fall	average ±SE	13.18±1.27	17.94±1.94*	20.33±2.99*	11.59±1.57
	min ÷ max	2÷31	5÷48	8÷52	2÷29
	coefficient of variation,%	55.44	63.30	62.55	55.93
Spring	average ±SE	12.55±0.97	11.93±0.85	12.38±1.68	14.44±1.24
	min ÷ max	2÷29	0÷31	0÷30	5÷33
	coefficient of variation,%	58.08	54.13	66.33	43.02
Undeveloped inflorescence					
Fall	average ±SE	4.64±0.67	6.29±0.70	11.56±1.77***	9.41±2.07**
	min ÷ max	0÷19	0÷16	0÷31	0÷30
	coefficient of variation,%	82.45	65.28	65.11	90.63
Spring	average ±SE	7.84±0.91	7.95±0.56	10.58±1.36	12.08±1.58
	min ÷ max	1÷38	2÷24	3÷34	2÷29
	coefficient of variation,%	87.26	53.30	63.04	65.35

Note: *; **; ***- denotes the statistically significant difference in comparison with control at P≤0.05; P≤0.01; P≤0.001, respectively

The safflower seeds can be sown in the fall as well as in the spring gratitude to their tolerance to low temperatures. The fall sowing safflower resulted in earlier flowering and maturity and increased yield compared to spring sown plants (PETRIE et al., 2010). The analysis of bio-morphological characters of safflower from two variants of sowing indicated that the height of safflower plants from fall sowing seeds was 9-10cm higher than the spring sowing plants (Table 1). Similar results were reported by ESENDAL et al. (2008); in fall sown safflower the value of plant height was the tallest (207.5cm), and the lower height was 55.2cm for spring sown plant.

In addition the fall sowed plants had undeveloped inflorescences less by 1.67 times comparing to the spring-sown plant. The applied doses of radiation did not have significant influence on height of safflower plants both fall and spring sowing. However, the dose of 50Gy had the statistically significant impact at P≤0.05 on the number of developed

inflorescences per plant of fall sown safflower. Increasing the dose up to 100Gy for seeds irradiation led to statistically rising of secondary branches number and developed inflorescences per plants. It is important to mention, that the number of undeveloped inflorescences also increased significantly. The plants of fall sowing from seeds treated by 150Gy doses had the worst bio-morphological characters in comparison with other experimental variants (Table 1). Concerning the studied characters of plants obtained from irradiated and control safflower seeds sown in spring there were no statistically significant differences between the values. The safflower plants obtained from irradiated seeds which have been sown in fall in experimental field have shown a significant response to an abiotic factor (gamma radiation) by changing their bio-morphological characters in comparison to spring sown safflower.

The impact evaluation of factors such as radiation and seed sowing time (fall or spring) on bio-morphological characters of safflower plants was carried out by ANOVA analysis (Table 2). The time of seeds sowing statistically significantly influenced the height, number of developed and undeveloped inflorescences per safflower plants more than the radiation. However, the contribution of radiation to the number of undeveloped inflorescences per plant was approximately 7 times greater than the contribution of sowing time. The interaction between radiation and sowing time factors contributed to changes in number of secondary branches and developed inflorescences per plant (Table 2).

Table 2. Impact of radiation and seed sowing time on bio-morphological characters of safflower.

Source	Sum of squares	Df	Mean square	F-ratio	P-value	Source contribution,%
Height						
Radiation (R)	166.45	3	55.48	0.48	0.6976	ns
Time of sowing (T)	3508.98	1	3508.98	30.26***	0.0000	10.01
Interactions R-T	345.59	3	115.20	0.99	0.3965	ns
Total	35051.30	264				
Secondary branch						
Radiation (R)	23.24	3	9.75	1.36	0.2569	ns
Time of sowing (T)	13.80	1	13.80	1.92	0.1672	ns
Interactions R-T	86.80	3	28.93	4.02**	0.0080	4.41
Total	1967.04	264				
Developed inflorescence						
Radiation (R)	441.54	3	147.18	2.20	0.0881	ns
Time of sowing (T)	474.26	1	474.26	7.10**	0.0082	2.50
Interactions R-T	909.61	3	303.20	4.54**	0.0040	4.80
Total	18959.90	264				
Undeveloped inflorescence						
Radiation (R)	1015.95	3	338.65	9.39***	0.0000	9.62
Time of sowing (T)	147.64	1	147.64	4.09*	0.0441	1.39
Interactions R-T	127.37	3	42.46	1.18	0.3188	ns
Total	10560.00	264				

Note: *; **; ***- denotes the statistically significant difference at P≤0.05; P≤0.01; P≤0.001, respectively

The correlations between the bio-morphological characters of plants grown in same conditions were determined by Pearson coefficients (Table 3). The strong direct proportional dependences of developed inflorescences on number of secondary branches per plant were determined in all variants of fall sown safflower and in control plant of spring sowing. Similar correlation between the number of secondary branches and developed inflorescences of Turkish safflower cultivars was described by BEYYAVAS et al. (2011).The irradiation of spring sown seeds provoked the disturbance of this regularity.

Table 3. Pearson correlation coefficient between number of secondary branches and developed inflorescence.

Sowing time	Control	50Gy	100Gy	150Gy
Fall	0.7783	0.8763	0.8054	0.7680
Spring	0.8398	0.6419	0.5354	0.5817

The number of seeds harvested per each safflower plant varied in large limits from one seed to 1719 seeds (Table 4). Because of that the impact evaluation of growing conditions and doses of radiation was carried out by averages of yield attributed characters. The safflower cultivated from untreated seeds (control) in fall sown field had 1.35 times higher number of seeds per plant in comparison with spring sown. The maximum amount of seeds was determined in variant 50Gy –fall-sown and was equal to 570.27. This character was significant (P≤0.05) higher, by 43.0%, than similar character of control plant.

The weight of seeds per plant also modified in the wide range from 0.01 to 53.60 g (Table 4). The weight of seeds from control field of fall-sown plant was 11.89g and spring-sown - 7.63g. Under the influence of radiation, the values of seeds weight were fluctuated towards rising or diminishing, but no significant changes were observed in all experimental variants.

The most important character of plant productivity is the weight of 1000 seeds (Table 4). The minimum and maximum values of this character varied from 10.70 to 53.30g and depended on sowing time and doses of gamma

radiation. For comparison the 1000-seed weight in season of 2015 was 29.35g and in 2016 – 37.80g (IVANOVA, 2016; IVANOVA et al., 2017). The large dispersion of values of yield attributed characters of safflower plants was also reported by other scientists (KIZIL et al., 2008; BEYYAVAS et al., 2011; AHMADZADEH, 2013; SHINWARI et al., 2014; KHAKI-MOGHADAM & ROKHZADI, 2015). In our experiment the pre-sowing irradiation of seeds by 150Gy caused significant increase in 1000-seed weight, by 19% in the fall-sowing field ($P \leq 0.01$). One safflower capitula of control plants contained on average 21.62-30.26 seeds (Table 4). The significant impact of irradiation was established only in spring sowing field of seeds treated by 100Gy ($P \leq 0.1$). Safflower cultivated in semi-arid conditions of Turkey had 30.6-40.1 seeds per capitulum (KIZIL et al., 2008), but Iranian cultivars characterized by 8.4-13.6 seeds per capitulum (KHAKI-MOGHADAM & ROKHZADI, 2015). PATIL et al. (2001) discovered that this yield component (number of seeds per capitulum) due to gamma irradiation showed high variability in generation F_2M_2. Number of seed per capitulum in F_2 was 56.52; in M_2 - 36.26 and in F_2M_2- 77.00 (PATIL et al., 2001).

Table 4. Seed yield of safflower grown from seeds irradiated by different gamma dose.

Time of sowing	Index	Yield attributing characters of safflower plant from irradiated seeds			
		Control	50Gy	100Gy	150Gy
Number of seeds per plant					
Fall	average±SE	398.73±43.89	570.27±68.21*	535.18±111.21	265.12±35.62
	min ÷ max	35÷1102	103÷1719	137÷1625	75÷602
Spring	average±SE	295.41±29.05	296.57±25.47	374.61±60.63	344.68±40.58
	min ÷ max	6÷740	32÷721	1÷1051	45÷760
Weight of seeds per plant					
Fall	average ±SE	11.89±1.33	15.44±1.87	17.23±3.50	9.30±1.63
	min ÷ max	0.61÷33.85	2.49÷53.60	3.37÷53.51	2.85÷30.84
Spring	average ±SE	7.65±0.80	7.35±0.70	9.94±1.68	8.82±1.04
	min ÷ max	0.10÷20.36	0.71÷22.58	0.05÷32.80	0.78÷19.86
Weight of 1000 seeds					
Fall	average ±SE	29.63±0.91	28.39±1.02	33.65±1.94	35.39±2.62**
	min ÷ max	17.4÷38.6	14.1÷38.6	17.2÷47.8	17.0÷51.8
Spring	average ±SE	25.47±0.97	24.77±0.86	27.77±1.42	25.93±1.22
	min ÷ max	10.7÷53.3	10.9÷37.10	15.7÷50.00	13.4÷40.60
Number of seeds per capitulum					
Fall	average ±SE	30.26±1.28	32.15±2.42	25.01±3.20	23.95±1.89
	min ÷ max	2÷42	12÷83	11÷63	12÷46
Spring	average ±SE	21.62±1.16	22.83±0.99	26.08±2.10*	22.10±1.83
	min ÷ max	3÷43	8÷37	1÷49	4÷45

Note: *; ** - significant differences at $P \leq 0.1$; $P \leq 0.05$, respectively.

According to ARSLAN (2007) the yield of safflower seeds is determined by the number of developed inflorescences and number of seeds per capitulum since these characters had direct significant positive effects on seed yield. The results obtained by us showed that between the number of developed inflorescences per plant and the number of seeds per plant had a strong linear correlation ($r > 0.7$) which was established in all experimental variants (50, 100, 150Gy) and in control (Table 5). The exception was the variant 100Gy-fall sown plant, where the coefficient of linear correlation was equal to 0.6934. Thus, the correlation between the number of secondary branches and developed inflorescences as well as between developed inflorescences and number of seeds per safflower plant was determined. The results reported by BAHMANKAR et al. (2014) suggested that 1000-seed weight; developed inflorescences per plants have direct positive effect on seed yield. The researchers (BAHMANKAR et al., 2014) have concluded that the number of developed inflorescence per plant; 1000-seed weight and plant height are putative morphological markers which can be considered as the desirable tools for screening elite safflower genotype under the field conditions.

Table 5. Correlation coefficient of between developed inflorescence and number of seeds per plant.

Sowing time	Control	50Gy	100Gy	150Gy
Fall	0.9199	0.8763	0.6934	0.9433
Spring	0.9252	0.8781	0.9440	0.7596

Quantitative evaluation of bio-morphological characters of safflower plants obtained from gamma irradiated seeds was done in comparison to the control (Table 6). The analysis of variance for these indices by ANOVA test revealed specificity influences of cultivation conditions. The impact of gamma irradiation was not significant in the indices variation in field conditions of spring sowing safflower. At the same time, there was significant contribution of radiation to the variation of all characters analyzed at 90, 95 and 99% confidence intervals in fall sown plants (Table 6).

Table 6. Analysis of variance for bio-morphological characters (ANOVA test).

Source	Df	Characters	Sum of squares	Mean of squares	F-ratio	Contribution of source.%
Fall sowing						
Radiation	3	Number of seeds per capitulum	1094.3	364.765	2.94**	8.34
		Number of seeds per plant	1.27806E6	426019.0	3.77**	10.45
		Weight of seeds per plant	747.757	249.252	2.46*	7.06
		Weight of 1000 seeds	0.0007373	0.0002458	4.81***	12.96
Total	100	Number of seeds per capitulum	13122.7			
		Number of seeds per plant	1.22358E7			
		Weight of seeds per plant	10585.6			
		Weight of 1000 seeds	0.0056887			
Spring sowing						
Radiation	3	Number of seeds per capitulum	338.457	112.819	1.54	ns
		Number of seeds per plant	144252.0	48084.1	1.01	ns
		Weight of seeds per plant	133.132	44.3773	1.25	ns
		Weight of 1000 seeds	0.00015106	0.000503	1.08	ns
Total	159	Number of seeds per capitulum	11786.1			
		Number of seeds per plant	7.58969E6			
		Weight of seeds per plant	5674.05			
		Weight of 1000 seeds	0.0074015			

Note: *; **; *** - significant differences at P ≤0.1; P ≤ 0.05; P ≤0.01, respectively

CONCLUSIONS

The correlations between the bio-morphological characters of safflower growing under the same conditions demonstrated the strong direct proportional dependences of developed inflorescences on number of secondary branches per plant as well as the direct correlation between developed inflorescence and number of seeds per plant.

The safflower plants obtained from irradiated seeds which were sown in fall showed a more significant response to the abiotic factor (gamma radiation) by changing their bio-morphological characters in comparison with the spring sown safflower.

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LICHEN DIVERSITY OF GÖLCÜK NATURE PARK (ISPARTA), INCLUDING NEW RECORDS FOR TURKEY

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Abstract. In this study where the lichen biodiversity of Gölcük Nature Park and its surroundings in Isparta province located in the north-western part of Mediterranean Region of Turkey is assessed, a list of 189 lichenized fungi species (192 taxa) is reported. The most frequent 3 species in the study area are *Melanohalea exasperata*, *Anaptychia ciliaris* and *Lecidella elaeochroma*. Distribution of lichenized fungi depending on the substrate, respectively, is (41.67%) epiphytic, (36.56%) saxicolous, (14.52%) terricolous, (3.76%) muscicolous and (3.49%) lichenicolous. On a morphological basis, the crustose lichen taxa predominate in the area with 55.50% percentage, followed by the foliose (25.13%) and the squamulose (7.85%) taxa, while the leprose taxa are the fewest. Among the identified taxa, 73 species are firstly recorded for the research area and Isparta province. *Diplotomma pharcidium* (Ach.) M. Choisy, *Flavoplaca granulosa* (Müll. Arg.) Arup, Frödén & Søchting, and *Miriquidica pycnocarpa* (Körb.) are three new records for Turkish Lichen Mycota.

Keywords: Lichenized fungi, diversity, Mediterranean Turkey.

Rezumat. Diversitatea lichenilor din Parcul Natural Gölcük (Isparta), inclusiv înregistrări noi pentru Turcia. În acest studiu, este evaluată biodiversitatea lichenilor din Parcul Natural Gölcük și împrejurimile sale din provincia Isparta situată în partea de nord-vest a regiunii mediteraneene a Turciei, fiind raportată o listă cu 189 specii de fungi lichenizați (192 taxoni). Cele mai frecvente 3 specii din zona studiata sunt *Melanohalea exasperata*, *Anaptychia ciliaris* și *Lecidella elaeochroma*. Distribuția fungilor lichenizati, în funcție de substrat, este (41,67%) epifită, (36,56%) saxicolă, 14,52% tericolă, 3,76% muscicolă și 3,39% lichenicolă. Din punct de vedere morfologic, taxonomia lichenilor crustoși predomină în zonă cu un procent de 55,50%, urmată de taxonii foliați (25,13%) și scuamoși (7,85%), în timp ce restul taxonilor sunt reduși. Dintre taxonii identificați, 73 de specii sunt înregistrate în primul rând pentru zona de cercetare și provincia Isparta. *Diplotomma pharcidium* (Ach.) M. Choisy, *Flavoplaca granulosa* (Müll. Arg.) Arup, Frödén & Søchting și *Miriquidica pycnocarpa* (Körb.) sunt trei noi înregistrări pentru Lichen Mycota din Turcia.

Keywords: fungi lichenizaţi, diversitate, zona mediteraneană a Turciei.

INTRODUCTION

Lichens are by definition symbiotic organisms, usually composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont, which is most often either a green alga or a cyanobacterium (NASH, 2008). Being sensitive to environmental changes due to their physiological and ecological characteristics (HAWKSWORTH & ROSE, 1976; SALO et al., 2012) lichens have been used as indicators for air pollution, climatic changes, forest structures and dynamics regarding the quality of biodiversity (GIORDANI et al., 2012).

Studies on "lichen biodiversity of Turkey" have more importance and value than it had in past, since the country has a great degree of lichen biodiversity that must be protected as well as the plant biodiversity. In spite of the increased number of studies concerning the diversity of lichens in Turkey in recent decades, there is yet insufficient information regarding lichen mycota in many areas of the country.

Isparta is a province located in the northwestern part of Mediterranean Region of Turkey. Lichen mycota of Gölcük Nature Park, located in southwestern part of Isparta province, has never been comprehensively studied before, although there have been several studies already reporting lichens from the province of Isparta. The publications including lichen records from Isparta province are SZATALA (1960); MAYRHOFER (1984); MAYRHOFER et al. (1990); KAYNAK et al. 1997; ÖZTÜRK et al. (1998); ÖZTÜRK & KAYNAK (1997, 1999); ÖZTÜRK et al. (2005); ÇOBANOĞLU & YAVUZ (2006); CZEIKA & CZEIKA (2007); ORAN et al. (2007); ŞENKARDEŞLER (2009); HALICI et al. (2012, 2013); KOÇ et al. (2014); TÜRK et al. (2015); SEZER (2016); KOÇ et al. (2016), and VONDRAK et al. (2016), in chronological order. According to this relevant literature from previous researches, a total number of 347 taxa have been recorded from Isparta province.

The present study aims to determine the diversity of lichenized fungi of the study area "Gölcük Nature Park" in Isparta and to contribute to the Turkish lichen mycota.

MATERIALS AND METHODS

Study Area. The study area including the Gölcük Nature Park and its surroundings within the borders of Isparta province covers a surface area of 5,925 ha. The Gölcük Nature Park is situated in the southwestern part of the city located between 37°38' – 38°03' N and 30°22' – 30°45' E in the C3 square of grid system of Turkey. Isparta province and the study area indicated by a circle as well as Gölcük Nature Park (GNP) are shown in Fig. 1. The park and the study area are surrounded by mountains in south and hills in west and by Isparta city centre in north. Gölcük, which is a caldera lake formed in Early Pliocene, has an altitude of 1380 m and is surrounded by circles of volcanic cones ranging between 1480 m (in north) to 1630 m (in west). The climate of the study area has semi-arid

Mediterranean characters with total annual precipitation of 506 mm and average annual temperature of 12 °C (DEMIR, 2010). The drought period in the study area is between March and November as shown in the ombrothermic diagram given in Fig. 2.

The study area has a mixture of Irano-Turanian and Mediterranean phytogeographic elements with 28.2% endemism. The dominant taxa are *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe, *Pinus sylvestris* L., *Cedrus libani* Carr., *Juniperus oxycedrus* L. subsp. *oxycedrus*, *Robinia pseudoacacia* L., *Juglans regia* L., *Castanea sativa* Miller, *Quercus robur* L., *Quercus coccifera* L., *Populus alba* L., *Populus nigra* subsp. *caudina* (Ten.) Bugala (FAKIR, 1998).

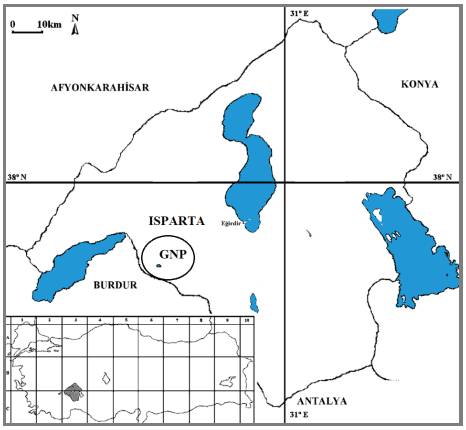

Figure 1. Isparta province and the study area (original).

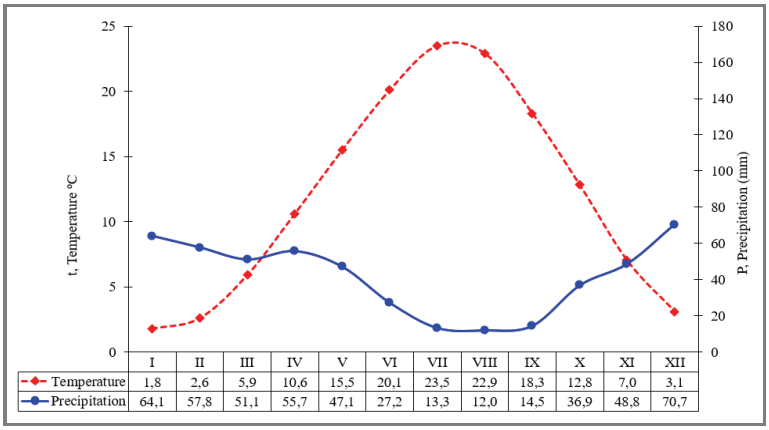

Figure 2. Ombrothermic diagram of Isparta (original).

The lichens were collected from 21 localities (Table 1; Fig. 3) in Gölcük Nature Park and its surroundings in Isparta between the dates April 11th, 2009 and July 22nd, 2010. The collected lichen samples were identified by using various identification keys and books (CLAUZADE & ROUX, 1985; PURVIS et al., 1992; WIRTH, 1995; BRODO et al., 2001; PURVIS, 2007; SMITH et al., 2009).

Microscopic observations were held through a stereomicroscope (Micros SZ45) and a light microscope (Olympus SZ40). The sections were examined in water, 10% KOH solution. Spot tests and UV Lamp (Merck Microbiology 4W 366 nm UV) test were applied to determine the lichen taxa according to identification keys when required.

Table 1. List of localities in the study area. (GNP: Gölcük Nature Park).

No	Locality	GPS Coordinates	Altitude (m)	Date
1	GNP, North-Western Slopes of Crater	37° 44’ 08.80” N 30° 29’ 09.90” E	1415	23.05.2009
2	GNP, North-Western Slopes of Crater	37° 44’ 07.70” N 30° 28’ 57.20” E	1460	23.05.2009
3	GNP, Western Slopes of Crater	37° 43’ 53.74” N 30° 28’ 47.32” E	1480	24.05.2009
4	GNP, Western Slopes of Crater	37° 43’ 48.40” N 30° 28’ 37.43” E	1632	24.05.2009
5	GNP, Western Slopes of Crater	37° 43’ 58.38” N 30° 28’ 35.48” E	1588	24.05.2009
6	Milas Promenade Area	37° 44’ 28.42” N 30° 29’ 14.80” E	1443	06.06.2009
7	GNP, South-Eastern Slopes of Crater	37° 42’ 57.58” N 30° 30’ 29.30” E	1577	07.06.2009
8	GNP, South-Eastern Slopes of Crater	37° 43’ 13.30” N 30° 30’ 13.70” E	1470	07.06.2009
9	GNP, Locust-Grove	37° 43’ 28.88” N 30° 30’ 17.82” E	1425	07.06.2009
10	Between Bezirgân and Hisartepe Hill	37° 44’ 26.65” N 30° 31’ 30.33” E	1296	14.06.2009
11	Path to Ağlasun (Sagalassos)	37° 42’ 48.30” N 30° 31’ 38.10” E	1408	16.06.2009
12	GNP, Southern Border	37° 42’ 16.00” N 30° 30’ 15.50” E	1620	20.06.2009
13	Karatepe Hill Western Slope	37° 43’ 53.00” N 30° 33’ 00.00” E	1605	22.07.2009
14	Summit of Karatepe Hill	37° 43’ 49.69” N 30° 33’ 15.31” E	1724	22.07.2009
15	GNP, Picnic Area	37° 44’ 04.70” N 30° 29’ 25.40” E	1397	01.05.2010
16	GNP, South-Western Slopes of Crater	37° 43’ 00.00” N 30° 29’ 00.00” E	1501	01.05.2010
17	GNP, South of the Lake	37° 42’ 53.00” N 30° 29’ 42.80” E	1462	01.05.2010
18	GNP, East Slopes of Crater	37° 43’ 43.27” N 30° 30’ 26.28” E	1573	22.07.2010
19	GNP, East Slopes of Crater	37° 43’ 49.60” N 30° 30’ 16.30” E	1460	22.07.2010
20	Sidre Hill, East Slope	37° 44’ 41.50” N 30° 34’ 00.00” E	1190	11.04.2009
21	Halife Sultan Cemetery	37° 44’ 44.13” N 30° 34’ 23.82” E	1128	11.04.2009

Current online information systems “ITALIC 5.0” (http://dryades.units.it/italic) (NIMIS & MARTELLOS, 2017) and “Mycobank Database” (http://www.mycobank.org) (ROBERT et al., 2015) were followed for the nomenclature of lichen taxa.

The specimens have been stored in the Herbarium of Marmara University (MUFE). At least one herbarium sample for every taxon has been recorded with a herbarium number between GÇ.2102-GÇ.2295.

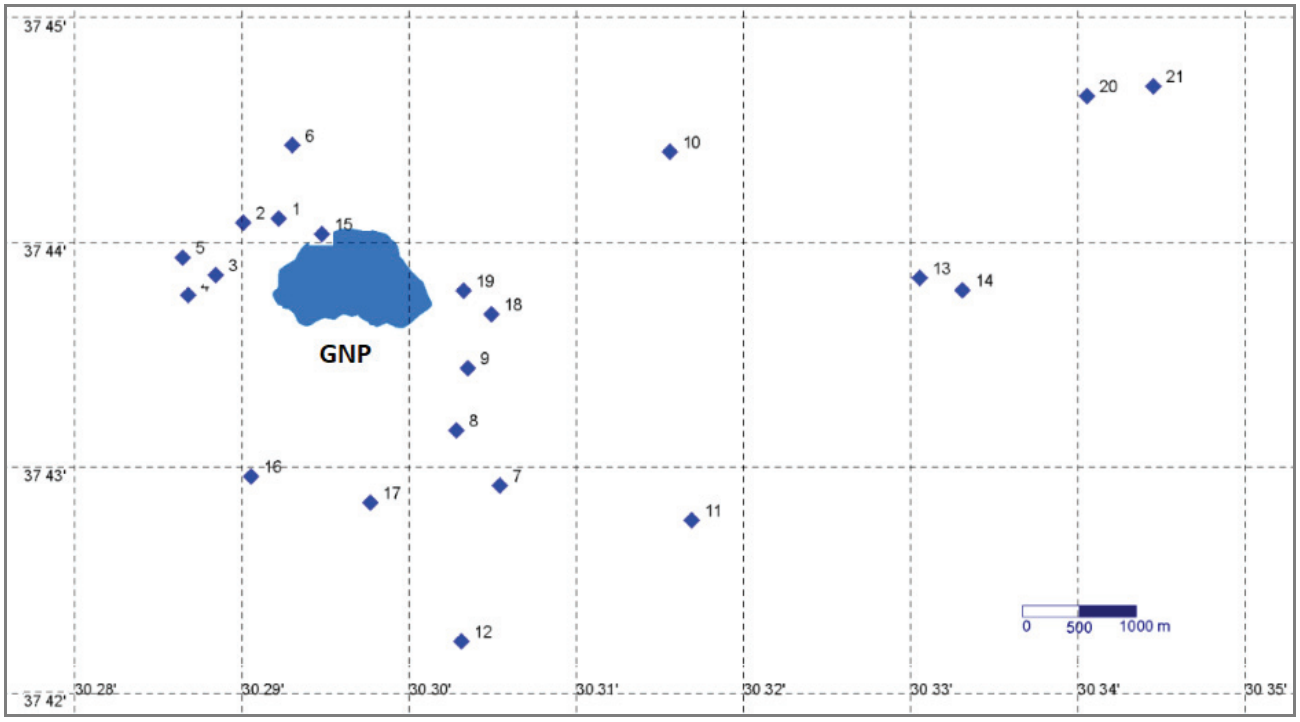

Figure 3. Localities (1-21) in the Study Area (original).

RESULTS

A total of 192 lichenized fungi taxa including 189 species, 3 subspecies and 3 varieties from Gölcük Nature Park and its surroundings are listed in alphabetical order. New records of taxa for Turkey are indicated by "*", and for Isparta Province are indicated by "+". The names of authors are abbreviated according to BRUMMITT & POWELL (1992). "Syn." is used for the synonym names of some taxa. Substrate abbreviations follow the locality numbers of the lichen samples in the list.

Aa: *Aspicilia caesiocinerea*, Af: *Acarospora fuscata*, Ag: *Amygdalus communis*, Ai: *Aspicilia cinerea*, Cl: *Cedrus libani*, CR: Calcareous rock, Cs: *Catapyrenium squamulosum*, Cv: *Candelariella vitellina*, Lr: *Lecanora rupicola*, M: Moss, Pa: *Populus alba*, Pc: *Placocarpus schaereri*, Pd: *Prunus domesticus*, Pg: *Pinus nigra*, Pm: *Protoparmeliopsis muralis*, Pn: *Populus nigra*, Pr: *Peltigera rufescens*, Ps: *Physcia stellaris*, Qr: *Quercus* sp., Rp: *Robinia pseudacacia*, S: Soil, SR: Silicious rock, So: *Sorbus* sp., Ta: *Tephromela atra.*

Acarospora cervina (Ach.) A.Massal.: 3, 4, 11, 20 CR; 6, 9, 10, 13, 14, 16, 17 SR; 19 Ai. GÇ.2102
Acarospora fuscata (Schrad.) Arnold: 5, 6, 10, 13, 14, 16 SR. GÇ.2103
Amandinea pelidna (Ach.) Fryday & Arcadia (Syn. *Amandinea lecideina* (H.Mayrhofer & Poelt) Scheid. & H. Mayrhofer): 6, 19 SR. GÇ.2106
Amandinea punctata (Hoffm.) Coppins & Scheid: 6 CR. GÇ.2107
+Amygdalaria pelobotryon (Wahlenb.) Norman: 10 S. GÇ.2108
Anaptychia ciliaris (L.) A.Massal.: 2, 7, 18 Pg; 4, 9, 12 Rp; 8 Qr; 10 S; 13 Cl; 15 Pn; 16, 17 SR; 20 Pd. GÇ.2109
Arthonia fusca (A.Massal.) Hepp (Syn. *Arthonia lapidicola* (Taylor) Branth & Rostr.): 20 CR. GÇ.2110
Aspicilia cinerea (L.) Körb.: 4 CR; 6, 7, 10, 13, 16, 17, 18, 19 SR. GÇ.2113
+Athallia cerinella (Nyl.) Arup, Frödén & Søchting (Syn. *Caloplaca cerinella* (Nyl.) Flagey): 7 Pg. GÇ.2124
Athallia cerinelloides (Erichsen) Arup, Frödén & Søchting (Syn. *Caloplaca cerinelloides* (Erichsen) Poelt): 7 Pg. GÇ.2125
Athallia holocarpa (Hoffm.) Arup, Frödén & Søchting (Syn. *Caloplaca holocarpa* (Hoffm.) A.E. Wade): 15 Pn; 18 Pg. GÇ.2140
+Blastenia ammiospila (Ach.) Arup, Søchting & Frödén (Syn. *Caloplaca cinnamomea* (Th.Fr.) H.Olivier): 5, 17 M. GÇ.2127
Blastenia crenularia (With.) Arup, Søchting & Frödén (Syn. *Caloplaca crenularia* (With.) J.R. Laundon): 4 CR; 13, 17, 18, 19 SR; 14 Aa, Pm. GÇ.2129
Blastenia herbidella (Arnold) Servít (Syn. *Caloplaca herbidella* (Hue) H.Magn.): 6, 7, 18 Pg; 14 Cl. GÇ.2139
Blennothallia crispa (Huds.) Otálora, P.M. Jørg. & Wedin (Syn. *Collema crispum* (Huds.) Weber ex F.H. Wigg.): 4, 11 SR; 20 CR. GÇ.2166
Buellia aethalea (Ach.) Th.Fr.: 4 CR; 8 SR. GÇ.2116

+*Buellia schaereri* De Not.: 8 Qr; 13 Cl; 15 Pn. GÇ.2117
+*Buellia spuria* (Schaer.) Anzi: 17 SR. GÇ.2118
+*Calogaya arnoldii* (Wedd.) Arup, Frödén & Søchting: 10, 16 SR. GÇ.2120
Calogaya decipiens (Arnold) Arup, Frödén & Søchting (Syn. *Caloplaca decipiens* (Arnold) Blomb. & Forssell): 3, 11 CR. GÇ.2132
Calogaya pusilla (A.Massal.) Arup, Frödén & Søchting (Syn. *Caloplaca saxicola* (Hoffm.) Nordin): 14 SR. GÇ.2145
+*Caloplaca aractina* (Fr.) Häyrén: 6, 16 SR. GÇ.2119
Caloplaca cerina (Hedw.) Th.Fr. s.lat (Syn. *Caloplaca cerina* var. *cerina* (Ehrh. ex Hedw.) Th.Fr.): 7, 8, 18 Pg; 11 M; 12 Rp; 13 Cl; 15 Pn; 16 SR. GÇ.2121
+*Caloplaca grimmiae* (Nyl.) H. Olivier: 13 Cv. GÇ.2137
Caloplaca haematites (Chaub.) Zwackh: 13 Cl; 14SR. GÇ.2138
+*Caloplaca nivalis* (Körb.) Th. Fr.: 5 M; 14 S. GÇ.2144
+*Caloplaca stillicidiorum* (Vahl) Lynge var. *muscorum* (A.Massal.) (Syn. *Caloplaca cerina* var. *muscorum* (A.Massal.) Jatta): 5 M; 14 S; 16 SR. GÇ.2123
Caloplaca stillicidiorum (Vahl) Lynge (Syn. *Caloplaca cerina* var. *chloroleuca* (Sm.) Th.Fr.): 5, 11 M; 5 Pr; 7 Pg; 14 SR; 15 Pn. GÇ.2122
+*Calvitimela aglaea* (Sommerf.) Hafellner: 13, 14 SR. GÇ.2147
Candelariella aurella (Hoffm.) Zahlbr: 2 Pg; 3, 4, 5 CR; 7, 14, 17 SR. GÇ.2148
+*Candelariella medians* (Nyl.) A.L. Sm.: 8, 14 SR; 20 CR. GÇ.2149
Candelariella vitellina (Hoffm.) Müll. Arg.: 4 Pc; 5, 8, 13, 16, 17, 18, 19 SR; 5, 10, 14 Pm; 5 Ta; 6 Pg; 11 Cs. GÇ.2150
Candelariella xanthostigma (Ach.) Lettau: 5 M; 6, 7, 8, 18, 21 Pg; 9, 12 Rp; 10 So; 13 Cl; 15 Pn. GÇ.2151
Carbonea vitellinaria (Nyl.) Hertel: 19 Cv. GÇ.2152
+*Carbonea vorticosa* (Flörke) Hertel: 13 SR. GÇ.2153
Cerothallia luteoalba (Turner) Arup, Frödén & Søchting (Syn. *Caloplaca luteoalba* (Turner) Th.Fr.): 4 Rp. GÇ.2142
Chrysothrix chlorina (Ach.) J.R. Laundon: 8 SR. GÇ.2156
Circinaria caesiocinerea (Malbr.) A.Nordin, Savić & Tibell (Syn. *Aspicilia caesiocinerea* (Nyl. ex Malbr.) Arnold): 4, 11 CR; 5, 6, 8, 10, 14, 17 SR. GÇ.2111
Circinaria calcarea (L.) A. Nordin, Savić & Tibell (Syn. *Aspicilia calcarea* (L.) Körb.): 3, 4, 20 CR. GÇ.2112
Circinaria contorta (Hoffm.) A.Nordin, Savić & Tibell (Syn. *Aspicilia contorta* (Hoffm.) Körb. subsp. *contorta)*: 3, 5, 11 CR; 13 SR. GÇ.2114
Circinaria contorta subsp. *hoffmanniana* (R.Sant.) I. Zhdanov (Syn. *Aspicilia contorta* (Hoffm.) Körb. subsp. *hoffmanniana)*: 3, 5, 11 CR; 13 SR. GÇ.2114
+*Cladonia chlorophaea* (Sommerf.) Spreng.: 10 S. GÇ.2157
Cladonia coniocraea (Flörke) Spreng: 5 S. GÇ.2158
Cladonia fimbriata (L.) Fr.: 1, 10 S. GÇ.2159
+*Cladonia foliacea* (Huds.) Willd.: 10, 11, 14 S. GÇ.2160
Cladonia pocillum (Ach.) Grognot.: 10 S. GÇ.2161
Cladonia pyxidata (L.) Hoffm.: 1, 4, 5, 8, 10, 11, 14, 16, 17, 19 S. GÇ.2162
+*Cladonia symphycarpia* (Flörke) Fr.: 10 S. GÇ.2163
Collema subflaccidum Degel.: 4 S. GÇ.2168
+*Diploschistes muscorum* (Scop.) R. Sant.: 5 M. GÇ.2170
+*Diploschistes scruposus* (Schreb.) Norman: 17 SR. GÇ.2171
Diplotomma alboatrum (Hoffm.) Flot (Syn. *Diplotomma epipolium* (Ach.) Arnold): 5, 13 SR; 15 Pn; 20 CR. GÇ.2174
+*Diplotomma chlorophaeum* (Leight.) Kr.P. Singh & S.R. Singh: 4 CR; 13 SR. GÇ.2173
+*Diplotomma hedinii* (H.Magn.) P. Clerc & Cl. Roux: 3 CR. GÇ.2175
**Diplotomma pharcidium* (Ach.) c: 15 Pn. GÇ.2176
+*Enchylium conglomeratum* (Hoffm.) Otálora, P.M. Jørg. & Wedin (Syn. *Collema conglomeratum* Hoffm.): 8 M. GÇ.2165
+ *Enchylium tenax* (Sw.) Gray (Syn. *Collema tenax* (Sw.) Ach.): 3, 4 CR; 10, 11 S GÇ.2169
Evernia prunastri (L.) Ach.: 1, 2, 7, Pg; 8 Qr; 12 Rp; 20 Pd. GÇ.2177
**Flavoplaca granulosa* (Müll.Arg.) Arup, Frödén & Søchting (Syn. *Caloplaca granulosa* (Müll.Arg.) J.Steiner): 13 SR; 20 CR. GÇ.2136
+*Flavoplaca microthallina* (Wedd.) Arup, Frödén & Søchting (Syn. *Caloplaca microthallina* Wedd.): 4, 11 CR; 14 Pg. GÇ.2143
Gyalolechia flavorubescens (Huds.) Søchting, Frödén & Arup (Syn. *Caloplaca flavorubescens* (Huds.) J.R. Laundon): 1, 2, 7, 8, 18 Pg; 12 Rp; 13 Cl; 15 Pn. GÇ.2134
Gyalolechia flavovirescens (Wulfen) Søchting, Frödén & Arup (Syn. *Caloplaca flavovirescens* (Wulfen) Dalla Torre & Sarnth.): 4 Rp; 6, 10, 14 SR; 7 Pg. GÇ.2135
Gyalolechia fulgens (Sw.) Søchting, Frödén & Arup (Syn. *Fulgensia fulgens* (Sw.) Elenkin): 11, 20 S. GÇ.2178
Hypogymnia farinacea Zopf: 1, 7 Pg. GÇ.2179
+*Hypogymnia physodes* (L.) Nyl.: 7 Pg. GÇ.2180

Hypogymnia tubulosa (Schaer.) Hav.: 2, 7, 18 Pg. GÇ.2181
Immersaria athroocarpa (Ach.) Rambold & Pietschm.: 6, 13, 19 SR. GÇ.2182
+*Lambiella insularis* (Nyl.) T.Sprib. (Syn. *Rimularia insularis* (Nyl.) Rambold & Hertel): 6, 7, 8, Pg; 10 So; 12 Rp; 13 Cl. GÇ.2273
+*Lathagrium auriforme* (With.) Otálora, P.M. Jørg. & Wedin (Syn. *Collema auriforme* (With.) Coppins & J.R. Laundon): 10 S. GÇ.2164
Lathagrium cristatum (L.) Otálora, P.M. Jørg. & Wedin (Syn. *Collema cristatum* (L.) Weber ex F.H. Wigg.): 4 S. GÇ.2167
Lecania naegelii (Hepp) Diederich & van den Boom: 15 Pn. GÇ.2183
+*Lecania subfuscula* (Nyl.) S.Ekman: 14, 16, 17 SR; 17 S. GÇ.2184
Lecanora albellula (Nyl.) Th.Fr.: 15 Pn; 18 Pg. GÇ.2185
+*Lecanora alpigena* (Ach.) Cl. Roux (Syn. *Lecanora polytropa* (Ehrh.) Rabenh.): 6, 8, 17 SR. GÇ.2198
Lecanora argentata (Ach.) Malme: 10 So; 18 Pg. GÇ.2187
+*Lecanora campestris* (Schaer.) Hue: 1, 2, 7, 8 Pg; 3 CR; 4, 12 Rp. GÇ.2189
Lecanora carpinea (L.) Vain.: 6, 7, 14 Pg; 8 Qr; 9 Rp; 10 So. GÇ.2190
+*Lecanora cenisia* Ach.: 4, 5, 8, 14, 17 SR. GÇ.2191
Lecanora chlarotera Nyl.: 7 Pg; 13 Cl. GÇ.2192
+*Lecanora filamentosa* (Stirt.) Elix & Palice (Syn. *Lecanora symmicta* (Ach.) Ach.): 4, 13 S. GÇ.2203
Lecanora intumescens (Rebent.) Rabenh.: 15 Pn. GÇ.2196
Lecanora rupicola (L.) Zahlbr. (Syn. *Lecanora rupicola* var. *rupicola* (L.) Zahlbr.): 4, 5, 6, 8, 13, 14, 17 SR. GÇ.2199
Lecanora saligna (Schrad.) Zahlbr.: 6, 7, 10, 13, 14 SR. GÇ.2200
+*Lecidea atrobrunnea* (DC.) Schaer.: 13, 14, 17 SR. GÇ.2205
+*Lecidea confluens* (Weber) Ach.: 10 SR. GÇ.2206
+*Lecidea fuscoatra* (L.) Ach.: 8, 17 SR. GÇ.2207
+*Lecidella anomaloides* (A.Massal.) Hertel & H.Kilias: 5 SR. GÇ.2209
Lecidella carpathica Körb.: 4 CR; 5, 6, 14, 18 SR. GÇ.2210
Lecidella elaeochroma (Ach.) M.Choisy: 1, 2, 6, 7, 8, 18, 21 Pg; 4, 9, 12 Rp; 8 Qr; 10 So; 13 Cl; 15 Pn. GÇ.2211
+*Lecidella scabra* (Taylor) Hertel & Leuckert: 13 SR. GÇ.2212
Lecidella stigmatea (Ach.) Hertel & Leuckert: 5, 17 SR. GÇ.2213
+*Lecidella wulfenii* (Ach.) Körb.: 4 S; 14 M. GÇ.2214
+*Lepra amara* (Ach.) Hafellner (Syn. *Pertusaria amara* (Ach.) Nyl.): 6 SR. GÇ.2237
+*Lepra corallina* (L.) Hafellner (Syn. *Pertusaria corallina* (L.) Arnold): 13, 16, 17, 19 SR. GÇ.2238
+*Lepra excludens* (Nyl.) Hafellner (Syn. *Pertusaria excludens* Nyl.): 8 SR. GÇ.2239
+*Lepraria alpina* (B. de Lesd.) Tretiach & Baruffo: 5 S. GÇ.2215
+*Lepraria caesioalba* (B. de Lesd.) J.R. Laundon: 5, 14, 17 S. GÇ.2216
+*Lepraria eburnea* J.R. Laundon: 4, 10, 14 S. GÇ.2217
Leproplaca cirrochroa (Ach.) Arup, Frödén & Søchting (Syn. *Caloplaca cirrochroa* (Ach.) Th.Fr.): 10 CR. GÇ.2128
Lobothallia radiosa (Hoffm.) Hafellner: 4, 11, 20 CR; 6 SR. GÇ.2220
Lobothallia recedens (Taylor) A.Nordin, Savić & Tibell (Syn. *Aspicilia recedens* (Taylor) Arnold): 4 CR; 5 SR. GÇ.2115
Megaspora verrucosa (Ach.) Arcadia & A.Nordin: 11, 14 S; 17 M. GÇ.2221
Melanohalea exasperata (De Not.) O.Blanco, A.Crespo, Divakar, Essl., D.Hawksw. & Lumbsch: 1, 2, 6, 7, 18, 21 Pg; 4, 12 Rp; 8 Qr; 10 So; 11 CR; 14, 16, 19 SR; 15 Pn. GÇ.2222
Melanohalea exasperatula (Nyl.) O.Blanco, A.Crespo, Divakar, Essl., D.Hawksw. & Lumbsch: 2, 7 Pg; 8 Qr; 10 So; 13 Cl; 14 SR; 15 Pn. GÇ.2223
**Miriquidica pycnocarpa* (Körb.) M.P. Andreev (Syn. *Lecidea pycnocarpa* (Körb.) Ohlert): 14 SR. GÇ.2208
+*Myriolecis albescens* (Hoffm.) Śliwa, Zhao Xin & Lumbsch (Syn. *Lecanora albescens* (Hoffm.) Branth & Rostr.): 13 S. GÇ.2186
Myriolecis crenulata (Hook.) Śliwa, Zhao Xin & Lumbsch (Syn. *Lecanora crenulata* Hook.): 13 SR. GÇ.2193
Myriolecis dispersa (Pers.) Śliwa, Zhao Xin & Lumbsch (Syn. *Lecanora dispersa* (Pers.) Röhl.): 3, 4, 11, 20 CR; 6, 14 SR. GÇ.2194
Myriolecis hagenii (Ach.) Sliwa, Zhao Xin & Lumbsch. (Syn. *Lecanora umbrina* (Ach.) A.Massal.): 15 Pn; 18 Pg. GÇ.2204
Myriolecis sambuci (Pers.) Clem. (Syn. *Lecanora sambuci* (Pers.) Nyl.): 4, 7, 18 Pg. GÇ.2201
+*Myriolecis semipallida* (H.Magn.) Śliwa, Zhao Xin & Lumbsch (Syn. *Lecanora semipallida* H.Magn.): 4 Rp; 13 Pg. GÇ.2202
Myriospora smaragdula (Ach.) Uloth (Syn. *Acarospora smaragdula* var. *lesdanii* (Harm.) H.Magn.): 4 SR. GÇ.2105
+*Nephromopsis chlorophylla* (Willd.) Divakar, A.Crespo & Lumbsch (Syn. *Tuckermanopsis chlorophylla* (Willd.) Hale): 1, 14 Pg. GÇ.2285
Parmelia saxatilis (L.) Ach.: 7 Pg; 10 S; 14 SR. GÇ.2226
Parmelia submontana Hale.: 18 Pg. GÇ.2227

Parmelina pastillifera (Harm.) Hale: 14 M. GÇ.2228
Parmelina tiliacea (Hoffm.) Hale: 2, 18 Pg; 10, 19 SR; 16 S. GÇ.2229
+*Peltigera degenii* Gyeln.: 1 S. GÇ.2230
+*Peltigera hymenina* (Ach.) Delise: 14 S. GÇ.2231
+*Peltigera lepidophora* (Vain.) Bitter: 1 S. GÇ.2232
+*Peltigera leucophlebia* (Nyl.) Gyeln.: 1, 14 S. GÇ.2233
+*Peltigera neckeri* Hepp ex Müll.Arg.: 17 S. GÇ.2234
Peltigera praetextata (Flörke ex Sommerf.) Zopf: 1, 11 S. GÇ.2235
Peltigera rufescens (Weiss) Humb.: 5, 10, 11, 16, 19 S. GÇ.2236
Phaeophyscia nigricans (Flörke) Moberg: 3, 4 CR; 19 SR. GÇ.2240
Phaeophyscia orbicularis (Neck.) Moberg: 3, 4, 11 CR; 6 SR; 7 Pg; 10 So; 12 Rp; 15 Pn. GÇ.2241
Phaeophyscia sciastra (Ach.) Moberg: 4 CR; 6, 13 SR. GÇ.2242
Physcia adscendens (Fr.) H.Olivier: 4, 9 Rp; 7, 18, 21 Pg; 10 So; 15 Pn. GÇ.2243
Physcia aipolia (Ehrh. ex Humb.) Fürnr.: 12 Rp; 14 Pg; 20 Pa; 20 Pd. GÇ.2244
Physcia dubia (Hoffm.) Lettau: 1, 7, 21 Pg; 4 S; 6 SR; 10, 13 SR; 11 S; 20 CR. GÇ.2245
Physcia leptalea (Ach.) DC. (Syn. *Physcia semipinnata* (J.F. Gmel.) Moberg): 1, 2, 7 Pg; 4, 12 Rp; 8 Qr; 20 Pd. GÇ.2246
Physcia stellaris (L.) Nyl.: 1, 7, 21 Pg; 4, 9, 12 Rp; 8 Qr; 10 So; 15 Pn. GÇ.2247
Physcia tenella (Scop.) DC.: 1, 2 Pg; 8 Qr; 10 So; 11 S. GÇ.2248
Physcia tribacia (Ach.) Nyl.: 6 Pg; 10 SR. GÇ.2249
Physconia distorta (With.) J.R. Laundon: 12 Rp; 14 Pg; 16 SR. GÇ.2250
Physconia enteroxantha (Nyl.) Poelt: 10 S; 13, 16 SR; 20 CR. GÇ.2251
Physconia grisea (Lam.) Poelt: 2 Pg; 4 CR; 14 Pg. GÇ.2252
Physconia perisidiosa (Erichsen) Moberg: 5, 11 S. GÇ.2253
+*Placidium rufescens* (Ach.) A.Massal. (Syn. *Catapyrenium rufescens* (Ach.) Breuss): 11, 14 S. GÇ.2154
+*Placidium squamulosum* (Ach.) Breuss (Syn. *Catapyrenium squamulosum* (Ach.) Breuss): 11, 14 S. GÇ.2155
Placocarpus schaereri (Fr.) Breuss: 4, 11, 12 CR. GÇ.2254
Pleurosticta acetabulum (Neck.) Elix & Lumbsch: 1, 2, 8, 14 Pg; 12 Rp; 15 Pn. GÇ.2255
+*Polycauliona polycarpa* (Hoffm.) Frödén, Arup & Søchting (Syn. *Xanthoria polycarpa* (Hoffm.) Rieber): 4 Rp; 8 Qr. GÇ.2295
+*Polychidium muscicola* (Sw.) Gray: 8, 17 S; 16 M. GÇ.2256
+*Protoparmelia badia* (Hoffm.) Hafellner: 10 SR. GÇ.2257
Protoparmeliopsis bolcana (Pollini) Lumbsch (Syn. *Lecanora bolcana* (Pollich) Poelt): 11CR; 13 SR. GÇ.2188
+*Protoparmeliopsis garovaglii* (Körb.) Arup, Zhao Xin & Lumbsch. (Syn. *Lecanora garovaglioi* (Körb.) Zahlbr.): 5, 10, 14, 17 SR. GÇ.2195
Protoparmeliopsis laatokkaensis (Räsänen) Moberg & R.Sant. (Syn. *Lecanora laatokkensis* (Räsänen) Poelt): 3, 4, 11 CR; 5, 6, 10, 13, 14, 16, 17, 18, 19 SR. GÇ.2197
Protoparmeliopsis muralis (Schreb.) M.Choisy: 3, 4, 11 CR; 5, 6, 10, 13, 14, 16, 17, 18, 19 SR. GÇ.2258
Pseudevernia furfuracea (L.) Zopf var. *furfuracea*: 1, 2, 7, 18 Pg; 10 S 12 Rp. GÇ.2260
Pseudevernia furfuracea var. *ceratea* (Ach.) D.Hawksw.: 1, 2, 7, 8 Pg; 10 S; 12 Rp; 20 Pd. GÇ.2259
Psora decipiens (Hedw.) Hoffm.: 11, 14 S. GÇ.2261
+*Psorotichia schaereri* (A.Massal.) Arnold: 4 S. GÇ.2262
Pyrenodesmia chalybaea (Fr.) A.Massal. (Syn. *Caloplaca chalybaea* (Fr.) Müll.Arg.): 4 CR. GÇ.2126
Pyrenodesmia variabilis (Pers.) A.Massal. (Syn. *Caloplaca variabilis* (Pers.) Müll.Arg.): 4, 20 CR; 14, 16 SR. GÇ.2146
+*Ramalina capitata* (Ach.) Nyl.: 14 SR. GÇ.2263
Ramalina farinacea (L.) Ach.: 1, 2, 7 Pg; 8 Qr; 9, 12 Rp; 13 Cl; 14 SR. GÇ.2264
Ramalina fraxinea (L.) Ach.: 12 Rp; 14 Pg; 15 Pn. GÇ.2265
+*Ramalina pollinaria* (Westr.) Ach.: 6 SR. GÇ.2266
+*Ramalina polymorpha* (Lilj.) Ach.: 1 Pg; 6 CR; 10, 14 SR. GÇ.2267
Rhizocarpon geminatum Körb.: 10, 13, 17 SR. GÇ.2268
Rhizocarpon geographicum (L.) DC.: 4, 5, 6, 7, 8, 10, 13, 16, 17, 18, 19, 20 SR. GÇ.2269
+*Rhizocarpon lecanorinum* Anders: 5, 6, 8, 14, 16, 17, 19 SR. GÇ.2270
+*Rhizocarpon simillimum* (Anzi) Lettau: 4, 6, 14 SR. GÇ.2271
+*Rhizocarpon subgeminatum* Eitner: 4, 5, 8, 10, 14 SR. GÇ.2272
Rinodina exigua (Ach.) Gray: 13 Lr. GÇ.2274
+*Rinodina interpolata* (Stirt.) Sheard: 5 SR. GÇ.2275
+*Rinodina milvina* (Wahlenb.) Th.Fr.: 7, 13, 14 SR. GÇ.2276
Rinodina pyrina (Ach.) Arnold: 18 Pg. GÇ.2277
Rinodina sophodes (Ach.) A.Massal.: 15 Pn. GÇ.2278
Rusavskia elegans (Link) S.Y. Kondr. & Kärnefelt subsp. *elegans* (Syn. *Xanthoria elegans* (Link) Th.Fr.): 4, 20 CR. GÇ.2293

+*Schaereria fuscocinerea* (Nyl.) Clauzade & Cl. Roux: 5 SR. GÇ.2279
Scytinium gelatinosum (With.) Otálora, P.M. Jørg. & Wedin (Syn. *Leptogium gelatinosum* (With.) J.R. Laundon): 10 SR; 11, 20 CR. GÇ.2218
Scytinium lichenoides (L.) Otálora, P.M. Jørg. & Wedin (Syn. *Leptogium lichenoides* (L.) Zahlbr.): 16 S. GÇ.2219
+*Silobia rufescens* (Turner ex Ach.) M.Westb. & Wedin (Syn. *Acarospora rufescens* (Ach.) Kremp.): 16 S. GÇ.2104
Squamarina cartilaginea (With.) P.James: 5, 6, 7, 8, 10, 11, 13, 14 SR. GÇ.2280
+*Tephromela atra* (Huds.) Hafellner: 4 CR; 5, 8, 13, 14 SR. GÇ.2281
Toninia sedifolia (Scop.) Timdal: 4, 10, 11 S 19 SR. GÇ.2282
+*Toninia taurica* (Szatala) Oxner: 4, 10, 11, 16 S. GÇ.2283
+*Toninia toniniana* (A.Massal.) Zahlbr.: 11, 14 S 20 CR. GÇ.2284
+*Umbilicaria crustulosa* (Ach.) Lamy: 14 SR. GÇ.2286
Variospora dolomiticola (Hue) Arup, Søchting & Frödén (Syn. *Caloplaca dalmatica* (A.Massal.) H.Olivier): 20 CR. GÇ.2131
Variospora flavescens (Huds.) Arup, Frödén & Søchting (Syn. *Caloplaca flavescens* (Huds.) J.R. Laundon): 4 CR. GÇ.2133
Verrucaria muralis Ach.: 3, 4 CR. GÇ.2287
Verrucaria nigrescens Pers.: 4, 20 CR 13 SR. GÇ.2288
Xanthocarpia crenulatella (Nyl.) Frödén, Arup & Søchting (Syn. *Caloplaca crenulatella* (Nyl.) H.Olivier): 9 Ps; 10 So; 12 Rp; 15 Pn. GÇ.2130
Xanthocarpia lactea (A.Massal.) A.Massal. (Syn. *Caloplaca lactea* (A.Massal.) Zahlbr.): 13 SR. GÇ.2141
Xanthomendoza fulva (Hoffm.) Søchting, Kärnefelt & S.Y. Kondr.: 13, 16 SR; 19 S. GÇ.2289
+*Xanthoparmelia loxodes* (Nyl.) O.Blanco, A.Crespo, Elix, D.Hawksw. & Lumbsch: 11 M. GÇ.2290
Xanthoparmelia pulla (Ach.) O.Blanco, A.Crespo, Elix, D.Hawksw. & Lumbsch: 6 CR; 10, 14, 16, 18, 19 SR. GÇ.2291
Xanthoparmelia verruculifera (Nyl.) O.Blanco, A.Crespo, Elix, D.Hawksw. & Lumbsch: 6, 10, 14 SR. GÇ.2292
Xanthoria parietina (L.) Beltr.: 1, 7, 21 Pg; 4, 9, 12 Rp; 8 Qr; 15 Pn. GÇ.2294

DISCUSSION

As a result of this study, 189 species, 3 subspecies and 3 varieties, a total of 192 taxa belonging to 79 genera, representing the lichen mycota of Gölcük Nature Park and surroundings of Isparta city were reported. Among these, 73 species were firstly recorded for the research area and the province of Isparta. *Diplotomma pharcidium* (Ach.) M.Choisy, *Flavoplaca granulosa* (Müll.Arg.) Arup, Frödén & Søchting, and *Miriquidica pycnocarpa* (Körb.) are 3 new records for Turkish Lichen Mycota.

According to the abundance of lichen species in 21 localities, epiphytic *Melanohalea exasperata* is the most frequent species (in 15 localities) while other epiphytics *Anaptychia ciliaris* and *Lecidella elaeochroma* (both in 13 localities) are the second frequent species together with crustose epilithic species *Acarospora cervina*, *Candelariella vitellina*, *Protoparmeliopsis muralis*, and *Rhizocarpon geographicum* (all in 12 localities) respectively have a prevalent abundance in the study ares. These frequent-taxa have been well adapted to the climate, habitat and substrate conditions of the research area. Ecological compliance is the most effective factor in lichen diversity in a region (HAUCK et al., 2007; LÕHMUS et al., 2007). A comparison of these results in this study to those of Yukarı Gökdere region in Isparta by ORAN et al. (2007) shows that *Anaptychia* ciliaris is the common frequent species between two studies, while a comparison to Barla Mountain in Isparta by KOÇ et al. (2016) indicates that *Acarospora cervina* is the common frequent species between two studies. The difference between these two frequency-based comparison of taxa-lists are due to the difference in ecological factors like habitat and substrate types among these studies.

The distribution of species in the region according to substrate types is as follows: 155 (41.67%) of the recorded taxa are epiphytic where 136 (36.56%) are saxicolous, 54 (14.52%) are terricolous, 14 (3.76%) are muscicolous, and 13 (3.49%) are lichenicolous (Fig. 4). *Pinus nigra* is substrate of 51 (13.7%) of the total taxa where *Populus nigra* and *Robinia pseudacacia* are that of 26 (6.99%). Siliceous rocks are prevailing with 87 (23.39%) taxa over calcareous rocks with that of 49 (13.17%) as dominating saxicolous substrates in the study area.

The distribution of species in the study area by thallus morphology is shown in Fig. 5. Among the recorded taxa, 106 (55.50%) are crustose, 48 (25.13%) are foliose, 15 (7.85%) are squamulose, 10 (5.24%) are placodioid, 9 (4.71%) are fruticose and 3 (1.57%) are leprose.

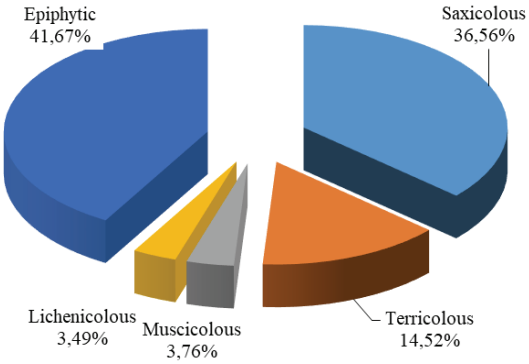

Figure 4. Taxon Distribution by Substrate Type.

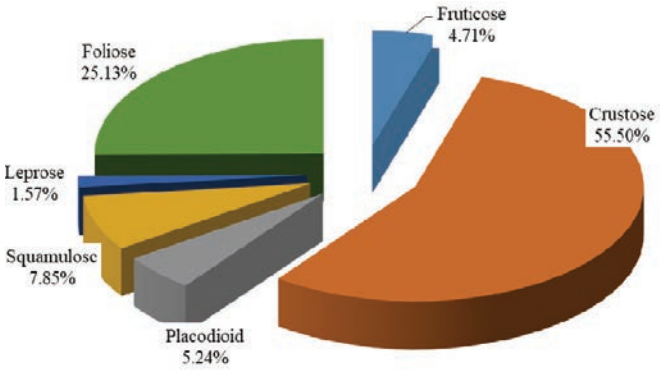

Figure 5. Taxon Distribution by Thallus Morphology.

The fruticose genera in the study area are *Evernia* (1 species), *Pseudevernia* (1 species), *Polychidium* (1 species), and *Ramalina* (5 species). The number and percentage of the fruticose taxa is lower compared to that of lichen records –which are mostly epiphytic as well- from *Quercus vulcanica* forests around Yukarı Gökdere in Isparta (ORAN et al., 2007). This is due to the fact that Gölcük Nature Park has a semi-arid climatic regime and inconvenient habitats for fruticose taxa.

The cyanolichens *Blennothallia crispa, Collema subflaccidum, Enchylium conglomeratum, Enchylium tenax, Lathagrium auriforme, Lathagrium cristatum* in the study are recorded only in moist microsites within the park area. Though the study area has a semi-arid Mediterranean climate, there are some habitats exposed to frequent dewfall in summer, as explained by HAUCK et al. (2007).

Altitude is an important factor besides climatic conditions on lichen diversity (ÇOBANOĞLU & SEVGI, 2009). In the present study, the altitude of the localities varies between 1128 m - 1724 m. Except for the localities II, III, IX and XII, there is an increase in the number of species recorded in every locality, directly proportional to the altitude of localities (Table 1). This proves that, with the increase of altitude, the lichen biodiversity increases as well. However, though visible in the above mentioned localities, the substrate type is a limiting factor. Localities over 1600 m are XIV, IV, X and XIII have higher number of lichen taxa compared to the rest of localities. Locality XIV which is the highest summit in the study area, has the maximum number of taxa (62) that is twice of the average specimen number, and one-third of the total taxa number in the given list. Since 640 lichen specimens have been collected from 21 localities in the study area, yields an average of 30.48 lichen taxa per locality. Besides locality XIV, there are 3 more localities that have higher number of taxa: locality IV (57 taxa), locality X (56 taxa) and locality XIII (47 taxa). Among these 3 localities, locality X (Between Bezirgân and Hisartepe Hill) bears a number of diversity in habitats as well as substrates whereas, locality XIV (Summit of Karatepe Hill) and locality IV (GNP, Western Slopes of Crater) around the caldera, have maximum altitude compared to the rest of localities in the study area. The lichen diversity in locality IV is significantly correlated with the altitude and presence of old conifers in habitat variables that indicate occurrence of forest conditions (COPPINS & COPPINS, 2002). Moreover, locality XXI which is at the north-east boundary of Gölcük Nature Park next to Halife Sultan Cemetery has the minimum number of taxa (7) depending on its altitude (1128 m) and close proximity to the city centre which can cause a decrease in lichen diversity in the study area.

HAUCK et al. (2007) reported that the number of lichen taxa in a study area corresponds with the variety of ecosystems and habitats in the region. Considering the present study, it is visible that, the lichen biodiversity and the number of taxa are parallel to the diversity of ecological diversity of the localities, since the present study reports a relatively higher number of taxa from a this-size natural park. In ORAN et al. (2007) study, that of 92 taxa from a Rim-Oak Nature Protection Area, Isparta that has a surface area of 1,300 ha is given, which yields an average-biodiversity ratio of 14.13 ha per taxon. KOÇ et al. (2016) study, a list of 241 infrageneric taxa from Barla Mountain, Isparta that has a surface area of 89,000 ha is given, which yields an average-biodiversity ratio of 369.29 ha per taxon. ÇOBANOĞLU et. al. (2009; 2010) study, a list of 115 taxa from Cozia National Park, Romania that has a surface area of 17,100 ha is given, which yields an average-biodiversity ratio of 148.70 ha per taxon. In the present study, a list of 192 taxa from Gölcük Nature Park, Isparta that has a surface area of 5,925 ha is given. This study yields an average-biodiversity ratio of 30.86 ha per taxon, in other words, this study reports a richer list of taxa compared to similar studies like KOÇ et al. (2016) and ÇOBANOĞLU et. al. (2010) which can be explained by the variety of ecosystems and habitats in the study area of this study.

CONCLUSIONS

Natural habitat characteristics and substrate diversity (LÕHMUS et al., 2007) as well as anthropogenic activities influence the distribution of lichen species (KAPUSTA et al., 2004). The high proportion of lichen biodiversity of the study area is directly related to this. It was also concluded that increase in altitude was found to be a decisive factor in lichen diversity. The number of lichen species differs with changing habitat related to the elevation

(SEVGI et al., 2016) consequently, the highest number of lichen species occurs at the highest altitudinal zone as mentioned in a previous study by ÇOBANOĞLU & SEVGI (2009).

Concerning the research area and Isparta province, the study by SZATALA (1960) is historically the first recording of lichens from various localities in Isparta, with a list of 46 taxa. The studies by ORAN et al. (2007) and KOÇ et al. (2016) are the only studies aiming to report lichen diversity of a certain, limited and protected area in Isparta, the rest of the studies have either random records or records from some pathways. The present study is one of those targeting a protecting area. The lichen biodiversity of Gölcük Nature Park located in Isparta, a Mediterranean city of Turkey is reported with a list of 189 species (192 taxa). At the same time, the distribution of taxa has been evaluated ecologically.

Notwithstanding that, there is not a single collection of "Lichen Mycota of Turkey", we can mention a recent work by JOHN & TÜRK (2017), a great attemp to compile a checklist of Turkey. However, it has many gaps to be filled. Wherefore, this study aims to make a contribution to taxonomic and mycotic studies on lichens in Turkey and provide a basis for further ecological, chemical and alike studies of lichenology that can be done in the region in the following years.

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MORPHOGENETIC POTENTIAL OF CALLUSES DERIVED FROM GAMMA IRRADIATED SAFFLOWER SEEDS

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Abstract. In order to induce morphological diversities in safflower experimental mutagenesis was applied in combination with *in vitro* culture. According to the tasks of inducing somaclonal variability seeds irradiated with gamma rays in doses of 50, 100, 150 Gy were used. Two types of explants (fragments of leaves cotyledons and hypocotyls) were applied for inducing *in vitro* morphogenesis. As callus-inducing mineral base medium was used by MURASHIGE & SKOOG (1962) with additions of growth regulators (6-Benzylaminopurine and 2,4-Dichlorophenoxyacetic acid). The derived calli were cultivated for induction of morphogenesis, been identified the hormones balance added to the culture media, the interval between subcultivation. Based on proved researches, a greater diversity in the safflower morphogenetic potential was revealed, generated by *in vitro* culture in a complex with gamma radiation.

Keywords: *in vitro* morphogenesis, callus, gamma radiation, safflower.

Rezumat. Potențialul morfogenetic al calusului obținut din semințe iradiate de șofrănel. În scopul inducerii diversități morfologice la șofrănel a fost propusă aplicarea mutagenezei experimentală în combinație cu cultura *in vitro*. Conform sarcinilor de sporire a variabilității somaclonale au fost folosite semințele iradiate cu raze gama în dozele 50, 100, 150 Gy. Pentru inducerea morfogenezei *in vitro* au fost utilizate două tipuri de explante (fragmente de frunze cotiledonate și hipocotil). Baza minerală a mediului de inducere a calusogenezei a servit MURASHIGE & SKOOG (1962) cu adaosuri a regulatorilor de creștere (6-benzilaminopurină și acid 2,4-diclorfenoxiacetic). Calusurile derivate au fost sub-cultivate în vederea inducerii morfogenezei, fiind identificată balanța hormonală, intervalul dintre pasaje. Pe baza cercetărilor a fost evidențiată majorarea diversității potențialului morfogenetic la șofrănel prin aplicarea culturii *in vitro* în complex cu radiația gama**.**

Cuvinte cheie: morfogeneză *in vitro,* calus, gama radiație, șofrănel.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an oilseed crop with multiple uses (CHAPMEN et al., 2010). Carthamidin (yellow dye, water-sol) and carthamin (red dye, water-insoluble), which are very important as a source of medicinal preparations, natural food colour and dyes for colouring fabrics, are extracted from safflower petals. Cultivated varieties are used as a source of quality oil (rich in linoleic acid).

However, there is a lack of information about safflower cultivars and their characteristics, so the objective of variety improvement is very important and actual.

In vitro technology has been known to be an effective procedure for diversification of crop plants. The genetic variability may be achieved by means of somaclonal variation or combination of *in vitro* culture with experimental mutagenesis. According to literature data, safflower regeneration through tissue culture has been limited by low frequency and lack of an efficient protocol that is suitable for most safflower cultivars. It has been limited by the many factors, namely genotype, age of seedling and callus (FAN & GUO, 2013); type of explants (CHAWLA, 2000); medium components, plant growth regulators and other additives (RAO et al., 2008; FAN & GUO, 2013; XUE et al., 2015).

The aim of the present work involved the study of impact of experimental mutagenesis associated with *in vitro* culture on morphogenetic potential of safflower calluses.

MATERIAL AND METHODS

The study involved the seeds irradiated with gamma rays in doses 50, 100, 150 Gy (as source of gamma rays was used the RXM-V-20 system, the radiation element - ^{60}Co). Untreated seeds were used for control. For the sterilization of materials, conditions were established which involved the rinsing in water with drops of Tween-80 (0.1%) and running under tap water for 15 min. Following, the seeds were surface sterilized for 1 min in 70% ethanol and then were disinfected with sodium hypochlorite solution (5.2%, as a commercial bleach, in dilution 1:1) for 17 min. After that, the seeds were rinses for three times 3 min each, in sterilized water to remove all traces of Clorox. The sterilization procedure and the incubation had been conducted in culture cabinet (laminar airflow hood).

The sterile seeds were inoculated in Magenta jars with MURASHIGE & SKOOG (MS, 1962) medium without hormones for inducing direct embriogenesis. Culture medium was solidified with 0.8% agar and adjusted to pH 5.7 and incubated at 25±2^{0}C under illuminated conditions (16h photoperiod). The fragments of cotyledon and hypocotyl were excised after 7-8 days of in vitro culture and have been inoculated on callus-inducing medium with mineral base after MURASHIGE & SKOOG (1962) and additions of growth regulators (6-Benzylaminopurine (BA) and 2,4-Dichlorophenoxyacetic acid (2,4-D)). pH of the medium was adjusted to 5.7 before sterilization by autoclaving for 20 minutes under the pressure of P = 1 atm, T=120^{0}C. Every 25 pieces per type of explant were inoculated on nutrient media in three repetitions. The explants were incubated in dark and temperature-controlled conditions (25 ± 2^{0}C) for 2-

3 weeks. After initiating callus vessels with explants were passed under 16 hour light and 8 hours dark for initiating morphogenesis. Serial passages were conducted every 2-3 weeks on initial and intermediate mediums.

As parameter it was assessed the frequency of explants (fragments of cotyledons leaves and hypocotyls) with positive response and calluses with morphogenetic potential. The software package Statgraphics Plus 2.1 was used for statistical analysis. ANOVA test it was applied for variance analysis of callusogenesis intensity, morphogenetic potential and Student test in assessment of statistically significant differences between treatments.

RESULTS AND DISCUSSION

For all variants the seeds germination, callus intensity were evaluated based on assessment system. According to the observations, the seeds germination had been identified during the first 24 hours after inoculation (Fig. 1).

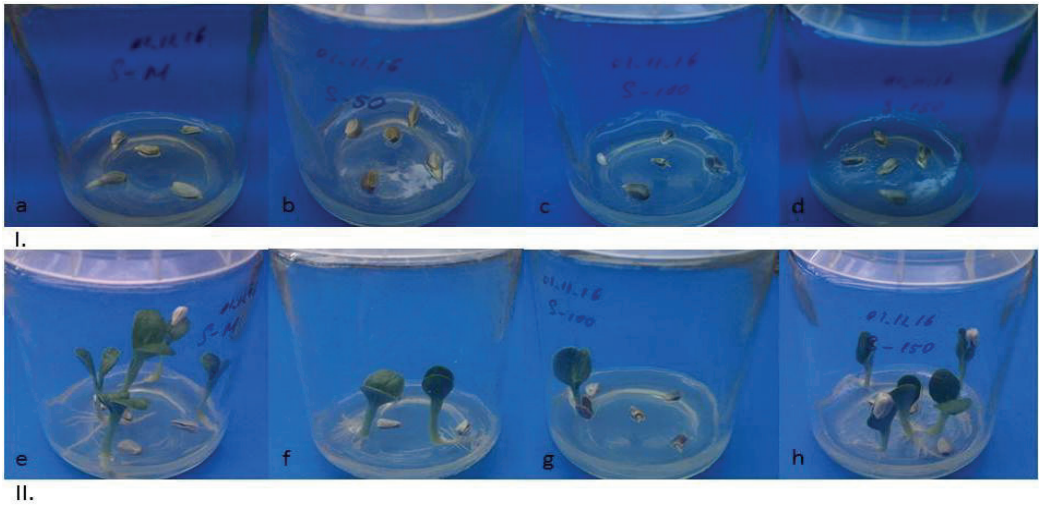

Figure 1. Safflower seeds germination (I) and direct regeneration (II): a) and e) control; b) and f) 50Gy; c) and g) 100Gy; d) and h) 150Gy.

At 2-3 days the impact of radiation on seed germination compared to the control (untreated) (Table 1) was appreciated. Thus, seeds irradiation doses 50 and 100Gy conducted to significant reduction of germination ($P \leq 0.01$) by 1.99 and 3.49 times respectively compared to the control. The impact of 150Gy dose was found insignificant.

Table 1. Safflower seeds germination (%) in dependence of irradiation doses.

Statistical parameters	Control	50Gy	100Gy	150Gy
Average value ± ES	93.33±6.67	46.67±6.67**	26.67±6.67**	81.10±1.10
min ÷ max	80÷100	40÷60	20÷40	80÷83.3
CV, %	12.37	24.74	43.30	2.34

**- significant at $P \leq 0.01$

Under dark and controlled conditions of temperature, intense proliferative processes were triggered in all experimental groups. Towards the end of the first passage (21 days after inoculation) the rate of explants with positive response varies between 90-94%, regardless of the type of explant or irradiation dose. It is necessary to be noted that the intensity of callus, estimated in degree, varies depending on the experimental variant studied. Based on bifactorial dispersion analysis, we find a significant 95% influence of gamma rays, while the explant type and their interaction have no significant impact on the intensity of callus (Table 2).

Table 2. Analysis of variance for callusogenesis intensity (ANOVA test).

Source of variance	Sum of Squares	Degrees of freedom	Mean Square	F-ratio
Radiation (R)	4.866	3	1.622	3.77*
Explant (E)	0.314	1	0.314	0.73
R-E interaction	0.260	3	0.086	0.20
Total	24.847	52		

* significant at $P \leq 0.05$.

The increased intensity was observed for hypocotyl / 50Gy - 2.86, and the lowest in the variant hypocotyl / 100Gy - 1.67 (Table 3).

Table 3. Dependence of callusogenesis values on the irradiation dose and type of explant.

Treatments	Explant	Callusogenesis intensity, note
Control	hypocotyl	2.71±0.22
	cotyledon leaves	2.51±0.19
50Gy	hypocotyl	2.84±0.46
	cotyledon leaves	2.33±0.38
100Gy	hypocotyl	1.67±0.37
	cotyledon leaves	1.72±0.29
150Gy	hypocotyl	2.52±0.19
	cotyledon leaves	2.43±0.23

Evaluating the data distribution, it was found that the mean values in control and experimental variants 100 or 150Gy range between 1-3 notes, but for 50 Gy represent 2-3 notes.

The morpho-structurally study of callus shows a slight difference regarding nuance and structure in dependence of type of explant and applied dose (Fig. 2a, b) and number of passages (Fig. 3).

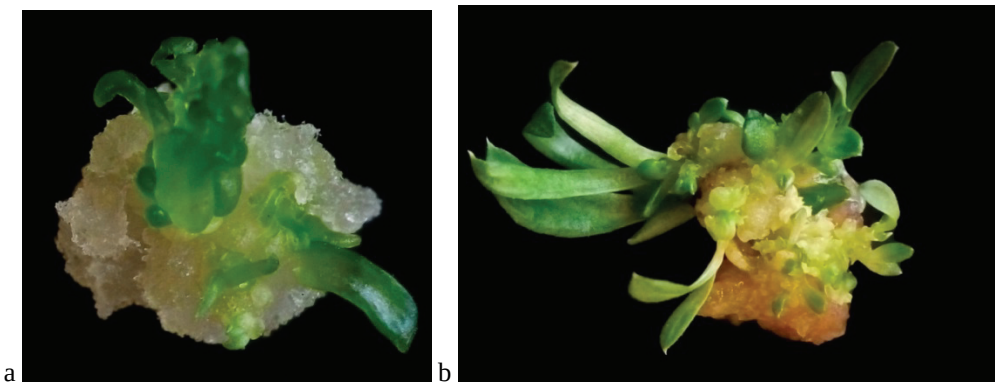

Figure 2. Types of morphogenic calluses. a – friable callus with organogenic potential obtained from safflower leaves, untreated; b – compact callus with embryogenic potential derived from safflower hypocotyl, 50Gy (original).

Thus, the initial callus of hypocotyl 50Gy had yellowish-white and friable structure (Fig. 3b), while leaf / 150Gy present compact structure of green color (Fig. 3c). After the second passage both histogene types had generated compact, green callus (Fig. 3h, i), which subsequently induce morphogenetic centers. It was mentioned that gamma irradiation accelerates the morphogenetic processes and induction of more shoots per explant (Fig. 3g) compared to the control. At the same period of subcultivation, only morphogenetic areas are initiated in untreated explants (Fig. 3f).

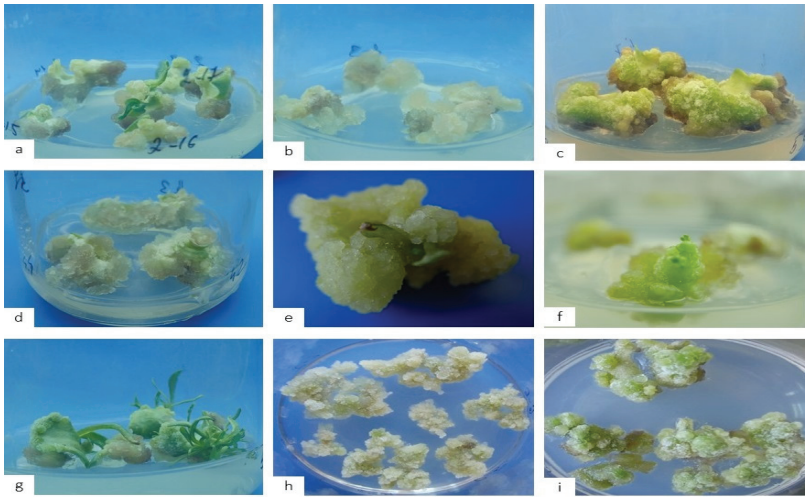

Figure 3. Callus initiation and regeneration for different types of safflower explants in dependence of irradiation doses and subcultivation duration: a) callus from cotyledon leaves; b) hypocotyl callus / 50Gy; c) callus from cotyledon leaves / 150Gy; d) callus from cotyledon leaves / control; e) callus from hypocotyl / 100Gy; f) induction of morphogenetic zones from cotyledon leaves / control; g) shots induction from hypocotyl callus / 50Gy; continually callus cultivation; h) hypocotyl / 50Gy; i) cotyledon leaves / 150Gy. Morphological aspect of callus at first passage (21 days of cultivation): a – e and second passage (after 30 days of cultivation): f – I (original).

The following assessment of morphogenetic intensity show that from an explant are obtained from 1 to 2.5 shoots. During the subcultivation, calluses with aerial rhizogenic capacity have been identified. In this case the subsequently cultivation conducted to the inhibition of the regenerative potential (Fig. 4a). Usually, the morphogenic areas, initiated from cotyledon leaves or hypocotyl, generated primordial shoots (Fig. 4b), which after elongation stage derived plantlets (Fig. 4c). According to the literature, explants of hypocotyl and cotyledons leaves have increased regenerative potential.

a b c

Figure 4. Induction of aerial roots (a); shoots (b) and shoot elongation (c) from morphogenic calluses of hypocotyl in control variant.

All these processes are primordially determined by the genotype (NIKAM & SHITOLE, 1999; BASALMA et al., 2008; RADHIKA et al., 2006; MOTAMEDI et al., 2011).

In our study, it was shown that the number of shoots per explant / callus varies depending on the experimental studied variant. Based on bifactorial dispersion analysis, a significant impact for interaction of gamma radiation and explant was found at 95% and alone gamma radiation at 90%, whereas the type of explant does not significantly influence the regenerative potential (Table 4).

Table 4. Analysis of variance of regenerative potential (ANOVA test).

Source of variance	Degrees of freedom	Sum of Squares	Mean Square	F-Ratio	Source contribution (%)
Radiation (R)	3	79.691	26.564	3.00*	21.61
Explant (E)	1	0.038	0.038	0.00	ns
R-E interaction	3	90.024	30.008	3.39**	24.41
Total	30	368.774			

*; ** significant difference from the control at $P \leq 0.05; 0.01$

After analyzing the interaction factors of gamma radiation / type of explant based on average values, it was fond that the highest regenerative potential (9.33) is attested in the variant 50Gy / cotyledon leaves and exceeds the control by about 9 times. The stimulatory or inhibitory impact of gamma rays has a tissue specificity of the explant.

The study carried out reveals a greater diversity in safflower morphogenetic processes generated by *in vitro* culture during subcultivation. Thus, if after passage IIIth was attested regeneration of plantlets from callus in all experimental treatments, while the fourth *in vitro* passage generates in the control group antogenic induction (direct formation of inflorescence) and antogenic induction associated with *albino* mutation in regenerants derived from irradiated seeds, 50Gy. At the same time, in the experimental treat (50Gy) sporadically were established callus with morphogenic activity which development has an atypical pathway of *in vitro* indirect regeneration: antogen induction and lack of elongation of the shoots.

According to the literature information, the most effective method of improvement, especially of qualitative characters, it is the complex use of *in vitro* culture with experimental mutagenesis, applying physical factors. Gamma rays are successfully combined with *in vitro* culture to ameliorate different species: apple, potato, pineapple, palm (AHLOOWALIA & MALUSZYNSKI, 2001), and for some species with vegetative multiplication this technique can be an important way for plant improvement (MALUSZYNSKI, 1995). In safflower, gamma irradiation fulfills variability induced *in vivo* at the level of quantitative and qualitative characters (RAMACHANDRAN & GOUD, 1983), but also chromosomal instability (KUMAR & SRIVASTAVA, 2010; VERMA & SHRIVASTAVA, 2014).

In our investigations, in the lots derived from irradiated explants were obtained regenerants with orange and yellow flowers, while the initial form was red. At the same time, there are differences in leaves shape and presence of spines: most of the obtained plants have spineless leaves, while some plantlets presented spines such original form.

CONCLUSIONS

1. The calluses initiated from fragments of leaves cotyledons and hypocotyls of safflower proved to *in vitro* cultivation the morphogenetic potential influenced by the type of explant and dose of gamma radiation.

2. The morphogenetic capacity express tissue specificity of the interaction explant / radiation dose, was dependent of calluses aspects (color, consistence - compact or fluid), duration of subcultivation.

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DISTRIBUTION OF THE *LEPTORHAPHIS* KÖRB. 1855 GENUS IN ROMANIA

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Abstract. The field work performed to find out the lichen species tabulated in the *Leptorhaphis* genus took place in a lot of habitats from Romania, especially within natural and seminatural forest habitats and also in man-made habitats such as orchards and shelterwoods. The lichen species taken into account were not identified in the studied sites. Based on the literature, it was found that the *Leptorhaphis* genus is rather weakly distributed on Romanian territory. Chorological data, the habitat type, substratum, cenotaxonomy, and taxonomy of the *Leptorhaphis* genus are presented in this paper. Also, the worldwide chorology of *Leptorhaphis* genus is presented. In conclusion, further field studies are needed for the identification of the lichen species of *Leptorhaphis* genus on an extended area from Romania.

Keywords: *Leptorhaphis* genus, chorology, Romania.

Rezumat. Distribuția genului *Leptorhaphis* Körb. 1855 în România. Activitatea de teren desfășurată pentru identificarea speciilor de licheni încadrate în genul *Leptorhaphis* s-a realizat in anumite habitate din România, în special în habitate forestiere naturale și seminaturale și deasemenea în habitate antropogene, cum sunt: livezile și perdelele forestiere. Speciile de licheni luate în considerare nu au fost identificate în ariile studiate. Pe baza literaturii de specialitate, genul *Leptorhaphis* este destul de slab distribuit pe teritoriul României. În acest articol sunt prezentate date corologice, tipul de habitat și substratul, cenotaxonomia și taxonomia genului *Leptorhaphis*. Deasemenea, este prezentată corologia generală a genului *Leptorhaphis*. În concluzie, este necesară continuarea studiilor pentru identificarea speciilor de licheni din genul *Leptorhaphis* pe o arie cât mai extinsă a teritoriului României.

Cuvinte cheie: genul *Leptorhaphis*, corologie, România.

INTRODUCTION

The *Leptorhaphis* genus is well known and widespread along European and American boreal and temperate areas (MARTÍNEZ & ARAGÓN, 2002) and also on the African and Asian continents (ALONSO & EGEA, 1997; KINALIOĞLU, 2009). The oldest information as regard the chorology of the *Leptorhaphis* genus in Romania dates back to 1922 (MORUZI et al., 1967). Data about the national spatial distribution of the mentioned above genus are few. All lichen species from *Leptorhaphis* genus were referred a lot in mountainous areas followed by hilly areas. Regarding the habitat where the lichen species considered within this study were found, the forest one is the most common (CODOREANU, 1966; BURLACU, 1967; BURLACU, 1969; CODOREANU, 1978).

The latest recorded data regarding the chorology of the *Leptorhaphis* genus is older, namely from the last century (CODOREANU, 1978). These historical data are important because they could help estimate the period when lichen species were not confirmed by field studies. In a similar study, *Lobaria amplissima* (Scop.) Forssell was recorded in 1870 in Latvia. Since that time and up to 2014, this species was not found again. In 2014, *L. amplissima*, a red-listed lichen species was found again on a dead wood in the North Vidzeme Biosphere Reserve of Latvia (JURCIŅŠ et al., 2014). In a study performed in London, it has been observed that a few lichen species, thoughe cited in literature, had not been discovered in the last 200 years (HAWKSWORTH & McMANUS, 1989).

The aim of this study is to update the chorological data of the *Leptorhaphis* genus in Romania by trying to find it in a field survey. The main objective of this study is the characterization of the spatial distribution of the *Leptorhaphis* genus in Romania and also its substrata, habitat type, cenotaxonomy, taxonomy and worldwide distribution.

MATERIALS AND METHODS

Studied area. The author performed research activities within natural and semi-natural forestry habitats. Also, habitats transformed by man such as orchards and shelterwoods were investigated. Field activities were performed between 2009-2017, both in rural and urban areas. Thus, semi-natural forestry habitats were predominantly investigated in the rural areas of the following counties: Alba, Arad, Bistrița-Năsăud, Buzău, Călărași, Cluj, Giurgiu, Gorj, Ialomița, Ilfov, Maramureș, Mehedinți, Sibiu, Vaslui, and Vrancea. The natural forestry habitats, especially protected areas were sampled in the following counties: Bacău, Bihor, Botoșani, Galați, Giurgiu, Hunedoara, Iași, Neamț, Prahova, Sibiu, Suceava, Tulcea, and Vaslui. One of the sampled semi-natural forests is situated in the urban area, namely the Bucharest Municipality. Some of the studied habitats are man-made, for instance: orchards and shelterwood sampled in the Sălaj and Vaslui counties (Fig. 1). The species tabulated in *Leptorhaphis* genus were not identified by the author in any of the studied sites.

Sampling procedure. Within each investigated habitat category, all available trees found in 10 by 10 m sampling units were sampled. The trees were sampled from 1 m up to 1.5 m above the ground. The field method complies with PRIGODINA-LUKOŠIENĖ & NAUJALIS (2006).

Literature data were used to highlight historical informations on the *Leptorhaphis* genus on Romania territory (CIURCHEA, 2004).

The nomenclature of the lichen species, their taxonomy and cenotaxonomy is according to www.speciesfungorum.org. Specimens included in the Collection of the Babeș-Bolyai University Herbarium from Cluj-Napoca are abbreviated in the text as H.U.C. Also, the Mycological Herbarium from Bucharest (BUCM) was consulted for identification of the studied specimens.

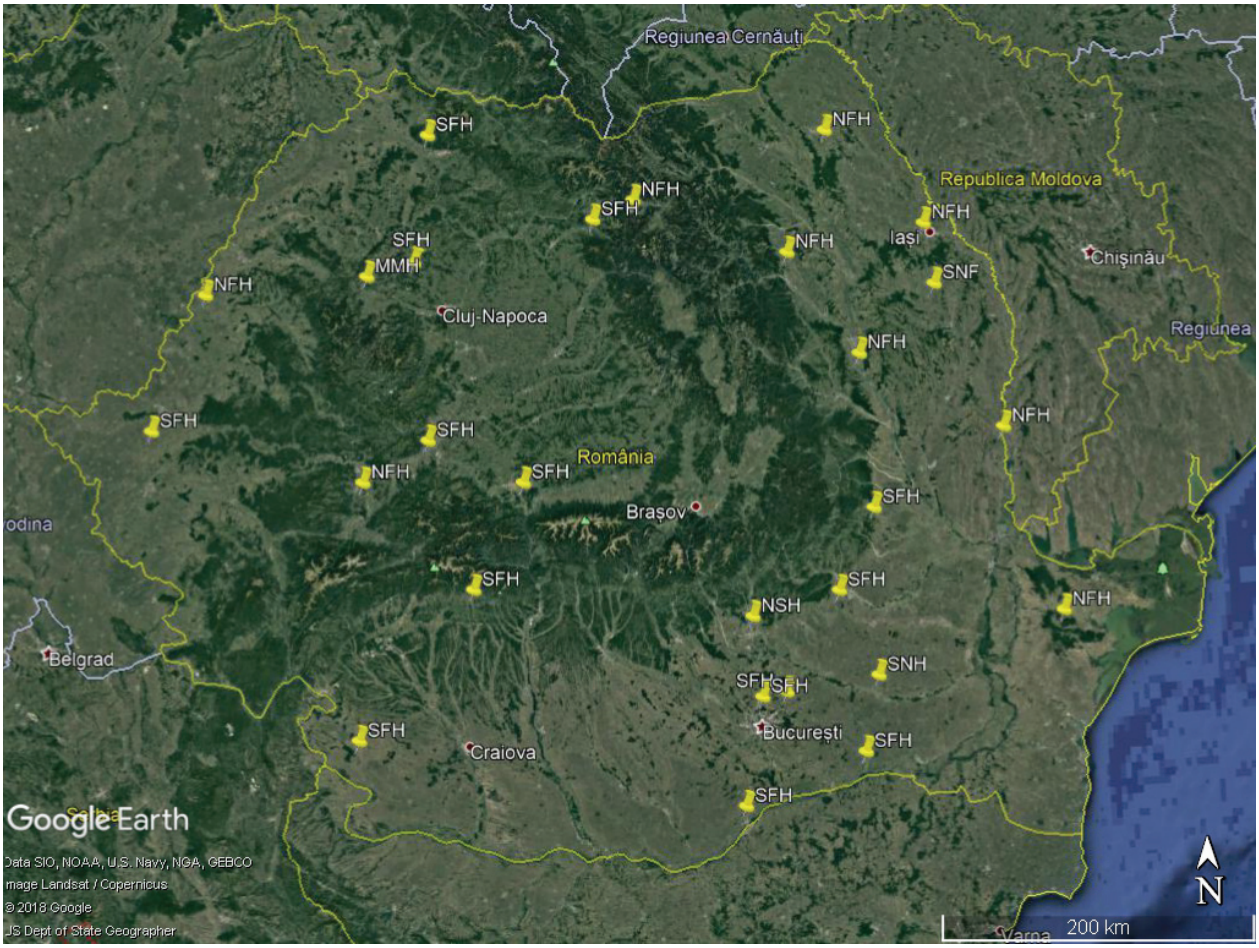

Figure 1. Original sampling sites performed in different counties from Romania (SFH-semi-natural forest habitats, NFH-natural forest habitats, MMH-man-made habitats).

RESULTS AND DISCUSSIONS

In Romania, the *Leptorhaphis* Körb. 1855 genus is represented by four species, especially epiphytic species, as follows: *Leptorhaphis atomaria* (Ach.) Szatala (1927), *Leptorhaphis epidermidis* (Ach.) Th. Fr. (1861), *Leptorhaphis quercus* (Beltr.) Körb. (1865), *Leptorhaphis tremulae* Körb. 1855 (CIURCHEA, 2004). None of these lichen species were identified during the field researches.

In literature, the distribution of the *Leptorhaphis* genus in Romania is weakly represented (CIURCHEA, 2004). Thus, only four species of this genus were identified in a few counties from Romania, as is presented below (CIURCHEA, 2004):

1) ***Leptorhaphis atomaria*** (Ach.) Szatala (Fig. 2)

Bihor County: Apuseni Mountains, Stâna de Vale, on corticolous substrata (CIURCHEA, 2004; H.U.C. nr. 553940), Defileul Crișului Repede, on corticolous substrata (CODOREANU, 1966; CIURCHEA, 2004); Caraș-Severin County: Banat Mountains, Danube Defile at Belobrești, Țigansca Reca Valley, near Remetea, Dubova Forest, on *Fraxinus* L. (CRETZOIU, 1941; MORUZI et al., 1967; CIURCHEA, 2004).

Figure 2. The spatial distribution of *Leptorhaphis atomaria* in Romania (original).

2) ***Leptorhaphis epidermidis*** (Ach.) Th. Fr. (Fig. 3)

Botoşani County: Moldova Plateau, Dersca and Lozna, on *Acer campestre* L. and *Tilia cordata* Mill. (BURLACU, 1967, CIURCHEA, 2004).

Figure 3. The spatial distribution of *Leptorhaphis epidermidis* in Romania (original).

3) ***Leptorhaphis quercus*** (Beltr.) Körb. (Fig. 4)

Arad County: Apuseni Mountains, Zarandului Mountains, Gurahonț, near Căsoaia Chalet, on corticolous substrata (CODOREANU, 1978); Bihor County: Stâna de Vale, on corticolous substrata (CIURCHEA, 2004, H.U.C. nr. 553940, 666853), Maramureș County: the locality is not indicated (MORUZI et al., 1967), Prahova County: Gârbova Mountain, Rea Valley, on corticolous substrata (CIURCHEA, 2004).

Figure 4. The spatial distribution of *Leptorhaphis quercus* in Romania (original). .

4**) *Leptorhaphis tremulae*** Körb. (Fig. 5)

Botoșani County: Moldova Plateau, Gorovei Forest, on corticolous substrata (BURLACU, 1969), Cluj County: Transilvania Basin, Cluj-Napoca, Botanical Garden, on *Berberis brachypoda* Maxim. (CODOREANU et al., 1960; MORUZI et al., 1967).

Figure 5. The spatial distribution of *Leptorhaphis tremulae* in Romania (original).

5) ***Leptorhaphis wienkampii*** J. Lahm ex Hazsl. (Fig. 6)
Cluj County: Ciucea, on corticolous substrata (CRETZOIU, 1941).

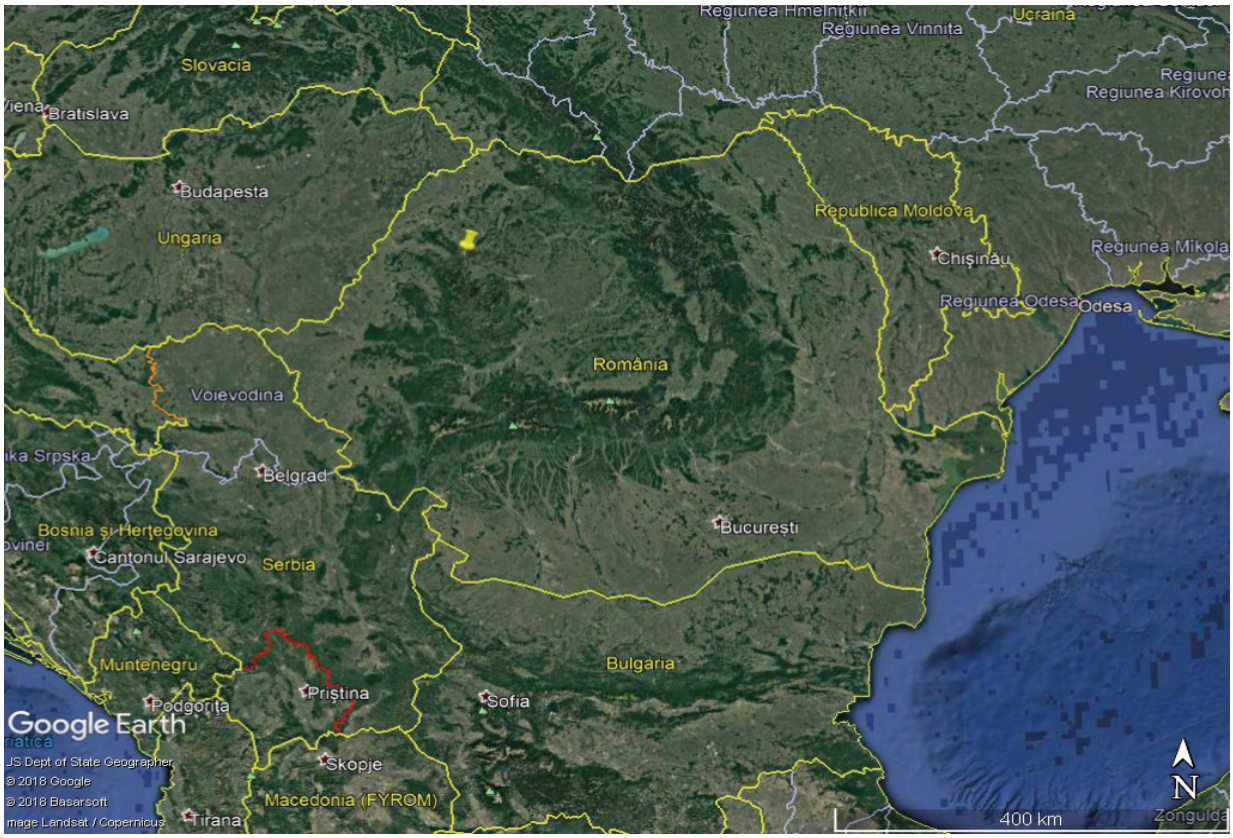

Figure 6. The spatial distribution of *Leptorhaphis wienkampii* J. Lahm ex Hazsl. in Romania (original).

The Romanian red-list of lichen species does not present any data regarding the sociological status of the species belonging to the *Leptorhaphis* genus (SÂRBU et al., 2007; ARDELEAN et al., 2013). Perhaps nowadays their distribution on the Romanian territory is limited by an intensive anthropogenic pressure on their forestry habitats.

The species of the *Leptorhaphis* genus are growing on various corticolous substrata especially from natural forestry habitats (Table 1). Although the species from the *Leptorhaphis* genus depend on wood substrata, none of the sampled trees in the research was colonized by these species. The main cause could be the conversion of primary forests into secondary ones. Lichen species are dependent especially on the substrata type. Thus, lichen species from *Leptorhaphis* genus prefer trees with a high value of the bark pH such as *Populus* L. and *Fraxinus* L. (SKYE, 1968). The substrata type is related both to bark pH and its organic enrichment. The two environmental factors are important to lichen sociology and their chorology. Based on the aspects mentioned above, the *Leptorhaphis* genus is tabulated in the *Xanthorion* community (SKYE, 1968).

Table 1. The substrata on which the species of the *Leptorhaphis* genus are growing and their habitat types (CIURCHEA, 2004).

Species	Substrata	Natural habitat	Anthropogenic habitat
Leptorhaphis atomaria	ash tree, willow, poplar	forestry	-
Leptorhaphis epidermidis	birch	forestry	Botanical garden
Leptorhaphis quercus	oak, birch	forestry	-
Leptorhaphis tremulae	poplar	forestry	Botanical garden
Leptorhaphis wienkampii	corticolous	-	-

Legend: - data are not available

From a sociological point of view, the lichen species taken into account are tabulated into two superior cenotaxons: *Arthonio-Lecidelletea elaeochromae* Drehwald 1993 and *Physcietea* Tomaselli et De Micheli 1957 (Table 2). All lichen species of *Leptorhaphis* genus are tabulated in nitrophilous communities (CIURCHEA, 2004) and their occurrence on oak, willow, and birch with a low pH of the trees bark indicates an eutrophication of substrata (LAUNDON, 1963). Also, in a study performed in Majorca (Spain), it was revealed that these lichen species are growing on nutrient enriched substrata associated to the other nitrophilous lichen species (AGUIRRE-HUDSON & FIOL, 1993). The taxonomy of *Leptorhaphis* genus is well known, but with doubtful taxons with respect to its order (Table 3).

In addition to the known species of this genus for Romania, there are other species widespread on the European, African, American and Asian continents, as follow: *Leptorhaphis amygdali* (A. Massal.) Zwackh 1862, identified in Croatia, Germany, Italy, and Hungary; *Leptorhaphis laricis* (J. Lahm) M. B. Aguirre 1991, identified in Slovakia, Spain, and Germany; *Leptorhaphis lucida* Körb. 1863, identified in Slovakia, Austria, Germany, Russia, USA, Czech Republic, Georgia, Hungary, Ukraine, Norway and Sweden; *Leptorhaphis parameca* (A. Massal.) Körb. 1865, identified in Bulgaria, Austria, Germany, Italy, Norway, Switzerland, USA, Hungary, Ukraine and Turkey. All this species are common on *Fraxinus* L., *Populus* L., and *Prunus* L. (AGUIRRE-HUDSON et al., 2005; KINALIOĞLU, 2009; INASHVILI & BATSATSASHVILI, 2010). The other species, for instance *Leptorhaphis opuntiicola* L. A. Fiol & M. B. Aguirre 1993, were found in Spain (AGUIRRE-HUDSON & FIOL, 1993) and Morocco (ALONSO & EGEA, 1997) on phorophytes; and *Leptorhaphis maggiana* (A. Massal.) Körb. 1865 was identified in Germany (DE BRUYN, 2001). Three of the four lichen species cited in Romania are also widely distributed, for instance *L. atomaria* is known from Russia, Austria, Finland, France, Germany, Italy, Norway, Poland, Slovakia, Sweden, Switzerland, UK, Bulgaria, Hungary, the Netherlands, Spain, Ukraine, USA, and Israel; *L. epidermidis* was found in Austria, Czech Republic, Slovakia, Belgium, Finland, France, Germany, Ireland, Italy, Norway, Poland, Sweden, Switzerland, UK, USA, Hungary, the Netherlands, Portugal, Spain, and Ukraine; *L. tremulae* was cited from Germany. These three lichen species were common on *Populus* and *Betula* (DE BRUYN, 2001; AGUIRRE-HUDSON et al., 2005; KONDRATYUK et al., 2005; EICHLER et al., 2010).

Table 2. The cenotaxons in which the studied lichen species occurs (CIURCHEA, 2004).

Species	Class	Order	Alliance	Association
Leptorhaphis atomaria	*Physcietea* Tomaselli et De Micheli 1957	*Physcietalia adscendentis* Hadač 1944 em Barkm. 1958	*Xanthorion parietinae* Ochsner 1928	N/A
Leptorhaphis epidermidis	*Physcietea* Tomaselli et De Micheli 1957	*Physcietalia adscendentis* Hadač 1944 em Barkm. 1958	*Xanthorion parietinae* Ochsner 1928	*Physcietum adscendentis* Frey et Ochsner 1926
Leptorhaphis quercus	*Arthonio-Lecidelletea elaeochromae* Drehwald 1993	*Graphidetalia scriptae* Hadač 1944	*Lecanorion subfuscae* Ochsner 1928	*Rinodinetum exiguae* Klem. 1951
	Physcietea Tomaselli et De Micheli 1957	*Physcietalia adscendentis* Hadač 1944 em Barkm. 1958	*Xanthorion parietinae* Ochsner 1928	*Physcietum adscendentis* Frey et Ochsner 1926
Leptorhaphis tremulae	N/A	N/A	N/A	N/A
Leptorhaphis wienkampii	N/A	N/A	N/A	N/A

Legend: N/A data are not available

Table 3. The taxonomy of the considered lichen species (www.speciesfungorum.org).

Species	Kingdom	Division	Class	Order	Family
Leptorhaphis atomaria	Fungi R. T. Moore 1980	Ascomycota Caval. Sm. 1998	Dothideomycetes O. E. Erikss. et Winka 1997	Incertae sedis	Naetrocymbaceae Höhn (1909)
Leptorhaphis epidermidis					
Leptorhaphis quercus					
Leptorhaphis tremulae					
Leptorhaphis wienkampii					

CONCLUSIONS

Although the field activities were performed on a vast area on the Romanian territory, no new records about the chorology of the *Leptorhaphis* genus were obtained. For this reason, further field studies are needed to find out which lichen species belong to the *Leptorhaphis* genus.

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PHENOLOGICAL, AUTECOLOGICAL AND PHYTOCOENOLOGICAL CHARACTERISTICS OF A *Sedum caespitosum* Cav (CRASSULACEAE) POPULATION FROM CENTRAL DOBROGEA (SE ROMANIA), LESSONS FOR BROADER, MORE EFFECTIVE CONSERVATION

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Abstract. *Sedum caespitosum* is a small 5(4)-merous and haplostemonous ephemeral Crassulacean, precociously inhabiting inhospitable habitats a few weeks in spring, vanishing before competitors progress in their lifecycles. As phenological shifts are responses to climate changes, and because the Dobrogean populations show phenological, autecological and phytocoenological characteristics differing from halophylous South-Eastern Central European populations, I report herein data about a population located near Gura Dobrogei in Central Romanian Dobrogea. The phenology of *Sedum caespitosum* is typical for ephemeral therophytes, with overwintering seeds (resting propagules). The active phase of the lifecycle begins in March and lasts about two months, as by June the plants are completely dry with ripe seeds. Particularly unusually though, *S. caespitosum* population studied shows a distinct, second blooming-phase, sometimes succeeding a brief apparent vegetative arrest/death of leaves and shoot parts which bloomed in the first blooming period, indicating the existence of two meristem subsets with anthesis decouple by brief endo-dormancy. If true, this hypothesis would be a highly unusual case in an annual plant, similar to some sort of condensed polycarpy (as Bârcă V suggested in personal communication). The ecological and phytocoenological data reported here show that *Sedum caespitosum* grows in Dobrogea in clearly non-halophylous plant associations developed on shallow topsoil covering the superficial limestone bedrock, and supporting the hypothesis that it is at most an opportunistic halophyte being just a salt-tolerant species exploiting ecological niches inaccesible for other plants, thus avoiding the competition which it is unable to withstand. Another explanation of the observed autecological and phytocoenological inconsistent preferences exhibited by *Sedum caespitosum*, is that the halophylous populations from South-Eastern Central Europe indeed belong to a distinct taxon, specialised in exploiting salt-rich flatlands and saltpans, as claimed by Simonkai when describing *Sedum deserti-hungarici* Simonkai (1890) based on specimens from one of those halophylous populations.

Keywords: *Sedum caespitosum*, phenology, autecology, conservation, Dobrogea.

Rezumat. Caracteristici fenologice, autecologice şi fitocenologice ale unei populaţii de *Sedum caespitosum* Cav (Crassulaceae) din Dobrogea Centrală (SE România), învăţăminte pentru o mai largă şi mai eficientă conservare. *Sedum caespitosum* este o Crassulacee mică, 5(4)-meră şi haplostemonă care ocupă precoce habitate neospitaliere câteva săptămâni primăvara, dispărând înaintea progresiei competitorilor prin ciclul lor de viaţă. Întrucât decalajele fenologice sunt adesea răspunsuri la modificări climatice, şi deoarece populaţiile dobrogene au caracteristici fenologice, autecologice şi fitocenologice diferite de cele ale populaţiilor halofile Sud-Est-Central Europene, prezint aici date despre o populaţie localizată lângă Gura Dobrogei în Dobrogea Centrală Română. Fenologia *Sedum caespitosum* e tipică therofitelor efemere, care iernează prin seminţe (propagulele de repaus). Faza activă a ciclului de viaţă începe în martie şi durează cam două luni, încât prin iunie plantele sunt complet uscate, cu seminţe mature. Foarte neobişnuit totuşi, populaţia de *S. caespitosum* studiată prezintă o a doua fază de înflorire distinctă, uneori succedând unei perioade de scurtă întrerupere vegetativă aparentă sau de moarte a frunzelor sau a unor părţi din lăstarii care înfloriseră în prima perioadă de înflorire, indicând existenţa a două subseturi de meristeme cu antheza decuplată de scurte perioade de endo-dormanţă. Dacă e adevărată această ipoteză ar fi un caz foarte neobişnuit la o plantă anuală, similar unui tip de policarpie condensată (aşa cum sugerează Bârcă V. într-o comunicare personală). Datele ecologice şi fitocenologice prezentate aici arată că *Sedum caespitosum* creşte în Dobrogea în asociaţii vegetale clar nehalofile pe cuverturi subţiri de sol acoperind superficial substrate calcaroase, şi susţinând ipoteza că este cel mult o halofită oportunistă fiind de fapt doar o specie halo-tolerantă exploatând nişe ecologice inaccesibile pentru alte plante, astfel evitând competiţia pe care e incapabilă să o suporte. Altă explicaţie a neconcordanţelor observate în preferinţele ecologice şi fitocenologice arătate de *Sedum caespitosum*, e aceea că populaţiile halofile Sud-Est-Central Europene aparţin într-adevăr unui taxon distinct, specializat în exploatarea sărăturilor joase, cum susţine Simonkai descriind *Sedum deserti-hungarici* Simonkai (1890) pe baza unor exemplare din aceste populaţii halofile.

Cuvinte cheie: *Sedum caespitosum*, fenologie, autecologie, conservare, Dobrogea.

INTRODUCTION

Sedum caespitosum (Cav.) D.C. is a small ephemeral Crassulacean described initially 1791 by CAVANILLES (1791), as *Crassula caespitosa*, it was described again some 40 years later by de Candolle under *Sedum caespitosum* in 1828: 405.

In my experience from Romanian habitats, it strikingly distinguishes itself by the peculiar short life-cycle specialized for avoiding competition by occupying the niche of inhospitable habitats for a few weeks in spring, vanishing before its competitors are early in their lifecycles.

Taxonomically it was a disputed species, with relatively many synonyms, of which I mention the ones which are more prevalently used: *Aithales caespitosa* (Cav.) Webb & Berth., *Crassula diffusa* Lam., *Crassula magnolii* DC., *Crassula verticillaris* Linné, *Procrassula caespitosa* (Cav.) Fourr., *Procrassula magnolii* (DC.) Griseb., *Sedum*

cespitosum Boiss., *Sedum erythrocarpum* Pau, *Sedum rubrum* (L.) Thell., *Tillaea rubra* L. (Marhold 2011); *Sedum rubro* (L.) Thell., (ICN), (HART 1991) .An interesting name for this species, now synonimized, is *Sedum deserti-hungarici* Simonkai which was given to plants from populations located near to the population in western Romania, which are relatively close to the one I studied .

Morphologically, (see also BÂRCĂ 2018b, NICULAE 2018b, where the morphological characteristics of the exact population studied here are detailed), it is characterized by some distinctive characters which together are of diagnostic importance: *S. caespitosum* has a small habitus, with erect, straight, un-branched or slightly branched glabrous stems about 5-8cm tall, growing in dense populations but without giving the appearance of dense mats like *S. hispanicum* or *S. sexangulare*. It is charaterised by 5(4)-merous and haplostemonous flowers wich usually have white to pinkish petals, with sometimes a reddish longitudinal medial vein.

Biogeographically, it has a wider circum-mediterranean distribution extending from Portugal and Morocco to Asia Minor, Syria, Israel and Iran. It develops best in the Mediterranean and sub-Mediterranean climatic zones, but its range stretches Northwards into Poland where it is adventive, via European Turkey and the Balkans into Romania, Hungary and Cszeck republic and Slovakia (but also see LIPPERT, 1995, JALAS et al, 1999).

In Romania, *Sedum caespitosum* (Cav.) D.C. is rare and localized, and relatively neglected in the recent years; unlike other Crassulacean species which were better studied both regarding their chorology (BÂRCĂ & NICULAE 2005, 2006, BÂRCĂ 2016a, NICULAE, 2018a, NICULAE & BÂRCĂ, 2005; 2006), and their morphology (BÂRCĂ & NICULAE, 2008; BÂRCĂ 2018b) their general biology (BÂRCĂ & NICULAE, 2011, BÂRCĂ et al., 2011), their ethnoyatric and medicinal proprieties (STANCIU et al., 2009); BÂRCĂ, 2015, 2018a; BÂRCĂ & NICULAE, 2018) sozological aspects (ARBUNE et al., 2009).producing even taxonomical surprises (BÂRCĂ, 2016b).

In the case of *Sedum caespitosum* (Cav.) D.C., from Romania despite it being a species of community interest for conservation (but also probably because of its rarity and inconspicuousness) no primary data from targeted studies were published in the mainstream literature in Romania. Most of the data available comes as collateral information, scattered occasionally in floristic lists or haphazordously in phytosociologic works about associations where this species happens to occur.

The Romanian distribution of *Sedum caespitosum* (Cav.) D.C., is relatively wide but localized, remaining virtually that published in the old monography of RĂVĂRUȚ in Flora of RSR edited by Săvulescu (RĂVĂRUȚ 1956), as I review below, in which I mention the sources only for the few recent additions, the rest just citing Răvăruț 1956:

Alba county: Zlatna on Piatra Caprei, Abrud on Mt. Vulcan; Bihor county: Mădăraș; AR: Șimand, Chișineu-Criș, Mâșca, Adea, Pilu, Socodor, Pecica, Grăniceri, Arad, Vulcan, Rubicioara; Timiș county: Foeni, Diniaș (STERE 1977), Distr. Timiș-Torontal without locality (BORZA 1944); Bacau county: Fântânele (MITITELU et al., 1993), Tepoaia (MITITELU et al., 1993), Vladnic (MITITELU et al., 1993); Tulcea county: Babadag (ȘTEFUREAC 1970), Denis Tepe Hill (ANDREI 1963), Beștepe (SÂRBU & ȘTEFAN 2005).

Later studies mention *Sedum caespitosum* D.C. again in Tulcea county at Beidaud (PETRESCU et al 2014).

More recent fieldwork research with Barca Valentin, following hints by respected older botanists (ANDREI 1963; Negrean G., Cristurean I.,and personal communications with all of them) pointed out the fact that the ecology and phytocoenology of *Sedum caespitosum* D.C. in Dobrogea differs considerably from those of the populations from the western part of the country, where the plant was considered typical halophyte, inhabiting in the spring the margins of the saltpans resulted from snowmelt and spring rains.

The phenology and general survival strategy was similar, but the autecology and phytocoenology was quite different in the two regions of the country.

Thus, while in Western areas of Romania (BORZA,1944) and, to perhaps some extent also in Moldova (MITITELU et al., 1993; SÂRBU & ȘTEFAN 2005), Slovakia (FEHÉR, 2007), Hungary (BÁTORI et al., 2014; BORHIDI, 2003; JAKAB & TÓTH, 2003; JAKAB, 2005; MOLNÁR et al 2012; KIRÁLY, 2007; TÓTH, 2003), Serbia (KNEŽEVIĆ et al., 2008), *Sedum caespitosum* D.C. is a hallophyte clearly associate with saltpans, in Dobrogea it grows in limestone, and in plant associations clearly non-halophylous, likewise some of the populations in Southern Europe and Bulgaria (where PAVLOVA et al (2003) reported it from serpentine substrate in Mt Rodopi).

This aspect prompted me to report here preliminary data about the penology, autecology and phytocoenology of one population of *Sedum caespitosum* D.C. from Central Dobrogea as a first step towards understanding the real ecological characteristics and physiological requirments and adaptations of this species concerning the saline content of the soil in the habitats it ocuppies in its vast natural distribution range.

The population studied is located close to the heart of the distribution range of the species in Dobrogea, being one of the last populations downstream on the river Casimcea, before this river flows into the coastal/litoral lake Tasaul.

MATERIAL AND METHODS

Study site of the population studied is located in the center of the distribution range of the species in Dobrogea, with the following coordinates (in WGS84 system): N 44 29' 11" E 28 29' 30".

Data were gathered during fieldwork observations in situ during the vegetation season of the local population of *S. caespitosum*, using a hand magnifying glass. Measurements in situ were made using a caliper, and some specimens were fruther measured in the lab using a stereo-microscope or a compound microscope with an eyepiece micrometer, calibrated with an objective/stage micrometer, both produced by IOR Bucharest.

Illustrative photographs of some individuals were taken in-situ and ex-situ using a 16MP digital camera (Sony NEX5n) equipped with a Macro 100mm f 2.8 lens with 1:1 macro capabilities or a lighter Sony DSCH3 with its native macro zoom lens at various focal lengths and magnifications. Photographs were post-processed using GIMP software to improve brightness and contrast and to improve color rendition, and image size by cropping and print resolution were adjusted.

Phenophase limits were taken as follows; -the beginning was marked when at least 10 individuals from a patch of more than 100 began the respective phenophase, the end of a phenophase was marked when most (more than 50%) of the individual plants in a plot with a populations of at least 100 made that transition.

For each phenophase the lenght and approximate dates are presented, and also the relative share of the lifecycle of each phenophase is presented in charts. Illustrative color pictures of plants representative for each phenophase are presented.

RESULTS

Sedum chaespitosum is a therophyte, and the seeds which represent the resting propagules, overwinter in the shallow soil on the rocky outcrops or in the dried follicles of the fruits. The active, vegetative and generative phase of the life cycle begins in March and lasts only about two months, as by June the seeds are ripe and the plants are completely dry.

The structure of the life cycle and phaenophases in Sedum caespitosum (Cav) DC. in a natural population in the proximity of Gura Dobrogei site, Central Dobrogea, Romania is documented for the first time herein and presented in a synthetic form in Table 1.

Table 1. Structure of the life cycle and phaenophases in Sedum caespitosum (Cav) DC. in a natural population in the proximity of Gura Dobrogei site, Central Dobrogea, Romania.

Phenophase			**Length (days)**		**Aproximative Dates**	**Activity Phase**	
		Winter/spring Seed phase	135		1.I-17.IV	Winter-Spring rest	**Dormant seeds / seed bank (multiannual)**
Vegetative + Generative prezygotic	Common preflower phase	Shoot Development	22		18.III-9.IV	Active phase	
		Flower buds	3		9.IV-11.IV		
Generative	1st Bloom	1st Flower phase	7		12.IV-18.IV		
		1st Fruit Maturation	14		19.IV-2.VI		
	2nd Bloom	2nd Flower phase		***7***	***15.V-21.V***		
		2nd Fruit Maturation		***9***	***22.V-30.V***		
Resting Seed Phase		Autumn/winter Seed phase	180		3.VI (***31***).V -31.XII	Autumn-winter rest	

The germination and emergence of the new plantlets. At the first annual visit to the site during the year of study the 25th of March, some tiny 2-leaved and 4-leaved plantlets were found, so the actual emergence of the first plantlets in situ was inferred to be March 18, fact supported by the long emergence period of plantlets in ex-situ culture in Bucharest, due to an unusual warm winter and a late cold spell in March.

The pre-flowering phase of shoot development lasted for about 22 days, between 18.III and 9.IV, so the first floral buds were visible on April 9-th. The buds matured in 3 days and the first flowers were open April 13th.

First blooming, The anthesis started April 12. The flower phase lasted about a week, between 12.IV-18.IV, the afternoon of April 17 a bit under 30% of the flowers turned to fruits, with the follicles still orthokarpic, fact that was used to infer 18.IV as the end of flower phase, fact supported also by the evolution of ex-situ cultures.

Fruit maturation phase lasted about 14 days between 19.IV-2.V, phase during which the fruits were first green and succulent, then turned yellow-light brown with some reddish speckles, until finally turning light brown and appearing completely exsiccated around May 2. After May 2, most of the plants had completely dry fruit, with divergent follicles, and the plants proper started to wither and their leaves become dry and shriveled. Then, after about 3 more weeks the fruits started to show some adaxial folds along the suture, resembling the well-known lips found in the fruits of other Sedum species like for example in S. *urvillei*, but the folds were very narrow and inconspicuous.

Second blooming. After about 2 weeks from the anthesis of the first flowers, i.e. around may 14 some plants (many of them being almost devoid of fleshy, succulent leaves at that moment) produced a second generation of floral buds which quickly proceeded to anthesis so that many individuals in the population showed a second blooming phase which tentatively started on May 14, but as most of the plants were in full bloom by May 15, I concluded that the second blooming lasted for about a week between 15-21.V as by may 21st almost all of the flowers were withered and the follicles were starting to tilt.

Fruit maturation phase of the fruits resulted from the second blooming phase lasted about 9 days between 22.V-30.V, so by may 30 the vast majority of follicles were brown and dry.

The fruits resulted from the second blooming phase evolved in a similar manner as the ones of the first blooming phase the fruits first green and succulent, then turned light brown with more reddish speckles, until finally turning light brown and appearing completely exsiccated before May 30. On May 30, most of the plants had completely dry fruits, with divergent follicles, and the stems and the leaves plants were apparently completely dry and shriveled.

I consider based on both the in-situ observations and the evolution of plants in ex-situ culture the beginning of June as the start of the summer/autumn seed phase, which is the resting phase of *S. caespitosum*.

The summer/autumn seed phase in which the seeds are first contained within the dry /ripe follicles, lasted for approximately 180 days between 3.V (***31***).V -31.XII. It is arbitrarily limited by the end-of year day of Dec 31, but physiologically and phenologically this phase is not broken in 2 sections or periods by the calendar year, as the life cycle is indeed cyclic.

The winter-spring seed phase continues the summer/autumn seed phase and lasts about 135 days between 1.I-17.IV, until the start of the next vegetative phase of the following year.

The Overall Resting Seed Phase which is divided in 2 parts only due to calendar year reasons, comprises the summer-autumn /winter seed phase and the winter-spring seed phase. At the beginning of next year's vegetative phase some dry fruits with open follicles are to be found in situ along the tiny plantlets newly germinated from seeds from previous years.

The Active phase / Resting Seed Phase periods of the life cycle were split approximately 42 / 213 days with an apparently short active phase being actually longer than usually displayed by the plants in ex-situ culture, due to the erratic weather pattern of the year studied.

I must additionally mention here that the seeds enter a resting phase which is continued for a proportion of the seeds with a dormancy period resulting in germination delayed by one or several years, and -by consequence- resulting in the actual provision of a seed-bank for the population, fact not apparent from this study but demonstrated experimentally via multi-annual artificial cultures (Barca V, personal communication).

The absolute lenght of the phenophases in the life cycle of *S. caespitosum* in a natural population in the proximity of Gura Dobrogei site, Central Dobrogea, Romania is presented in the chart in, together with the calendar dates of the actual limits of the phenophases discussed (Fig. 1).

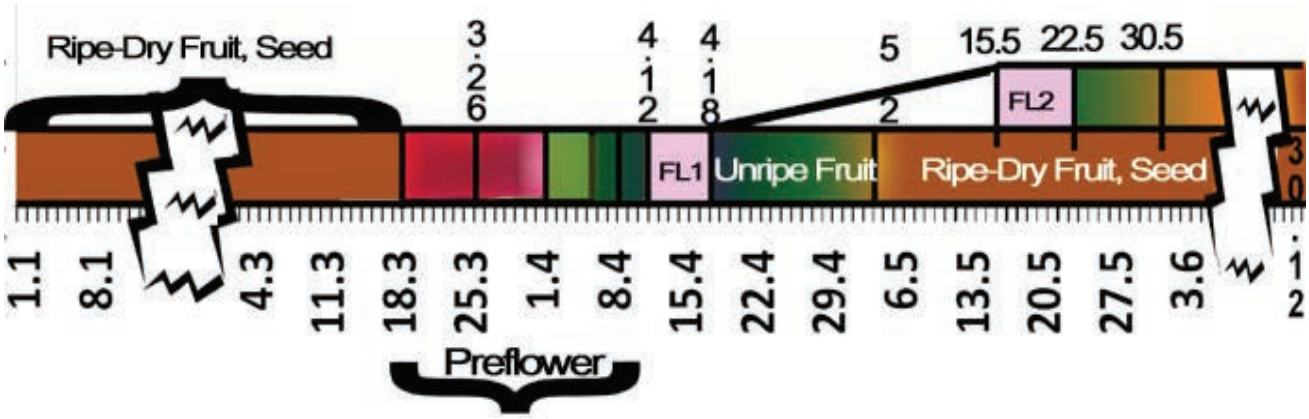

Figure 1. The absolute lenght of the phenophases in the life cycle of *S. caespitosum* in a natural population in the proximity of Gura Dobrogei site, Central Dobrogea, Romania. The horizontal axis is discontinuous, to improve the resolution of the time scale, parts of the resting phase of the life cycle were eliminated, with the gaps being figured as blank spaces between zigzagged lines. The approximate dates of the phenophase limits are marked in the system DD.M, above the chart.

To facilitate a better understanding of the relative contribution of each of the phases in the overall life cycle, the relative length of the Phenophases in the life cycle of *S. caespitosum* is presented in the chart if Fig. 2, with color pictures illustrating the aspect of representative plants for each phase described.

Figure 2. The relative lenght of the phenophases in the life cycle of *S. caespitosum* in a natural population in the proximity of Gura Dobrogei site, Central Dobrogea, Romania. In the center the life cycle is depicted in full (T) and a quarter slice of the cycle representing the active phases, with arrows linking the illustrative picture of plants in situ with the corresponding phases of the cycle.
A: Aspect of very young new plantlets in the 2nd week of shoot development in situ within the pytocoenosis;
B: close-up of young new plantlets in the 2nd week of shoot development in situ within the phytocoenosis, together with young shoots of *Sedum urvillei*; C: A young plant with advanced flower buds, ready to bloom;
D: Mature flower towards the end of the anthesis of the first blooming , the fertilized follicles start spreading,
E: maturing fruits of the first generation , with different ages, the older one with fully spread follicles is a rarer pentamerous form,
F: mature, dried fruit of the first blooming phase, and the primordium of the flower of the 2nd bloom;
G: mature, dried fruit of the first blooming phase, and a flower of the 2nd generation in full bloom; All photos made by Bârcă Valentin.

Autecological characteristics. The population studied herein grows in relatively dense colonies on shallow topsoil on skeletal, fragmented and/or intensely fractured superficial bedrock, in slight contrast with another habitat in a different place nearby, where it grows directly on exposed, unfragmented rocky calcareous outcrops or in pockets or crevices, formed by dissolution during exocarstic activity on probably Triassic or Jurassic limestones. Aspect of the habitat is depicted in Fig. 3, in which *Sedum caespitosum* is shown in situ on the first type of habitat.

Figure 3. The aspect of the phytocoenosis inhabited by *S. caespitosum* in a natural population in the proximity of Gura Dobrogei site, Central Dobrogea, Romania, 1: clearly visible dry fruits of the first blooming phase; 2: flowers of the second blooming phase just beginning the anthesis, on the same individuals; yS.u: young shoots of *Sedum urvillei* in situ within the phytocoenosis, together with; oS.u: an older (probably a 1-year old plant from the last year) with incipient flower buds; dS.u: a dry, dead, 2-year-old *Sedum urvillei* plant from previous years (original).

The population studied herein grows in relatively dense colonies on shallow topsoil on skeletal, intensely fractured superficial bedrock, in slight contrast with another habitat in a different place nearby, where it grows directly on exposed, unfragmented rocky calcareous outcrops or in pockets or crevices, formed by dissolution during exocarstic activity on probably Jurassic limestones.

The native, natural *Sedum caespitosum* population in the proximity of Gura Dobrogei site inhabits the gentle slopes of the left banks of Casimcea River. The topsoil is very shallowith dark richer soil, immobilized in place in crevices and cracks in the highly fractured substrate. The bedrock substrate is calcareous, composed of probably Jurassic limestone consisting often of narrow calcareous sheaths with fractures sealed with calcite inclusions.

This structure with the sheaths oriented in places perpendicular to the soil surface allows better fixation of alluvial soil and especially of the loess where apparent, and also allows better root penetration in the vertical cracks and fissures between the lamellar sheaths.

Phytocoenological characteristics. In the studied site, *S. caespitosum* occurs in the intensely grazed and trampled pasture situated on the left banks and slopes on the left side of river Casimcea, where it stood very strong pressure from hears of sheeps/goats from a sheep farm located in close proximity (less than 100m). The facies of the site corresponds to Assoc. *Artemisio austriacae-Poëtum bulbosae* Pop 1970, which is built by *Poa bulbos*, and much less by *Artemisia austriaca -which is surpassed by Thymus sp.(* probably *T. pannonicus.* In patches less disturbed, *S. caespitosum* thrived at Gura Dobrogei in *Assoc*. *Sedo hillebrandtii-Polytrichetum piliferi* Horeanu et Mihai 1974 included in Western Pontic thyme steppes with *Thymus zygioides* (code 34.9211). The Assoc. is built mainly by *Polytrichum piliferum* and *Sedum urvillei,* with reported acompaining species *Dichanthium ischaemum, Potentilla argentea, Thymus zygioides and Thymus pannonicus, Xeranthemum annuum Sanguisorba minor, Scleranthus perennis*. Acording to PETRESCU (2012), PETRESCU et al., (2014) this Assoc. also hosts the of European interest, like *Campanula romanica, Dianthus nardiformis, Moehringia grisebachii* and also other rare locally threatened species like *Festuca callieri* and *Gagea szovitzii,* the last species being also significant for the in situ blooming and vegetation period of *S. caespitos*.

DISCUSSIONS

The phenology of *Sedum caespitosum* is the typical one for an ephemeral therophyte, and the seeds which represent the resting propagules, overwinter in the shallow soil on the rocky outcrops or in the dried follicles of the fruits. The active phase of the life cycle begins in March and lasts only about two months, as by June the seeds are ripe and the plants are completely dry.

One interesting fact mentioned here for the first time is that *Sedum chaespitosum* Cav (Crassulaceae) population from Central Dobrogea studied shows a distinct, second blooming phase, sometimes succeeding a brief apparent vegetative arest or even death of parts of the shoots and leaves of the shoots which bloomed in the first blooming period. This second bloom occurs when enough water is available, and could be interpreted as a strategy to take advantage of extended favorable periods during the same vegetative season. This finding warrants further experimental research coupled with phylogenetic examination of similar behavior in other Sedum taxa exhibiting more than one blooming phase in the same season.

Normally, annual herbs are monocarpic and this second blooming phase would suggest existence of dormant buds, primordia derived from meristems dormant or with delayed development, which begin re-growth as the first flowers wither and dry. As suggested by (VOLAIRE & NORTON, 2006), for the case of perennial grasses of Mediterranean origin, this second blooming phase could be interpreted like a condensed period of summer dormancy (endodormancy) triggered by the longer days and warmer temperatures caused by an overall delay in lifecycle due to shift of the anthesis, coupled with a subsequent break of endodormancy. This hypothesis though lacks a trigger for the putative release from dormancy, which in the case of Mediterranean perennial grasses is represented by temperature decrease (or day length decrease!?) in autumn.

In annuals, flowering appears to be “direct” (GRAINGER 1939, meaning anthesis follows flower initiation without any intervening rest period), a process which exploits and exhausts all floral meristems of any given individual during the completion of the life cycle within one year.

In many of the *Sedum caespitosum* population studied, the second blooming phase occurred after the leaves and even the stems of those individuals were apparently completely dry, suggesting a brief resting period between blooming phases and indicating the existence of a subset of meristems which entered a delayed anthesis after a brief dormancy. If this hypothesis holds true, this would be a highly unusual case in an annual plant, and could be assimilated with some sort of condensed polycarpy (as Bârcă V. suggested in personal communication).

Another, more orthodox explanation for this observed phenomenon -as TOOKE & BATTEY (2010) suggest, could be that sub-optimal chilling during a long and unusually warm winter which could have made budbreak protracted (but only a for subset of meristems in the same individuals), followed by a brief late coldspell which could explain good synchronization among individuals and among the 2 meristem subsets, consistent with the report of (SUNLEY et al. 2006 apud TOOKE & BATTEY 2010) that increasing chilling of blackcurrants and raspberries leads to more synchronous flowering. Further detailed experimental work is warranted to verify this hypothesis.

The ecology and phytocoenology of *Sedum caespitosum* D.C. on the Dobrogean site studied was found to differ indeed to a considerable extent from those of the populations from the western part of the country where the plant was considered typical halophyte, inhabiting in the spring the margins of the saltpans resulted from snowmelt and spring rains.

this study showed that, while the phenology and general survival strategy was similar, the autecology and phytocoenology was quite different in the two regions of the country.

Thus, in Western areas of Romania (BORZA, 1944) an likewise in the East-Central European countries, *Sedum caespitosum* D.C. was found to be a hallophyte clearly associate with saltpans, as reported in many works, some quite recent.

In Hungary these ecological traits of *Sedum caespitosum* D.C. were reported in the recent years by (BÁTORI et al 2014; BORHIDI A., 2003; JAKAB, 2005; JAKAB & TÓTH 2003, MOLNÁR et al 2012; KIRÁLY, 2007; TÓTH, 2003) and all of them consider this species as a halophyte, and similar perception was expressed about the ecology and phytocoenology of *Sedum caespitosum* D.C. by researchers of other salt-rich habittas in Slovakia (FEHÉR, 2007) and Serbia (KNEŽEVIĆ et al., 2008).

Unlike the populations in Western Romania and in Hungary some of the populations in Bulgaria were reported in Mt Rodopi from serpentine substrate PAVLOVA et al (2003)).

Although I have no direct knowledge of the ecology and phytocoenology of *Sedum caespitosum* D.C in the mountain populations, I expect the ones in Moldova (MITITELU et al., 1993; SÂRBU & ȘTEFAN, 2005), to be less halophylous.

The data reported herein show that, like the populations in Westeren and Southwestern Europe where *Sedum caespitosum* D.C. is not a halophylous plant, in Dobrogea it grows in plant associations clearly non-halophylous developed on shallow topsoil covering superficial limestone bedrock without any halophylous traits.

The data reported here about the autecology and phytocoenology of one population of *Sedum caespitosum* D.C. from Central Dobrogea, support the hypothesis suggested by Barca Valentin (personal communication) that *Sedum caespitosum* D.C. is not necessarily a typical hallophylous but it tolerates higher concentrations of cations –not specifically Na-; and of anions (not only Cl^- or SO_4^-) but more likely Ca, Mg, CO3, this more general salt tolerance allowing *Sedum caespitosum* to ocupy and take advantage of ecological niches inhospitable for other plants, escaping in

this manner from the competition which it seems mostly unable to withstand. These data lead us to consider *Sedum caespitosum* at most an oportunistic halophyte.

Another explanation of the autecological and phytocoenological discrepancies exhibited by *Sedum caespitosum* populations from diferent ares is that the halophylous *Sedum caespitosum* populations from South-Eastern Central Europe indeed belong to a distinct taxon, specialised n exploiting salt-rich flatlands and saltpans, as claimed by Simonkai when describing *Sedum deserti-hungarici* Simonkai (1890) based on specimens from one of those halophylous populations.

All these aspects should be taken in consideration when devising conservation measures aimed not only at preserving this important Crassulacean specis, but also, when implementing measures for preservation of animal species which might critically depend for their survival on the wellbeing of this tiny plant which they might use as food-plant like perhaps other cases which were documented previously; of *Aizobius sedi* Germ. (Apionidae, Curculionoidaea) (BÂRCĂ & NICULAE, 2011), and *Scolitantides orion* (Pallas 1771) (Lepidoptera, Lycaenidae), (BÂRCĂ & NICULAE, 2018a) to use other Crassulaceans) and also see other cases of trophic interactions documented for *Alcea rosea* L. (Malvaceae) used by the weevil *Rhopalapion longirostre* Olivier, 1807as host plant (BÂRCĂ et al., 2011), or of *Aristolochia clematitis* L used by Zerynthia polyxena (Dennis et Schiffermuller, 1775), (Lepidoptera, Papilionidae) as food-plant (BÂRCĂ, 2018a; BÂRCĂ & NICULAE, 2018b).

CONCLUSIONS

The phenology of *Sedum caespitosum* is the typical one for an ephemeral therophyte, and the seeds which represent the resting propagules, overwinter in the shallow soil on the rocky outcrops or in the dried follicles of the fruits. The active phase of the life cycle begins in March and lasts only about two months, as by June the seeds are ripe and the plants are completely dry.

Particularly unusually though, *S. chaespitosum* population from Central Dobrogea studied shows a distinct, second blooming phase, sometimes succeeding a brief apparent vegetative arrest or even death of parts of the shoots and leaves of the shoots which bloomed in the first blooming period, indicating the existence of a subset of meristems which entered a delayed anthesis after a brief dormancy. If this hypothesis holds true, this would be a highly unusual case in an annual plant, and could be assimilated with some sort of condensed polycarpy (as Barca V suggested in personal communication).

The ecological and phytocoenological data reported here support the hypothesis that *Sedum caespitosum* D.C. in Dobrogea grows in clearly non-halophylous plant associations developed on shallow topsoil covering superficial limestone bedrock, so it is at most an oportunistic halophyte being just a salt-tolerant species exploiting ecological niches inhospitable for other plants, thus escaping the competition which it is unable to withstand.

Another explanation of the observed autecological and phytocoenological inconsistencies exhibited by *Sedum caespitosum*, is that the halophylous *Sedum caespitosum* populations from South-Eastern Central Europe indeed belong to a distinct taxon, specialised in exploiting salt-rich flatlands and saltpans, as claimed by Simonkai when describing *Sedum deserti-hungarici* Simonkai (1890) based on specimens from one of those halophylous populations.

All these aspects should be taken in consideration when devising conservation measures aimed at both preserving this important Crassulacean species, and, also, for preservation of animal species which depend somehow on this plant.

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COPROSCOPY OF WILD MAMMALS: THE CASE OF RED FOX *Vulpes vulpes* (Thomas Say, 1823), COMMON JACKAL *Canis aureus* (Linné, 1758) AND WILD BOAR *Sus scrofa* (Linné, 1758) IN MARSH OF RÉGHAIA (ALGIERS)

MARNICHE Faiza, MILLA Amel, TIMTAOUCINE Khaoula, BACHA Assia

Abstract. During our study around the marsh of Réghaia. we carried out a trip every fortnight from February 21 to May 2, 2016 and 58 droppings were collected in the surrounding area of Jebel, 22 feces for the red fox *Vulpes vulpes* and Wild boar *Sus scrofa* each and 14 droppings for the common jackal *Canis aureus*. The flotation method of 58 samples allowed us to identify 19 species belonging to the 4 phylums, 6 classes, 9 orders and 14 families. We were able to identify 12 species of parasites in red fox droppings. 10 genera for common jackals and 15 parasites in wild boar and with common species. The total number of parasites identified is 19 genera. The results obtained for red fox *Vulpes vulpes* show a dominance of *Strongyloides* sp. with a rate of 31.82% followed by *Uncinaria* with 22.73%. *Ancylostoma* and *Eimeria* with 22.73% for each. Concerning the common jackal *Canis aureus*, the nematod *Strongyloides* occupies the first place with 21.43% followed by *Ancylostoma* sp., *Ascaridia* sp. and *Teania* sp. with 14.29% the rest of the parasitic species occupies the same rank with a prevalence rate equal to 7.14%. As for wild boars *Sus scrofa*, we noted after examinations of 22 excreta that 54.5% are infested by *Strongyloides* sp. followed by *Ancylostoma* sp. with an infestation rate of 50% followed by *Eimeria* sp. with a rate of 45.5% followed by *Ascaris* sp. with a percentage equal to 27.3%.

Keywords: parasites, wild mammals, *Sus scrofa*, *Vulpes vulpes*, *Canis aureus*, coprology, Réghaia.

Rezumat. Coproscopia mamiferelor sălbatice: cazul vulpii roșii *Vulpes vulpes* (Thomas Say, 1823), șacalului comun *Canis aureus* (Linnaeus 1758) și mistrețului *Sus scrofa* (Linné, 1758) în mlaștina din Réghaia (Alger). În timpul studiului nostru în jurul mlaștinii Reghaïa am efectuat două săptămâni de colectări de la 21 februarie - 2 mai 2016, 58 de colecții de excremente în jurul ariei Jebel, 22 excremente de vulpe roșie *Vulpes vulpes* și mistreț *Sus scrofa* și 14 excremente pentru șacalul comun *Canis aureus*. Metoda de flotare a 58 de probe ne-a permis identificarea a 19 specii aparținând celor 4 încrengături, 6 clase, 9 ordine și 14 familii. Am identificat 12 tipuri de paraziți găsiți în excrementele de vulpe roșie. 10 genuri pentru șacalul comun și 15 genuri de paraziți în mistreți și specii comune. Totalul genurilor de paraziți identificați este de 19 genuri. Rezultatele pentru vulpea roșie *Vulpes vulpes* arată dominanța *Strongyloides* sp. cu o rată de 31,2% urmat de *Uncinaria* cu 22,73% *Ancylostoma* și *Eimeria* cu 22,73% pentru fiecare. Pe șacalul comun *Canis aureus*, nematodul *Strongyloides* ocupă primul loc cu 21,43% urmat de *Ancylostoma* sp., *Ascaridia* sp. și *Teania* sp. cu 14,29%, restul speciilor parazite ocupă același loc, cu o rată de prevalență egală cu 7,14%. În ceea ce privește mistrețul *Sus scrofa* am observat după 22 excremente examinate că 54,5% sunt infestate cu *Strongyloides* sp. urmate de *Ancylostom*a sp. cu o rată de infestare de 50%, apoi *Eimeria* sp. cu o rată de 45,5% și de *Ascaris* sp. cu un procent egal cu 27,3%.

Cuvinte cheie: paraziți, mamifere sălbatice, *Sus scrofa*, *Vulpes vulpes*, *Canis aureus*, coprologic, Reghaïa.

INTRODUCTION

Zoonoses are one of the greatest threats to overall health (JONES et al., 2008). Most of these diseases are caused by parasitic organisms whose hosts are animals that live in relation to the human species (ZAJAC & CONBOY, 2013). In fact over 75% of human diseases are zoonoses originating from wild animals (TAYLOR et al., 2001). Wild mammals are an important reservoir of parasitological fauna, which is why our study targeted three wild mammalsŞ wild boar *Sus scrofa*, red fox *Vulpes vulpes* and the common jackal *Canis aureus*. The objective of our work is to highlight the parasites in the intestinal tract (feces) carried and transmitted by: red fox, wild boar and common jackal at the marsh Réghaia and the risk that can reach man.

STUDY AREA

The Réghaia marsh is found at the northern edge of the plain of Metidja in Algeria, 30 km from Algiers with a heterogeneous environment with open presence of olive tree, pistachio and casuarina trees. It covers a total area of 1.500 hectares (ha) of which 75 hectares are occupied by a freshwater pool, 600 ha of land and 900 ha inland and marine space. Its geographic coordinates are 36 ° 45 'and 36 ° 48'North and 3 ° 19' and 3 ° 21 'East. It is located in the bioclimatic area, subhumid, with mild winters and a minimum temperature equal to -1.1 ° C and a maximum equal to 44 ° C. It is characterized by a stony and loamy soil (Fig. 1).

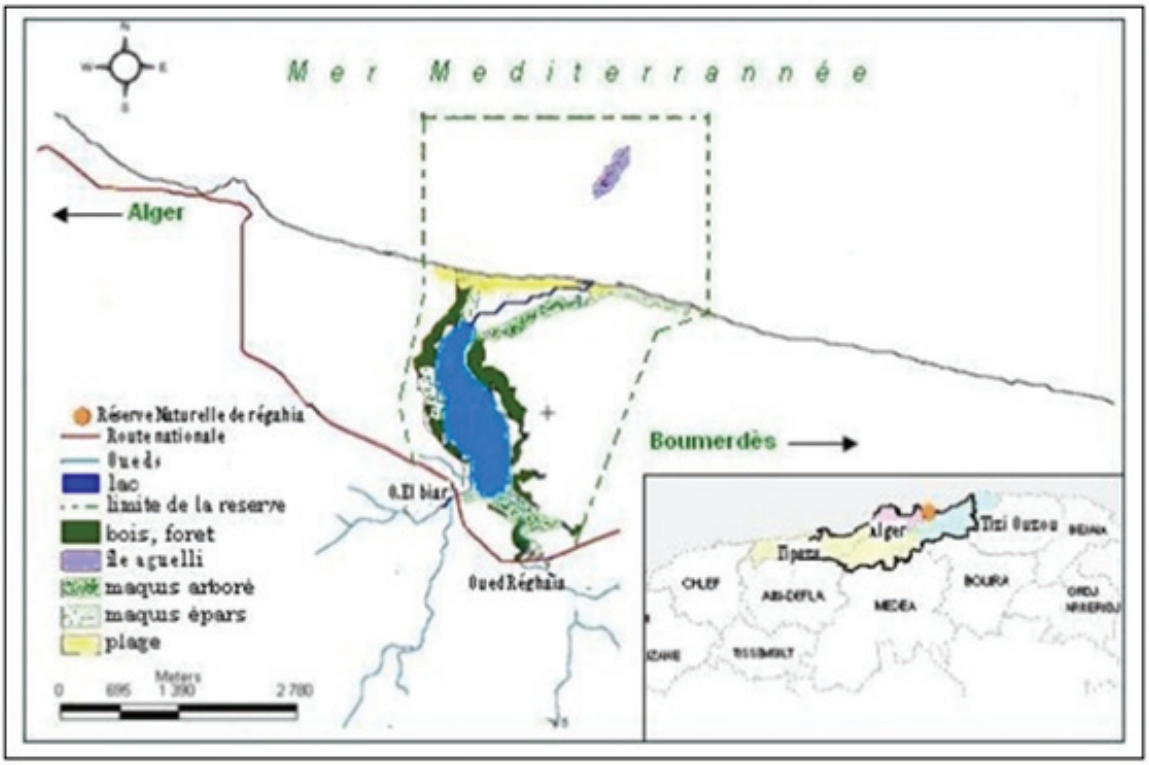


Figure 1. Study area (original).

MATERIALS AND METHODS

We collected 58 droppings during a trial period from February to May 2016 during the passage around the Djebel marsh of Réghaia, from wild mammals such as the red fox *Vulpes vulpes*, the common jackal *Canis aureus* and the wild boar *Sus scrofa.* Our study focused on 22 wild boar, 22 red fox and 14 jackal droppings. These were kept in sterile boxes or labeled coprology boxes with the date species name and station then brought back in the zoology laboratory at ENSV El Alia and then stored in the laboratory fridge (+ 4 ° C) until further processing (Fig. 2). The droppings were collected on the ground. To identify the droppings for each wild mammal tracks we used a guide for the recognition of droppings. These criteria (TEMPELE & CUTTELOD, 2009) are:

- For the wild boar the poop looks like a small ball in most cases depending on the freshness the balls are flattened or agglomerated between them by moisture which gives the excrement of wild boar a characteristic appearance.
- The waste contains many hairs remaining fruit.
- For the red fox the feces are colored from beige to black the variation of the color depends on the food ingested rounded at one end pointed or twisted by the hair at the other end.
- For the common jackal the dung is deposited on the tracks at ground level on the low vegetation's and on the stones; these excrements have a characteristic odor similar to that of the sulfur.

Analyzes of excrements three wild mammals were made by the qualitative method of flotation. This is a qualitative techniquesimple and fast the most used in veterinary medicine for the examination of droppings this process concentrates the parasitic elements from a small amount of excrement and traces those low density on the surface.The flotation method is based on a simple principle: the eggs have a shell that protects them. For a while the penetration of denser liquids; Dilution with these liquids will tend to float them on the surface while heavier residues or those that are rapidly absorbed fall into the bottom of the containers. This technique has the advantage of simplicity of execution speed and low cost (NaCl). Nevertheless this solution easily penetrates the egg which has the effect of distorting. for it must never exceed the prescribed time in the development of the technical (15 to 20 Min vicinity) also because the NaCl solution tends to crystallize quickly enough which would make the reading quite difficult after a certain time. We proceed to reading the blade under an optical microscope by scanning the latter in one direction horizontal or vertical at x10 magnificationwe increase it to 40 x when numbering or detection of a pest it proceeds to identify of it later.

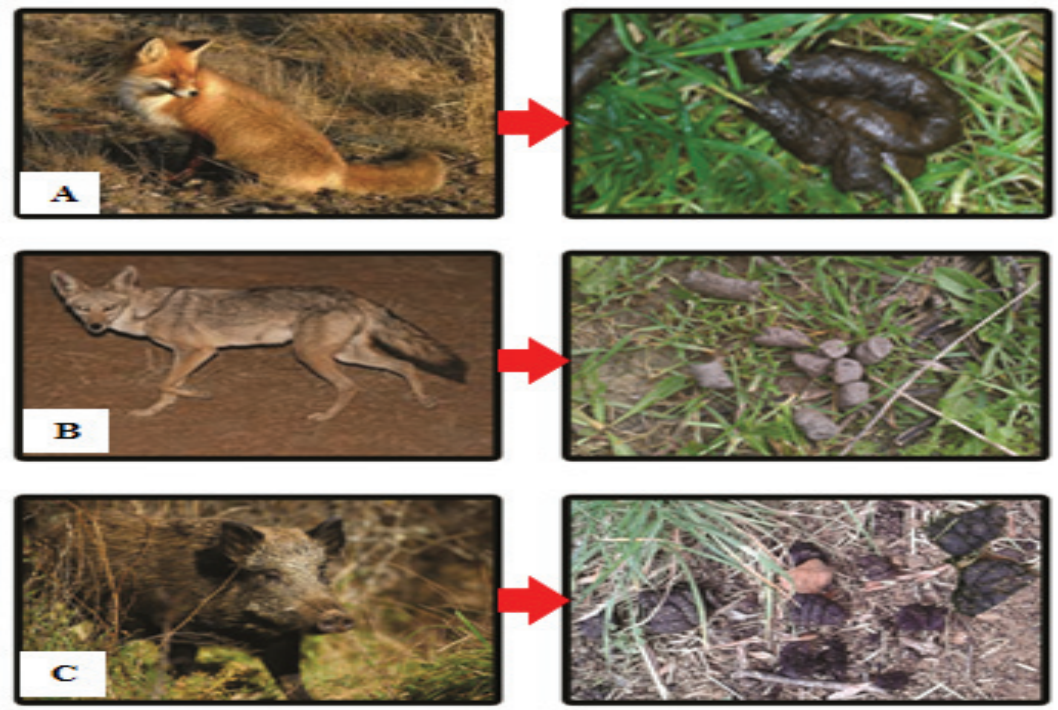


Figure 2. Collection of the excrement of the three wild mammals: A. Red fox; B. Jackal; C. Wild boar (original).

Methods of data analysis

Species noted are processed first by an ecological index of composition such that the relative abundance (AR%) is the percentage of individuals of a given species relative to the total number of the individual. It is expressed by the following formula: AR (%) = (ni / N) X 100 (or: number of individuals of species i; N: total number of individuals of all species) (DAJOZ, 1970). Parasitological analyzes used such as the status of the host the prevalence abundance and mean intensity by a statistical method parasite rate. These tests were performed using the Quantitative Parasitology V 3.0 software (ROZSA et al., 2000).

RESULTS AND DISCUSSIONS

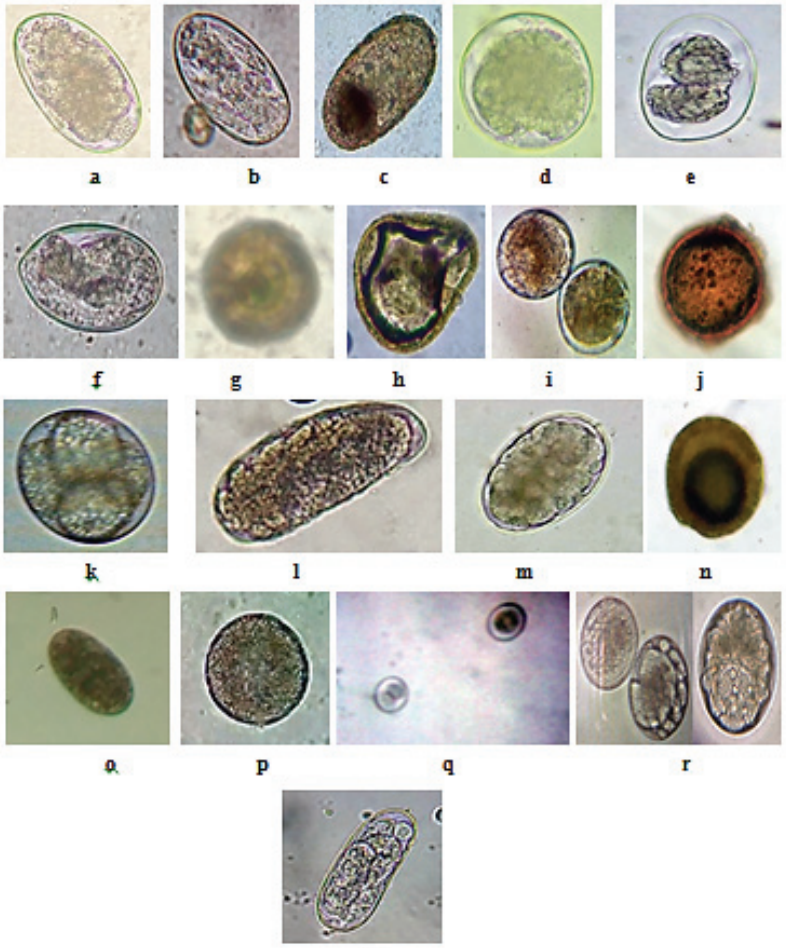
- **Coprological analysis results**

The results of the relative abundances (AR%) of the parasites found in the excrement of the 3 wild mammals are shown in Table 1. We noted a high relative abundance of *Eimeria* sp. in both wild mammals wild boar (60.80%) and red fox (14.06%). Followed by those of nematode eggs of *Ancylostoma* sp. with a rate of 30.67% in jackal in red fox is 18.92% and finally in wild boar is 8.52%. *Strongyloides* sp. with a rate of 26.67% in the jackal 21.08% in the red fox and 12.01% in the wild boar. While those of Plathelminth eggs oocysts of *Isospora* sp. and *Balantidium coli* cysts are poorly represented.

Table 1. Abundance relative (AR %) of parasites found in feces wild 3 mammals.

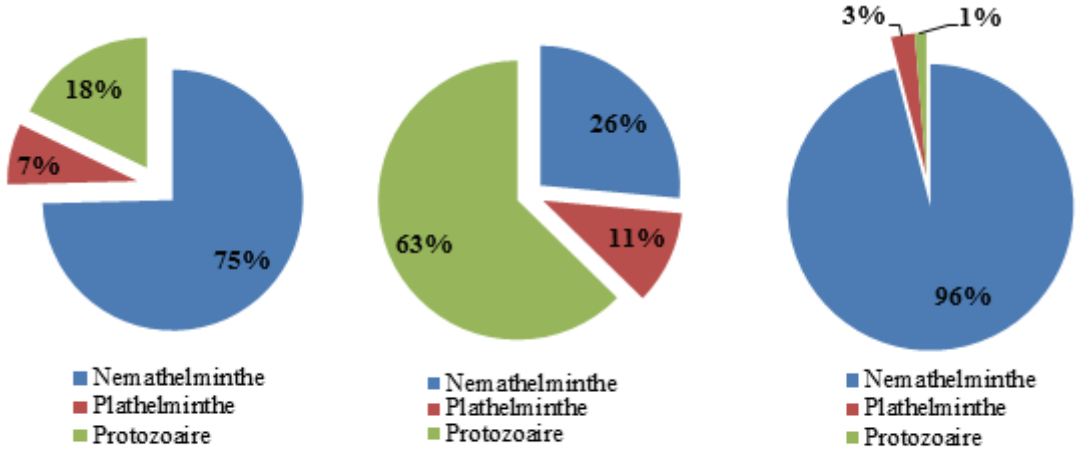
Category		**Aspect**	**Red Fox**		**Wild Boar**		**Common Jackal**	
Philum	**Species**		**ni**	**AR (%)**	**ni**	**AR (%)**	**ni**	**AR (%)**
Nemathelminthes	*Ancylostoma* sp.	egg	35	18.92	207	8.52	23	30.67
	Strongyloides sp.	egg	39	21.08	292	12.01	20	26.67
	Uncinaria sp.	egg	30	16.22	19	0.78	-	-
	Trychostrongylus sp.	egg	-	-	-	-	4	5.33
	Ascaridia sp.	egg	13	7.03	18	0.74	17	22.67
	Ascaris sp.	egg	0	0.00	13	0.53	1	1.33
	Toxocara sp.	egg	5	2.70	20	0.82	-	-
	Globocephalus sp.	egg	9	4.86	22	0.90	-	-
	Cooperia sp.	egg	7	3.78	3	0.12	4	5.33
	Physocephalus sp.	egg	-	-	49	2.02	-	-
	Physaloptera sp.	egg	-	-	-	-	3	4.00
Plathelminthes	*Teania* sp.	egg	2	1.08	5	0.21	1	1.33
	Monezia sp.	egg	7	3.78	216	8.89	-	-
	Cestoda sp.	egg	0	0.00	8	0.33	-	-
	Mesocestoides sp.	egg	5	2.70	35	1.44	-	-
	Fasciola sp.	egg	-	-	-	-	1	1.33
Apicomplexa	*Balantidium coli*	cyste	7	3.78	-	-	1	1.33
	Eimeria sp.	oocyste	26	14.05	1478	60.80	-	-
	Isospora sp.	oocyste	-	-	46	1.89	-	-
Totale	**19 species**		**185**	**100.00**	**2431**	**100.00**	**75**	**100.00**

a. Variations in the number of parasites found in the excrement according to the classes of each animal studied. From Figs. 3, 4 we noticed that the class of Nemathelminthes dominates two wild mammal Canidae, compared to pigs (Suidae). Protozoa in wild boar are better represented relative to the common red fox and jackal.



a. *Ankyl stoma* sp.; b. *Uncinaria* sp.; c. *Ascaridia* sp. ; d. *Cooperia* sp. ; e. *Globocephalus* sp.; f. *Strongyloides* sp. g. *Taenia* sp. ; h. *Moniezia* sp.: i. *Mesocestoides* sp.; j. *Balantidium coli* ; k. *Eimeria debliecki*; l. *Physaloptera* sp m. *Trichostrongylus* sp.; n. *Toxocara* sp. o. *Fasciola* sp.; p. *Ascaris* sp.; q. *Isospora* sp.; r. *Cestoda* sp.; s. œuf de *Physocephalus* sp.

Figure 3. Parasites found in the excrements of the three mammals seen under the light microscope GRx40 (original).



a. Red fox **b. Wild Boar** **c. Jackal**

Figure 4. Spectra of changes in effective parasites found in feces according to the classes**.**

b. *Variations of the number of parasites found in the excrement according to the species of each animal studied.* The variation of the numbers of parasites found in the excrement of these wild mammalsthe dominant parasite in the Red Fox is a nematode of the genus *Strongyloides* (egg) with a rate of 21.08% followed by the *Ancylostoma* (egg) with 18.92% then *Uncinaria* (egg) with 16.22% finally the Coccides with the genus *Eimeria* (egg) with a percentage of 14.05%. The other species are poorly represented with a level of from 1.08% to 7.03%. Unlike the wild boar coccids dominate with *Eimeria* (egg) with a rate equal to 60.80%followed by *Strongyloides* (egg) with a percentage of 12.01%.

By cons in the common Jackal dominant parasitic nematodes are hosting with the dominance of the genus *Ancylostoma* (egg) with 30.67% then comes the second *Strongyloides* (egg) with a rate of 26.67% and *Ascaridia* third with a percentage of 22.67%. Other genera are poorly represented at a rate which varies between 1.33% to 5.33% (Fig. 5).

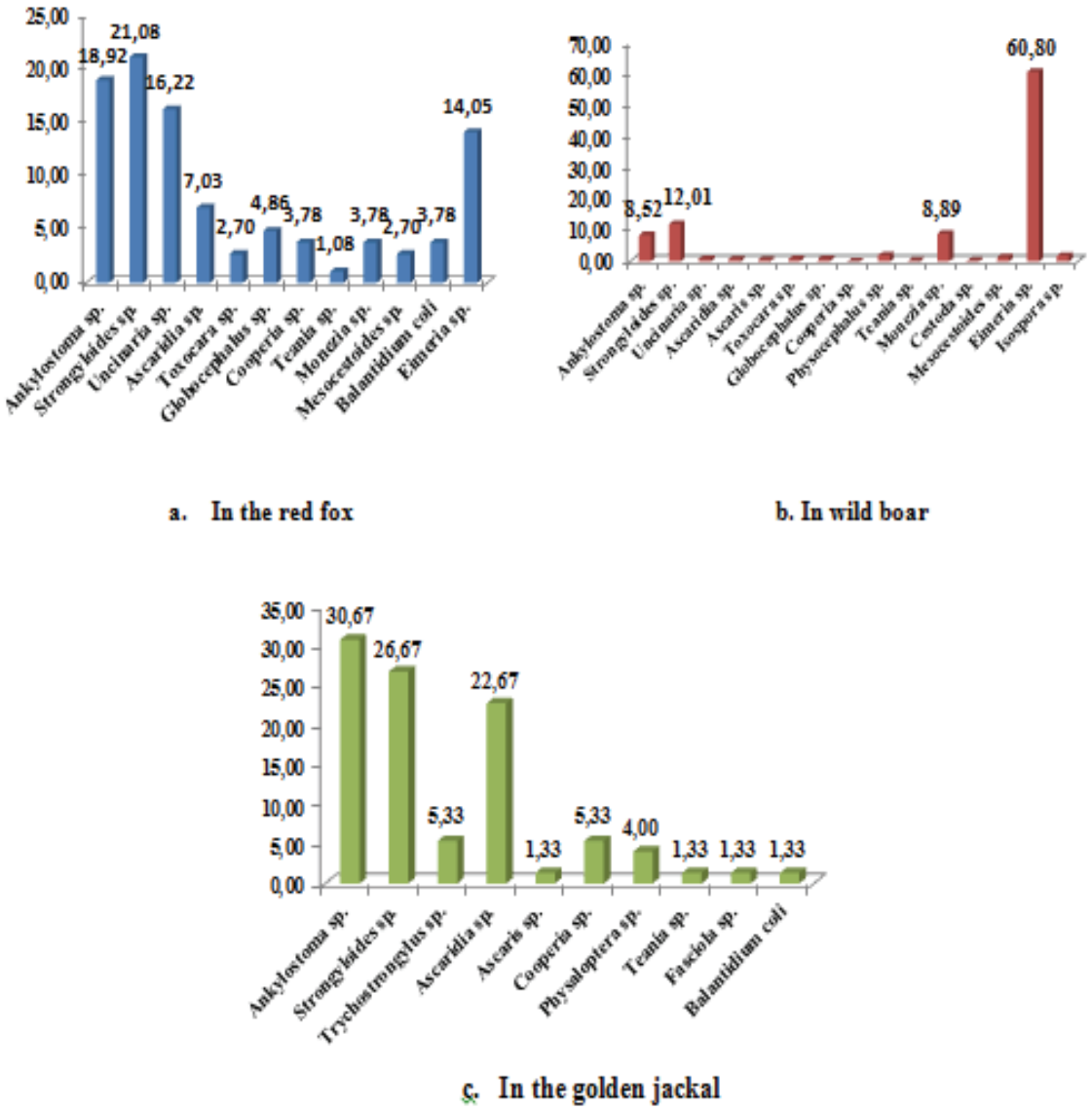


Figure 5. Changes effective parasites found in feces depending on the species of each animal studied.

c. *Exploitation of results by a statistical method: parasitic Index.* The prevalence and intensity of endoparasites found in the feces of three wild mammals studied are shown in Figs. 6, 7, 8.

On a total of 22 droppings 54.5% (12 droppings) are infested with *Strongyloides* sp. (egg) in wild boar. Followed by *Ancylostoma* sp. (egg) with an infestation rate of 50.00% (11 excrements) which belongs to the class of dominant species and to the species *Eimeria* sp. with a rate of 45.5% (10 droppings) which belongs to the class of less dominant species. Then we note a prevalence of 27.3% (6 droppings) that are infested with *Ascaris* sp. (Egg) and 18.2% (4 droppings) for *Ascarida* sp. and *Mesocestoides* sp. Each of which belongs to the class of satellite species. Finally for the other species have a rate varies between 4.50% and 13.6% (from 01-03 excrements) that gather to the class of rare species. Intensity data has undergone a logarithmic transformation in order to respect the rule of normality according to the law of variation of parasitisms as a function of size. As regards the mean intensity. it gradually increases between 1.00 and 2.00 (very low) for *Ancylostoma* sp. *Ascaridia* sp., *Ascaris* sp., Cestoda sp., *Cooperia* sp., *Eimeria* sp.,

Globocephalus sp., *Isospora* sp., *Mesocestoides* sp., *Moniezia* sp., *Physocephalus* sp., *Strongyloides* sp., *Taenia* sp., *Toxocara* sp., *Uncinaria* sp. with an intensity varying from 1.00 to 2.00 very low average (Fig. 6).

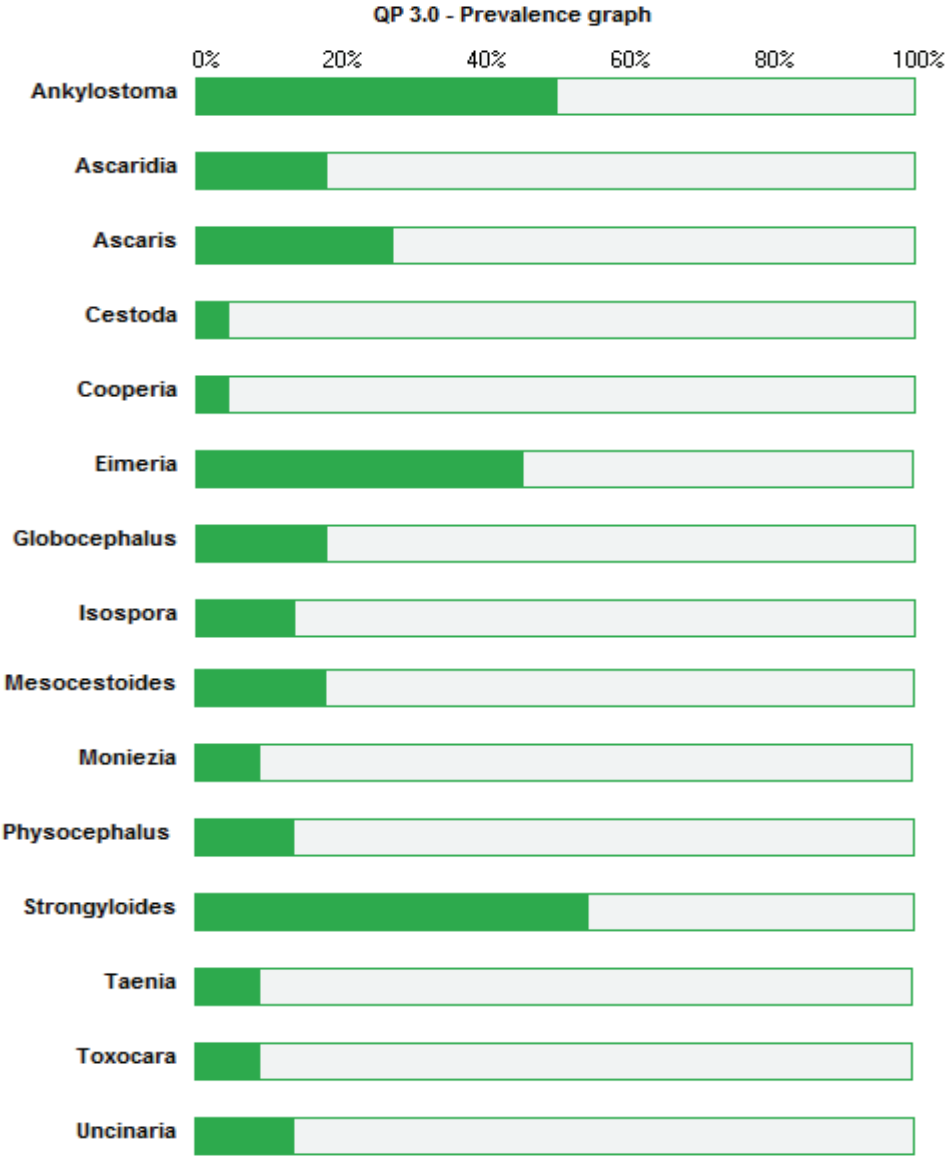


Figure 6. Plot of the prevalence of endoparasites found in the droppings of wild boar with the software (Quantitative Parasitology V 3.0.).

- **In canines red fox *Vulpes vulpes***

On a total of 22 Red Fox foxes 22.7% (5 droppings) are infested with *Ancylostoma* sp. (eggs)followed by *Ascaridia* sp., *Strongyloides* sp. and *Uncinaria* sp. with a rate of 18.2% (4 droppings) each belonging to the class of satellite species. For the other species the other species have a rate varies between 4.50% and 13.6% (from 01-03excrements) which gather to the class of rare species. Intensity data has undergone a logarithmic transformation in order to respect the rule of normality according to the law of variation of parasitisms as a function of size. With regard to the mean intensityit gradually increases between 1.00 and 2.00 (very low) for *Ancylostoma* sp., *Ascaridia* sp., *Balantidium coli*, *Cooperia* sp., *Eimeria* sp., *Globocephalus* sp., *Mesocestoides* sp., *Moniezia* sp., *Strongyloides* sp., *Taenia* sp., *Toxocara* sp., *Uncinaria* sp. with an intensity that varies from 1.00 to 2.00 very low average (Fig. 7).

- **At jackal common *Canis aureus***

Out of a total of 14 jackal droppings 14.3% (2 droppings) are infested with *Ancylostoma* sp. (eggs). *Ascaridia* sp. (eggs) and *Taenia* sp. (eggs) followed by *Ascaris* sp., *Strongyloides* sp., *Trychostrongylus* sp., *Physaloptera* sp., *Fasciola* sp., *Cooperia* sp. and *Balantidium coli* with a rate of 7.1% (1crottes) each belonging to the class of rare species. Intensity data has undergone a logarithmic transformation in order to respect the rule of normality according to the law of variation of parasitisms as a function of size. With regard to the mean intensity equal to 1.00 (very low) for *Ankylostoma* sp., *Ascaridia* sp., *Ascaris* sp., *Balantidium coli*, *Cooperia* sp., *Strongyloides* sp., *Taenia* sp., *Trychostrongylus* sp., *Physaloptera* sp., *Fasciola* sp. with an intensity equal to 1.00 very low average (Fig. 8).

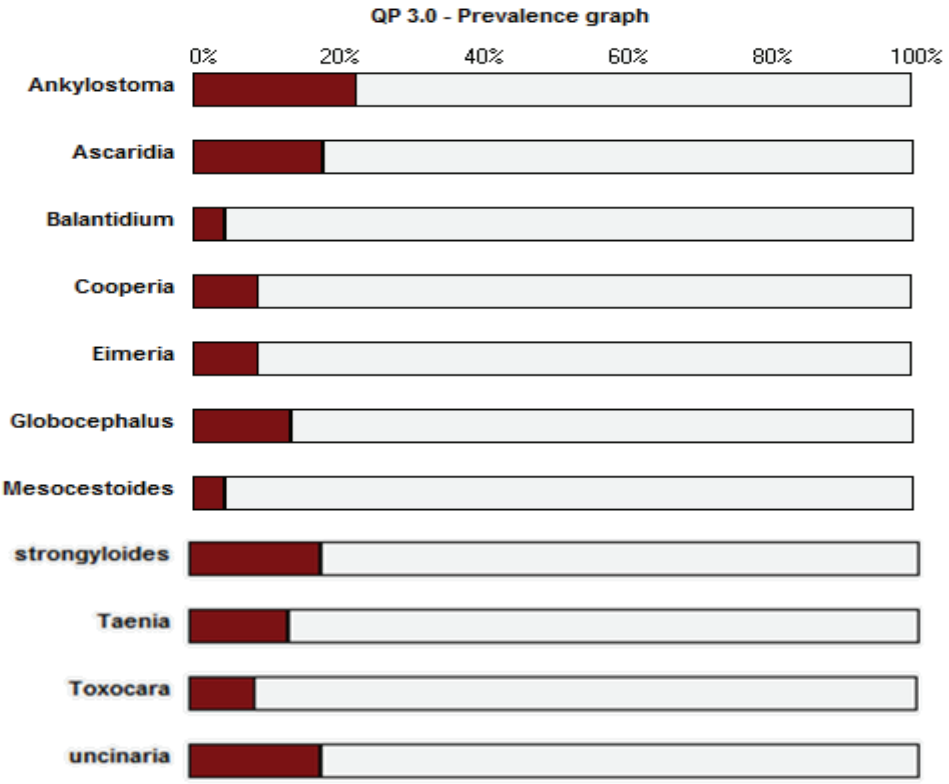


Figure 7. Graph of prevalence of endoparasites found in the droppings of red fox with software (Quantitative Parasitology V 3.0.).

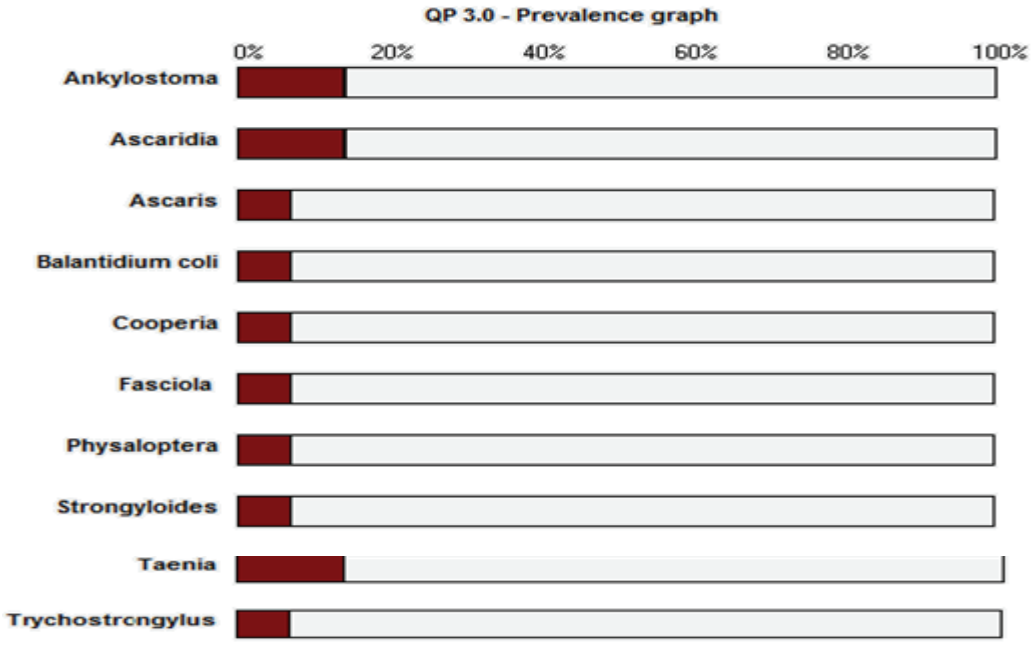


Figure 8. Graph prevalences of endoparasites found in the droppings of common jackal with the software (Quantitative Parasitology V 3.0.).

CONCLUSIONS

Intestinal parasitism takes an important place in the overall pathology of wildlife. The discussion focuses on the inventory of parasites found tract of red fox, common jackal and wild boar in the swamp of Réghaia during the period from late February until May 2016. Through the method of water samples we identified 19 species belonging to four branches six classes, 9 orders and 14 families belonging to the protozoa. Nemathelminthes and flatworms and nematodes remain the most dominant branch. The results obtained for the red fox show a dominance of *Strongyloides* sp. with a rate of 31.82% followed by *Uncinaria* with 22.73%. *Ancylostoma* and *Eimeria* with 22.73% for each for the rest of the parasites found in the red fox their percentage does not show a significant difference between 9% and 18%. The study on infestation *Uncinaria* and

Toxocara confirms the results of DEPPIERE (1999) obtained on the red fox with a prevalence of 40% and 24% for *Uncinaria, Toxocara*. Regarding the infestation due to *Eimeria* with a rate of 22.7% corresponding to studies by SADDAM (2015) on the golden jackal with 33.33%. Our results have directed us to detect two types of infestation: by specific parasites and other canids; by non-specific parasites which can be explained by food intake. These results are consistent with LABORDE (2008) on the wolf – a research conducted in France also found specific and nonspecific parasites in wolf represented by *Eimeria* spp. birds or herbivores as well as avian Ascaridae. *Strongyloides* occupy first place with 21.43% followed by *Ancylostoma* sp., *Ascaridia* sp. and *Teania* sp. with 14.29% the rest of pest species occupies the same rank with a prevalence rate equal à7.14%. Our results confirm those found by LABORDE (2008) which marked a low rate of infestation with trematodes, in a percentage of 10%. LESLIE (2005) found a rate of 11.2% for trematodes. We can see that the parasites affecting the common red fox and jackal are common with those found in wild canids in general. Based on our study, results after 22 examined droppings of wild boar 54.5% (12 excrements) infested with *Strongyloides* sp. followed by *Ancylostoma* sp. with a 50% infection rate (11 excrements) then *Eimeria* sp. with a rate of 45.5% (10 excrements) followed by *Ascaris* sp. with a percentage equal to 27.3% for other parasites found that their prevalence rates vary between 18.2% and 4.5%. To make a comparison of parasite diversity of the wild mammal we will compare our results with those of SADDAM (2015). The infestation *Strongyloides* ranks first with 48% followed by *Emeria* with a prevalence rate equal to 32.0% and these results are consistent with ours. GASSO et al. (2015) found in 59 samples of wild boar. The prevalences for parasites eggs state are represented by *Metasrongylus* sp. 58.9%, 36.2% with *Ascarissuum, Physocephalus sexalatus* and *Globocephalus urosubulatus* possess an unknown prevalence. As urbanization spreads and reconciliation with cash freedom is growing so there is an increasing risk of disease transmission to humans.

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TERRESTRIAL ISOPODS (ISOPODA, ONSICIDEA) IN SEBIȘ TOWN, ARAD COUNTY (ROMANIA)

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Abstract. In the Sebiș town from Arad County, western Romania, we recorded 17 terrestrial isopod species. The study was made in the autumn of 2017, with the direct collecting method. The most common isopod in Sebiș is a species linked to open areas (*Trachelipus nodulosus*). The most favorable habitat type for terrestrial isopods in Sebiș town was the periurban area, presenting the highest species richness. This habitat type is less affected by human activities compared to the other habitat types, thus it shelters species both with restrictive and larger ecological demands. Unlike another town from the region, Sebiș town is bordered north by forests. Native, forest and wetland species are present especially in the periurban area, some of them being present exclusively here. However, some of these species are present occasionally in the industrial area, which is mostly abandoned and situated at the town's outskirts. The terrestrial isopod fauna from Sebiș underlines the importance of natural and forested areas close to the town, at least in the case of this group.

Keywords: localities, region, forests, surrounding areas, anthropogenic disturbance

Rezumat. Izopode terestre (Isopoda, Oniscidea) în orașul Sebiș, județul Arad (România). In orașul Sebiș din județul Arad, vestul României, am identificat 17 specii de izopode terestre. Studiul a fost realizat in toamna anului 2017, cu metoda de colectare directă. Specia de izopod cea mai comună în Sebiș a fost una de zone deschise (*Trachelipus nodulosus*). Cel mai favorabil tip de habitat pentru izopodele terestre din Sebiș a fost zona periurbană, prezentând cel mai mare număr de specii. Acest tip de habitat este mai puțin afectat de activitățile umane în comparație cu alte tipuri de habitate, adăpostind astfel atât specii cu cerințe ecologice mai restrictive cât și cu cerințe largi. Spre deosebire de un alt oraș din regiune, Sebiș se învecinează la nord cu păduri. Speciile native, de pădure şi de zone umede, sunt prezente în special în zona periurbană, unele dintre acestea fiind prezente doar în aceasta. Totuşi, anumite specii din această categorie ajung ocazional şi în zona industrială, în mare parte abandonată şi situată la periferia oraşului. Fauna de izopode terestre din Șebiș demonstrează importanţa existenţei unor zone naturale, împădurite, la periferia oraşelor, cel puțin pentru acest grup.

Cuvinte cheie: localități, regiune, păduri, zone învecinate, afectare antropică.

INTRODUCTION

Habitats which are partially affected by humans can be considered transition zones both for terrestrial isopods characteristic to natural zones and for species linked to affected areas (e.g. FERENȚI et al., 2013a,b). Thus, a higher diversity and abundance was observed in areas with an intermediate disturbance like suburban habitats, compared with more urbanized habitats or even natural ones (e.g. VILISICS et al., 2007). For terrestrial isopods, urban areas seem very diverse environments, which can offer optimal conditions both for non-native species, but also for natives linked to natural areas, and even for the endemic ones (GIURGINCA, 2006; GIURGINCA et al., 2017; VILISICS & HORNUNG, 2009; VILISICS et al., 2012). The isopod assemblages differ greatly between different regions of the same locality (e.g. JĘDRYCZKOWSKI, 1981; VILISICS & HORNUNG, 2009; LAZA et al., 2017), but also between localities situated in different regions (VILISICS et al., 2012; LAZA et al., 2017). The effect of urban areas and urbanization on fauna proved to be negative in numerous occasions (e.g. MCKINEY, 2008; FATTORINI, 2011; MARTINS et al., 2013; RAMÍREZ-RESTREPO & MACGREGOR-FORS, 2017). In Romania the studies upon terrestrial isopods in urban areas are limited to the capital (GIURGINCA, 2006; GIURGINCA et al., 2017) and some small towns from the western part of the country (BODIN et al., 2013; FERENȚI et al., 2015; HERLE et al., 2016; LAZA et al., 2017). The first three cited studies were made in Bihor County. In Arad County the only study was performed in the Pâncota town, where the isopod fauna was poor because of the past human impact, expressed mainly by deforestation (LAZA et al., 2017). Nevertheless, near the small stream from the town native forest species had survived, despite the lack of forests from surroundings (LAZA et al., 2017).

Sebiș is a town localized in Arad County, close to Pâncota, having approximately the same size, and located at the same altitude. The only major difference is the fact that Sebiș is presently bordered north with a forest. We supposed that this fact will make a difference, determining a richer urban terrestrial isopod fauna. Thus, we investigated the terrestrial isopods from Sebiș, having two main objectives: 1. comparing the terrestrial isopods composition from Sebiș with the isopods previously identified in Pâncota, and 2. identifying the habitats, which shelter the richest isopod fauna in Sebiș.

MATERIAL AND METHODS

The Sebiș town is situated in Arad County, western Romania, at an altitude of approximately 150 m, being an important urban center for the Crișul Alb valley (VELCEA et al., 1979). Its population was, according to the last population census from 2011, 5831 inhabitants (http://www.recensamantromania.ro/rezultate-2). The town is located in

the Zărand Depression, on the southern flank of the Codru-Moma Mountains (MÂNDRUȚ, 2006). The region looks like a bay of the Crișuri Plain insinuated along the Crișul Alb River (MÂNDRUȚ, 2006), with a plain relief in the southern part of the locality. The north-eastern region of the town is bordered by a, mainly oak, forest, but some black locust plantations are also present here. There are also agricultural fields and grasslands, which surround the locality especially at its southern part. In the northern part of the town there are two artificial ponds. The town is crossed from east to west by the Moneasa River, which is a tributary of the Crișul Alb River (UJVÁRI, 1972). In the northern part of the town there are some small streams, surrounded by willows.

Sebiș hosts numerous traditional old buildings with green spaces and wide streets with trees. The downtown contains zones with crowded, multistory new blocks, without green spaces, but with wood storages. The town is crossed by numerous asphalted roads. Two small parks are located in the downtown. A cemetery and also some, mostly abandoned, industrial areas are situated in the northern and western parts of the town.

The samples were taken on October 7, 2017. We collected samples from 39 sampling points. They were classified in five habitat types: old buildings, parks, industrial zones, new buildings and periurban area. Isopods were collected directly by hand as well as in other urban studies (FERENȚI et al., 2015; LAZA et al., 2017), from under different shelters, debris, from the humid soil near wet areas. At each location we spent approximately 20 minutes. The isopods were conserved in test tubes with alcohol, separating in different test tubes the smaller fragile species from the larger ones. The species were identified in the laboratory, using the scientific literature (e.g. RADU, 1983, 1985). The data was analyzed both for the total and for the five habitat types. We calculated the percentage abundance and frequency of occurrence for each species. The other parameters were calculated using PAST (HAMMER et al., 2001). The species affinity to different habitat types taking into account their abundance was estimated by the correspondence analysis. The similarity between the assemblages from different habitat types was estimated by the Jaccard index, and the species diversity with the Shannon-Wiever index (SHANNON & WIEVER, 1949). With the Mann-Whitney test we estimated the significance of the differences between the terrestrial isopod assemblages from different habitat types.

RESULTS

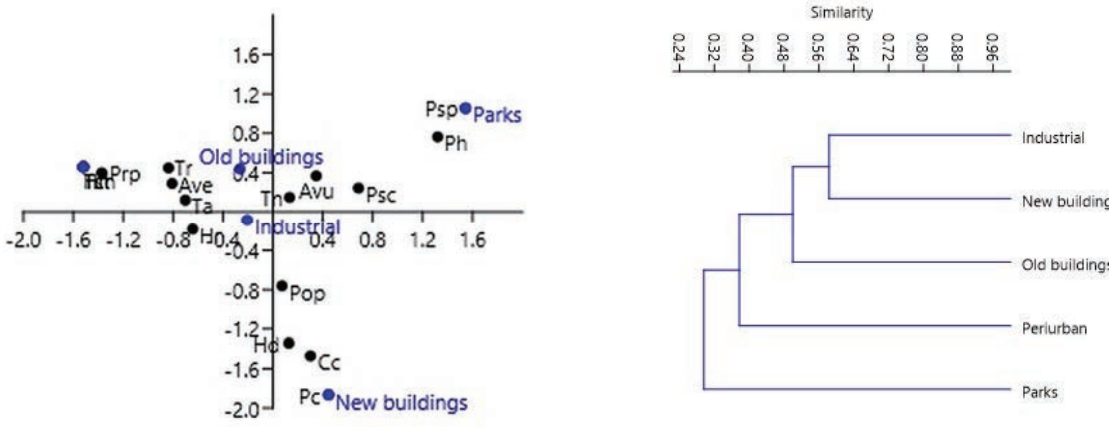
In the Sebiș town we collected 318 individuals belonging to 17 terrestrial isopod species: *Trichoniscus steinboecki* Verhoeff, 1931, *T. crassipes* Verhoeff, 1908, *Hyloniscus riparius* (C. Koch, 1838), *Haplophthalmus danicus* Budde-Lund, 1880, *H. mengii* (Zaddach, 1844), *Platyarthrus hoffmannseggii* Brandt, 1833, *Cylisticus convexus* (De Geer, 1778), *Porcellionides pruinosus* (Brandt, 1833), *Protracheoniscus politus* (C. Koch, 1841), *Trachelipus arcuatus* (Budde-Lund, 1885), *T. nodulosus* (C. Koch, 1838), *T. rathkii* (Brandt, 1833), *Porcellium collicola* (Verhoeff, 1907), *Porcellio scaber* Latreille, 1804, *P. spinicornis* Say 1818, *Armadillidium vulgare* (Latreille, 1804) and *A. versicolor* Stein, 1859. The most commonly encountered species was *T. nodulosus*, which was identified in 76.90 % of the sampling points, being also the most abundant species (125 individuals). This was followed by *A. versicolor*, both by percentage abundance and frequency of occurrence (Table 1). The third place, by both parameters, was occupied by *H. riparius*. *T. steinboecki*, *T. crassipes* and *P. collicola* were represented by the lowest number of individuals (one individual each). The highest number of species / sample was six, registered twice. In seven sampling points we found only one species.

Table 1. The percentage abundance (P%), frequency of occurrence (f%), species richness (S) and diversity (H) from Sebiș town.

Species	Total		Old buildings		Parks		Industrial area		New buildings		Periurban area	
	P%	f%	P%	f%	P%	f%	P%	f%	P%	f%	P%	f%
1. Trichoniscus steinboecki	0.31	2.56	-	-	-	-	-	-	-	-	0.96	10.00
2. Trichoniscus crassipes	0.31	2.56	-	-	-	-	-	-	-	-	0.96	10.00
3. Hyloniscus riparius	8.81	17.90	-	-	-	-	14.28	25.00	6.66	16.66	15.38	40.00
4. Haplophthalmus danicus	4.09	10.30	2.29	8.33	-	-	-	-	17.77	33.33	2.88	10.00
5. Haplophthalmus mengii	1.26	2.56	-	-	-	-	-	-	-	-	3.84	10.00
6. Platyarthrus hoffmannseggi	1.89	7.69	-	-	21.05	33.33	-	-	2.22	16.66	0.96	10.00
7. Cylisticus convexus	6.60	20.50	-	-	-	-	9.52	37.50	33.33	83.33	-	-
8. Porcellionides pruinosus	0.94	7.69	1.14	8.33	-	-	1.58	12.50	2.22	16.66	-	-
9. Protracheoniscus politus	4.40	7.69	-	-	-	-	1.58	12.50	-	-	12.50	20.00
10. Trachelipus arcuatus	0.63	5.13	-	-	-	-	1.58	12.50	-	-	0.96	10.00
11. Trachelipus nodulosus	39.30	76.90	56.32	91.66	47.36	100	52.38	87.50	26.66	66.66	21.15	50.00
12. Trachelipus rathkii	3.14	15.40	5.74	25.00	-	-	-	-	-	-	4.80	30.00
13. Porcellium collicola	0.31	2.56	-	-	-	-	-	-	2.22	16.66	-	-
14. Porcellio scaber	3.46	17.90	6.89	16.66	10.52	66.66	1.58	12.50	4.44	33.33	-	-
15. Porcellio spinicornis	0.63	2.56	-	-	10.52	33.33	-	-	-	-	-	-
16. Armadillidium vulgare	4.40	23.10	3.44	16.66	10.52	33.33	6.34	37.50	2.22	16.66	3.84	20.00
17. Armadillidium versicolor	19.50	41.00	24.13	50.00	-	-	11.11	50.00	2.22	16.66	31.73	50.00
S	17		7		5		9		10		12	
H	2.00		1.26		1.39		1.52		1.76		1.91	

The terrestrial isopod species` diversity from Sebiș was H=2.00. In terms of habitat types, the highest species diversity was registered in the periurban area, followed by new buildings, industrial area, parks and old buildings (Table 1). The differences between the assemblages of terrestrial isopod species from different habitat types, according to the Mann-Whitney index, were not significant ($p>0.05$). According to the correspondence analysis, *P. spinicornis* and *P. hoffmannseggii* showed affinity for parks, but *P. collicola, C. convexus, H. danicus* and *P. pruinosus* for new buildings (Fig. 1a). According to the Jaccard index, the most accentuated overlap is registered between the assemblages from new buildings and industrial areas, the most distinct assemblage being found in parks (Fig. 1b).

The differences between the five habitat types from Sebiș town were obvious. The highest species number (12) was registered in the periurban area, the lowest number (5) being present in public parks. Also, there was a variation of the species richness, diversity, percentage abundance and frequency of occurrence of the species from different habitat types (Table 1). The two Trichoniscus species were present only in the periurban zone, while *P. spinicornis* was found exclusively in parks. *T. nodulosus* was the best represented species in almost all habitat types, with the exception of the periurban area (where *A. versicolor* had higher percentage abundance) and new buildings (where *C. convexus* was on the top).



a b

Figure 1. (a) Correspondence analysis between isopod species and habitat types and (b) the Jaccard similarity between the isopod assemblages found in different habitat types.

DISCUSSIONS

The 17 terrestrial isopod species identified in Sebiș town confirmed the hypothesis of this study, Sebiș having a richer fauna than Pâncota town from the same region (LAZA et al., 2017). Moreover, in Sebiș town the species number is higher than in other towns from north-western Romania (BODIN et al., 2013; FERENȚI et al., 2015; HERLE et al., 2016). Also, the species richness from this small town is equal with the one present in the parks of Bucharest, the capital of Romania (GIURGINCA et al., 2017). This species richness is mainly caused by the periurban areas, where the highest species number and diversity was registered. This confirms the importance of some natural zones in the town's vicinity for the isopod assemblages from the town (HERLE et al., 2016). The more natural the zones close to the town are, the richer the terrestrial isopod assemblages will be. Thus, urbanization will reduce a fauna which was richer from the beginning. The existence of natural forested zones near towns and the management of the green spaces as natural as possible proved to be beneficial for the biodiversity (MÜLLER et al., 2018).

The differences between the two towns are expressed mainly by the higher abundance of the native fauna in the town surrounded with forest compared to the other without forest (LAZA et al., 2017), and do not necessarily imply the presence of some additional native species. Practically, the Sebiș town, having more natural forests in surroundings, preserves in many points species linked to natural forested areas, points, which are strictly localised in Pâncota (LAZA et al., 2017). Taking into account that the individuals from the Trichoniscus genus in Pâncota, being females, could not be determined (LAZA et al., 2017), it is hard to say whether the two species of this genus found in Sebiș are additional species or not. Anyway, *T. steinboecki* and *T. crassipes*, are novelty, they not being mentioned until now in the urban areas of western Romania (BODIN et al., 2013; HERLE et al., 2016; LAZA et al., 2017). Both species were collected from the periurban zone, fact which can indicate their native character in the region. *T. crassipes* was collected on humid soil near the pond from the quarry, while *T. steinboecki* was collected under debris from the humid soil on the bank of a small stream. *T. steinboecki* is a species considered characteristic to humid zones from deciduous forests (FARKAS & VILISICS, 2008). SCHMALFUSS (2003) had restricted the distribution of these two species to southeastern and eastern Austria, but afterwards they were found in Hungary (VILISICS, 2007). Both are considered to be rare species, which live in natural habitats, wet areas from mountain zones (HORNUNG et al., 2009), but VILISICS (2007) suggests that *T. steinboecki* could be a common species, since it was identified in numerous humid zones in Transdanubia. This species was also found in a forest from Croatia (FARKAS & VILISICS, 2008). In Sebiș, *T. steinboecki* was collected together with *H. riparius, H. danicus, H. mengii*, known for their

affinity with humidity (e.g. RADU, 1983; WIJNHOVEN, 2000; FERENȚI & COVACIU-MARCOV, 2016) and *P. hoffmannseggii*, a myrmecophilous species (RADU, 1985). *T. crassipes* was found in Sebiș in the same microhabitat as *H. riparius* and *A. versicolor*.

Another additional species in Sebiș compared to Pâncota (LAZA et al., 2017) is *T. rathkii*, which is a euritopic species, being widespread in Romania (e.g. TOMESCU et al., 2015), and very common in other urban areas (e.g. VILISICS & HORNUNG, 2009; ŠATKAUSKIENĖ et al., 2015; HORNUNG et al., 2015). *P. scaber* and *P. spinicornis* are also well represented species in localities from Romania (e.g. BODIN et al., 2013; FERENȚI et al., 2015). *P. scaber*, while being frequently mentioned in towns (e.g. JĘDRYCZKOWSKI, 1981; VILISICS & HORNUNG, 2009; VILISICS et al., 2012; ŠATKAUSKIENĖ et al., 2015), is missing from Pâncota (LAZA et al., 2017). Its identification in many samples from Sebiș confirms the fact that its absence from Pâncota was incidental. More precisely, the species was introduced by chance in town by urbanization, and sometimes, by the same chance, it was not introduced (LAZA et al., 2017). An additional confirmation of this fact is that in Sebiș this species was not found in the periurban area, being clearly linked to urbanization, confirming the affirmation that urban pressure increase leads to the increase of cosmopolitan species` abundance (JĘDRYCZKOWSKI, 1981).

The differences between the isopod assemblages from different town parts were determined by the surface of the habitats from them, by their link to periurban or natural zones and by the disturbance level. The lowest species number was registered in parks, habitats which also in other towns sheltered few species (e.g. FERENȚI et al., 2015). Unlike these, big cities have a high number of species in parks (e.g. JĘDRYCZKOWSKI, 1981; VILISICS & HORNUNG, 2009; GIURGINCA et al., 2017). In the case of Sebiș, these two small parks have no humid zones and are completely isolated in the downtown, similar to other small towns (FERENȚI et al., 2015). Thus the few species present here are common and synanthropic. On the contrary, in big cities the parks are large, with diverse humid habitats, offering appropriate conditions for a high number of species (e.g. JĘDRYCZKOWSKI, 1981; VILISICS & HORNUNG, 2009; GIURGINCA et al., 2017). The small parks from Sebiș are not remnants of the initial natural habitats, but they are recent arrangements situated in an already disturbed area. In Sebiș town even industrial zones shelter more species than parks.

In the Sebiș town, the identified isopods are mostly native, characteristic for the region and expected in the region. Unlike other cities (e.g. VILISICS & HORNUNG, 2009; VILISICS et al., 2012; FERENȚI et al., 2015), non-native, invasive or recently introduced species do not exist in Sebiș. This fact is probably a consequence of the reduced anthropogenic impact upon the region, many species being introduced passively by human (COCHARD et al., 2010), and their spreading is facilitated by anthropogenic activities, like highway networks (VONA-TÚRI et al., 2017). However, in Sebiș synanthropic species are also present, which are typical for towns, and were also reported in towns from northwestern Romania (FERENȚI et al., 2015; HERLE et al., 2016; LAZA et al., 2017). Terrestrial isopod species in Sebiș are distributed according to their previously known ecological demands (RADU, 1983, 1985; TOMESCU et al., 2011, 2015, 2016). Species linked to natural zones are present in the periurban zone and synanthropic ones are more numerous where the anthropogenic disturbance level is higher. Unlike Pâncota, species linked to natural forested areas are better represented; both *P. politus* and *T. arcuatus* are well represented in many sampling points, especially in the periurban area. However, both species were also present in the industrial zone, which is situated at the town's outskirt, and after some decades of functioning it is mostly abandoned now. In the past, they were also reported outside forests in wet areas (e.g. FERENȚI & DIMANCEA, 2012; LAZA et al., 2017), but in Sebiș they can survive in the former industrial zones situated near forests. However, some species linked to natural humid zones are present in the downtown, even in the newly constructed neighborhoods. For example, *H. danicus*, which was frequently observed in natural, humid zones (e.g GIURGINCA, 2006; CICORT-LUCACIU & SUCEA, 2015; FERENȚI et al., 2015), is advantaged by these new buildings because of the wood storages, under which they were found. This species is considered to be adapted to decomposed logs (RADU, 1983). Probably, in the past *H. danicus* was present along the Moneasa River, which flows near this town part, nowadays it being regularized and dammed.

In Sebiș, *T. nodulosus* has the highest percentage abundance and frequency of occurrence, as well as in other towns surrounding Sebiș: Pâncota (LAZA et al., 2017) and Salonta (FERENȚI et al., 2015). All three towns are situated in or at the limit of plain areas, and *T. nodulosus* is considered characteristic for plain and xeric zones with herbaceous vegetation (e.g. FARKAS, 2010; TOMESCU et al., 2015). In the intermountain depression areas from western Romania, *T. nodulosus* is not the most common species, even if it is present in the town (HERLE et al., 2016). Also, *T. nodulosus* lacks from other towns, like some from Switzerland, Zurich, Lucerne, Lugano (VILISICS et al., 2012), which are probably situated outside the species` distribution range (SCHMALFUSS, 2003).

The terrestrial isopods from Sebiș confirm our supposition based on the isopod assemblages from Pâncota town, from the same region (LAZA et al., 2017). Because of the more surrounding forests, the town shelters larger populations of native species, linked to forested or humid areas. In the same time, the lack of some synanthropic species from some towns is probably accidental. The more natural periurban zones near Sebiș permit the survival of a large number of species. Sebiș has a rich terrestrial isopod fauna because of the surrounding natural areas and the reduced anthropogenic pressure.

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ROAD MORTALITY ON TWO SECONDARY ROADS NEAR ABRĂMUŢ LOCALITY, WESTERN ROMANIA: EFFECTS OF YEAR PERIOD AND ROAD SURROUNDING HABITATS

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Abstract. On two local roads situated near Abrămuţ locality in western Romania we studied in the year 2017 the effect of the seasons and the roads` surrounding habitats upon the fauna road mortality. We analyzed both road-killed invertebrates and vertebrates in three seasons (spring, summer and autumn) and on two road segments of the same length, bordered with different habitat types, and situated at only few kilometers from each other. One of the road segments was surrounded with a forest and the other with agricultural terrains. Totally, on the two roads 571 animals were killed by cars, from which 555 were invertebrates and only 16 vertebrates. The number of taxons and the victims` diversity were higher on the road near the forest in each season. In spring and summer the individuals` number was higher near the forest, but in autumn the individuals` number was higher on the road surrounded by agricultural terrains because of the high number of road-killed Heteroptera Pyrrhocoridae. On the road bordered with the forest animals related to humid forested areas were killed, including protected amphibian species. On the road surrounded by agricultural terrains common and generalist animals were killed, but also animals, like bees, attracted by the blooming culture plants. These results clearly indicate the negative effect of the road traffic upon the native fauna, especially on the road bordered with forests, in contrast to the road that crosses agricultural terrains, a fact that should be taken into account when upgrading or constructing new roads.

Keywords: habitat type, season, road vicinity, road-killed animals.

Rezumat. Mortalitatea rutieră pe două drumuri secundare lângă localitatea Abrămuț, vestul României: efectele perioadei din an și al habitatelor înconjurătoare. Pe două drumuri locale de lângă localitatea Abrămuț din vestul României s-a studiat, în anul 2017, efectul perioadei din an şi al habitatelor limitrofe drumurilor asupra mortalităţii rutiere a faunei. Astfel, s-au analizat atât nevertebratele cât şi vertebratele ucise de maşini în trei perioade din an (primăvara, vara şi toamna), pe câte un segement de aceeaşi lungime din două drumuri delimitate de habitate diferite, situate la doar câţiva kilometri unul de altul. Unul dintre drumuri se învecinează pe o parte cu o pădure, iar celălalt cu terenuri agricole. În total pe cele două drumuri maşinile au ucis un număr de 571 de animale, dintre care 555 nevertebrate şi doar 16 vertebrate. Numărul de taxoni şi diversitatea victimelor a fost mai mare pe drumul de lângă pădure în fiecare perioadă de studiu. Primăvara şi vara numărul de indivizi a fost mai mare lângă pădure, dar toamna numărul de indivizi uciși a fost mai mare pe drumul dintre terenuri agricole, datorită numărului foarte mare de Heteroptere Pyrrhocoridae ucise. Pe drumul delimitat de pădure au fost ucise animale legate de zone umede împădurite, inlusiv indivizi din specii protejate de amfibieni. Pe drumul delimitat de terenuri agricole au fost ucise animale comune, generaliste, dar şi grupe atrase de plantele de cultură în floare, precum albinele. Aceste rezultate indică clar că circulaţia rutieră are efecte mult mai negative asupra faunei native pe drumuri care se învecinează cu păduri decât în cazul celor care traversează terenuri agricole, fapt de care ar trebui ţinut cont la modernizare sau realizarea de drumuri.

Cuvinte cheie: tip de habitat, sezon, vecinătatea drumului, animale ucise de maşini.

INTRODUCTION

Road traffic has numerous negative effects upon biodiversity (see in: COFFIN, 2007; BENNETT, 2017). Nevertheless, many road mortality differences are not determined by the road traffic, but by some road's surrounding areas` peculiarities and the behavior of the affected species (e.g. CANAL et al., 2018). The habitat type surrounding the roads and the year period seem to be the most important parameters, which modify the road mortality intensity (e.g. BRAZ & FRANÇA, 2016; DUTTA et al., 2016; COVACIU-MARCOV et al., 2017; CANAL at al., 2018; JEGANATHAN et al., 2018). The intensity of road mortality seems lower on local roads than on more important roads, fact proven in the case of vertebrates in a survey realized from a moving car (CANAL et al., 2018). Unlike this, invertebrates` road mortality was recorded to be very high on minor roads with low traffic (CICORT-LUCACIU et al., 2016; CIOLAN et al., 2017). Moreover, recent studies clearly indicated that it is better to study the road mortality on the entire fauna, not only on certain groups (e.g. CIOLAN et al., 2017; JEGANATHAN et al., 2018). Western Romania is a region where recently some studies regarding the road mortality on minor roads were conducted (CICORT-LUCACIU et al., 2012; CIOLAN et al., 2017; COVACIU-MARCOV et al., 2017). One of those studies analyzed the seasonal road mortality changes on the entire fauna (CIOLAN et al., 2017). Despite investigating secondary roads, a high number of road killed animals was reported (CICORT-LUCACIU et al., 2012; CIOLAN et al., 2017; COVACIU-MARCOV et al., 2017). Nevertheless, the study which analyzed the road mortality of the entire fauna was made in a homogenous wooded area (CIOLAN et al., 2017), and the study which highlighted road mortality differences determined by the road surrounding habitats was made only on amphibians and reptiles (COVACIU-MARCOV et al., 2017). Consequently, we have proposed to establish to what extent the roads surrounding habitats influence the road mortality and if this influences are equally obvious in the case of invertebrates like in the case of amphibians and

reptiles (COVACIU-MARCOV et al., 2017). Therefore we have analyzed the impact of road traffic upon the entire fauna on two roads situated near Abrămuț locality, western Romania. The first road borders to one side a forest, and the second, although situated at only few km distances from the first one, crosses an agricultural area. We supposed that on the road near the forest among the road-killed animals will prevail species related to forested areas, like in other cases (CIOLAN et al., 2017), and on the other road we will encounter species characteristic to agricultural areas. Also, we presumed that the road mortality intensity will be higher on the road near the forest, compared to the road surrounded by agricultural terrains. Thus, we proposed the following three objectives: 1) to establish the road-killed animals on the roads near Abrămuț; 2) to observe the road mortality seasonal differences; 3) to determine the effect of the habitats bordering the roads from Abrămuț upon road mortality.

MATERIALS AND METHODS

The two studied roads are situated close to Abrămuț locality, western Romania, in Barcău-Crasna Plain (MÂNDRUȚ, 2006), at approximately 150 de m altitude. One of the roads is situated north of Abrămuț, leading to Marghita town. The other one is situated south of Abrămuț, leading to Sânlazăr locality. The first road is bordered at one side with an oak forest, with old trees, but which was thinned in the past. At the other side the road borders an abandoned field and a railroad surrounded by bushes, parallel with the road. The second road, situated at few kilometers from the first one, crosses an agricultural area, being bordered with bushes. Both roads are asphalted, in good condition, with two lanes. On each road we investigated an approximately 1 km length segment. The study was made in the year 2017, involving three field trips. The first one took place in spring, in May, the second one in summer, in July, and the third one in autumn, in October. The roads were walked by foot, like in previous studies in western Romania (CICORT-LUCACIU et al., 2012; CIOLAN et al., 2017; COVACIU-MARCOV et al., 2017). The field trips were made in the fist part of the day. The corpses in good condition were determined to species level, but the degraded ones were appointed to higher taxonomic units, like in other cases (e.g. CIOLAN et al., 2017; JEGANATHAN et al., 2018).

For each field trip and road we calculated an average of the cars that crossed the road during an hour. We calculated the percentage abundance and the diversity of the road killed taxons by surroundings and study period. The diversity was estimated using the Shannon Wiever index (H), and the similarity using the Jaccard index and the Bray Curtis index. The significance of the differences between periods and habitats were calculated with the help of the Mann Whitney test. The correlation between the number of cars / hour and the number of road killed individuals, between the number of cars / hour and the diversity and between the number of cars / hour and the number of road-killed taxons were established using the linear regression model. The statistics were made with the help of the free software PAST (HAMMER et al., 2001).

RESULTS

During the three field trips we identified on the two roads from Abrămuț 571 road-killed animals. Among them only 16 were vertebrates and the other 555 were invertebrates. The highest number of corpses was registered in spring (226). On the second position was autumn (179 corpses) and then summer (166 corpses). More animals were killed by cars on the road surrounded by agricultural terrains (291 compared with 280 near the forest). More corpses were registered in autumn on the road near agricultural areas (139). Animals belonging to 38 taxons were killed by cars on the roads from Abrămuț (Table 1). Among vertebrates, amphibians, reptiles, birds and mammals felt victims to the road traffic. In the case of amphibians we could determine two species (*Triturus cristatus* (Laurenti, 1768) and *Rana dalmatina* Fitzinger, 1839), and in the case of reptiles only one species (*Natrix natrix* (Linnaeus, 1758)). In the case of birds the one killed in autumn could be determined, belonging to the species *Turdus merula* Linnaeus, 1758. The number of the road-killed amphibians near the forest was higher in autumn. The variation of corpses` number was higher near agricultural areas. The number of the road-killed individuals and taxons differed greatly between the two roads, being influenced by the neighboring habitats. On the road near the forest the number of road-killed taxons was higher (35 compare with 24), even if the number of corpses was lower in autumn (Table 1).

The highest percentage abundance was registered by Heteroptera, Coleoptera and Diptera (Table 1). Coleoptera (generally considered), Lepidoptera larvae and Hymenoptera Vespidae were the only taxa killed on each road in each period (Table 1). The total diversity of road-killed animals at Abrămuț was H=2.71. The diversity and percentage abundance varied a lot between periods. The highest diversity was registered in spring (H=2.75) and the lowest in autumn (H=1.42). The victims` diversity was much higher on the road near the forest (H=2.88) compared to the road surrounded by agricultural terrains (H=1.95). Diptera Brachicera had a high percentage abundance, both in spring (18.58) and in summer (28.91), but no flies were killed in autumn (Table 2). Heteroptera Pyrrhocoris were killed only on the road from the agricultural areas, representing almost half of the victims (49.82%). On the road near the forest none of the taxons registered such high percentage abundance, the taxon with the highest percentage abundance, Diptera Brahicera, registering a value of only 21.78%. The highest diversity was on the road near the forest (H=2.88).

The number of cars that crossed the roads during an hour varied both between roads and periods (Table 1), but this number did not affect significantly the abundance (r=-0.02, p=0.95), the taxons` richness (r=+0.45, p=0.36) or diversity (r=0.31, p=0.53). The variation of the road killed taxons by habitat types was significant (p=0.01). The overlap

between taxons identified on the two roads was 0.55 by the Jaccard index, and 0.38 by the Bray-Curtis index. Significant differences were registered between the road mortality in spring and autumn (p=0.005). The distinct taxonomic composition in autumn is reflected by the similarities between seasons: between spring and summer the Jaccard index showed a high similarity (0.57), but between spring and autumn (0.32), respectively summer and autumn (0.44) the similarity was lower. The same pattern can be observed when abundance is taken into account (Bray-Curtis index), the resemblance between the first two seasons being more obvious (0.51), than between summer or spring and autumn (0.28 an 0.23). The similarity between all samples shows the same thing (Fig. 1).

Table 1. The percentage abundance (P%), number of individuals, number of taxons, diversity and evenness of the taxons killed on the roads from Abrămuț (Agr. – agricultural field).

Season	**Spring**		**Summer**		**Autumn**		**P% total**
The road's surrounding habitat	**Forest**	**Agr.**	**Forest**	**Agr.**	**Forest**	**Agr.**	
Cars/h	42	22	69	13	61	31	
Oligochaeta Annelida	5.26	-	4.67	1.69	5.00	-	2.62
Mollusca Gasteropoda others	13.53	7.52	2.80	-	10.00	-	5.60
Mollusca Gasteropoda Limax	3.00	1.07	-	-	-	-	0.87
Arahnida Araneidea	3.00	4.30	1.86	-	-	0.71	1.92
Myriapoda Diplopoda	2.25	-	-	-	-	-	0.52
Myriapoda Chilopoda	0.75	-	0.93	-	-	-	0.35
Dermaptera	-	-	0.93	-	-	-	0.17
Orthoptera	-	-	20.56	10.16	10.00	3.59	6.47
Mantodea	-	-	-	-	12.50	-	0.87
Odonata	-	-	-	1.69	-	-	0.17
Blattoidea	1.50	-	-	-	-	-	0.35
Heteroptera others	-	-	1.86	1.69	10.00	-	1.22
Heteroptera Pyrrhochoris	-	19.35	-	10.16	-	87.05	25.39
Coleoptera Tenebrionidae	-	-	0.93	-	-	-	0.17
Coleoptera Cantharidae	6.76	-	-	-	-	-	1.57
Coleoptera Scarabeidae	12.78	4.30	5.60	-	-	-	4.72
Coleoptera Lucanus	-	1.07	0.93	-	-	-	0.35
Coleoptera Carabidae	11.27	11.82	4.67	-	5.00	1.43	6.12
Coleoptera Elateridae	0.75	-	-	-	-	-	0.17
Coleoptera Curculionidea	3.00	2.15	-	-	-	-	1.05
Coleoptera Cerambicida	1.50	-	0.93	-	-	-	0.52
Coleoptera Chrysomelidae	5.26	3.22	-	-	-	-	1.75
Coleoptera Coccinelida	0.75	-	0.93	3.38	2.50	-	0.87
Coleopotera Staphylinidae	-	-	-	-	5.00	0.71	0.52
Lepidoptera adults	0.75	-	1.86	1.69	-	-	0.70
Lepidoptera larvae	3.75	8.60	6.54	1.69	12.50	2.87	5.25
Diptera Brachicera	18.04	19.35	34.57	18.64	-	-	15.76
Diptera Nematocera Typulidae	0.75	5.37	-	-	-	-	1.05
Hymenoptera Formicidae	0.75	-	0.93	-	-	-	0.35
Hymenoptera Vespidae	0.75	4.30	0.93	10.16	12.50	2.87	3.67
Hymenoptera Apis	0.75	6.45	2.80	35.59	2.50	-	5.60
Hymenoptera others	0.75	-	-	1.69	-	-	0.35
Amphibia Anura	-	-	-	1.69	-	-	0.17
Amphibia *Rana dalmatina*	0.75	-	1.86	-	7.50	-	1.05
Amphibia *Triturus cristatus*	-	-	-	-	2.50	-	0.17
Reptilia *Natrix natrix*	0.75	-	-	-	-	-	0.17
Aves	-	1.07	1.86	-	2.50	0.71	0.87
Mammalia Rodentia	0.75	-	0.93	-	-	-	0.35
No. of individuals	133	93	107	59	40	139	571
P%	23.29	16.28	18.73	10.33	7.00	24.34	100
No. of taxons	26	15	22	13	14	8	38
H (Shannon-Wiever index)	2.67	2.37	2.28	1.97	2.48	0.61	2.71
H` (Pielou index)	0.81	0.87	0.71	0.77	0.91	0.27	0.72

Table 2. The variation by season and habitat of the percentage abundance, number of taxons and individuals, diversity and evenness of taxons killed on the roads from Abrămuț.

	Habitats		**Seasons**		
	Forest	**Agr.**	**Spring**	**Summer**	**Autumn**
Oligochaeta Annelida	5.00	0.34	3.09	3.61	1.11
Mollusca Gasteropoda others	8.92	2.40	11.06	1.80	2.23
Mollusca Gasteropoda Limax	1.42	0.34	2.21	-	-
Arahnida Araneidea	2.14	1.71	3.53	1.20	0.55
Myriapoda Diplopoda	1.07	-	1.32	-	-
Myriapoda Chilopoda	0.71	-	0.44	0.60	-
Dermaptera	0.35	-	-	0.60	-
Orthoptera	9.28	3.78	-	16.86	5.02
Mantodea	1.78	-	-	-	2.79
Odonata	-	0.34	-	0.60	-
Blattoidea	0.71	-	0.88	-	-
Heteroptera others	2.14	0.34	-	1.80	2.23
Heteroptera Pyrrhochoris	-	49.82	7.96	3.61	67.59
Coleoptera Tenebrionidae	0.35	-	-	0.60	-
Coleoptera Cantharidae	3.21	-	3.98	-	-
Coleoptera Scarabeidae	8.21	1.37	9.29	3.61	-
Coleoptera Lucanus	0.35	0.34	0.44	0.60	-
Coleoptera Carabidae	7.85	4.46	11.50	3.01	2.23
Coleoptera Elateridae	0.35	-	0.44	-	-
Coleoptera Curculionidea	1.42	0.68	2.65	-	-
Coleoptera Cerambicida	1.07	-	0.88	0.60	-
Coleoptera Chrysomelidae	2.50	1.03	4.42	-	-
Coleoptera Coccinelida	1.07	0.68	0.44	1.80	0.55
Coleopotera Staphilinidae	0.71	0.34	-	-	1.67
Lepidoptera adults	1.07	0.34	0.44	1.80	-
Lepidoptera larvae	6.07	4.46	5.75	4.81	5.02
Diptera Brachicera	21.78	9.96	18.58	28.91	-
Diptera Nematocera Typulidae	0.35	1.71	2.65	-	-
Hymenoptera Formicida	0.71	-	0.44	0.60	-
Hymenoptera Vespidae	2.50	4.81	2.21	4.21	5.02
Hymenoptera Apis	1.78	9.27	3.09	14.45	0.55
Hymenoptera others	0.35	0.34	0.44	0.60	-
Amphibia Anura	-	0.34	-	0.60	-
Amphibia *Rana dalmatina*	2.14	-	0.44	1.20	1.67
Amphibia *Triturus cristatus*	0.35	-	-	-	0.55
Reptilia *Natrix natrix*	0.35	-	0.44	-	-
Aves	1.07	0.68	0.44	1.20	1.11
Mammalia Rodentia	0.71	-	0.44	0.60	-
No. of individuals	280	291	226	166	179
P%	49.03	50.96	39.57	29.07	31.34
No. of taxons	35	24	29	26	16
H (Shannon-Wiever index)	2.88	1.95	2.75	2.44	1.42
H` (Pielou index)	0.81	0.61	0.81	0.74	0.51

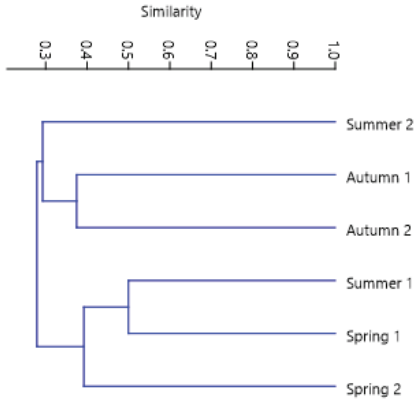

Figure 1. The similarity between the samples from different seasons and habitat types (1 – forest, 2 – agricultural area).

DISCUSSIONS

The hypothesis of our study was largely verified, the road mortality being influenced both by period and by the aspect of the habitats near the roads (e.g. D'AMICO et al., 2015; BRAZ & FRANÇA, 2016; DUTTA et al., 2016; COVACIU-MARCOV et al., 2017; CANAL et al., 2018; JEGANATHAN et al., 2018). Thus, on the road near the forest the number of road-killed taxa and diversity was much higher than on the road surrounded by agricultural areas, although the number of road-killed individuals was slightly higher on the last one. These differences were clear in each period, even very obvious in autumn in the case of diversity. Thus, although each season` conditions are modifying the road mortality intensity, the surrounding habitats` aspect is extremely important, the differences in roads mortality determined by them being clear in each season. The period influences the dynamics of the road mortality (e.g. GARRIGA et al., 2017; CIOLAN et al., 2017), but its composition is determined by the roads surrounding habitats. Even in areas where the road is surrounded with uniform habitats, road mortality differences determined by some habitat disparities were mentioned (CIOLAN et al., 2017).

The data from Abrămuţ confirms the negative impact of road modernization upon the fauna (e.g. JONES et al., 2014). On the road with the most road-killed individuals, the one from agricultural areas, the traveling speeds 10 years ago were much reduced because the road was not asphalted. After its modernization, the road became more circulated and the speed increased, nowadays reaching 80-90 de km/h, causing a high number of road killed animals. Nevertheless, because the road is surrounded by uniform habitats, which are highly affected by humans, like agricultural terrains, the victims` diversity is reduced. Like in other cases, at Abrămuţ the invertebrates' road mortality was much higher compared to the vertebrates (e.g. SEIBERT & CONOVER, 1991; CICORT-LUCACIU et al., 2016; CIOLAN et al., 2017).

The invertebrates registered on the road near the forest belonged to taxa linked with more humid and forested habitats, like on other road surrounded by forests (CIOLAN et al., 2017). On the road near the forest more Oligocheta and Gastropoda individuals were killed compared with the road from agricultural terrains. Nevertheless, in the case of some flying insects the number of road-killed individuals was lower on road surrounded by forests than on roads surrounded by open areas (KEILSOHN et al., 2018). At Abrămuţ however the road without forest borders with agricultural terrains not natural areas, thus the diversity is reduced. On the road surrounded by agricultural terrains were killed only two taxons that were missing on the road near the forest (Heteroptera Pyrrhocoris and Odonata). On the road near the forest only one Dermaptera individual was killed, probably at night, they generally being nocturnal animals which avoid daylight, in the daytime staying under different shelters (RADU & RADU, 1967). Ortoptera were killed by cars on both roads, but only in summer and autumn, like in other cases (CIOLAN et al., 2017). Nevertheless, they were killed in much higher number in the forested area than in the agricultural one. We found only one road-killed dragonfly in summer on the road in the agricultural area. It was identified at approximately 1 km from Barcău River and some ponds near its course; after the emergence adult dragonflies usually fly far from the water, not turning back before the reproduction period (e.g. CORBET, 1980). The existence of the river could explain its presence on this road. Contrary, on the road near the forest, where permanent waters are missing, no dragonfly was killed. On roads bordering with wet areas dragonflies were killed by cars in large numbers (RIFFELL, 1999). The five Mantodea individuals were killed in autumn, on the road near the forest, in a clear and relatively warm day, which corresponds to their demands (RADU & RADU, 1967). Heteroptera were killed in a high percentage especially in the agricultural area. Heteroptera Pyrrhocoris are adapted to feed on extremely dried out seeds (SOCHA, 1993), thus the harvest of sunflower seeds could explain their high number on the road from the agricultural area in autumn.

Among butterflies, the larvae were killed in higher number compared to adults. Like in other cases, the high number of larvae could be a consequence of the rich vegetation near the roads (CIOLAN et al., 2017), vegetation which they can feed on. Like in other cases (CICORT-LUCACIU et al., 2016; CIOLAN et al., 2017), adult butterflies were only accidentally killed, they being more frequent in grasslands areas (e.g. SKORKA et al., 2015; KEILSOHN et al., 2018). The number of bees was much higher in summer in the agricultural areas. The bees were probably attracted by sunflower crops near the road, becoming victims of the cars. Also in other cases the existence blooming plants, like black locust, near the roads greatly increased the bees` road mortality (CICORT-LUCACIU et al., 2016). The wasps were probably attracted by the corpses on the road, on which they were observed feeding (CICORT-LUCACIU et al., 2016). Wasps were killed in both habitats in each period unlike bees which lacked in autumn in the agricultural area. The road's negative impact on pollinating insects not only causes the alteration of biodiversity but also has negative economic consequences (BAXTER-GILBERT et al., 2015).

The vertebrates killed by cars on the roads from Abrămuţ were fewer than invertebrates, many individuals belonging to species related to forest areas. The most road-killed vertebrates belong to amphibians, like in other cases (e.g. ASHLEY & ROBINSON, 1996; D'AMICO et al., 2015; GARRIGA et al., 2017; CIOLAN et al., 2017; JEGANATHAN et al., 2018). At Abrămuţ *R. dalmatina* prevailed, a forest species well represented in Romania (FUHN, 1960). This species was killed in each period but also on the road near the forest. The first field trip was made at the beginning of May, after the reproduction period of this species (FUHN, 1960). Probably this is the explanation for the low individuals` number registered in spring. *R. dalmatina* is often killed on roads (e.g. CICORT-LUCACIU et al., 2016; COVACIU-MARCOV et al., 2017), roads with high traffic strongly affecting it in the reproduction season (HARTEL et al., 2009). The individuals killed in autumn could result from the more intense activity period before hibernation, the species using open habitats during spring and autumn migrations

(e.g. HARTEL et al., 2009). The negative impact of roads upon amphibians is very strong and well known for many years (e.g. FAHRIG et al., 1995; HELS & BUCHWALD, 2001). In Romania both *R. dalmatina* and *T. cristatus* are protected (O.U.G. 57/2007). Among reptiles, only one *N. natrix* individual was killed. In Europe this species is frequently killed on roads (e.g. CIESIOŁKIEWICZ et al., 2006; MEEK, 2009; KAMBOUROVA-IVANOVA et al., 2012; COVACIU-MARCOV et al., 2017). Because the road killed *N. natrix* was identified in a warm spring day, it may come to the road for thermoregulation, like in other cases (MCCARDLE & FONTENOT, 2016). Although they are faster than amphibians, snakes are disadvantaged on roads by their greater length (CICORT-LUCACIU et al., 2012), the number of road-killed individuals increasing in some regions because of the traffic increase (TÓTH et al., 2017). Asphalted roads have a negative impact also upon birds, both directly because of the road mortality (e.g. ASHLEY & ROBINSON, 1996; D'AMICO et al., 2015; CANAL et al., 2018) and indirectly because of the noise and habitats` alteration (e.g. BROTONS & HERRANDO, 2001; MCCLURE et al., 2013). At Abrămuț, birds were killed by cars on the entire study period, lacking in spring in the forested region and in autumn from the agricultural region. Although forests seems to reduce the birds road mortality intensity (CIOLAN et al., 2017), which is higher on roads surrounded by pastures (BRAZ & FRANCA, 2016), at Abrămuț birds were killed on both roads. At Abrămuț, the road from the forested area is bordered only on one side by forest, on the other side being neighbored with open areas. This could advantage birds, the more open area favoring their flight, on contrast to roads which cross dense forested areas, where not a single bird was killed (CIOLAN et al., 2017). Forest seems to reduce the road mortality of other flying vertebrates, like bats (SECCO et al., 2017). Mammals were represented by a very low number of rodents, which were killed in spring and summer in the forested area.

The number of road-killed animals on the two roads from Abrămuț is very high taking into account the short distance that we investigated and the fact that we made only three surveys. Although the surveys were made by walking on the roads, the most efficient methods in the case of small animals (e.g. SLATER, 2002; LANGREN et al., 2007), the number of road-killed animals was probably much higher, as in other cases (CIOLAN et al., 2017). Our results confirm the road mortality seasonal differences (e.g. ASHLEY & ROBINSON, 1996; GARRIGA et al., 2017; JEGANATHAN et al., 2018) and the influence of road`s surrounding habitats upon them. Because of the fact that on the road near the forest the diversity and number of road-killed taxons were much higher, it is clear that roads should not be constructed near forest, but only near degraded habitats like agricultural areas, where even if the number of road killed individuals can be high, the diversity and number of taxons are low.

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PRELIMINARY DATA REGARDING INTERSPECIFIC RELATIONSHIPS BEETLE SPECIES COLLECTED FROM DIFFERENT ECOSYSTEMS MET IN DOLJ COUNTY IN 2017. NOTE 2

LILA Gima

Abstract. The research studies on the diversity of interspecific relationships from Dolj County exposed in this paper were achieved in 2017. The beetle biological material (6 specimens, one with mites) was collected from terrestrial ecosystem – Bucovăț. The host, from the systematic viewpoint, belong to the order Coleoptera, respectively to family Scarabaeidae. The interspecific relationship identified is transport or spread, respectively forezy. The species on which mites were found are *Onthophagus taurus* (Schreber, 1759). From the systematic viewpoint, the identified mites are belong arachnids - *Macrocheles* sp. (Mesostigmata: Macrochelidae). The species by mites identified at *O. taurus* is new for Dolj, Romania.

Keywords: interspecific relationships, beetles, mites, ecosystem, foresy.

Rezumat. Date preliminare privind relații interspecifice la coleoptere din diferite ecosisteme din județul Dolj colectate în 2017. Nota 2. Cercetările privind diversitatea relațiilor interspecifice la coleoptere din județul Dolj expuse în lucrarea de față au fost realizate în anul 2017. Materialul biologic de coleoptere (6 exemplare, din care 1 exemplar prezintă acarieni) au fost colectate din ecosistemul terestru, satul Bucovăț. Gazda, din punct de vedere sistematic, aparţine ordinului Coleoptera încadrându-se în familia Scarabaeidae. Specia pe care s-au găsit acarieni este *Onthophagus taurus* (Schreber, 1759). Relația interspecifică identificată este de transport sau răspândire, respectiv forezie. Acarienii identificați în urma cercetărilor de specialitate, din punct de vedere sistematic, aparțin genului *Macrocheles* (Mesostigmata: Macrochelidae).

Cuvinte cheie: relații interspecifice, coleoptere, acarieni, ecosystem, forezie.

INTRODUCTION

The purpose of this paper is to present some contributions to the knowledge of the diversity of parasites, analyzing beetle species present in different types of ecosystems in Dolj County.

In recent years, insects have undergone the complex action of ecological factors (climatic, soil and biotic factors) affecting the biological cycles of insects, spread emergence of mass propagation or decrease the number of the specimens of certain species, the emergence of new pests, etc. As a result, the number of beetle specimens found in the studied ecosystems was low.

All the material found on land was identified and analysed; then, the level of infestation was assessed. The beetle biological material (6 specimens, one with mites) was collected from terrestrial ecosystem - Bistreț. The hosts, from the systematic viewpoint, belong to the order Coleoptera, respectively to Scarabaeidae family.

From the systematic viewpoint, the identified mites are *Macrocheles* sp. (Mesostigmata: Macrochelidae).

MATERIALS AND METHODS

The material used in this paper consisted in 6 specimens found in the field, which were identified, analysed and studied, three species having mites.

The species of beetles are presented in systematic order according to the year they were collected and there are mentioned the species of parasite identified for each of them.

The material was collected in 2017. Collections were made at different dates, each year in May. Collection date is mentioned for this species. Moreover, for every locality, there are rendered the geographical coordinates, flora and fauna information. Collection methods were different according to the analysed host species.

1. **Collection methods** for *Onthophagus taurus.*

The insect was sampled from the ground with a pair of tweezers and put in a jar containing filter paper soaked in alcohol 4%. There were taken photos and the material was transported to the museum, entomology laboratory, where the specialists took samples from the surface of the insect-body. To analyze the mites, after taking photos, they were placed in a solution of paraffin and sent to the expert for determination.

2. *Collection and research methods for mites.*

Using tweezers, mites were collected from *Onthophagus taurus*, more precisely from the feet and the ventral side of the abdomen. For identification, the mites were prepared in paraffin that was stored at room temperature until they were transported and examined carefully under the microscope.

To determine the collected material, there were used the works of PANIN (1955; 1957), the determination of the species of beetles in the entomology laboratory of the Department of Natural Sciences of the Museum of Oltenia Craiova. Some of the photos were taken with DMC-FZ62 Panasonic FullHD digital camera by Lila Gima and another category was taken by Mrs. Cristian Boicea, by means of the stereomicroscope OLYMPUS 3D.

The taxonomy and nomenclature of the identified species is made according to Fauna Europea.

RESULTS AND DISCUSSION

The analysed material was represented by 44 specimens of which 7 specimens had parasites. The material was collected in 2017 from Bistreț village. There are rendered the collection sites, the species of collected beetles and the identified mites on legs.
Host: *Onthophagus taurus* (Schreber, 1759)
Parasite: *Macrocheles* sp.
Collection site: Bistreț
Date of collection: May 17, 2017

***Onthophagus taurus* (Schreber, 1759)**
Scarabaeoidea: Scarabaeidae: Scarabaeinae: Onthophagini: *Onthophagus*

It is a coprophagous species common in all climatic conditions except for the alpine steppes (PANIN, 1955; 1957). It is frequent in cow and horse dung and human excreta, under which it digs galleries.

O. taurus can reach a length of 5.5-11 millimeters. These small beetles are an almost oval shape and their color is black or reddish. Sometimes, the pronotum has a faint metallic sheen. Males have a pair of long protrusions or horns (hence the name of the species) that they use to fight each other and gain access to the female.

Flight activity is during the day and seasonal activity is from spring to autumn.

The development from egg to adult takes 8-10 weeks, depending on the soil temperature. There are at least two generations per year (Fig. 1).

Figure 1. Deuteronymph fixed with uropod
on the leg of *O. taurus* (original).

The abundance of beetles depends on many criteria, but the most important ones are the available fresh manure amount and the manure quality. Chemical residues from animals can be harmful, but not essential, for the population growth.

This species is present in Europe, Morocco, Algeria, Tunisia, Syria, Iraq, Transcaucasia, Asia Minor, Iran, Afghanistan, Central Asia and the USA (Texas).

***Macrocheles* sp.** (Fig. 1)

Arachnida: Micrura: Acari: Anactinotrichida: Mesostigmata: Dermanyssina: Eviphidoidea: Macrochelidae: *Macrocheles*

Macrochelidae are is a cosmopolitan family of predatory mesostigmatic mites, many of which occupy specialized and often unstable habitats. Phoresy on co-occurring flying insects and Choleoptera plays a vital role in assuring niche continuity for macrochelids (KRANTZ, 1999).

As it regards their presence at Choleoptera, the specialized literature provides brief information. In the country, there were reports for the presence of *Macrocheles punctillatus* (Willm.), *Macrocheles plumiventris* Hull and *Anoetus ferroniarum* (Duf.) at the species of the genus *Onthophagus* Latr.; *Macrocheles glaber* Müll and *Anoetus ferroniarum* (Duf.) at *Aphodius* Illig. (BALTHASAR, 1963).

The olfactory receptors on the tarsi allow mites to find their hosts, while the receptors on the top are involved in the localization and attachment to the host and in the perception of the substrate during motion (FARISH & AXTELL, 1966; WICHT *et al.*, 1971; COONS & AXTELL, 1973; HUNTER & ROSARIO, 1988 - *In*: TATYANA SACCHI & PIRES DO PRADO, 2004).

Mites (Animalia: Arthropoda: Chelicerata: Arachnida: Acari) continuously reproduce in an appropriate environment, but they disperse when certain environmental factors adversely affect their presence in the habitat (KRANTZ, 1999). The specialization of the dispersed behaviour reveals the variability that contributes to the distribution and diversity of mites (MITCHELL, 1970 - *In*: TATYANA SACCHI & PIRES DO PRADO, 2004).

The particularities of phoresy (transport of mites from one place to another with the help of another organism) include active host search, recognition of attachment signs and host specificity, tranquility, recognition of host abandonment signs and, if necessary, synchronization with the life cycle of the host. The recognition of the ideal host is fundamental for transportation to a new location and is often based on chemical or olfactory stimuli produced by the host. *Phoresy* may be an adaptation for survival or it may be a parasitic manifestation as it involves displacement through interactions within the ecosystems.

Environmental variations, with their effect on intra and interspecific relationships, lead to displacement and persistence in different habitats. The difficulty in defining phoresy reflects the diversity of behavioral and ecological parameters of the involved species.

Phoresy is important for the maintenance of the species that may act as predators or parasites during different development stages and it serves to define the survival strategy of the mutualistic predatory and parasitic species (TATYANA SACCHI & PIRES DO PRADO, 2004).

In the present study, mites are represented by one species belonging to one order (Mesostigmata) and the family Macrochelidae.

CONCLUSIONS

The work joins the efforts of specialists who contribute to the knowledge of entomofauna diversity.

Locality for collection, Bistreț village, represent new collection site for species *Onthophagus taurus*.

This study only signals the presence of the mite *Macrocheles* sp. at the choleoptera. The mite identified in the studied beetle are specie reported by foreign authors, but there are no mentions of them in the Romanian specialized literature. On the other hand, it is difficult to draw firm conclusions about the specificity of the host, because the studies performed on them so far are brief.

The present study, for the time being, signals the presence of the mite in this species of choleoptera. We will continue to collect beetles and make observations.

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THE CATALOGUE OF THE COLLECTION BOUGHT BY GRIGORE ANTIPA FROM VÁCLAV FRIČ (CZECH REPUBLIC)

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Abstract. Václav Frič (Czech Republic) was one of the numerous sellers of zoological items for museums of natural history from Europe at the end of the 19th century and beginning of the 20th century, rewarded with several European prizes. Some of the pieces had a didactic purpose (gem imitations, Protozoa, models or real ones, models of fossils). The catalogue of 642 real or model specimens contains species of invertebrates, vertebrates, fossils, gem imitations bought by Grigore Antipa from Václav Frič at the beginning of the 20th century for the "Grigore Antipa" National Museum of Natural History from Bucharest, Romania.

Keywords: Václav Frič, collection, catalogue, Grigore Antipa, natural history.

Rezumat. Catalogul colecției cumpărate de Grigore Antipa de la Václav Frič (Republica Cehă). Václav Frič (Republica Cehă) a fost unul din numeroșii comercianți de preparate zoologice pentru muzeele de istorie naturală din Europa de la sfârșitul secolului al XIX-lea și începutul secolului al XX-lea, recompensat cu numeroase premii europene. Unele dintre piese au un caracter didactic (imitații de pietre prețioase, protozoare, modele și reale, modele de fosile). Catalogul conține 642 de exemplare reale sau mulaje, conține specii de nevertebrate, vertebrate, fosile, imitații de pietre prețioase, cumpărate de Grigore Antipa de la Václav Frič la începutul secolului al XX-lea, pentru Muzeul Național de Istorie Naturală „Grigore Antipa" din București, România.

Cuvinte cheie: Václav Frič, colecție, catalog, Grigore Antipa, istorie naturală.

INTRODUCTION

During the past decade, new researches based on the study of old papers, old inventory registers, archives and old labels revealed the evidence of the existence of a much vast patrimony in the collections of "Grigore Antipa" Museum. Part of these collections were donated, like the invertebrate collection of Ion Cantacuzino (PETRESCU & PETRESCU, 2016) or the ornithological collection of Monsignor Gabriel Foucher in 1913 (PETRESCU et al., 2017) and some were acquired from famous natural history dealers, such as a hummingbird collection bought from Wilhelm Schlüter (PETRESCU A., 2008) or various specimens, mostly invertebrates, from Gustav Schneider (PETRESCU & PETRESCU, 2018). These are testimony of the tireless efforts that Grigore Antipa had made to collect the most interesting and precious specimens, therefore contributing to a continuous expansion of the Museum's cultural heritage. Even from the beginning of his activity in 1893 as the director of the Natural History Museum, his outmost priority became the enrichment of the collections inherited from his predecessor, Gregoriu Ștefănescu.

At the end of the 19th century and beginning of the 20th century, Václav Frič (Czech Republic) was one of the numerous sellers of zoological items for museums of natural history from Europe, and his activity was rewarded with several European prizes. For his prodigious career as a "Naturalienhandlung" ("natural history dealer") Frič's activity had been rewarded with several European prizes as it is minutely detailed by REILING & SPUNAROVA (2005). No catalogue of items from Frič could be identified in Bucharest and also, until this date no other mentions of these acquisitions were made in the published chronology of the museum (MARINESCU & IONESCU-KONNERTH, 1985).

MATERIAL AND METHOD

From the Archive of the "Grigore Antipa" Museum we have studied the correspondence of Grigore Antipa with the Czech natural history dealer, Václav Frič. Five letters (no. 168-172), from February 1907 to November 1908, also old labels from collection jars, Grigore Antipa's inventory, other old museum registers and documents from the National Archives from Bucharest (Ministry of Culture and Public Instruction Fund) have been taken into study.

For each specimen the label had been checked, as well as the old/ present inventory number and the magnification number, in the case of the plaster models. The catalogue presents the specimens in taxonomical order.

Abbreviations: coll. no.: collection number; sp.: specimen

RESULTS AND DISCUSSIONS

The acquisitions were made from "Naturalien Handlung V. Frič in Prague, Wladislawgasse 21 a", before the opening of the museum in 1908 and before the event from 1914 when the name of the museum was changed from "Museum of Zoology" to "Museum of Natural History". We presume that the pieces from Frič were bought between 1905 and 1913.

The collection acquired from Frič comprises a total of 908 recorded items, nowadays only 642 of them are preserved in the "Grigore Antipa" Museum. The catalogue of the Frič collection is represented in several categories of the museological collections: models for educational purposes- mineral casts, gem and crystals imitations, also

meteorites, actual and fossil Protozoa (Foraminifera, Amoebozoa, Radiolaria), a collection of gypsum copies or models of amphibians, birds and mammals fossils, a small collection of microscopic foraminifera shells, numerous alcohol-preserved specimens of various taxa, fixed on a glass plate- Poriphera (Calcarea, Hexactinellida and Demospongiae), Vermes (Acantocephala, Plathelmynthes, Nematoda, Nematophora, Annelida and Onychophora), Crustacea (Copepoda, Cladocera, Phyllopda, Ostracoda, Isopoda, Amphipoda, Decapoda), Arachnida (Araneea and Scorpiones), Millipedes (Chilopoda and Diplopoda), Chaetognatha and Pisces, naturalized mounted animals (birds), skeleton pieces or mounted skeletons from different taxonomic groups. In time, more than three hundred specimens were lost or deteriorated (three termite nests, a holothurian, etc.).

CRISTAL AND MINERAL IMITATIONS

On March 17th 1913, Grigore Antipa bought 240 pieces of crystal and mineral imitations from Václav Frič. Nowadays, most of the models are still present in Museum's collection and the Mineralogy section of the public exhibition, although 45 pieces had been lost in time. The acquisition of crystals, minerals and meteorites models was extracted from a receipt from National Archives, Ministry of Culture and Public Education fund, valued at 550 crowns (in Romanian currency at that time, 596 lei and 15 bani).

Gem imitations are present in the third catalogue from 1873 found in the Archive of the Botanical Museum of Harvard University (REILING & SPUNAROVA, 2005).

The objects include imitations of precious stones (70 pieces) (Fig. 2 A); imitations of processed stones (84 pieces, 20 pieces lost); imitation of Cullinan rough diamond and processed brilliants from Cullinan diamond (9 pieces)- since their acquisition, seven of them are still displayed in the public exhibition; copies of the most famous diamonds (28 pieces); various gemstones with different types of processing executed in crystal glass (according to gemological advice of Prof. Braun) (32 pieces); diamonds with different types of processing (10 pieces); pieces of native gold of Kongsberg crystallized silver ore, a copy of the largest platinum nugget ever found (in 1913); plaster models of iron meteorites found in Bochum, Elbogen and Braunau (Bohemia, Czech Republic).

MODELS OF PROTOZOA (FORAMINIFERA, AMOEBOZOA, RADIOLARIA)

Models of **protozoa** were acquired in 1907 (Fig.1 A), as it presented in a 42 pieces list from a letter received from Frič, on October 16th 1907. Most of these models were displayed in the first hall of Invertebrates from the „Grigore Antipa" Museum, first floor, few of them were present there, in a new presentation, until 2004 (IONESCU & SCHNAPP, 1961) (Fig. 1 B, D).

The most consistent collection is the **Foraminifera** with 23 plaster models and microscopic shells of present and fossil species, acquired in 1907. Seven of the plaster models are enlarged copies of present species, with original labels: *Cyclolina cretacea* d'Orb. (coll. no. 9480/27); *Diplophrys archeri* Bark. (coll. no. 8112); *Lagena vulgaris* Williams (150 x; old coll. no. 32, present coll. no. 9480/32); *Pamphagus mutabilis* Bailey (coll. no. 8118); *Amphistegina quoai* d'Orb. (coll. no. 9480/83); *Cassidulina crassa* d'Orb. (180 x; old coll. no. 81, present coll. no. 9480/71); *Dentritina arbuscula* d'Orb. (old coll. no. 24, present coll. no. 9480/22). On the old label it is mentioned a series, 736-2, which indicates that the pieces belong from the first catalogue of Frič (1862).

Out of the **fossil Foraminifera**, there are eleven plaster models with original labels: *Ehrenbergia serrata* Reuss. (Miocen; old coll. no. 82, present coll. no. 9480/72) (Fig. 2B); *Haplostiche foedissima* Reuss. (Upper Cretacic; coll. no. 9480/2); *Haplophragmium irregulare* Roem. (Cretacic; old coll. no. 4, present coll. no. 9480/4); *Heterostegina* sp. (Pacific Ocean; old coll. no. 99, present coll. no. 9480/84); *Nonionima bulloides* d'Orb. (Neogen; old coll. no.95, present coll. no. 9480/81); *Numulites lucossanum* (coll. no. 8123); *Nummulites* sp. (section; coll. no. 9480/113); *Orthocerina quadrilatera* d'Orb. (old coll. no.39, present coll.no. 9480/37); *Siderolithes calcitrapoides* Lam. (Cretacic, Maastricht; old coll. no. 88, present coll. no. 9480/113); *Vulvulina gramen* d'Orb. (Adriatic Sea, fossil; old coll. no. 78, present coll. no. 9480/68;); *Verneulina spinulosa* Reuss. (Miocene; old coll. no. 6, present coll. no. 9480/6).

A small collection of five **microscopic foraminifera shells** in magnifying boxes is still preserved in the Museum's collection: *Quinqueloculina* d'Orb. (1 sp., coll. no. 8131); *Peneroplis* Fiume (1 sp., coll. no. 8132); *Globigerina* d'Orb. (many species, Arctic Sea, Wallrous Expedition, coll. no. 8133); *Polystomella* Lam. (1 sp., Venice, coll. no. 8136) (Fig. 2 D).

From **Amoebozoa,** ten plaster, enlarged models with four original labels complete the collection: *Amoeba proteus* Leidy (coll. no. 8120); *Amoeba* sp. (development, 4 pieces, coll. no. 8139); *Arcella vulgaris* Ehr. (1000x; old coll. no. 3, coll. no. 8110); *Difflugia piriformis* Pty. (coll. no. 8114); *Euglypha alveolata*, Duj. (coll. no. 8117); *Hyalosphenia cuneata* Stein (coll. no. 8115); *Lecquereusia spiralis* (Ehrbg.) (700x; old coll. no. 5, coll. no. 8119); *Nebela collaris* (Ehr.) (coll. no. 8116); *Pseudodifflugia gracilis* Schlumb. (coll. no. 8113); *Trynema enchelys* Duj. (1200 x; old coll. no. 9, coll. no. 8111).

From Messina, Václav Frič sends six enlarged models of **Radiolaria**: *Actinomma inerme*, Haeck. (coll. no. 8121); *Amphilonche messanensis* Haeck. (coll. no. 8109); *Diploconus fasces* Haeck. (coll. no.8108); *Eliosphaera inermis* Haeck. (coll. no. 8107); *Stylodictya multispina* Haeck. (coll. no. 8106) (Fig. 1 C).

97 of the 100 existing pieces of Foraminifera are also present in the University Museum of Utrecht, The Netherlands (Reiling & Spunarova, 2005). Microscopic collection of foraminifera shells in magnifying boxes are also present in Redpath Museum of McGill University, Montreal, Canada (REILING & SPUNAROVA, 2005).

PORIFERA

This collection is mentioned in the list from October 16th 1907 (Archive of "Grigore Antipa" Museum, no. 170), with only five specimens absent. 28 species from three major classes are present, Calcarea, Hexactinellida and Demospongiae, being the most representative (with 14 families): *Sycon raphanus* Schmidt. (1 sp., Adriatic Sea, coll. no. 8143); *Hyalonema sieboldi* Gray (1 sp., Japan, coll. no. 8156); *Geodia placenta* Schmidt., (1 sp., Adriatic Sea, coll. no. 9481/19); *G. conchilega* Schmidt (1 sp., Adriatic Sea, coll. no. 9481/18); *Geodia* Lam. (1 sp., Japan; coll. no. 8166); *Erylus discophorus* (Schmidt) (1 sp., Adriatic Sea, coll. no. 8161); *Ancorina cerebrum* Schmidt (1 sp., Adriatic Sea, coll. no. 8164); *Tethya aurantium* (Pall.) (1 sp., Adriatic Sea, coll. no. 8160); *Clathria (Clathria) coralloides* (Scop.) (1 sp., Adriatic Sea, coll. no. 8175); *Mycale (Mycale) massa* (Schmidt) (1 sp., Adriatic Sea, coll. no. 9481/27); *M.* (*Aegogropila) syrinx* (Schmidt) (1 sp., Adriatic Sea, coll. no. 8177); *Suberites domuncula* (Olivi) (1 sp., Adriatic Sea, coll. no. 8172); *Petrosia (Petrosia) ficiformis* (Poiret) (1 sp., Adriatic Sea, coll. no. 8184); *Haliclona (Rhizoniera) grossa* (Schmidt) (1 sp., Adriatic Sea, coll. no. 9481/30); *Spongilla lacustris* (L.) (1 sp., Bohemia, Czech Republic, coll. no. 9481/36); *Ephydatia fluviatilis* (L.) (2 sp., Bohemia, coll. no. 9481/35, 9481/34); *Ephydatia muelleri* (Lieberkühn, 1856) (1 sp., Bohemia, old coll. no. 73, coll. no. 9481/33); *Ulosa stuposa* (Esper) (1 sp., Adriatic Sea, coll. no. 8190); *Dysidea avara* (Schmidt) (1 sp., Adriatic Sea, coll. no. 8192); *Spongia (Spongia) officinalis* L. (1 sp., Adriatic Sea, coll. no. 8194); *Scalarispongia scalaris* (Schmidt) (1 sp., coll. no. 9481/42); *Cacospongia mollior* Schmidt (1 sp., Adriatic Sea, coll. no. 8197); *Sarcotragus spinosulus*, Schmidt (1 sp., Adriatic Sea, coll. no. 9481/60); *S. foetidus* Schmidt (Adriatic Sea, inv.no. 9481/48), *Ircinia variabilis* (Schmidt) (1 sp., Adriatic Sea, coll. no. 8202).

PLATYZOA

The original collection of human and animal parasitic worm species included 77 species from 42 genera, as indicated by the inventory of Grigore Antipa and the letter from October 16th 1907 (no. 170). Nowadays, the 55 specimens from six phyla, spiny-headed worms, flatworms, round worms, horsehair worms, polychets and velvet worms, are preserved in glass jars with their original labels, which specify the name of the species and the firm (V. Frič, Prague).

Phylum Acantocephala reunites in the Museum's collections only two parasitic species: *Acanthocephalus lucii* Mll. (1 sp., coll. no. 8419) and *A. anguillae* Mll. (1 sp., coll. no. 8420).

Phylum Plathelmynthes is represented by 32 specimens of flatworms from Cestoda (the most numerous), Trematoda and Monogenea: *Agamoema ovatum* Dias. (1 sp., coll. no. 9482/29); *Amphilina foliacea* (R.) Wag. (1 sp., coll. no. 8383); *Anthobothrium* sp. (1 sp., coll. no. 8378); *Eubothrium crassum* (Bloch) Nyb. (1 sp., coll. no. 9482/14); *Bathybothrium rectangulum* (Bloch) (1 sp., coll. no. 9482/12); *Caryophyllaeus mutabilis* Rud. (1 sp., coll. no. 8381); *Hymenolepis villosa* (Bloch) (1 sp. from hen intestine, coll. no. 8364); *Ligula intestinalis* L. (2 sp., immature and mature form, coll. no. 9482/17, 9482/18); *Anoplocephala perfoliata* Goeze (1 sp., coll. no. 9482/9); 13 specimens of larval stages of different Teniidae, immature and mature forms – *Coenurus cerebralis* (2 sp.- one from sheep brain, coll. no. 8369, 8370); *Cysticercus fasciolaris* Rud. (1 sp., coll. no. 8373); *Cysticercus tenuicollis* (1 sp., coll. no. 8366); *Diphyllobotrium latum* L. (from fish muscles, 1 sp., coll. no. 8382); *Schistocephalus solidus* Mll. (2 sp., immature and mature form, coll. no. 8375, 9482/15); *Drepanidotaenia lanceolata* (Bloch) (1 sp., coll. no. 9482/10); *Echinococcus granulosus* (Batsch) (2 sp. from dog intestine, coll. no. 8367, 8368); *Taenia saginata* Goeze (1 sp., coll. no. 9482/11); *Taenia microps* (1 sp., coll. no. 8365); *Taenia multiceps* (Leske) (1 sp.- from dog intestine, coll. no., 8360); Monogenea: *Nitzschia sturionis* Abild. (1 sp., coll. no. 8356); *Diplozoon paradoxum* Nord. (1 sp., coll. no. 8355) and *Mazocraes alosae* (Herman) (1 sp., coll. no. 9482/16). Trematods are represented by various fish and mammalian parasites: *Echinostoma revolutum* (Froelich) (1 sp., coll. no. 9482/5); *Dicrocoelium dendriticum* (Rud.) (1 sp., coll. no. 8352); *Bunodera luciopercae* Mll. (1 sp., coll. no. 9482/3); *Opisthorchis felineus* Rivolta (1 spec, coll. no. 9482/2); *Clonorchis sinensis* (Loos) (1 sp., coll. no. 9482/4); *Distomum terreticole* Rud. (1 sp., coll. no. 8353).

A various collection of round worms, **Phylum Nematoda**, comprises 17 specimens from Chromadorea, Secernentea and Enoplea classes: *Ascaris allenata* Molin (1 sp., coll. no. 9482/21); *A. compar* Schrank (1 sp., coll. no. 9482/33); *Heterotyphlum obtusocaudatum* (Zeder) (1 sp., coll. no. 9482/22); *Hysterothylacium aduncum* (Rud.) (1 sp., coll. no. 9482/23); *Cucullanus* sp. (1 sp., coll. no. 9482/37); *Ancylostoma duodenale* Dub. (1 sp., coll. no. 8353); *Gnathostoma hispidum* Fedch. (2 sp. - one from pig stomach, coll. no. 9482/30, 8412); *Dictyocaulus filaria* Rud. (1 sp., coll. no. 8403); *Ascaridia galli* Schrank (1 sp., coll. no. 8406); *A. columbae* Gmel.(1 sp., coll. no. 8407); *Physaloptera clausa* Rud. (1 sp., coll. no. 8413); *Strongylus equinus* Mll. (1 sp., coll. no. 8414); *Bunostomum trigonocephalum* (Rud.)(1 sp., coll. no. 8415); *Setaria equina* Abild. (1 sp. - from horse peritoneum, coll. no., 9482/34); *Trichiuris ovis* (1 sp., coll. no. 9482/27); *Trichocephalus unguicularis* Rud. (1 sp., coll. no. 9482/26).

Other phyla are represented in the collection, like Phylum **Nematomorpha** or the horsehair worms, which complete the vast collection of worms: *Gordius aquaticus* L. (1 sp., coll. no. 8416) and *G. tolasanus* Duj. (1 sp., coll. no. 8417), **Annelida**,with one palolo worm, *Palola viridis* Gray (1 sp., coll. no. 8444) and one peripatopsid or velvet worm, **Phylum Onychophora**, *Peripatopsis balfouri* (Sedgwick) (1 sp., coll. no. 8712) (Fig. 2F).

ARTHROPODA
CRUSTACEA

Crustacean collection reunites a wide variety of parasitic, marine, terrestrial and freshwater species from four classes: Hexanauplia (Subclass Copepoda), Branchiopoda, Ostracoda and Malacostraca (Amphipoda, Isopoda and Decapoda). In the museum's collection only 51 specimens are still present, while during time more than 100 specimens were lost.

Copepods are represented by three species, ectoparasites to commercial fish species: *Achtheres percarum* Nord. (2 sp., ♂, ♀, North Sea, parasite in perch, coll. no. 9490/32); *Lernaeocera branchialis* L. (1 sp., coll. no. 8824) and *Dichelesthium oblongum* Abildg. (1 sp., coll. no. 9490/33).

Class **Branchiopoda** is represented by phyllopods, by Notostraca: *Triops cancriformis* (Bosc) (2 sp.- one development coll. no. 8819, 9490/24); Cladoceran, water fleas, with only three species: *Brachiella thynni* Cuv.; *Daphnia magna* Strauss (1 sp., coll. no. 8821); *Leptodora kindtii* (F.) (1 sp., coll. no. 9490/28) and Spinicaudata, clam shrimps, *Leptestheria dahalacensis* (Rupp.) (1 sp., coll. no. 8820). Other two species from Anostraca complete the freshwater invertebrate collection: *Artemia franciscana* Kell. (1 sp., coll. no. 9490/26); *A. salina* (L.) (1 sp., coll. no. 9490/25). Other specimens from the fish lice group, Branchiura are less representative, with only two species: *Argulus coregoni* Thor. (2 sp.-♂, ♀, coll. no. 9490/35) and *A. foliaceus* (L.) (2 sp.- ♂, ♀, coll. no. 8826).

Ostracods are present with only one specimen, *Cypris pubera* Mll. (1 sp., coll. no. 9490/27).

Isopoda represent the greatest part of Frič's crustacean collection (acquired in 1908, no. 172), exactly 138 species from 28 genera,13 families and four suborders (Cymothoida, Oniscidea, Valvifera and Limnoriidea) of marine and mainly terrestrial isopods. 34 specimens are still present in the collection, preserved in alcohol (Fig. 2F).

Subord. Cymothoida: *Conilera cylindracea* (Montagu) (Napoli, 1 sp., coll. no. 9490/10, Napoli);

Subord. Oniscidea: *Cylisticus albomaculatus* Borutzkii (1 sp., coll. no. 9490/81); *Armadillidium albanicum* Verh. (1 sp., coll. no. 9490/59); *A. maculatum* (Risso) (1 sp., coll. no. 9490/54); *A. granulatum*, Br. (2 sp., coll. no. 8835, 9490/60); *A. pictum* Br. (1 sp., coll. no. 9490/64); *A. gestroi* Tua (1 sp., coll. no. 9490/55); *A. stagnoense* Verh. (1 sp., coll. no. 9490/56); *A. nasatum* Budde-Lund (1 sp., coll. no. 9490/63); *A. vulgare* (Lat.) (1 sp., coll. no. 9490/61); *Armadillo officinalis* Dum. (1 sp., coll. no. 9490/65) (Fig. 2F); *Chaetophiloscia glandulifera* Verh. (1 sp., coll. no. 9490/97); *Philoscia muscorum* (Scop.) (1 sp., coll. no. 9490/92); *P. muscorum var. nigrovittata* Verh. (1 sp., coll. no. 9490/94); *Tiroloscia exigua* (Budde-Lund) (1 sp., coll. no. 9490/95); *Halophiloscia couchii* (Kinahan) (1 sp., coll. no. 9490/96); *Oniscus asellus* L. (1 sp., coll. no. 9490/86); *Porcellio longicornis* Stein (1 sp., coll. no. 9490/79); *P. laevis* Lat. (1 sp., coll. no. 9490/76); *P. scaber* var. *marmoratus* Brandt (1 sp., coll. no. 9490/69); *P. flavomarginatus* (Lucas) (1 sp., coll. no. 9490/75); *P. imbutus trinacrius* Verh. (1 sp., coll. no. 8834); *P. monticola* Lereb. (1 sp., coll. no. 9490/72); *Trachelipus arcuatus* (Budde-Lundt) (1 sp., coll. no. 9490/74); *T. mostarensis* (Verh.) (1 sp., coll. no. 9490/73); *T. rathkii* (Brandt) (1 sp., coll. no. 9490/70); *T. ratzeburgii* (Brant) (1 sp., coll. no. 9490/77); *T. trachealis* (Budde-Lund) (1 sp., coll. no. 9490/71); *Porcellium conspersum* (C. Koch) (1 sp., coll. no. 9490/78); *Sphaerobathytropa ribauti* Verh. (1 sp., coll. no. 9490/83); *Oritoniscus flavus* (Budde-Lund) (1 sp., coll. no. 9490/87); *Tylos latreillei* Audouin (1 sp., coll. no. 9490/84); **Subord. Valvifera**: *Idotea balthica* (Pall.) (1 sp., coll. no. 9490/101, Napoli); **Suborder Limnoriidea**: *Limnoria lignorum* (Rathke) (1 sp., coll. no. 9490/105).

Amphipoda is less represented in the collection with only one species of ectoparasitic amphipod on whale: *Cyamus ceti* (L.) (1 sp., coll. no. 9490/302).

Decapoda with only one species is mentioned in Antipa's inventory from 1931, *Potamon fluviatile* (Herbst), missing from present collection.

ARACHNIDA
Araneea

Other great part of collection is represented by spiders from Palearctic and Holarctic regions. In the collection register of Grigore Antipa 122 species are mentioned from 1931, bought in 1907 according to the letter received on June 28th; 98 of them are still present with 118 specimens, from 67 genera and 28 families. For some of them is mentioned also the collecting place: Java, Borneo, St. Catherina (Canada).

Ord. Opiliones: *Lacinius horridus* (Panzer) (1 sp., 9491/102); *Asianellus festivus* (Koch) (4 sp. - 2♂♂, 2♀♀, coll. no. 9491/81, 9491/82, 9491/85); *Philaeus chrysops* (Poda) (2 sp. - ♂, ♀, 9491/80); *Pellenes tripunctatus* (Walck.) (2 sp. - ♂, ♀, coll. no. 9491/83); *Agroeca brunnea* Blackw. (1 sp., 9491/68); *Liocranum rupicola* (Walck) (1 sp., coll. no. 9491/125); *Amaurobius fenestralis* (Ström.)(2 sp. - ♂, ♀, coll. no. 9491/86); *Callobius claustrarius* (Hahn) (1 sp., coll.no. 9491/88); *Coelotes terrestris* (Wider) (1 sp., coll.no. 9491/89); *C. atropus* Welck. (1 sp., coll. no. 9491/45); *Tegenaria domestica* (Clerck) (1 sp., coll. no. 9491/93); *Histopona torpida* Koch (1 sp., coll. no. 9491/46); *Asagena phalerata* Panz.(1 sp., coll.no. 9491/12); *Steatoda bipunctata* L. (2 sp. - ♂, ♀, 9491/96); *S. castanea* (Clerck) L. (1 sp., coll. no. 9491/97); *S. albomaculata* (De Geer) (2 sp., coll. no. 9491/8, 9491/11); *S. grossa* (Koch) (1 sp., coll. no. 9491/9); *Parasteatoda lunata* (Clerck) (1 sp., coll. no. 9491/7); *Phylloneta impressa* Koch (1 sp., coll. no. 9491/5); *Theridium varians* Hahn (2 sp. - ♂, ♀, 9491/6); *Bathyphanthes alticeps* (1 sp., coll.no. 9491/18); *Clubiona pallidula* (Clerck) (1 sp., coll. no. 9491/63); *C. caerulescens* Koch (1 sp., coll. no. 9491/66); *C. germanica* Thor. (1 sp., coll. no. 9491/65); *C. holosericea* De Geer (1 sp., coll. no. 9491/64); *Cteniza sauvagesi* Rossi (1 sp., coll. no. 8756); *Cyrtocarenum cunicularium* Oliv. (1 sp., coll. no. 8757); *Diaea dorsata* Fab. (1 sp., coll. no. 9491/77); *Pistius truncatus* (Pall.) (1 sp., coll. no. 9491/79); *Misumena vatia* (Clerk) (3 sp. - ♂, ♀, coll. no. 9491/71, 9491/78); *Thomisus*

onustus Walck (2 sp., coll. no. 9491/72, 9491/73); *Xysticus bifasciatus* Koch (2 sp. - ♂, ♀, coll. no. 9491/76); *X. cristatus* Clerck (2 sp. - ♂, ♀, coll. no. 9491/75); *Dysdera erythrina* Walck. (1 sp., coll. no. 9491/2); *Scotophaeus quadripunctatus* L. (1 sp., coll. no. 8739); *S. scutulatus* Koch (1 sp., coll. no. 9491/61); *Haplodrassus signifer* Koch (1 sp., coll. no. 9491/62); *Zelotes subterraneus* (Koch) (1 sp., coll. no. 9491/98); *Z. petrensis* (Koch) (1 sp., coll. no. 9491/99); *Gnaphosa bicolor* Hahn (1 sp., coll. no. 9491/59); *G. lugubris* Hahn (1 sp., coll. no. 9491/60); *G. lucifuga* (Walck) (2 sp., coll. no. 8740, 9491/58); *Larinioides ixobolus* Thor. (1 sp., coll. no. 9491/107); *Araneus circe* Audouin (2 sp. - ♂ , ♀, coll. no. 8750); *Araniella cucurbitina* (Clerck) (2 sp. - ♂, ♀, coll. no. 9491/101); *Nephila* sp.(1 sp., Borneo, coll. no. 9491/39); *Singa hamata* (Clerk) (2 sp. - ♂, ♀, 9491/24); *Zygiella atrica* Koch (3 sp. - ♂, ♀, 9491/22, 9491/95); *Z. montana* Koch (1 sp., coll. no. 9491/21); *Z. x-notata* (Clerck) (2 sp. - ♂, ♀, coll. no. 9491/23); *Mangora acalypha* (Walck) (1 sp., coll. no. 9491/20); *Gonatium rubens* Blackw. (1 sp., coll. no. 9491/19); *Harpactea rubicunda* Koch (1 sp., coll. no. 9491/1); *Titanoeca quadriguttata* (Hahn) (1 sp., coll. no. 9491/10); *Tenuiphantes mengei* (Kulczyński) (1 sp., coll. no. 9491/17); *Neriene emphana* Walck. (2 sp.- ♂, ♀, coll. no. 9491/16); *Neriene montana* Clerk (2 sp. - ♂, ♀, coll. no. 9491/13); *Megalepthyphantes nebulosus* (Sund.) (2 sp. - ♂, ♀, coll. no. 9491/15); *Pityohyphantes costatus* (Hentz) (1 sp., coll. no. 9491/14); *Neriene montana* (Clerck) (1 sp., coll. no. 9491/124); *Trochosa ruricola* (De Geer) (2 sp. - ♂, ♀, coll. no. 8745); *T. terricola* Thor. (1 sp., coll. no. 8768); *T. robusta* (Simon) (2 sp. - ♂, ♀, coll. no. 9491/52); *Pardosa amentata* (Clerck) (1 sp., coll. no. 9491/53); *P. monticola* (Clerck) (2 sp. - ♂, ♀, coll. no. 9491/56); *P. morosa* (Koch) (1 sp., coll. no. 9491/55); *Lycosa tarantula* L. (2 sp., coll. no. 8741, 8742); *Alopecosa fabrilis* (Clerk) (1 sp., coll. no. 9491/54); *A. cuneata* (Clerck) (3 sp.- ♂, ♀, coll. no. 9491/48, 9491/57); *A. farinosa* (Herman) (2 sp. - ♂, ♀, 9491/47); *A. schmidti* (Hahn) (1 sp., coll. no. 9491/50); *A. inquilina* (Clerck) (2 sp. - ♂, ♀, coll. no. 8744); *Micromata virescens* (Clerk) (1 sp., coll. no. 9491/69); *Nemesia caementaria* (Lat.) ((1 sp., coll. no. 8758); *Pisaura mirabilis* (Clerk) (2 sp. - ♂, ♀, coll. no. 8759); *Pachygnatha clerki* Sund. (2 sp. - ♂, ♀, 9491/42); *Actinopus sp.* (1 sp., St. Catherina, coll. no. 8760); *Philodromus dispar* Walck. (1 sp., coll. no. 9491/74); *Pholcus opilionides* Sch. (1 sp., coll. no. 9491/38); *Eratigena agrestis* (Walck) (1 sp., coll. no. 9491/43); *Cicurina cicur* (Fab) (1 sp., coll. no. 9491/44); *Tetragnatha pinicola* Koch (1 sp., coll. no. 9491/126); *Tetragnatha extensa* (L.) (1 sp., coll. no. 9491/41); *Uroctea durandi* (Lat.) (1 sp., coll. no. 9491/4).

Scorpiones

Ten species from eight genera, nine of them still remained in the collections. The material was collected from Europe (Italy), Turkmenistan, SE Asia (Indonesia, Java, Celebes, Borneo), from Africa and America (Mexico and Venezuela) and sent to Grigore Antipa on October 15th 1907 (letter no. 170/ October 16th 1907).

Butheolus melanurus (1 sp., Turkmenistan, coll. no. 9491/141); *Euscorpius flavicaudis* De Geer (1 sp., Italy, coll. no. 9491/159); *Heterometrus cyaneus* Koch (1 sp., Java, coll. no. 8725); *H. longimanus* (Herbst) (1 sp., Borneo, coll. no. 9491/148); *H. phipsoni* (Pocock) (1 sp., East Indies, Indonesia, coll. no. 9491/149); *Opistacanthus lecontei* (1 sp., Africa, coll. no. 8722); *Pandinus imperator* Koch (1 sp., Africa, coll. no. 8723); *Theliphonus caudatus* L. (1 sp., Celebes, coll. no. 8731); *Tityus trinitatis* Pocock (1 sp., Venezuela, coll. no. 8718).

MILLIPEDES

Chilopoda

In a letter from February 11th 1908 (no. 171), Frič announced Grigore Antipa that he had sent the chilopod collection. 224 species and specimens from 49 genera are mentioned in the register of Grigore Antipa as being bought from Frič, 26 of them are present in our collection (Fig. 2 E). Most of them were collected or described from Central and Eastern Europe (Balkanic Peninsula), including Romania (Transylvania and Banat).

Bothriogaster signata Kessler (1 sp., coll. no. 8795); *Cryptops hortensis* (Donovan) (1 sp., coll. no. 9492/53); *C. anomalans* Newport (1 sp., coll. no. 9489/91); *Geophilus carpophagus* Leach (1 sp., coll. no. 9492/41); *G. longicornis* Leach (1 sp., coll. no. 9489/87); *Clinopodes flavidus* Koch (1 sp., coll. no. 9489/85); *Pleurogeophilus mediterraneus* (Mein) (1 sp., coll. no. 9489/83); *Eurypleuromeris conspersa* (Koch) (1 sp., coll. no. 8777); *Henia (Henia) illyrica* (Mein.) (1 sp., coll. no. 8796); *Himantarium gabrielis* L. (1 sp., coll. no. 8793); *Lithobius macilentus* Koch (1 sp., coll. no. 9489/96); *L. calcaratus* Koch (1 sp., coll. no. 9489/93); *L. dentatus* Koch (1 sp., coll. no. 9489/98); *L. erythrocephalus* Koch (1 sp., coll. no. 9492/39); *Lithobius forficatus* L. (1 juv., coll. no. 8803); *L. melanops* Newp.(1 sp., coll. no. 9489/94); *Lithobius mutabilis* Koch (1 sp., coll. no. 9492/38); *L. muticus* Koch (1 sp., coll. no. 9492/40); *L. nodulipes* Latz (1 sp., coll. no. 9489/97); *Lithobius (Lithobius) parietum* Verh. (1 sp., coll. no. 9489/99); *L. tricuspis* Mein (1 sp., coll. no. 9489/95); *L. validus* Mein. (1 sp., coll. no. 8802) (Fig. 2 E); *Pachymerium ferrugineum* Koch (1 sp., coll. no. 9492/47); *Schendyla nemorensis* Koch (1 sp., coll. no. 9489/84); *Scolopendra morsitans* L. (1 sp., coll. no. 8798); *Scutigera coleoptrata* L. (1 sp., coll. no. 8806).

Diplopoda

In the letters from Frič (from February 11th and November 26th 1908), the collection of Diplopoda is very vaste, 190 species which were sent to Grigore Antipa with different occasions, prior to the date of the letter or afterwards. In the collections of the Museum, 93 species from 43 genera (10 families), a total of 102 specimens are still present.

Superord. Juliformia, Ord. Julida: *Allajulus groedensis* Att. (1 sp., coll. no. 9489/54); *A. molybdinus* Koch (1 sp., coll. no. 9489/49); *Brachyiulus projectus* Verh. (1 sp., coll. no. 9489/74); *B. rosenauensis* Verh. (1 sp., coll. no. 9492/16); *B. silvaticus* Verh. (1 sp., coll. no. 9492/11); *B. silvaticus discolor* Verh. (1 sp., coll. no. 9489/69); *B.*

unilineatus Koch (1 sp., coll. no. 9489/72); *Choneiulus palmatus* (Nem.) (1 sp., coll. no. 9489/81); *Cylindroiulus horvathi* (Verh.) (1 sp., coll. no. 9492/18); *C. dicentrus* Latz. (1 sp., coll. no. 9492/22); *C. latzeli* (Berleze) (1 sp., coll. no. 9489/52); *C. luscus salicis* (Verh.) (1 sp., coll. no. 9489/50); *C. luridus* (Koch) (1 sp., coll. no. 9492/19); *C. punctatus* Leach (1 sp., coll. no. 9492/20); *C. nitidus* Verh. (1 sp., coll. no. 9489/51); *Enantiulus dentigerum* (Verh) (1 sp., coll. no. 9489/80); *E.nanus* Latz. (1 sp., coll. no. 9492/44); *Hypsoiulus alpivagus* (Verh) (1 sp., coll. no. 9492/4); *Hungaroiulus curvicornis* (Verh.) (1 sp., coll. no. 9489/56, 9489/66); *Julus* sp. (1 sp., Ceylon, coll . no. 9489/55); *J. barbatus* Verh. (1 sp., coll. no. 9489/63); *J. ciliatus* Verh. (1 sp., coll. no. 9489/59); *J. ciliatus liptauensis* Verh. (1 sp., coll. no. 9492/5); *J. ligulifer* Latz. (1 sp., coll. no. 9492/1); *Kryphioiulus occultus* (Koch) (1 sp., coll. no. 9492/21); *Leptoiulus deubeli* (Verh.) (1 sp., coll. no. 9492/9); *L. vagabundus baconyensis* (Verh.) (1 sp., coll. no. 9489/58); *L. alemannicus* (Verh.) (2 sp., 9489/60, 9489/62); *L. saltuvagus* Verh. (1 sp., coll. no. 9489/64); *L. silvivagus* Verh. (1 sp., coll. no. 9489/25); *L. trilineatus* Verh. (2 sp., coll. no. 9489/57, 9489/61); *Megaphyllum bosniense* Verh. (1 sp., coll. no. 9492/14); *M. projectum projectum* Verh. (2 sp., coll. no. 9492/13, 9492/15); *Mesoiulus bosniensis* (Verh) (1 sp., coll. no. 9492/25); *Microbrachyiulus littoralis* (Verh.) (1 sp., coll. no. 9492/24); *Ophyiulus germanicus* (Verh.) (1 sp., coll. no. 9492/8); *Pachybrachyiulus podabrus krohnii* Verh. (1 sp., coll. no. 9489/71); *Phylacodon fallax* (Mein.) (1 sp., coll. no. 9492/7); *Pachyiulus oenologus* (Berl.) (1 sp., coll. no. 9489/76); *P. fuscipes* Koch (2 sp., coll. no. 9489/77, 9489/78); *P. unicolor* Koch (1 sp., coll. no. 9489/75); *Schizophyllum mediterraneum* Latz. (1 sp., coll. no. 9489/83); *Styrioiulus pelidnus* (Latz) (1 sp., coll. no. 9492/43); *Stenophyllum hermannimuelleri* Verh. (1 sp., coll. no. 9489/79); *Typhloiulus strictus* Latz. (1 sp., coll. no. 9492/23).

Superord. Merocheta, Ord. Polydesmida: *Acanthotarsius edentulus* (Koch) (1 sp., coll. no. 9492/30); *Brachydesmus carniolensis* Verh. (1 sp., coll. no. , 9489/20); *B. dolinensis* Attems (1 sp., coll. no. 9492/34); *B. chyzeri* Daday (1 sp., coll. no. 9489/19); *B. dentatus* Verh. (1 sp., coll. no. 9489/73); *B. styricus* Verh. (1 sp., coll. no. 9489/22); *B. subterraneus* Hell. (1 sp., coll. no. 9489/17); *Basicentrus tridentinus* Latz. (1 sp., coll. no. 9489/28); *Eubrachydesmus superus* Latz. (1 sp., coll. no. 9489/16); *Polydesmus barberri* Latz. (1 sp., coll. no. 9489/24); *P.bolivari* Verh. (1 sp., coll. no. 9489/27); *P. collaris* Koch (1 sp., coll. no. 9492/32); *P. complanatus* L. (1 sp., coll. no. 8780); *P. denticulatus* Koch (1 sp., coll. no. 9489/32); *P. falcifer* Latz. (1 sp., coll. no. 9489/23); *P. fissilobus albanensis* Verh. (1 sp., coll. no. 9489/26); *P. germanicus* Verh. (1 sp., coll. no. 9489/30); *P.hamatus* Verh. (1 sp., coll. no. 9489/29); *P. herzegowinensis* Verh. (1 sp., coll. no. 9489/21); *P. illyricus* Verh. (1 sp., coll. no. 9489/31); *P. montanus* Daday (1 sp., coll. no. 9492/31); *Stylobrachydesmus dadayi* (Verh) (1 sp., coll. no. 9489/18); *Strongylosoma hispanicum* Verh. (1 sp., coll. no. 9489/36); *S. italicum* Latz. (1 sp., coll. no. 9489/34); *S. pallipes* Oliv. (2 sp., coll. no. 9489/33, 9489/34).

Superord. Nematomorpha, Ord. Chordeumatida: *Triakontazona pusillum* (Verh.) (1 sp., coll. no. 9489/41); *Craspedosoma simile* Verh. (1 sp., coll. no. 9489/40); *Microchordeuma gallicum* (Latz.) (1 sp., coll. no. 9489/38); *Melogona voigtii* (Verh) (1 sp., coll. no. 9489/37); *Orobainosoma flavescens* (Latz.) (1 sp., coll. no. 9489/39).

Ord. Callipodida: *Acanthopetalum albidicolle* Verh.(1 sp., coll. no. 9489/43); *A. carinatum* (Brandt) (1 sp., coll. no. 9489/44); *Callipodella fasciata* (Latz) (1 sp., coll. no. 9489/45); *Dischizopetalum illyricum* (Latz.) (1 sp., coll. no. 9492/46); *Heterocraspedum scabratum* (Koch) (1 sp., coll. no. 9489/46).

Superord. Oniscomorpha, Ord. Glomerida: *Trachysphaera acutula* (Latz.) (1 sp., coll. no. 9489/10); *T. costata* (Waga) (1 sp., coll. no. 9489/13); *T. cultrifera* Verh. (1 sp., coll. no. 9489/11); *T. schmidti* Hell. (1 sp., coll. no. 9489/14); *T. pyrenaica* Rilbaut (1 sp., coll. no. 9489/12); *Euglomeris connexa* (Koch) (1 sp., coll. no. 9489/6); *Glomeris formosa formosa* Latz. (1 sp., coll. no. 9489/5); *G. hexasticha* Brandt (1 sp., coll. no. 9489/7); *G. hexasticha* var. *theresiae* Verh. (1 sp., coll. no. 9489/9); *G. marginata* var. *perploxa* Lat. (1 sp., coll. no. 9489/3); G. *pustulata* (Fab.) (1 sp., coll. no. 9489/2); *G. trisulcata* Roth (1 sp., coll. no. 9489/4); *Typhloglomeris coeca* Verh. (1 sp., coll. no. 9489/15).

Infraclass Helminthomorpha, Ord. Polyzoniida: *Polyzonium germanicum illyricum* Verh. (1 sp., coll. no. 9489/101); *P. transsilvanicum* Verh. (1 sp., coll. no. 9492/54).

Superord. Oniscomorpha, Ord. Sphaerotheriida: *Sphaerotherium* sp. (1 sp., coll. no. 9489/1).

Other phyla, such as Chaetognatha, Pisces and Aves are poorly represented.

CHAETOGNATHA

It is present with only two species of *Sagitta* and *Flaccisagitta*: *Sagitta sp.* (1 sp., coll. no. 8500), *Flaccisagitta hexaptera* d'Orbigny (1 sp., coll. no. 8499).

PISCES

Fishes are represented by four species, mentioned in the list from 1907, two of them are present, *Ameiurus cattus* L. (1 sp., coll. no. 6324); *Leptocephalus conger* L., (1 sp., coll. no. 6534), preserved in alcohol in Fish Collection.

AVES

Birds are present in the collection by one naturalized mounted species, *Collocalia aesculenta* L. (Salangana) and her nest (1 sp., coll. no. 4420), a skeleton of kiwi bird, *Apteryx australis* Shaw (coll. no. AC 703), a skeleton of a hummingbird (coll. no. AC 183), all of them bought on October 16th 1907; a fossil skeleton of *Odontopteryx toliapica* Owen (1 sp., coll. no. 11008/5).

Different entire skeletons or pieces from **different groups** of animals were bought in October 1907: four pieces could be found in the collection of comparative anatomy, two of them being displayed for a long time in the museum, a skeleton in alcohol of *Beluga leucas* (coll. no. AC 586) and a baculum of *Megaptera novaeangliae* (coll. no.

AC 268) (in the Museum' s public exibition, for more than 100 years); a skeleton of *Draco volans* (coll. no. AC 192); three models of **fossil AMPHIBIA** (Caudata and Anura): *Audrias scheuchzeri*; *Branchiosaurus salamandroides* Fritsch (1 sp., coll. no. 1108/4) and *Palaeobatrachus dolfussi* Tschudi and seven of **fossil MAMMALS**: *Canis familiaris intermedius* (coll. no. 9971), *Hipparion elegans* Gromova (coll. no. 9644), posterior leg of *Hipparion* sp. (coll. no. 11010/13), *Anoplotherium commune*, Cuv. (coll. no. 11010/6), *Lophiodon parisiense* Gervais (coll. no. 11010/3), *Palaeotherium crassus* (coll.no. 9632), *P. medium* (coll. no. 9633).

Other pieces had been lost, like gyps copies of different skeletal parts of fossil mammals or galvano - plastic copies of fossils - present in the catalogue of Frič from 1889, in Museum für Naturkunde der Humboldt Universität, Berlin (REILING & SPUNAROVA, 2005).

CONCLUSIONS

Although there is no published catalogue of pieces acquired from Václav Frič, in the memoir from October 2nd 1907 for the Ministry of Culture and Public Instruction, Grigore Antipa mentions the acquisition of a collection from V. Frič from Prague valued at 2500 marks, along with other specimens that would serve in enriching a museum of European size.

The collections bought from Frič reunite numerous species, mostly from a wide collection of arachnids and millipedes. The catalogue of numerous specimens, 642 that are still present in the "Grigore Antipa" National Museum of Natural History collections of invertebrates, vertebrates, compared anatomy, mineralogy, geology and paleontology, stands as the true evidence of the tireless efforts of Dr. Grigore Antipa to organize a modern museum, a pillar for future generations of specialists, one which could survive centuries of history.

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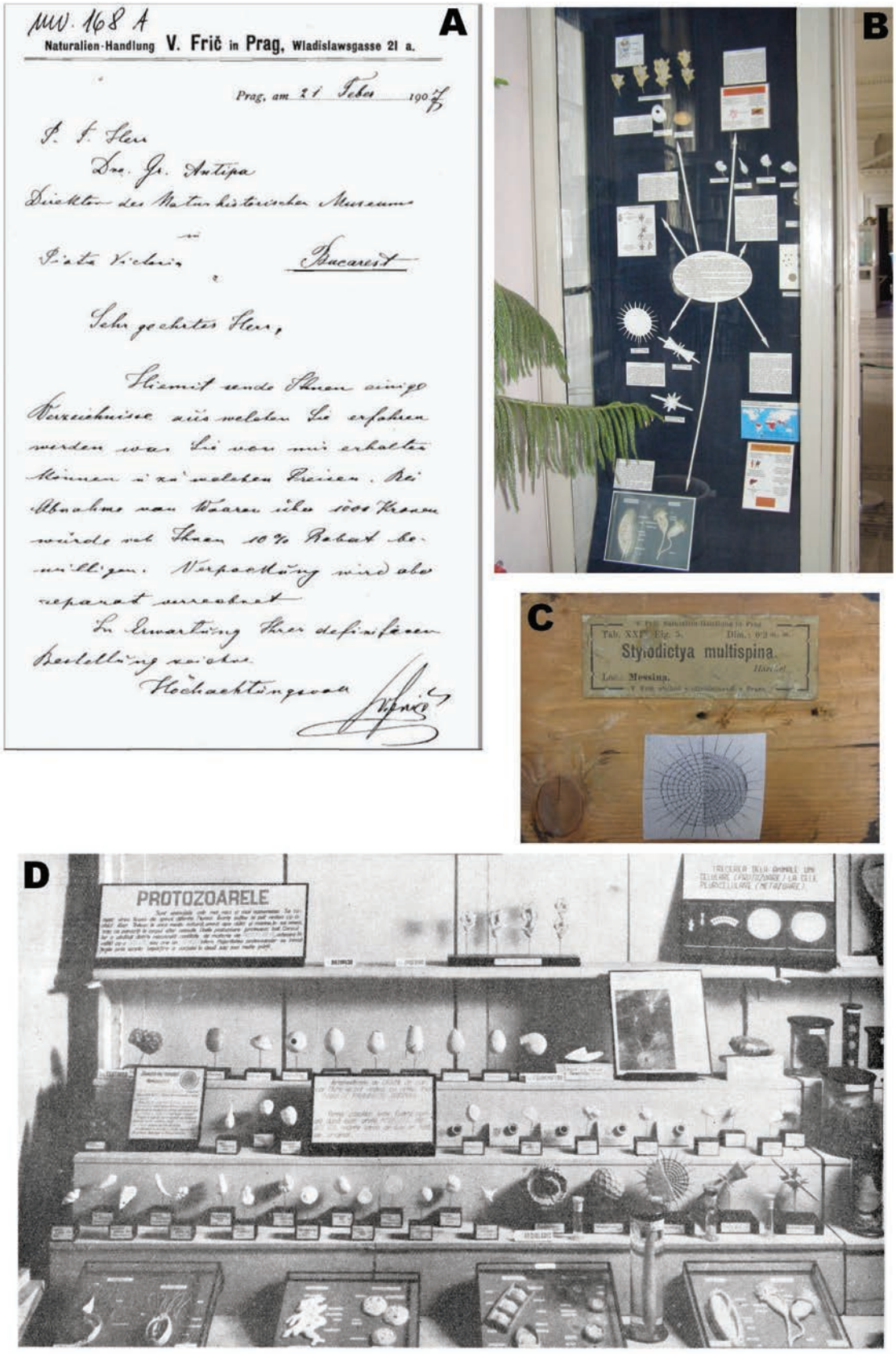

Figure 1. A, First letter from Frič to Grigore Antipa, February 21st 1907 (no. 168) from Museum's Archive; B, Showcase with Protozoa (Museum's public exhibition, after 2004); C, Original label on the back of the wooden stand of an enlarged model of radiolarian, *Stylodictya multispina*; D, Showcase of enlarged protozoa models from the first hall of Invertebrates - Public exhibition, ground floor (IONESCU & SCHNAPP, 1961).

Figure 2. A, Showcase with imitation of precious stones (cca. 1990); B, Magnified model of fossil Foraminifera (*Ehrenbergia serrata*); C, Label for enlarged model of radiolarian, *Diploconus fasces*; D, Magnifying box containing microscopic Foraminifera, *Polystomella* sp.; E, Chilopod, *Lithobius validus*; F, Peripatosid velvet worm (*Peripatopsis balfouri*) with original label from Frič; G, Trinidad thick-tailed scorpion (*Tityus trinitatis*) (original photos).

LIFE HISTORY AND PLURIVOLTINISM OF *Scolitantides orion* (PALLAS 1771), (LEPIDOPTERA, LYCAENIDAE) FROM ROMANIAN SOUTHERN DOBROGEA, IN CAPTIVE BREEDING ON *Hylotelephium telephium*, SOZOLOGICAL IMPLICATIONS

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Abstract. *Scolitantides orion* (Pallas, 1771) (Lepidoptera, Lycaenidae), is a Palaearctic species, spanning across Eurasia, in localized, locally threatened populations. It has a characteristic biology, being stenophagous on a few Crassulacean species, and facultative myrmecophylous. In Europe, it is declining, severely in some areas - mainly due to habitat loss, being red listed and legally protected in Fennoscandia and Central Europe. In Romania, it is not listed and endangered, but some of its populations are sharply declining due to multiple causes. Besides its own conservation concerns, it is interesting as a model for ethological, physiological and ecological studies. As it shares the food-plant with *Parnassius apollo* - another critically endangered species in some places, any knowledge gained about its biology and ecology could prove essential for conservation. The knowledge about the phenology and voltinism of this species -from other parts of Europe is scarce and contradictory, and no data are available about its ecology, phenology or voltinism in Romania. The aim of the experimental work presented herein was to investigate the life history and voltinism of this species under conditions in Southern Romania in captive breeding, with the larger scope of gaining knowledge about the biology and logistics involved in the conservation, (re)introduction and propagation of endangered lepidopteran species which use Crassulaceae as host/food plants. Under our conditions, and feeding exclusively on live *Hylotelephium telephium* (Crassulaceae), *Scolitantides orion* typically developed 4 generations/year, with an average generation turnover of 42-55 days, and a diapausal length of about 185 days. Such a long diapause and other personal data describing anomalous 5th generations more frequent during the last years, with unusually warm and excessively long autumns, suggested the perspective of more efficient mass breeding of this and similar lepidopteran species to obtain larger adult numbers for (re)introduction conservation programs.

Keywords: developmental plasticiy, *Hylotelephium telephium*, *Scolotantides orion*, conservation, life history.

Rezumat. Ciclul de viaţă şi plurivoltinismul la *Scolitantides orion* (Lepidoptera, Lycaenidae) din Dobrogea Sudică Română, crescut în captivitate pe *Hylotelephium telephium*, implicaţii sozologice. *Scolitantides orion* (Pallas, 1771) (Lepidoptera, Lycaenidae), este o specie Palaearctică, cu distribuţie Eurasiatică, în populaţii localizate, local ameninţate cu o biologie caracteristică, stenofagă pe câteva specii de Crassulaceae,este facultativ myrmecofilă. În Europa, este în declin - sever în unele regiuni - în principal, datorită degradării habitatului, fiind trecută pe listele roşii şi legal protejată în Fennoscandia şi Europa Centrală. În România, specia nu este trecută pe listele roşii si nici periclitată, dar unele populaţii sunt în declin abrupt datorită unor multiple cauze. În afara propriilor probleme de conservare, specia este interesantă ca model pentru studii etologice, fiziologice şi ecologice. Cum împărtăşeşte planta gazdă cu *Parnassius apollo* - altă specie critic periclitată în unele locuri, orice cunoştinţe obţinute despre biologia şi ecologia ei pot fi esenţiale pentru conservare. Datele privind fenologia şi voltinismul acestei specii, din alte părţi ale Europei, sunt puţine şi contradictorii, iar cele despre ecologia, fenologia sau voltinismul în România, lipsesc. Scopul acestui studiu experimental este investigarea ciclului de viaţă şi a voltinismului acestei specii în condiţiile din sudul României în creşterea în captivitate, cu scopul mai larg de a obţine cunoştinţe despre biologia şi logistica implicate în conservarea, (re)introducerea şi propagarea de specii de lepidoptere periclitate care folosesc Crassulaceaele, ca plante gazdă. În condiţiile noastre, în hrănire exclusivă pe *H. telephium* (Crassulaceae) viabil, *S. orion* dezvoltă tipic 4 generaţii/an, cu un turnover de 43-54 zile/generaţie, şi o durată a diapauzei de circa 185 de zile. O asemenea diapauză lungă şi alte date personale inedite care arată o a 5-a generaţie anormală mai frecventă în ultimii ani cu toamne neobişnuit de calde si îndelungate, au sugerat perspectiva unei culturi în masă mai eficientă a acestei specii de lepidoptere şi a altora similare pentru obţinerea unui număr mai mare de adulţi pentru programe de (re)introducere pentru conservare.

Cuvinte cheie: plasticitatea dezvoltării, *Hylotelephium telephium*, *Scolotantides orion*, conservare, ciclu de viaţă.

INTRODUCTION

Scolitantides orion (Pallas, 1771) (Lepidoptera, Lycaenidae), is a Palaearctic species, spanning across Europe and Asia, in localized populations confined to patchy habitats where it can find its specialized food plants, and is locally threatened in some areas (KUDRNA, 2002).

According to COULONDRE (1994) on such a vast range with multiple (meta)-populations, several subspecies are accepted as valid. In Europe, 3 subspecies are present; *S. orion parvula* (De Sagara 1926) in the Pyrenees and Iberian Peninsula, *S. orion ultraornata* (Verity 1937) = *S. o. wahlgreni* (Bryk, 1946) in Fennoscandia, while the populations from Central, South-Eastern Europe and Turkey being assigned to the nominotypical subspecies *S. orion.*

According to the IUCN evaluation from 2010 (VAN SWAAY et al., 2010), still valid through 2018, *S.orion* is reported extinct in the European part of Turkey while “Strong decline in distribution or population size of more than 30% has been reported from Germany, Norway, Poland and Ukraine”and a “decline in distribution or population size of 6-30% has been reported from Austria, Romania, Russia, Slovakia and Sweden”. In Finland, the species is protected under the Nature Conservation Decree (IUCN class Vulnerable; RASSI et al., 2001). Throughout Fennoscandia the distribution is patchy in disjunct (meta)-populations, in Finland the species existed in only 5 -10 patches (SOMERMA, 1997) but tends to somewhat recover following an apparently successful action of habitat restoration at one site

(MARTTILA et al., 2000). While the species was always been rare, the current steeper decline seems to be caused by shrub/forest succession after woodland pastures abandonment, scarcity of natural forest fires, residential development and quarrying of stone (MARTTILA et al., 2000).

The current threat status in Europe is LC (Least Concern) and according to European Red list of butterflies; (VAN SWAAY et al., 2010) NT (near threatened) in the European Union. In Sweden, due to steep population decline in the last 30 years, *S. orion* was classified as EN (Endangered) (Red list of Swedish species; Swedish Species Information Centre 2012).

Knowledge about *S. orion* in Romania is scarce at best, and it is considered not endangered (although declines between 10-30% have been reported, IUCN) and it is not explicitly protected. No ecological data from Romania were available at the beginning of this study and the situation did not improve in recent years.

S. orion is a stenophagous species throughout its distribution range, the larvae feed exclusively on a few species in a couple of Crassulaceaean genera, e.g. *Hylotelephium* in Finland (SAARINEN, 1995; MARTTILA et al., 2000; KOMONEN et al., 2008), Sweden (ELMQVIST, 2011; CARLSSON & ELMQUIST, 2009), Norway (ENDRESTØL et al., 2009), Germany (TRAENKNER & NUSS, 2005). In Romania, *S. orion* feeds on *Hylotelephium telephium* together with *Aizobius sedi* (BÂRCĂ & NICULAE, 2011), *Sedum*, *Jovibarba* and *Sempervivum* (BÂRCĂ V. unpublished data) and therefore its distribution conforms with that of its host-plants (also see BÂRCĂ & NICULAE 2005, 2006, BÂRCĂ 2016a, 2016b; NICULAE & BÂRCĂ, 2005; 2006). Due to its narrow stenophagy it faces the same threats as its host-plants, both in Romania and elsewhere (ARBUNE et al., 2009).

The species is considered as facultative myrmecophilous, according to TRAENKNER & NUSS, (2005); being attended by several ant species from at least 4 genera (*Camponotus*, *Formica* (*Serviformica*), *Lasius (Lasius)* and *Tetramorium*), but does not depend on association with ants for completing its life cycle.

The ecology of *S. orion* is quite complex, in Central and southern Europe the species inhabiting patchy habitats with xero-thermophylous rocky landscapes in close proximity with taller shrubs and forest margins. In Finland in Linnansaari National Park, at the Northern range limit of the species, it inhabits small unforested exposed bedrock areas occurring patchily within a true island network, where they feed on *H. telephium* (KOMONEN et al., 2008).

The phenological data available are controversial and suggest a remarkable phenological plasticity for this species, which shows differing numbers of generations per year in different areas. Thus, it has one generation per year over most of Europe TOLMAN & LEWINGTON, 1998, according to KUDLA, 1951, for Moravia, REINHARDT & KINKIER, 2004, for Rhineland-Palatinate, ELMQUIST & CARLSSON, 2009; CARLSSON & ELMQUIST, 2013 for Sweden, ENDRESTØL et al., 2009, for Norway, KOMONEN et al., 2008, for Finland.

In warmer regions it has 2 or 3 generations per year: in Switzerland (TOLMAN & LEWINGTON, 1998; TRAENKNER & NUSS, (1994, 2005); in Czech Republic Central Bohemia (SRDINKO, 1912), in Italian South Tyrol (HUEMER, 2004). In Germany, 2 generations were reported in Saxony (REINHARDT, 2003) and in Thuringia (BERGMANN, 1952), where also a partial second generation occurs, while TRAENKNER & NUSS, (2005) reported 3 generations.

In Central Europe, TOLMAN & LEWINGTON (1998) report *S. orion* has one generation per year while FORSTER & WOHLFAHRT (1955) reported two generations, with one at higher elevation in the Alps.

There is a wider consensus though on the fact that pupae of all generations could enter diapause and only hatch the next or the after-next year. No data are available about intraseasonal dormancy/diapause between generations of the same year.

MATERIAL AND METHODS

Insects: The *Scolitantides orion* insect female progenitors were collected from the vicinity of the village Sipotele, Constanța County in Southern Dobrogea, in April, and placed in "oviposition cages" where they laid eggs that were further used in the subsequent experimental breeding of larvae that produced the next generations.

Plants: In the field, under natural conditions, *S. orion* feeds on several genera of Crassulaceae, but our previous experience showed that the most biomass-efficient food-plant species in seminatural conditions is *Hyllotelephium telephium*, so we chose this plant species as food source.

The food/host plant for *S. orion* was *Hyllotelephium telephium* stock plants cultivated intensively from plant progenitors were collected during the previous years at the same site where the *S. orion* adults were collected. For the breeding experiments the plants were transplanted in small plastic bags approximately 10x10 cm, tied around the main stem(s) too avoid larval access into the bags. This allowed easy placement in the cages and easy replacement maneuvers with minimal disturbance to the larvae.

Captive breeding was performed in relatively smaller "rearing cages" and the resulting pupae were retrieved by hand and used for further observations.

To provide adults with more room for pairing and flight, the retrieved pupae were transferred, and adults left to emerge in relatively larger "oviposition cages" where they copulated and oviposited freely, and he eggs were retrieved daily.

Because our previous experience showed that the neonate and un-burrowed first instar larvae are more sensitive to environmental conditions and much more prone to getting lost in the litter, the eggs were placed in "hatching containers" where they were kept as the whole first instar developed.

The second instar larvae were transferred to the ”rearing cages” where the development of the rest of larval stage and the pupation occurred.

Cages:

The “oviposition cages” consisted of parallelepipedic tent with the approximate side dimensions 1.5x2x1m, made of plastic/fiberglass screen 10 mm mesh placed over a supporting frame of plastic construction PVC pipes. The bottom of the cage was made of a plastic sheet with margins raised 15 cm from the ground and fastened with velcro to the “cover section”, allowing relatively easy access to the interior of the cage and “insulating” the interior from the access of insect predators. Previous experience showed that the “insulation” is very important as spiders and wasps readily catch the adults and can ruin the experiment. Our setup was very efficient at keeping the predators and water out of the cage and at keeping the adults and even the neonates inside the cage.

The “rearing cages” consisted of cylindrical tubes made of 1x1.5 m sheets of the same plastic screen mesh sewn on a side, pulled over 2 metal wire circles and tied at the ends after the plant with the insects was placed inside. These cages were hung from a cord above ground to avoid access of Forficulla and Periplaneta which readily eat the late instars larvae.

The “hatching containers” consisted of 2 approximately cylindrical 3L plastic food containers with tightly fitting lids custom-fashioned to allow reversed stacking one on top of the other, fitted with a laptop fan to allow adequate ventilation. The second, reversed container was only placed on top of the first one after the oviposition took place.

The plants on which the adults were to lay the eggs were placed in the lower container of the “hatching containers” and 4 of these containers with oviposition plants were placed in the “oviposition cage”.

Every 2 days the eggs were removed together with segments pinched off the leaves or stems or together with the flowers to which they were attached and placed in containers on fresh food-plants so that the neonates could burrow into fresh leaves.

All the pupae that we could find were promptly transferred in the oviposition cages until the imagines hatched. The pupae were placed in plastic yogurt 1L containers with tight lid fitted with the same screen mesh, and having inside a rectangular metal screen mesh rolled into e cylinder onto which the imagines could climb and hang to extend their soft wings after hatching from the pupae.

The egg stage begun when the first eggs were found until the last eggs were found when inspecting the plants and the rest of the oviposition cage.

The larval stage begun when we could observe the first eggshells or eggshell marks on the leaves, or when observing neonates (but normally the neonates readily burrow into the leaf parenchyma so they could have easily been overlooked), and lasted until no more viable eggs remained. (The nonviable eggs change appearance after a couple of days, so they could easily be distinguished from viable eggs).

The pupal stage begun when we found the first pupae in the rearing cages and lasted until no more pupae or larvae could be seen in the rearing cages. (The pupae were readily removed from the cages every day).

The imago stage begun when we found the first imagines in the containers and lasted until the last day an imago was found. (The containers were checked every morning and evening and the newly hatched adults were released from the containers in the oviposition cage, but between inspections the containers were closed). All generations produced diapausing pupae, which were removed from the containers after 30 days and stored separately in a larger container similarly fitted with a hatching net made of metal screen mesh. Some adult hatched erratically after a short diapause, before autumn and these were not counted towards any generation.

RESULTS AND DISCUSSIONS

The 6 females introduced in the oviposition cage produced 58 eggs over a period of 8 days. Of this egg batch of the first generation 9 eggs were found dead and 49 produced neonates of the first generation. The females of the first generation oviposited on the upper leaves and stems down to the 3rd internode, the females of the second generation oviposited on the upper leaves and stems with a few down to the 3rd internode, and also on the inflorescences of SM food-plants, while the females of the subsequent generation oviposited on the top leaves and stems down to the 2nd internode, with a higher proportion of eggs on the upper leaves (data not shown, more thorough experimental work was needed to evaluate the significance and causes of the apparent preference differences shown by the females of different generations, and the results are reported elsewhere).

These were the founders which developed in the later stages giving 4 generations, with various lengths of developmental stages, as shown in Table 1. The absolute duration of each of the developmental stages of the 4 generations are shown in the chart in Figure 1, and the life history of *S. orion* as revealed by our present experimental study with the relative length of the developmental stages and their placement in the life cycle is depicted in the chart in Figure 2.

First generation. The first eggs were found on April 9, the eggs needed 12-16 days for full development and the first larvae hatched on April 20. The larvae needed 27-30 days for full development and the first pupated after 27days on May 18.T heactive pupal stage lasted 14 days and the first imagines of the first generation emerged from pupae on June 01. Some pupae entered diapause, of which 3 normal-looking imagines hatched erratically and were not

counted. These erratic emergences of imagines outside the "normal" life cycle of the majority of their generation suggest that under natural conditions the adult populations on wing at a certain time would comprise also individuals from other generations. The imagines of this first generation hatched syncronous over 4 days and started feeding the first day after wing expansion and started ovipositing the second day, and oviposition lasted for 12 days, while the last adult died after 12 days. The whole first generation lasted 69 days but the first oviposition started after 55 days.

Table 1. Absolute durations of each *Scolitantides orion* developmental stage of all generations in one year (days).

Developmental Stage	**Egg**	**Larva**	**Pupa**	**Imago**	**Generation length**	
					a	b
First Generation	12	27	14	16	69	55
2nd Generation	9	20	11	14	54	42
3rd Generation	10	21	10	15	56	43
4th Generation	11	25	185	16	237	225
Dev Stage Sum	42	93	220*	61	365	365

a- generation length including whole flight period of imagines
b- generation length until the day the first eggs were oviposited
*- pupal stage including the diapause
Dev Stage Sum = Summary of duration of each Developmental Stage n the life cycle; e.g. duration of all 4 Egg stages of one year life cycle

Second generation. The first eggs of the second generation were found on June 3rd, the eggs needed 9 days for full development and the first larvae hatched on June 12. The larvae needed just 20-24 days for full development and the first pupated after 20 days on July 12. The active pupal stage lasted 11 days and the first imagines of the first generation emerged from pupae on July 11. This generation also, some pupae entered diapause, of which 5 normal-looking imagines hatched erratically and were not counted. The imagines of this 2nd generation hatched synchronous over 3 days and started feeding the first day after wing expansion and started ovipositting the second day, and oviposition lasted for 13 days, while the last adult died after 14 days. The whole 2nd generation lasted 54 days but the first oviposition started after 42 days.

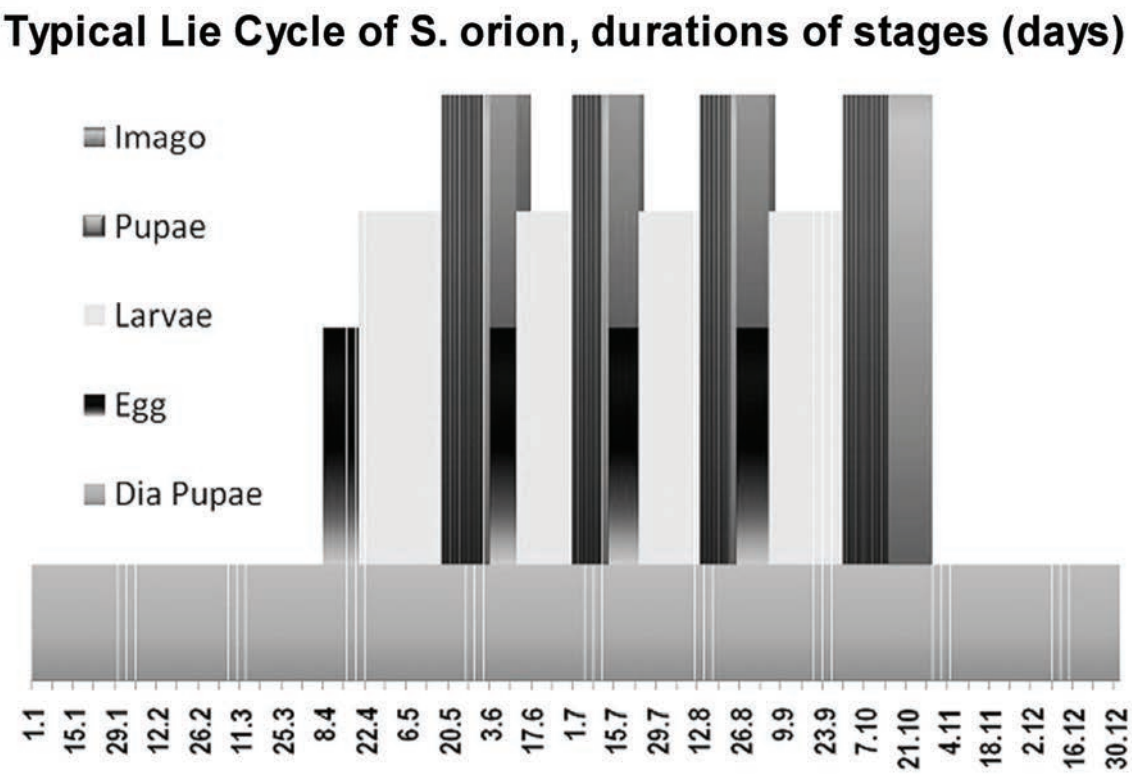

Figure 1. The absolute duration of each of the developmental stages of the four *S. orion* annual generations.

Third generation. The first eggs of the third generation were found on July 13, the eggs needed 10 days for full development and the first larvae hatched on July 23. The larvae needed 21-23 days for full development and the first pupated after 21days on August 13. The active pupal stage lasted 10 days and the first imagines of the first generation emerged from pupae on August 23. This generation also, some pupae entered diapause, of which 2 normal-looking imagines hatched erratically and were not counted. The imagines of this 3rd generation hatched syncronous over

2 days and started feeding the first day after wing expansion and started ovipositting the second day, and oviposition lasted for 14 days, while the last adult died after 15 days. The whole 3rd generation lasted 56 days but the first oviposition started after 48 days.

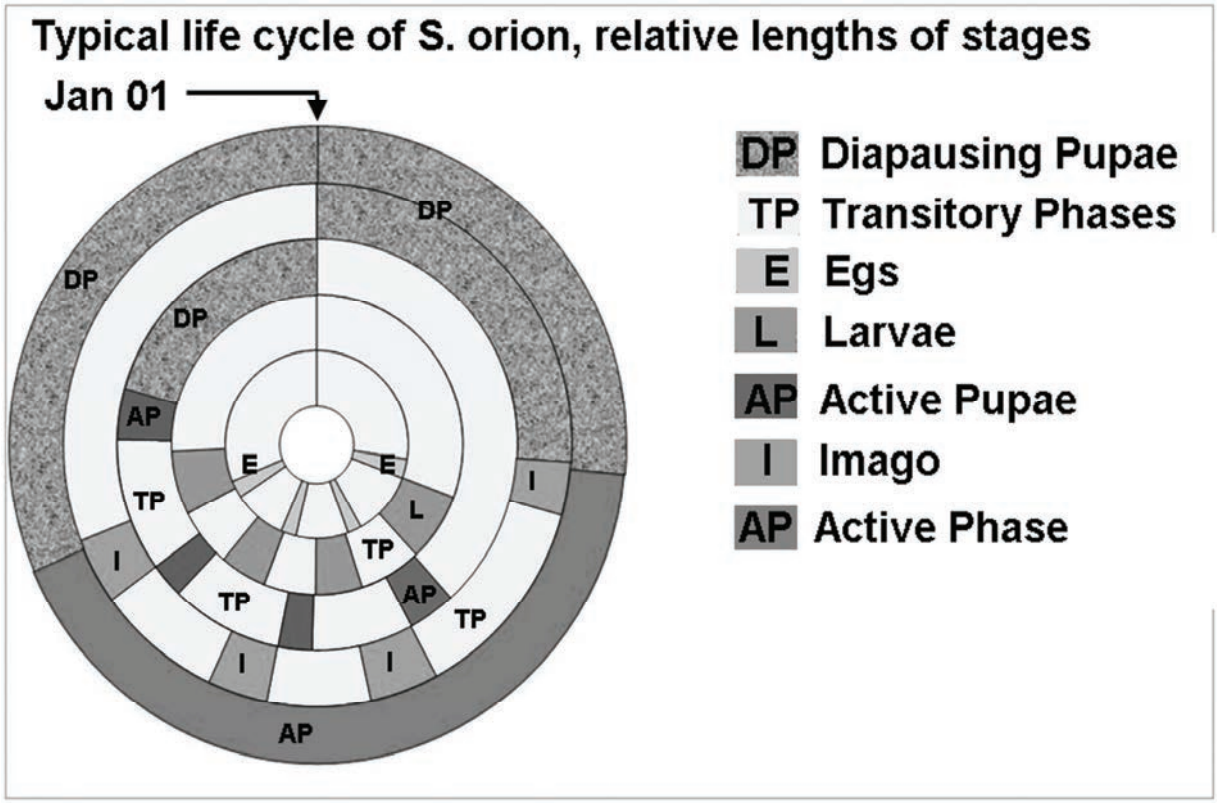

Figure 2. Life history of *S. orion* as revealed by our present experimental study with the relative length of the developmental stages and their placement in the life cycle.

Fourth generation. The first eggs of the third generation were found on August 25, the eggs needed 11 days for full development and the first larvae hatched on September 5. The larvae needed 25-29 days for full development and the first pupated after 25 days on September 30. The pupae entered diapause, of which 6 normal-looking imagines hatched erratically and were not counted. These diapausing pupae generated the imagines of the first generation of the subsequent year, after overwintering. The whole fourth generation needed 36 days to reach pupation of the first individuals.

Normally, the duration of the overwintering normally could only be inferred from previous observations. In our case, the pupae were washed, disinfected briskly with 5% bleach, kept outside on moss in a single layer in a cardboard box sheltered from direct sun and rain until December when they were checked and transferred to and stored in a refrigerator at 5^{o}C for overwintering. 10 pupae were placed outdoors for overwintering in the same cardboard box and the 7 live remaining pupae after overwintering were transferred to a hatching container placed outdoors, where 5 of them hatched between April 3rd and April 8. Judging from the emergence of these imagines, the diapause of the 4th generation lasted cca. 185 days.

Our results showed that *S. orion* life cycle in captive breeding on *H. telephium* under climatic conditions in Southern Romania consisted of 4 generations per year, with an effective generation turnover between 46 and 59 days between larval eclosion and oviposition, the last generation entering diapause and giving the imagines of the first generation of the subsequent year. This did seem normal to us but later, checking the published data of other authors we noticed that the largest number of generation per year reported was of 3 generations, in Southern Germany, by TRÄNKNER & NUSS (2005) with a comprehensive discussion in (REINHARDT, 2003). This discrepancy could be explained by differing climatic conditions, especially with regard to temperature. In our experiments, the generation turnover shortened, so one more generation could develop over a year, possibly also due to a longer period with favorable temperatures.

It also need to be noted that our experiment gave the larvae ad-libitum access to good quality food resources and provided shelter both from inclement weather and predator, and such optimal conditions may prove difficult to find under natural conditions. Also, during this experiment, after the hatching of the larvae of the first generation we did not count the survival rate of the larvae, so it is possible that cannibalism occurred and so a certain degree of selection and pressure towards faster development might have also occurred.

These facts suggest a developmental plasticity, allowing the individuals to adapt their phenology to the climatic condition of the moment and so exploit windows of opportunity open by favorable climatic (or nutritive) conditions.

Another interesting fact was a relatively good synchronization of developmental stages, the developmental stage length variation falling within 2-3 days for the vast majority of individuals. This made for a very clear-cut separation of generations on wing, while our observations in the field on multiple populations in various locations in Romania and Macedonia showed much more continuous flight periods with potentially overlapping generations.

The observed synchronization in our experiment might be a result of cannibalism and/or could be explained by a higher degree of genetic uniformity in our population than under natural conditions, factor that should not be neglected as it has been shown that inbreeding is an actual possibility and could represent a serious threat in small butterfly populations (SACCHERI et al., 1998). Moore experimental work is needed to check for this fact.

Some individuals of all generations entered diapause and some hatched erratically throughout the summer and autumn without any explanation of the triggers and mechanisms involved in *selective* inducement of diapause and then for the erratic "escape" from diapause *exhibited by only some individuals*, as reported by TRÄNKNER & NUSS (2005) and discussed by MÜLLER, 1992. These erratic emergences of imagines not complying with the "normal" life cycle of the majority of their generation suggest that under natural conditions the adult populations on wing at a certain time would comprise also individuals from other generations.

This also points to a lack of hormonal inhibition of reproduction in later generations as observed in some migratory Sphingidae species whose last generation developing at more northern latitudes, even if reached maturity and imagines emerged, they seem to be sterile and do not reproduce. Our findings seem to support such hypothesis and warrant more thorough experimental research towards its empirical validation or rejection.

The presumed voltinism elasticity and lack of hormonal inhibition of reproduction in late generations could, coupled with a very long diapause lasting almost half a year or more and other personal data describing anomalous 5^{th} generations more frequent during the last years, with unusually warm and excessively long autumns, suggest that the active life cycle could be prolonged and more generations could be pushed into the diapause period of the year, provided optimal conditions are given for longer period of time, even if the active life cycle could not be accelerated and no more generations could be compressed in the natural life cycle by shrinking the developmental stages into shorter generation turnover. This opens the perspective of more efficient mass breeding of this and similar lepidopteran species to obtain larger adult numbers for (re)introduction conservation programs.

CONCLUSIONS

a) - Captive breeding of *S. orion* on *H. telephium* under climatic conditions in Southern Romania is possible and could prove a viable option for generation of larger numbers of adults in artificial conditions.

b) - *S. orion* life cycle in captive breeding on *H. telephium* under climatic conditions in Southern Romania has 4 generations per year, with an effective generation turnover between 46 and 59 days our findings differ from other published experimental data reporting only 3 generations, in Southern Germany, by TRÄNKNER & NUSS (2005). This discrepancy could be explained by several factors:

i - presumably more favorable climatic conditions in our experiment;

ii - optimality of other ecological conditions may prove difficult to find under natural settings;

iii - a certain degree of selection and pressure towards faster development, cause by e.g. cannibalism, behavioral reproductive depression due to confinement;

c) - These discrepancies regarding voltinism and length of developmental stages under different environmental conditions suggest a developmental plasticity, allowing the individuals to adapt their phenology to the climatic condition of the moment and so exploit windows of opportunity opened by favorable climatic (or nutritive) conditions as suggested by HUNTER & MCNEIL (1996) and HOPPER (1999).

d) - A relatively good synchronization of developmental stages was observed, resulting in very distinct flight periods of subsequent generations on wing, contradicting our multiple observations in the field which showed much more continuous flight periods with potentially overlapping generations. The observed synchronization in our experiment might be a result of cannibalism and/or could be explained by a higher degree of genetic uniformity in our population than under natural conditions. Moore experimental work is needed to check for this fact.

e) - This also point to a lack of hormonal inhibition of reproduction in later generations as observed in some migratory Sphingidae species whose last generation developing at more northern latitudes, even if reached maturity and imagines emerged, they seem to be sterile and do not reproduce.

f) - The presumed voltinism elasticity and lack of hormonal inhibition of reproduction in late generations could, coupled with a very long diapause lasting almost half a year or more suggest that the active life cycle could be prolonged and more generations could be pushed into the diapause period of the year, provided optimal conditions are given for longer period of time, even if the active life cycle could not be accelerated and no more generations could be compressed in the natural life cycle by shrinking the developmental stages into shorter generation turnover. This opens the perspective of more efficient mass breeding of this and similar lepidopteran species to obtain larger adult numbers

for (re)introduction conservation programs. Our findings generated many hypotheses and prompted more thorough experimental research towards their empirical validation or rejection which will be reported elsewhere.

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THE SPECIES OF MACROLEPIDOPTERA COLLECTED FROM THE GUȘTERIȚA HILL, SIBIU, EXISTING WITHIN THE COLLECTION OF DR. VIKTOR WEINDEL

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Abstract. The present paper represents a contribution to the knowledge of the Macrolepidoptera collected in the past from the area around Sibiu. This material collected from the Gușterița Hill, near Sibiu is presented in according with the recent systematic list published about Romanian Lepidoptera (RAKOSY et al., 2003). Among those 10 families with a number of 152 species, the best represented species belong to the families: Papilionidae, Lycaenidae, Geometridae and Nymphalidae. The period of collected material is more than 54 years that represents an important contribution to the knowledge of Macrolepidoptera collected in the past from Gușterița Hill, near Sibiu.

Keywords: Lepidoptera, collection Dr. V. Weindel, Gușterița Hill, Sibiu, biodiversity.

Rezumat. Specii de macrolepidoptere colectate din Dealul Gușteriței-Sibiu, existente în cadrul colecției dr. V. Weindel. Prezenta lucrare reprezintă o contribuție importantă la cunoașterea lepidopterelor colectate în trecut din împrejurimile Sibiului. Cea mai mare parte a lucrării o reprezintă prezentarea sistematică a materialului colectat din Dealul Gușteriței folosindu-se nomenclatura din ultima listă publicată a fluturilor din România: Catalogul lepidopterelor României (RÁKOSY et al., 2003). Dintre cele 10 familii care cuprind un număr de 152 de specii, cel mai bine reprezentate sunt speciile aparținând Familiilor: Geometridae, Papilionidae, Lycaenidae și Nymphalidae. Perioada colectărilor acoperă un interval de timp de peste 54 de ani ceea ce reprezintă o contribuție importantă la cunoașterea speciilor de lepidoptere colectate în trecut din Dealul Gușteriței.

Cuvinte cheie: lepidoptere, colecție Dr. V. Weindel, Dealul Gușteriței, Sibiu, biodiversitate.

INTRODUCTION

The present study is a part of an ample project for an inventory of the Lepidoptera species collected in the past and in the present around Sibiu city. Along the time I collected and studied species from the Forest Dumbrava Sibiului and from around Sibiel village situated at 22 km distance from Sibiu (STANCĂ-MOISE, 2016).

In my project I also studied the Collection Dr. Viktor Weindel, one of the most important Macrolepidoptera collection preserved at the Natural History Museum in Sibiu.

Along the time, some specialists studied this material (SCHNEIDER, 1984; SCHNEIDER, 1996; SZÉKELY, 1996, 2003, 2008, 2014; VLAD-ANTONIE & CIOBANU, 2004; MOISE, 2011a,b,c,d; STANCĂ-MOISE, 2002, 2015, 2017). On remarks the paper of Dr. C. Bucșa and Dr. I. Tăușan that presents the entomological studies made around Sibiu, by many specialists and their collections during the time, inclusive Dr. Viktor Weindel collection (BUCȘA &TĂUȘAN, 2011).

In the material existing in the Dr. V. Weindel collection I selected the species collected near Sibiu, on the Gușterița Hill.

The Gușterița Hill is situated in the N-Eastern part of Sibiu city, and because of the favourable ground and climate, has a xerothermic steppic vegetation, similar with that in Transsylvanian Plain, and also in Blaj zone (SCHNEIDER-BINDER, 1971). The Gușterița Hill is characterized by mixed forests with a different specific to the other forest massifs from the Sibiu depression and its marginal hills, situated in the triangle formed by the confluence of the river Cibin with the river Olt, from Tălmaciu conglomerates till the Podu Olt (SCHNEIDER-BINDER, 1972).

These forest on the Gușterița Hill are similar, by their floristic composition (ANTONIE, 2016; MOISE, 2016), with those from the Olt narrow path, here are present species of: *Quercus robur, Quercus petraea, Tilia cordata, Cerasus avium, Sorbus torminalis,and Carpinus betulus*. The grassy vegetation is characterized by balcanic and mediterranaeans influences with species of: *Galium kitaibelianum, G. valantioides, Genista ovata, Primula columnae, Tamus communis* that give a southern character of these forests from the Gușterița Hill (SCHNEIDER-BINDER ,1973).

In the present day the Gușterița Hill sufferend a major transformation because of the man!s intervention that cleared a part of forest, and the grounds were transfered in the intravillan part destined the dwellings constructions.

All these changes of the ecosystem had a negativ effect on the flora that constitute a food source for larvas and a support to deposition of eggs for adults and also on the former lepidoptera fauna.

MATERIALS AND METHOD

Studying the collection V. Weindel preserved at the Natural History Museum in Sibiu, I found 152 Lepidoptera species, colected only on the Gușterița Hill, near Sibiu. I studied all data in this collection and for every species I noticed data about collected specimen with their day, month and the year of collection. I made also a verification of all data after the Catalogue: *Macrolepidopterele din colecția Dr. V. Weindel* published by SCHNEIDER

(1984). In this paper is mentioned that this collection of V. Weindel was taken by acquisition from Ntural History Museum in Sibiu in 1964 and contains 4.322 samples of Macro- and Microlepidoptera collected during the period 1900-1959. The species proceed from South of Transsylvania, but also from environs of Sibiu. Unfortunately some labels are incomplete, without information about the day, the month or the year of collection. The systematic list is brought up to date in accordance with the nomenclature presented by „*Catalogul lepidopterelor Romaniei*" (RÁKOSY et al., 2003), with an identification number for every species.

RESULTS AND DISCUSSIONS

The paper presents the systematic list of the Lepidoptera species collected during the years 1903-1957, on the Gușterița Hill near Sibiu city. These species are in the Lepidoptera Collection of *Dr. Victor Weindel*, preserved at the Natural History Museum in Sibiu.

1. Family HESPERIOIDEA

1. Erynis tages (Linnaeus, 1758): July 10, 1903, July 23, 1921, July 25,1921, July 20, 1924 (3410 Ro, 6879 K & R)
2. Carcharodus alceae (Esper, 1780): July 13, 1924 (3 ex.) (3412 Ro, 6882 K & R)
3. Pyrgus malvae malvae (Linnaeus, 1758): April 16, 1952 (3427 Ro, 6904 K & R)
4. Carterocephalus palaemon (Pallas, 1771): June 9 (without a year) (3435 Ro, 6919 K & R)
5. Thymelicus lineola (Ochsenheimer, 1808): July 13,1924 (3438 Ro, 6923 K & R)
6. Hesperia comma (Linnaeus, 1758): September 26, 1920 (2 ex.) (3442 Ro, 6928 K & R)
7. Ochlodes venatus (Bremer & Grey, 1853): July 9,VII.1907 (2 ex.), July 25, 1921 (3444 Ro, 6930 K & R)

2. Family PAPILIONIDAE

8. Iphiclides podalirius podalirius (Linnaeus, 1758): Aprlil 1920 (4 ex.), July 4,1904, July 20, 1917, July 26, 1921 (2 ex.), July 20, 1924 (2 ex.), July 10, 1938 (2 ex.), April 6, 1957, April 28, 1957 (3458 Ro, 6958 K & R)
9. Papilio machaon machaon Linnaeus, 1758: July 4, 1904 (2 ex.), July 28, 1904, April, 1920 (2 ex.) makes the day of collection, July 25, 1921, July 28, 1921 (4 ex.), September 26, 1920 (2 ex.), July 13, 1924, April 5, 1925, July 10, 1938, July 9, 1953, July 17, 1955 (3460 Ro, 6960 K & R)
10. Leptidea sinapis sinapis (Linnaeus, 1758): May 6, 1956, April 6, 1957, July 24, 1955 (3464 Ro, 6966 K & R)
11. Anthocharis cardamines (Linnaeus, 1758): April 28, 1957 (3469 Ro, 6973 K & R)
12. Pieris rapae (Linnaeus, 1758): July 7, 1922 (3 ex.), July 14, 1922, August 17, 1922, July 2, 1950 (3478 Ro, 6998 K&R)
13. Pontia daplidice auct. September 31, 1957 (3484 Ro, 7003 K & R)
14. Pieris rapae (Linnaeus, 1758): May 6, 1956, August 7, 1904, August 15, 1921 (3 ex.), September 25, 1921 (2 ex.) (3478 Ro, 6998 K & R)
15. Pieris napi napi (Linnaeus, 1758): July 28, 1904, August 18, 1921 (3480 Ro, 7000 K & R)
16. Colias hyale (Linnaeus, 1758): July 28, 1904, July 25, 1921, July 26, 1921, August 28, 1921 (5 ex.), July 23-27, 1921 (5 ex.), August 2, 1921, September 25, 1921 (6 ex.) September 28, 1919, 19.X.1921, July 24, 1955 (3492 Ro, 7021 K & R)
17. Colias croceus (Fourcroy, 1785): August 10, 1903, July 4, 1904, September 28, 1919 (2 ex.), September 26, 1920, September 31, 1957, 1925(3489 Ro, 7015 K & R)
18. Colias myrmidone myrmidone (Esper, 1780): August 4, 1925 (3490 Ro, 7017 K & R)
19. Colias chrysotheme chrysotheme (Esper, 1780): September 16, 1957 (2 ex.) (3491 Ro, 7018 K & R)

3. Family LYCAENIDAE

20. Hameris lucina (Linnaeus, 1758): July 25, 1921 (3499 Ro, 7030 K & R)
21. Callophrys rubi (Linnaeus, 1758): June15, 1903 (3518 Ro, 7058 K & R)
22. Satyrium ilicis (Esper, 1779): July 6, 1907 (3523 Ro, 7065 K & R)
23. Lycaena phlaeas phlaeas (Linnaeus, 1761): September 26, 1920, April 28, 1957 (3502 Ro, 7034 K & R)
24. Lycaena tityrus tityrus (Poda, 1761): July 26, 1921, August 21, 1921(3506 Ro, 7039 K & R)
25. Cupido minimus minimus (Fuessly, 1775=alsus [Denis & Schiffermüller], 1775: July 6, 1907, July 26, 1921 (3533 Ro, 7088 K & R)
26. Cupido osiris (Meigen, 1829): June 15, 1903 (3534 Ro, 7089 K & R)
27. Everes argiades (Pallas, 1771): July 25, 1921 (3 ex.), July 26, 1921, August 28, 1921 (2 ex.), April 30, 1950 (2 ex.), May 2, 1952 (3536 Ro, 7093 K & R)
28. Everes decolorata (Staudinger, 1886): July 13, 1924, July 20, 1924 (3537 Ro, 7094 K & R)
29. Celastrina argiolus (Linnaeus, 1761): July 6, 1907, July 25, 1921 (2 ex.) (3540 Ro, 7097 K & R)
30. Maculinea arion (Linnaeus, 1761): July 13, 1924, July 24, 1955 (2 ex.) (3551 Ro, 7112 K & R)

31. Plebeius argus argus (Linnaeus, 1761): August 10, 1903, July 28, 1904, August 7, 1904, July 25, 1921, July 26, 1921 (5 ex.), August 21, 1921, July 13, 1924 (2 ex.), July 20, 1924, July 20, 1952, June 17, 1956, July 19, 1956, July 24, 1955 (6 ex.) (3560 Ro, 7127 K & R)
32. Plebeius argyrognomon (Bergsträsser, 1779): July 20, 1952, July 19, 1956 (3562 Ro, 7129 K & R)
33. Polyommatus thersites (Cantener, 1835): September 31, 1951 (3577 Ro, 7162 K & R)
34. Polyommatus icarus (Rottemburg, 1775): August 3, 1904, July 25, 1921 (2 ex.), July 26, 1921, August 15, 1921 (5 ex.), August 28, 1921, June 30, 1957, September 16, 1951 (2 ex.) (3578 Ro, 7163 K & R)
35. Meleageria daphnis ([Denis & Schiffermüller], 1775): July 22-25, 1921 (5 ex.), June 30, 1957 (3580 Ro, 7171 K & R)
36. Meleargia bellargus (Rottemburg, 1755): June 15, 1903, August 21, 1921 (3581 Ro, 7172 K & R)

4. Family NYMPHALIDAE

37. Argynnis pandora ([Denis & Schiffermüller], 1775): August 3, 1904 (3594 Ro, 7203 K & R)
38. Argynnis adippe ([Denis & Schiffermüller], 1775): June 17, 1956 (3596 Ro, 7205 K & R)
39. Neptis sappho [Pallas, 1771]: July 13, 1924, August 28, 1956 (3652 Ro, 7290 K& R)
40. Issoria lathonia (Linnaeus, 1758): October 3, 1920, August 28, 1921 (3600 Ro, 7210 K & R)
41. Boloria euphrosyne (Linnaeus, 1758): May 2, 1952 (2 ex.) (3607 Ro, 7220 K & R)
42. Boloria selene ([Denis & Schiffermüller]: 1775): July 17, 1921 (3609 Ro, 7222 K & R)
43. Vanessa cardui (Linnaeus, 1758): July 6, 1907 (3617 Ro, 7245 K & R)
44. Aglais urticae (Linnaeus, 1758): August 27, 1921 (3621 Ro, 7250 K & R)
45. Polygonia c-album (Linnaeus, 1758): July 6, 1907 (2 ex.) (3623 Ro, 7252 K & R)
46. Melitaea phoebe ([Denis & Schiffermüller], 1775): August 19, 1956, August 23, 1956 (3637 Ro, 7271 K & R)
47. Melitaea trivia trivia ([Denis & Schiffermüller], 1775): July 16, 1921 (3640 Ro, 7274 K & R)
48. Melitaea didyma dydima (Esper, 1778): July 26, 1921 (4 ex.) (3641 Ro, 7276 K & R)
49. Melitaea aurelia aurelia Nickerl, 1850: July 30, 1952, July 24, 1955 (2 ex.), June 26, 1921 (3 ex.) (3643 Ro, 7280 K & R)
50. Melitaea athalia athalia (Rottemburg, 1775): June 26, 1921 (3 ex.), July 20, 1952 (3645 Ro, 7283 K & R)
51. Apatura iris (Linnaeus, 1758): August 4, 1923 (3658 Ro, 7299 K & R)
52. Pararge aegeria (Linnaeus, 1758): July 13, 1924, May 6, 1956, (3665 Ro, 7307 K & R)
53. Lasiommata megera megera (Linnaeus, 1767): September 19, 1920, September 26, 1920 (3 ex.), July 26, 1921, August 19, 1956, July 13, 1924, September 16, 1951 (3667 Ro, 7309 K & R)
54. Lasiommata maera maera (Linnaeus, 1758): August 3, 1904 (3668 Ro, 7312 K & R)
55. Lopinga achine achine (Scopoli, 1763): June 15, 1903, June 17, 1956 (3670 Ro, 7315 K & R)
56. Coenonympha glycerion glycwrion (Borkhaunsen, 1788): June 12, 1921, August 13, 1921, August 21, 1921, *Coenonympha pamphilus* (Linnaeus, 1758): July 28, 1904 (2 ex.), July 6, 1907, July 25, 1921, July 26, 1921, September 26, 1920, August 15, 1921, April 16, 1951, July 20, 1952, July 17, 1955 (3677 Ro, 7334 K & R)
57. Aphantopus hyperatus (Linnaeus, 1758): July 23, 1921, July 25, 1921, July 13, 1924, July 20, 1952 (3682 Ro, 7344 K & R)
58. Melanargia galathea (Linnaeus, 1758): June 30, 1903, July 6, 1907 (4 ex.), June 29, 1922 (2 ex.) (3704 Ro, 7415 K & R)
59. Minois dryas (Scopoli, 1763): July 25, 1921 (2 ex.), July 26, 1921, July 27, 1921, July 24, 1955 (3706 Ro, 7427 K & R)
60. Chazara briseis briseis (Linnaeus, 1764): July 28, 1904, July 20, 1914, July 26, 1921, August 15, 1921 (2 ex.), August 19, 1956 (4 ex.) (3718 Ro, 7449 K & R)

5. Family LEMONIIDAE

61. Lemonia taraxaci ([Denis&Schiffermüller], 1775): 9 ex. July 22, 1942, August (7) without day and year (3359 Ro, 6806 K & R)

6. Family SPHINGOIDEA

62. Mimas tiliae (Linnaeus, 1758): August 7, 1939 (3366 Ro, 6819 K & R)
63. Agrius convolvuli (Linnaeus, 1758): August 7, 1938 (3373 Ro, 6828 K & R)
64. Hemaris tityus (Linnaeus, 1758): May 1, 1927 (3384 Ro, 6839 K & R)

7. Family GEOMETROIDEA

65. Heliomata glarearia ([Denis & Schiffermüller]: April 14, 1921, May 8, 1921, July 23, 1921, July 28, 1921 (3774 Ro, 7537 K & R)
66. Macaria alternaria Hübner, [1805]: July 23, 1921, July 25, 1921, August 6, 1921, August (without day and year) (3777 Ro, 7540 K & R)
67. Chiasmia clathrata (Linnaeus, 1758): July 21-23, 1921(3784 Ro, 7547 K & R)

68. Plagodis pulveraria (Linnaeus, 1758): July 27, 1921 (2 ex.) (3806 Ro, 7606 K & R)
69. Plagodis dolabraria (Linnaeus, 1758): July 31, 1938 (3807 Ro, 7607 K & R)
70. Pseudopanthera macularia (Linnaeus, 1758): June 15, 1903 (3816 Ro, 7620 K & R)
71. Selenia teralunaria (Hufnagel, 1767): August 10, 1903 (3834 Ro, 7643 K & R)
72. Artiora evonymaria ([Denis & Schiffermüller]: 1775): August 28, 1921, August 1937, August 6, 1939 (3836 Ro, 7645 K & R)
73. Peribatodes rhomboidaria ([Denis & Schiffermüller]: 1775): August 21, 1921 (3885 Ro, 7754 K & R)
74. Ectropis bistortata Goeze, 1781: June 30, 1903 (3912 Ro, 7796 K & R)
75. Ematurga atomaria atomaria (Linnaeus, 1758): March 27, 1921 (3920 Ro, 7804 K & R)
76. Pseudoterpna pruinata (Hufnagel, 1767): August 2, 1925, August 28, 1938 (4006 Ro, 7965 K & R)
77. Thetidia smaragdaria (Fabricius, 1787): August 13, 1938 (4013 Ro, 7975 K & R)
78. Chlorissa viridata (Linnaeus, 1758): July 27¸ 1921(4017 Ro, 7982 K & R)
79. Chlorissa cloraria (Hübner, [1813]): July 23, 1921 (2 ex.), July 27, 1921, August 6, 1921, August 8, 1921 (2 ex.) (4018 Ro, 7983 K & R)
80. Hemistola chrysoprasaria (Esper, 1795): July 27, 1921, August 5, 1939 (4026 Ro, 8000 K & R)
81. Chylophora punctaria (Linnaeus, 1758): May 14, 1921(4040 Ro, 8022 K & R)
82. Timandra griseata auct. (*comae* A. Schmidt, 1931): July 23, 1921, August 28, 1921 (4044 Ro, 8028 K & R)
*83. Scopula orn*ata (Scopoli, 1763): July 27, 1921 (2 ex.), August 6, 1921 (2 ex.), August 10, 1921 (4054 Ro, 8045 K & R)
84. Scopula rubiginata (Hufnagel, 1767): August 8, 1921, August 1, 1939 (4057 Ro, 8054 K& R)
85. Scopula marginepunctata (Goeze, 1781): August (without day and year) (4059 Ro, 8059 K & R)
86. Scopula incanata (Linnaeus, 1758): July 25, 1921, August 25, 1921 (4060 Ro, 8060 K & R)
87. Idaea ochrata (Scopoli, 1763): July 25, 1921 (4077 Ro, 8099 K& R)
88. Idaea moniliata ([Denis & Schiffermüller]: 1775): July 23, 1921 (4085 Ro, 8120 K & R)
89. Idaea dilutaria (Hübner, 1799): July 10, 1921(4091 Ro, 8136 K& R)
90. Idaea seriata (Schrank, 1802): August 17, 1921 (4095 Ro, 8155 K& R)
91. Idaea pallidata ([Denis & Schiffermüller], 1775): May 14, 1921 (4099 Ro, 8168 K & R)
92. Idaea aversata aversata (Linnaeus, 1758): July 25, 1921 (4105 Ro, 8184 K & R)
93. Rhodostrophia vibricaria (Clerck, 1759): August 28, 1921 (2 ex.), August (without day and year) (4111 Ro, 8205 K& R)
94. Scotopteryx chenopodiata (Linnaeus, 1758): August 10, 1903, July 23, 1923, July 27, 1923 (2 ex.) (4129 Ro, 8239 K& R)
95. Scotopteryx bipunctaria ([Denis & Schiffermüller], 1775): August 10, 1903, July 27, 1921 (2 ex.), August 6, 1921, August 10, 1921 (4128 Ro, 8236 K & R)
96. Catarhoe rubidata ([Denis & Schiffermüller], 1775): July 13, 1934 (4150 Ro, 8268 K & R)
97. Catarhoe cuculata (Hufnagel, 1767): May 8, 1921 (4151 Ro, 8269 K & R)
98. Epirrhoe rivata (Hübner, 1813): August 9, 1921 (4156 Ro, 8277 K & R)
99. Epirrhoe galiata ([Denis & Schiffermüller], 1775): July 31, 1938, July (without day and year) (4158 Ro, 8279 K & R)
100. Camptogramma bilineata (Linnaeus, 1758): June 7, 1954 (4162 Ro, 8289 K & R)
101. Pelurga comitata (Linnaeus, 1758): August 25, 1921 (4177 Ro, 8314 K & R)
102. Cosmorhoe ocellata (Linnaeus, 1758): August 28, 1921(4182 Ro, 8319 K & R)
103. Colostygia pectinataria (Knoch, 1781): August 2, 1921, August 7, 1938 (4226 Ro, 8385 K & R)
105. Melanthia procellata ([Denis & Schiffermüller], 1775): August 1, 1938, August 14, 1938 (4242 Ro, 8411 K & R)
106. Triphosa dubitata (Linnaeus, 1758): June 12, 1921 (4255 Ro, 8428 K& R)
107. Perizoma lugdunaria (Herrich-Schäffer, 1855): August 8, 1921, August (without day and year) (4276 Ro, 8458 K & R)
108. Perizoma flavofasciata (Thunberg, 1792): July 16, 1928 (4281 Ro, 8464 K & R)
109. Eupithecia centaureata ([Denis & Schiffermüller], 1775): July 27, 1921, August (without day and year) (4317 Ro, 8509 K & R)
110. Eupithecia tripunctaria Herrich-Schäffer, 1852: July 25, 1921 (4334 Ro, 8535 K & R)
111. Minoa murinata (Scopoli, 1763): June (without day and year) (4413 Ro, 8663 K & R)
112. Clostera anastomosis (Linnaeus, 1758): June 31, 1938 (4440 Ro, 8701 K & R)
113. Notodonta ziczac (Linnaeus, 1758): August (without day and year) (4455 Ro, 8719 K & R)
114. Cryphia erepticula (Treitschke, 1825): August 4, 1939 (4529 Ro, 8806 K & R)
115. Idia calvaria ([Denis&Schiffermüller], 1775): July 23, 1939 (4540 Ro, 8835 K & R)
116. Polypogon tentacularia (Linnaeus, 1758): July 27, 1921, August 9, 1921, August 10, 1921 (4552 Ro, 8849 K & R)
117. Catocala elocata elocata (Esper, 1787): August 27, 1939, August 28, 1938 (4574 Ro, 8877 K & R)
118. Catocala electa electa (Vieweg, 1790): August 3, 1939 (4577 Ro, 8883 K & R)
119. Lygephila craccae ([Denis&Schiffermüller], 1775): June 1938 (without day) (4602 Ro, 8934 K & R)
120. Euclidia glyphica (Linnaeus, 1758): August 19, 1956 (4617 Ro, 8969 K & R)
121. Hypena proboscidalis (Linnaeus, 1758): July 3, 1950 (4633 Ro, 8994 K & R)

122. Phytometra viridaria (Clerck, 1759): July 25, 1921, August 6, 1921, August (without day and year) (4638 Ro, 9006 K & R)
123. Rivula sericealis (Scopoli, 1763): July 26, 1921 (4640 Ro, 9008 K & R)
124. Abrostola trigeminna Werneburg, 1864: June 12, 1921 (4692 Ro, 9093 K & R)
125. Trichoplusia ni (Hübner, 1803): August 1, 1939 (4686 Ro, 9081 K& R)
126. Emmelia trabealis (Scopoli, 1763): July 6, 1907, July 27, 1921 (2 ex.), August 6, 1921, August 15, 1921 (4696 Ro, 9097 K & R)
127. Pseudeustrotia candidula candidula ([Denis & Schiffermüller]: August 26, 1921 (4713 Ro, 9122 K & R)
128. Amphipyra pyramidea (Linnaeus, 1758): August (without day and year) (4803 Ro, 9307 K & R)
129. Amphipyra berbera (Rungs, 1949): August 31, 1921 (4804 Ro, 9308 K & R)
130. Heliothis viriplaca viriplaca (Hufnagel, 1766): August 27, 1939 (4830 Ro, 9364 K & R)
131. Heliothis peltigera ([Denis & Schiffermüller]: 1775): July 22, 1938 (4833 Ro, 9367 K & R)
132. Thalpophila matura (Hufnagel, 1767): August 10, 1921 (4901 Ro, 9496 K & R)
133. Phlogophora meticulosa (Linnaeus, 1758): August 1934 (without day)
134. Calamia tridens tridens (Hufnagel, 1766): July 30, 1939 (5013 Ro, 9848 K & R)
135. Dianobia contigua ([Denis & Schiffermüller], 1775): July 13, 1938 (5061 Ro, 9919 K & R)
136. Hada nana (Hufnagel, 1767): August 28, 1938 (5076 Ro, 9925 K& R)
137. Hadena irregularis (Hufnagel, 1766): August 1, 1939 (5118 Ro, 9964 K & R)
138. Tholera decimalis (Poda, 1761): August 1937 (without day) (5128 Ro, 10065 K & R)
139. Mythimna turca (Linnaeus, 1761): August 27, 1939 (5131 Ro, 9999 K & R)
140. Hyphilare albipuncta ([Denis & Schiffermüller], 1775): August 28, 1938, August 6, 1939 (5144 Ro, 10002 K & R)
141. Conistra rubiginosa (Scopoli, 1763): April 5, 1925 (5218 Ro, 9603 K & R)

8. Family NOCTUIDAE

142. Noctua pronuba (Linnaeus, 1758): August (without day and year)
143. Xestia ditapezium ([Denis & Schiffermüller], 1775): July 23, 1921 (5361 Ro, 10199 K & R)
144. Xestia c-nigrum (Linnaeus, 1758): August 27, 1921 (5362 Ro, 10200 K & R)
145. Euxoa obelisca ([Denis&Schiffermüller], 1775): August 13, 1921 (5405 Ro, 10282 K & R)
146. Agrotis segetum ([Denis&Schiffermüller], 1775): August (without day and year) (5427 Ro, 10351 K & R)

9. Family NOLIDAE

147. Nola cucullatella (Linnaeus, 1758): July 10, 1921 (5477 Ro, 10427 K & R)
148. Nycteola degenerana (Hübner, 1799): August 28, 1921 (5487 Ro, 10443 K & R)
149. Bena prasinana auct.: VII (without day and year)

10. Family ARCTIIDAE

150. Amata phegea (Linnaeus, 1758): July 20, 1952 (5531 Ro, 10517 K & R)
151. Eilema complana (Linnaeus, 1758): July 25, 1921 (5519 Ro, 10490 K & R)
152. Euplagia quadripunctaria (Poda, 1761): July 20, 1924 (2 ex.) (5584 Ro, 10605 K & R)

CONCLUSIONS

In this list, there are mentioned 152 species, belonging to 10 families of Lepidoptera from the scientific collection of DR. Victor Weindel.

The city Sibiu and the environmental zones, because of their geographical position, constituted a faunal region, well delimited, where there were collected different species of insects, during a long period of time.

So it is possible to study the evolution of the insect fauna in this area. The Lepidoptera collections began early in 1900 and they were continued until the present day (2018). The present study centralizes the collected species still from the year 1903 until in the year 1957 in the Lepidoptera Collection of Dr. Viktor Weindel.

Those 152 species belonging to 10 families constitute an important documentary and scientific material that characterizes biogeographically the marginal zones of the Sibiu city.

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THE EXPEDITION OF HUMBERTO DOS PASSOS-FREITAS IN THE DANUBE DELTA (ROMANIA) - 1922 AND THE "GRIGORE ANTIPA" NATIONAL MUSEUM OF NATURAL HISTORY FROM BUCHAREST

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Abstract. Between April 19th and May 19th, 1922 Humberto dos Passos-Freitas, a businessman and sportsman from Madeira, made an expedition to the Danube Delta, Romania. Rudolph Pop, the taxidermist of the Museum of Natural History in Bucharest, accompanied him during the expedition,. The results of the observations and collections made in the Danube Delta are recorded in a 41 page paper, published in Bucharest in 1922, at Socec Publishing House. In this expedition, 127 species of birds belonging to 36 families and 16 orders were observed. At that time, the authors called this report as preliminary, that is, the first part; unfortunately, the second part never appeared.

Keywords: Expedition, Danube Delta, Romania, Birds, Madeira, Funchal, Rudolph Pop, Grigore Antipa.

Rezumat. Expediția lui Humberto dos Passos Freitas în Delta Dunării (România) - 1922 și Muzeul Național de Istorie Naturală "Grigore Antipa" din București. În perioada 19 aprilie-19 mai 1922 Humberto dos Passos Freitas, afacerist și sportiv din Madeira face o expediție în Delta Dunării, România. Pe tot parcursul expediției este însoțit de taxidermistul Muzeului de Istorie Naturală din Bucuresti, Rudolph Pop. Rezultatele observațiilor si ale colectărilor din Delta Dunării sunt consemnate într-o lucrare de 41 de pagini, apărută în București, în 1922, la Editura Socec. În această expediție au fost observate 127 de specii de păsări din 36 de familii și 16 ordine. La vremea aceea autorii au numit acest raport ca preliminar, adică prima parte, din păcate cea de-a doua parte nu a mai apărut niciodată.

Cuvinte cheie: Expediție, Delta Dunării, România, păsări, Madeira, Funchal, Rudolph Pop, Grigore Antipa.

INTRODUCTION

Personal life. Humberto dos Passos-Freitas (1893-1926) was born in Funchal, Madeira, and he was the heir of a considerable fortune that allowed him to be a passionate practitioner of sports, travel and literature. Humberto dos Passos Freitas was an active character linked to Funchal, where he took part in all sports or social events. Patriot and convinced monarchist, he made himself remarked by the active life within the community of Madeira Island. His name is linked to the establishment of the first sports club in Madeira. On November 8th, 1909, he set up Madeira Sports Club (Club Sports da Madeira) (RODRIGUES, 2000), when he brought a football from England and the taste for the game. He wanted to set up a club to bring together a group of athletes to practice activities "against alcohol consumption and a good development of morality and hygiene" (NASCIMENTO, 2011). He even participated in an 18 km long cross, which became traditional, between Funchal and Câmara de Lobos, called "I Marathona a Câmara de Lobos – ida e volta", where he won the third place, representing Club Sports da Madeira (MOTA, 1945).

Humberto dos Passos Freitas was married to Maria Glafira Gomes de Freitas and had a daughter, Dalila Rohena, married Pereira. Dalila Rohena was born on November 16th, 1914 in Funchal. She graduated from primary studies in Sintra, Portugal. She lived in London where she studied piano and foreign languages. She collaborated with Madeira publications like "*Diário da Madeira*" and the magazine "*Os nossos filhos*". As a writer, she is known under the pseudonyms: Diana Passos Freitas, Teresa Maria or Dalila Rohena Diana (GUERRA ANDRADE, 1999). She published: "*Fogo entre cinzas*" (1946), "*Retalhos*" (1949), etc. (ASCENSÃO DE MACEDO, 2013).

World War I. During the First World War, two bombardments of the German submarines hit the bay and the city of Funchal. The first one took place in the morning of December 3rd, 1916, and the target was the sinking of the "*Dacia*" ship under the English flag and its escorts, "*Surprise*" and "*Kanguroo*", under the French pavilion. "*Dacia*" had the task of supervising submarine cables. Although the damages to the buildings were insignificant, 34 sailors from the „*Surprise*" ship died and many were injured (RODRIGUES, 2017). The Red Cross of Madeira (Cruz Vermelha na Madeira) was directly involved in rescuing the wounded andmany civilians voluntarily participated in damage reduction. For the transport of the wounded, Humberto dos Passos Freitas made available to the Red Cross his yacht, his car and also a donation of a large sum of money for logistics. , his. These dramatic moments, as well as the involvement of the Funchal community, were mentioned in the press and noted in the reports of the Red Cross of Madeira (FREITAS GOMES, 2017): *Diário Da Madeira* (December 1916, 1917), *A Capital* (1916), *Republica* (1916), *O Secolo* (1916), *Ilustração Portuguesa* (1917).

The second bombardment began at 6.20 p.m., on December 12, 1917, when a German submarine targeted only land targets. The action lasted for 30 minutes and no targets were seriously damaged. There was a widespread panic. Five civilians died and others were injured. After the second bombardment, the U.S. consulate officer in Madeira, Humberto dos Passos Freitas, asked for help from the Americans. The request was honored and, two months later, a

ship from the U.S. Naval Division of the Azores was sent to patrol around Madeira and raise the morale of the population (RODRIGUES, 2014).

"Glafiberta" and the exile. "*Diario de Las Palmas*", Gran Canaria (Spain), dated June 7th, 1919, informed the readers about the arrival of the "*Glafiberta*" ship after a five-day trip from the island of Madeira. On board, there were eight Portuguese politicians, who took part in the revolutionary movement attempting to reinstate the monarchy in Portugal, called "Revolta do Monsanto". There were 289 people arrested, exiled on the island of Madeira and locked in Lazareto Castle, in Funchal. During the night of June 1st, , eight of them succeeded to escape by descending on ropes from a height of 15 m, while Humberto dos Passos-Freitas waited for them on the beach with his "*Glafiberta*" ship (RUI CARITA, 2017). In September 1919, while he was in Las Palmas, Humberto dos Passos-Freitas was invited to participate in the sailing race organized by the Gran Canaria Royal Yacht Club.. He won this race with "*Glafiberta*" yacht, on September 15th, as it is mentioned by a comment in "*Diario de Las Palmas*" on the same day. The heroic act of Humberto Passos-Freitas was not without consequences, as he was sentenced to exile. "*Porto Da Cruz*" (1953) wrote that, during his exile, he visited all Europe, from the Middle East to England, and RODRIGUEZ (2016) said that, after traveling half of the world, H. dos Passos-Freitas returned to Madeira in 1923 and published a book, "*Vinte e um Dias num Bote*", about an expedition around the Madeira Archipelago, which he dedicated to his wife. But this book is not the only one, as in 1922, he published in Bucharest, at Socec Publishing House, a volume of 41 pages, "*Birds observed by Passos - Freitas Ornithological Expedition to the Delta of the Danube April - May 1922*", which helps us reconstitute the exile life of this untiring traveler, lover of nature and sports (Fig. 1).

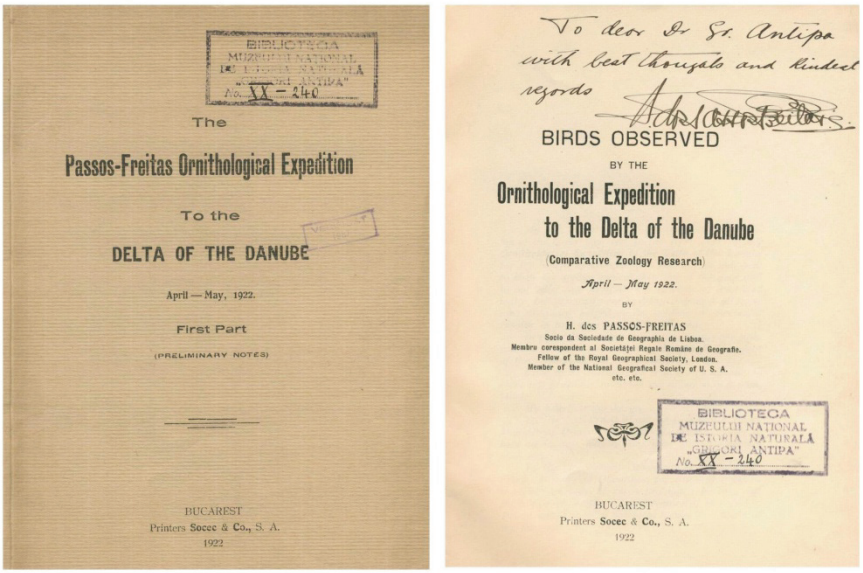

Figure 1. Cover of the book published by Humberto dos Passos-Freitas in Bucharest (Romania), at Socec Publishing House, 1922, about the Expedition in the Danube Delta (originals).

Geographical societies. He was a member of several geographical societies in the world, perhaps as a recognition of the expeditions he made in various areas of the globe, on his yachts, "*Glafiberta*" and then "*Physalia*". We find him as: Member of the Sócio da Sociedad de Geographia de Lisboa; Corresponding member of the Romanian Royal Society of Geography, Fellow of the Royal Geographic Society, London and Member of the Natural Geographic Society of U. S. A., etc. (PORTO DA CRUZ, 1953).

Our work renders different aspects of the 1922 one-month expedition in the Danube Delta. Here we present the routes, the people who accompanied him and the results of this expedition.

MATERIAL AND METHODS

Our study was based on the documents found in the Archive of the "Grigore Antipa" National Museum of Natural History (MNINGA Archive), where 15 files from 3 dossiers (1921, 1922 and 1923) were deposited and refer to Rudolph Pop, the taxidermist of the Museum; MNINGA collections; documents, books from the Museum's library, and articles on this subject from the press. The biographical references about Humberto Passos-Freitas have been obtained from several bibliographic sources, encyclopedias, works on the history of Madeira, or articles published in Madeira and Portugal.

The volume "*Birds observed by Passos - Freitas Ornithological Expedition to the Delta of the Danube April - May 1922*" from the collection of MNINGA Library, with dedication to Grigore Antipa (Fig. 1), helped us establish the route of this expedition in the Danube Delta and prepare a map of the trips (Fig. 2).

For a better understanding of the results of this expedition, we divided the trip on four routes marked from A to D, and the reference localities were numbered. To simplify the explanation in the table, the observation and collection places were marked with the following numbers: **A: Brăila Şerbanu Lake**: **●1**. Brăila, April 19, 1922; **● 2**. Şerbanu Lake, April 20; **● 3**. Aurelu Canal, Turcoaia, Dunărea Veche Lake, April 21; **● 4**. Şerbanu Lake, April 21; **● 5**. Brăila, April 23; **B: Brăila – Sulina ● 6.**Brăila, April 24 & 25; **● 7**. Chilia Nouă, April 26; **● 8.** Chilia Veche, April 27; **● 9.** Matiţa Lake, April 28; **● 10**. Lunga and Bogdaproste Lakes, April 29; **● 11**. Letea, Merheiu Mare, Merheiul Mic and Gârla Sulimanca, April 30; **● 12**. Letea Forest, May 1; **● 13**. Periprava Forest, departure, May 2; **● 14**. Vâlcov, May 3; **● 15**. Departure to Sulina, Stambul Island and entry into the Black Sea, May 4, 1922; **C: Sulina – Tulcea ● 16**. Sulina, April 5-13, 1922; **● 17**. Tulcea, May 13, arrival in Tulcea; **D: Tulcea – Razelm Lake ● 18.** May 14, 1922, they left Tulcea to reach Jurilovca, crossing the Queen Elizabeth channel, which connects with Babadag Lake and Razelm Lake. At 5 p.m., they arrived at Jurilovca; **● 19**. Razelm, May15, they arrived at Goloviţa Island and Bisericuţa Island; **● 20**. Popina Island, May16, they spent the night at Sarichioi, because Popina did not offer a good camping spot; **● 21**. May 17, they left Sarichioi and overnighted at Jurilovca; **● 22**. Sinoe Lake, May18; **23**. Return to Tulcea, May 19; **● 24**. Brăila, by ship, May 20, 1922.

Abbreviations: MNINGA ("Grigore Antipa" National Museum of Natural History); St. Obs. b. (Stations of observed birds); St. n./e. (Stations of nest/eggs); St. Coll. b. (Stations of collected bird specimens) (Fig. 2).

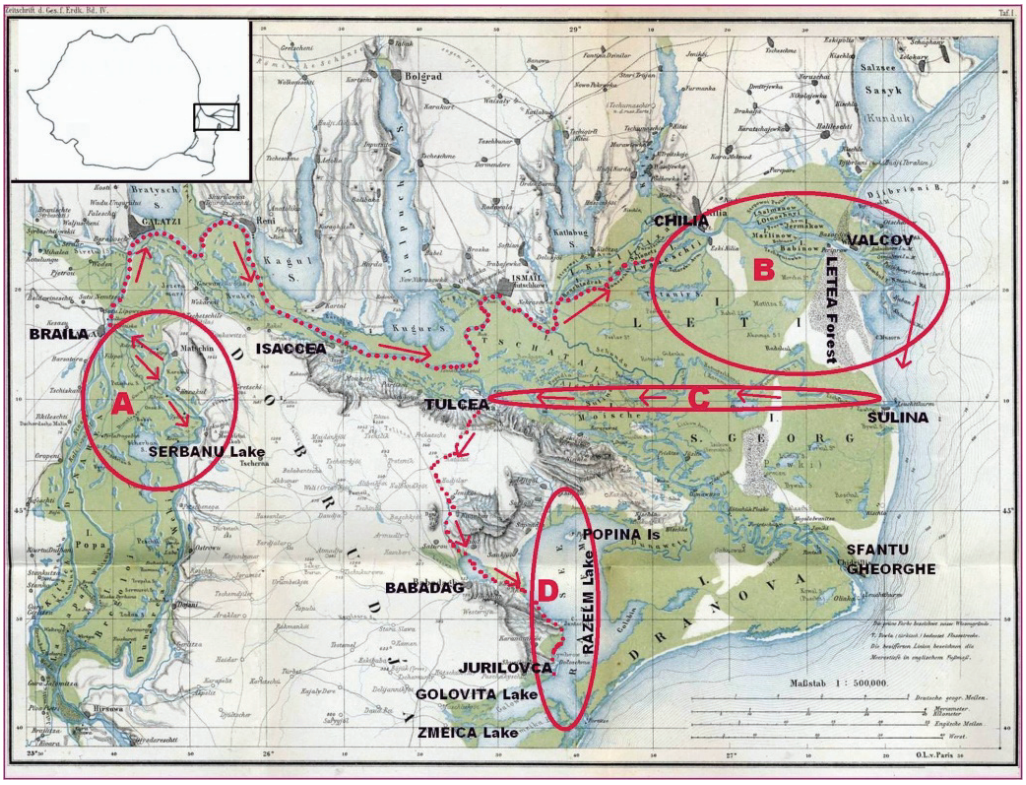

Figure 2. Map of the routes followed by Humberto dos Passos Freitas in the 1922 expedition in the Danube Delta. (modified after SPRATT & KIEPERT, 1867).

RESULTS AND DISCUSSION

The expedition from the Danube Delta (Romania, April 19th-May 20th, 1922) was possible with the help of some important people, whom Humberto dos Passos-Freitas thanked in his book. Among them, we mention: **Constantin Gheorghe Banu** (1873-1940), Minister of Domains at that time, Romanian lawyer, politician and minister, Romanian writer, journalist and politician who served as the Arts and Religious Affairs Minister (1922 -1923); **Alexandru C. Constantinescu** (1859-1926), law PhD, who attended his studies in Paris, later on deputy (since 1901) and then liberal senator, with a successful political career, Minister of the Interior for several times, Minister of Agriculture and Domains and of Industry and Commerce; **Martinho de Brederode** (**Martinho Maria Teixeira Homem de Brederode de Cunha)** (1866-1952), Portuguese Minister, Foreign Office; he entered diplomacy at the age of 23 and carried out various diplomatic missions at the request of the Ministry of Foreign Affairs of Lisbon, in Tangier, Canton, Peking, Paris, Rome and finally, in 1919, in the Balkans (Romania, the Kingdom of Serbia-Croatia-Slovenia and Greece) (STOICA, 2011; MOCANU, 2009); in 1934 he retired and died in Braşov (Romania) in 1952, without seeing Lisbon again in the last 25 years of his life. In addition to his diplomatic skills, demonstrated on the occasion of his assigned missions, Martinho de Brederode was passionate about literature and poetry. He published under the pseudonym Marco Sponti, probably due to

diplomatic discretion. His other passion was bullfighting (tauromaquia). He was one of the founders of the Portuguese Bullfighting Royal Club (Real Clube Tauromáquico Português) (STOICA, 2010).

Grigore Antipa (1867-1944), the director of the Museum of Natural History in Bucharest and of the State Fisheries, helped Humberto dos Passos-Freitas to obtain the necessary permits for the expedition in the Danube Delta. Fascinated by the distinction and erudition of this educated foreigner, Grigore Antipa gives him access to the collection and the specialized staff to help him, guide him and, eventually, defend him from various unforeseen dangers. Those who accompanied him in the expedition and helped him with the logistics during the Danube Delta trip were Rudolph Pop, the taxidermist of the museum, Dr. D. G. Ionescu, Administrator of Fisheries at Braila and Mr. Polihroniadi. It is possible that Grigore Antipa and Ludovic Mrazec (1867-1944), the director of the Geology Institute, a specialist in geology and petroleum exploitation, to have recommended him as a correspondent member of the Romanian Royal Society of Geography and the Bene Merenti Order, the first class gold medal for scientific merits.

Initially, we thought that Humberto dos Passos-Freitas spent only a month in Romania, but STOICA (2010), in a study on the life and work of Martinho de Brederode, reminds of a Romanian businessman's complaint against Martinho de Brederode to the Portuguese Masonry, quoting dos Passos-Freitas as a witness, who would have spent a year in Romania and knew Brederode. Knowing that he had the status of exile, thus the extension of his stay in Romania could be explained, during which we suppose he visited Transylvania and Bessarabia. There are no documents in this respect, but in the collections of the Museum in Bucharest there is a bird collected from Transylvania in December 1922, donated by Rudolph Pop, and in the preface of his book about the expedition in the Danube Delta, published in Bucharest, Humberto Passos-Freitas thanks Rudolph Pop for the letters sent to Tulcea and Bessarabia. During the expedition in the Danube Delta, he wanted to visit Odessa, but was prevented by the Russian Bolshevik officials, who stopped him at the border.

The itinerary included **four areas** in Dobrogea and the Danube Delta: **Brăila - Șerbanu Lake (A), Brăila – Chilia Nouă and Chilia Veche - Sulina (B), Sulina - Tulcea (C)** and **Tulcea - Razelm Lake (D)** (Fig. 2). The route of the expedition and the main stations per day are found in Table 1.

The first visited itinerary was the wetlands around Brăila, Șerbanu Lake and the Old Danube, connected by Filipoiu and Aurelu Canals **(A: Brăila - Șerbanu Lake)** (Fig. 2). The information noted by Humberto dos Passos Freitas for this wetland, which disappeared since then, is very important. Today, about 100 years after this expedition, the wetland is completely transformed and, the area has become a productive agroecosystem. The rich biodiversity of this ecosystem was researched between 1896 and 1916 by Robert Ritter von Dombrowski, the ornithologist of the Museum of Natural History in Bucharest, who collected several species of birds from this area and published data on them in the volume "*Ornis Romaniae*". These preparations were exhibited in the museum, in dioramas, at the official opening in 1908, in the new building, on Kiseleff Road, no. 1, today's headquarters. Since his departure from Romania, during the First World War, in 1916, no observations and other ornithological studies had been published from this area.

Filipoiu Canal, with a length of 27 kilometers, impressed Passos-Freitas through the richness of the avifauna, its wildness, beauty and the trees that come out of the water. He visited an island where he discovered the ruins of a house, called by the locals "Șerban Palace". These were the ruins of the Royal Family vacation home, where King Carol I of Romania spent one month of vacation each summer, until his death in 1914. Every summer, the Royal Family left from Bucharest on a cruise from Giurgiu to the Old Danube Branch. "Șerban Palace" was abandoned after the King's death, destroyed by time and vandalized during the war.

On Filipoiu Canal, Aurelu Canal and Dunărea Veche, there were large colonies of hundreds of birds – Gray Herons (*Ardea cinerea*), Great Cormorants (*Phalacrocorax carbo*) and rooks (*Corvus frugilegus*). He observed 50 species of birds and collected over 20 species. We do not know whether they were prepared or not by Rudolph Pop or whether Humberto Passos shot them only for pleasure or he was a collector. In the ornithological collection of the "Grigore Antipa" National Museum of Natural History in Bucharest, there is a single piece, a Common Buzzard (*Buteo buteo*) collected from this expedition as a naturalized-mounted piece and a chiropter, a bat that has a label with the name of Humberto Passos-Freitas (Fig. 3). Among the observed species, we mention the White-tailed Eagle (*Haliaeetus albicilla*), nowadays very rare in Romania, whith only 3-4 nesting pairs being present; *Neophron percnopterus*, the Egyptian vulture, nested in Dobruja, but always in a small number of pairs, while presently it disappeared from Romania's avifauna. For Humberto Passos-Freitas the four nights spent here were not very comfortable because it rained and there were floods, thousands of insects, mosquitoes and orthopters, but also mice. In fact, he even caught a mouse that had hidden from the wetness in his clothes and kept it as a memory.

The second route Brăila-Chilia Nouă and Chilia Veche (B) (Fig. 2).

He spent two days in Brăila (April 24th-25th) for supplies. He made observations on the birds in the parks and gardens of the city. The avifauna was interesting, made up of small singing birds and many crows. We believe that these observations are also the first on the urban avifauna in Brăila. On April 26th, 1922, he crossed Chilia Branch of the Danube with the "*Borcea*" ship to the town of Chilia Nouă. On this route, he observed and was delighted with the stork nests (*Ciconia ciconia*), built on the reed roofs of the houses. He found out that, in Romania, people loved storks and protected them, and those who had stork nests on the house did not destroy them, because they believed they would bring good luck and protect the houses against fire. He was interested in storks and wanted to see such a nest nearly. As there was a delay of one day, because of the documents and permits, he paid a local guide who led him to a nest where the stork was nesting.

Figure 3. Mammals collected in the Danube Delta with the label Humberto Passos-Freitas (originals - MNINGA Collection).

He visited Gârla Pardina and Gârla Jidan and set up the camping site at the Fishing Station of the Government at Matița Lake. The rooms were very simple, just a bed put directly on the ground, however very hospitable (Fig. 4). In this area, he observed over 20 species of birds and shot 10 species of which: Black Kite (*Milvus migrans*), very rare today in Romania, the Smew (*Mergus albellus*) and Red-breasted Merganser (*M. serrator*). It is interesting to mention that at the beginning of the XXth century, the Smew nestled in the Danube Delta, while today, 100 years after these observations, it no longer nests there, it winters at the shore of the Black Sea, but in small numbers in recent years. At European level, the nesting population of this species counts only 1,500-2,000 pairs.

Figure 4. Images from the Danube Delta (1920-1930) (originals - MNINGA Archive).

On April 29, he continued the ornithological observations on Lake Lunga, Sahuna and Sfistofca. The next day, he continued on Gârla Sulimanca, Merheiul Mic and Merheiul Mare where he found nests of *Mergus merganser*, *Anas strepera* and a colony of hundreds of nests of *Ardea cinerea*. He collected *Phalacrocorax pygmaeus*, *Melanitta nigra* and *M. fusca*, northern species, very rare in Romania, probably still in migration. He left the ship and walked through Letea Forest, where he discovered an enormous nest of White-tailed Eagle, *Haliaeetus albicilla*, and after waiting for two hours, he shot the female, who died on the nest. He was amazed at the agility of a 50-year-old fisherman who climbed the tree, which seemed inaccessible, to recover the bird and the two chicks from the nest. The man reminded him of the monkeys he had seen in South America and who were not so agile. He was preoccupied to collect an *Eryx*

jaculus, for Dr. Ilchev in Sofia, but, unfortunately, he did not succeed. Delcho Ilchev (Ilcheff - in Passos-Freitas book), a Bulgarian zoologist, was the head of the Entomological Station at the Royal Institutes of Natural History. As a researcher, D. Ilchev had a significant contribution to the investigation of the butterfly fauna of Bulgaria. He published over 35 scientific and popular papers on Bulgarian fauna. On April 14th, 1925, he was killed in an assassination attempt against King Boris III, while trying to save the King's life (LAZAROV, 2011). We do not know the circumstances in which Humberto dos Passos Freitas met the Bulgarian scientist; he might also have visited Bulgaria. In Letea Forest, he observed and collected 10 bird species.

On May 2nd, 1922, he arrived at Periprava and visited the forest located a few kilometers away from the locality. He found a nest of *Aquila clanga*, but the birds left the nest and watched him from the distance. He did not have the patience to wait and gave up. He observed, but also shot some species of falcons: *Falco peregrinus*, *F. vespertinus*, *F. tinnunculus* and *Pernis apivorus*. The day was very hot and Passos-Freitas noted: "*The day was very hot and dry, the sand of which the soil of the Forest Letea was composed in its intense heat penetrated through the soles of our boots burning the feet, I can only compare it to the heat I experienced in the deserts of Morocco in 1915*". After these experiences, he decided to go to Vâlcov, where he spent one night. He wanted to visit Odessa, but for this trip, there were necessary many formalities that would have taken a long time. It was possible to waste precious time, and the hostile Bolshevik authorities did not allow him to enter the country. He decided to go to Sulina. The route from Vâlcov to Sulina passed near Stambul Island, where he observed a large group of *Pelecanus onocrotalus*. He shot a few specimens, those that could not get up in the air because of the large ingested prey. They had swallowed 1-2 kg carps!

The third route Sulina-Tulcea (C) (Fig. 2) was supposed to begin on May 5th, 1922, but Passos-Freitas felt sick, as written in his diary, because of a snake bite from those collected at Periprava: "*I was not able to go to Tulcea until May 13, 1922 as in changing one of the snakes caught at Periprava I was bitten by one of these, which gave me a bad time for several days*". On May 13th, he arrived in Tulcea, with some bird blood samples collected on glass blades for Professor George Zotta, later used in .his studies of malaria. Zotta George (1886-1942) was a Professor at the Faculty of Medicine (University of Bucharest), Department of Medical Zoology and Parasitology; he was the one who published the first studies on paludism in Romania in the "*Bulletin de la Société de Pathologie exotique*"in 1927, 1932 and 1938.

The fourth route Tulcea-Jurilovca (**D)** (Fig. 2) started on May 14th, when Passos-Freitas traveled on Elisabeth Canal, then on Babadag Lake and Razelm Lake to Jurilovca. He spent six days at Jurilovca and Razelm Lake. He visited Goloviţa Island, the island that rises a few inches above the water. He was impressed by the large number of birds and the fact that Romanians do not use this land for rice crops: "*I often wondered why some Romanians do not try to grow rice in these wilder land - as the soil and climate seem to be the ideal for that crop*". Here he observed *Grus grus*, *Aythya fuligula*, *Plegadis falcinellus* and *Platalea leucorodia*. On Bisericuţa Island, he observed 10 species of birds, of which we mention: *Egretta garzetta*, *Recurvirostra avosetta*, *Podiceps nigricollis*. At a great distance, but not sure, he also observed *Phoenicopterus roseus*, which was indeed observed and collected from that area in those years.

The trip to Popina Island, the largest island in Razelm Lake, did not offer any special remarks. Firstly, because of the strong wind, there were just a few birds, but they observed a lot of snakes (*Tropidonatus tesselata*) and found traces and feathers of the great bustard (*Otis tarda*), a large, rare bird, disappeared from the fauna of Romania, which appears accidentally during migrations. In the last days of the expedition, on May 17th-18th, he collected a few rare species at Sarichioi: *Tadorna tadorna*, *T. ferruginea*, *Bubo bubo* and a few eggs of *Riparia riparia*. On the North of Sinoe Lake, he found a very large colony of *Larus*, with flooded nests and hundreds of eggs floating above the water. This image is quite common in Laridae colonies from wetlands, when the weather is unfavorable and there are frequent rainfalls. On May 19th, the travelers returned to Tulcea, ending an adventure that lasted for a month.

Initially began as a hunting trip, although it was completed as a true ornithological expedition. The results are among the best, as over 120 species from 36 families and 16 orders were observed and collected (Table 1). The preliminary notes printed at the Socec Publishing House (Fig. 1) are now, a hundred years later, a precious document illustrating the rich biodiversity of this less-known area at that time. The text is clear, the explanations short and concise, as a true scientific work. The correct scientific names, precisely used, the correct determination of the species of birds, mammals, insects found on the ground prove that Rudolph Pop, who had the necessary knowledge of zoology, participated in the elaboration of the text.

RUDOLPH POP

The closest person on this trip was Rudolph J. Pop, an employee of the Museum in Bucharest, since December 1921. From two documents in the form of a table with the employees of the museum, drafted for the Ministry, one by Grigore Antipa on July 11th, 1923 (MNINGA Archives, file 1923: 132) and another table compiled by Richard Canisius, Antipa's assistant and substitute when Antipa was not in the country (MNINGA Archives, file 1923: 182), we found some biographical data about this museum preparator. Rudolph J. Pop worked and trained as a taxidermist at Christiania (now Oslo), in Norway. At the request of Grigore Antipa to the Ministry of Public Instruction for a preparator position, from April 8th, 1921, the Ministry approved hiring Rudolph Pop (MNINGA Archives, file 1921: 16). He settled down in Bucharest in November 1921, with residence in Avedic Street, no. 9. He was not married. Rudolph J. Pop was hired at the museum in Bucharest on December 1st, 1921. He was born on December 18th, 1895, in Budapest, and his parents were from Răşinari (Sibiu), Transylvania, the historical region of Romania at that time in the Austro-Hungarian Empire. He was mobilized and participated in the First World War as a soldier in the 31st Regiment

in the former Austrian army, and was discharged as invalid according to Registry 32165/1923; the last visa on his military ID card dates back to 13 September 1923 (MNINGA Archives, file 1923: 156). He trained himself in some of the most important workshops in Europe. He had acquired a lot of oral knowledge as he travelled a lot and according to the models and photographs, he had sent to the director of the Bucharest Museum, it was clear that he possessed the art of dermatoplasty. Grigore Antipa was impressed and proposed to the Minister to give him a double salary and the preparator position to be called "*dermoplast preparator"* (MNINGA Archives, file 1921: 16). Antipa managed to raise money, amounting to 5,000 lei, through Decision no. 7268 of August 8th, 1921, to pay his way from Norway to Romania and his installation in Bucharest. He intervened with the Foreign Ministry to free him from customs payments for the equipments he brought, including a hunting gun. The support given by Grigore Antipa, before and after his arrival in Bucharest, was constant. He managed to obtain him a railway free pass, but also a special hunting permit, mentioning the exceptions from the "general prescriptions of hunting periods and hunting laws" (MNINGA Archives, file 1921). Initially, this request was not entirely accepted by the Ministry, and Grigore Antipa, dissatisfied, returned the permits."*/To the Minister of Agriculture and Domains, Hunting Directorate / Dear Minister, / In my request no. 91 of April 24, c.y., I had the honor to kindly ask you to release me two hunting permits in the name of the undersigned and the preparator R. J. Pop, to be able to collect for scientific purposes, for both studies and collections from different years throughout the country. The permits you have issued, only for the state unrented forests and pools, allowing us "to hunt only 2 specimens of each species" and "only in the season allowed by law" and then the "bond" to address to the superior agent to note on the back of the permits" cannot be of any use to us and we restitute them.Taking into account the high scientific goals we pursue: the study of the fauna of the country, the biology of the animals in this fauna, reproductive biology studies, their migration, studies on the utility and damages brought to agriculture, forestry and pisciculture, etc., I have asked to be granted special permits that stand out from the general prescriptions of the hunting periods and hunting law measures. Such permits are granted in all countries for scientific studies and, moreover, they should be granted in our country where, there are no such studies./ To the Minister of Agriculture and Domains, the Department of Hunting/".*

After this expedition in the Danube Delta, Rudolph Pop returned to the Museum in Bucharest. In the archives of the Museum, we found an application of Grigore Antipa to the Minister of Finance and the Customs Department asking for the exemption of customs taxes for R. Pop's hunting gun so that it could be repaired at a factory in Germany and the permission to introduce 200 bullets freely into the country:*"/ Bucharest, / no. 37, August 21, 1922 / To the Minister of Finance, Customs Department. Locco / Mr. Minister, / Mr. Rudolf J. Pop, the dermoplast preparator of the Museum of Natural History, owns an English hunting gun, Rygly, caliber 12x8 mm with telescope, which is used to hunt various animals needed to enrich the museum collections. As his gun was damaged, he looked for a workshop that could fix it, but he did not find any in the country, so he has to send it to a factory in Germany. I would kindly ask you to allow the gun to be sent abroad and then reintroduced into the country without the payment of customs duties, since it is a necessary instrument for its work, as a preparator and the Museum absolutely needs it. At the same time, I would kindly ask you to allow the introduction of 200 bullets, 8 mm caliber, necessary for this gun, with the exemption of customs duties, which are also not available in the country./ Director / ss* (MNINGAArchives, File 1922: 73).

In Bucharest, in the same year, at the end of October, Rudolph Pop asked the director of the Museum of Zoology in Bucharest, Grigore Antipa, to give him a few months leave to attend a scientific expedition in British Columbia and Alaska. / "*Bucharest, October 26, 1923 / Mr. Director, / Presenting my opportunity to participate in a scientific expedition in British Columbia and Alaska from where I can bring valuable material to the Institution you preside, I would kindly ask you to give me a leave of ..... months and Dr. Emil Wilke, Professor of Natural Sciences, will replace me. / Mr. Director, I assure you of my special esteem and considerations. / R.J. Pop, Preparator of the Museum of Natural History. / PS ... For the time being, I would kindly ask you to approve the request of Dr. Emil Wilke, who will replace me. / To the Director of the Museum of Natural History*."(MNINGA Archives, file 1923: 45). We believe that Rudolph Pop remained in close contact with his companion in the Danube Delta and that this expedition he was about to take part in was organized by Humberto dos Passos-Freitas himself.

On the same application, Grigore Antipa wrote an approval resolution on October 27th, 1923, document that is in copy (MNINGA Archives, file 1923: 155): "*A three-month leave is granted on the condition that he would be replaced by Dr. Wilke and bring collections from the expedition he is undertaking to the Museum. Permission begins on Nov. 1, 1923. / Director, Grigore Antipa*". We do not know why Rudolph Pop postponed his departure in January 1924, as evidenced by MNINGA Archives document of October 1923, where Grigore Antipa, the director of the museum gave an official decision with the following content: / *October 27, 1923 / Mr. Preparator/ with regard to your request dated October 26, current year, I inform you that you are granted the 3-month leave starting with January 1924 to go and take part in the scientific expedition in British Columbia and Alaska. According to your wish, we have approved that during your absence, to be replaced by Dr. Emil Wilke who will receive your honorarium. The Management of the Museum has taken note of your commitment that you will bring to the Museum some of the collections you will gather in this expedition. / Director, Gr. Antipa. / To Mr. R. Pop, the preparator of the Museum of Natural History in Bucharest* /"(MNINGAArchives, file 1923: 158) (Fig. 5). Since January 1924, there have no longer been any mentions about Rudolph Pop in MNINGA Archives, so he has never returned to the museum in Bucharest. Starting with 1924, the preparator position was temporarily occupied by Emil Wilke, professor of natural sciences at the Evangelical School in Bucharest, until 1926.

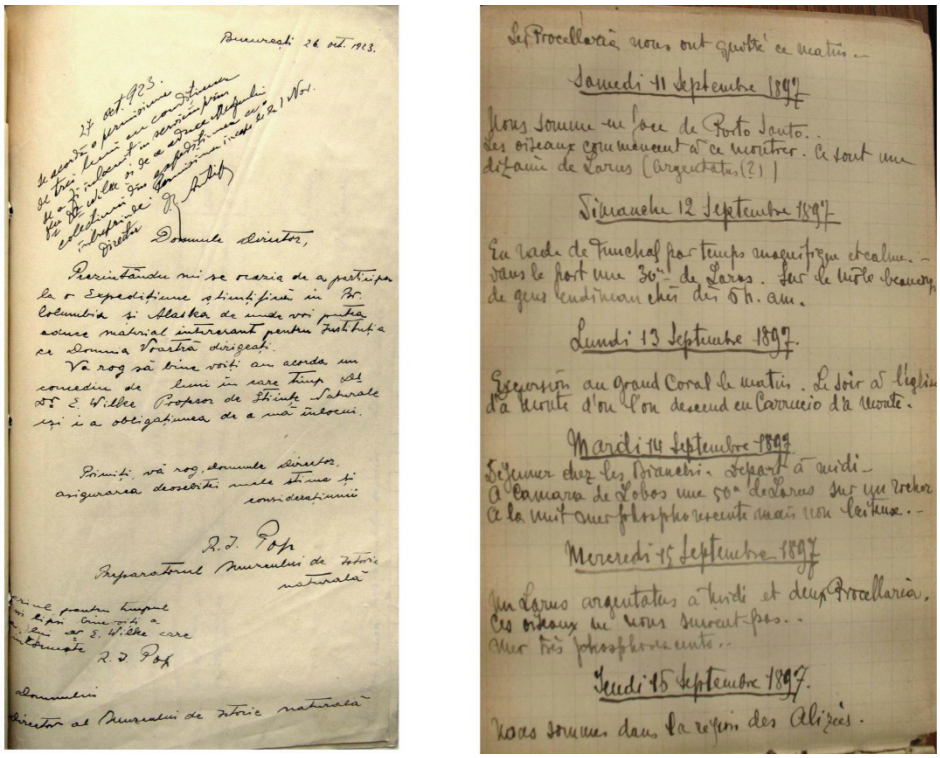

Figure 5. Rudolph Pop's request to Antipa for leaving in the Pacific-Alaska Expedition, left side (original: MNINGA Archive); The page from Emil Racovita diary where the Belgica ship made a stopover in Madeira, docking in Funchal Bay, right side (original: MNINGA Archive).

OTHER EXPEDITIONS. In September 1923, Humberto dos Passos-Freitas made a one-month expedition around Madeira with his yacht, an expedition completed with the publication of a book "*Vinte e um Dias num Bote*", describing the journey through the Deserted Islands, Porto Santo Island and his returning to Madeira and rendering the details of his researches and the conclusions reached, always with the style that no one else possesses (PORTO DA CRUZ, 1953; ASCENSÃO SILVA, 2018). In 1925, on June 7th, Humberto dos Passos-Freitas launched in Macicho his new yacht, built by Antonio Pedro Gonsalves, one of Madeira's best builder. It was 19 m long, 5.6 m wide at the bow and 3.80 at the stern. It had been built respecting the demands of its owner, who intended to make a scientific expedition in the Pacific. That is why the yacht was equipped with the top technique for that time: diesel engine, electrical and refrigeration installations, wireless telegraph, etc. (CAIRES, 2005; LEMOS SILVA, 2007). On December 15th, 1926, Funchal port of Madeira was hit by a powerful hurricane, a storm of extreme violence, with waves of 15 m, and the "*Physalia"* yacht anchored in the port was crushed; unfortunately, four crew members, along with Humberto Passos-Freitas and a woman were killed in this natural disaster. *Diário de Notícias*, Funchal, 16th December 1926, announced the shipwreck of "*Physalia da Expedição Portuguesa do Pacífico*" and the death of Humberto Passos-Freitas (LEMOS SILVA, 2007). Their bodies have never been found. A year later, on the beach of Funchal port, Passos Freitas'wedding ring was found, identified by the inscription "*Só Deus me vence*". Indeed, only God defeated this tireless traveler! But there remains only one question, to which only God will answer, was Rudolph Pop, on that fateful day, aboard the "*Physalia*" and shared the same fate with the rest of the crew?

EMIL RACOVIȚĂ AND MADEIRA

This year marks the 150th anniversary of the birth of the Romanian scientist Emil Racoviță (1868-1947). He studied in France, where he first graduated the *Faculty of Law* in Paris, then the *Faculty of Sciences* from Sorbonne; he also participated in the courses of the *School of Anthropology*. In 1896, he defended his Ph.D. thesis, entitled "*Le lobe céphalique et l'encéphale des annélides polychètes*". In 1897, at the age of 29, he was elected a member of the Zoological Society in France; after that, he was recommended to participate as a naturalist, alongside other international scientific personalities, aboard the "*Belgica"* ship, in the Belgian Antarctic Expedition (1897-1899). Emil Racoviță entered the universal consciousness as the founder of biospeology and as the naturalist of the "*Belgica*" expedition. On his way to the South Pole, after leaving the port of Antwerp, the Belgica ship made a stopover in Madeira, docking in Funchal Bay (AUGUSTO da SIVA & AZEVEDO de MENESES, 1921). On September 11th, 1897, Racoviță wrote to his parents "*Right now we stopped in the port of Madeira ... Sunday and Monday we stay in Madeira. We cannot land this evening, as the authorities do not allow this after eight in the evening. Tomorrow, I will leave early to visit Funchal, the white city that rises as an amphitheater above us; small, white houses, hanging at the foot of the mountain. They look happy and welcoming; it is said that women are beautiful, let's see* "... On September 13th, he made a trip to Coral Mountain, "*a too beautiful old crater. Riding for eight hours delighted me from all the points of view. Our stay was only a dream, but a*

beautiful dream" (Fig. 5). In this short trip, Emil Racoviță collected a few plants. The list of plants collected is now in the Archives of the "Grigore Antipa" Museum in Bucharest (Romania) (MARINESCU et al, 1999).

CONCLUSIONS

From April 19th to May 19th, 1922, Humberto dos Passos-Freitas, a businessman and sportsman from Madeira, made an expedition in the Danube Delta, Romania. During the expedition, he was accompanied by the taxidermist of the Museum of Natural History in Bucharest, Rudolph Pop. The expedition lasted for a month and wetlands with a wide variety of bird species were visited: Șerbanu Lake, Chilia Branch, Letea Forest, Periprava and the surrounding wetlands, the Black Sea shore between Vâlcov and Sulina, Sulina Branch and Razelm Lake.

The results of the observations and collections made in the Danube Delta are rendered in a 41-page paper, published in Bucharest in 1922, at Socec Publishing House. At that time, the authors called this report as preliminary, that is, the first part; unfortunately the second part never appeared.

Originally begun as a hunting trip, it was completed as a true ornithological expedition. The results are among the best, as over 120 species belonging to 36 families and 16 orders were observed and collected. The preliminary notes, published in 1922, are now, a hundred years later, a precious document illustrating the rich biodiversity of this less-known area at that time. The text is clear, the explanations concise, as a real scientific work. The correct scientific names, precisely used, the correct determination of the species of birds, mammals, insects found in the field prove that Rudolph Pop, who had the necessary knowledge of zoology, participated in the elaboration of the text.

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Table1. List of birds observed in 1922 by Humberto Passos Freitas and Rudolph Popin the Delta Danube (Romania).

No.	Species	Vernacular name	Synonym*	St. Obs. b.	St. n./e.	St. coll. B.
1.	*Corvus frugilegus*	Rook		1	1	
2.	*Corvus cornix*	Hooded crow		10, 11	10,11	
3.	*Corvus corone*	Carrion crow		2, 3		
4.	*Corvus monedula*	Jackdaw		1	1	
5.	*Pica pica*	Magpie	*Pica caudata*	2		
6.	*Pica pica*	Magpie	*Pica rustica*			
7.	*Garrulus glandarius*	Eurasian jay				
8.	*Oriolus oriolus*	Eurasian golden oriole	*Oriolus galbula*	12		12
9.	*Sturnus vulgaris*	Common starling		1, 2		
10.	*Passer montanus*	Eurasian tree sparrow		4		
11.	*Passer domesticus*	House sparrow		1		
12.	*Carduelis carduelis*	European goldfinch	*Carduelis elegans*	6		
13.	*Carduelis chloris*	European greenfinch	*Chloris ligurinus*	6		
14.	*Fringilla coelebs*	Common chaffinch		1		
15.	*Fringilla montifringilla*	Brambling		18		
16.	*Emberiza citrinella*	Yellowhammer		18		
17.	*Emberiza calandra*	Corn bunting	*Emberiza miliaria*	18		
18.	*Alauda arvensis*	Eurasian skylark		18		
19.	*Alauda arborea*	Woodlark	*Lullula arborea*	12		12
20.	*Galerida cristata*	Crested lark	*Alauda cristata*	18		
21.	*Anthus spinoletta*	Water pipit		2		
22.	*Anthus spinoletta petrosus*	Rock pipit	*Anthus aquaticus*	18		
23.	*Motacilla raii*	Yellow wagtail		1		
24.	*Motacilla alba*	White wagtail		2		
25.	*Motacilla flava*	Yellow wagtail		2		
26.	*Sylvia cinerea*	Lesser whitethroat	*Sylvia curucca*	6		
27.	*Sylvia atricapilla*	Orphean warbler	Wrong *Sylvia hortensis*	6		
28.	*Philloscopussibilatrix*	Wood warbler	*Phylloscopus sibilatrix*	12		12
29.	*Philloscopus trochilus*	Willow warbler	*Phylloscopus trochilus*	2		
30.	*Phylloscopuscollybita*	Chiffchaff	*Philloscopus rufus*	3, 12		12
31.	*Erithacus rubecula*	Robin		6		
32.	*Turdus merula*	Common Blackbird		2		
33.	*Turdus torquatus*	Ring Ouzel		2		
34.	*Oenanthe oenanthe*	Wheatear	*Saxicola oenanthe*	6		
35.	*Remiz pendulinus*	Eurasian penduline tit	*Parus pendulinus*	2, 3		
36.	*Parus major*	Great tit		2		
37.	*Parus caeruleus*	Eurasian blue tit	*Cyanistes caeruleus*	2, 6		
38.	*Lanius collurio*	Red-backed shrike		18		
39.	*Hirundo rustica*	Barn swallow		1	1	
40.	*Hirundo riparia*	European sand martin				
41.	*Delichon urbica*	House Martin	*Chelidon urbica*	2		
42.	*Upupa epops*	Eurasian hoopoe		2		
43.	*Merops apiaster*	Bee-eater		18		
44.	*Coracias garrulus*	European roller	*Coracias garrula*	12		12
45.	*Alcedoatthis*	Common kingfisher	*Alcedo ispida*	2		
46.	*Apus apus*	Common swift	*Cypselus apus*	6		
47.	*Cuculus canorus*	Common cuckoo		2, 12		12
48.	*Cuculus canorus*	Common cuckoo	*Cuculus romanicus*			
49.	*Picus viridis*	European green woodpecker	*Gecinus viridis*	2		
50.	*Dendrocopos medius*	Middle Spotted Woodpecker		12		12
51.	*Dendrocopos minor*	Lesser Spotted Woodpecker		2		
52.	*Strix aluco*	Tawny Owl	*Syrnium aluco*	11	11	
53.	*Bubo bubo*	Eurasian eagle-owl	*Bubo maximus*	2		
54.	*Gyps fulvus*	Eurasian griffon		22		
55.	*Neophron percnopterus*	Egyptian vulture				
56.	*Falco peregrinus*	Peregrine falcon		2, 13		13
57.	*Falco subbuteo*	Eurasian hobby		9		
58.	*Falco vespertinus*	Red-footed falcon	*Tinnunculus vespertinus*	13		
59.	*Falco tinnunculus*	Common kestrel	*Tinnunculus alaudarius*	13		13
60.	*Aquila clanga*	Greater spotted eagle	*Aquila imperialis*	11,13		
61.	*Aquila clanga fulvescens*	Greater spotted eagle	*Aquila fulvescens*			
62.	*Haliaeetus albicilla*	White-tailed Sea-eagle	*Haliaëthus albicilla*	2, 4, 12	12	
63.	*Milvus milvus*	Red kite	*Milvus ictinus*	9		
64.	*Milvus migrans*	Black kite		9		9
65.	*Pernis apivorus*	European honey buzzard		13		13
66.	*Buteo buteo*	Common buzzard	*Buteo vulgaris*	3		
67.	*Buteo buteo vulpinus*	Common buzzard	*Buteo desertorum*			
68.	*Circus aeruginosus*	Western marsh harrier				

69.	*Circusmacrourus*	Pallid harrier	*Circus pallidus*			
70.	*Pelecanus onocrotalus*	Great white pelican		11, 15		15
71.	*Pelecanus crispus*	Dalmatian pelican		1		
72.	*Phalacrocorax carbo*	Great cormorant		2, 11, 22	2	
73.	*Microcarbo pygmaeus*	Pygmy cormorant	*Phalacrocorax graculus*	11, 22		11
74.	*Mergus albellus*	Smew		9	9	
75.	*Mergus serrator*	Red-breasted Merganser		9	9	
76.	*Mergus merganser*	Goosander		11	11	
77.	*Melanitta nigra*	Common scoter	*Oedemia nigra*	11	11	
78.	*Melanitta fusca*	Velvet scoter	*Oedemia fusca*	11	11	
79.	*Aythya marila*	Greater scaup	*Fuligula marila*	19		
80.	*Aythya nyroca*	Ferruginous duck	*Fuligula nyroca*			
81.	*Netta rufina*	Red-crested pochard	*Fuligula rufina*			
82.	*Aythya fuligula*	Tufted duck	*Fuligula cristata*	4		
83.	*Anas platyrhynchos*	Mallard	*Anas boscas*	2, 9	9	
84.	*Anas penelope*	Eurasian wigeon	*Mareca penelope*	3		
85.	*Mareca strepera*	Gadwall	*Chaulelasnus streperus*	11	11	
86.	*Anas crecca*	Eurasian teal	*Querquedula crecca*	9		
87.	*Tadorna tadorna*	Common shelduck		21		21
88.	*Tadorna ferruginea*	Ruddy shelduck	*Tadorna casarca*	21		21
89.	*Anser anser*	Greylag goose	*Anser cinereus x*	2, 7		
90.	*Anser albifrons*	Greater white-fronted goose		7		
91.	*Cygnus cygnus*	Whooperswan	*Cygnus musicus*	3, 18		
92.	*Cygnuscolumbianusbewickii*	Tundra swan	*Cygnus bewicki*			
93.	*Ardea cinerea*	Grey heron		2, 11	2, 11	
94.	*Ardea purpurea*	Purple heron		2		
95.	*Ardea alba*	Great egret		4, 9		
96.	*Egretta garzetta*	Little egret	*Ardea garzetta*	9, 19		19
97.	*Ardea ralloides*	Squacco heron	*Ardeola ralloides*	12		12
98.	*Nycticorax nycticorax*	Black-crowned Night-heron	*Nycticorax griseus*	2		
99.	*Botaurus stellaris*	Great Bittern		10		
100.	*Ixobrychus minutus*	Little bittern	*Ardetta minuta*			
101.	*Ciconia alba*	White stork		4, 6, 7, 8	8	
102.	*Plegadis falcinellus*	Glossy ibis	*Ibis falcinellus*	19, 20		19
103.	*Platalea leucorodia*	Eurasian Spoonbill		19		
104.	*Grus grus*	Common Crane, Crane	*Grus communis*	12, 19		12
105.	*Graleola pratincola*	Collared pratincole		22		
106.	*Vanellus vanellus*	Northern lapwings	*Vanellus cristatus*	19		19
107.	*Pluvialis squatarola*	Grey plover	*Squatarola helvetica*	19		19
108.	*Recurvirostra avocetta*	Pied avocet, Avocet		19		
109.	*Tringa totanus*	Common redshank	*Totanus calidris*			
110.	*Calidris alba*	Sanderling	*Calidris arenaria*	19		19
111.	*Calidris alpina*	Dunlin	*Tringa alpina*	19		
112.	*Calidris minuta*	Little stint	*Tringa minuta*	19		
113.	*Lymnocryptes minimus*	Jack snipe	*Gallinago gallinula*			
114.	*Gallinago caelestis*			9		9
115.	*Hydroprogne caspia*	Caspian tern	*Sterna caspia*	9		
116.	*Sterna hirundo*	Common tern	*Sterna fluvitallis*	9		9
117.	*Chlidonias niger*	Black tern	*Hydrochelidon nigra*	22		
118.	*Chlidonias hybrida*	Whiskered tern	*Hydrochelidon hybrida*	22		
119.	*Chroicocephalus ridibundus*	Black-headed gull	*Larus ridibundus*	9		
120.	*Hydrocoloeus minutus*	Little gull	*Larus minutus*	9	9	
121.	*Larus canus*	Common gull		5		
122.	*Larus fuscus*	Lesser black-backed Gull		22	22	
123.	*Larus cachinnans*	Caspian gull	*Larus argentatus*	5, 9	9	
124.	*Podiceps nigricollis*	Black-necked grebe		3, 19		19
125.	*Podiceps grisegena*	Red-necked grebe	*Podiceps griseigena*	4		
126.	*Fulica atra*	Eurasian coot		2, 3		
127.	*Gallinula chloropus*	Common moorhen		2		
128.	*Otis tarda*	Great bustard		20		
129.	*Columba oenas*	Stock dove		18		
130.	*Columba palumbus*	Common wood pigeon		2		
131.	*Coturnix coturnix*	Common quail	*Coturnix communis*	19		

Abbreviations: St. Obs. b. (Stations of observed birds); St. N./e. (Stations of observed /collected Nest/eggs); St. Coll. b. (Stations of collected bird specimens). *** synonyms used by Passos Freitas in his book.**

Stations 1-24: 1, 5, 6, 24 - Brăila; **2** - Şerbanu Lake; **3** - Aurelu Canal, Turcoaia, Dunărea Veche Lake; **4** - Şerbanu Lake; **7** - Chilia Nouă; **8** - Chilia Veche; **9** - Matiţa Lake; **10** - Lunga Lake and Bogdaproste Lake; **11** - Letea, Merheiul Mare, Merheiul Mic and Gârla Sulimanca; **12** - Letea Forest; **13** - Periprava forest; **14** - Vâlcov; **15** - Sulina, Stambulul Island and the entrance to the Black Sea; **16** - Sulina; **17** - Tulcea; **18** - Jurilovca, Canal Regina Elisabeta ; **19** - Razelm, Goloviţa Island and Bisericuţa Island; **20** - Popina Sarichioi Island; **21** - Jurilovca; **22** - Sinoe Lake; **23** - Tulcea.

PRELIMINARY DATA ON SMALL AND MEDIUM-SIZED MAMMALS FROM CRAIOVA MUNICIPALITY (ROMANIA)

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Abstract. This paper presents preliminary data on mammal fauna from Craiova city, since the mammals in these anthropic ecosystems are insufficiently known. The preliminary list includes 19 species of mammals identified in the course of the years 2015 to 2018. From a systematic point of view, they belong to five orders: Eulipotyphla – 6 species, Chiroptera – 2 species, Lagomorpha – 1 species, Rodentia – 8 species, Carnivora – 2 species of mustelids. Among the highlighted mammals in the Craiova city, five species are vulnerable: *Crocidura leucodon, C. suaveolens, Plecotus auritus, Spermophilus citellus, Microtus agrestis*; they are mentioned in the Red Book of Vertebrates in Romania. Three of the observed species: *Lepus europaeus, Meles meles, Putorius putorius* are species of cynegetic interest.

Keywords: urban mammals, habitat, diversity, systematic.

Rezumat. Date preliminare despre fauna de mamifere mici și mijlocii din municipiul Craiova (România). În lucrarea de față sunt prezentate date preliminare cu privire la fauna de mamifere din orașul Craiova, cunoscându-se faptul că mamiferele din ecosistemele antropice ale orașului sunt insuficient cunoscute. Lista preliminară cuprinde 19 specii de mamifere identificate pe parcursul anilor 2015-2018. Din punct de vedere sistematic sunt distribuite în cinci ordine: Eulipotyphla – 6 specii, Chiroptera – 2 specii, Lagomorpha – 1 specie, Rodentia – 8 specii, Carnivora – 2 specii de mustelide. Dintre mamiferele evidențiate 5 specii au statut de vulnerabilitate: *Crocidura leucodon, C. suaveolens, Plecotus auritus, Spermophilus citellus, Microtus agrestis*, specii ce figurează în Cartea Roșie a Vertebratelor din România. Trei dintre speciile observate: *Lepus europaeus, Meles meles, Putorius putorius* sunt de interes cinegetic.

Cuvinte cheie: mamifere urbane, habitat, sistematică, diversitate.

INTRODUCTION

Mammals constitute a group of vertebrates with great adaptability and biologic diversity. Research about mammals in Romania, which include about 100 species (MURARIU, 1984), is reunited in two syntheses papers written by MURARIU (2003), and MURARIU & GEACU (2008) respectively. After the 90s, Romanian scientific literature has been enhanced in terms of research in mammalogy, which approach mammals from different points of view: morphological, anatomical, taxonomic, ecological, geographical, genetic, paleontological, hunting, etc.

Some of the scientific research also approaches the study of mammals from different rural and urban areas (ARDELEAN & BÉRES, 2000; BAZILESCU, 1971; BAZILESCU & PÎRVESCU, 1971; BOSTAN et al., 2015; CHACHULA et al., 2013; CHACHULA et al., 2017; DONE, 2007; IFRIM & VALENCIUC, 2006; MURARIU, 2006; PARASCHIV & ARDEI, 2011; SÁNDOR & KISS, 2004).

Studies related to the nutrition of birds of prey in urban areas have led to the identification of several species of mammals, specific to those anthropic areas (BANARU & COROIU, 1997; LAIU & MURARIU, 1998; LAIU et al., 2002; MOGA et al., 2005; NISTREANU, 2007).

What motivated us to approach a research regarding on the species of this group of vertebrates, was the lack of syntheses about mammals from Craiova. The first information about mammals in Craiova is provided by BARBU & SORESCU (1970) as a result of the studies conducted on the trophic spectrum of *Athene noctua*. Subsequently, new data were provided by BAZILESCU et al. (1980), which are revised and completed by GOGA (2012). Several species of mammals collected from Craiova are cited in the fauna of Romania (MURARIU, 2000; MURARIU & MUNTEANU, 2005).

Craiova is a city located in the South-West of Romania, in the Oltenia Plain, on the left bank of the Jiu River. The city has a surface of 81.41 km² and a continental climate, with Mediterranean influences.

The research was conducted in the main parks and gardens of the city: "Nicolae Romanescu" Park, the Youth Park, "Hanul Doctorului" Park, Craiovița Lake and Park, the Botanical Garden "Alexandru Buia" (CIOBOTEA et al., 1999), the area "Tanchiștilor Lake", in different neighbourhoods (1 Mai, Romanești, Craiovița, Brazda lui Novac, Rovine, Bariera Vâlcii, etc.). The peri-urban area was less studied (Valea Fetii, Mofleni, zona Banu Mărăcine, etc.) (Fig. 1).

The main purpose of this research paper is to publish a preliminary faunistic list regarding of the mammalian species which we have identified in different habitats in Craiova (intra-urban and peri-urban areas).

The list may constitute a starting point for other research studies which would follow changes in the faunistic community, caused by the increase in the degree of anthropization.

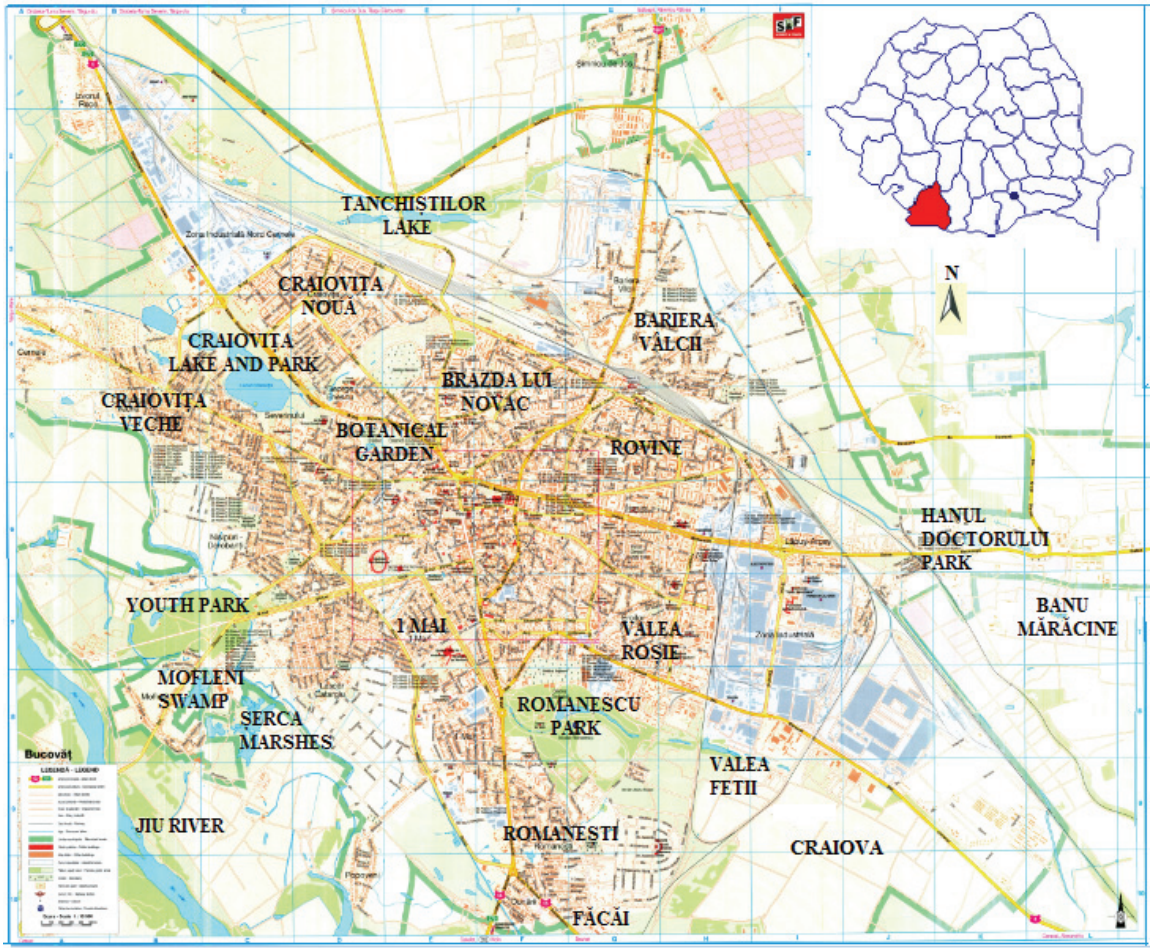

Figure 1. Map of Craiova Municipality, with the main studied areas (Printed by Druckerel Radinger; Impressum: SC Schubert & Franzke SRL, Cluj Napoca 2009). Processed. Scale 1: 16 500.

MATERIAL AND METHODS

For the research of mammals, classical methods have been used, applicable to all groups of mammals (field trips, observation, collection, photography).

The field trips in the main parks and in the main neighbourhoods of the city were performed monthly. The majority of them happened in the morning, between 7 AM and 2 PM, during spring, summer and autumn. In the winter season, the field trips were carried out between 10 AM and 2 PM. The evening research, although less frequent, was conducted between 7 PM and 9 PM. It took place in Rovine, Brazda lui Novac and Bariera Vâlcii neighbourhoods.

Direct observations were realised in the course of the years 2015 to 2018.

Part of the collected material (individuals from the families of Soricidae, Muridae and Cricetidae) was obtained with the help of barber traps. The traps were located randomly, at short distances of 3 to 7 metres one from the other, in different habitats: in forested habitat with trees and bushes, at their edge, on the edges of the paths, in open fields, meadows with ruderal vegetation, near lakes and rivulets. 70^0 ethyl alcohol was used as a preservative. The traps were set in spring and summer during 2015-2017. They were checked at an interval of 7 to 14 days. The mammals entered the traps accidentally.

Other types of mammals were collected directly by hand: moles, bats, hedgehogs. Small mammals, once collected, were preserved for didactic purposes (they can be found in the laboratory of vertebrates zoology). 70^0 ethyl alcohol and formalin 4% were used. Most individuals were collected by the authors, and a part was provided by students and colleagues. In the case of middle-sized mammals (rabbits, polecats, ground squirrels, etc.), observation and photography were employed as research methods. The field research and the information provided by the hunters were useful to complete the data about lagomorphs and carnivores mammals.

Small mammals were determined based on extern morphology, the aspect of the skull and the type of dentition. To identify and analyse mammals we have used the field guide (GAISLER & ZEJDA, 1995) and the scientific literature (IONESCU, 1968; MURARIU, 2000, 2004; MURARIU & MUNTEANU, 2005; POPESCU & MURARIU, 2001; VALENCIUC, 2002). The examination of the skull and dentition was realized with a stereomicroscope. A Cannon SX50 HS camera was used to photograph the species.

RESULTS AND DISCUSSIONS

Results

We hereby present the list of the identified mammals, with details regarding the places of observation and collection. For the systematics of mammals, we have used the information on the website: https://mammaldiversity.org/.

Order Eulipothyphla

Family Erinaceidae Bonaparte, 1838

1. Erinaceus concolor Martin, 1838 – The Southern white-breasted hedgehog or Eastern European Hedgehog

A discreet species, the hedgehog was observed in different neighbourhoods, during evening and night: Craiovița, Mofleni, Romanești, Valea Roșie, in the gardens and orchards of the locals, in the green areas between the buildings (Brazda lui Novac neighbourhood), in the Botanical Garden (2015), "Hanul Doctorului" Park, Romanescu Park, the Northern Ring Road of the city (the area with the groups of planted trees), Valea Fetii area, etc. It is important to mention that from 2012, there is a stable population of hedgehogs in Brazda lui Novac neighbourhood. During evening and night, they were observed in different parts of the neighbourhood, in the grassy areas between the blocks of flats.

Collecting points: Valea Roșie neighbourhood –1 individual in June 2015, from the courtyard of some locals. Brazda lui Novac – 1 individual in May 2016, from the grassy areas behind the Pedagogical High School.

Family Talpidae Gray, 1825

2. *Talpa europaea* Linnaeus, 1758 – The common mole, The European mole

The mole is one of the common and numerous species in Craiova city. It is a subterranean species, widespread in most of the researched habitats: Romanescu Park, the Youth Park, the Botanical Garden, the area Tanchiștilor Lake, Hanul Doctorului Park etc., with less occurrences in the area near the river Jiu. Its presence is revealed by a great number of molehills, observed in the areas studied. Most of the molehills were detected in Romanescu Park and in the Youth Park. This field survey has also attested the presence of this species in the yards of the locals from different neighbourhoods (mostly on the outskirts of the city: Romanescu neighbourhood, Valea Fetii, etc).

Collecting points: Youth Park – 1 individual in May 2016, along the alley. The area "Tankistilor Lake" – 1 individual the 28th of April, 2017, from a grassy area. The collected individuals were already dead.

Family Soricidae Gray, 1825

3. *Crocidura leucodon* (Herman 1780) – The bicolored shrew

Collecting points: Romanescu Park – 2 individuals, an adult and a young individual, on the 28th of July, 2015, in the open shrubbery area.

4. *Crocidura suaveolens* (Pallas, 1811) – The lesser white-toothed shrew

Collecting points: Romanescu Park – 1 individual on the 6th of August, 2015, 2 individuals on the 17th of July 2016 – on grassy field, at the edge of the path to the hippodrome, 1 individual on the 28th of May, 2017 – on forested areas, next to the oak trees (in the eastern side of the park, near the Ring Road of the city).

Hanul Doctorului Park – 2 individuals on the 15th of June, 2016 – on forested areas, next to the oak trees, near the water bodies.

5. *Sorex araneus* Linnaeus, 1758 – The common shrew

Collecting points: Romanescu Park – 1 individual on the 20th of June, 2015 –next to the clump of trees, in the eastern side of the park.

Youth Park – 1 individual on the 15th of June, 2016, 1 individual on the 19th of July 2017 – on forested areas, next to the oak trees, in grassy area.

6. *Sorex minutes* Linnaeus, 1766 – The Eurasian pygmy shrew

Collecting points: Youth Park – 2 individuals on the 15th of June, 2016 – on forested areas, next to oak trees, in grassy area.

Order Chiroptera Blumenbach, 1779

Family Vespertilionidae (Gray, 1821)

7. *Nyctalus noctula* Schreber, 1774 – The common noctule

The species was observed while flying, in Romanescu Park, at twilight, as well as during cloudy weather. A small population was also detected in Rovine neighbourhood, near the church and the blocks of flats around it. They took shelter in the attics of the buildings, in the bell tower of the church, as well as in tree hollows along the streets of the neighbourhood.

Collecting points: Rovine neighbourhood – 1 individual on the 28th of February, 2015 – on a grassy field, found at the bottom of a poplar tree, near the St. Constantin and Elena church.

Romanescu Park – 1 individual on the 24th of March, 2017 – collected from the grass, near an oak tree (fallen from the hollow tree).

8. *Plecotus auritus* Linnaeus, 1758 – The brown long-eared bat or common long-eared bat
We own individuals from the chambers of the Agronomic Students Complex (May 2015 – 1 individual, June 2016 – 2 individuals). Field research confirmed the presence of this species in the area, both in the attics of some buildings as in the hollow trees. Based on the field investigation and on our personal observations we can state that the species has adapted to this area.

Order Lagomorph Brandt, 1855
Family Leoporidae Gray, 1821

9. *Lepus europaeus* Pallas, 1778 – The European hare
For the urban area of the city, the European hare is a sporadic presence. *Lepus europaeus*, a terrestrial species, was observed in the eastern side of Romanescu Park, in the area with the clumps of trees, but also in the grassy habitat, near the hippodrome (in September 2015, August 2016, March 2018). It comes from the peripheral area Valea Fetii, located in the south part of the city, at the soud-eastern side of the park.
Moreover, on the outskirts of the Mofleni neighbourhood, the area of the Șerca marshes, solitary individuals were observed both in the reeds, as on uncultivated land with high grass and thistles, in June 2016 and March 2017. They probably come from the wastelands in Bucovăț, a village situated in the west of Craiova, on the right bank of the Jiu River.
Also, the species was observed in the didactic resort of Banu Mărăcine, in the area with fruit trees, and the individuals probably come from the agricultural lands of Cârcea. The locals chase them away because they gnaw the bark of trees and ruin the harvest. Individuals from the species were noticed during all seasons by the staff working at Banu Maracine resort. The discussions held with the hunters certified the presence of the hares, mostly in the peri-urban area: Făcăi area, Valea Fetii, the forested area in the north of the city, between the ring road and the outskirts Bariera Vâlcii neighbourhood, etc.

Order Rodentia Bowdich, 1821
Family Sciuridae Gray, 1821

10. *Sciurus vulgaris* Linnaeus, 1758 – The Eurasian red squirrel
It is an arboricolous species, common in the city, with numerous flocks in Romanescu Park. The coat of the red squirrel varies in colour, from red to dark brown. It is a delight for the locals to watch its spectacular leaps, the way in which it searches for and consumes the food, and the partners' games. Annually, in September and at the beginning of October, the red squirrel was observed burying its food in the ground (different kinds of fruit, like nuts, apples, acorns, etc). In the Botanical Garden and in the Youth Park the species is present in a smaller number.

11. *Spermophilus citellus* Linnaeus, 1766 – The European ground squirrel or The European souslik
It is a diurnal, terrestrial and gallericolous species, and it was observed in Făcăi (a peripheric area of Craiova city), near during the day, eating vegetal food. One or two specimens were observed on grassy areas, on meadows and near the foot paths, on the 12th of May 2015, the 11th of August 2015, the 28th of June 2016 and the 26th of May 2017.
Based on the field survey and on our personal observations (although scarce), we can claim that the species had a constant presence in Făcăi area throughout the years.

Family Muridae Gray, 1821

12. *Rattus norvegicus* (Berkenhout, 1769) – The brown rat
It is a common and frequent species in Craiova, which coexists with human beings. It was observed in all the areas that have been studied. It was mostly spotted on the shores of the lakes in the parks, in the Botanical Garden, Craiovița Lake; and throughout the public sewer system. The species was also observed in the basements of the blocks of flats, in the locals'cellars, in warehouses, landfills and dumpsters, etc.

13. *Mus musculus* Linnaeus, 1766 – The house mouse
Common and frequent species, the house mouse was observed in all the anthropic habitats of the city, mostly in cellars and residential annexes. Individuals of house mice also entered the traps placed in the parks.
Collecting points: Hanul Doctorului Park: 2 individuals on the 17th of August 2015; Youth Park – 1 individual on the 19th of July 2017.

14. *Apodemus sylvaticus* (Linnaeus, 1758) – The wood mouse
Collecting points: Romanescu Park: 1 individual of juvenile mouse on the 17th of July 2016 – at the line trees, at the outskirts of the park, in the south-east part of the park. Youth Park: 1 individual on the 19th of July, 2017 – in open grassy field, towards the nursery (near Șerca brook).

15. *Apodemus flavicollis* Melchior, 1834 – The yellow-necked mouse
Collecting points: Youth Park – 1 individual on the 19th of July 2017 – on forested areas, on area besides the oaks tree.

Family Cricetidae Rochebrune, 1883

16. *Microtus arvalis* (Pallas, 1799) – The common vole

Collecting points: Romanescu Park – 2 individuals on the 28th of July 2015, at the bottom of some shrubs, towards the hippodrome. Youth Park –1 individual on the 28th of May 2016 – on forested areas, near oak trees.

17. *Microtus agrestis* (Linnaeus, 1761) – The field vole or short-tailed vole

Collecting points: Romanescu Park – 1 individual the 12th of August 2016 – at the line trees towards the hippodrome. Youth Park: 1 individual on the 19th of July 2017 – on forested area with oak trees.

Order Carnivora Bowdich, 1821

Family Mustelidae Swainson, 1835

18. *Mustela putorius* Linnaeus, 1758 – The European polecat

The information about the European polecat is insufficient, because of its crepuscular and nocturnal activity. In Craiova, it was observed in the shrubbery area of the Jiu Meadow in November 2015. The field research confirmed the fact that this species has accidental occurrences near the households at the outskirts of the city, during colder seasons (autumn, winter), being in search of food.

19. ***Meles meles*** (Linnaeus, 1758) – The European badger

The presence of this species in the city was confirmed by an individual that was found dead in the Hanul Doctorului Park on the 31st of March 2018. We do not have data with regard to the place this individual originated from or about the time it appeared in the area.

Discussions

The preliminary systematic list of the mammals from the intra-urban and peri-urban area of Craiova city is based on a personal database and on the bibliographic references (Table 1).

Table 1. The faunistic list of the mammals from the habitats of Craiova city between 2015 and 2018.

No	Species	Records		Habitat					
		Literature	Personal database	Parks	Neighbourhoods, Buildings, Gardens, Green Areas	Peri-urban area	Open area Meadows	Forested area with trees and shrubs	Humid area
1.	*Erinaceus concolor*	BAZILESCU et al. (1980) MURARIU (2000)	collected	HDP BG	+		+	+	
2.	*Talpa europaea*	BAZILESCU et al. (1980) MURARIU (2000)	collected	YP	+	+	+	+	
3.	*Crocidura leucodon*	BARBU & SORESCU (1970) MURARIU (2000)	collected	RP			+	+	
4.	*Crocidura suaveolens*	BARBU & SORESCU (1970) MURARIU (2000)	collected	RP HDP			+	+	
5.	*Sorex araneus*		collected	RP YP			+	+	
6.	*Sorex minutus*		collected	YP				+	
7.	*Nyctalus noctula*	BAZILESCU et al (1980) GOGA (2012)	collected	+	+	+		+	
8.	*Plecotus auritus*	BAZILESCU et al. (1980)	collected	+	+	+		+	
9.	*Lepus europaeus*		observed	RP		+	+	+	
10.	*Sciurus vulgaris*	GOGA (2012)	observed	+	+			+	
11.	*Spermophilus citellus*	BAZILESCU et al (1980)	observed			+	+		
12.	*Rattus norvegicus*	BAZILESCU et al. (1980)	observed	+	+	+	+	+	+
13.	*Mus musculus*	BARBU & SORESCU (1970) BAZILESCU et al. (1980)	collected	+	+	+	+	+	+
14.	*Apodemus sylvaticus*	BARBU & SORESCU (1970)	collected	YP RP			+	+	
15.	*Apodemus flavicollis*		collected	YP			+	+	
16.	*Microtus arvalis*	BARBU & SORESCU (1970)	collected	RP YP			+	+	
17.	*Microtus agrestis*		collected	RP YP			+	+	
18.	*Mustela putorius*	BAZILESCU et al (1980)	observed			+	+		
19.	*Meles meles*	MURARIU & MUNTEANU (2005)	observed	HDP				+	

Legend: The names of the parks where the specimens have been collected; RP – Romanescu Park, YP – The Youth Park, HDP – Hanul Doctorului Park , BG – The Botanical Garden; + observed in the parks; common for those habitats.

Between the years 2015 and 2018, 19 species of mammals were identified, distributed in 5 orders and 9 families: Order Eulipotyphla – 6 species: *Erinaceus concolor* (Family Erinaceidae), *Talpa europaea* (Family Talpidae), *Crocidura leucodon, C. suaveolens*, *Sorex araneus, S. minutus* (Family Soricidae); Order Chiroptera – 2 species (*Nyctalus noctula, Plecotus auritus* (Family Vespertilionidae); Order Lagomorpha – 1 species: *Lepus europaeus* (Family Leporidae); Order Rodentia – 8 species: *Spermophilus citellus*, *Sciurus vulgaris* (Family Sciuridae), *Microtus arvalis, M. agrestis* (Family Cricetidae), *Rattus norvegicus, Mus musculus, Apodemus sylvaticus, A. flavicollis* (Family Muridae); Order Carnivora – 2 species: *Mustela putorius, Meles meles* (Family Mustelidae).

During the research, some species were also collected that have not been recorded yet: *Sorex araneus, S. minutus, Apodemus flavicollis, Microtus agrestis.* We did not find any records in the scientific literature regarding the presence of the species *Lepus europaeus* in Craiova city.

Among the species mentioned in scientific literature, but not identified on the field, we list the species: *Apodemus agrarius, Micromys minutus* (BARBU & SORESCU, 1970), *Myotis mystacinus, Plecotus austriacus* (BAZILESCU et al.,1980), *Myotis myotis* (GOGA, 2012). Regarding the bats, we mention that we received information from the locals regarding their presence in different neighbourhoods of the city: the city centre, Craiovița, Cornițoiu, George Enescu, Brazda lui Novac, 1 Mai, Valea Roșie, Lăpuș-Argeș etc. The species could not be determined due to a lack of catch and of a bat detector. It is possible that they were the same species that we collected, or some of the species cited by BAZILESCU (2014), BAZILESCU et al. (1980), and GOGA (2012).

Regarding the specimens from the Muridae Family, we often observed dead specimens of *Rattus norvegicus, Mus musculus* in different habitats of the city, as a result deratization**.** The shrews were also affected by the deratization carried out by specialised staff. Therefore pest controls of rodents should be realised in accordance with the existing legislation, by selective and efficient means directed to the targeted species, so that the biological equilibrium should not be disrupted.

Most of the species were observed and/or collected from parks: clumps of trees and shrubs, at their edges, in clearings of woods (Table 1). In the neighbourhood areas dominated by buildings we remarked species of bats, *Rattus norvegicus* and *Mus musculus.* We can also add to this category the *Erinaceus concolor*, with a limited distribution, and *Talpa europaea*, observed in the locals' gardens. Lately, we have often been noticed by the locals regarding the presence of moles in their gardens. In the peri-urban area of the city (meadows characteristic to the silvostepa area, fallow ground with grassy vegetation, shrubs, cultivated lands, etc.) we observed species like *Spermophilus citellus, Lepus europaeus* and *Mustela putorius.* In this area species of rodents like *Crocidura* sp., *Sorex* sp., *Microtus* sp., *Apodemus* sp. etc., it is possible to exist. This statement is supported by the opening of the galleries observed. In the future, the peri-urban area of the city should be researched more thoroughly.

The majority of species were observed/collected in forested areas. A few of the mammals were collected from open areas. Throughout the investigations we have concluded that the faunistic elements characteristic to the city and which have been frequently observed all over the years were the insectivores, rodents and the species of bats.

In accordance with the Law 407/2006, three species characteristic to the peri-urban area of Craiova, *Lepus europaeus, Mustella putorius* and *Meles meles*, are listed in Annex 1 as species of hunting interest. Among them, *Lepus europaeus* was observed in Romanescu Park.

Regarding the conservation status of the species, five of them have unfavourable status, as being vulnerable to the anthropic and climatic impact: *Crocidura leucodon, C. suaveleons, Plecotus auritus, Spermophilus citellus, Microtus agrestis* (MURARIU, 2005). Most of the mammals observed present secure status (they are not in need of special protection).

In recent years, the infrastructure of the city has been massively expanded towards the peri-urban areas, which brought major modifications in natural biocenosis. Many of the wild species near the city became part of the city.

The frequent adjustments of the parks in Craiova had minor effects on the existing mammals. Most of the identified mammals are terrestrial, gallericolous and nocturnal, and during the day they stay underground. The cutting of some massive hollow trees affected especially the microhabitat of the squirrels and the bats, and the depositing of rubble and waste attracted the rodents, especially *Rattus norvegicus* and *Mus musculus.*

It is already acknowledged that mammals are extremely mobile and they may move long distances in search of food. Food resources available in town determine some species from the rural area to move to urban areas. For instance, the fox is one of the species that got the closest to the city. It was observed on the agricultural lands in Ghercești, Cârcea, Preajba (localities near Craiova), as well as in Făcăi, Valea Fetii, Mofleni, Popoveni (peri-urban areas of Craiova). We consider that, parallelly with climate change, but also with socio-economic changes, the presence of some wild mammal species in the urban areas becomes possible.

Though the phenomenon of the urbanisation of mammals has been a debated issue during the last decades, the topic is wide and needs more thorough research. Cities have become favourable habitats for some species of mammals, providing shelter for them (especially in winter), as well as food resources and new microclimatic conditions; each species adapts to the new conditions in its own way.

CONCLUSIONS

The results of the study have shown that the mammals fauna in the habitats of the city of Craiova is quite diverse, gathering terrestrial, gallericolous-terrestrial, subterraneous, arboricolous and flying species which have adapted to the anthropic conditions.

As the data is preliminary, monitoring the mammals in Craiova must be continued for a better appreciation regarding: existent species, the dynamics in terms of the species population, the mammals'spatial distribution, the intra and interspecific relationships, the action of the disturbing factors on the populations of mammals, the mechanisms of the adaptation processes, etc.

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STATISTICAL EVALUATION OF SEMINAL PLASMA VS. BLOOD SERUM BIOCHEMICAL PARAMETERS IN BRUNA AND HOLSTEIN FRIESIAN BREEDING BULLS

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Abstract. A range of blood serum biochemical parameters (% protein, mg% fructose, IU - GOT, GPT and alkaline phosphatase enzyme activity) were analysed, as well as some seminal plasma biochemical parameters (% protein, mg% glucose, IU - GOT, GPT and alkaline phosphatase enzyme activity, mg% Ca, mg% P, mg% Mg) in two breeds of bulls, namely Bruna and Holstein Friesian. The t test revealed that the Friesian breed significantly differed from the Bruna breed with regard to seminal GOT activity (t=-3.175**), seminal alkaline phosphatase (t=2.848**), serum protein (t=3.813***), serum phosphorus and magnesium (t=-4.022**; -3.228**). Variability coefficients of enzymes activities were highly elevated in the Bruna breed (101.22% GPT and 83.76% alkaline phosphatase). Seminal enzymatic activities were not normally distributed to any of the breeds. In the Holstein Friesian breed, the significant correlations between seminal parameters (p <0.01 and p <0.001), were more numerous. Significant correlations between serum and seminal biochemical parameters, which were different from one breed to another, can be considered race characteristics. The same significant correlations between serum and seminal parameters that have been established in both breeds can be considered species characteristics, for example: serum protein-seminal protein (Bruna r=0.536**, p<0.01; Friesian r=0.673***, p<0.001), as well as serum alkaline phosphatase-seminal protein (Bruna r=-0.420*, p<0.05; Friesian r=-0.536**, p<0.01). Regressions revealed that in the Bruna breed the serum protein influenced 28% of the seminal protein variability, this percentage being 45% in the Friesian breed.

Keywords: blood serum parameters, Bruna breed, Holstein Friesian breed, seminal plasma parameters, statistical analysis.

Rezumat. Evaluarea statistică comparativă a parametrilor biochimici ai plasmei seminale şi ai serului sangvin la taurii de reproducţie din rasele Brună şi Holstein Friză. S-au analizat o serie de parametri biochimici ai serului sangvin (proteina %, fructoza mg%, activitatea enzimelor GOT, GPT si a fosfatazei alcaline UI) si a plasmei seminale (proteina %, glucoza mg%, activitatea enzimelor GOT, GPT si a fosfatazei alcaline UI, Ca mg%, P mg%, Mg mg%) la doua rase de tauri de reproducţie, Brună si Holstein Friză. Testul t a scos în evidenţă faptul că rasa Friză a diferit semnificativ de rasa Brună în privinţa activităţii GOT seminale (t=-3,175**), fosfatazei alcaline seminale (t=2,848**), proteinei serice (t=3,813***), fosforului şi magneziului seric (t=-4,022**; -3,228**). Coeficienţii de variabilitate la activităţile enzimatice seminale au fost extrem de crescuţi la rasa Brună, (101,22% GPT şi 83,76% fosfataza alcalină). Activităţile enzimatice seminale nu au fost distribuite normal, la niciuna dintre rase. Corelaţiile semnificative dintre parametri seminali (p<0,01 si p<0,001), au fost mai numeroase la rasa Holstein Friză. Corelaţiile semnificative dintre parametri biochimici, care au diferit de la o rasa la alta, pot fi considerate caracteristici de rasă. Numeroasele corelaţii semnificative dintre parametri serici si seminali care s-au stabilit la ambele rase, pot fi considerate caracteristici de specie, spre exemplu: proteina serică-proteina seminală (Brună r=0,536**, p<0,01; Friză r=0,673***, p<0,001), precum si fosfataza alcalină serică-proteină seminală (Brună r=-0,420*, p<0,05; Friză r=-0,536**, p<0,01). Regresiile au relevat faptul ca la rasa Brună proteina serică a influenţat variabilitatea proteinei seminale în proporţie de 28%, la rasa Friză acest procent a fost de 45%.

Cuvinte cheie:** analiză statistică, parametri plasmei seminale, parametri serului sangvin, rasa Brună, rasa Holstein Friză.

INTRODUCTION

Monitoring the biochemical parameters of blood or other biological fluids is a powerful tool for assessing the status of domestic animals health. Knowing the physiological limits of these parameters variation is a major concern for specialists in veterinary medicine. The relationship between blood biochemical parameters and seminal plasma in breeding bulls is less studied in literature and may be relevant not only for overseeing the animal health status, but also for establishing the mechanisms by which a number of phenotypic factors (diet, animal growth conditions, ambient temperatures etc.) are reflected in the body fluids biochemical variables. The data provided by literature on the normal variation intervals of blood biochemical parameters are very different from one author to another. These seem to depend both on specific experimental conditions, as well as on the breed. For example, the blood serum total protein concentration reported by KANEKO et al. (1997) ranged from 67.4 to 74.6 g/l, and STOJEVIĆ et al. (2008) communicated an average of 85.16±6.31 g/l). Some of the serum parameters, for instance calcium and magnesium concentrations vary more closely between different authors: Ca (mg%): 9.48 (PAYNE & PAYNE, 1987), 9.72-12.4 (KANEKO et al., 1997); 9.6±1.6 (STOJEVIĆ et al., 2008); Mg (mg%): 2.51(PAYNE & PAYNE, 1987), 1.8-4.31 (KANEKO et al., 1997), 2.21 (FREERKING, 1979), 1.97±0.34 (STOJEVIĆ et al., 2008). The enzymes activities of bovine serum vary significantly with different authors, the values being generally characterized by high variation ranges: alkaline phosphatase (IU): 96.81±1.94 - 162.36±193.25 (DIKOVIC, 2017), 89.6±7.95 (AL-FARTOSI, 2010), 0.3-114.3 (ALLCROFT & FOLLEY, 1941); GPT (U/l): 11-40 (KANEKO et al., 1997), 35.98-47.7 (MAZZULLO et al., 2014), 19.40±0.30 (ELIAZAB, 2015); GOT (U/l): 78-132 (KANEKO et al., 2008), 95.21-130.3 (MAZZULLO et al., 2014) and 45.05±3.04 (ELIAZAB, 2015).

Numerous studies have associated biochemical parameters of sperm with its quality. However, the values of these parameters should be interpreted with caution, being in many cases a breed characteristic. For example, in a

previous study concerning seminal plasma of Bruna bulls we obtained seminal protein values of 6.32±1.67% (TAMBA-BEREHOIU et al., 2017), lower than those specified in literature for other breeds: 13.5 g/l for the Nelore breed, [ASSUMPÇÃO et al., 2005] and similar values 7.04%, obtained by other researchers (GRAHAM, 1978). The content of seminal plasma in fructose, generally presents values ranging from 460-730 mg% (KIRTON et al., 1964; CUPPS et al., 1969). Enzymatic activity of semen also varies widely. Transaminase activity, represented by the enzymes glutamate-oxalacetate-transaminase (GOT) and pyruvate glutamate transaminase (GPT), presents in the literature values from 166.72 IU and respectively 34.56 IU, reported by CHAUHAN & SRIVASTAVA (1973) in bison semen, to 594.25 GOT (IU) and 40.93 GPT (IU), reported for Holstein bulls semen by HUSSAIN et al., (2016). The activity of alkaline phosphatase in seminal plasma also recorded different values in various authors. CHENOWETH & LORTON (2014) which quotes sources in the literature, reported a value of 1687.5 IU, MURDOCH & WHITE (1968) reported a value of 1990 UI and in previous studies conducted by us on Bruna bulls, we found values of 234.09-8947.56 IU (TAMBA-BEREHOIU, 2017). Differences in literature can highlight the breed characteristics as well as the influences of experimental conditions on seminal enzymatic systems.

The purpose of the paper was to highlight the way the variation of some semen biochemical parameters, from breeding bulls of Bruna and Friesian races, correlated with the dynamics of bovine serum biochemical parameters.

MATERIAL AND METHODS

The experiment consisted in the determination of some seminal plasma and blood serum biochemical parameters, taken from Bruna and Friesian breeding bulls. In this regard, 26 ejaculates were collected from every breed in the month of September (temperate climate). The sperm collection was done with the artificial vagina. The breeding bulls were kept under similar conditions, had close ages (3 yeas), and were used in the same regime for reproduction based on artificial inseminations. Blood samples were taken shortly after sperm collection. Seminal plasma (supernatant) was obtained by semen centrifugation, 20 min. at 2500 rpm. Blood serum was obtained by centrifuging the coagulated blood for 20 minutes at 2500 rpm. Parameters of seminal plasma were analysed by the following methods:

- determination of total seminal protein by the biuret method (%). 0.1 ml of seminal plasma was sampled, to which 5 ml of biuret reagent was added. The colour develops in 30' and the sample was occasionally stirred. The optical density (O.D.) was read at spectrophotometer (λ=570 nm) against a 1% bovine albumin standard (KRUEZIGER et al., 2009);

- **determination of seminal fructose (mg%)**. 2 ml of deproteinized seminal plasma (with zinc sulphate and sodium hydroxide solutions) was filtered. It was added 2 ml of 0.1% resorcinol in alcohol and 30 ml of 30% HCl. The sample was incubated 10' at 80° C in a water bath. After one hour rest in the dark, O.D. at 540 nm was read, against a control containing fructose solution, instead of seminal plasma (JIN-CHUN LU et al., 2007);

- **determination of seminal GOT activity (IU).** The enzyme glutamate oxalacetate transaminase catalyses a transamination reaction, namely: L-aspartate + α-ketoglutarate ↔ L-glutamate + oxaloacetate reaction. To 0.1 ml of seminal plasma was added 0.5 ml of enzyme substrate in phosphate buffer at pH 7.4 (aspartic acid, α-ketoglutaric) and it was incubated for 60 min. at 37° C. Subsequently, 0.5 ml reagent of 1 mM 2,4-dinitrophenylhydrazine solution in 1N HCl was added. After stirring and resting for 20 min., 5 ml of 0.4 N NaOH was added. After another 5 min rest, O.D. at 546 nm was read (REITMAN et al., 1957);

- determination of seminal GPT activity (IU). Pyruvate-glutamate transaminase catalyses the L-alanine + α-ketoglutarate ↔ L-glutamate + pyruvate reaction. 0.1 ml of seminal plasma was incubated for 30 min. at 37° C, with enzyme substrate (alanine, α-ketoglutaric acid in phosphate buffer pH 7.4). It was added 0.5 ml of 1 mM 2,4-dinitrophenyl hydrazine, prepared in 1N HCl. After stirring, the sample rested for 20 min, then 5 ml of 0.4 N NaOH was added. The sample was read after 5 min. to a spectrophotometer at 546 nm (REITMAN et al., 1957);

- determination of seminal alkaline phosphatase activity (IU). The reaction is the following: para-nitro-phenyl phosphate is hydrolyzed under the action of phosphatases (Bessey-Lowry-Brock method) and phenol is released. Phenol is yellow in alkaline medium and its concentration can be determined spectrophotometrically. 0.1 ml of seminal plasma, and 2 ml added substrate (7.6 mM sodium p-nitrophenyl phosphate solution in glycol buffer pH 10.4) were incubated for 30 min at 37° C. It was added 10 ml of 0.1 N NaOH and O.D. was read on the spectrophotometer at 405 nm. The control contained 2 mM para-nitrophenol solution (GALINDO, 2010);

Blood serum parameters were analysed according to the following methods:

- determination of blood serum protein, GOT, GPT and alkaline phosphatase activities. The same methods as for the determination of seminal plasma biochemical parameters were used.

- determination of blood serum glucose (mg%). The blood glucose in the presence of ortho-toluidine forms, in acidic medium, a green-blue complex. The colour intensity is proportional to the glucose concentration. To deproteinized serum, 6% trichloroacetic acid solution was added. It was centrifuged. To 1 ml supernatant 3 ml of ortho-toluidine reagent was added. The colour was developing in 20 minutes on a water bath. The O.D was read spectrophotometrically at λ=610 nm. (COOPER et al., 1970, based on the original method of HULTMAN, 1959).

- complexometric determination of Ca in blood serum (mg%). 1 ml serum, diluted with 25 ml distilled water was basified with 0.2 ml NaOH 9 N. Murexide crystals were used as indicator and the solution was titrated with 0.01 N

EDTA, until the colour turns from pink to purple. The control contains a standard solution of 2% $CaCO_3$ (ELDJARN, 2009, based on the original method of RAVINDRANATH, 1981).

- determination of blood serum P (mg%). To 0.1 ml of serum, sodium pyrosulfite and borax solution, ammonium molybdate solution and ascorbic acid solution were added. The control ws prepared similarly but contained instead of serum, 0.1 ml of phosphorus standard. The mixtures was allowed to rest 15 minutes. Then 2.5 ml of sulfite and sodium carbonate solution was added. It was stirred and O.D. was spectrophotometrically read at λ=660 nm (FOGG & WILKINSON, 1957).

- determination of blood serum Mg (mg%). To 2 ml serum, was added with stirring: 0.5 ml of polyvinyl alcohol, 0.5 ml of yellow titanium and 1 ml of 7.5% sodium hydroxide. After 5 minutes the sample and control O.D. were spectrophotometrically read at λ=540 nm. The colour was stable about one hour (HEAGY, 1948).

The analysed parameters were subjected to computer-assisted statistical calculations, using the professional IBM SPSS Statistics Program. In this regard, computing of descriptive statistics, t-test, Shapiro-Wilk normality test, correlation coefficients and regressions have been performed.

RESULTS AND DISCUSSIONS

The descriptive statistics of seminal plasma and blood serum biochemical parameters in Bruna and Holstein Friesian breeds are shown in Table 1.

Table 1. Bruna and Friesian breeds seminal plasma and blood serum biochemical parameters.

Parameters	Mean	Std.Dev.	Minimum	Maximum	CV%
Seminal plasma Bruna breed					
1. Protein (%)	4.205	1.345	1.98	8.66	31.98
2. Fructose (mg%)	678.377	144.054	314.68	975.50	21.23
3. GOT (IU)	104.928	19.540	29.60	136.70	18.62
4. GPT (IU)	20.513	20.764	1.78	85.16	**101.22**
5. Alkaline Phosphatase (IU)	2804.373	2349.199	590.10	11330.44	**83.76**
Blood serum Bruna breed					
6. Protein (%)	4.718	0.637	3.96	6.69	13.50
7. Glucose (mg%)	42.071	11.455	21.97	69.23	**27.22**
8. GOT (IU)	41.333	7.062	26.19	56.12	17.08
9. GPT (IU)	18.427	9.765	1.78	35.89	**52.99**
10. Alkaline Phosphatase (IU)	34.481	22.284	9.29	87.22	**64.62**
11. Ca mg%	9.327	1.263	7.60	12.00	13.54
12. P mg%	5.270	1.019	3.64	7.37	19.33
13. Mg mg%	2.669	0.377	1.85	3.60	14.12
Seminal plasma Friesian breed					
1. Protein (%)	4.473	0.719	3.120	6.58	16.00
2. Fructose (mg%)	699.593	189.474	398.590	1059.42	**27.08**
3. GOT (IU)	88.655	17.351	51.030	137.54	19.57
4. GPT (IU)	15.075	10.430	0.880	46.61	**69.18**
5. Alkaline Phosphatase (IU)	5176.582	3538.217	1498.180	14777.84	**68.35**
Blood serum Friesian breed					
6. Protein (%)	5.489	0.811	4.000	7.18	14.77
7. Glucose (mg%)	33.307	12.538	7.400	57.12	**37.64**
8. GOT (IU)	38.257	7.562	24.260	58.32	19.76
9. GPT (IU)	16.763	6.655	1.810	31.60	**39.70**
10. Alkaline Phosphatase (IU)	27.308	11.975	5.370	56.16	**43.85**
11. Ca mg%	9.492	1.241	7.600	12.80	13.07
12. P mg%	4.216	0.864	2.790	6.25	20.49
13. Mg mg%	2.294	0.457	1.430	2.91	19.92

Source: Own calculation based on the experiment results.

The parameters of semen and blood serum were consistent with the results of different authors who have treated similar subjects (TAMBA-BEREHOIU, 2017; STOJEVIĆ et al., 2008; HUSSAIN et al., 2016 and others). At a first data analysis, the values of the GOT, GPT, and alkaline phosphatase enzymes activities, reached significantly higher levels in seminal plasma than in blood serum, in both breeds. Also, the seminal plasma and blood serum carbohydrates values are extremely different. Seminal fructose, compared to serum glucose, reached a value of about 16 times higher in the Bruna breed and 21 times higher in the Friesian breed. Metabolically speaking, serum glucose is regulated by insulin, whereas seminal fructose is not influenced by this hormone, so it can provide in large quantities additional energy to gametes, to maintain their mobility.

From the variability coefficients point of view, we can state that the enzymatic activities of GPT and alkaline phosphatase had extremely high values in both breeds, especially in the seminal plasma, but also in the blood serum (Bruna breed: 101.22% seminal GPT and 52.99% serum GPT; 83.76% seminal alkaline phosphatase, 64.62% serum

alkaline phosphatase; Friesian breed: 69.18% seminal GPT and 39.70% serum GPT; 68.35% seminal alkaline phosphatase, 43.85% serum alkaline phosphatase). Relatively high variability coefficients had also other parameters, in particular seminal plasma fructose and blood serum glucose in both breeds. However, the variability was noticeably lower in the Friesian breed than in the Bruna breed. The variability in GOT activity was moderate and at the same values in seminal plasma and serum, regardless of breed. Enzymatic activity of semen depends on many factors (breed, amount of substrate, enzyme activators, certain hormones, temperature, nutrition and even quantity of semen etc.), fact which explains the increased variability in these biochemical parameters. Extremely high variability suggested that these parameters are not normally distributed. Generally, extreme variability of enzyme parameters reflects their sensitivity to environmental factors. At the same time, the large variation ranges of enzymatic activity can be explained by enzymes involvement in mechanisms of maintaining the normal biological constants of some parameters.

The results of the Shapiro-Wilk test and the differences between mean and median of the non-normal distributed parameters are outlined in Table 2.

Table 2. The Shapiro-Wilk test of seminal plasma and blood serum biochemical parameters in Bruna and Friesian breeds.

Parameter	Bread	Mean	Median	Mean-median differences	W	p
Seminal protein (%)	Bruna	4.205	3.960	0.245	0.872**	0.004
Seminal GOT activity (IU)		104.928	107.505	- 2.577	0.807***	0.000
Seminal GPT activity (IU)		20.513	15.775	4.738	0.662***	0.000
Seminal alkaline phosphatase activity (IU)		2804.373	2071.000	733.373	0.764***	0.000
Serum protein (%)		4.718	4.580	0.138	0.873**	0.004
Serum alkaline phosphatase activity (IU)		34.481	30.240	4.24	0.898*	0.014
Seminal GPT activity (IU)	Friesian	15.075	12.055	3.02	0.871**	0.003
Seminal alkaline phosphatase activity (IU)		5176.582	4378.430	798.152	0.765***	0.000
Serum protein (%)		5.489	5.245	0.244	0.891**	0.010
Serum Mg (mg%)		2.294	2.375	- 0.081	0.904*	0.019

*significant at p<0.05; **very significant p<0.01; ***extremely significant p<0.001

Significant deviations from normality, especially in the activity of seminal and serum GOT, GPT and alkaline phosphatase enzymes, are observed, in both breeds.

Significant differences between breeds of the means and standard deviations of certain seminal and serum biochemical parameters, were tested using the t (Student) test (Table 3).

Table 3. The significance of means and standard deviations differences of biochemical parameters between breeds.

Parameter	Breed	Mean	t-value	p	Std.Dev.	F-ratio variances	p
Seminal GOT activity (IU)	Friesian	88.654	-3.175**	0.0025	17.350	1.268	0.556
	Bruna	104.928			19.539		
Seminal alkaline phosphat. activity (IU)	Friesian	5176.582	2.848**	0.0063	3538.217	2.268*	0.045
	Bruna	2804.373			2349.199		
Serum protein (%)	Friesian	5.489	3.813***	0.0003	0.811	1.616	0.236
	Bruna	4.718			0.637		
Serum glucose (mg%)	Friesian	33.307	-2.631*	0.0112	12.538	1.192	0.655
	Bruna	42.071			11.455		
Serum P(mg%)	Friesian	4.216	-4.022***	0.0002	0.864	1.390	0.416
	Bruna	5.270			1.019		
Serum Mg (mg%)	Friesian	2.294	-3.228**	0.0022	0.457	1.470	0.347
	Bruna	2.669			0.377		

*significant at p<0.05; **very significant p<0.01; ***extremely significant p<0.001

Standard deviations are significantly different between breeds (p<0.05), only for seminal alkaline phosphatase activity. Instead, the Friesian breed differs extremely significant from the Bruna breed, regarding serum protein and serum phosphorus (t=3.813***, respectively t=-4.022***). At the same time, between the Friesian and Bruna breeds there were very significant differences between seminal GOT activity (t=-3.175**), seminal alkaline phosphatase activity (t=2.848**) and serum Mg (t=-3.228**). Serum glucose is also significantly different between Friesian and Bruna breeds (t=-2.631*). All these differences customize racial characteristics and can influence the reproductive behavior of breeding bulls and the quality of the semen collected for artificial inseminations.

Within each breed, specific metabolic correlations were established between the biochemical parameters of seminal plasma and blood serum. Table 4 shows Pearson significant correlations between seminal and serum biochemical parameters in Bruna bulls (n=26), which are not found in Friesian bulls (n=26).

Table 4. Pearson correlations coefficients, Bruna breed (n=26).

Parameters	r/p	pGOT	pGPT	pAlk	sP	sPhos
sGlu (mg%)	r	0.416*				
	p	0.034				
sGPT (IU)	r				**-0.574****	
	p				0.002	
sAlk (IU)	r			**0.562****		
	p			0.003		
sPhos (mg%)	r	0.427*	0.411*			
	p	0.029	0.037			
sMg (mg%)	r					0.439*
	p					0.025

*significant at p<0.05; **very significant p<0.01; pGOT-plasma GOT, pGPT-plasma GPT, pAlk-plasma alkaline phosphatase, sP-serum protein, sGlu-serum glucose, sGPT-serum GPT, sAlk-serum alkaline phosphatase, sPhos-serum phosphorus, sMg-serum magesium.

Serum alkaline phosphatase activity correlated very significantly (p<0.01) with seminal alkaline phosphatase activity (r=0.562**) and seminal protein correlated with seminal GPT activity (p<0.01), very significant (r=-0.574 **). These correlations are explicable, it is about the correlations between serum and seminal proteins. The increase of the magnesium level simultaneous with that of phosphorus (present in phosphate-based biological systems) can be explained by the role of magnesium in the most biological processes involving phosphates: synthesis and use of macroergic compounds (ADP, ATP, AMP), synthesis of hydrogen and electron transporters (di- and triphosphoropyridine nucleotides, DPN, TPN,), phosphorylation mechanisms, activation of glycolytic enzymes, bones metabolism (activation of enzymes such as ATP-aze, alkaline phosphatase, pyrophosphatase etc.). Therefore, it is not excluded that the relationship between the two parameters is part of a metabolic balance. In the Friesian breed, there have been also noticed a number of significant correlations between seminal and serum biochemical parameters that do not appear in Bruna breed (Table 5).

Table 5. Pearson correlations coefficients, Friesian breed (n=26).

Parameters	r/p	pP	pGOT	pGPT	sP	sAlk
pGPT (IU)	r		0.580**			
	p		0.002			
sGOT (IU)	r				-0.392*	
	p				0.047	
sAlk (IUI)	r		-0.425*		**-0.536****	
	p		0.030		0.005	
sMg (mg%)`	r	-0.527**	-0.392*	-0.456*	-0.880***	0.506**
	p	0.006	0.048	0.019	0.000	0.008

*significant at p<0.05; **very significant p<0.01; ***extremely significant p<0.001; Pp-plasma protein, pGOT-plasma GOT, pGPT-plasma GPT, sP-serum protein, sGOT-serum GOT, sAlk-serum alkaline phosphatase, sMg-serum magesium

It is observed that serum magnesium established significant positive and negative correlations with both seminal parameters, e.g. protein (r=-0.527**, p<0.01), GOT activity (r=-0.392*, p<0.05) and GPT activity (r=-0.456* , p<0.05) as well as with other serum parameters, such as protein (r=-0.880***, p<0.001) and alkaline phosphatase activity (r=0.506**, p<0.01). Serum magnesium exists in three forms: free or ionic (about 55% of total), bound to serum proteins (about 30%) and complexed with phosphate or citrate ions (15%) (FAVUS, 2006). The data in literature, concerning the relationship between serum proteins and magnesium suggest that serum magnesium levels are independent of that of albumin (EVANS et al., 1988). Furthermore, most authors consider that the serum magnesium level is not even a true indicator of the body total magnesium content (JAHNEN-DECHENT et al., 2012). Magnesium balance is determined by the absorption from the gastrointestinal tract and also, by the renal excretion. To these phenomena, bones depletion can be added under certain conditions (KANEKO et al., 2008). Numerous studies have shown, that besides the role of the diet, significant influence on the serum magnesium value is played by certain animal stressors like: transport, unfavourable thermal conditions, effort, reproductive exploitation, water regime or age (RAYSSIGUIER et al., 2013). Given the numerous factors involved in modulating the relationship between magnesium and serum proteins, we believe that the identified correlation can not be explained exclusively on the basis of available data in this paper. Seminal GOT activity also significantly correlated with seminal GPT activity (r=0.580**, p<0.01) and serum alkaline phosphatase activity (r=-0.425*, p<0.05). The serum protein established significant correlations with other serum enzymes, GOT (r=-0.392*, p<0.05) and alkaline phosphatase (-0.536**, p<0.01).

We have identified a number of significant correlations that are common to both races, but with different degrees of significance (Table 6).

Table 6. Similar correlations in both breeds.

Specification	r/p	Breed	pP	pGOT	sAlk
sP	r	Bruna	**0.536****	**-0.525****	**-0.420***
	p		0.005	0.006	0.033
	r	Friesian	**0.673*****	**0.389***	**-0.536****
	p		0.000	0.049	0.005

*significant at p<0.05; **very significant p<0.01; ***extremely significant p<0.001; Pp-plasma protein, pGOT-plasma GOT, sP-serum protein, sAlk-serum alkaline phosphatase

It was noted that the serum protein is the parameter which correlated positive with seminal protein, to both breeds (Bruna r=0.536**, p<0.01; Friesian r=0.673***, p<0.001) and also with serum GOT for Bruna and Friesian breeds (r=-0.525**, p<0.01; respectively r=0.389*, p<0.05). The activity of serum alkaline phosphatase established with seminal protein significant negative correlations in both breeds (Bruna r=-0.420*, p<0.05; Friesian r=-0.536**, p<0.01). The correlations in Table 6 can be considered mostly species characteristics, because they occurred between the same biochemical parameters in both breeds.

It is useful to observe regressions between blood serum and seminal plasma parameters, in each breed (probability level of p<0.01 and p<0.001). Figure 1 shows these regressions in Bruna breed (p<0.01).

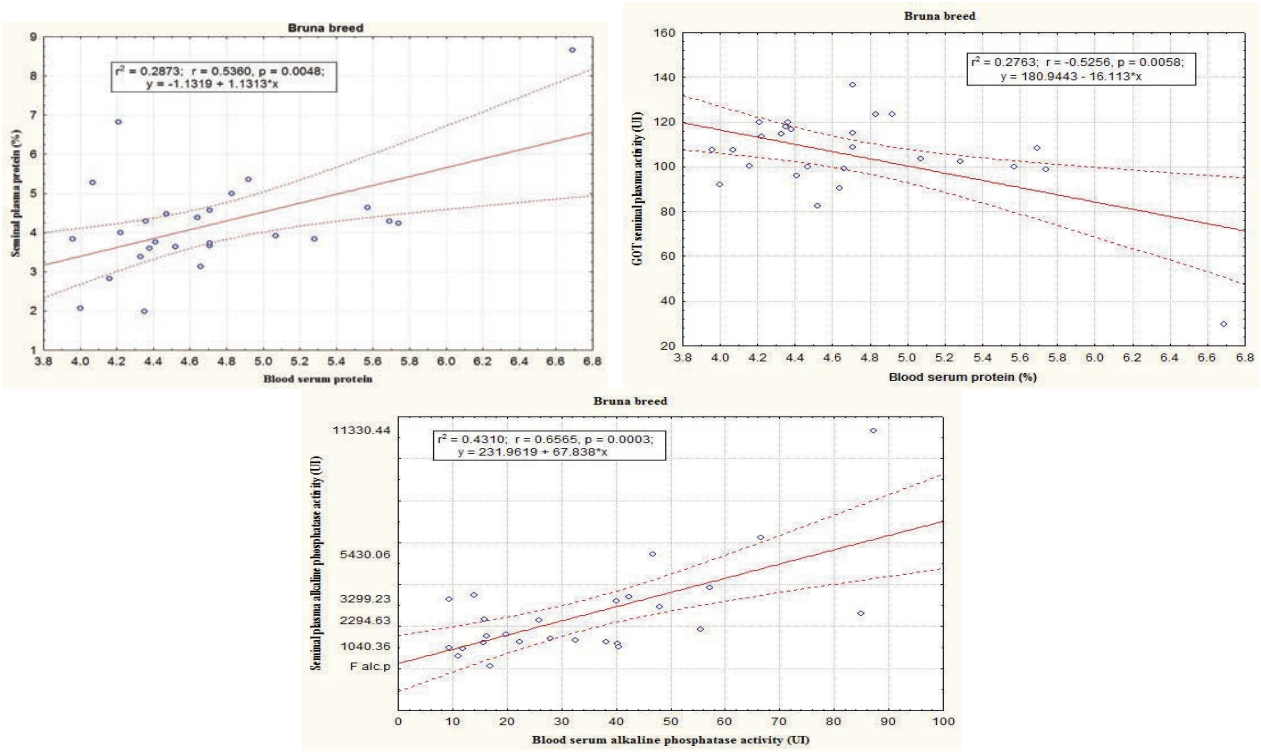

Figure 1. Blood serum – seminal plasma biochemical parameters regressions in Bruna bulls.

Serum protein determined 28% (r^2=0.287) of seminal protein variation and 27% of seminal GOT (r^2=0.276) variation. In contrast, the influence of serum alkaline phosphatase was noticeable on the increase of seminal alkaline phosphatase activity (43%), the coefficient of determination being increased in this case (r^2=0.431).

Figure 2 shows the regressions between blood serum and seminal plasma parameters in Friesian bulls breed.

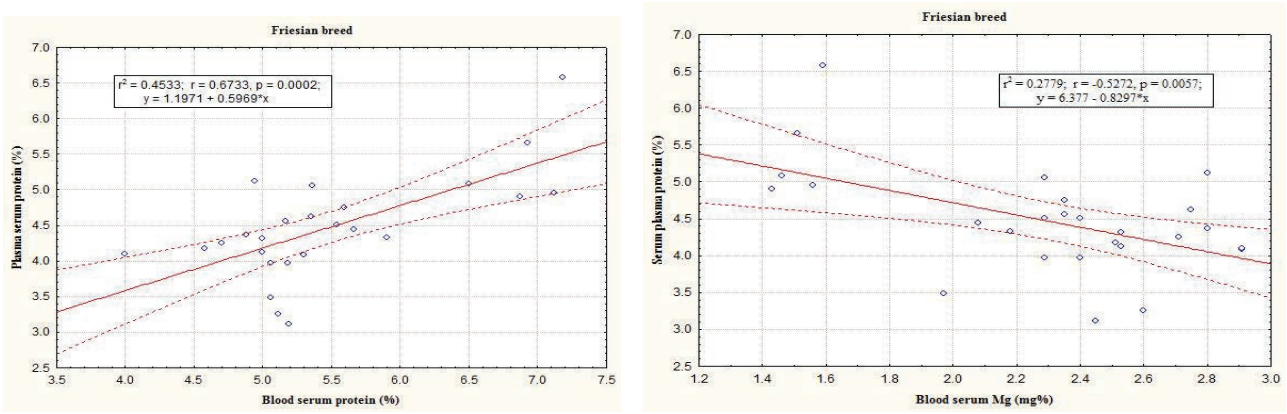

Figure 2. Blood serum - seminal plasma biochemical parameters regressions in Friesian bulls.

In Friesian breed, serum protein influences 45% of the seminal protein value (r^2=0.453). Seminal protein variability, or decrease in its concentration, is due to the blood serum Mg value, in proportion of 27.7% (r^2=0.277).

Biochemical analyses have reflected that the level of semen enzymes activities, namely GOT, GPT, and alkaline phosphatase, was dependent on how the blood serum protein varied. It had also been observed that the biochemical parameters have established stronger correlations in Friesian breed compared to Bruna breed and in larger

numbers. We must mention that the semen collection periods and various other factors, that is to say: temperatures, nutrition and reproductive exploitation, may partially modify the biochemical status of the two breeds semen.

CONCLUSIONS

The Friesian breed was significantly different from the Bruna breed, with respect to the values of seminal GOT (t=-3.175**), seminal alkaline phosphatase (t=2.848**), serum protein (t=3.813***), serum glucose (t=2.631*), serum phosphorus (t=-4.022***) and serum magnesium (-3.228**). Seminal GPT and alkaline phosphatase activities in particular, but also serum GPT and alkaline phosphatase activities, were extremely high in both breeds.

The Bruna breed revealed the highest coefficients of variability, eg: 101.22% seminal GPT and 83.76% alkaline phosphatase. The variability of seminal plasma and blood serum biochemical parameters was generally lower in Friesian breed than in Bruna breed.

The Shapiro-Wilk test demonstrated that not all the parameters had a normal distribution. The values of seminal GOT, GPT, alkaline phosphatase activities ($p<0.001$), phosphorus ($p<0.05$) and protein ($p<0.01$) deviated significantly from normal distribution in the Bruna breed. In the Friesian breed, seminal GPT ($p<0.01$) and alkaline phosphatase activities ($p<0.001$) as well as serum protein ($p<0.01$) showed significant deviations from normal distribution.

Pearson correlations between blood serum and seminal plasma parameters, which did not coincide to both breeds, at the probability levels of $p<0.05$ $p<0.01$ and $p<0.001$, were numerous. These correlations could be considered breed characteristics. Correlations between seminal biochemical parameters were more frequent in the Friesian breed and showed higher levels of significance. The same situation also emerged between biochemical parameters of blood serum. Certain correlations between the same biochemical parameters of blood serum and seminal plasma have occurred in both breeds. Thus, the serum protein correlated with seminal protein (Bruna r=0.536**; Friesian r=0.673***), with seminal GOT activity (Bruna r=-0.525**; Friesian r=0.389*) and with serum alkaline phosphatase activity (Bruna r=-0.420*; Friesian r=-0.536**), constituting species characteristics.

Regressions between serum and seminal parameters have revealed to what extent the serum parameters have influenced the seminal parameters. In Bruna breed, the serum protein influenced 28% of the seminal protein variability, 27% of the seminal GOT activity variability and serum alkaline phosphatase activity influenced the increase in seminal alkaline phosphatase activity by 43%. In Friesian breed, serum protein affects 45% of the seminal protein variability. Also, seminal protein varied, depending on the amount of serum Mg, in a proportion of 27.7%.

The results obtained by analysing the blood serum and seminal plasma of two breeds, Bruna and Holstein Friesian, highlighted some of the variability factors as well as the relationships patterns and the reciprocal influences of serum and semen biochemical parameters.

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ASPECTS ON THE IMPACT OF CLIMATE CHANGE ON VITICULTURE

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Abstract. Climate change is an incontestable phenomenon, one of the major threats to the environment and implicitly over humans. According to the European Environment Agency, climate changes mean global warming, increased sea and ocean temperatures, average global sea and ocean elevation, water acidification, shrinking glaciers due to the extensive melting of glaciers, desertification of green areas, and intensification of extreme meteorological phenomena (floods, long and prolonged drought, storms, etc.). Regarding vine culture, it is known that the main restrictive factors are climate factors, which have a decisive importance on this plant. Temperature and humidity, mainly determine the optimal run of the annual biological cycle of the vine, so of the phenophases. The main forecasts show that temperature variation will increase considerably in most wine regions in Europe. Thus, the increase in temperature will have serious implications for viticulture, especially as regards the plant-water relationship. Changes in the phenology of varieties are already recorded, with the forced triggering of the parga stage and the ripening of the grapes. Due to the fact that the grains of the grapes do not reach the varietal sizes, their must efficiency decreases. Some specialists also show that elevated temperatures and the lack of rainfall during the summer, to ripen grapes, prevent the attack of gray rot and favor the accumulation of sugars.

Keywords: climate change, vine, *Botrytis cinerea*.

Rezumat. Aspecte privind impactul schimbărilor climatice asupra viticulturii. Schimbările climatice reprezintă un fenomen incontestabil, una dintre marile ameninţări asupra mediului şi, implicit asupra omului. Potrivit Agenției Europene de Mediu modificările climatice implică creşterea temperaturilor medii la nivel global, creşterea temperaturii apei mărilor şi oceanelor, creşterea globală medie a nivelului mării şi oceanelor, acidifierea apei acestora, micşorarea calotelor glaciare datorată topirii extinse a gheţarilor, deşertificarea zonelor verzi, precum şi intensificarea fenomenelor meteorologice extreme (inundaţii, seceta dură şi prelungită, furtuni etc.). În ceea ce priveşte cultura viţei de vie se cunoaşte faptul că principalii factori restrictivi sunt factorii climatici, care au o importanţă hotărâtoare asupra acestei plante. Temperatura şi umiditatea, în principal determină derularea optimă a ciclului biologic anual al viţei de vie, deci a fenofazelor. Principalele previziuni arată că variaţia temperaturii va creşte considerabil în majoritatea regiunilor viticole din Europa. Astfel, creşterea temperaturii va avea implicaţii grave pentru viticultură, în special în ceea ce priveşte relaţia plantă-apă. Se inregistrează deja modificări ale fenologiei soiurilor, declanşarea forţată a etapei de pârgă şi de maturare a strugurilor. Datorită faptului că boabele strugurilor nu mai ajung la dimensiunile caracteristice soiurilor randamentul în must scade. Sunt şi unii specialişti care arată că temperaturile ridicate şi lipsa precipitaţiilor în cursul verii, spre maturarea strugurilor împiedică atacul putregaiului cenuşiu şi favorizează acumularea zaharurilor.

Cuvinte cheie: schimbări climatice, vița de vie, *Botrytis cinerea*.

INTRODUCTION

Climate changes affect fauna and flora, whose geographic distribution tends to head north. Climate deregulations have an impact on agriculture, human health, and generally on the social and economic framework. Although climate changes have been witnessed throughout the planet's history, present phenomena can have consequences that will overcome the responsiveness of natural and human systems. These systems risk being permanently altered or even destroyed.

According to numerous studies carried out over the past decades, temperature fluctuations and all extreme climatic phenomena are correlated with the CO_2 concentration in the atmosphere. The increasing concentration of carbon dioxide is a worrying phenomenon, and over the past decade scientists have confirmed that CO_2 has always been the main driver of climate change over the Earth's history. Carbon gas reduces atmospheric permeability for the Earth's reflected radiation to space, thus emphasizing the atmosphere's ability to keep the sun's heat longer than necessary through the greenhouse effect.

Studies on CO_2 in the Earth's atmosphere show that over the last 800 000 years, the concentration in this gas has never been higher than at present. According to PETIT et al. 1999, over the last 420 000 years, CO_2 concentration has peaked between 180 and 300 ppm.

If, for 500 years, the CO_2 concentration remained stable at about 270 ppm (EHLERINGER & CERLING, 1995) at the beginning of the nineteenth century (the start of the industrial revolution), CO_2 emissions increased by almost 50%, today the concentration value being about 400 ppm.

In addition to CO_2, other greenhouse gases are also covered by the Kyoto Protocol: methane (CH_4), nitrous oxide (N_2O) and anthropogenic fluorinated gases (so-called "F gases") grouped into: hydrofluorocarbons (HFC), perfluorocarbons (PFC) and sulphur hexafluoride (SF_6). In addition, we mention the ozone-depleting substances controlled by the Montreal Protocol: chlorofluorocarbons (CFCs), hydrochlorofluorocarbons (HCFCs) and halons (***. EPA www.epa.gov).

All these gases, which create stratospheric ozone layer imbalances, increase the equivalent concentration in CO_2, reaching carbon gas values between 470 and 490 ppm. According to SCHULTZ, 2000, if greenhouse gas emissions continue to rise at the same rate in the last part of our century, the equivalent CO_2 concentration will double. The carbon gas equivalent of the main greenhouse gases is shown in Table 1 (***. EPA www.epa.gov):

Table 1. The equivalent in CO_2 of the main greenhouse gases.

Greenhouse gas (other than CO_2)		Equivalent in CO_2
	Methane	1 tonne =28.36 tons CO_2
	Nitrous oxide	1 tonne = 265-298 tons CO_2
	Flux gases: sulphur hexafluoride	1 tonne = 23 500 tons CO_2

Being one of the major by-products of burning fossil fuels (for electricity, industrial, transport, households, etc.), billions of tons of CO_2 are emitted each year. Other sources of carbon dioxide, as well as other greenhouse gases, are: wood burning for food and heating, agriculture, industry, landfills, use of fluorinated industrial gases, human activity, etc. In addition to these man-made sources, natural gas sources must also be mentioned: volcanic erruptions, fires, rotting processes in oceans and swamps, etc. (***. ONERC, 2014; ***. EPA, 2017).

In addition, global warming produced by excess gas leads to other phenomena, producing in turn a significant increase in greenhouse gas concentrations, as in a vicious circle. An example is permafrost, which has collected enormous quantities of methane in its course over millions of years. Through its melting due to global warming, huge amounts of methane can be released into the atmosphere, with a devastating effect on the environment.

According to experts in the field (***. IPCC, 2014), carbon dioxide emissions will show spectacular increases along with the technological progress of some emerging countries, such as China, India and Brazil, which account for about 40% of the world's population.

A problem that is raised with consistency when it comes to CO_2 emissions is the need for a complete review of the ways in which society is organized; thus limiting the waste and establishing optimal ways to solve it, limiting the excessive consumption of food and non-food, unnecessary transportation of goods, etc.

MATERIAL AND METHOD

In order to assess the main aspects of the impact of climate change on the environment, in general and on vine in particular, a number of bibliographic databases have been extensively studied, including the Intergovernmental Panel on Climate Change (IPCC), the Intergovernmental Oceanographic Commission of UNESCO, the United States Environmental Protection Agency (EPA), the European Environment Agency, etc. Also, the Kyoto and Montreal Greenhouse Gas Regulations have been highlighted (***. EUROPEAN ENVIRONMENT AGENCY, 2016).

RESULTS AND DISCUSSIONS

CONSEQUENCES OF CLIMATE CHANGE

Temperature. As a consequence of the increase in greenhouse gas concentrations over the last 150 years, the continental average temperature has increased by about 0.8°C and Europe by about 1°C (***. A.E.M., 2016).

According to the UN, global average temperature has increased by about 1 degree in 2015, for the first time since the pre-industrial era. As a result of the double accumulation of greenhouse gases, a temperature increase in Central and Western Europe is expected to range between 2.5 and 5°C by the end of the 21st century; at a global level, the annual average temperature is expected to increase by about 1.8°C - 4°C (***. www.greenfacts.org). In Europe, the highest temperature increases occur in the southern continent and in the Arctic region.

The IPCC report for 2014 highlights the consequences of climate change in terms of temperature: in general, thermal differences between seasons and continents will be lower; also, the temperature increase will be stronger at the poles than at the equator, also, the temperature increase will be stronger at the poles than at the equator, on continents than in oceans, during nighttime than during daytime, in winter than in summer.

Rainfall. Although future precipitation changes are more difficult to establish, changes are foreseen in the total amount of rainfall, but especially changes in their annual distribution. Climate patterns predict an increase in precipitations in winter, the result of an acceleration of the hydrological cycle at European level. This increase will, however, be associated with a decrease in the amount of precipitations in the summer season for most territories. Also, rainfall is expected to be more quantitatively significant at higher and lower latitudes in most subtropical regions. In Europe, precipitations decrease in the southern regions and grow in the north and northwest of the continent.

Levels of seas and oceans. A consequence of the increase in global temperature is the melting of the ice and, implicitly, the increase of the sea level; so floods will be recorded in low-altitude areas, as well as altered geography of coastal areas (TOWLE et al., 2015a; b).

If during the last 50 years the planetary ocean level has increased by about 10 cm, some climate models indicate an increase ranging from 25 to 98 cm by the end of the century (***. I.O.C.U, 2017). One thing worth noting when it comes to raising sea and ocean levels is that out of the 20 metropoles of the world, 16 are located near different

seas or oceans. Also, according to the same climate models, about 20,000 islands are estimated to disappear from the geography of the planet (5th IPCC Report).

Acidification of sea and ocean water. The planetary ocean (which accounts for 90% of the planet's water) is the main source of precipitation, absorbing most heat from continents; it seems that the marine environment absorbs about 90% of this heat, mitigating the impact of global temperature (EKSTROM et al., 2015).

In terms of CO_2 emissions, the ocean is a regulator of carbonic acid concentration, more effective than forests (MANZELLO et al., 2008). Since the beginning of the industrial age, the global ocean absorbs more than 30% of CO_2 emissions, the amount absorbed annually nowadays being unprecedented. According to Christopher Sabine of the United States Oceanic and Atmospheric Administration (***. NOAA, 2017), the ocean absorbed about 120 billion tonnes of carbon produced from human activities in 1800. The same agency reports that about 20-25 million tonnes of CO2 is added every day to the planetary ocean (***. www.notre-planete.info).

So, by the end of the nineteenth century, the pH value of marine waters has remained constant. However, nowadays, the acidity of the ocean is rising, with a pH value less than 0.1 times the value since the start of the industrial revolution. It seems that by the mid-21st century, the amount of CO_2 that is absorbed by this ecosystem will lead to changes in the acidity of the upper layers of the ocean, the pH of water dropping even by 0.3; these changes are recorded for the first time in 20 million years of earth history (TOWLE et al., 2015a; b) and will have serious repercussions on biodiversity.

As far as marine life is concerned, it is already found that more than 25% of the corals are affected, as it is estimated that by the middle of our century about 50% of them will disappear. Other marine organisms containing calcium carbonate, including some plankton species, as well as non-carbonated organisms, will be affected if the CO_2 concentration increases and decreases the pH level (TRIBOLLET et al., 2006a; b; EZZAT et al., 2015). Increasing temperatures, combined with acidification of water, pose a serious threat to coral reefs; in general, all marine species, from bacteria to marine mammals will be affected by climate change (TOWLE et al., 2015a; b).

In terms of oxygen production the situation is worrying, too. The planktonic ecosystem produces oxygen through photosynthesis, consuming carbon dioxide. However, there are many oxygen-free "dead" areas, especially in some coastal areas, such as the Gulf of Mexico. These areas began to be mapped after massive fish deaths were recorded in the early 1990s due to crossing these oxygen-free areas (SAMBROTTO et al., 2003). The lack of oxygen is related to the huge amounts of waste from human activities evacuated in these areas as well as to the rise in temperature (***. http://www.lemonde.fr/climat).

Consequences on human society. Climate changes that will primarily impact the environment and biodiversity will have a significant impact on human societies. Increased temperatures will result in higher levels of evaporation at ground and plant level, which will increase the frequency and severity of drought periods. On the other hand, floods and other extreme phenomena will have adverse effects on agricultural production and water resources will drop. According to the UN, climate change could have as a consequence, by 2080, that over 100 million people could live in extreme poverty and nearly 600 million could suffer from malnutrition (***. FUTURA SCIENCE. 2014).

THE INFLUENCE OF CLIMATE CHANGE ON VINEYARD CULTURE

Temperature changes will entail variations in the hydrological cycle, regimes, frequency and duration of precipitations. Since most traditional European vineyards are not irrigated, one of the future challenges will be to ensure access to water and irrigation of plantations.

The increase in greenhouse gas concentrations will have, according to many specialists (LONG et al., 2004), a strong impact on agricultural and natural ecosystems, including on vines, an impact already observed in most plants, annual and perennial. A few examples are: the intensification of photosynthesis process, the increase of biomass production, better water use (BINDI et al., 1996; BINDI et al., 2001; SALAZAR-PARRA et al., 2012); these phenomena are sometimes associated with a decrease in the weight of grape grains (JONES, 2006).

Achieving a common strategy on European viticulture will be very difficult due to the many factors that need to be taken into account: the diversity of vine plantations, climatic conditions of vineyards, specific microclimates, different soil types.

If we refer generally to the temperate climate vineyards, much of them will be exposed to varying climatic conditions, depending on the harvest year, conditions with a global warming trend. The variability of the rainfall regime will have significant consequences for the vineyard culture. The warm and humid climate during the maturation period will affect many of the varieties that are part of the traditional range of a vineyard. Also, periods of intense drought and heavy rain will lead, on the one hand, to competition between the vineyards for water during drought and, on the other hand, to erosion, strong leakage and increased water infiltration during the the precipitations period.

A great challenge will be the prophylaxis and treatment of vines for partially known diseases and pests that will threaten the development of vine and the quality of grapes. The varieties of white vines will be most affected, especially as they have a limited plasticity (JONES, 2006).

According to the general data on the influence of climate change on vines, there is a gap in the phenological stages: dewlap, flowering, grain formation, prick and maturation; grape harvesting is advanced, depending on the area and the harvest year by 20, even 30 days compared to the 1970s. Changing phenology is the result of increasing the temperature value, the heat requirements that trigger the phenological phases being met earlier. The phenomenon has

repercussions not only for the loss of traditional landmarks (traditional "100-day harvesting" rule), but also for the harvesting dates for early and late varieties.

On the other hand, the periods of drought recorded during maturation are less favorable for the development of the *Botrytis cinerea* fungus and therefore to the gray rot attack, one of the major problems of world viticulture. Increased temperatures and lack of precipitation during maturation are conducive to the accumulation of sugars in grapes, so the addition of sugar will no longer be necessary. Instead, the accumulations of organic acids will record very low values, which will lead to unbalanced, "burned" wines with a weak typicality.

High temperatures during the harvest period will lead to an increase in the temperature of the grapes, the thermal dynamics of the winemaking process being modified and very difficult to monitor (SCHULTZ, 2000).

The data from the CLIMATOR (2007-2010) research project shows that in the French wine region Bourgogne there was an increase in the grain weight and therefore an increased yield of the harvest of over 50% compared to 1994 and about 200% over the period between 1070 and 1999 (AdCC & VIGNE, 2012). The factors involved are the increase in CO_2 concentration associated with plant vigor caused by: an important floral induction due to higher temperatures and thermohygrometric variations; an increase in the amount of reserve substances in the vine plant, as the period of time between grape harvesting and the fall of leaves (storage period by the plant) has increased; the application of different cultural practices (removing some of the flowers or grapes in order to grow better the remaining ones).

The increasing yields of grape harvest may, however, have repercussions on the concentration in aromatic compounds and, generally, on the content of volatile compounds which contribute to the specificity and typicality of the variety.

Climate change can affect vine plantations and the risks associated with climatic extremes. These risks are related, first of all, to the loss of or significant reduction of the harvest, and are due to:

- spring frost: although the average number of days of frost is lower, the rise in temperature values has led to the advancing of deforestation and blossom; at the same time, although before breaking leaf buds a lower temperature is needed (vegetative rest, dormancy), it is difficult to establish the dominance of a parameter to the detriment of another; also in this context, the duration of vegetative development stages has decreased;

- severe drought: although a period of drought recorded during grapes maturation is favorable to the accumulation of sugars and the reduction of the risk of cryptogamic attack, however, a period of severe drought, i.e. a water deficit, has repercussions on both vine growth and forced maturation of grapes, negatively influencing the production and quality of grapes and wines;

- excessive temperatures lead to forced firing of grape stage and maturation. In recent years, there has been a tendency for varieties to enter early in the parga (end of July and early August), and the sowing of varieties is far less obvious. The sudden and forced entry of grapes in parga leads to stopping the growth of grapes, and they no longer meet the dimensions of the variety, which negatively influences the yield of the must. Also, in black grape varieties, high temperatures cause the grain to be colored before they reach the typical variety.

Excessive heat also causes partial withering and drying of the foliar appliance, its reduced surface having negative repercussions on the growth of grapes and the accumulation of sugars, and therefore on the quantity and quality of the wine.

Another problem is the increase in temperature during the voluntary rest period of the vine. As it is known, during this period (in the temperate climate: end of December, early January to late March) the vine is ready to resume its vegetation cycle, but remains in a state of rest only due to unfavorable conditions. Alternatively, alternating temperatures during this period can lead to significant losses of buds, more so than in the deep rest of vineyards due to low resistance to frost. Also, if the average temperatures in this period exceed the biological threshold of the vine, it can enter vegetation (ALBUQUERQUE, 1993). If cold weather occurs later, the vine can be affected even at temperatures of -10, -15 ºC -episodes of extreme precipitations - frequent and aggressive rains - can lead to soil erosion phenomena and loss of fertility in the hilly area; unfortunately, these degradation phenomena are irreversible.

In general, the precipitation regime tends to decrease, as well as have an uneven distribution throughout the year. Drought periods, alternating with periods of abundant rainfall, sometimes torrential and often cold, have a negative impact on the main physiological processes of vines.

There is an increasing tendency of extreme phenomena in recent years, such as hail, strong winds, storms, torrential rains that affect plants. Strong grain can often lead to compromising grape harvest, but also damage to the foliar appliance and even the destruction of wood - the changes to the diseases and pests of the vine represent an important phenomenon and complex, the evolution of the diseases, their intensity, their impact being related to many factors, difficult to analyze in a short period of time (DUTEAU et al., 1981).

There has been an increase in the incidence of pests and diseases caused by vectors that love heat: oidium, wood diseases, cicadas (hemiptera), etc. (SALINARI et al., 2007; MIRA DE ORDUNA, 2010; PANGGA et al., 2011).

Another issue related to the influence of climate changes on the vine concerns the quality of the harvest and, consequently, of the wine; thus, elevated temperatures increase the accumulation of sugars but also the intensity of the process (DUCHÊNE & SCHNEIDER, 2005), a phenomenon involving a disagreement between the accumulation of metabolites: sugars accumulate faster than aromatic compounds and phenolic compounds, the risk of imbalance aromatic and color (BERLI et al., 2008; SADRAS & MORAN, 2012).

The evolution of the main climatic parameters, confronted with the optimal conditions necessary for the development and maturation of the vine, are factors with particularly important effects on a possible other geographic distribution of the vineyards (JONES & WEBB, 2010). The evolution of climatic indicators will play an increasingly important role in the distribution of vine varieties and the simulation of potential plantations (PIERI, 2010).

There is a possibility that, in order to obtain a high quality grape harvest, as well as for the viability and optimal development of vineyards, the geographical distribution of vineyards and vine varieties will be greatly changed. MALHEIRO et al., 2010 shows that new wine regions in northern France may be more suitable for quality viticulture than established vineyards. These geographical changes could have significant indirect impacts on water resources and ecosystems (HANNAH et al., 2013).

From an economic point of view, major changes can be made: for example, increasing alcohol content and changing the aromatic and chromatic profiles of wines can change the hierarchy of consumer preferences and influence prices (PICHERY & BOURDON, 2007). Also, DOCs (Designations of Origin) are particularly important factors in the wine economy by codifying the use of varieties, placing plantings and technical practices to guarantee the quality of origin. The entire economic and institutional system linked to the vine may be affected.

CONCLUSIONS

Changing temperature and hydrological variations will lead to long periods of drought. In this context, where most traditional European vineyards are not irrigated, access to water and planting irrigation must be ensured.

Increasing greenhouse gas concentrations will have an impact on vineyards, especially as regards the intensification of photosynthesis, increased biomass production, better water use, phenomena associated with a decrease in the weight of grapes.

Climate change already leads to a gap in the phenological stages: flourishing, flowering, grain formation, pruning and maturation; grape harvesting is advanced, depending on the area and the harvest year by 20, even 30 days compared to the 1970s. Also, the duration of the vegetative development stages has decreased and the sowing of the varieties is much less obvious.

Seasons of severe drought, with a pronounced water shortage, have repercussions on both vine growth and forced maturation of grapes, negatively influencing the production and quality of grapes and wines.

There has been an increase in the incidence of pests and diseases caused by vectors that love heat: oidium, wood diseases, cicadas, etc.

On the other hand, experts say that the drought periods recorded during maturation are less favorable to the development of the *Botrytis cinerea* fungus and thus to the gray rot attack, one of the major problems of world viticulture. Increased temperatures and lack of precipitation during maturation are favorable to the accumulation of sugars in grapes; however, elevated temperatures leading to increased sugar accumulation, but also to the intensity of the process, imply a disagreement between the accumulations of metabolites: sugars accumulate faster than aromatic compounds and phenolic compounds, the risk of aromatic imbalance and color.

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CURRENT STATUS AND FUTURE PERSPECTIVES IN ROMANIA ON BIOLOGICAL CONTROL OF SWEET POTATO FUNGAL PATHOGEN

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Abstract. Sweet potato (*Ipomoea batatas* (L) Lam.) is a less grown vegetable in Romania, mostly due to its growth requirements. This crop demands for warm weather and well-drained soils for its best growth. In Romania, such growth conditions are found in the south area of the country, especially on the sandy soils of the Dolj County. At the Research-Development Station for Field Crops on Sandy Soils (RDSFCSS) – Dăbuleni, the sweet potato was successfully acclimatized and established in the crop rotation, mostly due to the pedo-climatic conditions of the area. These vegetables increased crop diversification and ensured a good productivity under the current climate change conditions. In countries were sweet potato is a traditional vegetable, a wide range of plant pathogens is mentioned for this crop. Until now, no major phytosanitary problems have been encountered in Romania, allowing this culture to be successfully introduced into the organic production system. However, some fungal pathogens, like *Alternaria* spp., *Fusarium* spp., *Botrytis cinerea*, *Pythium* spp., *Penicillum* spp. and *Rhizopus stolonifer*, were previously isolated from sweet potato plants and tubers cultivated in SCDCPN - Dăbuleni, against which several bacterial antagonists were previously tested. Based on these results, the aim of present study is to analyze the mechanisms by which different biocontrol strains suppress sweet potato pathogens in order to establish the best measures of biological control. The studies were performed *in vitro* both against pathogenic species encountered for this vegetable in Romania, and against pathogens that are present in our country without infecting sweet potato (*Macrophomina phaseolina, Rhizoctonia solani*), although they induce economic losses in other country conditions. Fungal disease suppression was found to be related with microbial competitors of plant beneficial bacteria, able to produce lytic enzymes and antibiotic compounds. The studied biocontrol bacterial strains were able to induce, *in vitro*, mycelial growth inhibition, fungal cells lysis and other alterations of the hyphae.

Keywords: *Ipomoea batatas*, biological control, microbial interactions.

Rezumat. Stadiul actual şi perspective privind controlul biologic al cartofului dulce cultivat în România. Cartoful dulce (*Ipomoea batatas* (L) Lam.) este o legumă mai puţin cultivată în România, în special datorită cerinţelor de creştere pe care le are. Această specie este iubitoare de căldură şi necesită soluri bine drenate. În România, astfel de condiţii de cultivare sunt întâlnite în zona de sud a ţării, în special pe solurile nisipoase din judeţul Dolj. La Staţiunea de Cercetare - Dezvoltare pentru Cultura Plantelor pe Nisipuri (RDSFCSS / SCDCPN) – Dăbuleni, cartoful dulce a fost aclimatizat şi introdus cu succes în asolament, în special datorită condiţiilor pedo-climatice din zonă. Ca urmare, această legumă a permis diversificarea spectrului de culturi, cu o bună productivitate în condiţiile actuale ale schimbărilor climatice. În ţările cu tradiţie pentru această cultură, este menţionat un spectru larg de fungi fitopatogeni. Cu toate acestea, în România nu au fost întâmpinate probleme fitosanitare grave. Acest lucru ar permite introducerea cu succes a acestei culturi în sistemul de producţie ecologică. Cu toate acestea, o serie de fungi fitopatogeni, precum: *Alternaria* spp., *Fusarium* spp., *Botrytis cinerea*, *Pythium* spp., *Penicillum* spp. şi *Rhizopus stolonifer*, au fost izolaţi de la plante sau de pe tuberculi de cartof dulce cultivaţi la SCDCPN – Dăbuleni, împotriva cărora au fost testate diferite izolate bacteriene autohtone. Din aceste considerente, scopul prezentei lucrări este de a analiza mecanismele prin care diferite tulpini de biocontrol acţionează asupra unor potenţiali patogeni ai cartofului dulce, în ideea de a stabili cele mai eficiente măsuri de combatere biologică. Astfel au fost realizate studii *in vitro* faţă de diferite specii microbiene fitopatogene. Spectrul de boli analizat, a cuprins atât specii patogene întâlnite în ţara noastră la această cultură, cât şi specii de patogeni care sunt prezenţi în România (*Macrophomina phaseolina, Rhizoctonia solani*) fără a fi întâlniţi la cultura de cartof dulce, dar care în ţări cu climat diferit produc pagube economice. Reducerea infecţiilor cu fungi fitopatogeni a fost corelată competiţiei microbiene cu bacterii benefice plantelor, capabile să producă enzime litice şi compuşi cu activitate antimicrobiană. Tulpinile bacteriene studiate au prezentat activitate de control biologic. *In vitro*, acestea au fost capabile să inhibe creşterile miceliene, să inducă liză celulară şi să modifice morfologia micelian a ciupercilor fitopatogene.

Cuvinte cheie: *Ipomoea batatas*, combatere biologică, interacţiuni microbiene.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L) Lam.) is among world's most important crops. Due to its productivity it is considered the seventh most important food crop worldwide (LIU, 2017). For its best growth, this crop demands for warm weather, with optimum temperatures between 21 and 29°C, and well-drained soils, such as the sandy loam (***. DAFF, 2011). In Romania, similar growth conditions are found in the south area of the country, especially on the sandy soils of Dolj County. At the RDSFCSS – Dăbuleni, the sweet potato was successfully acclimatized and established in the crop rotation, mostly due to the pedo-climatic conditions of the area. These vegetable increased crop diversification and ensured a good productivity (up to 53.3 t/ha in KSP1 cultivar) under the current climate change conditions (DINU & SOARE, 2015).

In countries where sweet potato is a traditional crop, a wide range of plant and tuber fungal pathogens is mentioned for this vegetable. The frequently mentioned plant diseases (AMES et al., 1997; CLARK et al., 2015; EKMAN & LOVATT, 2015) are listed in table 1.

Table 1. Worldwide plant diseases of sweet potato.

Plant disease	Plant pathogen
Alternaria leaf spot, or leaf, petiole and stem blight	*Alternaria* species (mainly *A.alternata, A.bataticola, and A.tenuissima)*
black rot	*Ceratocystis fimbriata*
Fusarium wilt	*F.oxysporum* f.sp. *batatas*
violet root rot	*Helicobasidium mompa*
leaf and stem scab	*Sphaceloma batatas* (syn. *Elsinoe batatas*)
sclerotial blight	*Sclerotium rolfsii*
Rhizoctonia stem canker	*Rhizoctonia solani*
Minor fungal pathogens	
chlorotic leaf distortions	*Fusarium lateritium*
leaves spots	*Phomopsis ipomoea-batatas* (syn. *Phyllosticta batatas), Cercospora* sp., *Septoria* sp., *Ascochyta* sp., *Curvularia* sp., *Colletotrichum* sp., and *Pestalotia batatae*
Storage fungal diseases	
foot rot	*Plenodomus destruens*
java black rot	*Lasiodiplodia theobromae* (syn. *Diplodia gossypina*)
charcoal rot	*Macrophomina phaseolina* (syn. *Sclerotium bataticola*)
grey mold rot	*Botrytis cinerea*
soft rot	*Rhizopus stolonifer* and *Mucor* sp.
blue mold	*Penicillium expansum* and other *Penicillium* species
dry rot	*Diaporthe phaseolorum* (syn. *Phomopsis phaseoli*)
Fusarium root rot	*F.solani* and *F.javanicum*

In the past few years, several pathogenic infections extended their areal of infections along the sweet potato growth regions. LEE et al. (2016) mentioned the first report of dry rot caused by *Diaporthe batatas* (formerly *D. phaseolorum*) in Korea, at 'Juwhangmi' cultivar of sweet potato. PAUL et al. (2017) mentioned the first report of *Rhizopus microsporus* causing Rhizopus soft rot of sweet potato, also in Korea. The same research group also mentioned the first report in South Korea, of Fusarium root rot caused by *F.solani* in sweet potato, during storage of 2016 and 2017 (YANG et al., 2018). Such notes are of great importance for Romania, since at the RDSFCSS - Dăbuleni, which is the biggest center for growing sweet potato in our country, almost all sweet potato cultivars are Korean varieties. This vegetal material exchange between Romania and South Korea is based on the collaboration protocol between the two countries.

Although, worldwide, the spectrum of sweet potato pathogens is relatively large, in our country only a few pathogens were noticed in the field conditions of RDSFCSS - Dăbuleni. No management problems or economical important losses were registered before harvest in the research and production plots of sweet potato in that region. However, it is expected that, due to the climatic changing conditions and the exchange of biological material, several pathogens that currently do not attack sweet potato, might find appropriate conditions to infect. It is also believed that when growers will extend this vegetable's culture, phytopathogenic attacks will be increased, and species of pathogens that are present in our country at different other crops will find appropriate conditions to infect also sweet potato. The plant diseases and tuber storage rot of which we consider to extend their spectrum of infection are *Pectobacterium carotovorum*, *Fusarium solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani*.

Sweet potato pathogens, isolated during 2015 to 2017 were *Alternaria* spp., *Fusarium* spp., *Botrytis cinerea, Pythium* spp., *Penicillum* spp. and *Rhizopus stolonifer*, most of them from unappropriated storage conditions. Against these pathogens, we previously tested 52 bacterial antagonists (BOIU-SICUIA et al., 2016; 2017b). Based on these results, the aim of present study is to analyze the mechanisms by which different biological control strains suppress sweet potato pathogens in order to establish the best measures of biological diseases control. These studies were performed *in vitro* against pathogenic species encountered for this vegetable in Romania, and against pathogens that are present in our country but did not infected sweet potato until now (*Macrophomina phaseolina*, *Rhizoctonia solani*), although they induce economic losses in other country conditions.

MATERIAL AND METHODS

Plant pathogens. Several phytopathogenic fungi (Table 2) were used in this study in order to examine the direct interaction mechanisms involved in microbial biocontrol. All plant pathogenic fungi were grown and maintained on potato-dextrose-agar during the study.

Beneficial bacteria. The bacterial strains used in this study were: *Bacillus amyloliquefaciens* OS17, *B. endophyticus* 1T2, *B. atrophaeus / subtilis* 6T4, *B.subtilis* ssp. *subtilis* Dj3, *B. subtilis*/ *mojavensis* Dj6, and *Pseudomonas chlororaphis* Sal.c2. These strains are Romanian native, and were previously isolated and characterized at the RDIPP as potential biocontrol agents (DINU et al., 2012; SICUIA, 2013; BOIU-SICUIA et al., 2017a; b). Routinely, these bacteria were grown on Luria Bertani (LB) agar, at 28°C. However, for longer preservation, they were stored at -80°C, in LB broth with 30% glycerol.

Table 2. Filamentous fungal species.

Phytopathogenic fungi	Provenience
Alternaria sp.	USAMV Bucharest, Faculty of Biotechnology collection
Botrytis cinerea	Isolated from harvested sweet potato, of local production (RDIPP - Bucharest collection)
Fusarium oxysporum	RDIPP - Bucharest collection
Fusarium solani	RDIPP - Bucharest collection
Macrophomina phaseolina (syn. *Sclerotium bataticola*)	RDIPP - Bucharest collection
Rhizoctonia solani DSM 63002	DSMZ Collection, Germany

Study on microbial interactions. Direct interaction among the biocontrol and plant pathogenic microorganisms was analysed by optical microscopy in dual cultures, using MC1 microscope. Both microorganisms, the phytopathogenic fungi and the biocontrol bacteria, were inoculated simultaneously, in the same plates, at 2cm distance from each other. The fungal inoculum consisted of calibrated plugs (6mm in diameter) of 7 to 10 days old cultures. The beneficial strains were inoculated in spots, using two days old bacterial biomass. Microbial co-cultivation was performed on PDA medium. Plates were incubated at 28°C and analysed after 3 to 14 days.

RESULTS AND DISCUSSION

In order to understand the effect of bacterial inoculants during the biocontrol process, we studied the fungal growth and its morphology in the presence of beneficial bacteria. Fungal modification obtained in pathogenic growth due biocontrol treatment, was analysed by comparing fungal growth from the untreated control plates to the one developed in the presence of some plant beneficial bacteria. Some correlations were possible between fungal modifications and the biocontrol traits of the beneficial bacteria.

Alternaria sp. growth was inhibited by all tested bacterial strains. The pathogen increased its sporulation at 2÷3 millimetres from the colony edge exposed to the biocontrol bacterial (Fig. 1a). In the mentioned area, the sporulation process was rushed compared with the rest of the colony. In only three days of incubation, an intense brown line, correlated with conidia formation (Fig. 1b) could be seen near the edge of the colony at the interaction zone with any biocontrol bacteria tested.

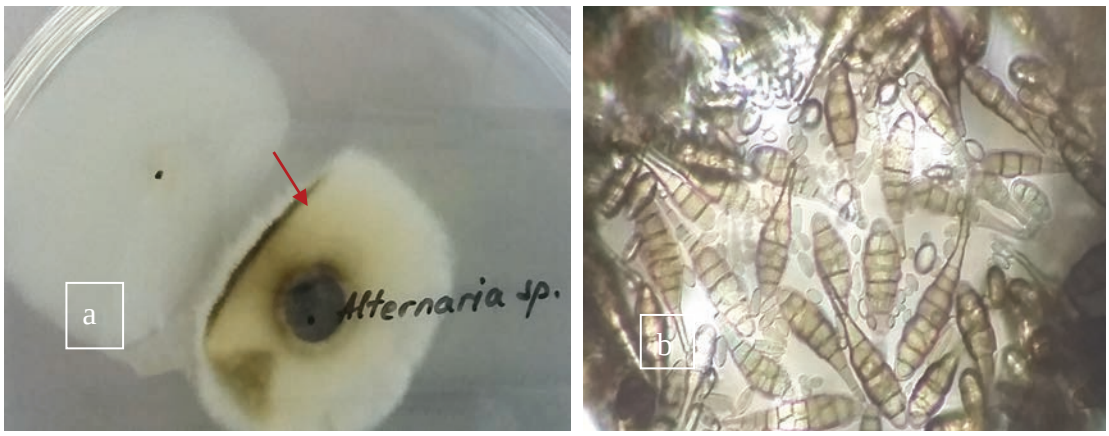

Figure 1**.** Co-cultivation of *Alternaria* sp. and biocontrol bacteria. **a.** Double culture technique of *Bacillus* sp. and *Alternaria* sp. (the arrow indicates the line with abundant conidia formation) **b.** *Alternaria* sp. conidia.

In the interaction zone with *Pseudomonas chlororaphis* Sal.c2, *Alternaria* sp. presented irregular cells along the mycelial growth (Fig. 2a). Although the tested *Bacillus* spp. strains showed *Alternaria* sp. growth inhibition (Fig. 2b), there were no clear mycelial modification at the microscopic level, only fungal colonisation of the hyphae by the bacterial strains, which express *in vitro* swimming and swarming motility (SICUIA, 2013).

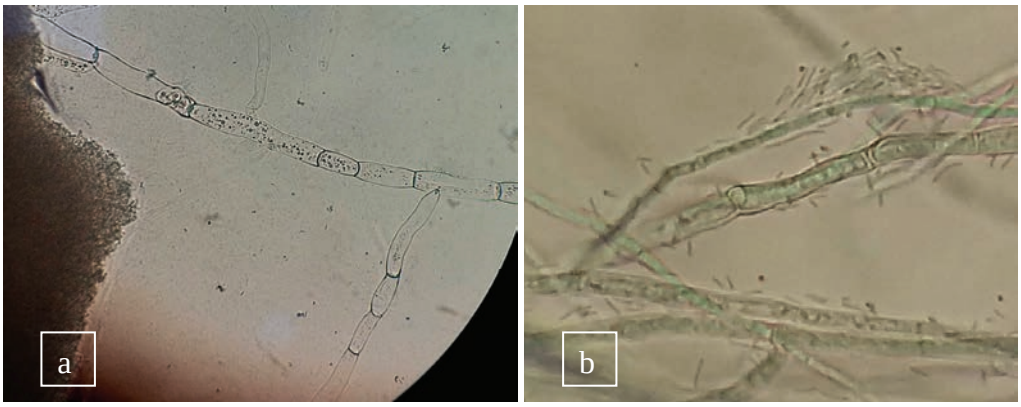

Figure 2. Microbial interaction between *Alternaria* sp. and biocontrol bacteria.
a. Irregular cells in *Alternaria* sp. growth near *Pseudomonas chlororaphis* Sal.c2 strain;
b. Hyphae colonized by *Bacillus amyloliquefaciens* OS17 bacterial cells.

In *Botrytis cinerea* interactions with the biocontrol bacteria, swelling of the apical fungal cells were seen, in the first days of co-cultivation. After 3 to 5 days of incubation, these fungal cells were lysed. The cellular coating was degraded and leakages of the cytoplasmic content were seen (Fig. 3). These could be due to bacteria lytic enzymes production, as the tested strains are known to produce chitinases, cellulases, proteases, and/or lipases (DINU et al., 2012; SICUIA, 2013; BOIU-SICUIA et al., 2017a; b).

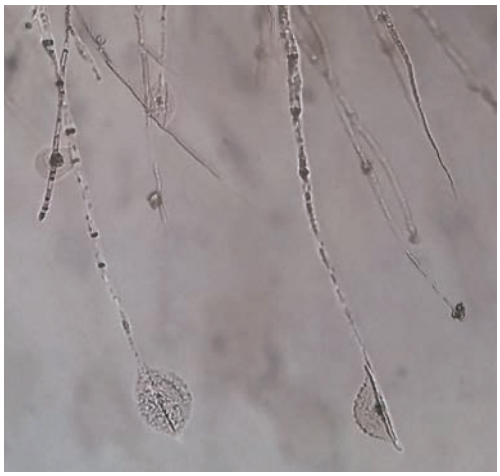

Figure 3. Fungal cell lysis and cytoplasm leaks in *Botrytis cinerea* biocontrol.

Swellings of the fungal cells (Fig. 4) were also noticed in *Fusarium oxysporum* and *F. solani*, when *B.subtilis* ssp. *subtilis* Dj3 was used as biocontrol strain. Similar aspects were also described in *F.oxysporum* biocontrol with strains of *Bacillus brevis* (BAPAT & SHAH, 2000) or *Paenibacillus polymyxa* (DIJKSTERHUIS et al., 1999). It is mentioned that anti-fungal compounds produced by the biocontrol bacteria are counteracted by magnesium ions (DIJKSTERHUIS et al., 1999). This suggests that biocontrol bacteria could induce osmotic stress, probably by nutrient competition. The presence of living bacteria increases fungal repression compared with cell-free bacterial supernatant (DIJKSTERHUIS et al., 1999; SICUIA, 2013).

In *F. oxysporum* and *F. solani*, some biocontrol bacteria induced an increased number of vacuoles. As these vacuoles increased their volume, the hyphae architecture was modified (Fig. 5). It is believed that these modifications were generated due to osmotic stress caused by the antifungal bacterial strains. As vacuoles are involved in maintaining cellular homeostasis (RICHARDS et al., 2012), the osmotic stress could be responsible for a higher accumulation of ions and molecules inside the vacuoles in order to maintain the integrity of the cell and avoid fungal cell lysis in the presence of biocontrol bacteria.

If we refer to *Rhizoctonia solani*, this pathogen is present in Romania, however it has not yet been reported to infect sweet potato grown in our country. As Rhizoctonia stem canker is mentioned to infect this crop in USA (CLARK et al., 2015) and China (LIFEI et al., 2016) we considered important to study the impact of autochthon biocontrol-bacteria treatment on such pathogen. Therefore, we compared the fungal growth morphology with and without the presence of biocontrol bacteria from *in vitro* cultures. Typical *Rhizoctonia solani* branches in right angles from the main hypha, having a septum near the branch origin (Fig. 6). In order to survive adverse conditions, Rhizoctonia produce specialized hyphae with compact monilioid cells, that fuse together in order to produce sclerotia (TREDWAY & BURPEE, 2001).

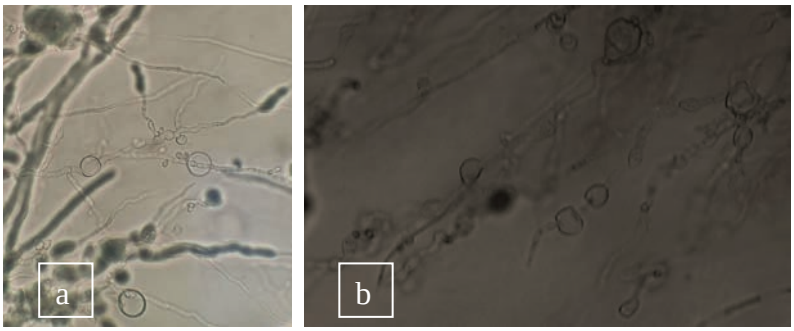

Figure 4. Fungal cells swelling in the presence of *B.subtilis* Dj3 biocontrol bacteria.
a. *Fusarium oxysporum*, **b.** *Fusarium solani*

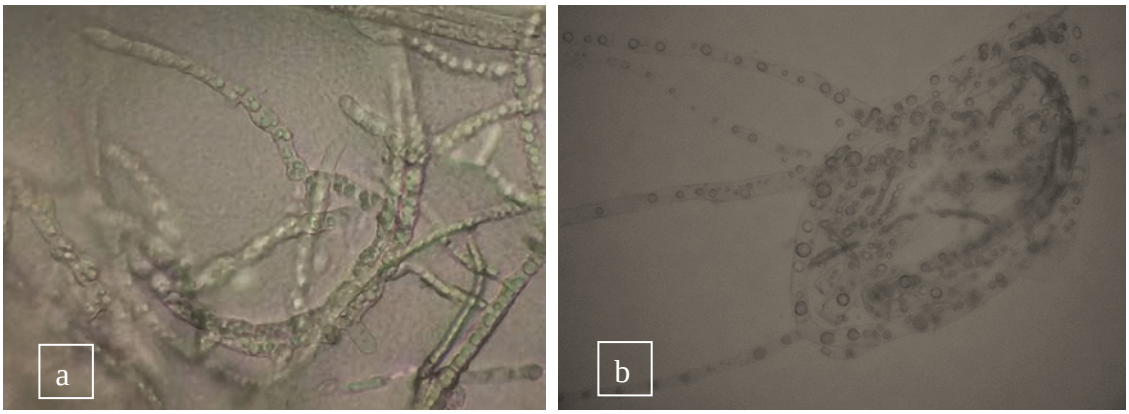

Figure 5. Increased number of vacuoles and vacuole size in *Fusarium oxysporum* cells in the presence of some biocontrol bacteria.
a. *Bacillus atrophaeus / subtilis* 6T4, **b.** *B.amyloliquefaciens* OS17

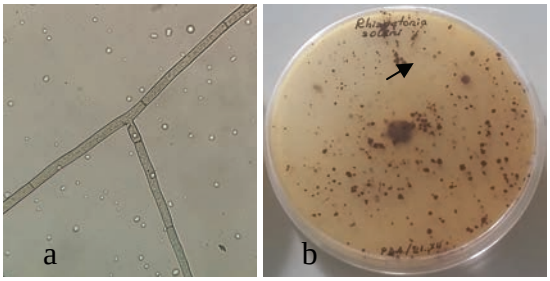

Figure 6. *Rhizoctonia solani* pathogen. **a.** mycelia branching in right angles from the main hypha,
b. six weeks old culture of *R.solani* on PDA medium (sclerotia are indicated by arrow).

Microbial interactions among *Rh. solani* and bacterial antagonists revealed different biocontrol mechanisms. In the presence of *Bacillus endophyticus* 1T2, hyphal morphology was changed. Fungal cells were shorter and swollen (Fig. 7A). Similar aspects were also seen in the presence of *B. amyloliquefaciens* OS17. Moreover, due to the high motility of this biocontrol bacteria strain, the fungal hyphae were colonized with bacterial cells (Fig. 7B). Hyphal growth deformation was induced by *B..subtilis* Dj3, consisting in mycelial curling (Fig. 7D). Likewise, in dual culture of *Rh. solani* with *B. atrophaeus / subtilis* 6T4 an increased number of vacuoles were observed in the fungal cells.

Dual culture technique of *Rh. solani* and *Pseudomonas chlororaphis* Sal.c2, revealed an increased number of vacuoles in the fungal cell, increased vacuole size, and cytoplasmic coagulation within the hyphae (Fig. 8). Similar changes induced by another *Ps. chlororaphis* biocontrol strain were also reported in *Rosellinia necatrix* (CALDERÓN et al., 2014). Moreover, different *Pseudomonas* species were mentioned to induce cytoplasmic coagulation not only in Rh. Solani, but also in other pathogens like *Botrytis cinerea*, *Macrophomina phaseolina* or *Phytophthora capsici* (BARKA et al., 2000; KUMAR et al., 2005; DIBY et al., 2005).

Macrophomina phaseolina (syn. *Sclerotium bataticola*) is a soil-borne plant pathogen with a broad spectrum of plant hosts, where it can express various disease symptoms. On sweet potato, it produces charcoal rot, and the disease evolves during storage. Although this pathogen is present in Romania, it has not been reported yet on locally produced sweet potatoes. Despite of this, we preventively analyze the possibility to biologically control this pathogen with plant beneficial bacteria. *In vitro* studies of microbial interactions showed that *Macrophomina phaseolina* could be severely affected by the biocontrol strains. Fungal growth was inhibited by all tested bacterial strains. The microscopic analysis of the interaction zone revealed fungal cell swelling, cell lysis and cytoplasm likings (Fig. 9).

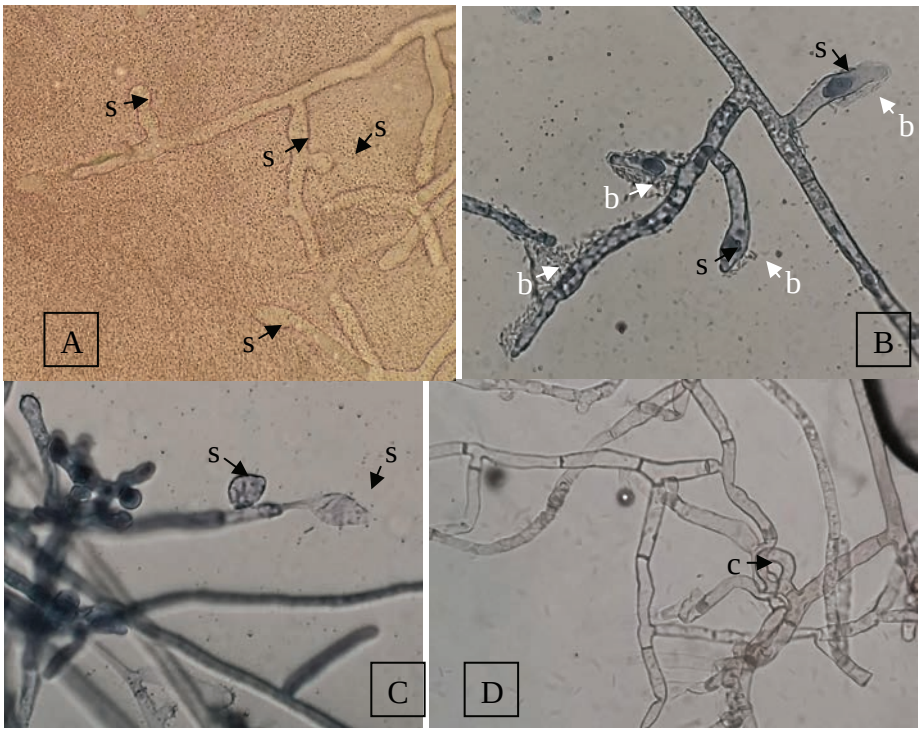

Figure 7. Fungal growth deformations of *Rhizoctonia solani* due to some biocontrol bacteria like *Bacillus endophyticus* 1T2 (**A**), *B.amyloliquefaciens* OS17 (**B**), *B. subtilis*/ *mojavensis* Dj6 (**C**), and *B.subtilis* Dj3 (**D**) c = mycelial curling, s = fungal cell swelling, b = bacterial cells colonizing the mycelia.

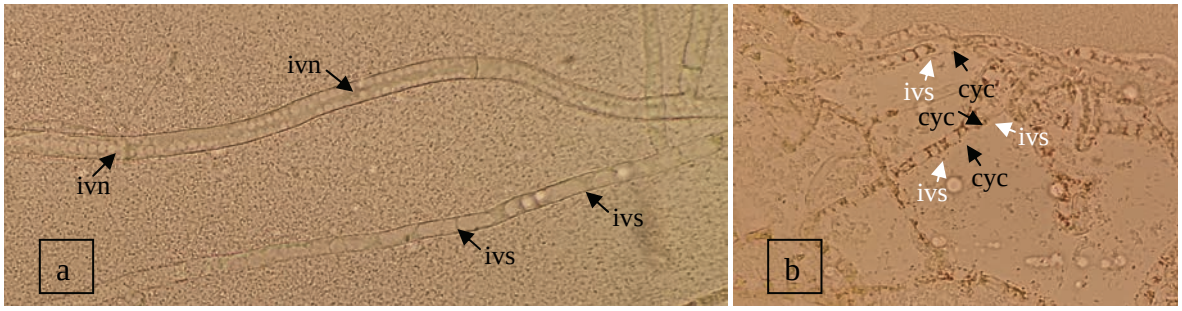

Figure 8. Bacterial interaction of *Pseudomonas chlororaphis* Sal.c2 with *Rhizoctonia solani* hyphae observed under the optical light microscope. Details in **a)** illustrate an increased number of vacuoles (ivn) in the fungal hyphae and an increased vacuole size (ivs), **b)** illustrate the fungal cells with cytoplasmic coagulation (cyc) within the hyphae and vacuoles with an increased size.

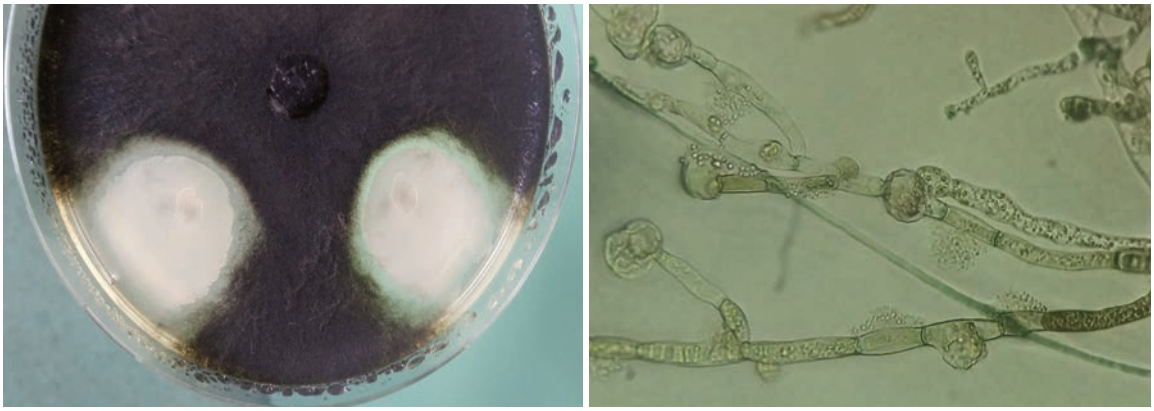

Figure 9**.** Microbial interaction between *Macrophomina phaseolina* and biocontrol bacteria.
a. Double culture technique of *Bacillus* sp. and *M. phaseolina*;
b. Fungal cell swelling, cell lysis and cytoplasm likings due to *Bacillus atrophaeus / subtilis* 6T4 biocontrol activity.

Biocontrol studies on *Macrophomina phaseolina* suggest that lytic enzymes, like chitinase and β-1,3-glucanase, produced by biocontrol bacteria are involved in hyphal degradation and cell wall digestion of *M. phaseolina* (SINGH et al., 2008).

CONCLUSIONS

Sweet potato is a relatively new crop for Romanian growers. This vegetable was successfully acclimatized and established in the crop rotation, mostly on the sandy soils of the Dolj county. No phytosanitary problems of economical important losses were registered on sweet potato, before harvest, in our country. However, it is expected that due to changing climate conditions and biological material transfer among states, several pathogens that currently do not attack sweet potato could find appropriate conditions to infect. It is also believed that when growers will expand this vegetable's culture, the incidence of diseases will be increased, and the spectrum of pathogens will be enlarged. We consider that fungal diseases like *Fusarium* spp., *Macrophomina phaseolina*, and *Rhizoctonia solani* will extend their spectrum of infection also to sweet potato.

Several fungal pathogens of sweet potato and pathogens mentioned worldwide to infect this vegetable were analyszed in dual culture, with several biocontrol agents (*Bacillus amyloliquefaciens* OS17, *B. endophyticus* 1T2, *B. atrophaeus / subtilis* 6T4, *B.subtilis* ssp. *subtilis* Dj3, *B. subtilis*/ *mojavensis* Dj6, and *Pseudomonas chlororaphis* Sal.c2). The purpose of these studies was to evaluate the potential of these Romanian native biocontrol strains to suppress pathogenic growth, and to evaluate the microbial interaction involved in the biological control process. Several mechanisms are supposed to be involved in the biocontrol process: the lytic enzyme production, antifungal compounds and competition with pathogens. The studied biocontrol strains were able to induced, *in vitro*, mycelial growth inhibition, fungal cells lysis and other alterations of the hyphae.

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THE ROLE OF THE FLORA IN AGRICULTURAL SYSTEM DEVELOPMENT IN MOUNTAIN AREAS - CASE STUDY: TAFRAOUTE IDA OUZDOUTE, MOROCCO

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Abstract. The open areas in the Western Moroccan Anti Atlas Mountains constitute a diversified natural potential. However, this wealth is doomed to disappearance under the growing impact of natural and anthropogenic hazards - an example being the changes introduced on the agricultural production system. Hence, the importance of having a look at these territories in terms of participative sustainable development is great. This paper provides the first elements of a work-in-progress research, which aims at setting up a geographical information system integrating the components of geo-bio-diversity, the local know-how, the heritage, and the risks that are associated with them. It is to present the results of the study of the flora throughout the Tafraoute Ida Ouzdoute Valley (Ighrem, Taroudant). The exploration, sampling, and the semi-structured interviews are the main methodological tools adopted. The inventory and analysis of the data on the local flora confirm the diversity of plants, their importance in the development of agriculture and the enrichment of the ecosystem services of the mountains. In addition, a great need has been detected in terms of communication on the enhancement and preservation of this component of biodiversity.

Keywords: plants diversity, agricultural system, ecosystem services in mountain areas, sustainable development participatory, Tafraoute Ida Ouzdoute (Morocco).

Rezumat. Rolul florei în dezvoltarea sistemului agricol din zonele montane – studiu de caz: Tafraoute Ida Ouzdoute, Maroc. Zonele deschise din Munții Anti Atlas din vestul Marocului constituie un potențial natural diversificat. Cu toate acestea, această bogăție este sortită dispariției sub impactul tot mai mare al pericolelor naturale și antropice – un exemplu fiind modificările introduse în sistemul de producție agricolă. Prin urmare, importanța de a avea o privire asupra acestor teritorii în ceea ce privește dezvoltarea durabilă participativă este mare. Acest document oferă primele elemente ale unei cercetări în curs de desfășurare, care vizează crearea unui sistem informațional geografic care să integreze componentele geo-bio-diversității, know-how-ului local, patrimoniul și riscurile asociate acestora. Este vorba de prezentarea rezultatelor studiului florei în cazul Văii Tafraoute Ida Ouzdoute (Ighrem, Taroudant). Explorarea, eșantionarea și interviurile semistructurate sunt principalele instrumente metodologice adoptate. Inventarul și analiza datelor privind flora locală confirmă diversitatea plantelor, importanța acestora în dezvoltarea agriculturii și îmbogățirea serviciilor ecosistemice ale munților. În plus, a fost detectată o mare nevoie în ceea ce privește comunicarea privind sporirea și conservarea acestei componente a biodiversității.

Cuvinte cheie: diversitatea plantelor, sistem agricol, servicii ecosistemice în zone montane, dezvoltare durabilă participativă, Tafraoute Ida Ouzdoute (Maroc).

INTRODUCTION

The Anti Atlas Mountains of Western Morocco constitute one of the ecosystem complexes of the Globe with major scientific importance, and which require systematic research (MICHARD et al., 2008). The Ighrem Region in Taroudant, Morocco (9°23'W, 30°25'N, elevation 115 m) is a good example of this complexity (Fig. 1).

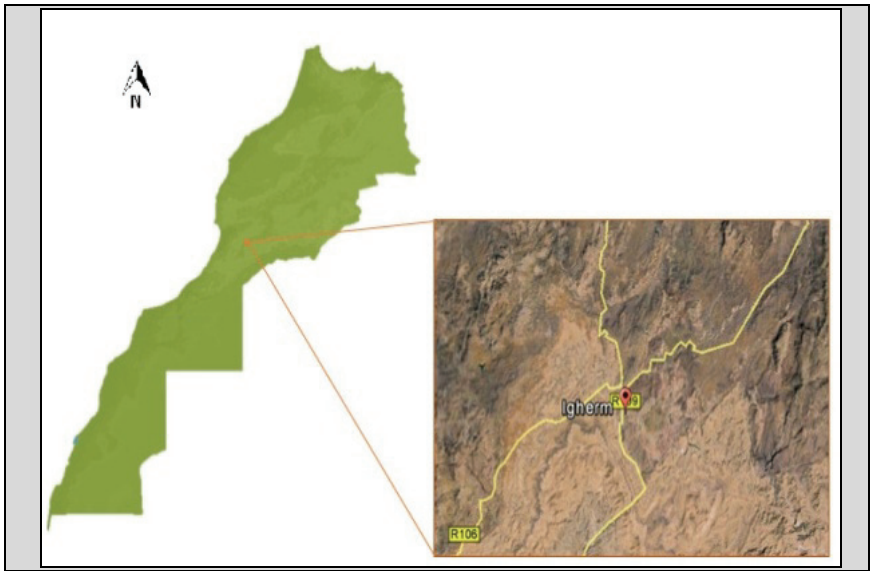

Figure 1. Location of the study area (original).

This entire territory is mainly formed of landforms and valleys which make life difficult for its inhabitants (AL-AZKI 2003; 2006, 2008; 2010-2011a; b). This geography imposes a special agricultural system. The zones of exploitation are landscaped in the form of levels where the trees, shrubs and herbaceous plants are grown successively on plots which are very fragmented (Fig. 2).

The management of water resources has undergone a great change. The traditional ancestral system based on the distribution of in shares has been changed with a modern system in terms of integration of new techniques of collection and irrigation. This change accompanied the dynamic of development recorded in many communities of the Ighrem (BERCU, 2015).

Study area. The geography of this territory is characterized by the abundance of landforms and valleys, which makes life difficult and imposes a special agricultural system. The trees, the other plants and the market gardening succeed one another in holdings that are generally very fragmented and distributed in floors (Fig. 2); the management of the water resource has changed from a traditional system ancestral database on the distribution of shared water rights, to a modern system with the integration of new techniques of collection and irrigation (BERCU et al., 2015).

Figure 2. Agriculture on levels at Targa, Tafraoute Ida Ouzdoute Valley (original photo).

However, its mountain ecosystems are facing extinction by natural hazards (drought, erosion, etc.) and anthropological impacts (human migration and neglect of the territory, excessive exploitation of natural resources). The present study of the Tafraoute Valley aims at presenting the results of the efforts undertaken to enhance and preserve the floristic richness as a major component of the biodiversity of the valley. In the different stages of this study, an interest was given to the involvement of the local population in order to ensure a sustainable development participation (PARRIS & KATES, 2003). This development is necessarily linked to the maintenance of mountain agricultural system, which requires a rigorous and responsible management of natural resources, as well of ecosystem goods and services derived from it. (Fig. 3).

In fact, this study first proposes to establish an inventory of the main plant species of the valley and to determine their uses depending on the local traditions and practices. Then, it aims at checking to what extent the development projects put in place in the valley have contributed to the preservation of the flora (TANCOIGNE et al, 2014; GRÊT-REGAMEY et al., 2015).

Conceptual Framework. The mountain ecosystems of Ighrem are threats to destruction under the effect of natural (drought, erosion, etc.) and anthropogenic risks (migration of men, excessive exploitation of natural resources). The present study in Tafraoute Ida Ouzdoute aims at presenting the results of the efforts undertaken to enhance and preserve the floristic richness (KÖRNER et al., 2011). The ecosystem services, biodiversity and sustainable development are the concepts that govern this work (Fig. 3).

Ecosystem is the foundation upon which human beings survive and their community civilization develops (O'FARRELL et al., 2007). One of these key components is the biodiversity, which was defined as all species of microorganisms, plants and animals, as well as ecosystems where they live and interact (KAENNEL, 1998; ***. COM, 2001; ***. UNEP, 2010).

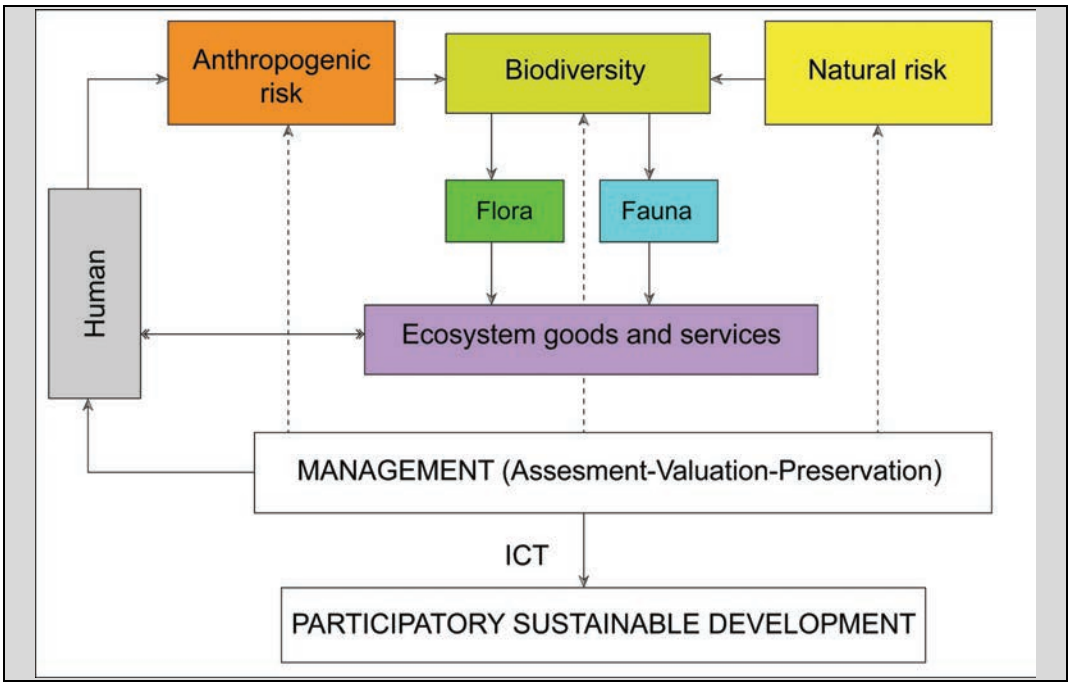

Figure 3. Research model (original).

The products and services provided to the ecosystem service function furnish the necessary environmental conditions and process in order to meet the requirements of human beings (COSTANZA et al., 1997; HUANG et al., 2010). Therefore, the maintenance and protection of ecosystem service function is essential for achieving harmony and balance between human community and ecosystem (HUANG et al., 2011a; b). So, a sustainable development participation seems necessary to maintain life in these isolated rural areas. As it was defined by the report of 2001, sustainable development implicitly requires the participation of the local population to ensure the sustainability of resources and the continuity of human life.

This paper presents the first elements of an ongoing research project that aims to develop a geographic information system incorporating elements of geo-bio-diversity, local expertise, as well as the risks associated with them in the territorial development (GRAY, 2011).

MATERIALS AND METHODS

The approach undertaken to develop this study was a multidisciplinary one. Through exploration and sampling, 34 local plants were collected. This work was complemented by semi-structured maintenance. Thus, with 30 record maintenance guides, an ethno-botanical survey of land was used to determine the local use of these plants. In parallel, regular monitoring of the plant nursery was used to evaluate the two local species production capacity. The two main criteria to undertake this evaluation were: the number of plants (seedlings) who survive and the number of plants who die of the total number cultivated.

The farm where the works were carried out, with an area of 600 m² and a fenced area of 4,000 m², has been an agricultural project in partnership with the Government Programme "National Initiative for Human Development" in 2010.

The methods of prospecting and sampling were supplemented with interviews. Thus, with 30 record maintenance guides, an ethno-botanical survey of land was used to determine the local use of these plants. This sample represented 15.87% of the population of the locality. These people were selected on the basis of 1) their knowledge of the medicinal plants of the forest, and 2) their experiences with traditional uses of plants.

RESULTS AND DISCUSSION

The inventory of the flora from plantations. The work done in the Tafraoute Ida Ouzdoute Valley allowed the collecting of the main species of local flora. 28 species were identified belonging to 24 families (Table 1; Fig. 4) (PRODAN & BUIA, 1966; MORARIU & TODOR, 1972; BENABID & BELLAKHDAR, 1987; DRĂGULESCU, 1995; BENABID, 2000; 2002; FĂGĂRAȘ, 2005; CHIFU et al., 2006; BENABID & MELHAOUI, 2011; AXINI, 2012).

Table 1. The systematic analysis of the plant species identified in the Tafraoute N'Ida Ouzdoute Valley.

NO.	ORDER	FAMILY	TAXA
1.	**Rosales**	Rosaceae	*Prunus amygdalus* Batsch
2.		Rhamnaceae	*Ziziphus lotus* (L.) Lam.
3.	**Fabales**	Fabaceae	*Ceratonia siliqua* L.
4.	**Apiales**	Apiaceae	*Ferula communis* L.
5.	**Malvales**	Cistaceae	*Cistus villosus* L.
6.	**Malpighiales**	Euphorbiaceae	*Euphorbia echinus* Hook. f. & Coss.
7.	**Sapindales**	Anacardiaceae	*Pistacia lentiscus* L.
8.			*Schinus molle* L.
9.	**Lamiales**	Oleaceae	*Olea europaea* L.
10.		Lamiaceae	*Thymus leptobotrys* Murb.
11.			*Lavandula suaveolens* (L.) Poiteau
12.			*Lavandula multifida* L.
13.	**Brassicales**	Brassicaceae	*Moricandia suffruticosa* (Desf.) Coss. & Durieu
14.	**Gentianales**	Apocynaceae	*Nerium oleander* L.
15.	**Boraginales**	Boraginaceae	*Echium horridum* Batt.
16.	**Solanales**	Solanaceae	*Withania frutescens* (L.) Pauquy
17.	**Asterales**	Asteraceae	*Senecio* sp. L.
18.			*Cladanthus arabicus* (L.) Cass.
19.			*Centaurea* sp. L.
20.			*Launaea arborescens* Murb.
21.			*Carthamus fruticosus* Maire
22.	**Ericales**	Sapotaceae	*Argania spinosa* (L.) Skeels
23.	**Caryophyllales**	Cactaceae	*Opuntia ficus-indica* (L.) Mill.
24.		Polygonaceae	*Rumex vesicarius* L.
25.	**Asparagales**	Asphodelaceae	*Asphodelus tenuifolius* Cav.
26.		Asparagaceae	*Urginea maritime* L.
27.			*Asparagus stipularis* Forssk.
28.	**Liliales**	Liliaceae	*Asparagus albus* L.

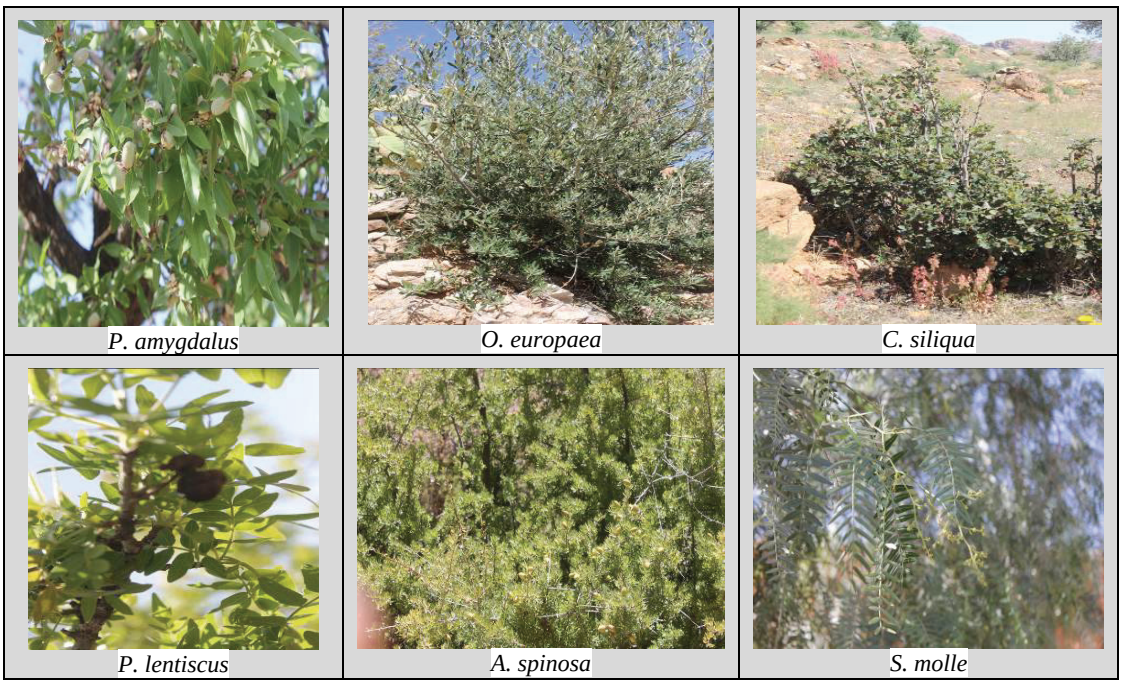

Figure 4. The main trees species of Tafraoute Ida Ouzdoute Valley (original photos).

Mainly, the valley was dominated by 6 vegetable-tree species (Fig. 4; Table 2).

Shrubs and herbaceous plants identified in the valley have developed plant associations with the tree species above mentioned. 4 shrubs species and 18 herbaceous plant species were identified and determined in the valley (Fig. 5; Table 2).

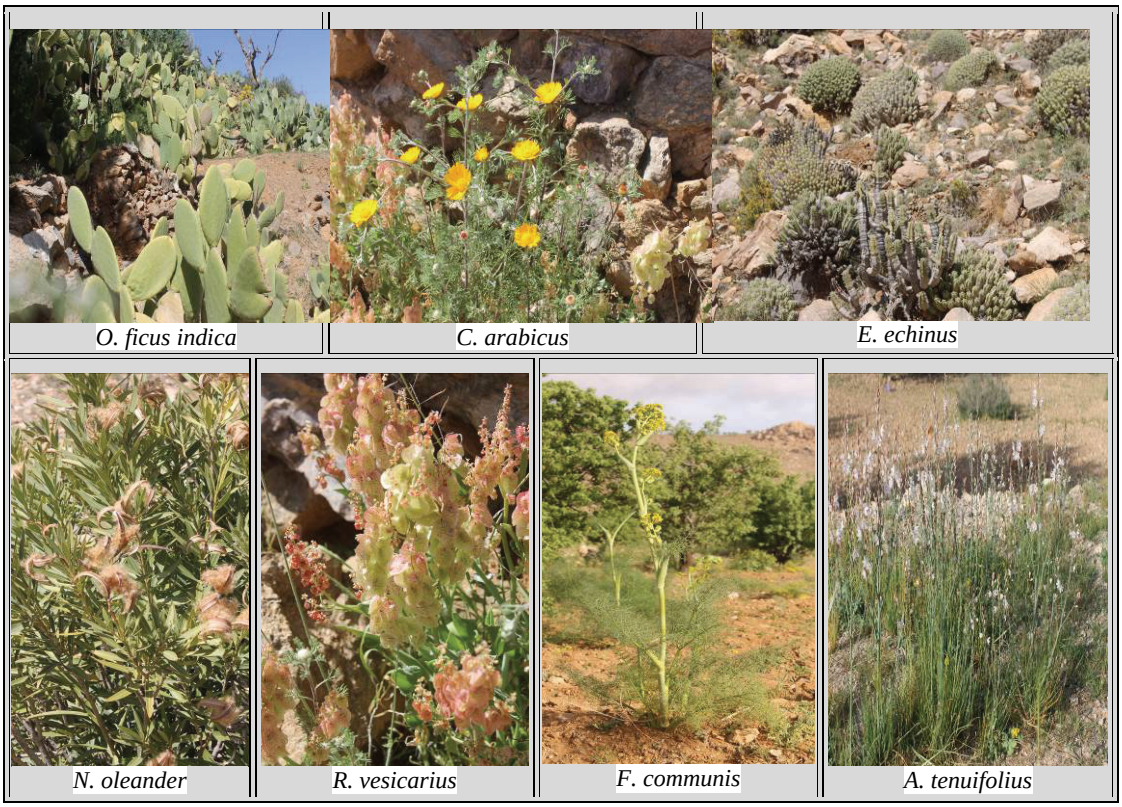

Figure 5. A sample of the floristic procession at Tafraoute n'Ida Ouzdoute Valley (original photos).

Table 2. The biological type and flora uses from the Tafraoute N'Ida Ouzdoute Valley.

NO.	SPECIES	BIO-TYPE	USES
1	*P. amygdalus*	tree	food, medical, cosmetic
2	*O. europaea*		food, medical, cosmetic
3	*C. siliqua*		food, medical, cosmetic
4	*P. lentiscus*		medical, tonic
5	*A. spinosa*		food, medical, cosmetic
6	*S. molle*		fuel and barrier in grazing
7	*N. oleander*	shrub	moderate toxic
8	*Z. lotus*		medical
9	*L. arborescens*		-
10	*A. albus*		food, after cooking
11	*T. leptobotrys*	herbaceous plant	medical, tonic
12	*C. villosus*		medical
13	*C. fruticosus*		nectariferous plant
14	*W. frutescens*		pasture plant
15	*M. suffruticesa*		medical
16	*E. horridum*		medical
17	*E. echinus*		medical, ornamental
18	*L. suaveolens*		cosmetic
	L. multifida		cosmetic
19	*A. tenuifolius*		medical
21	*U. maritima*		medical
22	*O. ficus indica*		food, medical
23	*R. vesicarius*		medical, cosmetic
24	*A. stipularis*		medical (vegetable powder used on the wounds)
25	*Senecio* sp.		medical
26	*C. arabicus*		medical
27	*Centaurea* sp.		-
28	*F. communis*		medical (domestic use and hives)

The flora uses of the Ida Ouzdoute Valley. The flora of Tafraoute Ida Ouzdoute Valley (Ighrem, Taroudant) is generally used for food, medicinal, aromatic or cosmetics purposes, according to interviewees in this study (Table 2). However, a laboratory work is needed to confirm or affirm these statements, and to determine the doses of use of each of the species (FISCHER, 1941; VASILCA-MOZĂCENI, 2003; HSEINI & KAHOUADJI, 2007; BENKHNIGUE et al., 2014).

The preservation of the flora in plantations. Starting from 2010, the aboriginal population has begun preservation projects of the flora in Tafraoute Ida Ouzdoute Valley. The experience of the Tigmi N'targa locality was a successful example. This experiment is unique in the eastern of Anti-Atlas Mountains (Fig. 6).

The efforts were directed to planting the local species *O. europaea* (olive trees) and *P. amygdalus* (almond trees). In a period of two years, the achieved results were 9200 seedlings of *O. europaea* located in the valley`s fields. The success rate was lower for *P. amygdalus*; only 4578 seedlings survived, of which 2500 were sold, the rest were located in the valley.

Figure 6. The plant nursery at Targa, Tafraoute Ida Ouzdoute (original photo).

CONCLUSIONS

The present study has enabled us to draw up the floristic listing of a mountainous area enclave of the Anti Atlas Mountains in the Occidental Morocco. An extra work is recommended to complement the vernacular names of species and itemize their uses.

The culture of the two species *P. amygdalus* and *O. europaea* in the valley of Targa has proven its effectiveness. The new plantations have strengthened the vegetation cover. A lack was noticed with regard to the improvement of the living conditions of the seedlings to decrease the losses during the culture (34.22% for *P. amygdalus* and 8% for *O. europaea*).

It has become mandatory to raise awareness on the importance of biodiversity in the survival of these rural areas and for the integration of modern technology in agriculture.

All these efforts will not have a significant impact unless the main actor, the farmer, is at the center of every project of territorial development.

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ULTRASTRUCTURAL ASPECTS OF CELLULAR RESISTANCE TO POLLUTION. CASE STUDY - *Phragmites australis* (CAV.) TRIN. EX STEUD.

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Abstract. Hiperaccumulator plants possess specific pathways for adaptation and growth in hostile environments, in the presence of large amounts of exogenous material, with harmful action. Reed (*Phragmites australis* (Cav.) Trin. ex Steud), a hyperaccumulator plant for cadmium, is also resistant to other heavy metals and radionuclides. It is a species widespread on Earth's surface with a great capacity to adapt to different environment conditions. After the analysis of the ultrastructural characteristics of three reed populations, developed in natural conditions, in different locations in the Middle Jiu Valley (Gorj, Romania), containing slightly different amounts of heavy metals and radionuclides, was established a possible resistance mechanism to heavy metals. Exogenous particles of different shapes, enter in the leaf through the stomata or penetrate the cuticle and the cell wall. In leaf tissue, the exogenous material is distributed through plasmodesmata to the neighbouring cells. Reed manifests a natural resistance to some stress factors achieved by: ferritin granules synthesis, as well as of chelating substances, with role in binding metal and in the transport of exogenous materials and active metabolic pathways, that allow a cell intense activity, even in the presence of exogenous material in the nucleus or mitochondria. In addition to this, all exogenous materials can be accumulated and stored in large vacuoles, located in some deposit cells, from the lacunar parenchyma, near the vascular system and stomata annex cells.

Keywords: *Phragmites australis*, coal exploitation, cell ultrastructure, cellular adaptations for resistance to pollution.

Rezumat. Aspecte ultrastructurale ale rezistenței celulare la poluare. Studiu de caz - *Phragmites australis* (Cav.) Trin. ex Steud. Plantele de tip hiperacumulator posedă căi specifice de adaptare şi creştere în medii ostile, în prezența unor cantități mari de material exogen, cu acțiune dăunătoare. Stuful (*Phragmites australis* (Cav.) Trin. ex Steud.), o plantă hiperacumulatoare pentru cadmiu, este, de asemenea, rezistentă la alte metale grele și la radionuclizi. Este o specie larg răspândită pe suprafața Pământului, cu o mare capacitate de a se adapta la diferite condiții de mediu. După analiza caracteristicilor ultrastructurale ale celor trei populații de stuf, dezvoltate în condiții naturale, în diferite locații din Bazinul Mijlociu al Jiului (Gorj, România), conținând cantități ușor diferite de metale grele și radionuclizi, s-a stabilit un posibil mecanism de rezistență la metalele grele. Particulele exogene de diferite forme, pătrund în frunză prin stomate sau penetrează cuticula și peretele celular. În țesutul frunzelor, materialul exogen este distribuit prin plasmodesmata celulelor vecine. Stuful manifestă o rezistență naturală față de anumiți factori de stres realizată prin: sinteza granulelor de feritină, precum și a substanțelor chelatoare, cu rol în legarea metalului și în transportul materialelor exogene și căilor metabolice active, care permit o activitate intensă a celulelor, chiar și în prezența materialului exogen în nucleu sau mitocondrii. În plus, toate materialele exogene pot fi acumulate și stocate în vacuole mari, localizate în unele celule de depozitare, din parenchimul lacunar, în apropierea celulelor vasculare și celulelor anexe ale stomatelor.

Cuvinte cheie: *Phragmites australis*, exploatări carbonifere, ultrastructura celulei, adaptare celulară pentru rezistența la poluare.

INTRODUCTION

The existing life forms on planet Earth are adapted to different environmental conditions (ecological niches) and in different geochemical conditions, that allow differential development. In time, some species have acquired resistance and can grow on media that are unsuitable for other species (high activity of radionuclides, toxic amounts of heavy metals, etc.). As a result of experimental investigations, many species of plants involved in the process of phytoremediation have been established, able to reduce the soil pollutants level, and thus modify their geochemical composition, so as to be favourable for human health and for the health of other species (AIT ALI et al., 2004; ALKORTA et al., 2004; WEIS & WEIS, 2004; BRAGATO et al., 2006; RAJAKARUNA et al., 2006; WANG & JIA, 2009; KUMARI & TRIPATHI, 2015; PARDO et al., 2016). These techniques were used to "clean" the environment after some natural or anthropogenic disasters (explosions at Chernobyl or Fukushima nuclear plants, earthquakes, and tsunamis, etc.). After a series of experiments conducted by IANNELLI et al. (2002), PIETRINI et al. (2003) was established that the species *Phragmites australis* (Cav.) Trin. ex Steud is a hyperaccumulator species for cadmium (Cd). In a comparative experiment on the decontamination capacity of the wastewater by *P. australis* and *Thyfa latifolia* L., it was concluded that reed is more efficient for adsorbtion of copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), iron (Fe), lead (Pb) and zinc (Zn); superior results can be obtained by combining the two species (KUMARI & TRIPATHI, 2015). PARDO et al. (2016) demonstrated the enhanced capacity of rhizofiltration for arsenic (As) of the reed, after external iron addition and iron plaque formation at the root level, in a hydroponic experiment. Researches in molecular biology have led to the discovery of genes involved in phytoremediation processes, functioning of specific metabolic pathways, synthesis of stress-protective substances. Thus, DAVIES et al. (2006) using transcriptomics technology, have identified the genes responsible for phytoremediation activity in *P. australis*. Through gradual adaptation of the parental cell line (HJ16) at a high concentration of H2O2, AL-QENAEI et al. (2013) have obtained a new resistant cell line H_2O_2 Jurkat.

Adaptation of the organisms to environmental conditions, with different geochemical composition and through

different metabolic pathways, is possible through the synthesis of stress-protective substances (ferritin, etc.), genes amplification or gene mutations. Cell biology studies have shown exogenous polluting particles interaction with organelles, modification of metabolic pathways (CORNEANU, 2011).

Reed occupies disturbed, primary places and can also thrive in damp places with high acidity. It is particularly common along roads, ditches and roadside debris piles deployed, wherever there is a small depression with water. PENKO (1993) observed stunted reed plants on acidic wastes from abandoned copper mines in Vermont. Also were found near waste dumps on middle Jiu valley, in Romania (CORNEANU, 2011), and near abandoned mines in Upper Silesia, Poland (CISZEWSKI et al., 2013). Different types of changes and / or man-made damages may contribute to the spread of reed. For example, a flooding due to tidal restrictions may lead to a decrease in the mass of water that may favour reed (ROMAN et al., 1984). Also, sedimentation may promote the spread of reed, by raising the water level and effective reduction in the frequency of floods.

P. australis is a clonal herbaceous species with a concave woody stem that can grow up to 6 m tall. The leaves are lanceolate, frequently 20-40 cm long and 1-4 cm width. The flowers that form in midsummer, are arranged in reddish spikelets. Ripening seeds is highly variable and occurs in autumn and winter, being important for colonization of new areas. In nature, there are cytotypes with different somatic chromosomes numbers (4x - 16x), with a good adaptation to various environmental conditions, tetraploid (4x) and octoploid forms (8x) being the most common. Different degree of polyploidy leads to differences in ontogeny, stem morphology, leaf anatomy and morphological differences between clones. These differences are sustained when the plants are grown in a common environment (HANSEN et al., 2007; LAMBERTINI et al., 2008). WEIS & WEIS (2004) consider that the reed is an invasive species, accumulating a greater amount of heavy metal compared to some native species and makes it suitable for use in wet locations, for phytoremediation and restoration of wetlands. Researches of LAMBERTINI et al. (2008) on numerous reed populations of various origin and ploidy level underlined that "populations of *Phragmites australis* in Europe can be considered as part of a single meta-population."

This experiment was conducted on *P. australis*, phytoremediator species that naturally vegetated in three different sites regarding the content of heavy metals and radionuclides. The three locations are part of the same areas situated in the Middle Jiu Valley, fragmented by surface coal exploitations, at a distance of 30 km between them (Oltenia region, Gorj county, Romania). There were performed investigations regarding: soil contents in heavy metals and radionuclides activity, analysis of ultrastructural characteristics of mature plant leaf cells, determination of ultrastructural characteristics that support the adaptation of the plants to a substrate with a high content of pollutants.

MATERIALS AND METHODS

Site description. The middle basin of the river Jiu is located in the Getic Piedmont. In the mining basin of North Oltenia is the main reserve of coal, valued at about 3 million tons, providing one-third of the total electricity produced annually in Romania. An important role in this regard of the mines and surface coal (lignite) exploitation and the two existing thermoelectric power plants (TEPP) (Turceni and Rovinari) in the Middle Jiu Valley (Fig. 1). Mining and energy production leads to environmental degradation through pollution and habitat fragmentation. The area is heavily populated and the villages in the area are affected by coal dust and ash from coal power plants, as well as from ash pits.

Biological material. Investigations were performed on three populations of reed harvested from three wetlands located in the Middle Jiu Valley (Gorj county, Romania), currently separated (but originating from a single initial population spread over a length of 30 km):

- the population nearby the village Țânțăreni (air pollution from ash pit and fly ash from TEPP-Turceni, as well as from the European road E66);
- the population from a wetland nearby TEPP-Turceni (200m) (air pollution from ash pit and fly ash from TEPP-Turceni);
- the population located at the base of a closed sterile waste dump, 30 years old, near the village Cocoreni. The site is about 15 km North of TEPP Turceni and at the same distance from TEPP Rovinari. The location is in the vicinity of a coal deposit (500 m) (air pollution from ash pit and fly ash from TEPP-Turceni, as well as coal dust from coal deposit).

In Fig. 1 are presented the sample locations, as well as the potential pollution sources.

Biological material samples were collected from mature leaves, from plants located in the middle of the three areas.

Amounts of heavy metals and radionuclides activity from the soil. The soil samples were harvested from the rhisosphere horizon (the 5-20 cm).

The heavy metals analysis were done in National Research and Development Institute for Soil Science, Agrochemistry and Environment Protection Bucharest, Romania. The amount of heavy metals in soil (expressed in mg / kg soil) was determined using flame atomic mass spectrometry method (LĂCĂTUȘU et al., 2011).

Radionuclides activity was done in Radioactivity Monitoring Station, Environment Protection Agency Craiova, Dolj, Romania Radionuclides activity was determined by the Duggan method (1988), (IAEA TECDOC 1092 directives), with a gamma spectrometry system, analyzer SPECTRUM-MASTER-ADCAM, model 92X. For the energy and efficiency of calibration standard gamma punctiform and volume sources with energies of the gamma radiation in

the range of interest (5 – 20.000 keV) were used; Am241, Cs137, Co60, Eu152, Ba133. The collecting time of the natural background amounted to 2000.000 s. Radionuclides activity was expressed in Bq/kg, confidence level 95%.

Ultrastructural investigations. Leaf samples were harvested from mature plants. Pieces of about 1 mm^3 taken from the middle of the leaf were prefixed in a solution of 2.7% Glutaraldehyde solution (2 ½ hours), postfixed in a 1% solution of osmium tetroxide (1 ½ hours), infiltrated and soaked in EPON 812 resin. Serial sections of about 80-90 nm thick were contrasted with uranyl acetate and lead citrate. Analysis of leaf ultrastructural characteristics were determined with a TEM JEOL JEM 1010 electron microscope (Electron Microscopy Center, Babeș-Bolyai University, Cluj-Napoca, Romania.

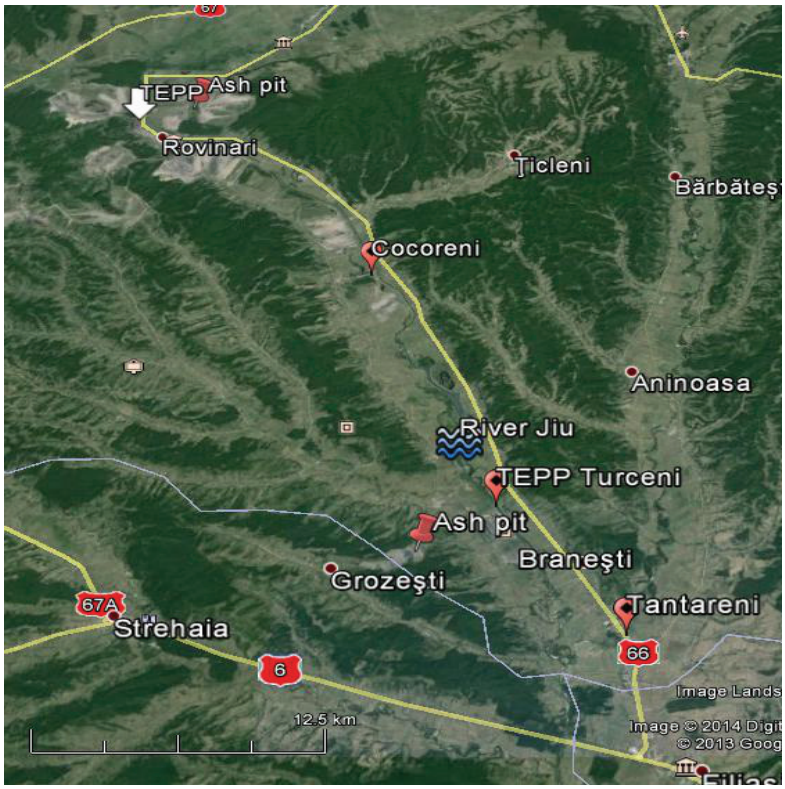

Figure 1. Sample location and the potential pollution sources (original).

RESULTS AND DISCUSSION

The levels of pollutants in soil (radionuclides activity and the amount of heavy metals in topsoil). Coal, like any ore, contains a discrete quantity of radionuclides, existing primary naturally such as: K-40, U-238, U-235, Th-232, Ra-226. Natural radionuclides are released in the environment from by-products or wastes, resulted from energy production. They have physico-chemical properties similar to some constituent chemical elements of living organisms, are metabolized and arrive finally, through different natural trophic chains, into the human organism (CHIOSILĂ, 2004). During coal combustion, most uranium, thorium and their decay products are released from the coal matrix and distributed in gas phase as well as in solid combustion products (fly ash and bottom ash).

Soil analysis revealed the presence of natural radionuclides, belonging from ash and coal dust, as well as of Cs-137 (artificial radionuclide), of Chernobyl provenance. Radionuclides activity was dependent on the considered site and the distance from the source of pollution. Values over the accepted limits for Romania were recorded for U-238, U-235 and Cs-137 (artificial radionuclide) in Țânțăreni site, and U-238, U-235, Pb-210, Cs-137 in Cocoreni site. The soil samples harvested near TEPP-Turceni were recorded the highest values for U- 238, Pb-210, Pb-214, U-235 and Cs-137 (Table 1). The radionuclides activity in samples from the ash pit (bottom ash) was 10-20 times higher than in soil and the ash participate to air pollution, being spread by the wind, over the entire considered area.

The amount of heavy metals recorded values slightly over the normal content in soil, but under the alert limits for: Pb, Ni Cr, Cu in the three sites. Thus, the highest values for Pb, Ni and Cr were recorded in the Țânțăreni site, while values slightly over normal for Cu, Pb and Ni were recorded at the basis of sterile waste dump from Cocoreni. Nearby TEPP-Turceni the highest values were recorded for Zn, Cu, Pb, Ni and Cr (Table 2).

Table 1. Radionuclides activity in topsoil, ash and coal dust (Bq · kg^{-1} soil)
(bold letters mark the values over the limit for Romania).

Radionuclide	Țânțăreni village Mean ± SD	TEPP-Turceni Mean ± SD	Cocoreni Mean ± SD	Limits for Romania	Bottom ash (ash pit) Mean ± SD	Lignite dust Mean ± SD
U-238/Th-234	**43.20 ± 5.55**	**51.30 ± 6.47**	**34.10 ± 4.51**	25.0	**656.60± 76.90**	<10.5
Ra-226	25.50 ± 2.00	38.90 ± 2.50	22.50 ± 1.40	10.0-90.0	**509.0± 186.60**	19.1±1.75
Pb-210	33.40 ± 1.47	**79.15 ± 6.46**	**44.78 ± 1.54**	20.0-40.0	**568.60± 42.70**	0
Bi-214	26.70 ± 2.37	36.96 ± 3.22	22.0 ± 1.40	20.0-40.0	**267.40± 13.10**	23.50±1.91
Pb-214	24.10 ± 1.18	**41.50 ± 2.15**	23.30 ± 1.10	20.0-40.0	**385.70± 19.40**	14.80±1.28
U-235	**4.49 ± 0.41**	**5.26 ± 0.54**	**3.33 ± 1.23**	2.0	**32.90± 8.26**	2.18±1.83
Ac-228/Th-232	35.80 ± 7.72	34.80± 1.90	35.60 ± 1.80	13.0-65.0	**207.31 ± 24.0**	19.10±1.72
Pb-212	4.15 ± 1.39	49.3± 1.82	30.0 ± 1.65	20.0-50.0	**324.90± 20.70**	0
K-40	485.40 ± 22.0	464.0 ± 25.9	388.90± 21.60	330.0-800.0	929.30± 76.0	<43.0
Cs-137	**15.90 ± 0.83**	**6.54 ± 1.96**	**5.77 ± 0.55**	0	**114.70± 10.10**	<1.9

Table 2. The amount of heavy metal in topsoil and bottom ash (mg · kg^{-1} soil)
(bold letters mark the values over normal content in soil).

Heavy metal	Țânțăreni village	TEPP-Turceni	Cocoreni	Normal content in soil	Allert limits	Bottom ash (Ash pit)
Zn	49.1	**306.0**	58.7	100	300-700	63.0
Cu	13.8	**45.2**	**22.4**	20	100-250	19.0
Fe	20,899	22,214	21,205	*	*	25,004
Mn	593.0	363.0	310	900	1500-2000	252.0
Pb	**22.5**	**42.6**	**22.1**	20	50-250	2.53
Ni	**68.6**	**35.1**	**25.7**	20	75-200	**50.0**
Cr	**47.8**	**40.9**	21.2	30	100-300	20.0
Co	9.8	8.8	8.28	*	*	7.38
Cd	Traces	0.9	Traces	1.0	3.0-5.0	0.154

*There are no available data in Romanian standards

Ultrastructural characteristics of leaf. The ultrastructural leaf analysis of the mature plants reveals some minor differences between the three genotypes of *P. australis*. Ultrastructural investigations showed that species adapts to the presence in the environment of the stressors (heavy metals and radionuclides).

Penetration and accumulation of exogenous material in leaf parenchyma cells. In the three sites were recorded similar issues regarding the penetrating paths of the exogenous pollutant particles, their dissemination and accumulation. Exogenous particles, acicular shape, granular or squamous, enter in the leaf tissue through stomata or through penetration of cuticle and epidermal cell wall (Fig. 2).

Similar to other species found in the same site (*T. latypholia*), they can be extracted from the environment by the plant root system and spread through the vascular circulation (unpublished data). In the parenchymal tissue adjacent cells, exogenous particles are scattered by plasmodesmata (Fig. 3). In the cell vacuole (Fig. 4) exogenous particles are usually aggregate, acicular particles are usually found in the form of needle-shaped aggregates of particles. Squamous particles are also aggregate, especially in the circulatory system into the inner wall of the leading vessel. Granular particles form different sized granules, by aggregation. Also, in the cells in this area, near plasmodesmata, multivesicular bodies (MVB) can be present (Fig. 4).

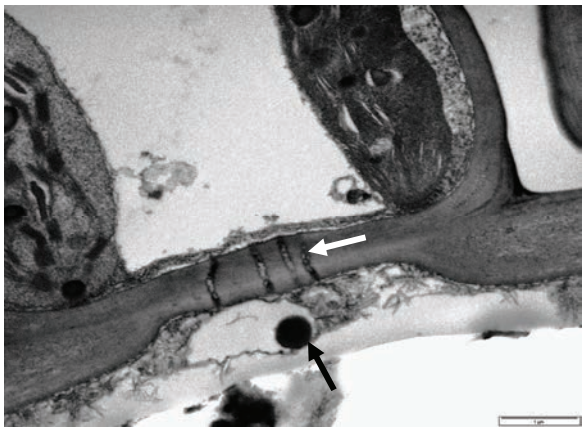

Figure 2. Longitudinal section through a plasmodesma, on its tract being visible exogenous material (white arrow). In the vesicle situated under the cuticle, there are exogenous material and anthocyanin granules (black arrow) (scale bar 1µm) (original).

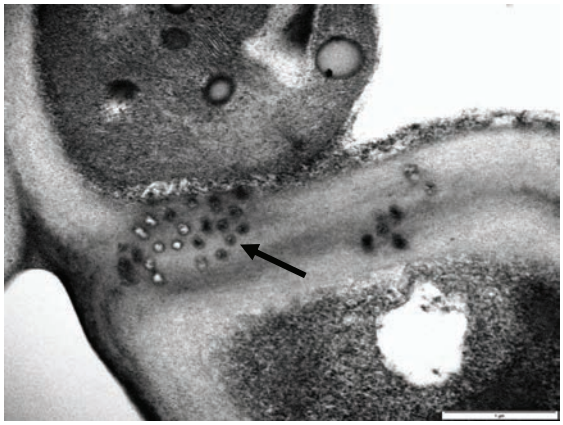

Figure 3. Transversal section through a plasmodesma. In some canalicles there is exogenous matter, which migrate from one cell to another (arrow) (scale bar 1µm) (original).

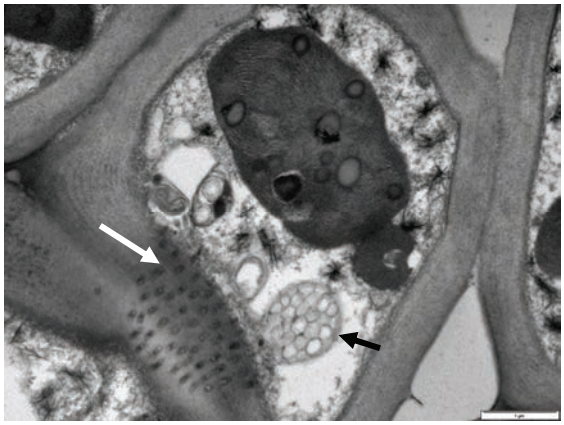

Figure 4. Transversal section through a plasmodesma (white arrow). In the cell cytoplasm, there is a MVB (black arrow) and accumulation of exogenous matter (scale bar 1µm) (original).

Exogenous particle interaction with organelles. Acicular particles are usually grouped near plasmalemma, on the tonoplast surface (Fig. 5) or within it, in the vacuole (Fig. 6), on the surface or inside the cell wall (Fig. 7), and in intercellular space, which forms aeriferous circulatory system, where they reach by crossing stomata ostiole (Fig. 8). They can also be found on the external surface of the endoplasmic reticulum channels, inside the nucleus and of some organelles. The plasmodesmata presence, as well as of the exogenous particles in the cell wall, confirm that exogenous particles end up in the leaf tissue by penetrating the cuticle and the epidermal cell wall. From the leaf tissue cells, they are spread mainly through the vascular circulation, exogenous particles being present in libber and ligneous cells, as well as in fundamental parenchyma cells (Figs. 9, 10).

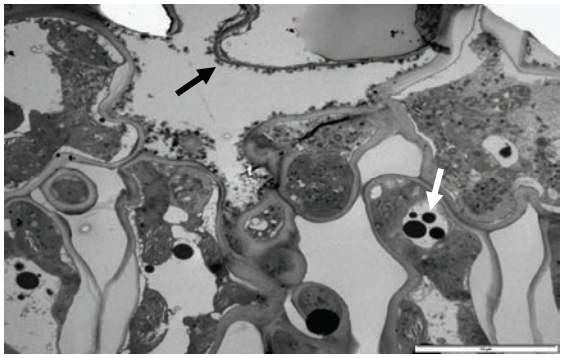

Figure 5. Acicular exogenous matter accumulated and disposed on the vacuole (black arrow) tonoplast. In some vacuole there are anthocyanin granules (white arrow) (scale bar 10µm) (original).

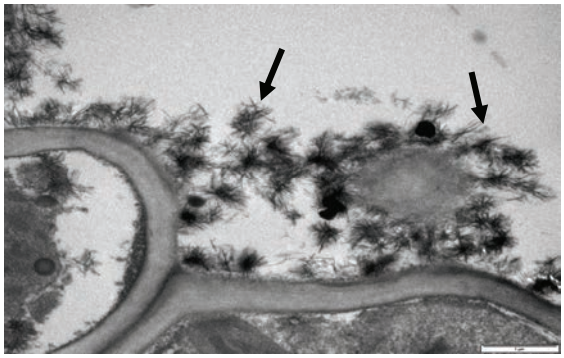

Figure 6. Exogenous acicular matter, disposed in vacuole and on tonoplast (detail), (scale bar 1µm) (original).

Another pathway for their presence in leaf tissue is their penetration through the stomata. In Fig. 8 in the stomata annex cells (which are in "open" position) there are vesicles with a chelating substance having an active defense action of the plant, in the presence of exogenous material. Exogenous particles were also found in some cell organelles, especially in the nucleus and mitochondria. In the nucleus, in the presence of small quantities of exogenous acicular particles, which are accumulated in karyolymph, the ultrastructure is relatively normal (Fig. 11).

Figure 7. Acicular exogenous particles arranged on the exterior walls of the cell, in the intercellular space (black arrow) and in the cell walls (white arrow), (scale bar 1µm) (original).

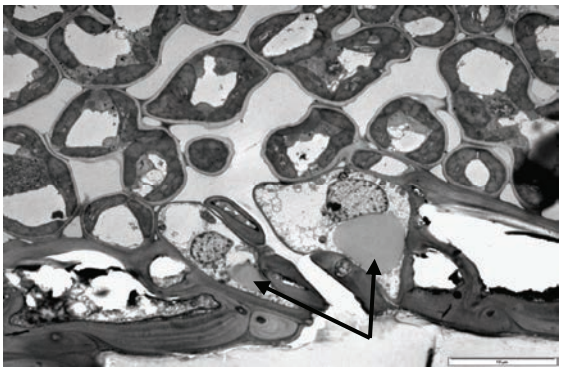

Figure 8. Stomata in "open" position, with chelating substance (arrow) in the annexes cells (scale bar 10µm) (original).

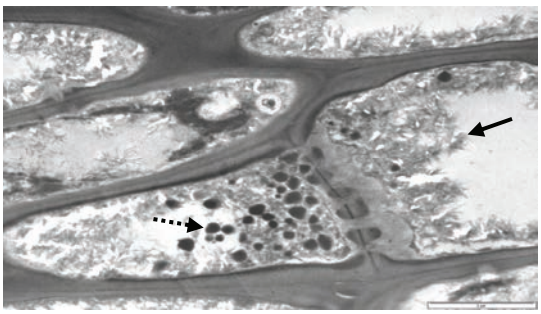

Figure 9. Massive accumulation of acicular exogenous particles (simple arrow) in libberian cells, together forming an accumulation of antyhocyanin granules (dots arrow) (scale bar 2µm) (original).

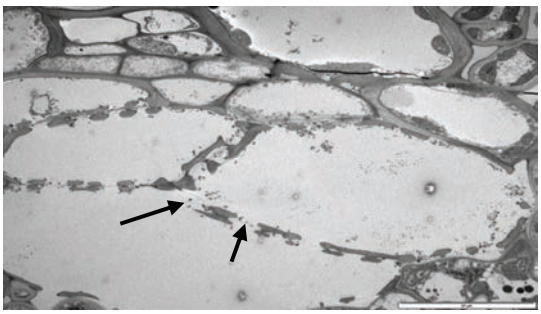

Figure 10. The cells from the ligneous vessels with a very lax constitution, which permit an easy penetration of the exogenous material (arrow) (scale bar 20µm) (original).

If the amount of acicular exogenous material is great, karyolymph shows varying degrees of alteration. In addition, the exogenous material is present in the cell vacuole (Fig. 12). The defensive cell reaction is the presence in the vacuole of the anthocyanin vesicles, as well as with chelating substance (Fig. 12).

In mitochondria, exogenous particles were found on the surface of mitochondrial crysta and in the matrix (Fig. 13). As a result of the accumulation of a big quantity of exogenous particles in chloroplast, its ultrastructure is altered, the main aspects of the alterations being the presence of a small amount of plastoglobuli and the grana fragmentation (Fig. 14).

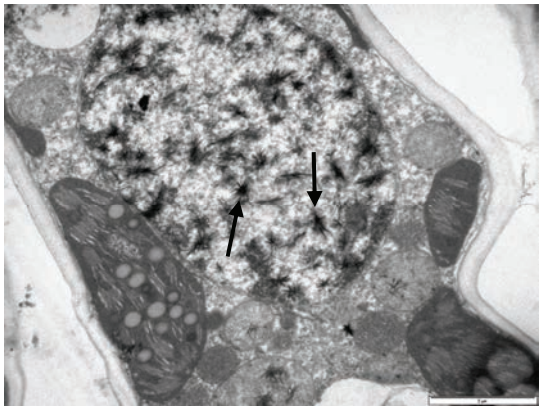

Figure 11. Nucleus with a moderate amount of acicular particles (arrow), accumulated in karyolymphe (scale bar 2µm) (original).

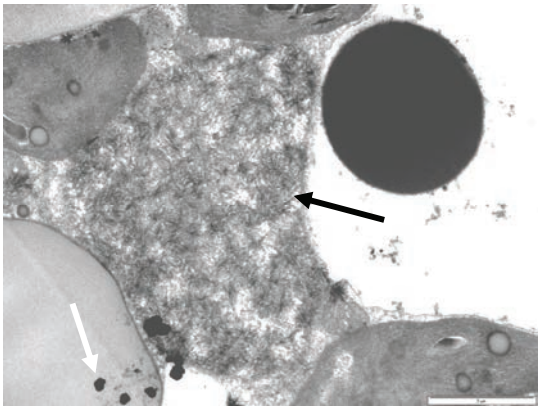

Figure 12. Nucleus with an altered structure (black arrow). In a vacuole (right) there is a giant anthocyanin granule. In left there are vesicles with chelating substance in which there are exogenous particles (white arrow) (scale bar 2µm) (original).

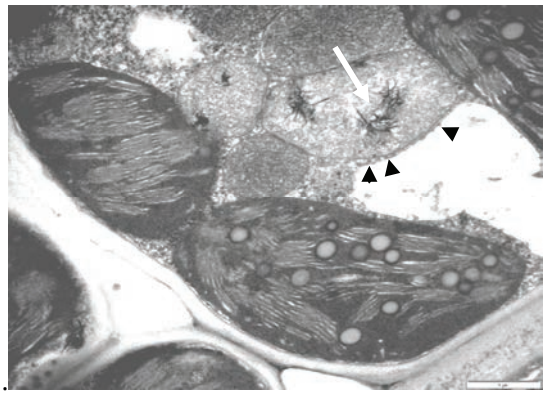

Figure 13. Mitochondria with an accumulation of exogenous acicular particles (white arrow). The chloroplast contains numerous plastoglobules. In mitochondria take place the ferritin synthesis, disposed as granules in cytoplasm (black arrows), (scale bar 1µm) (original).

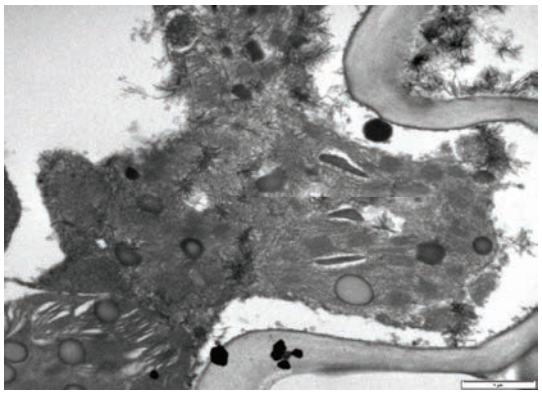

Figure 14. Chloroplast with altered structure as a result of the exogenous particles accumulation in the cell (scale bar 1µm) (original).

Defense reaction of the plant cell. In the presence of exogenous particles (of heavy metals and radionuclides), the reaction of the plants from the three sites was similar, with some particularities.

Synthesis of chelating substances (probably phytochelatins) accumulated in vesicles of different sizes usually accompanied by granules with exogenous substances, or by electron-dense (osmiophilic) granules. These vesicles are present in the plants from all three sites. These vesicles can be merged (Fig. 15, several small vesicles located in the vacuole arranged around the exogenous acicular particles), resulting electron-light vesicles, relatively uniform in size (5.0 to 7.6 µm), present mainly in aerial circulatory system (Fig. 8) or in circulatory system, in addition to electron-dense granules (Turceni site), or in addition of exogenous aggregate particles (sites Ţânţăreni and Cocoreni). The exogenous particles presence on the surface or inside vesicles with chelating substance, especially in the both circulatory systems (liberrian and ligneous), highlights their involvement in the chelating and the transport of this exogenous material (Fig. 16).

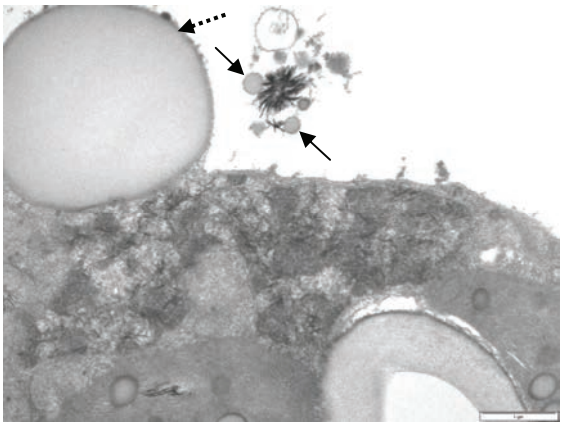

Figure 15. Many small vesicles with chelating substance present in a vacuole, around of an acicular exogenous particle (simple arrow). As result of their merging, result big vesicles full with chelating substance (dot arrow), (scale bar 1µm) (original).

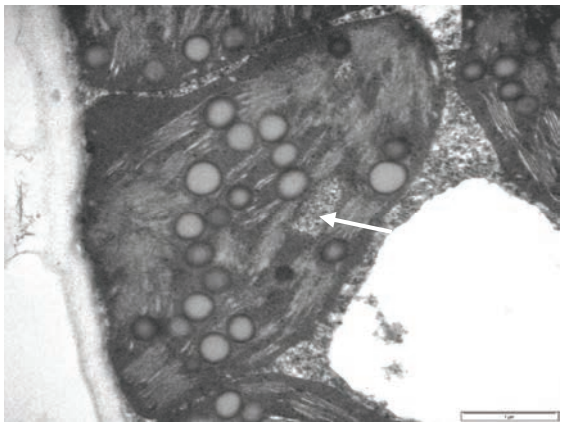

Figure 16. Chloroplast with many plastoglobuls. In stroma there is the “crystal-like bodies” area (arrow) in which take place the ferritin synthesis (scale bar 1µm) (original).

Ferritin synthesis has a role in defense and adaptogenic properties. Iron is an essential element for all life forms and limits oxidation to the ferric form, having a profound impact on the productivity of the organisms. As a consequence of an inappropriate diet, about 30% of the global population suffers from different forms of iron deficiency (GOTO et al., 2001). In all organisms are ubiquitous but Ferritin, a metalloprotein of 450 kDa, rich in iron, composed of 24 subunits (THEIL, 1987). Ferritin in plants shows an important role in redox reactions. The function of ferritin in plants consists of keeping the iron for a short or long time, to protect the cell from the toxic effects of free iron, thus serving as a primary antioxidant. PRASAD & NIRUPAM (2007) considers that ferritin acts against biotic and abiotic stress factors, accumulating heavy metals, as well as a protector of the genome. Ferritin genes have been found at different species of plants, especially vegetables, being reported ferritin synthesis in the chloroplast and mitochondria. HARRISON & AROSIO (1996) reported the presence in the chloroplast stroma of the regions without thylakoid (named "crystal-like bodies"), formed by the accumulation of ferritin (Fig. 16). DUY et al. (2007) reported the synthesis of ferritin in the chloroplast of the species *Phragmites australis*. In plants from Ţânţăreni site, where it was recorded a large amount of iron in the substrate, in the chloroplast was revealed the existence of ferritin granules, spherical or small rod-shaped. Histochemical methods were not used in this research to identify ferritin

features. However, it is possible that in Fig. 13, to be evidenced ferritin synthesis, in mitochondria. In mitochondria matrix and cytoplasm around mitocondria are located some electron-dense particles of similar size. They are synthesized in the mitochondria and subsequently migrate into the cytoplasm.

Granules of anthocyanin. Anthocyanins are water-soluble pigments, substances belonging to the group of flavonoids, found in all tissues of higher plants (leaves, stems, roots, flowers and fruits). In leaves and sometimes in stem, they serve to protect the cells from the damage "high-light" by absorbing blue-green and ultraviolet light (high-light stress). Anthocyanin synthesis occurs in chloroplast, anthocyanin molecules being arranged on the thylakoid surface (Fig. 17). They migrate to the periphery of the chloroplast and then into the cytoplasm. After their aggregation, resulted small granules virions shape like, disposed on internal surface of the tonoplast (Fig. 18). They arrived in the in vacuole, merges resulting initially small granules, and then large anthocyanins granules in the cell vacuole (Fig. 12).

In the vacuoles there is a different amount of exogenous material, free or stored in multivesicular bodies (together or not with the remnants of the destroyed cells) vesicles with chelating substance, a.o. The presence of the anthocyanins granules in these cells blocks the action (toxic or not) of exogenous material and so the plant can accumulate a large amount of toxic substances in the cells (Fig. 19).

Multivesicular Bodies (MVB) are endosomal organelles containing small vesicles (exosome), formed following the inward budding of the outer endosomal membrane. Studies on the biogenesis, structure and function of multivesicular bodies were carried out by different authors (AN et al., 2007; PIPER & KATZMANN, 2007; OTEGUI & REYES, 2010). Their presence in the cell is associated with exchanges between neighbouring cells by plasmodesmata, as it shows in Fig. 4 (Ţânţăreni site). DUY et al. (2007) found that plant cells can secrete endosomes derived from multivesicular bodies. After OTEGUI (2014): "MVBs are endosomes that consist of a limiting membrane and internal vesicles". "The internal vesicles arise from invaginations of the limiting membrane and carry membrane proteins targeted for degradation in the lysosome/vacuole. MVBs play a crucial role in both the endocytic and the secretory pathways of all eukaryotic cells, sorting proteins for degradation or recycling, down -regulating receptors, and mediating the transport of proteins to the vacuole/lysosome. Thus, MVB functions are tightly related to cell signalling, differentiation, and transport of vacuole cargoes" (Figs. 20, 21).

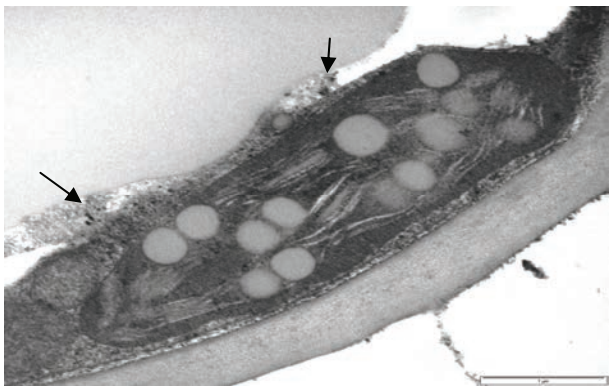

Figure 17. Young chloroplast with plastoglobules and electron-dense granules (anthocyanin granules), synthesized at the thylakoids level (scale bar 1µm) (original).

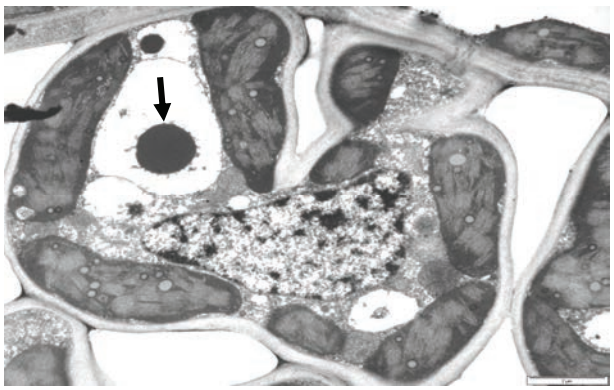

Figure 18. The synthesis and formation of the anthocyanin granules. After synthesis in the chloroplast, the small anthocyanin granules will be aggregated, resulting big granules which accumulated in the vacuole (arrow), (scale bar 2µm) (original).

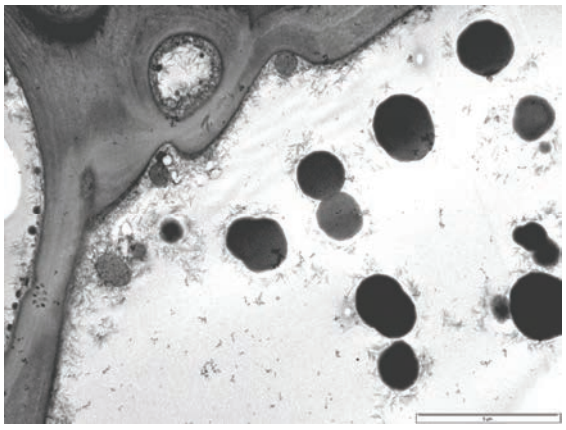

Figure 19. Acicular exogenous particles and anthocyanin granules, accumulated in the vacuole of a lacuna parenchyma cell (scale bar 5µm) (original).

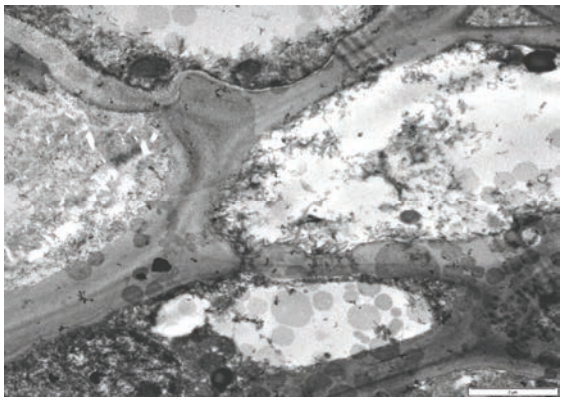

Figure 20. Accumulation of acicular exogenous material and alogen granules in the cells of libberian vessels, which will become deposit cells. The nucleus and cytoplasm present alterations (scale bar 5µm) (original).

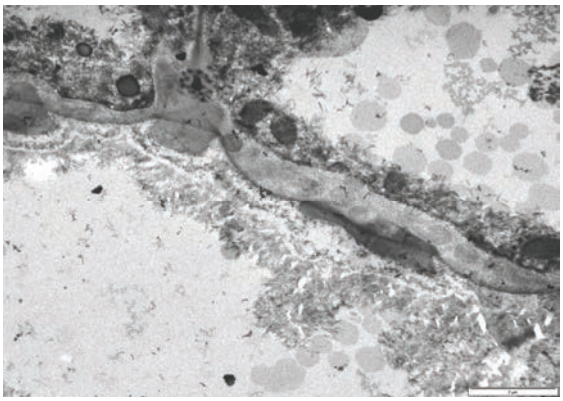

Figure 21. Accumulation of acicular exogenous particles and destroyed cell vestigial, in the cells situated near central cylinder. These cells will become also deposit cells (scale bar 2µm) (original).

Deposit cells. Exogenous material, along with the remnants of the destroyed cells is accumulated in deposit cells, located on the underside of the leaf, near the lower epidermis, circulatory system (libberian cells or ligneous cells) and aeriferrous circulatory system (formed from intercellular spaces) or in the fundamental parenchyma from the central cylinder, represent deposit cells of exogenous material, and/or remnants of the organelles (Figs. 20, 21). In these cells, multivesicular bodies are also, involved in exocytosis processes. Between deposit cells, there are plasmodesmata, that facilitate the transport of this material. In the core of plasmodesmata canalicles or in transit, through the cell wall, there is present this exogenous material. Also in the stomata annex cells, located in the vicinity, there are vesicles with chelating substances (Fig. 8). The presence of all these factors in the deposit cells (exogenous material, multivesicular bodies, vesicles with chelating substances, anthocyanins granules and other structures and substances such as Ferritin, a.o.) situated near the lower epidermis and the circulatory system, supporting their involvement in the inactivation and / or exocytosis of the toxic waste materials from the plant. These findings are in accord with the ones described in a review, regarding the complexation of the metals with different ligands and their mechanisms by LEITENMAIER & KÜPPER (2013).

CONCLUSIONS

After the distribution of the various pollutant elements in the soil in the area under consideration, the major sources of pollution are TEPP Turceni, TEPP Rovinari (fly ash), ash pits (bottom ash) and the coal deposit Cocoreni (coal dust). In the analyzed soil samples was registered an activity of the natural and artificial radionuclides over the normal limits for Romania (U- 238, Pb-210, Pb-214, U-235 and respectively Cs-137), but the content in heavy metals, even is over the normal soil content (Pb, Ni, Cr, Cu), is still under the alert limits. The most polluted site is placed nearby TEEP Turceni.

P. australis cosmopolitan, hyperaccumulator species is very suitable for the study of the penetration of exogenous particles and various cellular strategies of containment, neutralization and their storage at the tissue level. Exogenous particles entering in the plant, on the one hand by absorption, at the root level, and arrive in leaf tissue through the leading vessels and on the other hand, arrive directly through the cuticle and penetration of the cell wall or through stomata. They are spread in leaf tissue through plasmodesmata, located between adjacent cells. In the cell, the exogenous particles are visible on the tonoplast or in the vacuoles, near endoplasmic reticulum tubules and in some organelles like the nucleus, mitochondria, and chloroplasts.

The active cell reaction is demonstrated by the presence of specific structure and synthesis of stress-protective substances (multivesicular bodies, vesicles with a chelating substance, anthocyanin granules, ferritin particles, a/o). Chelators synthesized in the chloroplast thylakoids, gather and incorporate exogenous particles, transporting them through the intercellular spaces and the circulatory system, in cells located near the lower epidermis.

The exogenous particles and remnants of cell organelles are clustered in cell vacuoles, located in the lower epidermis, and nearby leader system vessels, which become deposit cells. Anthocyanin granules accumulated in cell vacuoles block the action of exogenous toxic particles. The ferritin molecules, synthesized in mitochondria and chloroplasts, have an important role in many important redox reactions. They keep the iron in the cell and act as primary antioxidants. The presence of multivesicular bodies (MVB), in cells located near the lower epidermis, suggests the existence of an exocytosis activity, through which are probably removed the accumulations of deposit cells.

ACKNOWLEDGEMENTS

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PURIFICATION AND CHARACTERIZATION OF DECARBOXYLASE FROM A HALOPHILIC MICROORGANISM WITH POTENTIAL IN BIOTRANSFORMATION OF WASTE GLYCEROL FROM BIODIESEL INDUSTRY

NEAGU Simona, COJOC Roxana, GOMOIU Ioana, TUDORACHE Mădălina, ENACHE Mădălin

Abstract. The present study reveals the influence of the culture medium composition on decarboxylase production by a strain of halotolerant bacteria isolated from the Letea salt lake in the Danube Delta, namely strain LN 1-17, the purification of the enzyme obtained on the culture medium that offers optimal conditions for its production, as well as the enzyme characterization. The results of this study confirm that the investigated strain harbors the capacity to produce lysine decarboxylase. Related to the influence of culture medium composition the best result was recorded on MH medium supplemented with lysine and casamino-acids. The purification of the enzyme by precipitation with acetone followed by gel filtration was considered appropriate due to the increase of the enzymatic specific activity even if it has led to a decrease in the protein content. The biochemical characterization of the enzyme showed an optimal activity at neutral pH (7), in the absence of NaCl and at a temperature of 60^0C.

Keywords: decarboxylase, halophilic microorganisms, waste glycerol, biodiesel.

Rezumat. Purificarea şi caracterizarea decarboxilazei de la un microorganism halofil cu potenţial în biotransformarea glicerolului rezidual din industria biodiesel. Studiul de faţă se referă la influenţa compoziţiei mediului de cultură asupra producerii de decarboxilaze de către o tulpină bacteriană halotolerantă izolată din lacul sărat Letea din Delta Dunării, respectiv tulpina LN 1-17, la purificarea enzimei obţinute pe mediu de cultură care oferă condiţii optime pentru obţinerea acesteia precum şi la caracterizarea enzimei. Rezultatele acestui studiu confirmă că tulpina investigată are capacitatea de a produce lizin decarboxilază. În ceea ce priveşte influenţa compoziţiei mediului de cultură, cele mai bune rezultate au fost obţinute pe mediul de cultură MH suplimentat cu lizină şi acizi cazaminici. Purificarea enzimei prin precipitarea cu acetonă, urmată de gel filtrare, chiar dacă a condus la scăderea conţinutului de proteină este adecvată, deoarece conduce la creşterea activităţii specifice a enzimei. Caracterizarea biochimică a enzimei a arătat că aceasta are activitate optimă la pH neutru (7), în absenţa NaCl şi la 60^0C.

Cuvinte cheie: decarboxilaze, microorganisme halofile, glicerol rezidual, biodiesel.

INTRODUCTION

Waste glycerol is the by-product obtained in large amounts in the manufacturing process of biodiesel (KALIA et al., 2016; NEAGU et al., 2018b in press). This product began to represent a problem related to the ratio efficiency/price of the biodiesel industry processes. In spite of the fact that the glycerol molecule by its structure has huge application, the waste glycerol has no value for the synthesis industry due to the contained impurities like methanol, water, salts, soaps (HIRSCHMANN et al., 2005). On the other hand, the purification of waste glycerol is not a feasible alternative. The lack of proper management for waste glycerol entails the lowering of the biodiesel industry's interest due to high production costs and an increased environmental pollution by large glycerol stocks (NEAGU et al., 2018).

Residual glycerol is the most important by-product in biodiesel production from biomass (DASARI et al., 2005). Lately, worldwide biodiesel production has substantially grown. As a direct consequence, residual glycerol (unpurified) has been produced in quantities that will soon outweigh the current market requirements, constituting a serious environmental problem by its storage. The idea of producing an enormous amount of residual glycerol is automatically associated with enormous losses of energy and material resources. Moreover, a hyperproduction of glycerol may result in a significant decrease in its price. Another aspect of the problem of residual glycerol is that its applicability is reduced compared to that of purified glycerol. This is due to numerous impurities found in the residual glycerol matrix (i.e. water, salts, soaps, etc.) (HIRSCHMANN et al., 2005). As a consequence, residual glycerol is used in industry only for the production of dried pet food (LEONETI et al., 2012). In literature, attempts are made to use residual glycerol but in a mimic laboratory form, i.e. pure glycerol contaminated with supposed impurities, for developing the glycerol industry on the residual glycerol branch by designing and applying new synthesis technologies (YUAN et al., 2010; LAURIOL-GARBEY et al., 2011).

Bioconversion of residual glycerol can be accomplished through several paths like its transformation into glycerol carbonate by carbonylating, glycidol and glycerol carbonate compounds which, under the action of decarboxylase, can be converted to polyglycerol (TUDORACHE et al., 2017).

Decarboxylases are produced by most microorganisms in response to their development in acidic environments, by acting to protect the microbial cell from the noxious action of acid media (GALE & EPPS, 1944; ALVAREZ-ORDÓÑEZ et al., 2010). These are enzymes of the carboxylases class, subclass E.C. 4.1.1, which catalyze the removal of the carboxyl group from an organic compound with carbon dioxide and proton forming. In bacteria, the amino acids decarboxylases appear to be either inducible or constitutive. On the other hand, if a multiple copy of the gene which codify the synthesis of lysine decarboxylase are present, the level of enzymatic activity increase (VENTOSA et al., 1989; KIKUCHI et al., 1997).

The purpose of this study was to analyze the influence of the culture medium composition on decarboxylase production by a strain of halotolerant bacteria isolated from Letea salt lake in the Danube Delta, to purify the enzyme obtained on the culture medium that offers optimal conditions for its production, as well to characterize the enzyme.

MATERIALS AND METHODS

The influence of the culture medium composition on decarboxylase production. In a first step, it was attempted to stimulate the production of lysine-decarboxylase by the halotolerant bacterial strain. Three variants of the culture medium for enzyme biosynthesis were used. Thus, the first variant of the culture medium (GSM) was prepared starting from 3 stock solutions: solution A containing 4g Na_2HPO_4 x $12H_2O$, 5g KH_2PO_4, dissolved in 100 mL distilled water; solution B containing 10g NH_4Cl, 5g NaCl, 4.1g $MgSO_4$ x$7H_2O$, dissolved in 100 mL distilled water; solution C formed by 8g glucose, 4g lysine and 8g casamino-acids dissolved in 315 mL distilled water.

Solutions A and B were sterilized by autoclaving at 121^0C for 20 minutes, and solution C was sterilized by filtration on a Millipore membrane with 0.22 µm pore size. A volume of 20 mL from solution A was mixed in sterile conditions with 1.1 mL solution B and 78,9 mL solution C (WANDA LU & MALLETTE, 1970). A negative control of the three stock solutions was also carried out without adding the casamino-acids to the culture medium, which would contribute to the stimulation of enzyme synthesis. The second variant of the culture medium contained (g/L): yeast extract 10, NaCl 100, $MgCl_2$x$6H_2O$ 7, $MgSO_4$x$7H_2O$ 6, $CaCl_2$x$2H_2O$ 0.36, KCl 2, $NaHCO_3$ 0.06, NaBr 0.026 (MH medium, VENTOSA et al., 1972), supplemented with cu 1% lysine and 2.5% casamino-acids.

The third variant of the culture medium was MH medium, supplemented with 1% lysine. For these culture medium variants, a volume of 10 mL of bacterial inoculum was used, whose optical density at 660 nm was 0.4. Bacterial cultivation was performed at 30°C, 140 rpm. After 24 hours, the strain grew on all culture media, except for the negative control of the first variant of culture medium. The decarboxylase activity was determined after 24h and 72h of incubation of the strain in the tested culture media.

Decarboxylase activity assay. Decarboxylase activity has been determined by the Lenhoff method (PHAN et al., 1982). Each test tube contained: 20 µL enzymatic extract, 256 µL of 10 mM phosphate buffer, pH 7 and 24 µL of 100 mM lysine solution. The mixture was incubated for 30 minutes at 30°C. The reaction was stopped with 300 µL K_2CO_3, for five minutes at 30°C. After this step, 300 µL of 20 mM 2,4,6-trinitrobenzene sulfonic acid (TNBS) were added. The obtained reaction product (cadaverine) was extracted with 600 µL of toluene. The mixture was vortexed for 1 min and the final product separated into two phases: an orange aqueous phase and an organic phase, colorless to yellow. Aliquots of 200 µL of the organic layer were taken and absorbance was determined at 340 nm with toluene as blank. The amount of cadaverine released per time unit, determined at 340 nm, is a measure of lysine-decarboxylase activity. Catalytic activity was expressed as µmoles/min/mL.

Enzyme purification. After the growth of the bacterial culture and the enzyme biosynthesis, the culture liquid was centrifuged at 9500 g for 10 minutes at 4°C. The supernatant was removed, and the biomass was stored at -80 °C until the next step. The cells were treated with 40 mL of 0.9% NaCl solution and centrifuged. The washed cells were suspended in 30 mL of 0.02 M phosphate buffer, pH 7. Ten mL of this suspension were sonicated (15 repeats/5sec). The resulting mixture was centrifuged at 4°C for 20 minutes, 4000g, to remove cell debris. The obtained supernatant was considered crude enzyme extract. A volume of 10 mL was subjected to partial purification by precipitation with 80% acetone. This purification step was carried out at negative temperatures (-5°C), the ratio of acetone: enzymatic extract being 2: 1 (v/v). Acetone was added gradually, under continuous stirring, taking care that the temperature shall exceed -2°C, after which it was left in the refrigerator for 24h. The obtained precipitate was separated by centrifugation at 4°C, 9500 rpm, 20 minutes. The obtained acetone powder was stored and dried at room temperature for 60 minutes, then suspended in a volume of 10 mL of 50 mM Tris-HCl buffer, pH 7.5.

The resulting protein extract was purified by gel filtration in 1 mL aliquots. The work protocol required, in a first step, the preparation of the gel filtration column by washing it with MilliQ water and 96% ethanol and charging it with 2 mL of gel obtained by hydrating 2 g of BioGel P-Biorad copolymer P100 with 50 mL of MilliQ water for 12h at 20°C. The equilibration was performed with 0.05 M Tris-HCl buffer pH 7.5, containing 0.003 M EDTA and 0.001 M β-mercaptoethanol. Elution was performed with 0.1M, 0.2M, 0.5M and 1M Tris-HCl, pH 7.5, in three repetitions for each concentration. Protein concentration (Lowry method – LOWRY et al., 1951) and enzymatic activity were determined at each step by the method described by Lenhoff, with some modifications (KIM et al., 2015). The decarboxylase activity was expressed as µmoles/min/mg of protein.

Biochemical characterization:

The pH influence on lysine decarboxylase activity. The decarboxylase activity of the purified extract was determined in reaction media with pH values between 4-10, the other incubation conditions remaining constant (30°C, 0% NaCl, 100 mM lysine).

The influence of temperature on lysine decarboxylase activity. In order to determine the optimal reaction temperature of the purified protein extract, it was incubated at various temperature values between 4-60°C, the other incubation conditions remaining constant (pH 7, 0% NaCl, 100 mM lysine).

The influence of NaCl concentration on lysine decarboxylase activity. In order to determine the optimum concentration of NaCl for the catalytic activity of the protein extract, the salt concentration was varied from 0 to 3 M NaCl in the reaction medium.

RESULTS AND DISCUSSION

The influence of culture medium composition on the decarboxylase production. Following the qualitative study, which aimed to identify strains of halophilic/halotolerant bacteria able to synthesize lysine-decarboxylase, the strain LN1-17 was selected to obtain a purified enzyme preparation and to establish the optimal catalytic activity parameters. The strain was isolated from Letea Salt Lake located in the Danube Delta, whose salinity is influenced by environmental conditions ranging from 7 g/L in springtime to 32 g/L during summer and autumn (NEAGU et al., 2018b in press).

The preliminary characterization of the strain revealed a large yellow-orange, glossy, flat, reniform colony, Gram negative staining, short, isolated rods. The strain grew between 0-4M NaCl with optimal from 0 to 3M. The strain was tested for decarboxylase production following the method previously described (NEAGU et al., 2016) and positive results were recorded. In order to estimate the influence of culture medium composition on decarboxylase production, the strain was growth on GSM and MH media with various chemical compositions as described in materials and methods and the enzymatic activity has been evaluated at 24 and 72 hours of cultivation.

The data obtained showed that the highest decarboxylase activity was obtained 24 hours after the inoculation in the second culture variant (MH medium with lysine addition and casamino acids – Table 1). Thus, for the subsequent steps of enzymatic biosynthesis, purification and biochemical characterization of lysine decarboxylase, MH culture medium supplemented with 1% lysine and 2.5% casamino acids was used.

Table 1. Decarboxylase activity of the strain LN1-17 after 24h and 72h of growth on different culture media; I (GSM) – medium with glucose, salts, lysine and casamino-acids; II – MH medium supplemented with lysine and casamino-acids; III- MH medium supplemented with lysine.

Culture medium variant / Enzyme activity (µmoles/min/mL)	I (GSM24h)	I (GSM48h)	II (24h)	II (72h)	III (24h)	III (72h)
LN1-17	0.0043	0.0038	0.012	0.0076	0.0085	0.0058

Enzyme purification. The enzyme purification protocol has been detailed in materials and methods area. In a first step of the method the enzyme has been purified with 80% cold acetone at negative temperature and in a final step a gel filtration using BioGel P-Biorad P100 gel for column preparation, was applied.

The results presented in Table 2 showed that the amount of enzyme decreased in each step of the purification starting from 13,5 mg/mL in the first step until to 0,27 mg/mL in last step of the gel filtration. On the other hand, the specific activity of the enzyme increased up to 0.034 µmoles/min/mg (Table 2). The partially purified enzyme preparation was biochemically characterized by determining the optimal physicochemical parameters of catalytic activity.

Table 2. The purification steps of lysine-decarboxylase synthesized by the halotolerant bacterium strain LN1-17.

No.	Purification step	Volume (mL)	Protein content (mg/mL)	Specific activity (µmoles/min/mg)
1	Culture filtrate	100	13.5	0.0005
2	Acetone precipitation 80%	10	6.69	0.0008
3	Gel filtration	1	0.27	0.034

Biochemical characterization of the purified enzyme

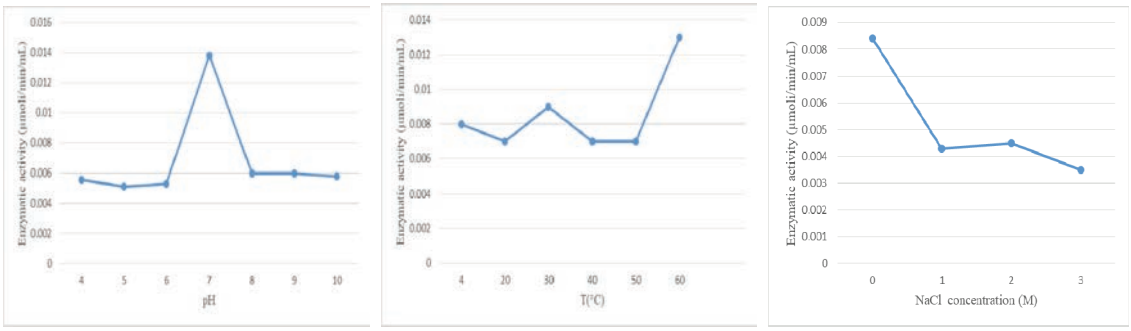
The pH influence on lysine decarboxylase activity. As presented in Table 3 and Fig. 1a, the partially purified decarboxylase exhibited catalytic activity at pH values between 4-10, with a maximum at pH 7.

Table 3. The decarboxylase activity of the purified protein extract, at different pH values (µmoles/min/mL).

pH / Strain	4	5	6	7	8	9	10
LN1-17	0.0056	0.0051	0.0053	0.0138	0.006	0.006	0.0058

The influence of temperature on lysine decarboxylase activity. From the data presented in Table 4 and Fig. 1b, the presence of catalytic activity is observed in the temperature range 4-60˚C, the optimal activity being observed at 60˚C.

The influence of NaCl concentration on lysine decarboxylase activity. It was found that the decarboxylase synthesized by strain LN1-17 showed catalytic activity at values of 0-3 M NaCl concentration, the activity being maximum at the concentration of 0 M NaCl, which confirms the halotolerant character of the enzyme that is correlated with the growth of the bacterial strain (Table 5 and Fig. 1c).



(a) (b) (c)

Figure 1. Influence of pH (a), temperature (b) and NaCl concentration (c) on the catalytic activity.

Table 4. The decarboxylase activity of the purified protein extract, at different values of temperature (μmoles/min/mL).

Temperature (˚C) / Strain	4	20	30	40	50	60
LN1-17	0.008	0.007	0.009	0.007	0.007	0.013

Table 5. The decarboxylase activity of the purified protein extract, at different NaCl concentrations (μmoles/min/mL).

NaCl concentration (M) / Strain	0	1	2	3
LN1-17	0.005	0.0043	0.0045	0.0035

CONCLUSIONS

The results from this study evidence the capacity of the strain LN 1-17 to produce extracellular protein with lysin-decarboxylase activity. The enzymatic activity appears to be influenced by the composition of the culture medium, the best result being recorded on MH medium supplemented with lysine and casamino-acids. The purification of the enzyme by precipitation with acetone followed by gel filtration was considered appropriate due to the increase of the enzymatic specific activity even if it has led to a decrease in protein content. The biochemical characterization of the enzyme showed optimal activity at neutral pH (7), in the absence of NaCl and at 60^0C. The activity at increased temperature could be an advantage for using the enzyme in various industrial and biotechnological processes and for obtaining biosensors for monitoring technological processes at high temperature or monitoring parameters in extreme environments.

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STUDIES ON ENVIRONMENTAL POLLUTION IN LARGE INSTALLATION OF COMBUSTION IN REGARD WITH INTERNATIONAL STANDARDS

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Abstract. Industrial development and globalization forces the European Union to reconsider immediate needs for energy production and transport. The study refers mainly to the impact Craiova II Thermal Power Complex has on vector environment - air. Due to the technological process that pollutes the environment, we have monitored some of the physical-chemical parameters of the air, with implications for the ecosystems. The coal waste deposited in various places has a high content of pyrite. The most important environmental pollution problem is oxidation of the pyrite and the generation of acidity. The coal waste is deposited and constitutes an active source of acid (H_2SO_4) that generates soil contamination at surface, as well as groundwater contamination, endangering ecosystems. Pyrite oxidation results in Fe^{2+}, SO_4^{2-} and H^+. Coal mining drainages are characterized by low pH, a highly varied composition that prevail in high concentrations of sulphate, iron, manganese, aluminum and other toxic and radioactive ions, as well as solid particles in excess. This drainage is one of the oldest problems arising from coal mining.

Keywords: environmental protection, air quality, physico-chemical parameters, coal.

Rezumat. Studiul privind poluarea mediului de către instalaţiile mari de ardere în acord cu standardele internaționale. Dezvoltarea industrială şi fenomenul de globalizare obligă Uniunea Europeană la o reconsiderare imediată a necesităţilor de energie, producere şi transport. Studiul se referă în principal la impactul pe care îl are Complexul Termoenergetic Craiova II asupra mediului-vector aer. Datorită procesului tehnologic care poluează mediu, am monitorizat o parte din parametrii fizico-chimici ai aerului, cu implicaţii asupra ecosistemelor. Deşeurile de cărbune depozitate în diverse locuri, au un conţinut ridicat de pirită. Cea mai importantă problemă legată de poluarea mediului, o reprezintă oxidarea piritei şi generarea de aciditate. Deşeurile sunt depozitate şi constituie o sursă activă de acid (H_2SO_4) care generează contaminarea solului la suprafaţă şi a apei freatice, periclitând ecosistemele. Din oxidarea piritei rezultă Fe^{2+}, SO_4^{2-} şi H^+. Drenajele din minele de cărbuni sunt caracterizate de pH scăzut, o compoziţie foarte variată în care predomină concentraţii mari de sulfaţi, fier, mangan, aluminiu şi alţi ioni toxici şi radioactivi, ca şi particule solide în exces. Aceste drenaje constituie una din cele mai vechi probleme ce apar ca urmare a exploatării cărbunelui.

Cuvinte cheie: protecția mediului, calitatea aerului, parametrii fizico-chimici, cărbuni.

INTRODUCTION

In order to protect the atmosphere and improve air quality, measures are needed to control pollutant emissions. To assess the degree of air pollution, pollutant emissions are calculated and the quality of the ambient air is determined. Emissions are measured by appropriate assessment methods, specific to each pollutant, based on emission factors and activity indicators. The analysis of emissions at national level, sectorial distribution, spatial and temporal targets are key elements in setting environmental priorities, identifying the targets to be achieved and policies to be adopted, both locally and nationally. The indicators selected must meet the identification criteria and be relevant for the main issues related to the atmosphere.

The main objectives of environmental policy in Romania are designed to ensure a clean environment and aim to ensure a healthy life for the population, to lead to the elimination of poverty and environmental degradation, to regenerate the economy based on sustainable development principles and to harmonize national legislation on protection of the environment with that of the European Union.

Coal power plant in Romania and European countries are responsible for more than half of the negative effects on health. Directive 84/360 / EEC on the control of environmental pollution from industrial sources has been amended by Directive 2009/31 / EC on integrated pollution prevention and control and transposed into Law no. 205/2010 improved by Law no. 278/2013 on industrial emissions (***, 2013). The objective of the above-mentioned legislation is to reduce pollution to the admissible limits. For the environmental factor air, there are considered measures to prevent, reduce, offset, as far as technically possible, measures generated by implementing legislation in force (RUSU & ROJANSCHI, 1980; BRÎNDUȘA & KOVACS, 2007; GAVRILESCU & POPESCU, 2012).

Different types of coal, coming from several basins, can have high sulfur content. In its structure, there are two forms of sulfur, namely: organic and inorganic sulfur. In coals from Russia and the United States, for example, inorganic sulfur is the major component, which is present between 0.5% and 11% in the form of iron disulfide (pyrite and marcasite). Other forms of inorganic sulfur are elemental sulfur and sulphides, which contain different metals. Organic sulphide is present in the form of thiols or mercaptans, sulphides or thioethers and thiophene compounds, these being integrated into the coal matrix. For the removal of organic sulfur from coal covalent bonds (C-S), which are resistant to the chemical treatment, must be broken (CISMAȘIU 2010a; b; TOMUȘ et al., 2015; ATHRESH et al., 2017).

MATERIALS AND METHODOLOGY

The branch of Electrocentrale Craiova II is located in the N-E area of Craiova, about 1 km away from the lower railway passage between Bariera Vâlcii Street and Craiova-Filiaşi railway line. The main road access to the Craiova II SE is located on the eastern side, namely on Bariera Vâlcii Street. The thermal power plant SE Craiova II occupies an area of approx. 433,727 sqm. The site of slag and ash storage (landfill of non-hazardous waste - slag and ash) in the area of approx. 153 ha is located approx. 5.5 km of the premises (plant), S-E of the commune of Şimnicu de Sus, at approx. 1 km N-E from the village of Jieni (Fig. 1).

CE Oltenia SA - SE Craiova II, located on the southern platform of the city, produces electricity for the national energy system and heat for Craiova. The flow of fuel depends on the momentary load of the boiler and fuel quality. SE Craiova II using coal, 97% of the total quantity of fuel (Oltenia lignite basin) with the carrier oil (0.20% of the amount of fuel used) or natural gas (approximately 3% of the amount of fuel used) (Fig. 2).

Studies were undertaken during the period 2017-2018 and aimed at the following parameters: temperature, humidity, SO_2, NO_2, NH_3, carbon monoxide, carbon dioxide, dust, fly ash, PM_{10}, sediment particles, etc. As a result of the technological process within CET II Craiova, there results ash and slag, which represents debris consisting mainly of calcite, pyrite and other minerals, which during the combustion of coal subdivide and decompose. Clay particles remaining in the combustion zone for a sufficient time become complex silicates, molten glass-like. The mineralogical analyzes of slag and ash from the burning of inferior coals indicate the presence of compact, round aggregates along with other laced sponge aggregates.

In accordance with the mineralogical data, the ashes show a 10-16% crystalline phase (quartz, mullite, hematite and magnetite) and a vitreous phase of 50-70%. The global analyzes show that the lignite ash produced in the Oltenia carboniferous zone has the following oxidic composition: SiO_2 48%, Al_2O_3 23%, Fe_2O_3 8,1%, CaO 9,2%, MnO 3%, SO_3 3,7%, ash is silicoaluminous.

The research carried out on the level of radioactivity of the ashes produced by CET Craiova II is as follows (Eq/kg): Ra-226 137,4±16,5, Th-232 82,9±14,9, K-40 520,9±52, α-global 554±100, β-global 1070±117, which revealed a radionuclide content 2-4 times higher than standard materials (POPA et al., 2008).

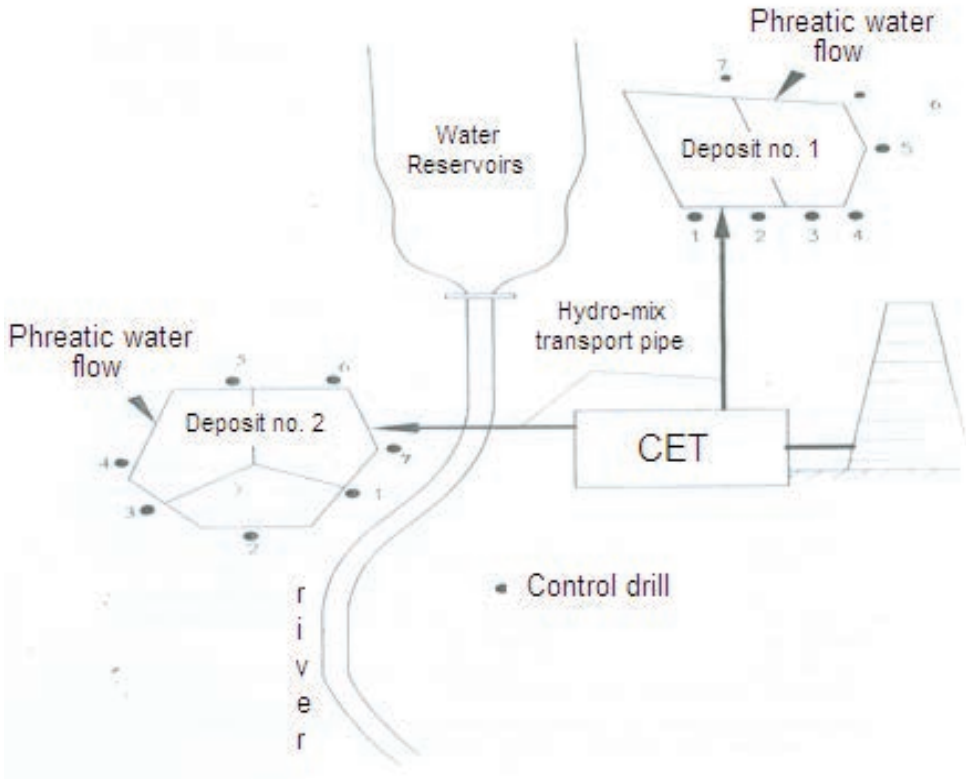

Figure 1. Scheme for operation of CET II Craiova Thermoelectric Power Plant (after GAVRILESCU, 2007).

Figure 2. The satellite image of the Deposit from Monastery Valley within Oltenia Power Complex (from Google Earth, accessed: March 11, 2018).

RESULTS AND DISCUSSIONS

The legal framework of environmental protection aims at preventing and reducing pollution of any kind, conservation and preservation of environmental quality, management responsible for natural resources and avoid overexploitation of ecological reconstruction of the areas affected by pollution from human activities and natural phenomena destructive and not last, maintaining a balance between the natural environment and the quality of life.

In the case of SE Craiova II, air quality indicators have been studied, this being the most important environmental factor for the transport of pollutants, as they represent the support of the fastest transport to the environment, so that air quality monitoring is on first place in monitoring activity.

The gaseous pollutant concentration determined in 2017 is influenced by the ability of plant tissue to convert SO_2 into relatively non-toxic forms. Sulfite (SO_3^{2-}) and sulphite acid (HSO_3) are the main compounds formed by the dissolution of SO_2 in aqueous solutions. Phytotoxic effects are diminished by their conversion by enzymatic and non-enzymatic mechanisms into sulfate, which is much less toxic than sulfite. As it can be seen, MAC (Medium concentration) in case of SO_2, 0.17 (mg/m^3) is not found in any month (Tables 1; 2). Up to certain levels, nitrogen oxides (MAC-0.30 mg/m^3) have a beneficial effect on plants, contributing to growth. In these cases, however, it has been observed an increase in the susceptibility to insect infestation and environmental conditions. As for ammonia, the values are below MAC-0.30 mg / m^3. Sometimes the gases can trap dust and other particles that reach the ground in dry form. Acid deposits can also occur at variable distances, generally difficult to sample (GAVRILESCU & GAVRILESCU, 2009; RODRÍGUEZ-MAROTO et al., 2009).

Table 1. Concentrations of gaseous pollutants determined in 2017.

Month for the determination of MAC	Gaseous pollutants – average samples for 30 min. – MAC (mg/m^3)		
	SO_2	NO_2	NH_3
Medium concentration I	0.0057	0.0103	0.0525
Medium concentration II	0.0048	0.0138	0.0467
Medium concentration III	0.0049	0.0070	0.0189
Medium concentration IV	0.0050	0.0046	0.0145
Medium concentration V	0.0049	0.0044	0.0220
Medium concentration VI	0.0037	0.0106	0.0528
Medium concentration VII	0.0036	0.0095	0.0779
Medium concentration VIII	0.0044	0.0045	0.0904
Medium concentration IX	0.0039	0.0082	0.0696
Medium concentration X	0.0050	0.0089	0.0466
Total MAC	0.75	0.30	0.30

Table 2. Content in sedimentable dusts recorded in 2017.

Harvesting points	Quantity of powders (g/m^2/month)									
	I	II	III	IV	V	VI	VII	VIII	IX	X
P1	3.76	3.62	7.39	6.73	14.66	6.41	7.89	8.07	10.02	6.17
P2	4.40	3.66	5.86	7.57	9.60	6.84	7.20	7.67	7.77	-
P3	5.32	3.31	12.48	11.26	18.08	11.55	22.82	11.99	18.52	6.57
P4	5.45	3.27	6.61	13.18	5.00	4.58	13.84	6.72	9.97	3.70
P5	14.66	2.85	11.33	8.72	13.63	15.12	25.29	5.70	14.15	7.34
MAC 17 $g/m^2/l$										

The maximum concentration of SO_2 recorded in 24 h to 200 m has a maximum CET of 18 µg/m^2. As the distance from the source increases, the concentration decreases, reaching up to 2 µg/m^3, exceeding the MAC 125 µg/m^3.

The total concentration of particulate matter TSP does not exceed the maximum permissible concentration (MAC), but it is above the threshold for alert over a distance of 1000 m (NE and SW). The average concentration exceeds MAC 24 h over a distance of 1000 m (NE) and the alert threshold PA, the distance of 750 m SV (Table 3). The temperature is between 54.2 and 60.8^{0}C and humidity between 10 and 13-19.2% V (Fig. 3).

Table 3. Total concentrations of TSP suspension powders.

Distance to the perimeter limit of the heap and the sector (m - sector)	**Concentration / concentration range (µg/m^3)**	**Health alert threshold (PA) (µg/m^3)**	**Limit value = Health Intervention Threshold (VL/PI) (µg/m^3)**	**Vegetation Protection Limit Value (VLV) / Ecosystems**	**Observations**
	2	3	4	5	6
Mediation time 1 h					
0-500 m, NE	430-410	350	500	-	< MAC > PA
0-1000 m, SV	400-350				< MAC > PA
500-1000 m, NE	410-380				< MAC > PA
1000-1500 m, SV	350-320				< MAC < PA
1000-3000 m, NE	380-280				< MAC > PA
1500-2500 m, SV	320-260				< MAC < PA
> 3000 m, NE, >2500 m, SV	<280, <260				< MAC < PA
0-500 m NV, SE	400-350				< MAC > PA
500-1500 m, NV, SE	350-290				< MAC < PA

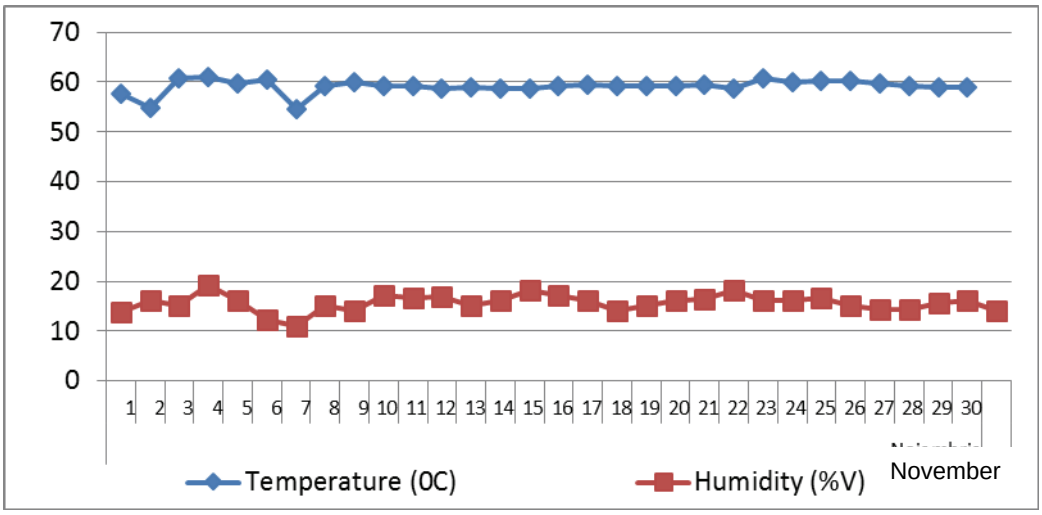

Figure 3. Temperature and humidity variation in November 2017.

NOx, SO_2 and CO do not exceed the allowed MAC (Fig. 4). CO_2 emissions from plant activity for the period 2013-2020 are covered by GHG Authorization (greenhouse gases) issued by the Ministry of the Environment and are within the permissible limits. Dust has values of 5.21-9.66 mg/Nm^3 (Fig. 5).

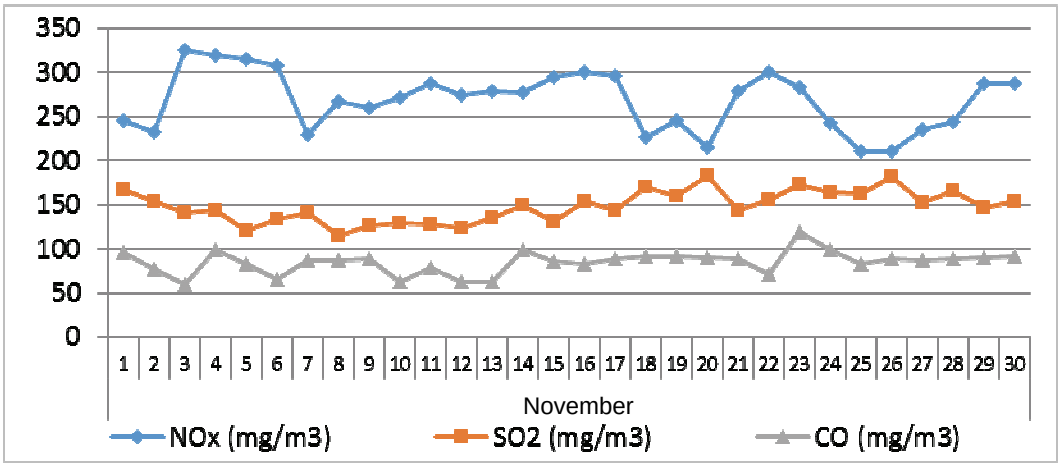

Figure 4. Variation of nitrogen oxides (NOx), SO_2 and CO sulfur in November 2017.

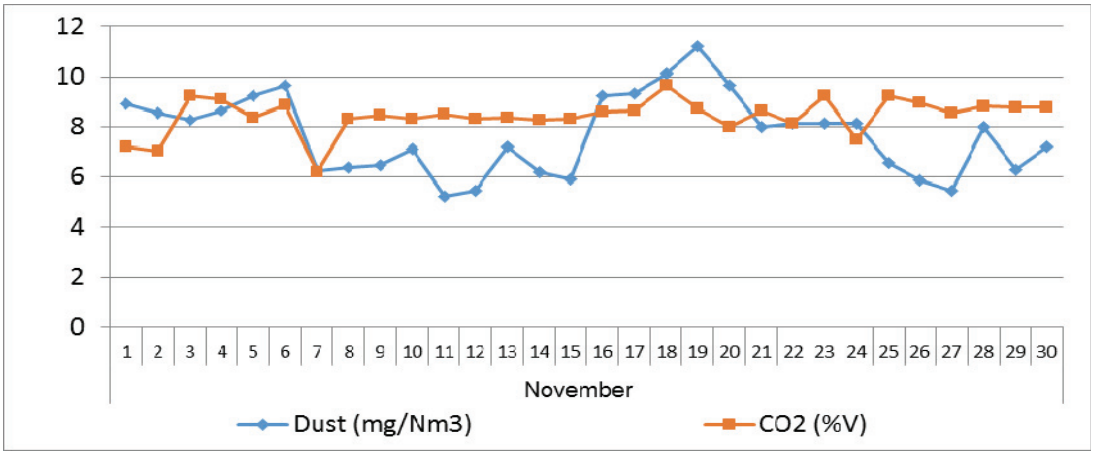

Figure 5. Variation of dust and CO_2 in November 2017.

Ash mg/Nm^3 is between 712 and 987 KNm^3/h. Ash contains also PM_{10}, which are aggregates containing hundreds of individual compounds, compounds with different chemical and thermodynamic properties. The main compounds found in PM_{10} are sulfates, nitrates, organic carbon, elemental carbon, soot, ammonium, etc. However, neither the primary biological fraction should be omitted. Organic compounds represent about 21-39% of the material particles (Figs 6, 7).

The STAS 12574-87 provisions shall be complied with at the site of the power plant, as follows:

- sedimentable dusts - max. 17 g/m^2/month;
- powder in suspension: 0.5 mg/m^3 at 30 minutes (short-term average) and 0.15 mg/m^3 at 24 h (long-term average).

Activity of the site or operation and exploitation must be carried out in such a way that the emission of pollutants that may affect directly or indirectly the soil quality at the site and in the immediate vicinity, to respect the maximum permitted concentrations of heavy metals (Co, Cr, Cu, Mn, Ni, Pb, Zn) provided by OMAPPM 756/1997.

The environmental impact assessment is based on: the quality indices of environmental factors (Ic), which method uses a matrix; the global pollution index (IPG), for which V. Rojanschi method is used (ROJANSCHI et al., 1997).

The impact assessment matrix, the establishment of the Ic quality index. The quality of an environmental factor or element environment fall against the permissible limits STAS - sites or regulatory basis, or expected effects of the project on the environment, based on - the size of which is determined taking into account the level of indicators quality that characterizes the effects. The impact assessment is thus carried out by a quantitative analytical method based on the Global Pollution Index (IPG), resulting from the ratio between the ideal state (natural) and the real (polluted) state (RUSU & ROJANSCHI, 1980; GAVRILESCU, 2007). According to the IPG, there is a quality scale (Table 4).

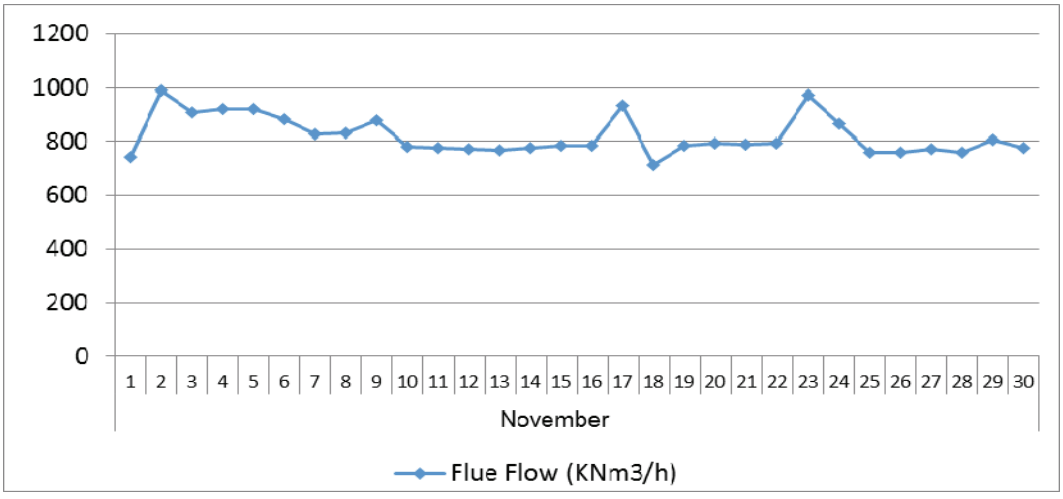

Figure 6. Variation of ash in November 2017.

Table 4. Emissions directed into the atmosphere.

Combustion plant	**Pollutant**	**Fuel type**	**Location of the broadcasting point**	**Va Emission limit values ** (mg / Nm^3) according to Act 278/2013, Annex V, Part 1, P ≥ 300 MWt**
IMA A 1 – existing installation (boilers K_1, K_2)	**SO ₂**	Sol Solid (lower lignite)	Co Dispersion point for the desulphurization plant	**20**
				20 (starting from 01.07.2020 to 30.06.2020
	P P Powders			**20**

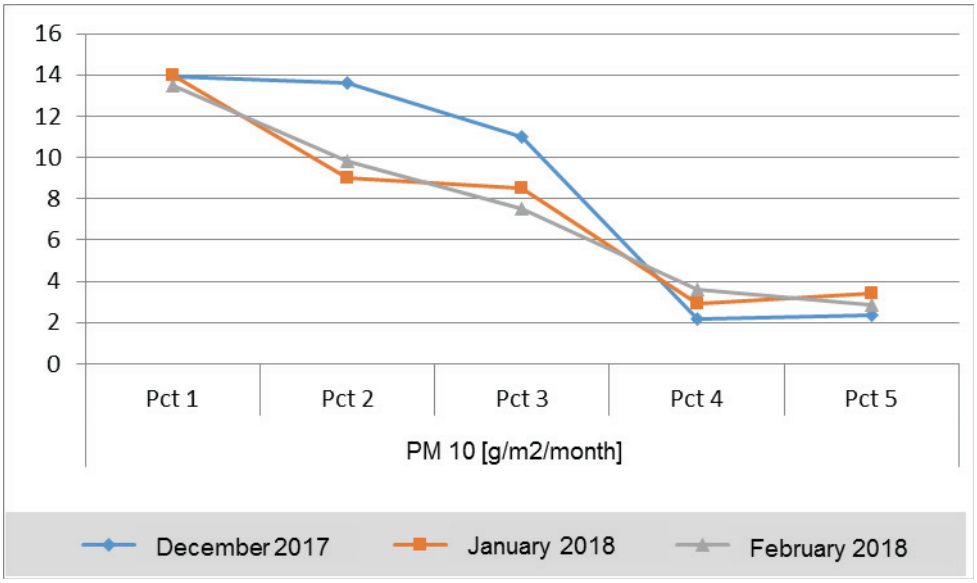

Figure 7. Variation of material powders in December 2017, January-February 2018.

The air quality. Emissions from work carried out in the installation must not cause air quality alteration by falling below the limit values set at Activity-Specific Indicators, in accordance with Law 104/2010 on air quality. The use of dense fluid deposition technology leads to a significant decrease in the surface of the slag and ash deposits of the dips. Measures will be taken to reduce spillages from the site by aspiration and / or spraying of dust deposition areas.

The STAS 12574-87 provisions shall be observed at the limit of the power plant location, as follows:

- sedimentable dust - max. 17 g/m^2/month;
- powder in suspension: 0.5 mg/m^3 at 30 minutes (short-term average) and 0.15 mg/m^3 at 24 h (long-term average).

The microbiological process for removing sulfur from coal requires simple equipment, low amount of reagents, but a long time of water treatment resulting from this process, water with a high ferrous sulphate content. The microbiological process is feasible in reactors when the particle size is less than 0.5 mm. At particle sizes greater than 0.5 mm, coal can be treated in piles.

Conventional technologies for the removal of pyrite from coal have as a general principle the elimination of pyritic inclusions by physical separation, especially of large inclusions that require a long biodegradation period. Pyrite sulphide (FeS_2) initially can be in the form of 2 crystal crystalline structures: pyrite and marcasite. They are different from the crystallographic point of view, chemically and thermodynamically, but have the same chemical formula. From the chemical point of view, pyrite is closer to the ideal formula FeS_2 than marcasite, in which sulfur deficiency was observed. At the same time, both pyrite and marcasite are attacked and decomposed by concentrated H_2O_2, but deposition of colloidal sulfur is observed only at pyrite.

In coal, pyritic sulfur is present in different amounts and forms, from very fine microscopic particles dispersed in the coal mass to large granules of several millimeters. The pyrite is deposited on the cleavage planes, fills the vertical cracks, spreads very finely, filling the walls and the inside of the fused cells. The larger particles, the coarser pyrite tend to sit or lie close to the roof of the coating. According to their appearance, the coal pyrites were classified as crystalline, massive, nodular, lamellar and globular. Pyrites have a high degree of purity; those unrelated to the carbon or silicon material are rarely found in coal and only in relatively large pieces. Massive nodules and additive pyrite can be easily removed by washing, while finely disseminated pyrite may be removed by the process substantially. This is particularly addressed to biological treatment.

Following the quality protection of all monitored environmental factors, we have proceeded to the elaboration of strategies and programs for the refurbishment and modernization of the electrical and thermal energy installations and equipment, respectively for the optimization of the existing technological processes or for the adoption and implementation of the best available techniques without the cost of excessive costs, as is provided by the current legislation (Romanian and European Union) (ZARNEA, 1994, ROJANSCHI et al., 1997, MIHAI et al., 2008, DEAK et al., 2009; GHIORGHIȚĂ et al., 2014).

In order to prevent the limitation and elimination of the impact of fossil fuel (coal, fuel oil, methane gas) production of electricity and heat, it is necessary to supply and use fuels, fuels and lubricants with low content of pollutants (S, N, P, heavy metals) according to standards and technical requirements, namely that of the legal regulations in force (quality standards, norms, specific instructions) (CISMAȘIU 2003; POPEA et al., 2004; CISMAȘIU 2010a, b; JACOB et al., 2018).

The optimization of the existing production processes consists of:

- upgrading and re-technologization the neutralization dilution and dispersion facilities and equipment in the environmental factors;
- adoption of best available techniques without involvement of excessive costs in the drafting and development programs;
- monitoring quality parameters of all environmental factors both on site and in its immediate vicinity;
- compliance measures and the conditions imposed by the competent organisms during checks conducted at the site, and the provisions of plans to prevent and limit pollution accidents, or of the action in the event of disasters or natural phenomena special (earthquakes, floods, abundant rainfall, strong winds, drought, etc.).

As possibilities to reduce the phenomenon of drifting, it is expected to stabilize dry crust that forms on the surface of the active deposit (bituminization, polymerization, silicification), during the periods when the conditions for the manifestation of this phenomenon are fulfilled, in order to prevent its occurrence. Placing trees around the deposition site is considered sufficient to reduce the amount of slag and ash scattered by the wind to a level that presents minimal health risks to the population. Also after the storage capacity is exhausted, the deposit area will be returned to the productive circuit, is the technical mining and the biological recultivation for the establishment of the tree plantations (***. 2013; TOMUȘ et al., 2015; CIOBOIU et al., 2017; CISMAȘIU et al., 2017; GAVRILESCU et al., 2017).

CONCLUSIONS

The emissions resulted from the activity in the system should not result in alteration of air quality by falling below the limit values set for activity-specific indicators, according to Law 104/2010 on air quality. Measures will be taken to reduce spillages from the site by aspiration and / or spraying of the soil deposition areas.

The provisions of STAS 12574 - 87 shall be respected at the site of the power plant: sedimentable dusts - max. 17 g/m^2/month, suspension powders: 0.5 mg/m^3 at 30 minutes (short-term average) and 0.15 mg/m^3 at 24 h (long-term average).

The operation of large combustion plants is permitted in compliance with the special provisions and the conditions stipulated in Law no. 278/2013 on industrial emissions, which transposes Directive 2010/75 / EC.

The operator shall, in normal operating conditions, emission limit values for large combustion plant IMA 1-existing - type I set given by Law no. 278 / 24.10.2013 on industrial emissions. No emissions to the air shall exceed the set emission limit value. It is mandatory that there is no other significant air emissions to the environment except for those legally accepted.

The CO_2 emissions from the plant's activity for the period 2013-2020 are covered by GHG Authorization (greenhouse gases) issued by the Ministry of the Environment.

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A REVIEW OF GENERAL ASPECTS OF MICROORGANISMS INVOLVED IN THE CORROSION PROCESS

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Abstract. The microbial corrosion process is the deterioration of metals under the influence of microbial metabolism. Due to the negative effects that resulted in massive economic losses and environmental pollution, studies on this complex phenomenon have developed during the last 80 years. All this resulted in an understanding of corrosion microbial organisms involved in this process and, not least, the key role of bacterial biofilm in biocorrosion. Thus, in this analysis we aim to highlight some aspects related to the involvement of microorganisms in the corrosion process.

Keywords: bacterial strains, corrosion process, biofilms.

Rezumat. O revizuire a aspectelor generale ale microorganismelor implicate în procesul de coroziune. Procesul de coroziune microbiană reprezintă deteriorarea metalelor sub influenţa metabolismului microbian. Datorită efectelor negative soldate cu masive pierderi economice, dar şi cu poluarea mediului, au condus la intensifcarea studiilor, pe durata a 80 de ani, asupra acestui fenomen complex. Toate aceste aspecte au avut drept rezultat înţelegerea fenomenului de coroziune microbiană, implicarea microorganismelor în acest proces şi nu în ultimul rând rolul cheie deţinut de biofilmul bacterian în biocoroziune. Astfel, prin prezenta analiză ne propunem să punctăm unele aspecte legate de implicarea microorganismelor în procesul de coroziune.

Cuvinte cheie: tulpini bacteriene, proces de coroziune, biofilm.

INTRODUCTION

Corrosion is a natural process, which converts fine metal to its oxide or hydroxide or another compound in a more stable form (MANSOUR & ELSHAFEIA, 2016). Different types of corrosion were reported such as galvanic, pitting, uniform, erosion, lamellar, crevice and microbial corrosions (RASHIDI et al., 2007; MANSOUR & ELSHAFEIA, 2016).

Microbial corrosion or biocorrosion refers to the accelerated deterioration of metals owing to the presence of biofilms on their surfaces (BEECH & SUNNER, 2004), biocorrosion is a importants category of the corrosion process, leading to important economic losses in many industries (WARSCHEID & BRAAMS, 2000; DALL'AGNOL & MOURA, 2014; USHER et al., 2014), including in the oil fild, offshore, pipelines, armaments, etc. (GU et al., 2000; MARTIN-GIL et al., 2004), potentially leading to environmental pollution (MANSOUR & ELSHAFEIA, 2016). KOCH et al., 2002 has estimated that the microbial corrosion process is responsible for 20% of the total damage to the corrosion process.

The involvement of microorganisms in the process of corrosion was observed since the 19th century, when in 1830 De la Rive suggested the existence of microcells on the surface of zinc (MANSOUR & ELSHAFEIA, 2016). Approximately 80 years ago von Wolzogen Kurh and Vander Vlugt identified sulphur reducing bacteria as responsible for metal corrosion under anaerobic conditions. The corrosion process is caused or promoted by microorganisms, usually chemoautotrophs, indicated as belonging to the genera: *Desulfovibrio, Pelobacter, Pseudomonas, Firmicutes, Archaeoglobales, Shewanella,* methanogenic archaea, etc.

MICROORGANISMS INVOLVED IN THE CORROSION PROCESS

Microorganisms which have been associated with corrosion involve many genera and species. They may be divided into three groups: bacteria, fungi, and algae (WARREN, 1987). EMDE et al., 1992 signalled a heterogeneous population of potentially corrosive microorganisms present in untreated water supply, treated water and corrosion tubercles, so we can talk about a corrosion consortium. Several groups of microorganisms are recognized for their role in corrosion, including the: sulphite-reducing bacteria (*Clostridium* sp.) (ENNING et al., 2012; ENNING et al., 2016), iron-reducing bacteria (*Bacillus* sp., *Clostridium* sp., *Escherichia coli, Enterobacter aerogenes, Klebsiella oxytoca, K. pneumoniae, Pseudomonas aeruginosa, P. cepecia, P. fluorescens*) (HERRERA & VIDELA, 2009), exopolymer producing bacteria (FORD et al., 1991; ZUO, 2007), sulphur-oxidizing bacteria (*Thiobacillus thiooxidans, Th. thioparus*) (LI et al., 2008), iron-oxidizing bacteria, fungi (*Penicillium, Rhizopus, Aspergillus*) (VIDELA, 1996).

Scientific works on the direct corrosion process by algae are quite scarce. They would to have the potential for inducing corrosion by their role in the ecosystem in the production of oxygen, nutrients, organic acids (PRESCOTT, 1969; WARREN, 1987).

Moreover, VERMA and KHAN, 2016 reported that green algae *Spirogyra* are used as an inexpensive and efficient mild steel corrosion inhibitor. However, some algae species are directly involved in the corrosion process as well, such as *Nostoc* sp., *Spirulina* sp. (MERT et al., 2011). From a biological corrosion viewpoint, significant fungi are

Cladosporium resinee, *Penicillum* sp., *Fusarium* sp., *Aspergillus* sp., *Hormoconis* sp., acid producing fungi (LITTLE et al., 2001; ROVETTA et al., 2013).

The microorganisms most commonly associated with the corrosion process are bacteria. Most of the time microorganisms implicated in biocorrosion are classified by the type of breathing respiration techniques (Table 1). Two types of single-celled bacteria exist, namely aerobic and anaerobic bacteria. The aerobic bacteria have the ability to use and detoxify oxygen, whereas anaerobic bacteria can survive without the presence of oxygen (MANSOUR & ELSHAFEIA, 2016).

Table 1. Bacteria involved in the corrosion process.

Respiration	**Aerobic respiration** **(have the ability to use oxygen)**	**Electon acceptor**	**Microorganisms**
		O_2	*Pseudomonas aeruginosa*, *Escherichia coli*, these bacterial strains can be all strictly or facultative aerobic organisms, corrode the carbon steel (JAYARAMAN et al., 1997; KIP & VAV VEEN, 2015)
	Anaerobic respiration **(have the ability to use S, CO_2, etc. like electron acceptor)**	NO_3^-	Denitrification: *Paracoccus denitrificans, Pseudomonas stutzeri*
		CO_2	Acetogenic bacteria: *Clostridium aceticum, Clostridium* sp. Methanogenicbacteria : *Methanobacterium* sp., *Methanosarcina* sp. (USHER et al., 2014)
		S	Sulphur respiration: facultative and obligate anaerobe bacteria *Thiobacillus thioparus*, acid producing (LI et al. 2008), *Desulfuromonas* sp.
		S^{2-}	Sulphate respiration: obligate anaerobe bacteria *Desulfovibrio desulfuricans* (VOICU et al., 2005), *Desulfovibrio vulgaris* (XU & GU, 2014)
		Fe^{3+}	Iron respiration: *Gallionella* sp., *Leptothrix* sp., *Clonothrix* sp., *Sphaerotilus* sp., etc. (VOICU et al., 2005)
		MnO_2	Manganese respirations: *Shewanella putrfaciens* (MARTIN-GIL et al., 2004)
		Fumarate	Fumarate respiration: *E. colli*

The groups of bacteria responsible for the corrosion process most frequently studied were: sulphate-reducing bacteria, sulphur-oxidizing bacteria, iron bacteria.

Sulphate-reducing bacteria, strictly anaerobic, are commonly considered to be the main originators of this microbiologically influenced corrosion (VOICU et al., 2005), including *Desulfuromonas* sp., *Desulfobacter* sp. and *Desulfococcus* sp. Sulphate-reducing bacteria gain their biochemical energy for growth by reducing sulphate to sulphide (HAMILTON, 1985).

Sulphur-oxidizing bacteria are primarily of the genus *Thiobacillus*, aerobic chemolithoautotrophs bacteria. These organisms notably form sulphuric acid during oxidation, and are capable of oxidizing both sulphur and ferrous iron, at a very low pH (WARREN, 1987).

Aerobic iron bacteria have also been associated with biocorrosion; this group of microorganisms is divided into two types: stalked (*Gallionella* sp.) and filamentous (*Leptothrix* sp., *Clonothrix* sp., *Sphaerotilus* sp.). These bacteria primarily oxidize or reduce iron species during respiration (WARREN, 1987).

BIOFILMS

Studies conducted over time on microbial corrosion have not fully disclosed mechanisms (KIP & VAV VEN, 2015) the underlying mechanism of microbial corrosion remains an open question. The mechanisms by which microorganisms induce corrosion essentially involve the basic electrochemical mechanisms of corrosion, based on the removal of electrons via oxygen or hydrogen ions (WARREN, 1987). We are focusing on biofilm and its role in microbial corrosion.

A biofilm is a complex structure (BEECH & SUNNER, 2004), and an essential part of the degradation process. Many elements of these processes are still unknown. In general, the process involves such mechanisms as: acidic degradation, electron movement, metal depolarization, polymerization and attachment of biofilms.

The biofilm involved in the corrosion process consists of a microbial consortium, a unique composition, influenced by environmental conditions: salinity, temperature, pH, biocide treatment, oil composition, nitrate, nutrient availability, surface of the substratum (VINGERON et al., 2016; 2018).

According to DONLAN 2002, biofilm is an assembly of surface-associated microbial cells that is enclosed in an extracellular polymeric, substance matrix, biofilm frequently enhances corrosion, facilitates exchange between metal and environment, and leads to deterioration of the metal (XU et al., 2014).

The origin of biofilm formation is attributed to indigenous microorganisms in the environment where this process takes place (LENHART et al., 2014). The microbial consortium may express different metabolic characteristics depending on the physico-chemical properties of the biofilm (VIGNERON et al., 2018). Within biofilm, interactions, cooperation, competition, intra and interspecific are established (ANDERSSON et al. 2008; VIGNERON et al., 2016). Thus, it can be concluded that the corrosion rate can be correlated with increasing biofilm.

The growth of biofilm is considered to be a result of complex processes involving the transport of organic and inorganic molecules and microbial cells to the surface, the adsorption of molecules to the surface and the initial attachment of microbial cells followed by their irreversible adhesion facilitated by the production of extracellular polymeric substances (BEECH et al., 2005).

Extracellular polymeric substances are molecules of different sizes, conformations and physical/chemical properties; they can be: polysaccharides, proteins, lipids, and even nucleic acids are actively secreted components (DECHO & GUTIERREZ, 2017).

CONCLUSIONS

Reducing microbial corrosion process involves understanding complex phenomena that take place within it. Also, it is necessary to study the microbiotic zone so as to evaluate the corrosion potential of the microorganisms present in the environment. An essential role in the corrosion process is played by biofilm. Therefore, taking measures to limit the formation of biofilm will lead to the reduction of microbial corrosion and less economic damages.

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STUDIES ON THE DISTRIBUTION OF ZOOPLANKTONIC COMMUNITIES IN THE PRUT - DANUBE ECOSYSTEMS

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Abstract. The goal of this paper was to study the distribution of zooplankton communities in the Lower Prut, a tributary of the Danube river. In this paper, data from the period of 2014-2015 were analysed, for both Prut and Danube rivers ecosystems. The complex expeditions were carried out on the Lower Prut ecosystems (Goteşti - Giurgiuleşti) and the Lower Danube area (Galaţi - Vilcovo). The value of the similarity coefficient of the zooplankton communities in Prut and Danube rivers was 0.40 (2014) and 0.33 (2015), the taxonomic difference being related to the different hydrological conditions. Although more than 85% of the identified taxa were recorded relatively rarely, considering their role in ecosystems, the Prut river deserves an important attention for the maintenance of zooplankton communities.

Keywords: zooplanktonic communities, Lower Prut, Danube river, biodiversity.

Rezumat. Studii privind distribuția comunităților zooplanctonice în ecosistemele Prut – Dunăre. Scopul acestei lucrări a fost studierea distribuției comunităților zooplanctonice din Prutul de Jos al Dunării, ca afluent al fluviului Dunărea. În această lucrare s-au analizat datele din perioada anilor 2014-2015, in același timp din ecosistemele Prut și Dunăre. Expedițiile complexe s-au desfășurat pe sectorul Prutului Inferior (Goteşti - Giurgiuleşti) și în zona Dunării de Jos (Galați - Vilcovo). Valoarea coeficientului de similaritate al comunităților zooplanctonice din râurile Prut și Dunăre a fost 0,40 (2014) și 0,33 (2015), diferența taxonomică fiind legată de diferitele condiții hidrologice. Cu toate că 85% din taxonii identificați s-au întâlnit relativ rar, având în vedere rolul acestora in ecosistem, râul Prut necesită o atenție sporită pentru menținerea comunităților zooplanctonice.

Cuvinte cheie: comunitățile zooplanctonice, Prutul Inferior, fluviul Dunărea, biodiversitate.

INTRODUCTION

The zooplankton, with its structure and functions, is an important component of aquatic biodiversity. This group of aquatic organisms has a non-uniform distribution mainly due to the phenomena of active aggregation and migration of planktonic species and also under the influence of biotic and abiotic factors. In aquatic ecosystems, the horizontal distribution of zooplankton is influenced by many factors such as the morphological and hydrological characteristics of the ecosystem or the structure of the tributary network.

In recent years, a considerable attention has been paid to the Danube basin, which has a regional importance, for the purpose of the protection and sustainable use of water resources (ANON, 2009). Prut is the second river according to its length and importance on the territory of the Republic of Moldova. It springs from the Oriental Carpathians, from the North-Eastern slope of Mount Goverla. Prut is the last major tributary of the Danube river, flowing to the southwest of the village Giurgiulești.

The goal of this paper was to study the distribution of zooplankton communities in the Lower Prut as the tributary of the Danube river. The paper was elaborated on the basis of data obtained under MIS ETC 1676 "Cross-border Interdisciplinary Cooperation for the Prevention of Natural Disasters and the Mitigation of Environmental Pollution in the Lower Danube Euroregion".

MATERIALS AND METHODS

In the current paper the data on the structural and quantitative parameters of the zooplancton communities are presented during the vegetation period of 2014 – 2015 in the Lower Danube Basin within the boundaries of Moldova, Ukraine and Romania. Sample collection was carried out in accordance to unified methods for hydrobiological sample collection and processing (DEREVENSKAIA, 2015; JURMINSKAIA et al., 2015; LEBEDENCO & JURMINSKAIA, 2015). A total number of 50 zooplankton samples were collected and analysed. The complex expeditions were carried out on the Lower Prut ecosystems (Goteşti - Giurgiuleşti) and the Lower Danube area (Galaţi - Vilcovo). The samples were collected using the Apstein zooplankton net (№ 55) by filtering a quantity of 100 l of water. The collected zooplankton material was fixed immediately in the field with formalin solution (40 %). The quantitative counting of zooplankton was carried out using Bogorov counting chamber and the binocular stereo zoom Discovery V8 ZEISS, using three replications. The density (N – ind/m^3) of the organisms was reported to cubic meter. Identification of the main zooplankton groups (Rotatoria, Copepoda, Cladocera) was carried out up to the highest possible level, with the use of the microscope Axio Imager A.2 (Zeiss). The determination of the taxonomic structure of the zooplankton groups was carried out with the use of identification guides (KUTIKOVA, 1970; NEGREA, 1983; NABEREJNII, 1984; ALEXEEV & TALOLICHIN, 2010). In order to determine the contribution of the Lower Prut biodiversity to the Danube river the taxonomic complex was analyzed in the Danube, and the Jaccard similarity coefficient was calculated to compare the diversity between the two communities of organisms of the Prut and Danube river, this being expressed in the following equation : $Qs = 2\,c / a + b$.

REZULTS AND DISCUSSION

In the taxonomic complex of the Lower Prut, 24 (2014) and 18 (2015) taxa were registered (Table 1), composed mainly of rotifers, which constituted 38% and 67%, respectively. In the Danube sector, the major contribution of the taxonomic complex of zooplankton belonged to copepods, and constituted 21 (52%) in 2014 and 12 taxa (67%) in 2015.

Table 1. The taxonomic complex in the Lower Prut and the Lower Danube ecosystems during 2014 – 2015 period.

Taxon	2014		2015	
	Lower Prut	Lower Danube	Lower Prut	Lower Danube
Rotatoria				
Asplanchna priodonta Gosse, 1850	+			
Asplanchna herricki Guerne, 1888			+	
Asplanchna sp. Gosse, 1850	+			
Brachionus bidentata Anderson, 1889			+	
Brachionus budapestinensis Daday, 1885		+		
Brachionus calyciflorus Pallas, 1776	+		+	
Brachionus quadridentatus, Hermann, 1783	+		+	+
Brachionus sp. Pallas, 1766			+	
Cephalodella gibba (Ehrenberg, 1832)			+	
Cephalodella eva (Gosse, 1887)		+		
Cephalodella sp. Bory de St. Vincent			+	
Euchlanis dilatata Ehrenberg, 1832	+	+		
Epiphanes senta (Műller, 1773)			+	
Epiphanes sp. Ehrenberg, 1832			+	
Filinia longiseta (Ehrenberg, 1834)	+		+	
Keratella quadrata (Műller, 1786)		+	+	
Lecane (Monostyla) sp. Ehrenberg, 1832	+			
Notommata sp. Ehrenberg, 1830		+		
Platyias patulus (Műller, 1786)		+		
Polyarthra luminosa Kutikova, 1962	+			
Polyarthra vulgaris Carlin,1943	+			
Rotatoria gen. sp.		+	+	+
Copepoda				
Acanthocyclops vernalis (Fischer, 1853)	+	+		
Copepodit Copepoda		+		
Copepodit Cyclopoida	+	+	+	+
Copepodit Harpacticoida			+	
Cyclopoida gen. sp.	+	+		+
Eurytemora lacustris (Poppe, 1887)				+
Eurytemora velox (Lilljerborg, 1853)		+		
Harpacticoida gen. sp.	+	+	+	
Mesocyclops asiaticus Kierfer, 1835		+		
Mesocyclops leuckarti (Claus, 1857)	+		+	
Mesocyclops sp. Sars, 1913				+
Microcyclops gracilis (Lilljeborg)		+		
Nauplii Copepoda	+	+	+	+
Nauplii Cyclopoida	+	+	+	+
Thermocyclops dybowskii (Lande, 1890)				+
Thermocyclops crassus (Fischer, 1953)	+	+		+
Thermocyclops oithonoides (Sars, 1863)	+			
Cladocera				
Alona rectangula Sars, 1862	+			
Bosmina longirostris (O. F. Műller, 1785)		+		+
Chydorus gibbus Sars,1891	+			
Chydorus sphaericus (O. F. Műller, 1785)	+			
Chydorus sp. Leach,1816				+
Daphnia longispina O. F. Műller, 1785	+	+		
Daphnia sp. O. F. Műller, 1785		+		
Scapholeberis mucronata (O. F. Műller, 1776)	+			
Sida crystallina (O. F. Műller, 1776)	+			
Total	24	21	18	12

An important feature of the Prut river basin is its mountainous hydrologic origin, which contributes to a relatively large variety of taxonomic composition of the ecosystem, including zooplankton species, which on its side adds to the Danube biodiversity, especially in the confluence area (ZUBCOV et al., 2014; LEBEDENCO et al., 2017). Although more than 85% of the identified taxa were recorded relatively rarely, this ecosystem deserve an important attention for the maintenance of its biodiversity.

The common complex of taxa recorded in the analysed ecosystems was smaller in 2015, constituting only 5 (Table 2) taxonomic units: *B. quadridentatus*, *Rotatoria* gen. sp., - rotifers, Copepodit *Cyclopoida*, *Nauplii*, *Copepoda*, *Nauplii Cyclopoida* - copepodele. For the period of 2014 the complex taxonomic unit was represented by rotifers – *E. dilatata*, copepode – *A. vernalis*, *Copepodit Cyclopoida*, *Cyclopoida* gen. sp., *Harpacticoida* gen. sp., *T. crassus*, Nauplii *Copepoda*, Nauplii *Cyclopoida*, and cladocera – *D. longispina*, constituting 9 units. The value (tab. 2) of the similarity coefficient of the zooplankton communities in Prut and Danube rivers was 0.40 and 0.33, the taxonomic difference being related to the different hydrological conditions.

Table 2. The dynamics of the similarity coefficient (Jaccard) of the taxonomic diversity complex of zooplankton in the lowland Prut and Danube ecosystems.

Ecosystem sector	**2014**	**2015**
Inferior Prut	24	18
Danube (inferior sector)	21	12
The number of common taxa	9	5
Similarity coefficient, Q_s	**0,40**	**0,33**

In the Lower Danube, downstream of the delta, the abundance of all zooplankton groups was low due to the backwater effect, which is confirmed in earlier studies (LEBEDENCO et al., 2016). The number of rotifers was very small, the development of the copepode group was the highest, especially of adult individuals, when the development stage of copepodites predominates in the Lower Prut. This was also conditioned by the climatic conditions in Lower Danube. Cladocers do not make a significant contribution to the zooplankton taxonomic complex formation in investigated ecosystems, with low values (14-20%) or even absence (Lower Prut 2015). At the same time, *B. longirostris* in the Danube was the dominant species with a range of 60 (St. Reni) to 360 ind/m^3 (St. Chilia). The zooplankton diversity of the Lower Prut and Lower Danube is described in figure 1.

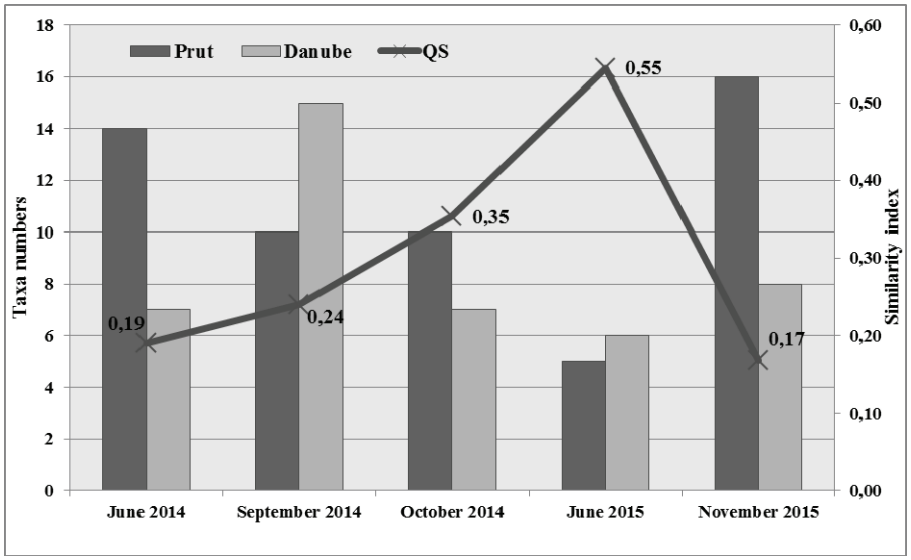

Figure 1. The number of taxonomic units in the ecosystems of Lower Prut and Lower Danube with changes over time and similarity coefficient.

The highest similarity index of the zooplankton communities between the Danube and Prut rivers with a value of 0.55 was recorded in June 2015, when the number of taxa in the given ecosystems has the lowest values. It has been noticed that the taxonomic diversity of the investigated ecosystems is largely high, while the similarity coefficient has low values (0.17 - 0.24). This demonstrates the low generality of these ecosystems and the existence of different habitats for the development of the zooplankton communities, caused by various factors such as the Giurgiulești port, the frequent floods in this area or other factors. The Shanon-Wiener diversity index in the studied ecosystems differs insignificantly and is 2.40 - in the Lower Prut and 2.43 - in the Lower Danube. The trophic level based on the diversity of zooplankton of the studied ecosystems is mesotrophic. The parameters of the zooplankton density in this area varied considerably, so that in the Lower Prut had values of 1.32 thousand ind/ m3 (June 2015) - 55.11 thousand in m3 / m3 (June 2014) and 1.25 in the Lower Danube 1.25 thousand ind / m^3 (November 2015) - 17,30 thousand ind /m^3 (September 2014). The fluctuations of the zooplankton biomass depend on taxonomic structure, when there is a direct dependence on the dominant complex and main groups included in its composition, therefore the biomass of zooplankton formed during the investigated period was considerable higher in the Danube sector than in the Prut river. For a saprobiological assessment of the monitored ecosystem, the species richness was analysed as a function of saprobity. In these communities the most indicators are representatives of β- mezasoprobe and o-β- mezasoprobe areas.

CONCLUSIONS

At the level of systematic groups, the zooplankton structure of the Lower Prut in 2014 includes: rotifers - 33 %, copepods – 52 %, cladocerans – 14 % and in 2015 year rotifers 67 %, copepods – 33 % and cladocerans - was absence. For the Lower Danube, the percent ratio of the taxa number is: rotifers - 38 %, copepods - 37 %, cladocerans – 25 % in 2014, and in 2015 year is: rotifers – 17 %, copepods – 67 % and cladocerans 16 %.

The value of the similarity coefficient of the zooplankton communities in Prut and Danube rivers was 0.40 and 0.33, the taxonomic difference being related to the different hydrological conditions. The common complex of taxa recorded in the analysed ecosystems was smaller in 2015, constituting only 5 units, while 9 were registered in 2014.

Despite the fact that the taxonomic diversity of the investigated ecosystems is very high, the similarity coefficient has low values (0.17 - 0.24). Analysing all the results of the investigated parameters, it can be concluded that the distribution of zooplankton communities of Lower Prut does not have a direct influence on the formation of biodiversity of Lower Danube. These two rivers present different habitats for zooplankton development. This demonstrates the low generality of these ecosystems and the existence of different habitats for the development of the zooplanktonic communities, caused by various factors such as the Giurgiulești port, the frequent floods in this area or other factors.

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OBSERVATIONS REGARDING THE ENTRY IN THE HIEMAL DIAPAUSE OF *Chrysolina fastuosa* (Scopoli, 1763) (CHOLEOPTERA, CHRYSOMELIDAE) IN THE NORTH-WESTERN PART OF ROMANIA (TINCA AREA, BIHOR COUNTY)

ILIE Aurelian Leonardo, MARINESCU Mariana, ILIE Lorena Cosma

Abstract. The paper presents the results of the observations performed by the authors on the entry in the hiemal diapause of *Chrysolina fastuosa* Scop., in the Tinca area, Bihor county, during September 2017 – January 2018. The overwintering can be achieved usually as an adult, sometimes as a larva.

Keywords: *Chrysolina fastuosa*, overwintering, Tinca area.

Rezumat. Observații privind intrarea în diapauza hiemală (iernare) la *Chrysolina fastuosa* (Scopoli 1763) (Coleoptera, Chrysomelidae) în partea nord-vestică a României (zona Tinca, județul Bihor). Lucrarea prezintă rezultatele observațiilor realizate de autori asupra intrării în diapauza hiemală (iernare) la *Chrysolina fastuosa* Scop., în zona Tinca, județul Bihor, în perioada septembrie 2017 – ianuarie 2018. Iernarea poate fi realizată,în general, în stadiul de adult, uneori în stadiul de larvă.

Cuvinte cheie: *Chrysolina fastuosa*, iernare, zona Tinca.

INTRODUCTION

Tinca area is located in the south-western part of Bihor County, in the north-western part of Romania. The climate is temperate-continental, the average altitude is 110 m, the vegetation belongs to the oak stage (BERINDEI & POP, 1972).

Data about the biology and the ecology of the species *Chrysolina fastuosa* at the Bihor county level and in Europe were published by different authors (FUSS et al., 2005; BARDIN & TIMRALEEV, 2007; BIENKOWSKI, 2010; BOZSIK, 2014; ILIE, 2017) (Fig.1).

Figure 1. *Chrysolina fastuosa* Scop. - larvae (after ILIE, 2017).

The host plants belong to Urticaceae and Lamiaceae families. The overwintering could be achieved usually in the adult stage, sometimes even in the larval stage, having different ages (ILIE, 2017). The present paper follows the influence of temperature and amount of incoming food on the overwintering of this species.

MATERIAL AND METHODS

The researches on the entry in hibernation of *Chrysolina fastuosa* were achieved in the Tinca area, on a lot situated in the proximity of the personal farm of the Ilie family, during September 2017 – January 2018.

This lot has a surface of $3m^2$ and presents a vegetation formed by *Lamium maculatum* Linnaeus 1758, *Urtica dioica* Linnaeus 1758, along with sporadic specimens of *Stellaria media* Villers1796, *Veronica didyma* Linnaeus 1758, *Prunella vulgaris* Linnaeus 1758, *Cichorium intybus* Linnaeus 1758 and *Erigeron annuus* Linnaeus 1758. The observations were performed on a daily basis, measuring nocturnal and diurnal temperatures, the velocity of wind (with the help of an anemometer).

RESULTS AND DISCUSSIONS

Generally, the entry in the hiemal diapause takes place during autumn, depending on temperature, in October, because of the high temperatures registered during October – November in the last years (2014-2016), the entry in overwintering was achieved at a very late date. Example – one specimen of larva of the third age, November 27, 2016, t=7°C (the last appearance in nature from 2016 (ILIE, 2017).

The high temperatures registered in autumn have determined the beginning of new series of adult in copula:

- Five pairs in copula, September 24, 2017, t=14°C.
- One pair in copula, October 6, 2017, t=11°C.
- One pair in copula, October 11, 2017, t=14°C.

The daily dynamics of the presence of *Chrysolina fastuosa*, during October 2017 – January 2018, is presented in the following table (Table 1).

Table 1. The daily dynamics of the presence of *Chrysolina fastuosa* in the Tinca area, in the analysed period.

October, 2017									
Day	**Number of specimens**				**Temperatures**		**Wind**	**Time**	
	L1	**L2**	**L3**	**A**	**Night**	**Day**	**Beaufort Degrees**	**Sunny**	**Dull**
6	-	2	2	6	3	11	-	x	-
7	-	1	2	5	5	15	-	x	-
8	-	1	1	6	5	15	-	x	-
9	1	4	-	5	4	15	-	x	-
10	-	2	-	6	1	14	-	x	-
11	1	2	1	5	4	14	-	x	-
12	-	2	2	4	4	15	-	x	-
13	-	2	2	4	5	16	-	x	-
14	-	2	3	1	7	17	-	x	-
15	-	2	5	2	7	17	-	x	-
16	-	1	7	2	9	23	-	x	-
17	-	3	6	1	10	25	-	x	-
18	1	3	6	1	9	25	-	x	-
19	-	2	19	1	8	23	-	x	-
20	-	2	14	1	4	21	-	x	-
21	-	3	16	1	5	17	-	x	-
22	-	1	12	-	6	16	-	x	-
23	-	-	11	-	10	13	2	-	Rain
24	-	-	10	-	3	12	4	-	Rain
25	-	2	10	-	4	18	2	x	-
26	-	3	9	-	7	14	3	-	X
27	-	3	13	-	5	14	4	-	Rain
28	-	2	12	-	4	12	-	-	X
29	-	2	5	-	4	11	3	-	Rain
30	-	1	5	-	-1	12	2	x	X
31	-	1	3	-	-6	9	-	x	Hoar-frost
November, 2017									
1	-	2	4	-	-6	9	-	-	Hoar-frost
2	1	2	6	-	5	13	-	x	X
3	1	2	14	-	7	13	-	x	Rain
4	1	3	22	-	7	14	-	x	-
5	3	4	23	-	3	17	2	x	-
6	1	3	10	-	-1	16	-	x	-
7	5	8	13	-	-3	15	-	x	-
8	2	8	19	-	5	12	-	-	Rain
9	1	4	11	-	7	12	-	-	Rain
10	5	24	7	-	8	12	-	-	X
11	7	10	21	-	7	13	2	x	-
12	5	19	6	-	7	11	2	x	-

13	3	15	5	-	10	15	2	-	X
14	4	11	3	-	0	6	3	-	Rain
15	4	12	3	-	3	9	-	-	X
16	4	26	3	-	4	12	-	x	-
17	6	41	3	1	5	8	-	-	X
18	4	18	4	1	6	8	-	-	X
19	4	22	4	-	3	7	2	x	X
20	3	32	3	-	-1	6	-	-	Hoar-frost
21	1	13	1	-	-3	4	-	-	Rain and sleet
22	3	11	1	-	3	8	-	-	Rain
23	2	18	3	-	4	12	2	x	-
24	2	16	2	-	5	9	-	-	X
25	6	11	3	-	1	13	-	x	Hoar-frost
26	8	33	7	-	7	12	-	-	Rain
27	4	10	4	-	2	6	2	-	Rain
28	4	17	3	-	-2	6	-	x	Hoar-frost
29	2	5	-	-	-5	7	-	x	Hoar-frost, frozen ground
30	-	5	-	-	2	8	3	-	Rain
December, 2017									
1	1	12	-	-	1	4	-	-	X
2	4	5	1	-	-1	4	3	-	X
3	4	15	4	-	1	3	2	-	Rain sleet, snow
4	4	5	-	-	0	1	3	-	Snow
5	-	2	-	-	-1	3	-	-	X
6	1	5	1	-	1	5	-	-	X
7	5	7	-	-	0	5	2	x	X
8	2	5	-	-	3	8	3	-	X
9	1	9	-	-	3	8,5	-	x	X
10	-	4	-	-	-1	2	-	x	Snow
11	-	2	-	-	0	9	3	-	Rain
12	-	5	-	-	8	17	3	x	X
13	-	6	3	-	5	7	-	-	Rain
14	1	4	1	-	2	7	2	-	Rain
15	-	5	1	-	6	10	2	-	Rain, mana (one minute)
16	1	3	-	-	4	7	2	-	X
17	1	10	1	-	2	4	-	-	X
18	4	5	2	-	-2	7	-	x	X, frozen, ground
19	1	1	-	-	-4	2	-	x	X, frozen, ground
20	1	3	-	-	-5	3,5	-	-	X, frozen, ground
21	-	-	-	-	-5	0	-	-	X
22	-	-	-	-	-3	1	-	-	X
23	-	-	-	-	0	3	-	-	X
24	-	1	-	-	3	6	2	-	X
25	-	4	-	-	2	8	2	x	-
26	1	2	2	-	-2	8	-	X	Hoar-frost (in winter)
27	-	3	1	-	3	13	-	x	-
28	-	4	1	-	5	13	3	-	X
29	-	3	-	-	3	6	-	-	X
30	-	3	-	-	0	5	-	x	X
31	-	-	-	-	1	4	-	-	X
January, 2018									
1	-	-	-	-	3	8	-	x	-
2	-	1	-	-	4	7	2	-	X
3	-	1	-	-	4	6	3	x	-
4	-	-	-	-	-1	3	-	x	-
5	-	1	-	-	7	10	-	X	-
6	-	1	1	-	7	10	-	x	-
7	-	2	-	-	6	13	-	X	-
8	-	2	1	-	5	13	-	x	-
9	-	2	-	-	3	11	-	x	-
10	-	2	1	-	1	10	-	-	X
11	-	2	1	-	1	10	-	x	X
12	-	2	1	-	1	10	-	x	X hoar-frost
13	-	2	1	-	2	5	-	-	X rain

Legend: L_1 =larva of first age; L_2=larva of second age; L_3 = larva of third age; A= adult; xx = partial sunny, partial dull (in the same day).

After January 13, 2018, the larvae were not observed at all, although in the following days the temperatures were relatively high for this period, both at night and day. The reason is, probably, the diminution of food, because the leaves of the hostplants were generally devoured and partial withered. Analysing the data rendered in the aforementioned table, we find that the appearance of a new generation of larvae takes place at the beginning of October (the first week).

The high temperatures during October (up to 25°C) and November (up to17°C) have determined an increase of the larvae's number (up to 50, in November 17) and even the hostplant (*Lanium maculatum* L.) flowered (November 5 till December 21).

Also, it is surprising that the number of hoarfrosts during autumn was very little (6) permitting to the host plants to remain fresh, as food for larvae.

Other consequences of global heating: the absence of snow (only three sprinkles of snow) and the presence of manna (rain and sun at the same time!) on December 15, a phenomenon specific to summer and autumn. At low temperatures (-5°C) in daytime, the larvae disappeared.

Wind speed was relatively low (2.3, very rarely 4 Beaufort degrees), having no influence on the larvae or adults activity.

The existence of larvae of first and second age proves that temperatures during the analyzed period have not been high enough and the number of days with high temperatures was not enough to transform these larvae into larvae of third age or even in pupa.

The feeding of the larvae was achieved only in the evening and particularly in the night, as in daytime they were only found on the ventral part of leaves or very rarely on the plants stems, remaining immobile. The feeding of adults was achieved only in the day time; after the feeding these were found only on the dorsal part of the leaves. The refuge of the adults on the ventral part of the leaves or at the bottom of the plant was achieved in the case of strong and persistent light, as adults preferred the shade.

CONCLUSIONS

The hibernation of *Chrysolina fastuosa* in the Tinca area begins, depending on temperature, during October – December. The hibernation can be achieved as adult, sometimes as a larva.

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ASSESSING THE PUBLIC AWARENESS LEVEL ON THE PRESERVATION OF CORAL REEFS (THE CASE STUDY IN BIAK NUMFOR, PAPUA, INDONESIA)

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Abstract. The aim of this study was to assess the level of public awareness on the importance of coral reefs preservation in Biak Numfor, Province of Papua, Indonesia. The study employed the descriptive qualitative research method. For data collection, techniques such as questionnaires and interviews, as well as documents, were used. The result of this study showed that the level of public awareness on the coral reefs preservation is as follows: (1) in the district of Oridek with a population of 4,665 people, a percentage of 52% is aware of the necessity to regulate the management of marine resources corals; (2) in the district of Amaindo (population of 2,209 people) the level of awareness was high, with a total of 18% concerned with the need for regulation management of marine resources, and the Padaido county, with a population of 1,707 inhabitants that have high levels of awareness about the need to regulate the management of marine resource utilization at 15%, as well as in the districts Biak East with a population of 6,698 inhabitants that has a level of consciousness regarding the need for management of marine resources especially coral reefs by 15%. In terms of public knowledge about the things that destroy coral reefs, the Aimando region has the highest percentage, namely 50% of people already know all that can damage coral reefs. While people in the Aimando district are aware of things that can damage coral reefs by 21%, the percentage corresponding to the Padaido district is about 16%, in East Biak district-level people's knowledge to cause damage to coral reefs is at 13%. People in the region of Oridek have a high level of awareness. The Core map programme impacts most notably the increased well-being of coastal communities. In order to maintain the balance and preservation of coral reefs, a law regulating these issues should be passed. Core map has to continue existing, in order to preserve the existence of coral reef ecosystems to sustain life aquatic biota.

Keywords: public awareness, coral reefs, preservation, Papua, Indonesia.

Rezumat. Evaluarea nivelului de conștientizare a publicului privind conservarea recifelor de corali (Studiul de caz în Biak Numfor, Papua, Indonezia). Scopul acestui studiu a fost de a evalua gradul de conștientizare a opiniei publice cu privire la importanța conservării recifelor de corali din Biak Numfor, provincia Papua, Indonezia. Studiul a utilizat metoda descriptivă de cercetare calitativă. Tehnicile de colectare a datelor folosesc chestionare și interviuri, precum și documente. Rezultatul acestui studiu a arătat că nivelul de conștientizare a populației cu privire la conservarea recifelor de corali este următorul: (1) în raionul Oridek cu o populație de 4.665 de persoane, 52% sunt conștienți de necesitatea de a reglementa gestionarea resurselor marine coraliere; (2) în districtul Amaindo (populație de 2.209 de persoane) nivelul de conștientizare a fost ridicat, cu o preocupare totală de 18% privind necesitatea gestionării reglementării resurselor marine și a județelor Padaido cu o populație de 1.707 de locuitori care au un nivel ridicat de conștientizare, necesitatea de a reglementa gestionarea utilizării resurselor marine este de 15%, precum și în raioanele Biak-Est, cu o populație de 6.698 de locuitori, cu un anumit nivel de conștiință unde ar trebui să se stabilească gestionarea resurselor marine în special recifele de corali la 15%. În ceea ce privește cunoașterea de către public a lucrurilor care distrug recifele de corali, districtul Aimando are cel mai mare procentaj, și anume 50% dintre oameni cunosc deja toate lucrurile care pot deteriora recifele de corali. În timp ce la nivelul districtului Aimando oamenii au cunostință de lucrurile care pot deteriora recifele de corali în proporție de 21%, dar în districtul Padaido este de aproximativ 16%, în districtul East Biak cunoștințele oamenilor despre cauzele care provoacă daune recifelor de corali este în procent de 13%. Oamenii din regiunea Oridek au un nivel ridicat de conștientizare. Programul Core map afectează în special creșterea bunei stări a comunităților de coastă. Pentru a menține echilibrul și conservarea recifelor de corali trebuie să se adopte o lege care să reglementeze aceste probleme. Ar trebui continuată găsirea fisurilor în Core map pentru a menține existența ecosistemului recifelor de corali și pentru a susține viața acvatică.

Cuvinte cheie: conștientizarea publică, recifele de corali, conservarea, Papua, Indonezia.

INTRODUCTION

Environmental issues have been the foremost concern today's people have to cope with, and it is only growing more complex as they advance. It is believed that human beings are the ones to blame for their slightest footprints causing damages to the environment (KUMURUR, 2008). However, it is not that the people themselves are unaware of the issues they generate. The notion is confirmed by individuals, realizing that most of the environmental issues such as coastal ecosystem and forest damage primarily root from their daily activities.

The issues also take place in coastal area, where the area is an essential life-supporting vessel providing resources and commodities for the surrounding people, if utilized properly. In the coastal area, most of the resources are renewable, e.g. fishes, shrimps, mollusks, pearl oysters, crabs, seaweed, mangrove forest, and coral animals, whose existence depends on efforts of preservation by a human. Moreover, the coastal area also possesses potential prospects as a space of environmental services, such as sites and habitats for recreational activities and medium of transportation. This is in line with HARYANTO (2008) who argues that the potency of marine and coastal area embodies hidden economic value for everyone, particularly communities in coastal areas.

On the contrary, the promising perspective lacks support from efforts of preservation by the surrounding community, shown by their state of ignorance towards the environment, e.g. littering, the preference for burning trash as the most efficient way of cleaning, illegal logging without efforts of reforestation, and construction of housing and offices with no concern of soil infiltration (DILISTI, 2011). This is possibly due to the insufficient information on

preserving the environment or the people's lack of motivation for maintaining the existence of the environment.

One cannot simply impose the burden of coral reef management solely to the government. The community is expected to integrate with the government in efforts of maintenance to generate an optimal result to preserve coral reefs. To maintain it, the slightest contribution from both sides counts. The government is responsible for disseminating information and knowledge about coral reef to the community – on the kinds and benefits of coral reef, its function, its preservation efforts, and possible impacts if the damage to coral reef gets worse – to be further practiced by the community in actions of utilization and conservation. Given that, the community will develop a sense of belonging to the coral reef, resulting in optimal preservation efforts.

Literature Review. Coral reef plays a significant contribution to the surrounding community, either viewed from a social, economic, or cultural aspect. It is the foremost primary underwater ecosystem which supports almost everything to the community: supporting the community's livelihood, as a habitat for commercial commodities, a support for tourism industry, providing sand for the beach, and as a barrier for waves and coastal erosion (WESTMACOTT et al., 2011). Moreover, DAHURI (2003) asserts that coral reef is a productive spot acting as a spawning ground, nursery ground and feeding ground for the fishes. With that in mind, it is deduced that the breed of fishes surrounding the coral reef is highly productive. Also, Dawes states that coral reef also acts as a medium for other organisms, such as oysters, lobsters, and tortoises. It is mentioned by DAHURI (2003) that a coral reef ma provide high organic/primary productivity due to the reef's ability to hold nutrients within the system and to act as a pool, accommodating every input from outside. As a result, the nutrients generated by the coral reef as a metabolic excess are utilized by plants without having to spread them to the water beforehand. There are eight general benefits of coral reef, i.e. (a) a barrier for the beach against the sea breeze, tidal waves, ocean current, and storm; (b) a resource of germplasm and biodiversity essential for food, bio technology, and health industries; (c) a medium for the fishes to breed (decorative fishes and target fishes living within the coral reef); (d) a shelter for underwater organisms; (e) a source of organic materials, enabling fishes to disguise to hunt for food; (f) as construction material for roads and buildings, raw material for industries and jewelry, such as coral rock; (g) as a potential spot for capture fisheries and coral tourism; and (h) a barrier for beach against wave erosion. Furthermore, loss of food security and values of biodiversity are the possible impacts happening in consequence of damage to the coral reef.

Contributing Factors of Damage to Coral Reefs. As an ecosystem, the coral reef is highly susceptible to changes in surrounding environments including human activities, and it requires a long time to recover. BURKE et al. (2002) mention factors contributing to the damage of coral reef, i.e.: (1) poor management of construction within the coastal area; (2) marine activities, such as ship from port and direct damage from anchoring; (3) illegal logging and changes in land use, causing an increase of soil sedimentation; (4) over-fishing activities, disturbing the balance within food chain in the coral reef ecosystem; (5) the involvement of bombs and poisons in fishing activities; and (6) global climate change.

Moreover, IUCN points out five objectives of underwater conservation sites, i.e. (1) protecting and maintaining underwater and estuary system, ergo, resulting in long-term sustainable resource of living and preservation of genetic biodiversity; (2) to prevent the decrease and as a conservation habitat of rare species; (3) to protect the underwater ecosystem from human activities which may damage it; (4) to provide sustainable welfare for the community; (5) as an appropriate management of underwater environment, with a broad spectrum for human activities primarily aimed at marine and estuary maintenance. It is essential for the surrounding community to develop mutual understanding, cooperation, and sense of belonging, as a preventive way to minimize the impact of damages to the coral reef.

On top of that, WESTMACOT et al. (2011) asserts that to prevent the widespread damage to coral reef, the government has to disseminate these policies to the coastal and fishermen community, i.e.: (1) setting a no-fishing zone and limitation of allowed fishing tools to use; (2) educating the fishermen to take specific protection for algae-eating and coral-eating fishes into consideration; (3) regulating the fishermen to not perform destructive fishing activities; (4) monitoring the composition and size of catches; (5) developing alternative livelihood sources for the fishermen community (if needed); (6) setting limitations for foreign fishermen through the permission granting system; and (7) setting regulations limiting coral reef biota harvesting for aquarium and souvenirs.

Dissemination to raise the community's awareness of coral reef conservation is vital, for them to experience sustainable benefits as a result of the preservation efforts. Henceforth, contributions from the community need to be focused on the identification, planning, and implementation of conservation in the coastal area to gain numerous possible benefits (SUPRIHARYONO, 2007). Without contributions from the community, it is almost impossible for the government to execute the "Codes of Conduct for Responsible Fisheries".

This is in accordance with the obligation of the government to conduct the MCS (Monitoring, Control, and Surveillance) system, aimed to maintain rationality of fish and environment resources management and harmony within utilization and sustainability of fish resources (SUPRIHARYONO, 2007). Furthermore, the government has several alternatives for encouraging the community to get involved in the management and maintenance of marine resources and environment. Through the persuasive way, consultation and intimate approach are available to persuade the community to participate in the programme implementation. Despite the long duration needed for the initial process to work effectively, persuasion is able to calm down tensions and likelihood of conflicts. Furthermore, MITCHELL et al. (2007) point out that consultation with the community is preferred to (1) address any upcoming issues more efficiently; (2) obtain information and understanding which is beyond science; (3) formulate socially-accepted alternatives; and (4) develop a sense of belonging to optimize the implementation. The awareness raising programme is mainly aimed at the

fishermen community; however, the government needs to consider executing the program to other stakeholders too, since the fishermen only take the role of actors, not policy makers.

The key aspects of sustainable development comprise local empowerment, self-support, and social justice. One effort of implementation is to progress from traditional environmental management of fish resources dominated by professionals from government and private sectors, to the approach which involves different layers of the community (MITCHELL et al., 2007). In addition, WESTMACOTT et al. (2011) argue that the efforts of developing sustainable fish resources are somehow tricky to execute, considering the numbers of people participating, the local community will have to strive to adapt to the new regulations and to cope with limited sources and options of livelihood.

As a consequence, the development of cooperation within the community and availability of alternative sources of income is critical to support sustainable livelihood. Moreover, the government is urged to conduct an empowerment in economic and social aspects of the local community within the coastal area with the intention to enhance the community's welfare, by utilization of coral reef potential. If executed properly, the programme enables the community to experience sustainable resources of commodities within the coral reef ecosystem and to opt for alternative ways of fishing, leaving the coral reef undamaged (NOTOATMODJO, 2005; WESTMACOTT et al., 2011; MALAU, 2013).

RESEARCH METHOD

The research combined quantitative and qualitative methods where quantitative data were obtained based on a survey of 180-240 households in the sample villages in Biak Numfor regency. Of 19 districts in the Biak Numfor Regency, this study involved four districts, namely Oridek, Padaido, Biak Timur, and Aimando; all are located in coastal area and island. The districts were preferred due to a higher rate of utilization of the potential natural resources among others. The quantitative data comprised a demographic characteristic of household members and economic standing of the households. Concurrently, the qualitative data were acquired by observation, comprehensive interview, focus group discussions, and forum, aimed to gain better understanding beyond the social and economic condition of the community and its relation to the utilization of marine resources, particularly coral reef.

RESULTS AND DISCUSSION

The Biak Numfor islands regency is located in Papua province, consisting of three main islands: Biak Island (1,833.86 km^2), Supiori Island (437.11 km^2), Numfor Island (331.26 km^2); and 41 smaller islands, e.g. Padaido Island and Mapia Island. The regency has a total area of 4,010 km^2, with a land area of 2,602.23 km^2. Biak Numfor is one of the regions with a tropical climate and tropical rain forest, with an average of 2,228 mm yearly. This is due to the fact that the regency geographically faces the Pacific Ocean. In addition, it is quite difficult to identify and differentiate between a wet and dry season in Biak Numfor. Based on data of 2004, the maximum temperature recorded is 30.5^0 Celsius on average, while the minimum temperature and average daily temperature have been registered at 23.9^0 Celsius and 27.2^0 Celsius respectively.

Number of Household Members Involving in Coremap Activities. The survey result elucidates the awareness level of society of Biak Numfor as follows: 47 percent of household members in Oridek district involved in Coremap activities, while in Biak Timur, Aimando, and Padaido, the rate of people involving Core map activities is 24 percent, 21 percent, and eight percent respectively (Fig. 1).

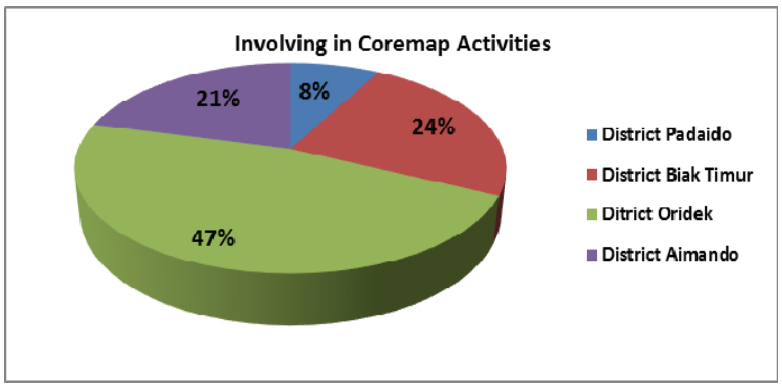

Figure 1.The percentage variation of the household members' involvement in Core map program.

Management of Marine Resources Utilization. As obtained from the survey, the awareness level of Biak Numfor society on the significance of regulations for management and utilization of coral reef is elucidated as follows: 52 percent from 4,665 people in the Oridek district are aware that management and use of coral reefs need to be regulated. Moreover, in Aimando, 18 percent of 2,209 people have the awareness that the community needs to be regulated in terms of management and utilization of coral reef. Furthermore, only 15 percent in both Padaido and Biak Timur district (from 1,707 and 6,698 people respectively) are aware of the need for rules to regulate the management and utilization of coral reef (Fig. 2).

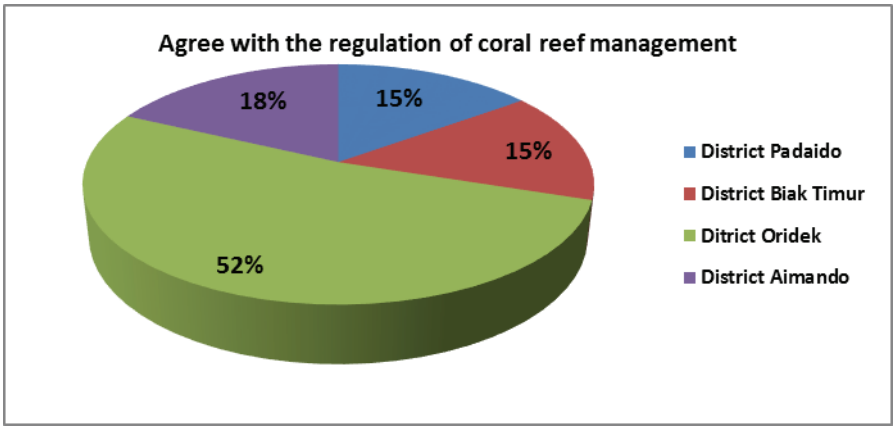

Figure 2. The percentage awareness of Biak Numfor society towards regulation of coral reef management.

Knowledge of Coral Reefs. People in every district in the Biak Numfor Regency have different knowledge of coral reefs. Among all members of society with adequate knowledge within the Biak Numfor Regency, 49 percent are from Oridek district, while 21 percent of the group are from Aimando. Moreover, the society in Biak Timur and Padaido needs further dissemination of coral reef information, since only 15 from each district have sufficient knowledge about the coral reef (Fig. 3).

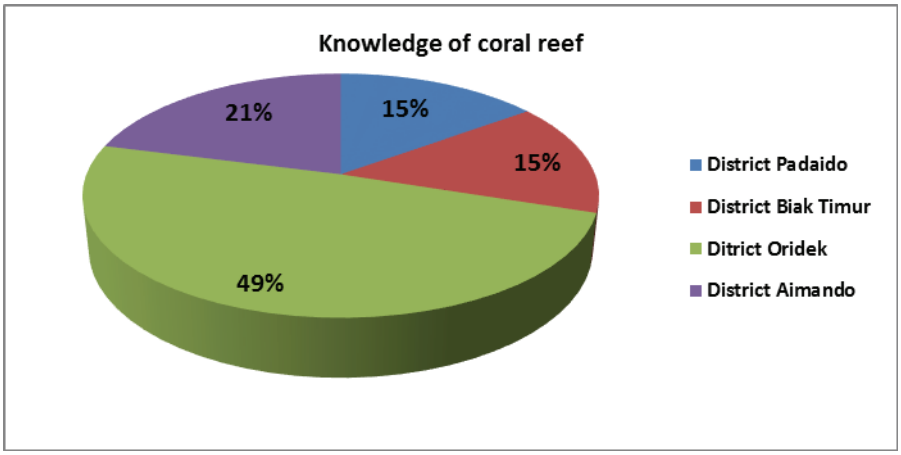

Figure 3.Tthe percentage variation of society within Biak Numfor who knows the coral reef.

Knowledge of Benefits of Coral Reef. The research discovered that a particular group of people in Biak Numfor have already known of the benefits of coral reef prior to the Core map program. The distribution of community members within Biak Numfor whose knowledge of coral reef benefits is displayed as follows: of all community members within Biak Numfor who understand the coral reef benefits, 49 percent are from Oridek district. Moreover, there is 21 percent of the society of Aimando district who understands the benefits of coral reef, while the rest 16 and 14 percent of the group are from the Padaido and Biak Timur district respectively (Fig. 4).

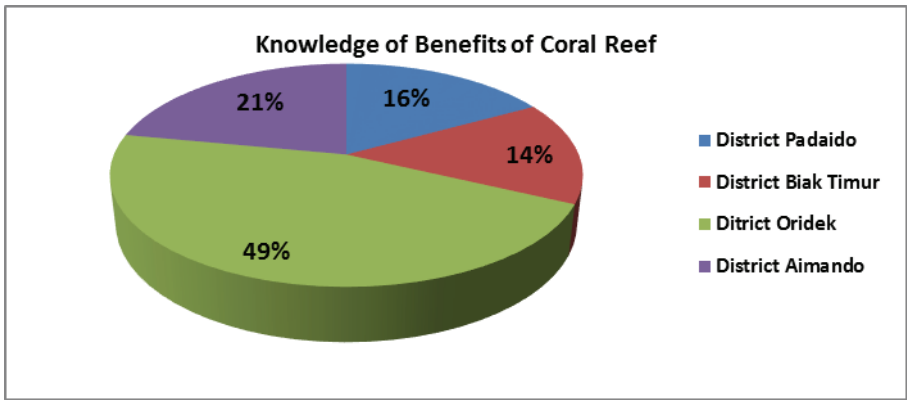

Figure 4. The percentage variation of society within Biak Numfor who know the benefits of coral reef.

Knowledge of Factors Contributing to the Damage of the Coral Reef. The observation reveals that the society in Biak Numfor needs more dissemination on coral reef and factors causing damage to it. This is due to the fact that knowledge is not distributed evenly to every district in the Biak Numfor regency. Of all community members with knowledge on the factors damaging coral reef, 50 percent are from the Oridek district, while 21 percent of the group are from the Aimando district. Moreover, the rest 16 percent and 13 percent of the group belong to the Padaido and Biak Timur district respectively (Fig. 5).

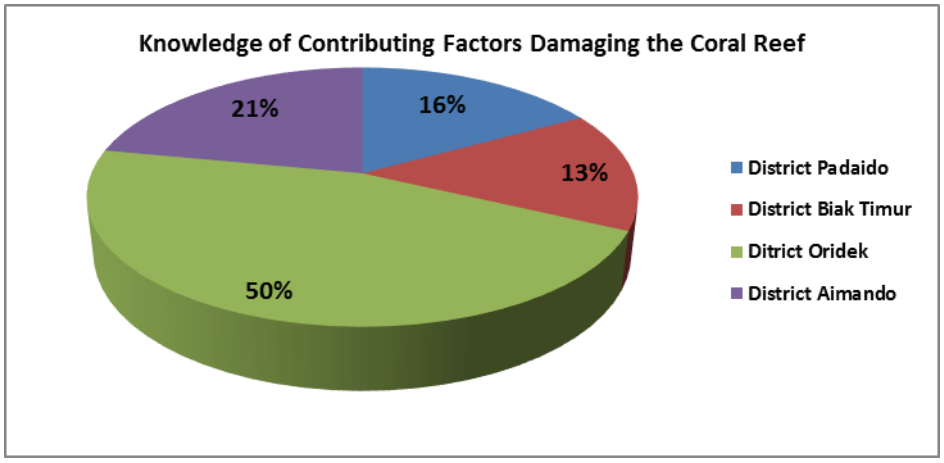

Figure 5. The percentage variation of community members in Biak Numfor who understand the factors damaging the coral reef.

Use of Fishing Gears that Damage Coral Reef. The following Fig. 6 illustrates that some community members in Biak Numfor have realized of the impact of damages to coral reef and thus, opposing the use of dangerous fishing gears to the coral reef. 52 percent of the group are from Oridek district, while 16 percent are from Aimando. Additionally, 16 percent and 14 percent of the group belong to the Padaido and Biak Timur district respectively.

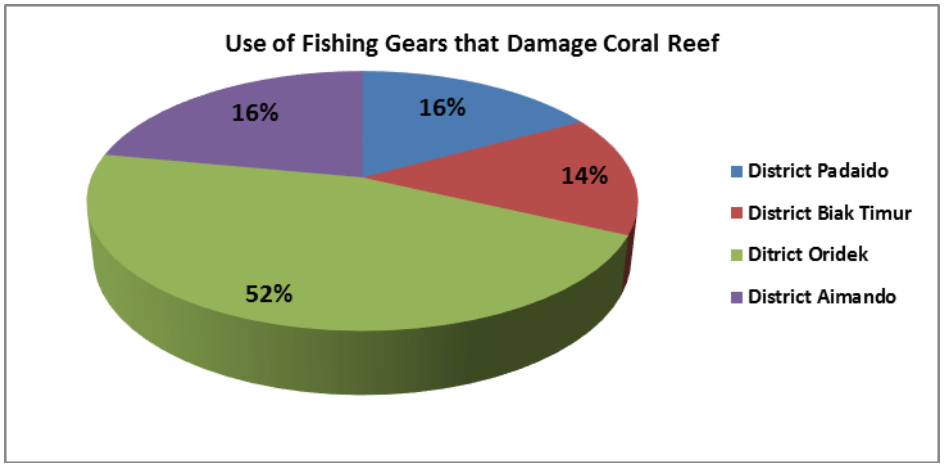

Figure 6. The percentage variation of community members within Biak Numfor opposing the use of dangerous fishing tools to the coral reef.

Knowledge of Ban of Explosives Use in Fishing Activity. There are some community members in Biak Numfor who understand of the destructive force of explosives in fishing activity, and the damage to the coral reef. However, the information needs to be disseminated more comprehensively, as it is unevenly distributed to each member within the community. Of all people whose knowledge of damage of explosives to the coral reef, 47 percent, and 21 percent belong to Oridek and Aimando district respectively. Moreover, 17 percent of the group are from Padaido, while 15 percent are from Biak Timur (Fig. 7).

Knowledge of Coral Reef Prior to the Core map Program. Previous to the core map program, there are some community members in Biak Numfor whose basic understanding of coral reef. Aimando is the district with most members whose basic knowledge of coral reef before the implementation of core map program, with 70 percent, while in Oridek, there are 30 percent of community members with basic understanding of coral reef. On the contrary, community members in Padaido and Biak Timur have no initial knowledge of the coral reef before the core map program.

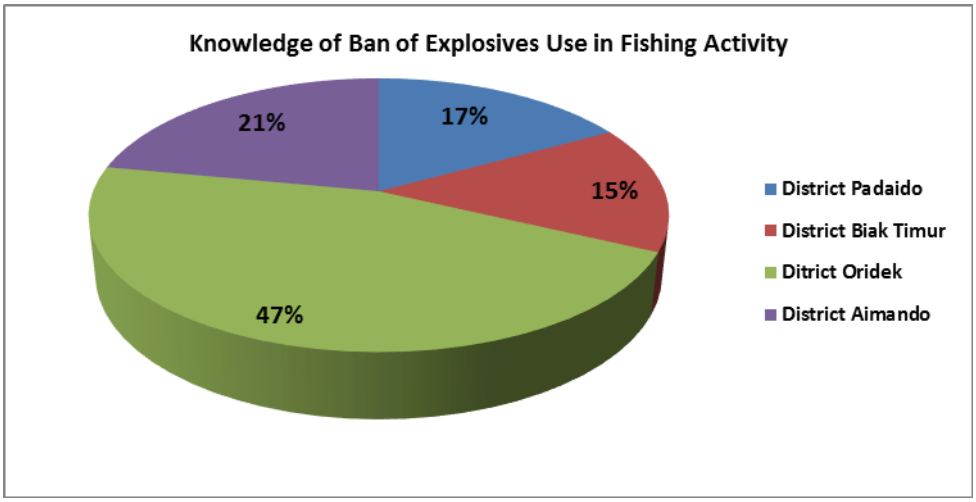

Figure 7. The percentage variation of community members who understand the damage of explosives in fishing activity.

Knowledge of the Ban of Fish Anaesthetic on Coral Reefs. People's knowledge regarding the ban of anesthetic on coral reefs in each district is different; in Oridek district, the percentage of people's understanding is at 41 percent preceded by Biak Timur district with 23 percent. Furthermore, the percentage of Aimando district is at 21 percent and the Padaido district has the lowest percentage among all with 15 percent. The following Fig. 8 illustrates the explanation of people's understanding regarding such an issue:

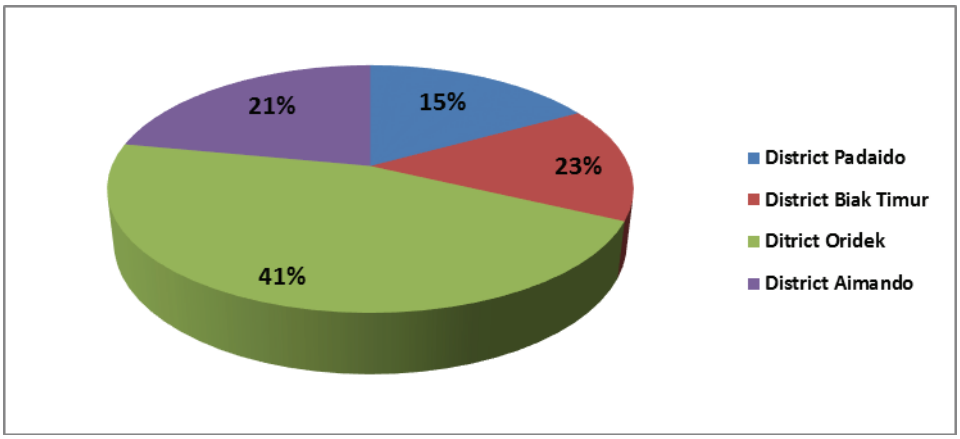

Figure 8. The percentage understanding of the Ban of Fish Anaesthetic on Coral Reefs.

Knowledge regarding the Conservation of Coral Reef. The percentage of the understanding of people of Biak Numfor Regency on the conservation of coral reef is varied. The percentage of the Oridek district is at 48 percent, Aimando with 21 percent, and the lowest districts are Padaido and Biak Timur with a percentage of 16 % and 15 % respectively (Fig. 9).

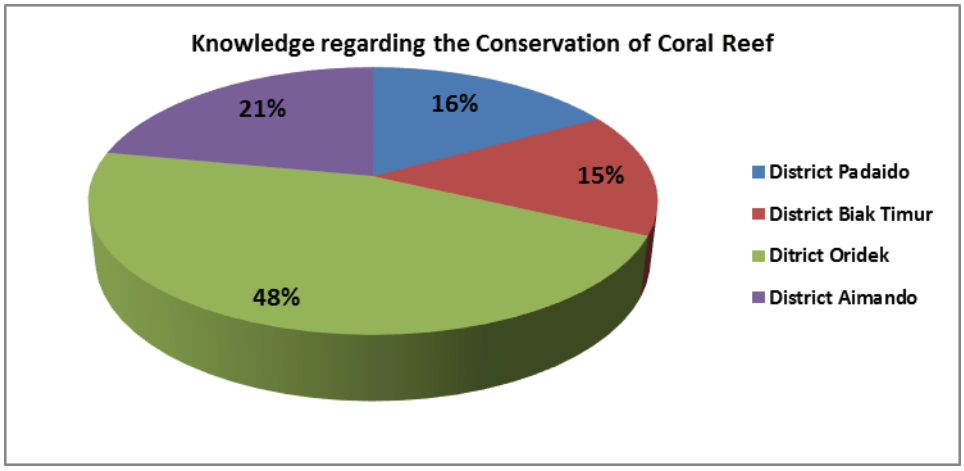

Figure 9. The percentage variation of understanding of the Ban of Fish anaesthetic on Coral Reefs.

Knowledge of the Core map Programme. People's knowledge of the core map programme in each district is different; in Oridek district, the percentage of people's knowledge is at 48 percent, followed by the Aimando district with 21 percent and Padaido district with 16 percent. Further, the lowest percentage among all is Biak Timur district with 15 percent (Fig. 10).

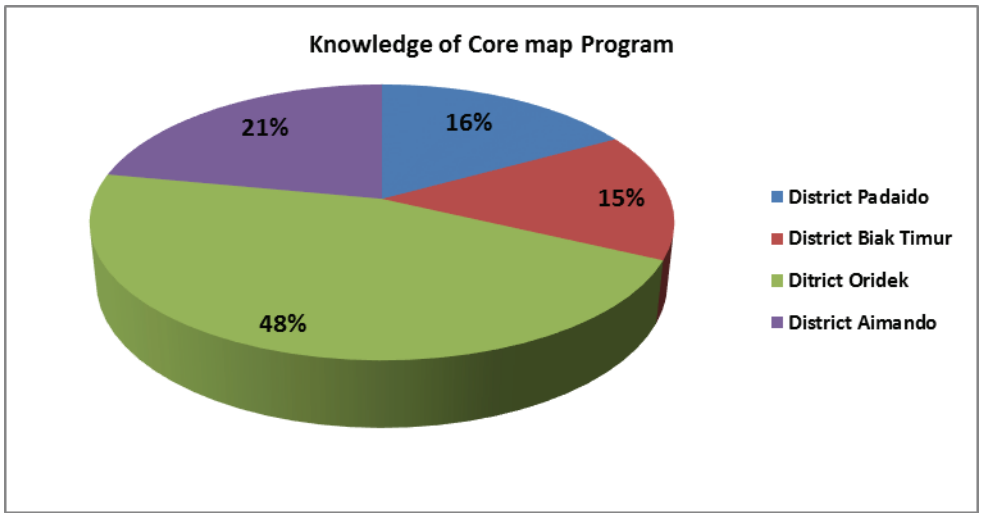

Figure 10. The percentage Knowledge of the Core map Program.

Eagerness to Involve in Core map Activity. People's eagerness to involve in core map activities in Biak Numfor Regency is at a different level. Each district has its own percentage, in which Padaido district gets 17 percent of its total population who wants to involve in core map activities, and then it is followed by Biak Timur district with 15 percent and Oridek district with 46 percent. Aimando district, however, arrives at 22 percent of its total population. Fig. 11 illustrates people's eagerness to involve in core map activities.

Knowledge of Coral Reef Supervision. Coral reef supervision should be importantly conducted to avoid its damage that caused by people's negligence in managing marine resources. Most people in Biak Numfor have understood the marine resources supervision with different levels of knowledge in each district, which Oridek district reaches 41 percent of its total population, followed by Aimando district with 33 percent and Biak Timur district with 22 percent. The lowest percentage of 4 percent, on the other hand, is from Padaido district of its total population.

Sustainability of the Core map Programme. The Core map programme gives a positive impact on people's life improvement in Biak Numfor Regency that they can live independently and preserve the natural resources. On top of it, people are eager to sustain this core map programme to maintain its good impact (HANING, 2008). From Fig. 12, it illustrates that people in Oridek district want to sustain the programme with 50 percent, followed by the Aimando district with 20 percent, the Padaido and Biak Timur district with 15 percent.

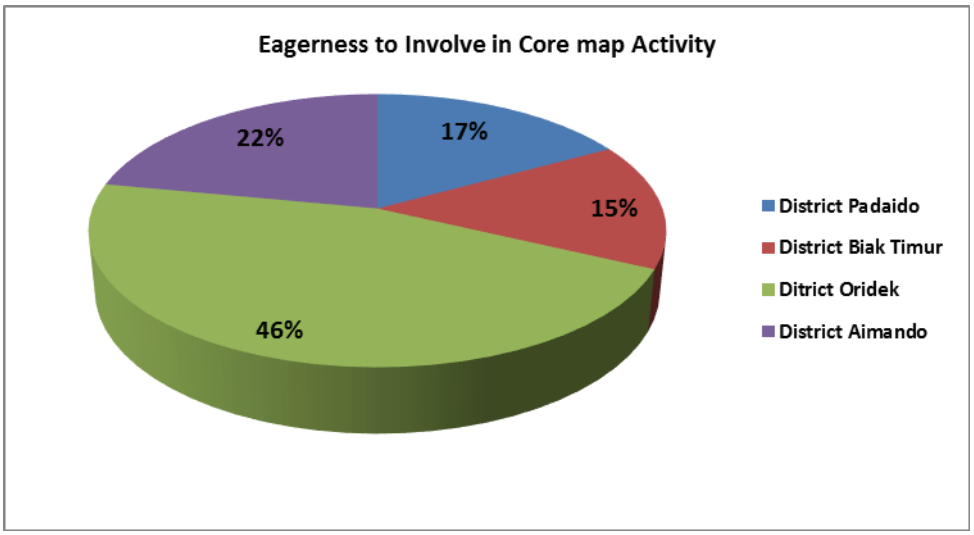

Figure 11. The percentage variation of People's eagerness to Involve itself in core map Activity.

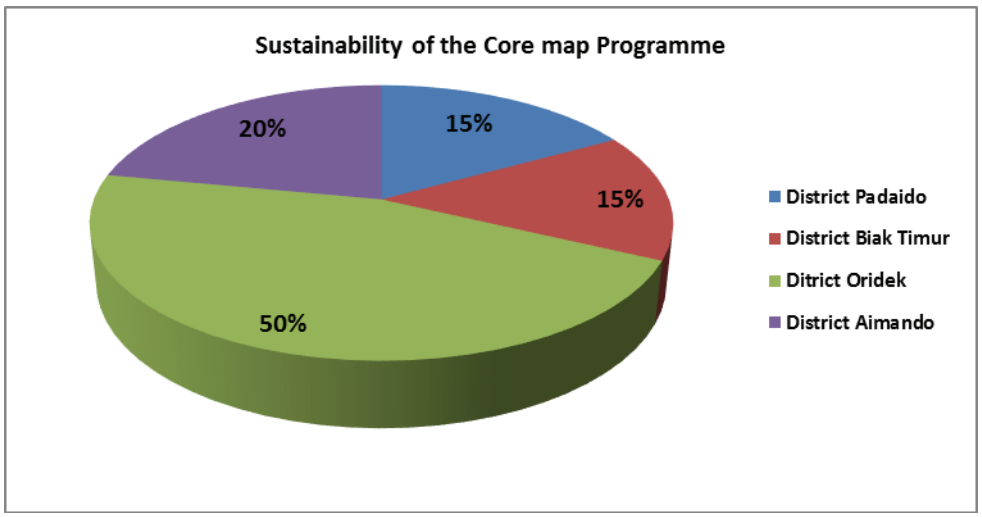

Figure 12. People's Eagerness to sustain the Core map Programme.

Supervision Activities in Each District. Developing core map programme requires community's involvement in which people in Oridek district have involved at 64 percent, Padaido district at 20 percent and Aimando district at 15 percent. Unfortunately, there is no programme involvement from people in Biak Timur district.

Punishment Approval for Violation. It is necessary to punish people who break the rule for its enforcement around core map location in which community obedience can support the programme development. The awareness level of individuals towards the rule in Biak Numfor Regency is different; 15 percent of people in Paraido district are aware of the rule, while in Biak Timur, Oridek and Aimando district, the rate of individuals who are aware of the rule is 14 percent, 51 percent, and 20 percent respectively.

Knowledge of Coral Reef Harvesting Ban. Coral reef as a marine resource should be preserved due to the ecosystem balance in its marine. If there is damage to the coral reef, the other habitats will be affected by its damage as well (NOVACZEK, 1997). Several coral reefs have been damaged in these days era, and it caused by surrounding people's daily activity that they have continually harvested coral reef as a building material. For that reason, there should be a regulation regarding the ban of coral reef harvesting in any ways (ASEP, 2001).

Based on survey results, some respondents from Biak Numfor Regency agree with the implementation of the rule on the ban of coral reef harvesting. It is expected that it can help preserving coral reef ecosystem in the region. The data from the survey reveal that people in the Oridek district agree with the regulation, and this gets the highest percentage, of 53 percent, followed by the Biak Timur district and the Aimando district that rate of people who approve the ban of coral reef harvesting is 30 percent and 12 percent consecutively.

The Thinking Level of Society about Exhausting Marine Resources. As obtained from the survey, most people argue that marine resources will be slowly exhausted (SIREGAR, 2010; WASKITO & MUGI, 2012). A survey in each district acquires various results; 59 percent of people in Oridek district claim that marine resources will be run out, and then the percentage in Biak Timur district is 27 percent. Furthermore, the rate of people in Aimando and Padaido district who believe in exhausting marine resources is only 11 % and 3 % respectively.

CONCLUSIONS

The purpose of this study is to understand the people's role in preserving coral reef through the core map programme and to discuss the impact of the programme on community welfare improvement around East Region in Indonesia. Environmental preservation is crucial for people to care about; therefore, actual action and broad knowledge are required to conserve coastal area. The results show that: (1) people in Oridek district have bigger awareness and thinking level than the other districts, (2) better coral reef is successfully achieved by implementing the core map programme, (3) the most dominant impact of the core map programme is on coastal community's welfare in Biak Numfor Regency, (4) it should be a coral reef balance and preservation after the core map programne implementation by setting a law of regulation, (5) the core map programne sustainability is required to preserve coral reef ecosystem and its marine biota.

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AUGUST VON SPIESS - UNDER THE SIGN OF THE DESTINY

ANTONIE Iuliana

Abstract. August von Spiess was born on the 6th of August 1864. He spent his childhood on his mother's estate in the Fiume region, Poland. The mother inspired him with the first feelings of love for nature. He attended junior school in several garrisons, following, as all his family, his father's displacements as an active military officer. Irrespective of the place, the child August preferred to wander in nature, instead of acquiring school knowledge. At the age of eleven he was enrolled in the Military School in Saint Poelten. After leaving the school, he graduated the Theresianum Military Academy in Vienna and was given the rank of second lieutenant. A schoolmate described Transylvania to him as a dream world in terms of relief and abundance of fauna. From this moment on, he did his best to come to Transylvania. He succeeded in being appointed to a regiment in Orastie and then in Sibiu. Here he made it a duty to improve himself in the art of hunting. After the First World War, under favourable circumstances, he was named by the Romanian king Ferdinand I, on the first of July 1921, in the position of manager of the royal hunting. In this position he developed a fruitful activity to organize royal hunting parties, to protect the fauna and also to build roads, huts, shelters and refuges. He proved his literary talent by writing articles inspired by the world of cynegetics and also writing specialized works, which are authentic monographs of the most known hunting fields. During his lifelong efforts, he succeeded to collect a large number of trophies from the hunting areas in Romania and also in the Central and Eastern Europe. His collection of trophies and hunting guns comes to one thousand pieces and this is the base of the Hunting Museum in Sibiu, denominated after his name: August von Spiess.

Keywords: biography, naturalist, hunting master, trophies, August von Spiess.

Rezumat. Sub semnul destinului-August von Spiess. Cel ce avea să devină col. A von Spiess s-a născut în ziua de 6 august 1864. Prima copilărie și-o petrece pe proprietatea mamei, în regiunea Fiume, Polonia. Mama, îi insuflă copilului cele dintâi sentimente privind natura. Cursul școlii primare îl parcurge în mai multe garnizoane, urmând, cu întreaga familie, mutările tatălui, ca ofițer activ. Peste tot, copilul August se dovedește mai aplecat pentru hoinărelile din natură, decât pentru asimilarea cunoștințelor școlare. La unsprezece ani este înscris la "Școala Militară" din Sant Pölten, iar după absolvire trece la Academia Militară Terezіană din Viena de unde iese cu gradul de sublocotenent. Un coleg îi descrie Transilvania ca un tărâm de basm sub raportul reliefului și a bogăției faunei. De aici înainte, depune toate strădaniile pentru a ajunge în Transilvania. Reușește să fie numit la un regiment din Orăștie și apoi la un regiment din Sibiu. Aici dezvoltă un larg program de perfecționare în arta cinegetică. După prima conflagrație mondială, prin împrejurări favorabile, este numit de către M. S. Regele Ferdinand I, pe data de 1 iulie 1921, în funcția de director al vânătorilor regale. În acestă funcție desfășoară o rodnică activitate pentru organizarea vânătorilor regale, pentru protejarea faunei, pentru construirea de drumuri, cabane, adăposturi și refugii. Își demonstrează talentul literar prin articole inspirate din lumea cinegetică și cărți de specialitate, adevărate monografii ale celor mai cunoscute terenuri de vânătoare. Prin strădaniile de o viață reușește să strângă un mare număr de trofee din terenurile de vânătoare din România și din Europa Centrală și de Est. Colecția sa de trofee și arme de vânătoare ajunge la o mie de exemplare și formează baza muzeului din Sibiu care îi poartă numele.

Cuvinte cheie: biografie, naturalist, trofee, maestru de vânătoare August von Spiess.

INTRODUCTION

The Natural History Museum in Sibiu has a very rich patrimony that was studied by a large number of specialists; it consists in botanical collections (DRĂGULESCU et al., 2015), ornithological collections (PRIPON, 2015), mineralogical and palaeonthological collections (CIOBANU, 2003; CODREA & CIOBANU, 2003) and also in the famous entomological collections studied in time by many entomologists (ANTONIE, 2015, 2016; CUPEZAN et al., 2015; IENIȘTEA, 1970; MOISE 2011a, b, c).

The present paper refers to the collections of the trophies and hunting guns, a part of the Brukenthal National Museum collections. The custodian of these collections is the Hunting, Trophies and Hunting Guns Museum "August von Spiess". This paper is an homage to its founder, August Roland von Spiess von Braccioforte, a noteworthy person, a naturalist, a writer, a great personality among the old days' famous hunters.

THE LIFE OF THE COLONEL AUGUST VON SPIESS

August von Spiess was born on August the 6th, 1864 in Prjemysl, a locality in the Austrian province Galitia, at present Przemysl in Poland.

The Parces traced his destiny under the divine protection of the goddess Artemisa. He fulfilled his destiny as August Roland von Spiess von Braccioforte zu Portner und Hoeflein and later, after he was ennobled, as colonel August von Spiess.

The colonel himself explained his great love for nature as a combination of two factors: his genetic inheritance and his education. He had plenty of both of them. His mother owned an estate at the Adriatic seashore, near Fiume. She taught him to love the greatness of the nature and inspired him with passion for observation and for study of the little creatures like crickets, butterflies, scorpions, cicadas, etc. As concerns his education, his father, as a military officer in

the Austrian Imperial Army, did his utmost to initiate his son in the secrets of the wild nature and particularly in those of hunting.

Until the age of ten he spent his childhood in this paradise. A wide panorama that looked out on the mountains but also on the Adriatic Sea, was like a great amphitheater. It influenced his natural instinct, which was enriched by initiatic elements of education that will be the base for his personality. His father stimulated his inclination towards nature. He gave him a halberd as a present and trained him to use it for hunting some interesting pieces from the local fauna.

After ten years of wandering in this mirific world, his father was transferred from Fiume to the Agram garrison and soon after that to another one, the Rudolfswerth-Krein garrison in Slovenia. The family followed him, and so, the little August passed from one school to another, with different languages of teaching, not having the possibility to absorb the knowledge at the class of his level. By lucky coincidence, the little schoolboy had a classmate with the same habits and they wandered together around the garrison, playing with the nests of the little birds, fishing and capturing crabs, having a life in accordance with his wishes, namely having ”an absolute freedom in the nature” (SPIESS, 1931a).

Such a life had two facets: the favourable one, that intensified his sense for observation that will influence his future features of hunter and the other one, less favourable, that brought about an insufficient learning and even a school delay concerning his knowledge. In order to solve this problem, the family decided:””the boy must be sent to a military school” (SPIESS, 1931a).

At the age of eleven, he was enrolled for the Military School in Sant Poelten. This was very unpleasant for the child August who was crying with tears of sadness. His father, holding the rank of a major now, was transferred to the Tarnopol garrison in Galitia.

Little August spent his holidays wandering around the city, having also a small shot hunting rifle, as a present from his father. His experience and the hunting knowledge received from his father were decisive for the young cadet. His father was moved again to the Lemberg garrison in Bohemia; Spiess junior, after finishing the military school, was enrolled and graduated the “Theresianum Military Academy” in Vienna. As a young second lieutenant he was commissioned in the same Lemberg garrison. By now, a stage of Spiess’ life came to an end and it began another one, which allowed him to be closer to his dreams of being closer to nature.

With his Lancaster rifle, he took part in the autumn and winter huntings around Lemberg. He was very impressed about stories heard from a Transylvanian friend, who described Transylvania as a wonderful natural area, as a fabulous realm.

Under the influence of this friend, the desire to move to a garrison in Transylvania, the region that was in his imagination like an El Dorado for hunting, germinated in his mind. His next steps led him closer to the fulfilment of his destiny. In August 1885 he was sent to the Orăștie garrison of the regiment 64 infantry. He improved his skills in hunting, taking part in numerous hunting outings on the areas near the Mureș river. But his mind was set on hunting large animals in the Carpathian Mountains, with their great crests and peaks, with endless forests and a legendary fauna.

The destiny helped him meet the imperial military officer, the colonel Berger of the garrison of Sibiu, an enthusiastic and perfect hunter. Their long friendship came to an end only when one of them passed away. He took a leave of absence from his unity’s commander and one day, with his Tyroler rifle up on his back, went to Sibiu by his bicycle with one big wheel, in order to take part, together with his friend, to a hunting party in the mountains.

He took constant steps to be transferred to Sibiu and finally he was removed to the Sibiu garrison – 31^{-st} Regiment, on the first of May 1889. At the end of his military career, he held the rank of colonel and the commanding officer of the Regiment 2 Infantry in Sibiu.

In his memories book about the adventure in Africa, the colonel August von Spiess confessed that: Țmy destiny brought me to Transylvania where I was welcomed by the hospitable inhabitants and I found here, among the numerous hunters, friendly and paternal advisers. I was interested particularly in the inhabitants of Romanian nationality, natural human beings for which the mountains had no secrets; when I learnt their harmonious language, they accepted me and they took me to their huntings and expeditions, as a schoolboy eager to learn. I spent many wonderful hunting moments in the company of those old mountain hunters. In their endless forests and in their high mountains I hunted a lot of wild beasts. So, I was able to appreciate their matchless hunting sense, their ability for orientation in the field and in their natural vocation for hunting and fishing” (SPIESS, 1942).

That was the time named “La Belle Epoque” and the young captain was determined to use all opportunities which appeared, to cultivate friendships and relationships all over the Central Europe. He graduated a school to improve his skills in target shooting and he was known as a sharp shooter.

Having so many talents, he made acquaintance with the manager of the hunting fields of the Imperial Court E.S., the Earl Thun, who offered him the favour to visit the hunting fields, the hunting park Lainz and also the administration bureaus of the hunting fields of the Imperial Court in Asper Lobau.

During his participation in the hunting parties in Neuberg and Muerzsteg he met the most important personalities in the world of hunting of the Habsburg Empire.

On a local-wide scale, together with his friend Berger, took the concession for hunting of five mountains in the Carpathians where they organized huntings of bears, wolfs, stags, foxes, black goats and birds.

He collected a large number of trophies, intending to found a hunting museum, which would keep his memory after his physical disappearance. His friendship with Berger went on. Berger was a witness at the Spiess’ wedding, he

was also the godfather of his children. Their friendship ended only after thirty years, when, in a tragic hunting accident, colonel Berger suffered a surgical operation that was fatal for him.

In his memoires, August von Spiess related about a hunting party in the mountains in 1890, when a violent storm took place, "a cloud burst" that produced large floods and damages, among them being also the fall of the salt mines in Ocna Sibiului, which were transformed in lakes. These are the salt lakes that are being used now for different treatments, mainly against rheumatism (SPIESS, 1931b).

At the end of the first World War, the Habsburg Empire collapsed and the way of life changed, the relationships disappeared and also the opportunities for the officers of the Sibiu garrison. The Association of the Hunters in Sibiu continued to exist, in spite of all difficulties, with Spiess as a president. He related that at that time hunting was not organized and was only an additional way to earn one's living.

His destiny interfered again in his life and directed him towards the Parces' bewitched words. Among the provisional state structures was also "The Troops Commandment in Transylvania" that in 1918 chose to establish its headquarters in Sibiu. The commander of these troops was appointed the general Arthur Văitoianu, a good connoisseur of the hunting art and a passionate hunter. The result of the likeness between of the two hunters followed in a natural logic. These two persons understood each other perfectly as concerns the hunting. Văitoianu found in the collocutor Spiess a hunting master, a good organizer and a good militant for the nature protection. But the general Vaitoianu didn't remain for a long time in Sibiu. On the 27th of September 1918 a new government was established in Bucharest and the general Arthur Văitoianu was appointed prime minister (GIURESCU, 1971).

It seemed that the key thread of his good destiny would be interrupted. But, on the contrary. The general Văitoianu related to M. S. the King Ferdinand I about August von Spiess and his qualities. The King was convinced and after a short time, on the first of July 1921, the colonel August von Spiess was appointed as Master of the Royal Hunting, holding this position until 1939.

August von Spiess related in his work "55 years of hunting": "For ten years I had under my responsibility twenty one royal hunting fields, from the crests of the Carpathian Mountains to the Black Sea", among them the Gurghiu area with 47.000 ha., Broşteni with 48.000 ha., the Danube Delta with 250.000 ha., etc. In these areas no one could hunt, except H. M. the King and his high guests (SPIESS, 1931c).

In his new position, August von Spiess started the work with a total abnegation and great ability. He initiated the construction of access roads, hunting huts, chalets, shelters, refuges; particularly he elaborated and put into practice the conceptions and theories concerning the protection of the nature and of the cynegetic fund all over the country. King Ferdinand I appreciated and upheld his work. The empathy between them was evident and August von Spiess accompanied the King in every hunting party.

His native and educational qualities, his competence and his merits, promoted him as an honorary member of many cynegetic societies in Romania and abroad. He was a member of the Commission for Nature Protection and for the National Park.

He accomplished his tasks with the same loyalty also during the rule of the King Carol II.

At the age of 72, his lifelong dream came true. At that time, a hunter was considered fully fulfilled only after hunting in Africa (a kind of: "et in Arcadia Ego!"). He took part in 1936 to a hunting in Kenya and in 1938 in Tanganyika, where he collected many valuable African trophies.

THE ACTIVITY AS A NATURALIST AND A WRITER

The personality of colonel August von Spiess expressed itself in an admirable way also in the sphere of writing. His innovative ideas were reported in the publications of that time, mainly in the Hunting Review ("Revista Vânătorilor"), a specialized review of the Hunters Union of Romania.

His books are certain monographs of the hunting fields, dealing with the climate, relief and mainly the species of the local fauna. The books were written mainly in the German language; they were published at the publishing houses in Berlin, Munich and Sibiu. Besides his scientific rigorous descriptions of the fauna species, colonel August von Spiess displayed also an indisputable literary talent. In his work of memoirs "55 years of hunting" he recounts about his evolution as a hunter and also about the dearest and the most significant recollections of his hunting parties. His style is rich in descriptions of landscapes and full of sentiments of total adhesion to the beauties of the nature: "Slowly, little by little, the full moon disk is rising over the sharp crags of Negoi, astounding, overflowing its silvery light over the whole realm" (SPIESS, 1931b).

His hunting expeditions are presented purely and simply as mythological expeditions where the proportions, the strictly technical elements are interwoven harmoniously with the human sentiments and also with the diversity of behavior of the hunters, forced frequently to take rapid decisions.

In his book "The black goats in the Retezat Mountains" ("Caprele negre din Masivul Retezat") he reported- among other things - about two famous hunting expeditions performed in the hunting field of the Retezat Mountains, which belonged to the Earl Kenderffy (Hungarian modification of the Romanian name for Cândea). This field, with a surface of 27.620 acres in the Retezat Mountains was taken in concession by the Romanian State according to the order of the King Ferdinand I in 1921 and was destined to royal hunting parties (SPIESS, 2005).

The narration of one hunting party which took place in 1882 is a proof of his literary talent, that utilized picturesque details interwoven with objective information, that made a description particularly interesting. That year, the Baron Tomya, as organizer, invited Rudolf, the heir prince of the Habsburg Crown, to take part at the hunting. Rudolf, together with his wife, ministers and high dignitaries of the Imperial Court, hunted black goats and bears in one week's time.

The baron Tomya, the master of that hunting party, was dressed in a coat of wolf skin that Prince Rudolph liked so much that he immediately ordered a few skins of wolf. A craftsman from Hateg manufactured a coat identical with Tomya's coat. The prince Rudolf, strongly impressed by that wolfskin coat, asked the painter of the Royal Court- who came especially from Vienna - to paint Tomya dressed in the famous coat. The painting, masterly executed and framed, was offered as a present to the Emperor.

As a reward, "the man with the wolfskin coat" was appointed as manager of the whole hunting field. The archduke Rudolf had also other similar initiatives, but they were abandoned after the tragedy of Mayerling.

Another episode, worthy of a man of letters' pen, was the great hunting party in 1923 of H. M. the King Ferdinand. Now, colonel August von Spiess was member of the organizing staff of the hunting. That hunting took place on the area taken in concession from the earl Kenderffy in year 1921, when the Retezat Mountains become the hunting royal fund, that later will be a part of the Retezat Park, the first National Park in Romania. The success of that hunting was proven by the numerous trophies won. The description of this hunting has plenty of enchantment and suspense. At that hunting took part, besides the notabilities of the Royal Court, His Royal Highness Carol and also the prince George, the heir of the Greek throne and the future King of Greece. The narration of the colonel August von Spiess does not confine itself to the facts that happened in that hunting area, but also to the King's visits in the cities, villages and historical monuments of Hunedoara county.

The King visited Hațeg, Sarmisegetuza Ulpia Traiana, the church of Densusi, Hunedoara, etc. Everywhere the King was welcomed with ovations. Colonel August von Spiess was enthusiastic by all he saw: "there are no many regions in the Central Europe like Hunedoara county and particularly Hațeg district, where every place, every valley, every cave and almost every mountain could present vestiges and monuments from the remote past" (SPIESS, 2005).

His book "The black goats from the Retezat Mountain", had the following dedication: "with veneration, to the brilliant hunter H. M. the King Carol II of Romania". This book was edited in Romanian language only in 2005, at the publishing house "Hora" in Sibiu, by the efforts of Walter Frank, who made the translation from German language and of a granddaughter of August von Spiess, Helga Stein, who lives in Dortmund, Germany. In the "Introduction", Helga Stein affirms that this book is "a valuable document about the history of hunting in Romania". The book is abundant in information, first of all about hunting, but also about geographical information about Hunedoara county, climate elements, art monuments and mainly historical, ethnographical and archaeological information. The author used a foreign bibliography of the most known authority in the matter.

Among the hunting works of reference written by August von Spiess, one could emphasize the following: "Gurghiu - a history of Gurghiu", a monograph, at the publishing house Krafft and Drotleff, Sibiu, "The Carpathian Stags" (Karpathenhirsch), the publishing house Paul Parey, Berlin, 1925, "The hunting reserves in the Retezat Mountain", "Under the magic of Carpathians" (In Zauber der Karpathen), the publishing house Paul Parey, Berlin, 1933, "The four seasons in the bird paradise in Mânastirea", "Seventeen years in the service of the Royal Hunting House of Romania" (Siebzehn Jahre in rumanischen Hofjagddienst), the publishing house F. K. Maye, Munchen, 1940, "From Ardeal to Kilimandjaro - Hunter in Africa the publishing house "The Royal Foundation for Literature and Art", 1942, "Fifty five years of hunting" (the memoires of colonel August von Spiess, the manager of the royal hunting). This work appeared in Romanian language and it was printed as a "serial story" in the "Hunter Review" in 1931.

August von Spiess was present in the specialized reviews, having an intense contribution with " Revista Vânătorilor" (The Hunter Review), "Carpații" (The Carpathians), "Wild und Hund", "Der Deutscher Jaeger", "Karpathen-Weidwerk", etc.

As a result of his prestige in the area of the Central Europe, he was asked to co-operate in the staff of specialists that elaborated the famous hunting handbook: "Die Hohe Jagd" printed in Berlin in the year 1905. August von Spiess elaborated the chapters about bear and lynx. Also, as a proof of his appreciation, he was elected as an honorary member of the jury at the International Hunting Exhibition in Leipzig, 1930.

In a retrospective look marked by nostalgia and the feeling of ephemeral, the colonel August von Spiess closed his historical adventures in the hunting fields with the following words: "The copper nails on the butt of my rifles are the distinctions of a lifelong tenacious hunter. These honor signs were collected by hunter since he was a child, up to an older age, in the great area between the Alps of the Central Europe, the Black See, the Russian steppes and forests, in the legendary Bialovies, the splendid hunting fields of Germany to Bulgaria, Romania and Dobrudja" (SPIESS, 1931d).

The Hunters' Review nr.12/1937 praised him: "We know Mr. Colonel August von Spiess from his multiple works offered to the cynegetic literature, but only in his work "The Carpathian Stags" he revealed the secrets of his heart. We thank wholeheartedly to the manager of the royal hunting and we assure him that no one of the Romanian writers found such accents that covered in a fine texture, associated with such an affectionate feeling, the crests of our old mountains" (SOLITARUL, 1937).

What made the memory of colonel August von Spiess unforgettable is, undoubtedly, his collection of the trophies and hunting guns that are in the patrimony of the Museum which bear his name. This valuable collection is

formed of 1000 pieces of trophies, mainly from Carpathian Mountains and from Africa. His collection was donated in 1963 to the Romanian State by his daughters, together with their house in Sibiu, that now shelters the Museum. This house where lived the colonel August von Spiess's family, is a building in Romanian style and it forms a distinct, agreeable spot in the context of the all-around buildings. The Museum was opened for the public in 1966, in accordance with the conditions stipulated in the donation act. The name of the Museum is: "The Museum of Hunting, Trophies and Hunting Guns - August von Spiess", in Sibiu.

At present, the Museum has a cynegetic patrimony of 1577 pieces that results from the collection of August von Spiess, plus the collection of the forestry expert Emil Witing and the collection of the Transsylvanian Society for Natural Sciences (Siebenbüergischer Verein für Naturwissenschaften zu Hermannstadt).

In addition to the hunting trophies, the Museum lodges also a large collection of hunting guns, hunting accessories and a splendid photographic original material. The circuit in Museum is conceived in such a way so that the visitors could make an idea about the hunting art, beginning with the Stone Age.

The museum consist in five showrooms, each of them with the pieces, well arranged in order to get an extensive general impression; among them, the third showroom is dedicated to the memory of the colonel August von Spiess and includes the photographs with explanatories texts, his hunting rifle, the most valuable personal trophies, the books written by him, and also the reviews which published his articles. On the whole, this third showroom is a supreme homage to the man, the hunter and the collector, the colonel August von Spiess.

The colonel August von Spiess passed away in 1953 at the age of 89 and his last resting place is the Municipal Graveyard in Sibiu.

CONCLUSIONS

Colonel August von Spiess's whole existence seemed to be under the sign of a favourable destiny, that influenced the progress of the events in his life, that gave him a valuable way in life and also conferred to his personality an unforgettable posthumous remembrance.

His mother inculcated him the love for nature and the interest to knowledge. His father explained to him the complicated secrets of hunting.

His father's transfers to different garrisons helped August as a child to know the great diversity of the nature, to meet friends with the same aspirations

At the "Theresianum Military Academy" in Wien, a schoolmate described him the province of Transylvania as a fabulous region in terms of nature, relief and fauna. In his mind appeared the thought which induced him the desire to meet that magic realm.

As a second lieutenant, he was appointed to a regiment in Orăștie and then in Sibiu. Here he had an intense hunting activity in accordance with his aspirations. He made his military career as a colonel, commandant of the Regiment.

At the end of the First World War he was mistrustful about his future. But his good destiny intervened again and made it possible to meet the commander of the Romanian Troops in Transylvania, the general Arthur Văitoianu. At his proposal in 1921, King Ferdinand I emitted a Royal Decree for appointment of the colonel August von Spiess in the position of the Manager of the Royal Hunting.

From this moment, the destiny of the colonel August von Spiess evolved in a straight line. His position permitted himself to prove not only his loyalty to Romanian Crown but also his mastership in hunting.

All his organizational accomplishments, the elaboration of specialized works, of monographs of the hunting fields, the collection of the trophies and the hunting guns represented the proof that the colonel August von Spiess did his best to recompense the fulfilments of his special destiny.

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FROM IDEA TO ACHIEVEMENT - FROM THE EXPERIENCE OF A BIOLOGIST

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Abstract. The present paper is a guide addressed to young faculty graduates, meant to help them to develop until reaching maturity and professional fulfilment. Some concepts, statements and guidelines from the lives of reputed scientists and people of culture are presented initially. There are discussed aspects of professional training in different branches of biological sciences: hydrobiology, environmental mutagenesis, medical genetics, cytogenetics, biotechnologies, evolutionism, etc. Each one presents the mentors who contributed to the formation of the young scientist, the scientific priorities achieved, and so on.

Keywords: biology, short biography, principles in scientific research.

Rezumat. De la idee la realizare - Din experiența unui biolog. Prezenta lucrare este un îndrumar adresat tânărului absolvent de facultate, în scopul dezvoltării sale până la maturitate și implinirea sa profesională. Inițial sunt prezentate câteva concepte, afirmații și îndrumări din viața unor reputați oameni de știință și cultură. Sunt discutate aspecte din pregătirea profesională în diferite ramuri ale științelor biologice: hidrobiologie, mutageneza mediului ambiant, genetica medicală, citogenetica, biotehnologii, evoluționism, etc. La fiecare sunt prezentați mentorii care au contribuit la formarea tânărului om de știință, prioritățile științifice realizate, și altele.

Cuvinte cheie: biologie, scurtă biografie, principii în cercetarea științifică.

In the thesaurus of different peoples, personalities and their culture, there are many recommendations on how to behave. Their knowledge will guide our behaviour in difficult situations and will enable the elaboration of appropriate responses. You will be pleased.

* Establish milestones with accurate and reasonable achievements over time, with appropriate answers for each action and be pleased (G. C. Corneanu, Biology graduate, 1965).

* With my life, I defend your life (Universita degli Studi di Torino, Italy; a similar thinking is found at NATO, Biology Dept., BNL, Upton, USA);

* Never the second! (the motto of the Kennedy family);

* Better to do and to repent than to not do and repent (King Baudion, Belgium, the beginning of the 20th century);

* If you want, you can! (applied within fair moral limits, multiple origins);

* Better his mother cry than my mother (originating in Oltenia);

* Do not regret having missed an opportunity; soon you will meet a better one (Vasile Alecsandri);

* Do not regret that you took a road and broke your leg; on another road, you could have broken both of them (diverse origins, etc.):

* Bad, but good; characterization made by one of the first generations of students in Craiova: bad because I did not accept any favours; good because I favored the worthy;

* Believe in yourself and your actions; do not abdicate, because hope dies the last (urge!).

During our professional training, we need to know as many aspects as possible about the topic we want to address and have reputable specialists as mentors, because they can guide our work. It is not enough to confirm the researches or their discoveries, but to bring new arguments and applications. It is preferable to continue over time the researches from the beginning of our activity, ensuring their completion. In this case, family, age, social conditions, all display a great importance. In my case, I consider I was favoured by fate. In my childhood, the ambiance offered by the family was made up of remarkable personalities of the Romanian culture, such as Elena Ivănescu, the niece of Vasile Alecsandri, who gave me important information about the great writer's life (the conspiratorial home from Mircești from the Siret meadow; the place of his birth in 1821, the year of Tudor Vladimirescu's 1821 revolution, some of the writer's personal objects and the remaining manuscripts). By the middle of the 20th century, one of his nephews (the forest engineer, Ivănescu) was a neighbour of General Ion Antonescu, the future marshal and politician, at Curtea de Argeș. I am the nephew of renowned men of culture - Vasile G. Paleolog, friend of the famous sculptor Brâncuși and Acad. Prof. Dr. Nicolae Corneanu, Metropolitan of Banat. In my childhood, my father with Dr. Ion Firu (Head of Natural Sciences Section and Director of the Oltenia Museum) conducted the first diorama at the museum. I saw then variability and adaptation of organisms to the environment and I decided I would be a biologist.

Activities in the social field. The activities carried out in the social field during the secondary school (grades 5 to 7, General School no.12, Craiova) and the very good results obtained during this period represented a recommendation document (or a passport) for future activities. Our school won the first place in the city for the quality of education, the household activities in the school and its neighborhood, for gathering building materials from the 4st district (the name of the regiment that defended the city in the First World War). Thus, the school was rewarded with a weekly free subscription for 2 years offered by Craiova School Inspectorate. In addition, for many years, photographs of the event were exposed at the monumental entrance in Romanescu-Bibescu Park. Moreover, it was not commented

upon the fact that my father was arrested and imprisoned for several months after the Communists took charge of the Prefecture of Dolj County, Craiova (January 24, 1946). After his liberation (action involving friends and relatives), he was employed at the Russian Military Airport in Craiova. There, I made my first flight in a Russian biplane, taking the place of the co-pilot who remained on the ground (I was not even seven years old!). I graduated from the high school (*Nicolae Bălcescu* Popular College, presently known as *Carol I* National College Craiova) in 1965. I also had the chance to have wonderful teachers and colleagues, the school leadership organizing meetings of the school students with alumni who came from abroad, such as Ion Petrescu Maciste, then Prof. Dr. Andrei Ion, etc.). Among my college colleagues, I mention Costin Georgescu (former director of RSS), Cornel Popescu (former BCR director), Octav Calleya (conductor of the Symphonic Opera in Murcia, Spain), and others.

The quantitative accumulations during the high school years and, then, the **Faculty of Biology, University of Bucharest**, represented the basis for my further development and training as a ***biologist***. Due to the limited study time, which involves a consistent effort, I gradually renounced to some practical activities (performance sports, etc.). During our training, we benefited from an exceptional teaching staff (the best in Romania), the facilities offered by the University of Bucharest, which had two Didactic Stations in Brăila and Sinaia, the Research Institutes located in Bucharest and other neighbouring localities, together with numerous memorial houses, in which various specialists lived and worked, from whom I received information (Gh. Tătărăscu, Bucharest, painter; Nicolae Grigorescu, Câmpina, painter, etc.).

* **Activities and social implications that have attracted the attention of security organs**:

- The altercation in the US embassy yard in Bucharest, with foreign students and recovery of the US flag from their hands;
- Presentation of Condolence at the US Embassy in Bucharest, after the assassination of President J. F. Kennedy, together with some faculty colleagues, personally invited by me;
- Obtaining materials from the US Embassy in Bucharest (leaflets and posters) related to the Kennedy family;
- Personal reception of a thank-you letter for my attitude from the US Ambassador and US Secretary of State.

Events or actions that I attended after graduating from the faculty, becoming a scientific researcher at *Stejarul* Research Station Pângărați, Faculty of Biology and Geology, Iași:

- Cancellation of the union elections at Stejarul Resort, due to the vitiation of legal procedures (Pângărați, October 1965). My request was drafted and submitted from the office of the President of Bacău Region Council, who was my sister-in-law's uncle from Plopeni.
- Photographing some tourist aspects in Iron Gates area. When I arrived in Craiova, the security organs checked my camera (fortunately, I was with my brother, engineer Costinel Baciu, Director at one of the underground plants at UM-Plopeni);
- Visiting a factory at U.M. Plopeni with the approval of that time General Manager, Minister Ion Dinca.
- Participation in some family events at my brother, engineer Baciu Costinel (the death of his adoptive parents, the baptism of a niece), etc.

Field investigations and documentation, carried out in the first years after I obtained the position of teaching assistant at the University of Craiova (Biology-Genetics, then Plant Genetics and Breeding), until my doctoral admission (Cluj, January 24, 1970):

- Initiating certain research regarding the effect of exogenous factors (ionizing radiation and other stressors) on genetic material (plants and humans);
- Visiting the underground energy unit in Tismana, Gorj;
- Investigations in the area with endemic nephropathy (1962-1970, Erghevița, Mehedinți (approval from the Health Ministry, Bucharest);
- Investigations in the mining area of Ciudanovita (approval from the Ministry of Mines, Rare Metals Directorate (one month every year, in 1968, 1969, 1970), etc.;
- Approval of the security organs to have collaborative relationships with foreign specialists in research projects: Prof. Dr. A. H. Sparrow, BNL, Upton, USA; Prof. Dr. Harold H. Smith, BNL Upton, USA; Prof. Dr. Silvano Scannerini, University of Torino, Italy; Institute of Plant Genetics, Gatersleben, Germany, etc.). Through this action, we introduced in Romania valuable genotypes of cultivated plants (tomatoes, cucumbers, lettuce, etc.) that were greatly appreciated by the specialists from the country. Among them, I mention especially those who worked in Craiova, which became a unit of excellence in Romania in the field of horticulture (some of them were my professors in Bucharest): Prof. Dr. Ion Maier, Prof. Dr. Ștefan Teodorescu, Prof. Dr. Mircea Oprean, Prof. Dr. Mircea Bălașa; Prof. Dr. Ion Brad; Prof. Dr. Ion Safta, Prof. Dr. Doc. Ion F. Radu, Prof. Dr. Liviu Pop, Prof. Dr. Vasile Sonea, Prof. Dr. Ion Ceaușescu (USA Bucharest), etc. I was also provided an optimum research material base at Ișalnița Vegetable Research Station (Research Unit) and another at the Ișalnița-Almăj Greenhouse Enterprise (cultivation in industrial greenhouses). All these were achieved by applying the principle ***if you want, you can***!
- Communicating and publishing the research results in different national and international publications.

Transfer and didactic and research activity at the University of Craiova (activities developed successively at the Faculties of Biology, Agronomy, Horticulture and General Medicine). At the Faculty of Agronomy and Horticulture, where I had shown passion and qualities for research, I was provided two optimum research facilities in the field of vegetables: one at the Isalnița Vegetable Research Station (director, Dr. Gh. Bulugiu) and the other at the Işalnița Greenhouse Enterprise (director, Dr. Ion Sandu), "being protected" from the inherent social problems. The Faculty of General Medicine provided me with another material base that was also very well equipped, where I conducted studies on animals (laboratory mice) regarding the effects of some bioactive mutagenic or radioprotective substances, at ultrastructural, cytogenetic and biochemical level.

Fields of activity to which I contributed with priorities.

1. Hydrobiology.

Mentors. Acad. Prof. Dr. Ștefan Procopiu, *Gh. Asachi* Technical University, Iași, Research Group for the Nobel Prize (Bârlad 1890 - Iași, 1972).

Gh. Asachi Technical University, Iași, Research Group for the Nobel Prize, Bârlad, Faculty of Chemistry, Acad. Prof. Dr. Doc. Ștefan Procopiu (Bârlad 1890 – Iași 1972), a member in the Nobel Commission, discovered the magneton and calculated its value a year before Niels Bohr (Bohr-Procopiu magneton). I received information about the factors that interact with ionizing radiation and alter their action. We also discussed changes in the physical constants of the environment during the sun's partial eclipse of May 20, 1966. Information about the solar eclipses was received from all the specialists approached: Prof. Dr. Sergiu Carăușu, UAIC Iași, Biology Department; Prof. Dr. Dumitru Carăușu, UAIC Iași, Biology Department; Acad. Dr. Rodewald Rudescu, the Romanian Academy, Bucharest; Dr. Alexandrina Negrea, the Institute of Speleology, Bucharest; Dr. Ștefan Negrea, the Institute of Speleology, Bucharest. I also approached the staff of the Hydrobiology Department of *Stejarul* Station, Piatra Neamț: Gabriel C. Corneanu, Ionel Carăușu – Phytoplankton; Dr. Ionel Miron – benthos; Constantin Grasu – Geology; Valerian Ciaglic – Hydrology; Ioniță Ichim – Geochemistry; Dan Munteanu – trophic chains; Constantin Crăciun – Chemistry. The investigations followed two aspects: (a) the presence of new phytoplankton species (*Rotifera*, *Cladocera*, *Copepoda* or *Harpacticoida*); (b) Vertical migration of the zooplankton during the partial solar eclipse of May 20, 1966.

Research fields.

(a) **New zooplankton species in the reservoirs located on the Bistrița valley** (Poiana Teiului – Piatra Neamț sector).

(b) **Partial solar eclipse (May 20, 1966)**.

The modifications appeared during the partial solar eclipse (May 12, 1966) were analyzed from the *Emil Racoviță* research ship (A. I. Cuza University, Iași, Faculty of Biology and Geology). The observations were made in the areas of the Barrage and Ruginești golf, at various depths: 0-5 m, 5-10 m, 10-20 m, 20-30 m, 30-40 m, 40-50 m. The water samples containing zooplankton were taken at five different times: (a) one hour before the eclipse; (b) at the beginning of the eclipse; (c) during the maximum phase; (d) at the end of the eclipse; (e) one hour after eclipse. In case of each depth, we measured: brightness; water temperature; air temperature. Samples were brought to the laboratory of "Stejarul" Station, being sorted by groups of zooplankton, processed and, then, sent to systematician specialists, from the country and from abroad. The final values were processed by analysing the average and the variance.

Total solar eclipse. In the last part of the 20th century, our team conducted a study on the effects of the total solar eclipse on the biochemical and haematological changes recorded in a population of laboratory mice (*Mus musculus* L.). Investigations took place at Curtea de Argeș, where, during the eclipse (including its maximum phase), the solar disk was covered for 12 minutes, the "crown of diamond" around it being thus visible. During the eclipse, there was a clear sky (0% nebulosity). A summary of these investigations was printed in the volume **Abstracts** edited by the Romanian Society of Biophysics.

Recognition / Citation in Reference Works

a. **Citation, the presence of a new species of *Copepoda*** in the analyzed area. C. B. Wilson, 1968 – the Smithsonian National Museum of Natural History, The World of Copepoda. Monograph.

b. **The Effect of the Partial Solar Eclipse**, May 20, 1966. Similar results were obtained by the specialists of the Research Station from Sevastopol, Crimea, in the study of the same eclipse.

2. Radiobiology

Mentors: Acad. Prof. Ștefan Procopiu, UAIC Iași; *Gh. Asachi* Technical University, Iași Faculty of Chemistry; the effect of ionizing radiation and the magnetic field on cytogenetic changes in plant species; Prof. Dr. C. C. Zolyneak, UAIC Iași, Faculty of Biology and Geology, Genetics, cytogenetic changes induced by different stress factors; the interaction between stress factors; Prof. Dr. I. Tudose, UAIC Iași, Genetics, cytogenetic changes induced by stress factors; Prof. Dr. Arnold H. Sparrow, BNL Upton, USA, Chromosomes and cellular radiosensitivity; Prof. Dr. Harold H. Smith, BNL Upton, USA; Radiobiology; Prof. Dr. Andreas Lazanyi, ICB, Cluj-Napoca, radioprotectors and chemical radiosensitizers; mutagenesis in plant breeding; changes induced by radiation and other stress factors; radioprotective substances; radiotaxa, cellular sensitivity, etc.

*** Recognition: Citation in Reference Works**

- The effect of radiation on *Lactuca sativa* species; Works quoted and commented: *M. Z. Haque, M.B.E. Godward, 1984 – Genetica **65**: 179-186; *M. Z. Haque, M.B.E. Godward, 1984 – Rev. Brasil. Genet. **8**: 709-721; *M. Z. Haque, M.B.E. Godward, 1984 – Seed. Sci. Technol. **14**: 611-617.

3. Human cytogenetics.

*** Mentors**: Prof. Dr. Doc. Ioan Moraru, IML Bucharest; Prof. Dr. Doc. Alexandru Caratzalis, IML Bucharest; Prof. Dr. Ștefan Antohi, IML Bucharest: mutagen factors and the reparatory process.

*** Cytogenetics**: mentors: Prof. Dr. Arnold H. Sparrow, BNL, Upton, USA; Prof. Dr. Harold H. Smith, BNL, Upton, USA; Prof. Dr. Andreas Lazanyi, the Romanian Academy, Cluj Branch, Romania; Italy, Prof. Dr. Silvano Scannnerini; Prof. Dr. Doc. Ștefan Procopiu; Prof. Dr. C. C. Zolyneak; Prof. Dr. I. Tudose; Prof. Dr. A. H. Sparrow, Brookhaven National Laboratory, Upton, USA; Prof. Dr. Harold H. Smith, Upton, BNL, USA; Prof. Dr. Ioan Moraru, IML Bucharest (Nobel Prize for Peace, 1985); Prof. Dr. Docent Alexandru Caratazalis, UMF Bucharest; Prof. Dr. Ștefan Antohi, IML Bucharest.

Specialization Course at UMF Bucharest, Faculty of Specialization of Physicians and Pharmacists, Bucharest (1968-1970).

Priorities:

- **Chromosomes and cellular radiosensitivity:** ICV (interphase chromosome volume); Q DNA / chromosome; the presence of eu- and heterochromatin; particular types of chromosomes; the position of centromeres in the nucleus; calculating the ICV value according to the presence of eu- and heterochromatin.
- **Natural antimutagenic substances and the reparatory process in humans** (subject in the practical work at the graduation of the specialization course in Human Cytogenetics, Prof. Dr. I. Moraru, Dr. A. Caratzalis, Prof. Dr. Ștefan Antohi).
- **Adaptation of organisms to the stress factors**.

4. Paleontology and Evolution. The emergence and evolution of life.

*** Mentor:** Prof. Dr. Ioan Bucur, *Babeș-Bolyai* University, Cluj-Napoca, Department of Geology Paleontology.

*** Theoretical Aspects in Human Evolution. Ways of monkey humanization. Traces of the humanization process.** Some of the oldest traces were found on the current territory of Romania, at Bugiulesti, Tetoiu, Vâlcea. In the middle of the 19th century (1956), the archaeologist C.S. Nicolăescu-Plopșor discovered traces of the presence of pre-human species, ***Australopithecus alutensis, A. olteniensis***, characterized by the use of long bones as tools and to extract bone marrow, which was a food source. The discovery was confirmed by the famous anthropologist, Raimond Dart, arrived in Romania, in Craiova, where I had the honor of meeting him at my uncle's house, Professor Petre Fetoiu (I was a child at that moment). Subsequently, various other personalities were involved in the confirmation or the refutation of this material evidence (Dr. Dardu Nicolăescu-Plopșor from Romania, specialists from Cluj-Napoca, Belgium, etc.). Based on these material traces, some anthropologists considered the traces of the pre-human activity discovered at Bugiulești, also belong to other species (***Australanthropus carpathicus,*** Dardu Nicolăescu-Plopșor). These traces are believed to be around 1,800,000-2,000,000 years old and are preserved in the collection of the Museum of Natural Sciences (History) from Craiova.

5. Cellular Biology

Mentors and collaborators: Prof. Dr. Constantin Craciun, UBB Cluj-Napoca, Acad. Prof. Dr. Gheorghe Benga, UMF Cluj-Napoca. In this field, the Nobel scientist George Emil Palade affirmed himself.

Research fields:

- Ultrastructural features under normal and stress conditions;
- Cellular structures involved in the adaptation processes to environmental factors: Loose nucleolar bodies (LNB's), body-guard, cell division
- Water channels and ions channels, etc.

Research priorities:

- ultrastructural changes in simulated extraterrestrial environment;
- cell division process;
- ultrastructural changes in the process of adaptation of plant and animal populations to stress;

6. Biotechnologies

*** Mentors**: Prof. Dr. Dorina Cachița-Cosma, Vasile Goldis West University of Arad; Prof. Dr. Doru Pamfil, USAMV Cluj-Napoca, Prof. dr. Ana Roșu, USAMV Bucharest.

***** Cell cultures and vegetal tissues on classical environments.

*** Bioactive substances:** Prof. Dr. Gabriel Racz, UMF Targu Mures; Prof. Dr. Ursula Stanescu, UMF Iași; Prof. Dr. Emilian Grigorescu, UMF Iași; Prof. Dr. Paul Atyim, UVVG-Arad, Satu Mare Branch, etc.

*** Priorities**:

- new regulators of growth in processes of organogenesis and callogenesis;
- new gelification substances;
- explant response under simulated extraterrestrial conditions;
- cultivated plant populations adapted to stress factors: wastes with a high content of heavy metals and radionuclides, mineral salts, etc.
- environmental factors involved in the development of plant explants: magnetic field; electro-magnetic field; acceleration force; force of gravity; different bioactive substances.

*__Citations, international recognition__: E. B. Herman, 1995 - Agricel Report, **25** (5): 33.E. B. Herman, 1997 - Agricel Report, **30** (3): 36.

Achievements in scientific research

*** Exchanges of specialists**.

* Reputed scientists accepted the invitation to lecture in Craiova:

Prof. Dr. Mike Smith, North Carolina University USA; Prof. Dr. Hideyuki Furukawa, Nagoya University; Prof. Dr. Junko Ebata, Osaka University, Japan; Prof. Dr. Nelo Bagni, Bologna University, Italy; Prof. Dr. Silvano Scanerini, Torino Degli Study University; Prof. Dr. Italo Barray, Ferarra University, Italy; Prof. Dr. Traianos Iupsanis, Aristotle University, Greece; Prof. Dr. Aristotle Scaltsoyanis, Aristotle University, Greece; Prof. Dr. Ivan Iliev, Sofia Forrest University, Bulgaria; Acad. Prof. Dr. Boris Matienco, Moldova Science Academy, Chişinău; .Prof. Dr. Petru Tarhon, Chişinău University, Prof. Dr. Sirichai Kanlayanarat, Bankok, Thailand, Prof. Dr. Valentin Boju, Montreal University, Canada, etc. (Figs. 1-4)

Figure 1. Prof. dr. Corneanu Gabriel in his lab in Craiova (2008).

Figure 2. Prof. dr. Gabriel Corneanu and Acad. Dan Munteanu in the Scientific Conference on Environment protection (Râmnicu Vâlcea, 2008).

Figure 3. Ass. prof. Maria Tzakira, Prof. Gabriel Corneanu, Prof. dr. Ivan Iliev, Prof. Dr. Aristotle Scaltoyanes, Prof. dr. Corneanu Mihaela in the Fifth International Conference "Propagation of Ornamental Plants" (Sofia, Bulgaria, 2007).

Figure 4. Prof. dr. Corneanu Gabriel, Prof. dr. Corneanu Mihaela, Prof. Dr. Sirichai Kanlayanarat (vice-president ISH) and dr. Wilawan Kanlayanarat in the International Conference "Horticulture in quality and culture of life" (Lednice, Czech Republic, 2014).

* From Romania, different specialists also held conferences: from Cluj-Napoca (Prof. dr. Nicolae Coman, Prof. dr. Dorina Cachiţa-Cosma, Prof. dr. Constantin Crăciun), from Iaşi (Prof. dr. Gheorghe Mustaţă, Prof. dr. Ion I.

Bâra), from Bacău (Prof. dr. Gogu Ghiorghiță), from Bucharest (Prof. dr. Ana Roșu, dr. Ioan Blada, Prof. dr. Gheorghe Mohan), from Constanța (Prof. dr. Rodica Bercu), from Timișoara (Prof. dr. hc. Gallia Butnaru), etc.

- We were also invited to hold conferences in other countries: University of Chișinău, Moldova; Osaka University, Japan; Aristotle University, Thessaloniki, Greece; Sofia University of Forestry, Bulgaria; University of Ferarra, University of Torino, Italy. Prof. Dr. Silvano Scanerini offered us a very nice surprise at Lions Club from Torino. The all assistance in full dress, with their hands on their hearts have intonated the club hymn for us.
- In addition, we had the honor of being part of international teams, alongside reputed researchers, to carry out research projects.
- At a national level, I have run numerous research grants.

Collaboration with specialists from territorial research associations (forestry, teaching, medical, environment protection and other), represents a source for research and knowledge of organism adaptation.

Specialist: Prof. Dr. Constantin Nețoiu, Prof. Dr. Viorel Lazăr, Prof. Dr. Petre Ploaie.

CONCLUSIONS

Individual development is dependent on the efforts made to obtain solid theoretical and practical knowledge in particular areas of biological science. The synthesis of these actions is presented in the parables transmitted by the forerunners. Different specializations can be followed after graduation. Examples of the activity carried out are accompanied by numerous milestones: the field of activity, the mentors who contributed to their formation, the priorities and the special achievements, their capitalization, and so on.

ACKNOWLEDGMENTS

In the achievement of this synthesis regarding the monitoring of the activity of young specialists who, meanwhile, became reputable scientists, the author has received the support of numerous scientists he has known since the mid-twentieth century. To all these mentors and collaborators, the author thanks for their trust and support over time.

Beside those reputable mentors and scientists, the author addresses sincere thanks to the numerous young people who have surrounded and helped him in his laboratory activities and in the field. Among them, I would like to mention the teams of specialists from the University of Craiova (Faculties of Agriculture and Horticulture), faculties and research institutes from Iași, Cluj-Napoca, etc.

Special thanks to Prof. Dr. Mihaela Corneanu, Banat University of Agricultural Sciences and Veterinary Medicine King Michael I of Romania from Timișoara (my wife and collaborator). We have been working together, full of hopes, looking for new achievements and opening new roads and horizons in science, during the last decades.

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RECOMMENDATIONS
regarding the elaboration of the papers for the scientific journal
"Oltenia. Studii şi comunicări. Ştiinţele Naturii"

The journal is edited by the Oltenia Museum Craiova and it publishes original papers in the fields of vegetal and animal biology, ecology-environment protection, mineralogy-palaeontology, as well as scientific reports, reviews, anniversary or commemoration papers.

It appears annually, it is ISI indexed (http://science.thomsonreuters.com/cgi-bin/jrnlst/jlresults.cgi?PC=MASTER &Word=oltenia**) and accredited by CNCSIS as a B+ Journal.**

I. Structure (format) for original papers, scientific reports and reviews:

A	***Original papers***	will be structured according to the information rendered in the Table 1.
B	***Scientific reports***	will be structured according to the author's (authors') preferences, but it has to include abstract and key words, both in English and Romanian.
C	***Reviews***	there will be mentioned: author (authors) of the book (name and first name – CAPITAL LETTERS), comma, the title of the book, lowercase letters (Italic), publishing house, publishing location, year, number of pages. Use a free space and then render the text of the review with as fewer paragraphs as possible and the same characters as in the case of original papers.

Table 1.

STRUCTURE OF THE PAPER	CHARACTERISTICS	OBSERVATIONS
TITLE	capital letters, 12 pt., bold, centred	
two spare rows (12 pt.) between the title and the name of the author/s		
Author/Authors	name, capital letters, first name, noncapital, 11 pt., bold, normal, aligned right	between two or many authors, use comma
One spare row, 10 pt.		
Abstract (English)	from the beginning of the line, without tab, 9 pt., bold, normal	the abstract will be written with 9 pt., normal, maximum 300 words
One spare row, 9 pt.		
Keywords (English)	from the beginning of the line, without tab, maximum 5 words, 9 pt., normal	
One spare row, 9 pt.		
Rezumat (Romanian)	from the beginning of the line, without tab, 9 pt., bold, normal	**Complete translation of the title in Romanian** (no capital letters, except for the first letter of the title; 10 pt., bold). The content of the abstract – 9 pt., normal, maximum 300 words
One spare row, 9 pt.		
Cuvinte cheie (Romanian)	from the beginning of the line, without tab, maximum 5 words, 9 pt., normal	
One spare row, 14 pt.		
INTRODUCTION	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
MATERIAL AND METHODS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
RESULTS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
DISCUSSIONS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
CONCLUSIONS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
ACKNOWLEDGEMENTS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
REFERENCES	10 pt. (capital letters, bold)	content – see bibliographical references
One spare row, 10 pt.		
Bibliography enumeration	see the detailed explanations at the references heading	
Personal data	Name and surname – 8 pt., bold, normal, centred Institution and e-mail address – 8 pt., normal, centred	

Other details related to the papers:

Publishing language	English
Page format	A4 (21 x 29.7 cm), margins: top – 2.5 cm; bottom – 2.0 cm; left – 2.0 cm; right – 2.0 cm; gutter – 0 cm; header, footer – 1.27 cm. The papers will be elaborated in Microsoft Word, justified; font: Times New Roman, 10 pt., normal; single space.

- Latin names (genus, subgenus, species, subspecies) will be written with italic characters; - Suprageneric names are **not** written with italic characters. The same procedure is used when they are mentioned within figures, graphs and tables.
The first mention of a taxon in the text will be followed by the taxon author's name and the publishing year of the description, according to the zoological nomenclature code (e.g.: *Cossus cossus* (Linnaeus 1758), afterwards, it will be used abbreviated.
The name of the authors quoted in the text will be written **normal, capital letters,** while the names of the authors of the taxa will be written **normal, lowercase letters**
For the names of Romanian authors and settlements diacritics must be used.
The materials sent for publication (printed and in electronic format) has to be between 2 and 8 pages (the number of pages must be **even**).

II. References

- **References** in the text (quotation) includes only the author's/authors' names (CAPITAL LETTERS) and publishing year. For example:
  - when it is a single author: IONESCU (1965) or (IONESCU, 1965);
  - when there are two authors, it is used "&": RĂDULESCU & SAMSON (1990) or (RĂDULESCU & SAMSON, 1990);
  - when there are more than two authors: IONESCU et al., (1992) or (IONESCU et al., 1992);
  - in case there are many papers written by the same author/authors, published in the same year, use the letters a, b, c, etc. after the year (e.g.: IONESCU, 2000; IONESCU, 2000a, …, 2000g);
  - authors are rendered alphabetically and, in case there are many papers written by the same author, they are introduced chronologically.

- **References** will include **only** the papers quoted in the text (10 pt.), alphabetically rendered, without numbers, as it follows:

- author (CAPITAL LETTERS), publishing year (normal), (**do not use** comma between the author and the publishing year or between the name and first name of the same author; use comma between different authors, when there are more than two), *the title of the paper* (*italic*), name of the journal, publishing house, volume number (bold), the number of fascicle (normal), number of pages (normal). When there are two authors, use "**&**"; if there are many authors, mention **all** of them. The ladies' first name is completely written; for gentlemen, use only the first letter.

The reference titles will be aligned as it follows: the first line from the beginning (no tab), the second at 1.27. For example**:**

EXEMPLE
Book reference:
BOŞCAIU N. 1971. *Flora şi vegetaţia Munţilor Ţarcu, Godeanu şi Cernei*. Edit. Academiei R. S. R. Bucureşti. 300 pp.
Paper published in a journal:
GULII V. & PAMUJAC M. 1994. Elemente ale protecţiei integrate a culturilor agricole de dăunători şi boli. *Protecţia integrată a plantelor*. Edit. „Ştiinţa". Chişinău: 112-118. STAN MELANIA & BACAL SVETLANA 2006. New contributions to knowledge stafilinidelor (Coleoptera: Staphylinidae) of the landscape reserve "Codrii Tigheci" (Moldova). *Oltenia. Studii şi comunicări. Ştiinţele Naturii*. Muzeul Olteniei Craiova. **22**: 155-159.
Reference to a part of a collective paper; volume (with editors):
IFTIME Al. 2005. Amfibieni şi Reptile. In: Botnariuc & Tatole (Eds.) *Cartea Roşie a Vertebratelor din România*. Edit. Academiei Române. Bucureşti: 1-325.
Papers presented at scientific manifestations and published in a volume without editors:
CIOCHIA V. & STANCĂ-MOISE CRISTINA. 2001. Contributions to the knowledge of the Macrolepidoptera from natural complex "Dumbrava Sibiului". *Sesiunea Ştiinţifică dedicată împlinirii a 75 de ani de la înfiinţarea Staţiunii Biologice Marine „Prof. Dr. I. Borcea"*. Agigea-Constanţa. 19-20 octombrie 2001: 125-131.
Official publications (laws, decrees, official reports):
***. România. Legea nr. 13 / 1993 pentru aderarea României la Convenţia privind conservarea vieţii sălbatice şi a habitatelor naturale din Europa, adoptată la Berna la 19 septembrie 1979. *Monitorul Oficial al României*. An V, nr. 62/25 martie 1993. Bucureşti: 1-20.
PhD thesis:
COSTACHE I. 2005. *Flora and vegetation Motru River Lower Basin.* Ph. D. Thesis, University of Bucharest. Romania. 290 pp., 8 Pl.
Web pages:
Muzeul Olteniei Craiova. Secţia Ştiinţele Naturii. *Oltenia. Studii şi comunicări. Ştiinţele Naturii.* (onlline). 2011. Publisher: Museum of Oltenia Craiova, Romania. www.olteniastudii.3x.ro (accessed: May 8, 2012).

Entire electronic document or service (data base):
***. Fauna Europaea: Chironomidae. In: *Fauna Europaea: Chironomidae, Diptera, Nemathocera*. (Ed. H. de Jong) Fauna Europaea version 1.5, http://www.faunaeur.org. (accessed: June 23, 2012).
E-book:
AHMADJIAN V. 1967. *The Lichen Symbiosis*. Blaisdell Publishing Company. Massachusetts. Available from: http://books.google.ro/books?id=at7uXMn8iMC&printsec=frontcover&hl=ro&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false. 152 pp. (accessed: January 15, 2013).
Electronic publication (papers):
DANILEVSKY M. L. 2007. A check-list of Longicorn Beetles (Coleoptera, Cerambycoidea) of Europe. Available online at: http://www.coleoptera-literatura.ic.cz./literatura/checklist_cerambycidae_2007.doc. (accessed: May 20, 2009).
Note: The papers published with other characters than the Latin ones, will be re-written with Latin characters, both in the text and at references, mentioning the original language of publication between square brackets at the end:
ALEXANDROVICH O. R. 1995. Reconstruction of the ways of the ground beetles (Coleoptera, Carabidae) fauna forming at the West of the Russian plain. In: I. K. Lopatin, Pisanenko A. D., Shklyarov L. P. (Eds.). *Fauna and taxonomy: Proceedings of Zoological Museum of the Byelorussian University*, Minsk: Nauka Tekhnika. **1**: 52-68. [In Russian].

III. Illustration

▪ Images (white/black or colour), tables, graphs and maps are inserted into the manuscript, but **the original versions have to be sent also separately**: high contrast photographs, electronic images in TIFF format at a minimum resolution of 300 dpi.	
▪ The references to the illustrations (tables, images, photographs) will be made in the text as it follows: (Fig. 1), (Figs. 1a, b), (Figs. 3; 5); (Table 1); (Photo 1).	
▪ **Graphs must be achieved in Microsoft Excel.**	
The title of a table (in English) will be placed above the table (aligned right), 9 pt., normal.	The title of a figure, photo, map (in English) will be placed below, centred, 9 pt., normal.
▪ For photographs and maps: - It will be mentioned the word 'original' in case it is achieved by the author/'s of the paper; - It will be mentioned the name of the author/'s between brackets in case it is achieved by another or other persons; - It will be mentioned the web source (if it is the case), which has to be entirely rendered at references, also mentioning the access date.	
Legend is placed below a table, graph, etc., 8 pt., normal (English)	
!!!!!!For graphs and diagrams, use white, black, grey, different tones and textures. **They will not be published in colours.**	

Illustrations references (tables, images) (in English), will be made in the text as it follows: (Fig. 1), (Fig. 1a, b), (Figs. 3; 5); (Table 1). The title of a figure (in English), will be centered below the figure; as for tables, put it above (aligned right), both 9 pt., normal. Examples: a) Table 1. List of the identified staphylinids from the Lower Dniester. b) Figure 1. Distribution of the butterfly *Papilio machaon* L.

The paper will be accepted for publishing if:

- **it respects** the aforementioned **requirements**;
- **it acquires a favourable review** from the scientific reviewers of the journal;
- **it is sustained** within the framework of the International Conference "*Museum and scientific research*";
- **the publication fee is paid**;
- **it is respected** the deadline, **March 31** of the current year.

The editorial board *has the right to reduce the number of figures and photos (if there are too many as compared to the text of the paper or if they do not correspond to the requirements) and not to accept papers sent after deadline,* **March 31, 2019**.

With all the respect for the authors, papers that do not correspond to the recommendations will be sent back.

The responsibility of the scientific content of the papers depends entirely on the authors. Authors must revise the papers reviewed by the reviewers.

The Editorial Board

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