MUZEUL OLTENIEI CRAIOVA

OLTENIA
STUDII ȘI COMUNICĂRI
ȘTIINȚELE NATURII

Vol. XXXII/1

CRAIOVA 2016

OLTENIA

STUDII ŞI COMUNICĂRI ŞTIINŢELE NATURII

Oltenia Journal for Studies in Natural Sciences

(Proceedings of the 23rd International Conference of the Oltenia Museum)

Tom. XXXII, No. 1 / 2016

MUZEUL OLTENIEI CRAIOVA

Oltenia. Studii şi comunicări. Ştiinţele Naturii

ISSN 1454 – 6914

2016, Tom. 32, no. 1

Cover Image: *The Building of the Section of Sciences of Nature of the Museum of Oltenia Craiova*

Journal coverage:
- Zoological Record (by Thomson Reuters, former ISI): http://science.thomsonreuters.com/cgi-bin/jrnlst/jlresults.cgi?PC=MASTER&Word=oltenia
- CNCSIS (The National University Research Council, Romania) – **„B+" category**
- SCIPIO: http://scipio.ro/web/oltenia.-studii-si-comunicari.-stiintele-naturii

Available On-line:
Oltenia. Studii şi comunicări. Ştiinţele Naturii with full text articles available on-line: http://biozoojournals.ro/oscsn/; http://www.olteniastudii.3x.ro/; http://biozoojournals.ro/

Publisher: Museum of Oltenia Craiova, Str. Popa Şapcă, No. 8 – 200 410, Craiova, Romania

Financial Support by: The Council of Dolj County, Romania

CONTENTS / CUPRINS

I. GEOLOGY / GEOLOGIE

Ion STELEA - Comments on the Supragetic Nappe in the Central-Eastern South Carpathians / Observații asupra Pânzei Supragetice din Carpații Meridionali central-estici... 7

Nicolae TRIF, Davit VASILYAN, Aurelian POPESCU - Fossil fish remains from the Romanian of Podari, SW of Romania. The first report / Pești fosili din Romanianul localităţii Podari, SV României. Prima semnalare.......................... 12

Vlad Aurel CODREA, Bogdan Gabriel RĂȚOI, Laurențiu URSACHI, Cristina FĂRCAȘ - A large deinothere (*Mammalia: Proboscidea*) in the Late Miocene of the Moldavian Platform at Huși (Vaslui County) / Un deinother (*Mammalia: Proboscidea*) de talie mare din Miocenul Superior al Platformei Moldovenești la Huși (Județul Vaslui)... 20

II. VEGETAL BIOLOGY / BIOLOGIE VEGETALĂ

Maria GONCEARIUC, Zinaida BALMUŞ, Ludmila COTELEA - Genetic diversification of *Salvia sclarea* L. quality by increasing the storage capacity of the essential oil / Diversificarea bazei genetice a calităţii la *Salvia sclarea* L. prin creşterea capacităţii de acumulare a uleiului esenţial.................................. 29

Luminiţa ROMAN, Anamaria Delia HOSU, Cristiana Andreea VASILIU, Horațiu ROMAN, Gențiana PREDAN, Ilda CZOBOR, Grigore MIHĂESCU - Potentiation of antibiotics by the hydroethanolic extract of *Juglans nigra* L. / Potențarea antibioticelor de către extractul hidroetanolic de *Juglans nigra* L. 37

Nadejda MIHNEA, Galina LUPAȘCU, Sofia GRIGORCEA, Irina ZAMORZAEVA – The reaction of some valuable tomato sorts (varieties) to filtrates of *Fusarium* spp. and *Alternaria alternata* cultures / Reacția unor soiuri valoroase de tomate la filtratele de cultură *Fusarium* spp. și *Alternaria alternata*.. 41

Sofia GRIGORCEA, Galina LUPAȘCU, Nadejda MIHNEA, Irina ZAMORZAEVA - Causative agents of brown staining of leaves and root rot of tomatoes in conditions of the Republic of Moldova / Agenţii cauzali ai pătării brune a frunzelor şi putrezirii rădăcinii la tomate în condiţiile Republicii Moldova... 45

Beatrice Agneta SZILAGYI, Florin STĂNICĂ, Silvana Mihaela DĂNĂILĂ-GUIDEA - Flowering of *Asimina triloba* (L.) Dunal in the conditions of Transylvania / Înflorirea la *Asimina triloba* (L.) Dunal în condiţiile din Transilvania.. 50

III. ANIMAL BIOLOGY / BIOLOGIE ANIMALĂ

III.a. INVERTEBRATES VARIOUS / NEVERTEBRATE DIVERSE

Alexandra Ioana HERLE, Severus-Daniel COVACIU-MARCOV, Sára FERENȚI - Past industry vs. nature: which one influences more the terrestrial isopod assemblages from a town in western Romania? / Industria din trecut sau natura: cine influenţează mai mult comunităţile de izopode terestre dintr-un oraş mic din vestul României? 55

Liana Mihaela FERICEAN, Mihaela CORNEANU - The variability of some phenotypic features and life cycle in two *Aphis pomi* populations from western Romania / Variabilitatea unor caractere fenotipice şi ciclul de viaţă în două populaţii de *Aphis pomi* din vestul României.. 61

Dina ELISOVEŢCAIA, Valeriu DERJANSCHI - Hibernation of the predatory stink bug *Perillus bioculatus* F. (Hemiptera, Pentatomidae) under laboratory conditions / Hibernarea ploşniţei răpitoare *Perillus bioculatus* F. (Hemiptera, Pentatomidae) în condiţii de laborator... 67

Lidia GAVRILIŢA - Interspecific competition of *Trichogramma* sp. at its mass rearing / Concurenţa interspecifică la *Trichogramma* sp. la înmulţirea în masă................ 71

Gheorghe MANIC - Notes on certain new genera and species of Pteromalidae (Hymenoptera, Chalcidoidea, Pteromalidae) identified within Plaiul Fagului Nature Reserve / Note privind unele genuri şi specii noi de Pteromalidae (Hymenoptera, Chalcidoidea, Pteromalidae) semnalate în Rezervaţia naturală Plaiul Fagului.. 77

Mircea VARVARA - Relative abundance and dominance of families of epigeic coleoptera (Order Coleoptera) in maize fields, Brăila (Brăila County) and Moldova (Romania) 1978-2010 (12 seasons) / Abundenţa relativă şi dominanţa familiilor de coleoptere (Order Coleoptera) în culturile de porumb, Brăila (Judeţul Brăila) şi Moldova (România) 1978-2010 (12 sezoane)............ 80

III.b. VERTEBRATES / VERTEBRATE

Mirela Sabina RIDICHE – Qualitative and quantitative analysis of the avifauna within the area of International Airport Craiova, Romania / Analiza calitativă și cantitativă a avifaunei din zona Aeroportului Internaţional Craiova, România.. 91

Adrian MESTECĂNEANU, Radu GAVA - A year of ornithological observations on Vâlcele, Budeasa, Bascov, Piteşti, and Goleşti reservoirs from ROSPA0062 Lacurile de acumulare de pe Argeş / Un an de observaţii ornitologice pe lacurile de acumulare Vâlcele, Budeasa, Bascov, Piteşti şi Goleşti din ROSPA0062 Lacurile de acumulare de pe Argeş.. 97

Ovidiu PAVEL, Ioan COROIU - Seasonal dynamics of bat fauna in Comarnic Cave, Romania / Dinamica sezonieră a faunei de lilieci din peştera Comarnic, România.. 110

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

Alina VLĂDUŢ - Thermal Comfort within Oltenia Plain / Confortul termic în Câmpia Olteniei.. 115

Olivia CIOBOIU, Carmen Mădălina CISMAŞIU - Structural and functional characteristics of biocoenoses in the flooded area of the Danube for the biotope reconstruction from industrial contaminated habitats / Caracteristicile structurale și funcționale ale biocenozelor din zona inundabilă a Dunării pentru reconstrucția biotopului din habitate contaminate industrial............................... 122

Oana Alexandra DRĂGHICEANU, Liliana Cristina SOARE, Monica POPESCU - The influence of nickel and cadmium compounds on gametophyte differentiation in *Dryopteris affinis* (Lowe) Fraser-Jenkins and *Dryopteris filix-mas* (L.) Schott / Influența compușilor cu nichel și cadmiu asupra diferențierii gametofitului la *Dryopteris affinis* (Lowe) Fraser-Jenkins și *Dryopteris filix-mas* (L.) Schott... 131

Simona NEAGU, Roxana COJOC, Ioana GOMOIU, Mădălin ENACHE - The production of lipases and decarboxylases by halophilic bacteria able to grow in the presence of waste glycerol / Producerea de lipaze și decarboxilaze de către bacterii halofile capabile să se dezvolte în prezența glicerolului rezidual........... 137

Larisa FLORESCU, Rodica CATANA, Mirela MOLDOVEANU - How do the stagnant and flowing systems influence the production of rotifers in the Danube Delta? / Cum influențează apele stagnante și curgătoare producția rotiferelor în Delta Dunării? 143

Olivia CIOBOIU, Carmen Mădălina CISMAȘIU - The impact of anthropogenic factors on the biocenotic reconstruction of industrial ecosystems from Oltenia Plain / Impactul factorilor antropici asupra reconstrucției biocenotice a ecosistemelor industriale din Câmpia Olteniei.. 149

Diana CUPȘA, Camelia GOILEAN - Studies upon the dynamics of the macrozoobenthic invertebrate communities from Valea Roșia River (Bihor County) / Studii privind dinamica asociațiilor de nevertebrate macrozoobentice din râul Valea Roșia (Județul Bihor).. 159

Nensi LALAJ, Irakli PRIFTI - Balance of produced waste in lagoons of the former oil refinery in Kuçova town, Albania / Balanța deșeurilor produse în lagunele fostelor rafinării petroliere din orașul Kuçova, Albania...................................... 166

Minodora MANU, Marilena ONETE - Comparative ecological characterization of the soil mite populations (Acari: Mesostigmata) from some anthropogenic ecosystems, Romania / Caracterizarea ecologică comparativă a populațiilor de acarieni edafici (Acari: Mesostigmata) din câteva ecosisteme antropice, România.. 173
.

Maria-Mihaela ANTOFIE - Potato resistance to cyst nematodes - peculiarities for Romania / Rezistența cartofului la nematozii cu chiști - particularități pentru România.. 181

Camelia SAVA SAND - *Gentiana lutea* L. - Considerations for a successful protocol on micropropagation / *Gențiana lutea* L. - Considerații privind un protocol de succes pentru micropropagare.. 187

Alexandru-Ionuţ PETRIŞOR - Assessing the efficiency of the Romanian Natural Protected Areas in conserving priority habitats / Evaluarea eficienţei ariilor protejate din România în conservarea habitatelor prioritare................................ 191

V. SCIENTIFIC ESSAYS / REFERATE ŞTIINŢIFICE

Constantin ENACHE - A citizen of Craiova, the geologist Ludovic Mrazec / Un cetățean al Craiovei, geologul Ludovic Mrazec.. 195

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"*.. 197

COMMENTS ON THE SUPRAGETIC NAPPE IN THE CENTRAL-EASTERN SOUTH CARPATHIANS

STELEA Ion

Abstract. Within the double-arcuate orogen of the Romanian Carpathians, the central-eastern South Carpathians connect the two bends formed as a result of the Cretaceous and Miocene compressions exerted by the eastward drifting of the pre-Apulian block inside the orogenic arc and the synchronous westward drifting of the outside Moesian block. The parallel sliding of the two crustal blocks involves a transcurrent tectonic regime in the central-eastern South Carpathians, routinely seen as a stack of thrust nappes. The existence of a large Supragetic Nappe on this orogenic segment is questioned in geodynamic context.

Keywords: South Carpathians, geodynamic context, Supragetic Nappe.

Rezumat. Observaţii asupra Pânzei Supragetice din Carpaţii Meridionali central-estici. În orogenul dublu arcuit al Carpaţilor româneşti, Carpaţii Meridionali central-estici racordează cele două curburi formate sub acţiunea compresiilor exercitate în timpul Cretacicului şi Miocenului de migrarea spre est a blocului preapulian de la interiorul arcului carpatic, simultan cu migrarea spre vest a blocului moesic de la exterior. Alunecarea paralelă a celor două blocuri crustale implică un regim tectonic transcurent în Carpaţii Meridionali central-estici, văzuţi de regulă ca o stivă de pânze de şariaj. Existenţa unei Pânzei Supragetice de amploare este pusă în discuţie în context geodinamic.

Cuvinte cheie: Carpaţii Meridionali, context geodinamic, Pânza Supragetică.

INTRODUCTION

The Supragetic Nappe is the most controversial tectonic unit in the South Carpathians. In contrast to the Mid Cretaceous Getic Nappe (MURGOCI, 1910a), practically unchanged until today, the Supragetic Nappe has been from the start an inconsistent tectonic concept, difficult to define despite many attempts.

A nappe over the Getic Nappe was defined for the first time by POPESCU-VOITEŞTI (1911) in the Iezer-Leaota Mountains area, later being extended over the internal margin of the South Carpathians (POPESCU-VOITEŞTI, 1929). This nappe was redefined on the Olt Valley by SCHMIDT (1930), and STRECKEISEN (1934) gave it another cartographic contour by including the entire eastern South Carpathians in a large Upper Nappe. Contested by GHIKA-BUDEŞTI (1940) and ignored for a while, the Upper Nappe has been once again redefined by CODARCEA et al. (1967) under the name of Supragetic Nappe, with a reduced cartographic contour by excluding the Iezer-Leaota Mountains from the nappe body. Finally, the north Sebeş-Cibin Massif was also excluded from the nappe by SĂNDULESCU (1980, 1984). In its current contour (Fig. 1), the Supragetic Nappe in the central-eastern South Carpathians includes the eastern border of the Lotru Mountains (the western slope of the Olt Valley), the Făgăraş and the Cozia Mountains.

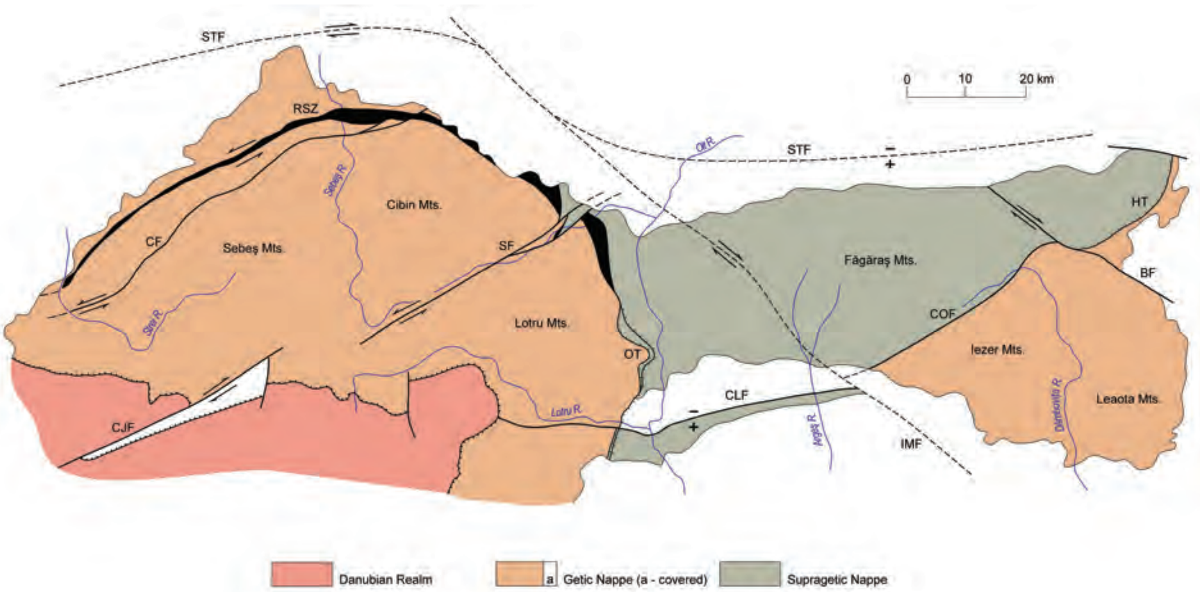

Figure 1. Main Alpine tectonic lineaments in the central-eastern South Carpathians: STF-South Transylvanian Fault; RSZ-Răşinari Shear Zone; CF-Cioclovina Fault; OT- Olt Valley thrust faults; CJF-Cerna-Jiu Fault; SF-Sadu Fault; IMF-Intramoesian Fault; BF-Bârsa Fault; HT-Holbav thrust; COF- Curmătura Oticului Fault CLF-Cozia-Lotru Fault.

The Alpine nappe-structure of the central-eastern South Carpathians is however difficult to explain given their position within the double-arcuate Carpathian belt. According to SĂNDULESCU (1980, 1984), the two bends of the Romanian Carpathians are the result of the Cretaceous and Miocene compressions on E-W direction exercised by the westward drifting of the Moesian block outside of the orogenic arc, at the same time with the eastward drifting of the pre-Apulian block inside the orogenic arc and the subsequent clockwise rotation of its transport direction. Moving parallel to the central-eastern South Carpathians, the two continental blocks have exerted compressions only on the orogenic segments in front of them, i.e. the Eastern Carpathians and the western South Carpathians.

Now widely accepted, this geodynamic scenario requires a transcurrent tectonic regime along the orogenic segment connecting the bends and a subsequent transpressive one in the north-eastern Făgăraş Mountains, closer to Vrancea bend. Consequently, a revaluation of the compressional tectonics role in the Alpine structuring of the central-eastern South Carpathians is necessary, the more so as no structural studies have been done on the assumed thrust planes in this region, the nappes being defined on the basis of the stratigraphic and lithofacial characters of their sedimentary cover. This paper is a short history of the Supragetic Nappe as a tectonic concept, commented from the perspective of the Alpine structures in the crystalline basement of the central-eastern South Carpathians.

THE TRANSCURRENT TECTONICS IN THE SOUTH CARPATHIANS

STILLE (1953) was the first author with an overall mobilistic vision on the Romanian Carpathians geotectonics. Like MURGOCI (1910a), Stille considered that the Getic Nappe emplacement started in the Palaeozoic as a result of the Moesian Platform underthrust beneath the western South Carpathians. A predicted deep fault on its northern margin allowed the platform translation towards WNW. This hypothesis was based on the observation that the pre-Alpine basement of the western South Carpathians was regenerated during the Alpine orogenesis, while the basement of the central-eastern South Carpathians preserved its Hercynian structure. That is true, except the northern slope of the Făgăraş Mountains, restructured by tight-folding in low-grade metamorphic conditions. Subsequent geophysical researches confirmed the Moesian Platform displacement towards WNW (e.g. AIRINEI, 1983) and the existence of some fault systems that accommodated this movement (e.g. VISARION et al., 1988).

PAVELESCU & NITU (1977) distinguished two stages of different tectonic regimes in the evolution of the Carpatho-Balkan chain, first of Cretaceous compressions, when the thrust nappes were emplaced, and second of post-Cretaceous strike-slips, when the pre-existing structures were aligned parallel to the chain axis, arcuate around the Moesian Promontory. The strike-slips in the South Carpathians are explained by the westward translation of the Moesian Platform simultaneously with the eastward translation of the crustal blocks inside the Romanian Carpathians. One of the most important transcurrent faults in the western South Carpathians is the Cerna-Jiu Fault, with dextral strike-slip of 30-40 km during the Eocene (BERZA & DRĂGĂNESCU, 1988).

In his geodynamic model, SĂNDULESCU (1980, 1984) does not make explicit references to the transcurrent tectonics of the South Carpathians, but admits that the transport of the continental blocks in this area was accommodated by the South Transylvanian, Intramoesian, and Peceneaga-Camena transcurrent faults. The dextral strike-slip on the South Transylvanian Fault also involves a transcurrent tectonic regime on the northern margin of the central-eastern South Carpathians. A transcurrent tectonic lineament on the northern Sebeş-Cibin Massif is Răşinari Shear Zone (Fig. 1). This is a pre-Alpine vertical fault intermittently reactivated as sinistral strike-slip fault from the Early Jurassic to the Mid Cretaceous (STELEA, 2000). The net horizontal displacement on the central segment of the shear zone is of 12 km.

However, the folded structure of the crystalline basement in the Făgăraş Mountains east of the Intramoesian Fault shows a transpressive tectonic regime decreasing from NE (tight-folded area) to SW (gently-folded area). Following the Late Cretaceous rotational motions of some tectonic blocks inside the pre-Apulian area (SURMONT et al., 1990), the eastern segment of the South Transylvanian Fault was probably locked, the dextral strike-slip on its western segment being transferred on the Intramoesian Fault, oblique to the orogen axis. Because the Intramoesian Fault is a long-lived fault, and still active, the Cretaceous horizontal displacement is difficult to quantify.

THE SUPRAGETIC NAPPE IN THE CENTRAL-EASTERN SOUTH CARPATHIANS

The first Supragetic Nappe was the Bucegi Conglomerates Nappe, of Miocene age, presumed by POPESCU-VOITEŞTI (1911) in the eastern South Carpathians. Based on stratigraphic and structural correlations between the Tertiary deposits in the Getic Depression and the East Carpathians, the author included in this nappe the Iezer-Leaota Mountains and the north-eastern part of the Făgăraş Mountains.

Later, POPESCU-VOITEŞTI (1929) separated three nappes within the Getic Nappe, the upper one including, beside the Iezer-Leaota Mountains, the north Sebeş-Cibin Massif and the entire northern part of the Făgăraş Mountains. All the vertical faults in the South Carpathians were considered as overthrust except for those in the north Sebeş-Cibin Massif and north Iezer Mountains, correctly seen as uncertain thrusts. The nappe was argued by an inaccurate observation regarding the lower metamorphic grade of its crystalline basement as compared to the Getic Nappe basement, which is only partly true. This second Supragetic Nappe was initially correlated with the Bucegi Conglomerates Nappe and the Bucovinic Nappe in the East Carpathians, then only with the Bucovinic Nappe (POPESCU-VOITEŞTI, 1942).

On similar criteria, SCHMIDT (1930) theorized the existence of a Supragetic Nappe in the eastern South Carpathians in order to explain the differences between the crystalline basement of the Lotru Mountains, then considered monometamorphic, and the polymetamorphic basement of the Făgăraş Mountains. The thrust fault outcrops on the Valley of Stan, where the augen gneisses of the Cumpăna Series are overlaying on the Getic Nappe. The thrust fault was continued northward on the north-eastern margin of the Sebeş-Cibin Massif up to Căpâlna Village on the Sebeş Valley.

At that time, the author was referring to the polymetamorphism of the Făgăraş Series in the northern Făgăraş Mountains, where a low-grade dynamic metamorphism is superimposed on the pre-Alpine medium-grade regional metamorphism. The tectonic relationship on the Valley of Stan involves the gently-folded augen gneisses in the southern Făgăraş Mountains. Representing the deepest structural level of the nappe basement, just the augen gneisses should be the most affected by the thrust related deformations.

Starting from these assertions, STRECKEISEN (1934) included the entire crystalline basement east of the Olt River in his Upper Nappe, the thrust fault being represented by the tectonic lineament Valley of Stan-Răşinari-Căpâlna. The author brought two arguments in support of the Upper Nappe in the central-eastern South Carpathians, both erroneous: (a) the low-grade schists along the thrust fault represent the deformed sedimentary cover of the Getic Nappe and (b) the supragetic basement contains augen gneisses, in contrast to the getic basement, which does not contain such rocks.

GHIKA-BUDEŞTI (1940) demonstrated that the supposed sedimentary cover represents the low-grade facies (actually mylonitic facies) of the Getic Crystalline and the augen gneisses are also present in the Getic Nappe basement. Concerning the Mesozoic sedimentary formations outcropping on the Valley of Stan, the author mentions the interesting hypothesis of MURGOCI (1910b), according to which these deposits would be autochthonous (i.e. Danubian) sedimentary cover outcropping in a tectonic window beneath the Getic Nappe. Following the paper of Ghika-Budeşti, the Upper Nappe was forgotten for a while.

CODARCEA et al. (1967) brought into discussion the existence of the Upper Nappe, renamed Supragetic Nappe, starting from some lithofacial considerations on the few Triassic sedimentary sequences in the South Carpathians, compared with the Triassic deposits in the East Carpathians. By correlating the Lower Triassic limestones on the Valley of Stan with the Lower-Middle Triassic bituminous limestones near Braşov, the authors outlined a large getic sedimentation basin from which the Făgăraş Mountains were excluded. With Lower-Middle Triassic dolomites in their north-eastern extremity, the Făgăraş Mountains would represent a supragetic sedimentation domain correlated with the bucovinic domain in the East Carpathians. Like POPESCU-VOITEŞTI (1929), the authors consider as uncertain the thrust faults in the north Sebeş-Cibin Massif and north Iezer Mountains. The nappe emplacement is Mid-Cretaceous, the thrust in the western South Carpathians being reactivated during Miocene.

SĂNDULESCU (1984) defines the Supragetic Nappe as crustal shearing nappe, which means that the getic and supragetic domains have formed a single crustal block before the shearing. Based on the sedimentary cover analysis, the author deduces that the nappe emplacement took place during the Mid and Late Cretaceous tectogenetic phases. The Late Cretaceous movements were recognized on the thrust along the Olt Valley and the Holbav thrust in the eastern Făgăraş Mountains. Săndulescu correlates the Supragetic Nappe with the Subbucovinic Nappe in the East Carpathians.

These are the main attempts in defining the Supragetic Nappe in the central-eastern South Carpathians. Despite the uncertainties regarding the thrust faults, the idea of a large Supragetic Nappe in this region is still accepted as proposed by CODARCEA et al. (1967) and adjusted by SĂNDULESCU (1980; 1984). Although many geologists agree with the geodynamic pattern developed by Săndulescu, the required transcurrent tectonic context in the central-eastern half of the South Carpathians was never discussed.

DISCUSSIONS

The structural correlations made by CODARCEA et al. (1967) at the level of the Triassic sedimentary cover are structurally irrelevant, first because this cover is not significant in the South Carpathians, and second because many facies variations without any structural relevance can arise within a large sedimentation domain. Moreover, the few Triassic sequences in the north-eastern Făgăraş Mountains (SĂNDULESCU et al. 1972a, b) show both dolomitic (supragetic) and bituminous (getic) facies. Bituminous limestones 'of getic type' also appear removed in the Paleogene breccias (Fig. 2a) laying on the southern border of the 'supragetic' Cozia Mountains, on the Argeş Valley (DIMITRESCU et al., 1985). Most likely, it was a single sedimentation basin during the Triassic, with the same crystalline basement.

At regional scale, the tectonic lineament Valley of Stan-Răşinari is a branch of Răşinari Shear Zone in the north Sebeş-Cibin Massif. As against the Alpine sinistral displacement on the shear zone, the Olt Valley branch is a restraining bend so that the transcurrent tectonic regime in the north Sebeş-Cibin Massif gradually became transpressive on the eastern border of the Lotru Mountains. The pre-Alpine mylonitic foliation and the Alpine faults within the shear zone are still subvertical on the Sadu Valley (Fig. 2b) and remain subvertical up to the Vadului River, the transpressive regime becoming dominant south of this river.

Transpression in restraining bend can generate folds, reverse faults, and thrusts. In this case one can speak about high-angle reverse faults and strike-slip related upthrusts. Even CODARCEA et al. (1967) considered that the displacement on the Olt Valley thrusts do not exceed 2 Km. Anyway, the crystalline basement on both sides of this tectonic lineament has the same pre-Alpine metamorphic history and the metamorphic formations west of the Olt River are found in the same lithostructural position on the southern slope of the Făgăraş Mountains, (STELEA, 2006). The getic lithologies east of the Olt River observed by STRECKEISEN (1934) are not simple convergence phenomena, as were considered the author, but represent just the Getic Crystalline.

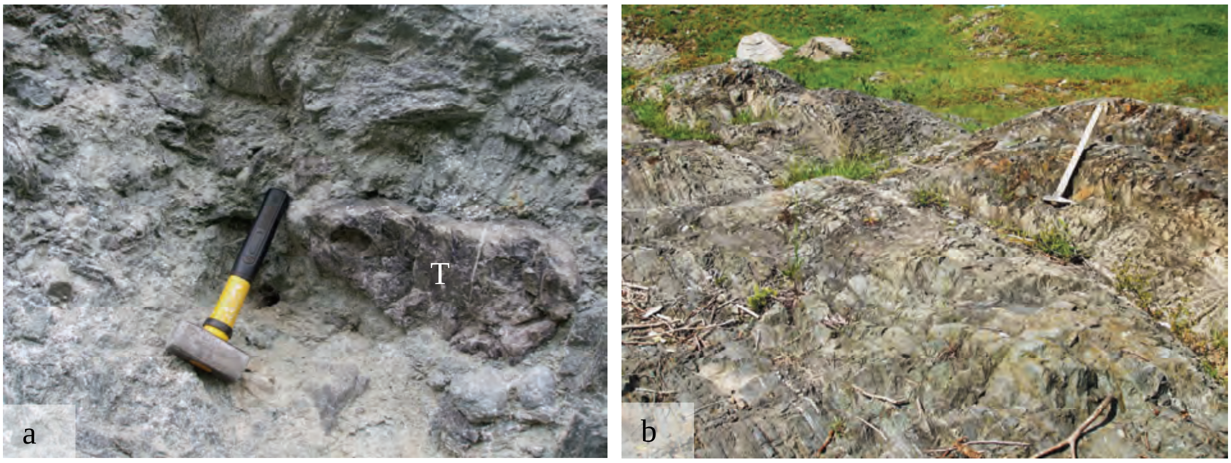

Figure 2. Outcrop photographs: a) block of Triassic bituminous limestones (T) in the Paleocene-Ypresian breccias laying on the crystalline basement of the Cozia Mountains (the Argeş Valley); b) subvertical mylonitic foliation within Răşinari Shear Zone on the Sadu Valley (original).

The tectonic lineament on the south-eastern border of the Făgăraş Mountains was the first time defined as thrust (i.e. Holbav Thrust) in the eastern extremity of the Făgăraş Mountains (SĂNDULESCU et al., 1972b). A post-Cretaceous transpressive tectonic regime in the eastern Făgăraş Mountains (NW-SE compression) occurred from the Burdigalian to the Early Badenian (e.g. HIPPOLYTE et al., 1999). Westward of Bârsa Fault, this lineament was mapped as vertical fault with unclear kinematics (DIMITRESCU et al., 1974, 1978), then interpreted as Mid-Cretaceous thrust by SĂNDULESCU (1984) and Mediterranean retrothrust by BALINTONI et al., (1986).

The same metamorphic formations, in the same structural position, outcrop in the Iezer and Făgăraş Mountains on both sides of this fault, named herein Curmătura Oticului Fault. Moreover, the same dikes of lamprophyres cut across the crystalline basement on both sides of Holbav-Curmătura Oticului lineament, including the Liassic getic cover in the Holbav sedimentary basin, whence their Liassic age (MANILICI & VÂLCEANU, 1962). If there were two sedimentation domains during the Triassic, with different getic and supragetic basements, then how to explain their joint magmatic activity during the Liassic?

Another problem regarding the existence of a large Supragetic Nappe in the eastern South Carpathians is the post-thrust erosion. It is assumed that the Supragetic Nappe was largely eroded in the central South Carpathians (SĂNDULESCU, 1984). Then why the nappe was not eroded in the Făgăraş Mountains? The Făgăraş Mountains area underwent erosion more than the central South Carpathians due to the up-lift subsequent to the Tertiary tectogenetic phases, especially to the Wallachian one. The supposed erosion contour of the Supragetic Nappe west of the Olt River outcrops at altitudes of 500-800 m while the nappe east of the Olt River is not eroded neither at altitudes of 2500 m. A tectonic window in the Getic Crystalline should be occurred along the main crest of the Făgăraş Mountains if there would be a Supragetic Nappe.

CONCLUSIONS

The geodynamic model of the Carpathians belt evolution during the Cretaceous-Miocene time span (SĂNDULESCU, 1984) involves a prevalent transcurrent tectonic regime in the central-eastern South Carpathians. The Alpine structures in this region show a transcurrent regime in the north Sebeş-Cibin Massif and a transpressive one in the Făgăraş Mountains, decreasing from NE to SW. The Late Cretaceous crust-shortening in the Făgăraş Mountains east of the Intramoesian Fault was accommodated by folding. East of Bârsa Fault, where an additional transpressive regime occurred in the Early Miocene (e.g. HIPPOLYTE et al., 1999), the crust-shortening was accommodated by folds and thrust faults.

The thrust faults on the Olt Valley are related to the Alpine sinistral strike-slip on Răşinari Shear Zone in the north Sebeş-Cibin Massif. Perpendicular to the orogen axis, the Olt Valley tectonic lineament has the significance of an intra-getic structural discordance that separates the crystalline basement of the central South Carpathians, with Hercynian tabular structure, from the crystalline basement of the eastern South Carpathians, partly reworked during the Alpine orogeny.

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Received: April 15, 2016
Accepted: July 20, 2016

FOSSIL FISH REMAINS FROM THE ROMANIAN OF PODARI, SW OF ROMANIA. THE FIRST REPORT.

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Abstract. The present work presents the first report of fossil fish fauna from the Pliocene of the Dacic Basin. So far, the fish remains from the sediments of the Dacic Basin were scarcely studied. Recently, during a field campaign, fossil material from the locality Podari, Craiova region of the Dacic Basin has been collected. In this work, we present the fish remains from the vertebrate assemblage. The fish fauna includes *Esox* sp., *Tinca* sp., *Scardinius* sp. and *Silurus* sp. Ecological interpretations and taphonomic observations on fishes suggest presence of standing or slow flowing well-vegetated water body and was buried in a near-shore environment or an alluvial plain close to the water in the Poldari site.

Keywords: Podari, Pliocene, Romanian, Dacic Basin, fish fossils.

Rezumat. Peşti fosili din Romanianul localităţii Podari, SV României. Prima semnalare. Acest articol constituie prima semnalare a faunei fosile de peşti din Pliocenul Bazinului Dacic. Până acum fosilele de peşti din Bazinul Dacic au fost studiate foarte rar. Recent, în timpul unei campanii de teren s-a colectat material fosil din localitatea Podari, regiunea Craiovei. În prezentul articol prezentăm peşti din asamblul faunei de vertebrate fosile. Fauna de peşti include taxoni aparţinând la *Esox* sp., *Tinca* sp., *Scardinius* sp. and *Silurus* sp. Interpretarea ecologică şi observaţiile tafonomice sugerează că fauna de peşti a populat o apă stătătoare sau încet curgătoare, cu vegetaţie acvatică bogată şi că fosilizarea s-a produs în apropierea unei zone de ţărm sau pe o câmpie aluvială apropiată de această apă.

Cuvinte cheie: Podari, Pliocen, Romanian, Bazinul Dacic, peşti fosili.

INTRODUCTION

Numerous localities with fossil vertebrates are known from the territory of Romania and especially from the Dacic Basin, Southern Romania. Many of them yielded microvertebrate faunas of Pliocene age, especially micromammals. Until now, fish faunas were not reported among this Pliocene remains. The only earlier report mentioned fish fossils from the Lower Pleistocene formations of this basin (VASILE et al., 2013). Fish remains of *Carassius, Tinca, Rutilus, Squalius, Scardinius, Esox* and *Silurus* were listed, but not described or illustrated.

The recent field work at the well-known vertebrate locality, Podari (Pliocene) yielded fossil fish material. In the present article, we describe and illustrate the collected taxa and make assessments on the paleoenvironment and taphonomic situation of this locality.

The Podari fossil site is located about 7 km south from the city of Craiova, on the right bank of the Jiu River and northwest from the village with the same name, on the eastern slope of Solomon Hill, about 1 km south-west of the Jiu River (Fig. 1).

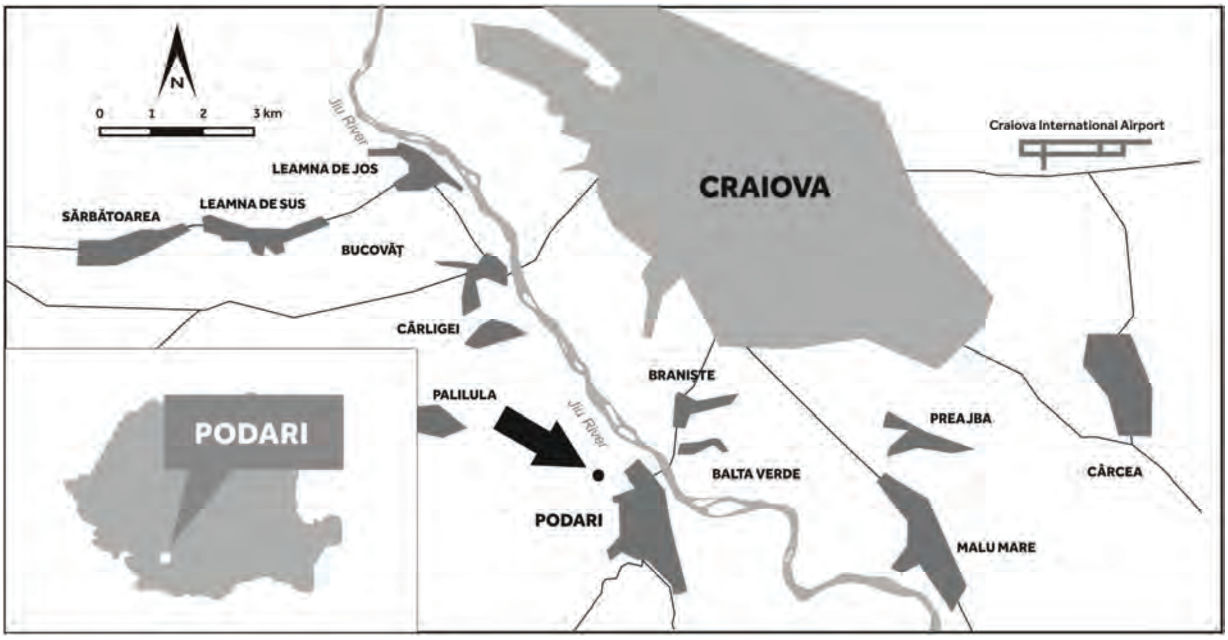

Figure 1. The location of the Podari outcrop (indicated with an arrow) near Craiova, SW of Romania.

GEOLOGICAL SETTINGS

The Podari fossil site was first mentioned by BANDRABUR (1971), which notified the presence of Pliocene age mollusks. These mollusks were described in a later article by ANDREESCU et al. (1981). The micromammals were reported by FERU et al. (1979) and studied by a team from ISER (Institute of Speleology Emil Racovița), IGR (Romanian Institute of Geology) and Oltenia Museum, team led by Costin Rădulescu and Petre Samson (RĂDULESCU et al., 1993, 1993a, 1999). As a result of the research, PANĂ et al. (2004) published a study on the small gastropods, and ȘTIUCĂ et al. (2003) described a new species of the water-mole family Talpidae.

The studied section has a thickness of ca. 20 m. The basal part of the sediments is represented by grayish-blue clays with scarce fossils. The deposits above, of approximately 14 m height, are made up of fine to coarse sands, gravel lenses, followed by sands with oblique lamination and sands with parallel lamination. All layers contain ferruginous and calcareous concretions. The fossil fauna of the sands, overlying the greyish-blue clays, contains mainly gastropod shells, where Viviparidae is the dominant family. The following upper part (5-6 m thick) of the described section is represented by a sequence of clays and clayey sands, with layers rich in fossil remains of unionid bivalve mollusks with sculptured shells. In this upper section, Viviparidae are present as a secondary group. The uppermost layer of the profile is represented by a 10 cm thick black clay with remains of coal and contains pellicles of iron oxides and gypsum crystals. This thin level provides the vertebrate fauna, including fishes, which are described in the present works. The section is covered by recent soil.

The described sediments are part of the western side of the Dacic Basin (PANĂ et al. 2004) and represent the Romanian stage based on palaeomagnetic measurements, micromammal biochronology and mollusk zonation. The palaeomagnetic age corresponds to the middle Gauss epoch (ANDREESCU et al., 1981), recently calibrated to the C2An Chron, subchrones C2An2n and C2An-1r. ANDREESCU et al. (2011, 2013) indicated an absolute age of 3.2 to 3.0 Ma. The age of the uppermost layer with fish remains can be correlated to the Mammal Zone MN16 (RZEBIK-KOWALSKA, 2002) or MN16a (ȘTIUCĂ et al., 2003) based on the occurrences of *Micromys praeminutus (*KRETZOI, 1959) and *Propliomys hungaricus* (KORMOS, 1934). ENCIU (2007) showed that this association of micromammals from Podari is similar with the one from Arondelli-Villafranca d'Asti in Italy of middle Piacenzian age, and partially with the Hungarian locality Csarnóta 2 dated to the Early Pliocene. In the Dacic Basin, the Podari locality is considered as an equivalent of the Cernătești and Tulucești micromammals sites (ANDREESCU et al., 2013). The fossil mollusk fauna of the underlying horizon of the level with the vertebrate fauna is very rich at the Podari. About 45 species of gastropods and 40 species of bivalves were found (ENCIU, 2007). Among them the sculptured unionids are the most important for age estimation, *Pristinunio pristinus* (Bielz), *Rytia brandzai* (Sabba), and *R. lenticularis,* being characteristic for the middle part of the Romanian (OLTEANU, 2006).

The Dacic Basin was the most western part of the Eastern Paratethian epicontinental sea. It existed from the Sarmatian s.l. until the Pleistocene, partially as a gulf and isolated basin. The basin was bordered by the Carpathians in the north and by the Balkan orogene in the south (ANDREESCU et al., 2013). With intermittent variations from marine to brackish and freshwater environments, it turned finally to a freshwater basin with pronounced endemism. The Romanian stage of the basin is characterized by habitats rich in carbonates and nutrients (JIPA & OLARIU, 2009).

MATERIAL AND METHODS

Bulk collecting of sediment was performed at Podari quarry. The screen washing of more than 300 kg of sediments revealed heterogenic fossil material composed mostly of fish skeletal remains, which includes vertebrae, pharyngeal teeth both isolated and associated with pharyngeal bone and elements of the postcranial skeleton. Apart from fishes, the fossil material contains other vertebrate remains e.g. snakes, turtles and mammals. Here, we present only the results of the fish material.

The material was compared with photographs published in the database of the Department of Archaeology at The University of Nottingham (DATUN), as well as with skeletons of *Esox lucius* (Figs. 2 B, D', and D'') (Collection of the Natural History Museum, Sibiu) and available literature (e.g. BÖHME, 2002, 2010, 2010a; GAUDANT, 1977, 1994, 2015; RÜCKERT-ÜLKÜMEN & YIGITBAS, 2007).

The measurements of the fossil bones were done using the Unior calliper (model 271) with a measurement error of 0.02 mm. Some of the fossils were measured on digital images, using a standard scale reference of 1 cm. The images were taken with a Nikon D700 camera mounted on a tripod, using a Sigma lense of 105 mm and extension tubes. The close observation of bone morphology was done using an Optika stereomicroscope (model S-10-L). All material described herein is stored in the Oltenia Museum, Craiova, Romania.

Systematic paleontology

Class: Actinopterygii Cope, 1887
Order: Esociformes Nelson, 1994
Family: Esocidae Cuvier, 1817
Genus: *Esox* Linnaeus, 1758
Esox sp.

Figure 2. Fossil fishes from the locality Podari. *Esox* sp. (A, C) and recent *Esox lucius* (B, D): A - isolated teeth, B – dentary with teeth, C – fragments of palatine, D´ – vomerine tooth, D´´ – palatine bone. *Scardinius* sp. (E-G): pharyngeal teeth from different positions. *Tinca* sp. (H-J): H, I, – isolated pharyngeal teeth and J – pharyngeal teeth articulated with pharyngeal bone. *Silurus* sp. (K-O): K – dentary, L – praemaxillae, M – proximal and N – distal portions of the pectoral spine, O – vertebra. Scale bars 5mm. For D' scale bar is 2 mm.

Material: One anterior fragment of the left palatine (Figs. 2 C1 and C2) and two dentary teeth (Figs. 2 A1, A2, A3 and A4).

Description: The preserved fragment of palatine is small, 5.4 mm wide and 9.9 mm long. The palatine bone presents a very characteristic aspect for the pikes, the tooth bases are arranged in longitudinally parallel to each other rows; in the preserved bone, four of these rows are observable. The tooth bases are round with thick tooth wall and rather small dental cavity in the centre. The tooth base rows have a distinct side marked by collagen ruptures of the hinge-like building a "C"-shaped structure (Fig. 2 C2). The two *Esox* teeth come from the dentary and are few millimetres in length (the larger 3.9 mm long, 1.5 mm wide at the base; the smaller 2.8 mm long, 1.2 mm wide at the base). The dentary teeth differ completely from the vomerine or palatal ones. The dentary teeth are straight, laterally compressed with a cutting ridge present on the anterior and posterior edges, which are well pronounced in the lower half of the crown. The vomerine and palatal teeth are slender, curved, in the cross-section almost circular and lack any cutting ridge (e.g. Fig. 2 D'). The dorsal surface of the bone is smooth and convex.

Remarks: The bone has small surface uplifts on one side and smooth surface of the other side. These uplifts are forming approximately four unevenly distributed rows, "C"-shaped structures that are the support bases for the palatine teeth of the pike. The opening that can be seen in the "C"-shape structures indicates the rupture of the attachments of the predentine or collagen formation that form the hinge-like teeth of the pike. FINK (1981) showed that the *Esox* genus has this flexible type of attachment. This flexible attachment type can be found in the palatine, vomer and pharyngeal bones of the genus *Esox*. A similar attachment was described for the Palaeocene species *Esox tiemani* (WILSON, 1984). The preserved fossil material (palatine fragment, teeth) of the pike from Podari can be identified only at the generic level.

Stratigraphic distribution: The order Esociformes that includes *Esox* genera - has its origin during the Mesozoic (NEWBREY et al., 2008). North American fossils demonstrate that the pikes first occurred in the early Campanian (WILSON et al., 1992). In Europe, this genus is known from the early Oligocene of Western and Eastern Europe (*Esox* sp.; GAUDANT, 1979a). So far in Romania, the genus is reported only from the late Oligocene – early Miocene of the Eastern Carpathians (loc. Gura Humorului, *Esox moldavicus,* SYCHEVSKAYA, 1974) (CONSTANTIN, 1999) and the Pleistocene of Southern Romania (VASILE et al., 2013, 2015), (loc. Copăceni, *Esox* sp.). During the Pliocene, they are known from the locality Priozernoe, Republic of Moldavia by *Esox moldavicus* (KOVALCHUK et al., 2014a), as well as from West Mongolia, *Esox sibiricus*, (SYTCHEVSKAYA, 1989) and late Pleistocene of Siberia, *Esox* sp. (SYTCHEVSKAYA et al., 2015; BÖHME & ILG, 2003).

Ecology: The distribution of the Recent pikes, with *Esox lucius* as model, shows their circumpolar distribution with a wide range of habitats. Pikes are found in rivers, lakes and even in weakly saline waters (CRAIG, 2008). In these environments, *Esox* has a key role in the structure of the entire freshwater communities. Here it can be responsible sometimes for the local influence or even disappearance of almost all other fish species (CRAIG, 2008). *Esox* is linked to a dense water plants environment since plants offer a spawning habitat (KOTTELAT & FREYHOF, 2007). The presence of dense water vegetation is a major environmental requirement for this fish (RAAT, 1988; BRY, 1996).

Order: Cypriniformes Bleeker, 1859
Family: Cyprinidae Bonaparte, 1832
Subfamily: Tincinae Jordan, 1878
Genus: *Tinca* Cuvier, 1816
Tinca sp.

Material: Six pharyngeal teeth, two isolated and four articulated with pharyngeal bones. Figs. 2 H1, H2, I1, I2, J1, J2, J3 and J4.

Description: The isolated teeth are similar in size, they measure 4.2 and 3.7 mm in height, about 1.5 and 1.4 mm wide and they are 2.2 and 1.9 mm long (Figs. 2 H1, H2 and I1, I2 respectively). The pedicle and neck of the tooth are not present in these loose teeth. The tooth root is short and compact. The tooth crown is compressed anterodorsally. An elongated and shallow depression spans the entire length of the grinding surface; it terminates laterally in a small hook. The other two teeth are complete and still articulate on a fragment of the pharyngeal bone (Figs. 2 J1, J2, J3 and J4). They are of similar size with the length of 3.2 and 3.2 mm, thickness of 1.3 and 1.1 mm and height of 4.7 and 5.18 mm. At the distal end, a small hook is present. Along the upper part of the grinding surface, they have an elongated, not very deep, depression. The masticatory surface makes with the tooth axis an angle of about 60-65°.

Remarks: The general shape of the pharyngeal teeth corresponds to descriptions and figures in e.g., BÖHME, 2002; GAUDANT 1979, 1994; RÜCKERT-ÜLKÜMEN & YIGITBAS, 2007, KOVALCHUK, 2015.

Stratigraphic distribution: Fossil forms of the genus *Tinca* are described from several localities in Europe. *Tinca* cf. *furcata,* Agassiz, 1843, is mentioned from the late Miocene of Germany (GAUDANT, 2015), *Tinca sp.* from the late Miocene of the Republic of Moldavia and Ukraine (KOVALCHUK, 2014, KOVALCHUK et al. 2014) and also in formations of the late Miocene to the early Pliocene of Turkey (RÜCKERT-ÜLKÜMEN et al. 2006, RÜCKERT-ÜLKÜMEN & YIGITBAS, 2007), Ukraine (KOVALCHUK, 2013) and the Pannonian Basin in Austria from Götzendorf (GAUDANT, 1994). *Tinca sayanica,* Sytcheskaya, 1989, is described for the first time in the late Miocene to the early Pliocene deposits from Mongolia (SYTCHEVSKAYA, 1989), while *Tinca tinca* is described from the late Pliocene of Netherlands (GAUDANT, 1979) and the

Pliocene of Croatia (KUREČIĆ & LENARDIĆ, 2015); *Tinca* sp. appears in the early Pleistocene of Romania (VASILE et al. 2013, 2015) and also in the middle Pleistocene of England (BÖHME, 2010).

Ecology: The typical habitat of the recent species of *Tinca* is densely vegetated shallow waters of lakes or still-water channels. The spawning areas are also closely linked to dense water vegetation (KOTTELAT & FREYHOF, 2007).

Subfamily: Leuciscinae Howes, 1991
Genus: *Scardinius* Bonaparte, 1837
Scardinius sp.

Material: five isolated pharyngeal teeth (Figs. 2 E1, E2, E3, E4, F1, F2, G1, and G2).

Description: Pharyngeal teeth of individuals of different sizes are present in the material. The most complete and largest one from Podari site is 10.2 mm high and 3.7 mm in its widest part. The tooth is strongly bent (nearly S-shaped) and with a high crown that makes about 50% of the total height of the tooth. The hook is rather small and sharp. The grinding surface is composed of seven protuberances located on its medial border and extremely reduced flat surface, running dorsoventrally and parallel to the medial border. In medial view, the protuberances have convex outline. The tooth belly is smooth and convex. The tooth pedicle is broken at its base.

Remarks: The teeth described here resemble the characteristics (grinding surface, the shape of the protuberances) of the recent genus *Scardinius* (RUTTE, 1962).

Stratigraphic distribution: The genus *Scardinius* is known in the fossil record since the late Miocene of Europe and Western Asia (BÖHME & ILG, 2003; KOVALCHUK, 2015). The Recent distribution of the genus is restricted mainly to Europe as well as partially to Western Asia (KOTTELAT & FREYHOF, 2007) with less than 10 species.

Ecology: The genus *Scardinius* prefers water bodies with low water energy, e.g. lakes, ponds, slowly flowing large rivers, usually associated with submerged vegetation (BÖHME & ILG, 2003, KOTTELAT & FREYHOF, 2007).

The cypriniform fishes are documented since the early Paleocene deposits of North America and early Eocene of Asia and Europe. They are the most diverse freshwater fish group in the world with more than 3.000 valid species (PASCO-VIEL et al., 2010; REICHENBACHER et al., 2011). This diversity leads to an amazing variety of the skeletal morphology. Within Cyprinidae, which are frequently presented in the fossil record by pharyngeal teeth, the identification is difficult due to heterodont dentition. The tooth morphology varies even within the same tooth raw. Due to this, the identification below the generic level is extremely difficult.

Order: Siluriformes Rafinesque, 1820
Family: Siluridae Cuvier, 1816
Genus: *Silurus* Linnaeus, 1758
Silurus sp.

Material: one dentary, one premaxilla, two fragments of the pectoral spine - the distal and proximal portions, one vertebra. (Figs. 2 K1, K2, L1, L2, M1, M2, M3, M4, N, O1, O2).

Description: The dentary and premaxilla are fragmentary preserved. The dental shelfs of both bones possess the bases of the teeth. They are round, have the same size and arranged irregularly close to each other. The dorsal process and body of the spine are preserved at the proximal portion of the pectoral spine. Our fossil is a fragment of the anterior part of these pectoral spines. The fragment preserves the articular head and a small part from the body of the spine. The well-preserved dorsal process is massive. Striae or ripples cannot be observed due to the bad preservation of the bone. The dorsal process makes with the body of the spine an angle close to 80°. The body of the spine at the base is almost cylindrical in cross-section and bears irregular striations along its surface, without dentition.

The distal part of the spine is anterodorsally compressed. The bone surface possesses hardly visible irregular striations. At both lateral sides of the bone, a serration is preserved. On one side, the spines are longer than in another one.

The vertebra centrum is encased partially in a sandstone concretion and only one side can be observed. The diameter of the vertebra centrum is 23 mm, the thickness - app. 7.4 mm. The exposed cotyle is large and shallow.

Remarks: The pattern on the dental shelf, the shape and structure of the pectoral spine, as well as the vertebra dimension, resemble the genus *Silurus* (BÖHME, 2002). Due to fragmentary preservation of the bone material, any further identification is impossible.

Stratigraphic distribution: The genus *Silurus* is known in the fossil record since the earliest Tortonian (late Miocene) of Europe (Hammerschmiede, BÖHME & ILG, 2003), the late Miocene of Ukraine (KOVALCHUK, 2011), and the Pliocene deposits of the Republic of Moldavia and Ukraine (KOVALCHUK et al. 2014, 2015). Most of the papers refer the material only to the genus level as *Silurus* sp.

Ecology: In present days, only two species of *Silurus* live in Europe: *Silurus glanis*, Linnaeus, 1758, and *Silurus aristotelis* Garman, 1890. They occupy rather similar habitats characterized by large and medium-sized rivers and nutrient-rich lakes with well-developed vegetation (KOTTELAT & FREYHOF, 2007) and can tolerate brackish waters (RÜCKERT-ÜLKÜMEN & YIGITBAS, 2007).

DISCUSSION AND CONCLUSIONS

The Podari locality yielded a fossil fish fauna consisting of four taxa, *Esox* sp., *Tinca* sp., *Scardinius* sp. and *Silurus* sp.. Unfortunately, the available material and its preservation does not allow identification at the species level. All four genera are found in the recent ichthyofauna of the water bodies of Romania. They are the frequent elements of the late Neogene ichthyofaunal assemblages from Central (Germany, Hungary) to Southeast and Eastern Europe (Greece, Turkey, Ukraine, Russia) (BÖHME & ILG, 2003). Following an actualistic approach to evaluate the palaeoenvironmental reconstruction we conclude that the presence of *Tinca*, *Scardinius*, *Silurus* and *Esox* suggests the presence of standing water body (lake, pond) or slow flowing large rivers, rich in nutrients and well-developed, dense vegetation.

We provide also some tentative taphonomic observations on a few preserved cyprinid pharyngeal teeth. None of the teeth shows resorption traces on their bases. The roots of the teeth are always broken. The cyprinids change their teeth during the entire lifetime. The teeth replace due to resorption of its foot and later removal of the remaining tooth crown. These remains can be accumulated in the sediments. In contrary to this, the teeth showing traces of breakage or fragments of the pharyngeal bone with teeth originate from fish as a result of post mortem breakage and of allochthonous origin (BÖHME, 2010a). Taking this into account, we suggest deposition in allochthonous environment for the fossil cyprinid (*Scardinius* sp., *Tinca* sp.) remains. In addition to this, all fossil bones are found in the organic rich clays containing coal and iron pellicles, interpreted to be a nearshore environment. The state of preservation of the fossil material does not allow us the identification beyond the generic level.

Further investigations might reveal additional and better-preserved material. We consider the present article to be just a first step in the research of the fossil fish fauna from the Pliocene of Dacic Basin. The recent industrial extension of the outcrop opens new possibilities for collecting. We also consider that close-by existing outcrops need to be investigated and the results synchronized.

ACKNOWLEDGMENTS

The authors wish to thank the reviewers, Mr. Oleksandr M. Kovalchuk (National Museum of Natural History, National Academy of Sciences of Ukraine), Mr. Werner Schwarzhans (Natural History Museum of Denmark, Zoological Museum, Copenhagen) and Mr. Marton Venczel, (Senior Researcher at the Ţării Crişurilor Museum, Oradea), for useful comments and corrections, which improved our manuscript. We would like to thank also to Mr. Silviu Georgescu for providing the source material for the *Esox lucius* skeleton that is now preserved in the Collection of the Natural History Museum, Sibiu and Ms. Alexandra Vişan, a student at the Faculty of Geography, University of Craiova, for their contribution to the field activity.

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Received: March 15, 2016
Accepted: August 12, 2016

A LARGE DEINOTHERE (*MAMMALIA: PROBOSCIDEA*) IN THE LATE MIOCENE OF THE MOLDAVIAN PLATFORM AT HUȘI (VASLUI COUNTY)

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URSACHI Laurențiu, FĂRCAȘ Cristina**

Abstract. Large deinotheres are known from only a few Late Miocene localities in Romania. Herein the new locality Huși is reported in the Moldavian Platform (northeastern Romania). On Dobrina Creek, a metatarsal bone was found *ex situ*, in the alluvium. However, based on the local geology one presumes that the bone originated either from the Khersonian (MN9) or the Meotian (MN9-MN12) deposits - rather from the last ones -, the only ones exposed in the area. Therefore, the Huși deinothere is considered to be a Vallesian or Turolian representative. The bone is assigned to *Deinotherium proavum* (= *D. gigantissimum*). This find reinforces the knowledge of the stratigraphical range of large deinotheres in Romania, all assigned to a single species, *D. proavum*. They were present since the lower Late Miocene until the uppermost Miocene and went extinct in the country before the Pliocene. The Huși deinothere is considered to be a Turolian representative.

Keywords: vertebrate palaeontology, regional geology, proboscideans, deinotheres, Late Miocene, Romania.

Rezumat. Un deinother (*Mammalia: Proboscidea*) de talie mare din Miocenul Superior al Platformei Moldovenești la Huși (Județul Vaslui). Deinotheri de talie mare sunt semnalați în mai multe localități românești, toate din Miocenul superior. Această lucrare semnalează o nouă astfel de localitate din Platforma Moldovenească (nord-estul României), Huși. Pe ogașul Dobrina a fost descoperit *ex situ* un metatarsian, în aluviuni. Totuși, privind contextul geologic local se poate presupune că acest os provine fie din depozitele Khersoniene (MN9), fie din cele Meoțiene (MN9-MN12) – mai degrabă din cele din urmă -, acestea fiind singurele care apar expuse la zi în această zonă. În consecință, deinotheriul de la Huși este considerat ca reprezentant al faunelor vallesiene sau turoliene. Osul a fost atribuit speciei *Deinotherium proavum* (= *D. gigantissimum*). Această descoperire consolidează cunoașterea distribuției stratigrafice a deinotherilor de talie mare din România, atribuiți unei singure specii, *D. proavum*. Aceștia au fost prezenți între baza și partea terminală a Miocenului superior, dispărând ante-Pliocen.

Cuvinte cheie: paleontologia vertebratelor, geologie regională, proboscidieni, deinotheri, Miocen superior, România.

INTRODUCTION

In Romania, Middle and Late Miocene terrestrial vertebrates are recorded in a lot of localities in both the Inner and Outer Carpathian regions. A different situation concerns the Lower Miocene deposits, where such fossils are almost absent. This situation could easily be explained by the specific palaeogeographic evolution of the region, where large areas were covered by the marine waters of the Paratethys Sea.

In the Middle and Upper Miocene deposits, terrestrial mammal communities of large herbivores are usual. Among other large mammals, proboscideans are present, both mastodons (e.g. NICORICI, 1976; CODREA & CIOBANU, 2008; ȚIBULEAC et al., 2015) and deinotheres (CODREA, 1994; CODREA & CIOBANU, 2008) being reported. However, they are not common: the vast majority of the finds concerns only a single fossil at each locality, usually an isolated tooth or bone. Deinotheres are reported from a few localities, but they still remain poorly known in our country. Even if their remains are rather scarce, it is clear that over this time span the deinothere lineage increased in dimensions, starting from medium sized and ending up much larger. In the latest Miocene (Pontian *s.s.*), the last largest representatives are recorded, but after the Miocene/Pliocene boundary there were no survivors, the only Pliocene proboscideans reported from Romania being the mastodon species: *Anancus arvernensis* (Croizet & Jobert, 1828), *Mammut borsoni* (Hays, 1834) and *M. praetypicum* (Schlesinger, 1919), (FERU et al. 1983; RĂDULESCU & SAMSON, 1985; CODREA et al., 2005).

This paper deals with a large deinothere limb bone found in the region of Moldova (Eastern Romania), in the Upper Miocene deposits of the Moldavian Platform (a local name for the southwestern sector of the larger Oriental European Platform; SĂNDULESCU, 1984). The hind leg bone was found *ex situ*, reworked from its original rock into the alluvium of the Dobrina Creek in the neighbourhood of Huși town, not far from its confluence with the Lohan Valley (near the location so-called “La Făzănărie”). The discoverer was Mr. Grigore Apostol, a native of Huși (MERLAN, 2010). He donated this fossil to the Huși Municipal Museum (abbreviated: HMM).

GEOLOGICAL SETTING

In Romania, the Moldavian Platform is exposed in the northeastern region of the country, bounded either by non-geologic limits such as the national borders to the north and east (with Ukraine and Republic of Moldova), or by geological ones, *i.e.* faults to the west and south; but the latter remain controversial (SĂNDULESCU, 1984; IONESI, 1994; BALINTONI, 1997). The main controversies concern the geological age and lithology of Rădăuți-Pașcani block, bounded by the faults Siretului (continuing in Ukraine under the name Ustilug-Rogatin) and Solca (=Rava Ruska).

According to some geologists the basement of this block could be at least Middle Proterozoic or even older (Karelian), while others estimate that it is much younger, Neoproterozoic (epi-Cadomian). This uncertainty is a result of the lack of drilling data, as no borehole has intercepted this basement. The nature of the platform basement is not important for this study although its tectonics could have played a role in the overlapping sediment distribution and thicknesses, mainly the Palaeozoic ones. Apart from this still poorly known block, the remaining areas of the platform have a metamorphic Proterozoic basement intruded by magmatic rocks (details in IONESI, 1994**;** BALINTONI, 1997).

This old basement of the Moldavian Platform is covered by three sedimentary megasequences (‘sedimentary cycles’, in IONESI, 1994). The first two megasequences involve Neoproterozoic-Palaeozoic and Mesozoic-Early Cenozoic sediments. The sedimentation of the last one started in the Middle Miocene (Late Badenian) and ended in the Late Miocene (in Meotian, according IONESI, 1994), although one cannot exclude the possibility that deposits younger than the Meotian could once have existed (mainly on the southern platform margin), but were subsequently removed by erosion.

In Huși area, the last sedimentary megasequence is of interest due to the presence of Khersonian and Meotian terrestrial deposits. These continental environments occurred due to the regression of the waters in the Dacian Basin during the Late Miocene (SAULEA et al., 1967, 1969; JIPA & OLARIU, 2009). Although the sedimentary deposit which yielded the deinothere fossil is unknown, the geology of the Dobrina Creek is indicative enough to support presumptions: the bone was reworked either from the Meotian or Khersonian deposits, the only ones exposed in this area (Fig. 1). Both are of continental origin and comprise sedimentary rocks (mudstones, sand channel fillings, etc.) which accumulated in fluvial palaeoenvironments.

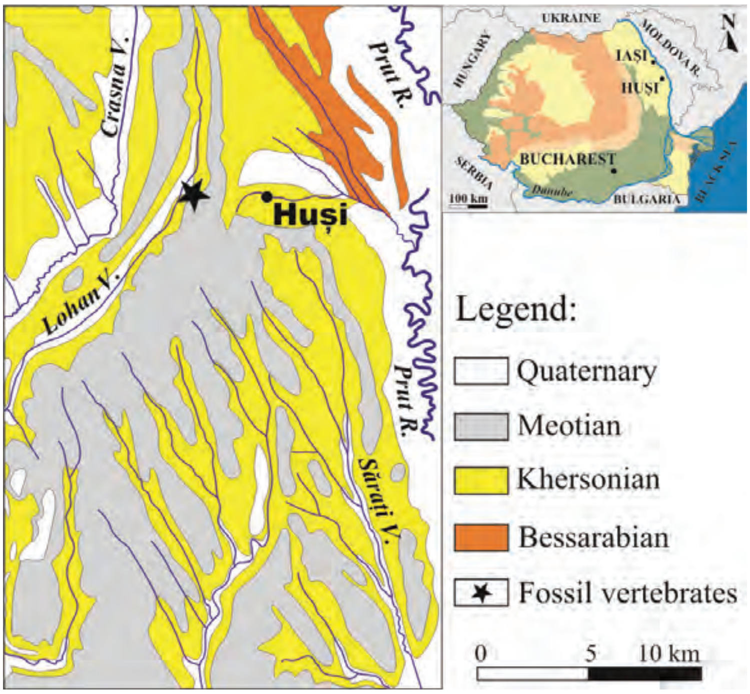

Figure 1. Geological map of the Huși area (modified after JEANRENAUD, 1971)**.**

The oldest sedimentary unit is named the ‘Huși Formation’ (in IONESI et al., 2005). It is Khersonian (‘Hersonian’ in IONESI et al., 2005), but in the absence of a type section (‘we grouped the deposits from this area under the name the ‘Huși Formation’, *being unable to mention a type-section*’ - our translation and underlining; IONESI et al., 2005, p. 422) in our opinion this makes the name invalid and this formation needs a reassessment of its definition. The authors underlined the dominance of brackish facies in these deposits, but such environments completely miss in some outcrops near Huși, as is that of Crețești-Dobrina 1 (URSACHI et al., 2015). This is not surprising because the same authors reported that south of Huși the brackish facies is specific just for the basal part of the Khersonian succession, the upper part having accumulated in freshwater environments with *Unio wetzleri* (p. 423). But for instance, we should notice that at Crețești-Dobrina 1 deinotheres miss, the only proboscidean being the mastodon *Choerolophodon pentelici* (Gaudry & Lartet, 1856).

The younger formation overlying the previous one comprises Meotian clay interleaved with sandy and sandstone deposits. According to JEANRENAUD (1971), the Khersonian/Meotian boundary would be marked by the Nuțasca-Ruseni andesitic tuff. However, this tuff was never dated and sometimes - mainly in eastern and southeastern areas of the platform - it can be difficult to locate in several outcrops. For instance, there is not a Meotian lithostratigraphic unit correctly defined in the area. It can be noticed that MARINESCU et al. (1998) published correlation charts for the Neogene deposits of Romania, including the Moldavian Platform. For the Meotian (included in the age they named “Malvensian”) eventually one could notice the so-called “Murgeni Formation”, but following the rules of the International Stratigraphic Guide (http://www.stratigraphy.org/index.php/ics-stratigraphicguide) there lacks a clear definition of this ‘formation’ and we cannot take it into consideration. Moreover as a whole, their paper concerns strictly regional stratigraphic charts, without any description and reference. Even the platforms are poorly defined (*e.g.* the Scythian Platform visibly is included into the Moldavian one!).

Concerning the topic of this study, we presume that the deinothere bone more likely originated from the Meotian deposits than from the Khersonian ones. Because most of the outcrops expose the basal section of the Meotian deposits, the deinothere could eventually belong to MN10-MN11 units or eventually even to uppermost MN9. This time span comprises the mammal ages Vallesian-Turolian.

MATERIAL AND METHODS

The single fossil from Huși documenting the presence of deinotheres is a right metatarsal III (HMM 6694). The bone did not need special preparation because the fossilization is fair and no matrix remained on the bone surface. It was measured with professional calipers, then photographed with Nikon Coolpix P520 (18.1 megapixels). The photos were processed in Adobe Photoshop CS2. Measurements are following GÖHLICH (1998).

RESULTS

SYSTEMATIC PALAEONTOLOGY
Order Proboscidea Illiger, 1811
Suborder Deinotheriidae Bonaparte, 1845
Genus *Deinotherium* Kaup, 1829
Deinotherium proavum Eichwald, 1831, 1835 (= *D. gigantissimum*, Ștefănescu, 1895)
(Fig. 2a-d)

Although the fossilization is fair, some damage should be mentioned, with broken parts in the following sectors of the bone: *facies articularis cuneiforme lateralis* (*tarsalis tertium*), the anterior-lateral and postero-medial areas and on the lateral surface and trochlea (lateral and postero-medial).

The bone is unevenly tetragonal-prism shaped, broader in the cranial direction and narrower in the plantar one. The articular surface for the tarsal *cuneiforme lateralis* is plain-concave. The proximal anterior margin is convex; the lateral one is sinuous, due to the alternation of the prominences of the articular facets with MtIV; their linking portion, is concave. The articular facets with MtIV are distinctly outlined and well preserved. The dorsal one has a half-ellipsoidal contour, and the smaller plantar one is ellipsoidal elongate, sub-parallel to the lateral margin of the proximal articular surface.

Figure 2. *Deinotherium proavum* right MtIII:
a - view of proximal articulation surface; b - medial view; c - cranial view; d - plantar view.

The medial surface is acute concave, sharply limited by a prominent vertical ridge, while the posterior limit is much more gradual and diffuse. *Facies articularis medialis* with MtII exposes in its anterior portion an obvious concavity, oriented front to rear. The trochlea is asymmetric, laterally lowered.

Measurements (mm): maximal length (physiologic) – 216.0; proximal width – 98.0; maximal proximal depth – 122.5; depth of *facies articularis proximalis* with Mt.III - ca. 117.0; length of *facies articularis medialis* with MtII - ca. 62.0; depth of same facet – 33.0; length of the plantar *facies articularis lateralis* with MtIV – 14.0; depth of same facet – 29.0; length of *facies articularis lateralis* – 31.0; depth of same facet – 34.0; maximal width of the diaphysis – 92.0; maximal depth of diaphysis – 92.0; maximal depth of trochlea – 117.0.

COMPARISONS AND DISCUSSIONS

When we compare the Huși deinothere metatarsal with other similar finds, the first thing that emerges is the scarcity of data related to the post-cranial bones of the large deinotheres, not only from Romania, but also from the neighbouring countries. For instance, the only deinothere metatarsals ever reported in our country are those of the notorious Mânzați deinothere (ȘTEFĂNESCU, 1899; pp. 136-138). ȘTEFĂNESCU reported smaller dimensions for Mt.III: length – 15.2; proximal width – 87.0. The cranial view of the bone reflects a pronounced arched line of the lateral margin of the MtIII ("Tabla" IV, A).

In the vicinity of Romania, in Bulgaria, KOVACHEV & NIKOLOV (2006) named a distinct large deinothere species, *D. thraceiensis* documented by a nearly complete skeleton unearthed from the Meotian deposits at Ezerovo. But, the arguments for a distinct species are much too meagre to defend such an assignation. There is no evidence to disassociate Ștefănescu's specimen from Mânzați from the one from Ezerovo, an opinion also defended by MARKOV (2008). The Ezerovo deinothere is indeed a big one (MtIII length – 225), but the difference is not large enough to document a distinct species. The differences in size may be interpreted as intraspecific variation. Therefore, the Huși deinothere bone is intermediate in dimensions between Mânzați and Ezerovo specimens. It doubtlessly belongs to a large deinothere, probably also sharing the same geological age with the ones from Mânzați and Ezerevo (MN11) or is perhaps, a bit older.

Deinothere systematics is a controversial topic. In Romania, already at the end of the 19th and beginning of the 20th centuries, there were two viewpoints: while Ștefănescu (see references in Tab. 1) agreed with the distinct species *D. gigantissimum*, ATHANASIU (1907) considered that the large deinothere should be only a variety, i.e. *D. giganteum* var. *gigantissimum*.

Over a half century ago, GRÄF (1957) proposed an evolutionary lineage of the European deinotheres, involving (from oldest to younger ones) the species "*D. bavaricum - D. levius - D. giganteum - D. gigantissimum*", showing a size increase over geological time, the last representatives in the latest Miocene being the biggest ones. Actually, there are various opinions arguing either for a larger species' diversity (e.g. GASPARIK, 1993, 2001; MARKOV, 2008, 2008a; PICKFORD & POURABRISHAMI, 2013) or an opposite one, keeping in Europe a couple of genera (*Prodeinotherium* Ehik, 1930 and *Deinotherium* Kaup, 1829) with fewer species (HUTUNNEN, 2002). Hutunnen placed into synonymy the middle-sized *Deinotherim giganteum* Kaup, 1829 and the large-sized *D. proavum* Eichwald 1831, 1835 (= *D. gigantissimum* Ștefănescu, 1891; a discussion on this synonymy in CODREA, 1994 and PICKFORD & POURABRISHAMI, 2013 with references hitherto), keeping valid only the first species. Although Hutunnen's viewpoint was accepted by some palaeontologists that studied this group (e.g. ATHANASSIOU, 2004), there are others such as CODREA (1994), MARKOV (2008, 2008a), GASPARIK (2001), VERGIEV & MARKOV (2010), PICKFORD & POURABRISHAMI (2013) and ourselves herein, who argue for the retention of the large deinotheres as distinct species. Concerning the largest deinothere species, there are also different viewpoints, several palaeontologists accepting the validity of Ștefănescu's name *D. gigantissimum* (MARKOV 2008, 2008a; VERGIEV & MARKOV, 2010; GAREVSKI & MARKOV, 2011), while others such as GASPARIK (2001), TÓTH (2010), PICKFORD & POURABRISHAMI (2013) and ourselves, concur on Eichwald's priority with *D. proavum*, as one of us pointed out more than two decades ago (CODREA, 1994).

Alternatively, VAN DER MADE & MAZO (2003) considered that all deinotheres should be classified in a single genus, *Deinotherium*, excluding *Prodeinotherium*. We will not discuss herein the problems related to the latter genus because this problem has already been debated for rather a long time (ANTUNES & GINSBURG, 2003) and because this genus is much older than the one that is of interest for us.

Although the genus *Prodeinotherium* has occurred in Europe since the Early Miocene (MN4a, GÖHLICH, 1999**;** VAN DER MADE & MAZO, 2003), in Romania the oldest deinotheres are documented rather late, in Middle Miocene deposits (Early Sarmatian, i.e. Astaracian, MN7+8, CODREA et al. 1991, 2007**;** CODREA & CIOBANU, 2008; Fig. 3, Table 1), these and all the younger ones belonging to the genus *Deinotherium*.

Nowhere in Romania has *D. proavum* been reliably reported from deposits older than the Meotian (Table 1). However, one can presume its presence even in the latest Sarmatian, but until now no such locality has been clearly documented in our country. Concerning deinothere extinction, in this area the latest ones went extinct in the Late Miocene (Turolian, MN13, CODREA et al., 2002), since there is no evidence about any Pliocene survivor, either in the Lower Pliocene (Dacian), or in the Upper Pliocene (Romanian, FERU et al., 1983; RĂDULESCU & SAMSON, 1985; ANDREESCU et al., 2011, 2013). It is obvious that the last Miocene representatives were the largest recorded in our country, following a rule confirmed elsewhere in Europe.

In eastern and southeastern Europe, palaeontologists such as VISLOBOKOVA et al., (2001) and VISLOBOKOVA (2005) reported a longer deinothere time span, persisting until MN15 (Late Pliocene). If we accept a wide distribution of large deinotheres in Central-Eastern Europe in the Late Miocene, without physiographic barriers to prevent dispersal, as was pointed out some decades ago by RÖGL & STEININGER (1984), in such a specific palaeogeography of this region, the extinction of deinotheres probably occurred gradually from west to east. This eventually could explain the survival of deinotheres in the above mentioned areas, in Ukraina and Russia, into the Pliocene, just as they persisted in Africa, where they survived even in the Pleistocene represented by *D. bozasi* ARAMBOURG, 1934 (ARAMBOURG, 1934; HARRIS, 1983**;** HARRIS et al., 2003**)**, a smaller sized deinothere compared to *D. proavum* (PICKFORD & POURABRISHAMI, 2013). However, in our opinion the stratigraphy of at least some of these Pliocene Eurasian localities should be carefully reassessed.

Table 1**.** Regional and stratigraphic distribution of deinotheres in Romania.

Species	**Locality**	**Geological age**	**References**
1. *D. giganteum*	Minişu de Sus Arad County	Early Sarmatian MN7+8	CODREA et al., 1991, 2007
2. *D. giganteum*	Deleni-Hârlău Iaşi County	Middle Sarmatian MN7+8 or MN9	MACAROVICI & ZAHARIA, 1967
3. *D. giganteum*	Vurpăr Sibiu County	?Pannonian *s.s.* ?MN9-MN12	CODREA & CIOBANU, 2008
4. *?Deinotherium* sp. (« *Tapirus giganteus* »)	Guşteriţa Sibiu County	?Pannonian *s.s.* ?MN9-MN12	ANONYMOUS, 1850
5. *D. giganteum*	Drăgeşti Bacău County	Khersonian or Meotian ?MN7+8 – MN12	ŞOVA, 1963
6. *D. giganteum*	Derna Bihor County	Late Pannonian *s.s.*- Early Pontian *s.s.* (?MN12)	JURCSAK, 1973, 1973a CODREA, 1989
7. *D. giganteum*	Brusturi Bihor County	?Pontian or Pannonian *s.s.* ?MN11 or ?MN12	CODREA, 1989
8. *D. giganteum*	Găiceana	?Meotian	ŞTEFĂNESCU, 1879, 1895
9. D. proavum	Bacău County	?Meotian	ŞTEFĂNESCU, 1891, 1895
10. *D. giganteum*	Supuru de Sus Satu Mare County	Pannonian *s.l.*	CODREA & ANDREICA, 1988
11. *D. giganteum*	Verneşti Argeş County	?Meotian	ATHANASIU, 1907
12. *D. proavum*	Mânzaţi Vaslui County	Meotian MN9-MN12	ŞTEFĂNESCU, 1895, 1899, 1910 CODREA, 1994
13. *D. proavum*	Gherghești Vaslui County	Meotian MN9-MN12	RĂŢOI et al., 2015 CODREA et al., 2015
14. *D. proavum*	Rediu Vaslui County	Uncertain ?Khersonian	Unpublished data
15. *D. proavum*	Huşi Vaslui County	Probably Meotian ?MN10	MERLAN, 2010; this paper
16. *D. proavum*	Derşida Sălaj County	Pontian *s.s.* MN13	JURCSÁK, 1973, 1983 CODREA et al., 2002 CODREA & MARGIN, 2009
17. ?*Deinotherium* sp.	Neamţ Monastry Neamţ County	Unknown	ŞTEFĂNESCU, 1905 ATHANASIU, 1907**;** MOROŞAN, 1936
18. *Deinotherium* sp.	Elanului Valley Vaslui County	Probably Meotian	JURCSÁK T. (*personal communication*)
19. *Deinotherium* sp.	Ghida Bihor County	Pannonian *s.l.*	KRETZOI, 1982
20. ??*Deinotherium* sp.	Comăneşti Galaţi County	?Pontian *s.s.*-Dacian	MOROŞAN, 1936

An interesting different viewpoint was raised by LUNGU & OBADĂ (2001, 2011). In their first paper (2001), they indicate in Table 1 a stratigraphic range for *D. gigantissimum* exclusively in the basal part of the stratigraphic unit that they named ‘Pliocene’ (Pontian = MN13-MN14). However in the text, commenting the MN10 unit, they affirm (p. 120): ‘It is to be mentioned that *D. giganteum* Kaup reaches gigantic sizes similar with those of *D. gigantissimum* Stefănescu is a synonym of *D. giganteum* Kaup’. In this manner, a contradiction between the table and text occurs, because the table mentions the large deinothere as a distinct species. A decade later (2011) they expose a different stratigraphic range, where the largest deinothere species *D. gigantissimum* occurred in southeastern Europe in the Vallesian (latest Bessarabian-Khersonian), afterwards disappearing in the Khersonian (we notice that in their chronology, the Khersonian would represents the MN10 unit, although this unit in the majority of stratigraphic mammalian charts is not Khersonian, but Meotian; *e.g.* STEININGER, 1999). Anyhow, this stratigraphic range fits not at all with the situation recorded either in Romania (for example in Transylvania, where *D. proavum* is reported in the latest Pontian *s.s.* at Sărmăşag, in the Şimleu Basin; JURCSÁK, 1983**;** CODREA et al., 2002**;** CODREA & MARGIN, 2009), or Hungary, where *D. proavum* finds are in a huge majority in the Late Miocene (KRETZOI, 1982), except the problematic Pliocene locality Zalaegerszeg (Dacian, MN14, GASPARIK, 2001). Therefore, such finds document a longer existence of the large sized deinotheres in this region, at least until the MN13 unit, much longer than the one presumed by the Moldavian palaeontologists.

Figure 3**.** Regional distribution of deinotheres in Romania: 1 – Minișu de Sus; 2 – Deleni-Hârlău; 3 – Vurpăr; 4 – Gușterița; 5 – Drăgești; 6 – Derna; 7 – Brusturi; 8 – Găiceana; 9 – Supuru de Sus; 10 – Vernești; 11 – Mânzați; 12 – Ghergheşti; 13 – Rediu; 14 – Huși; 15 – Derșida; 16 – Neamț Monastery; 17 – Elanului Valley; 18 – Ghida; 19 – Comănești.

Concerning the coexistence of *D. giganteum* and *D. proavum* in Europe, the opinions are also divided. Some palaeontologists agree with a long coexistence (*e.g.* MATHISHOV & KALMYKOV, 2012; in their Fig. 3, at least some Romanian deinothere localities are strangely placed in Brașov Depression, where no deinothere has ever been recorded, as well at west of the Olt River where the same situation occurs), while others are rather sceptical (a recent discussion on this subject, in PICKFORD & POURABRISHAMI, 2013 and references herein). We underline here, based on the Romanian deinothere localities that evidence of the coexistence between the medium and large deinotheres is rather meager (Table 1) based on the current evidence. In our opinion if such coexistence existed, it should be not too long-lasting around the transition to one species to another (a pattern defended by palaeontologists such as GASPARIK (2001) or PICKFORD & POURABRISHAMI (2013). This rule can be checked in Hungary, where in the *Hipparion* faunas, there is no coexistence between these deinothere species (KRETZOI, 1982). For other areas such as the Republic of Moldova (LUNGU & RZEBIK-KOWALSKA, 2011), we consider that either the stratigraphy of some localities or the species identification of the deinotheres should be revised, because the occurrence of the large forms since the Middle Sarmatian (in "Bessarabian", MN9; *e.g* at Breila and Malye Mileshty = Mileștii Mici in Romanian) is worth at least a discussion.

CONCLUSIONS

Herein, we consider once again that *D. proavum* is a valid species and *D. gigantissimum* is only a junior synonym of it.

The Huși deinothere find is important as it enlarges the list of deinothere localities in Romania. It can support the research of the palaeontologists interested in these proboscideans, in particular because the deinothere locality lists from our country are sometimes based only on old references (*e.g.* HUTUNEN, 2002). It also supports the frequency of these proboscideans in Moldova throughout the Late Miocene. A recent similar Meotian deinothere find at Ghergheşti (Vaslui County), not very far from Mânzați, reinforces this aspect (CODREA et al., 2015; RĂȚOI et al., 2015). Before the Meotian, *D. proavum* was reported neither from Moldova, nor from any other locality in Romania.

The deinotheres are present in our country in the Astaracian, Vallesian and Turolian vertebrate communities, the oldest representative of *D. giganteum* being reported from the inner Carpathians, in Transylvania, at Minișu de Sus (MN7+8). The youngest known deinothere in Romania is the one from Derșida (MN13), assigned to *D. proavum*. Everywhere, they document either fluvial or riparian environments.

Excepting the old find from Găiceana (Bacău County; Table 1), a locality with poorly documented stratigraphy, there is no other locality where the coexistence of the two deinothere species could be proved. The last occurrence of these proboscideans in Romania is older than the Pliocene.

ACKNOWLEDGEMENTS

The authors thank Paul Salomeea and Vicu Merlan (Municipal Museum of Huși) for facilitating access to the fossil for study and for the data they provided. Mihai Dumbravă (Cluj-Napoca) made Fig. 2. Special thanks are addressed by one of us (VAC) to Theodor Obadă (Zoological Institute Chișinău, Republic of Moldova) for the photographs with details on the limb bones of the deinothere skeleton exposed in Chișinău, for the rich amount of references he generously gave us and for our the collegial dialogues. Thanks to Martin Pickford (Collège de France, Paris) for improving the English, for useful suggestions and review and to Evangelia Tsoukala (Aristotle University of Thessaloniki) for her detailed review.

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Received: February 05, 2016
Accepted: July 16, 2016

GENETIC DIVERSIFICATION OF SALVIA SCLAREA L. QUALITY BY INCREASING THE STORAGE CAPACITY OF THE ESSENTIAL OIL

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Abstract. Genetic diversity of the quality in *S. sclarea* and development of genotypes with enhanced essential oil (EO) content have been conducted using the varieties of hybrid origin Ambra Plus (AP) and Nataly Clary (NC), both characterised by manifestation of fixed heterosis in a number of quantitative traits, including the content of EO. One hundred and twenty four inbred S_2 lines in the second year of vegetation not affected by degeneration through inbreeding have been developed and assessed through forced pollination. The findings show that the improvement of quality and the development of new genotypes with extremely high content of EO supported by the valuable quantitative traits through inbreeding in *S. sclarea* are efficient, the inbreeding provoking phenotypic, genotypic segregation of complex hybrid populations into a wide range of genotypes, some of them being promising. The phenotype of the inbred lines derived from the studied varieties is diversified, attesting genetic segregations expressed in the diversity of the values of quantitative trait indices such as plant height, inflorescence length and structure and in the content of EO supported by these traits. Along with the inbred lines in which the content of essential oil is lower than that in the lines they originate from, lines have been produced with enhanced (1.001-1.350%) and very enhanced (1.351-1.958%) content, the latter ones making 11% of the lines derived from AP and 7% of the total number of the lines derived from NC. Inbreeding causes phenological changes: the S_2 inbred lines fall into early-, mid- and late-ripening that constitutes 24, 48, 28%, respectively, in the lines derived from AP and 33% in each group of the lines derived from NC. The inbred lines with enhanced, very enhanced content of EO are valuable genotypes in view of the improvement of raw material quality and the increase of the productivity of *S. sclarea* cultivars.

Keywords: *Salvia sclarea*, genotype, inbred lines, essential oil.

Rezumat. Diversificarea bazei genetice a calităţii la *Salvia sclarea* L. prin creşterea capacităţii de acumulare a uleiului esenţial. Diversificarea genetică a calităţii la *S. sclarea* şi crearea genotipurilor cu conţinut ridicat de ulei esenţial s-a efectuat cu concursul soiurilor de provenienţă hibridă Ambra Plus (AP) şi Nataly Clary (NC), ambele caracterizându-se prin manifestarea heterozisului fixat la un şir de caractere cantitative, inclusiv, la conţinutul de ulei esenţial (UE). Prin polenizări forţate au fost create, evaluate 124 linii consangvinizate S_2 în anul al doilea de vegetaţie neafectate de degenerarea prin consangvinizare. Rezultatele obţinute demonstrează că îmbunătăţirea calităţii, crearea genotipurilor cu conţinut foarte ridicat de UE susţinut de caractere cantitative valoroase, prin metoda consangvinizări la *S. sclarea* este efectivă, consangvinizarea provocând segregarea fenotipică, genotipică a populaţilor hibride complexe într-o gamă largă de genotipuri, unele din acestea fiind foarte perspective. Fenotipul liniilor consangvinizate derivate de la soiurile AP şi NC, este diversificat, atestându-se şi segregări genetice exprimate în diversificarea valorilor indicilor caracterelor cantitative, cum ar fi talia plantei, lungimea, structura inflorescenţei cât şi a conţinutului de UE, susţinut de aceste caractere. De rând cu linii consangvinizate la care conţinutul de UE este mai jos de cât la soiurile de la care provin, s-au obţinut linii cu conţinut ridicat (1.001-1.350 %(s.u.) şi foarte ridicat (1.351-1.958% (s.u), ultimele constituind 11% linii derivate de la soiul AP şi 7% din numărul total de linii obţinute de la soiul NC. În rezultatul consangvinizărilor au loc şi schimbări fenologice: liniile consangvinizate $\mathbf{S_2}$, obţinute se împart în 3 grupe de maturizare: timpurii, medii şi tardive, care constituie 24, 48 şi 28%, respectiv, la liniile derivate de la soiul AP şi câte 33% în fiecare grup la liniile derivate de la soiul NC. Liniile consangvinizate cu conţinut ridicat şi foarte ridicat de UE sunt genotipuri valoroase în vederea îmbunătăţirii calităţii materiei prime, ridicării randamentului soiurilor de *S. sclarea*.

Cuvinte cheie: *Salvia sclarea*, genotip, linii consagvinizate, ulei esenţial.

INTRODUCTION

Salvia sclarea L. (Clary Sage) is a medicinal and aromatic species in the family Lamiaceae of Mediterranean origin known and appreciated in Ancient Egypt and the Roman Empire where it was cultivated. The species synthesizes and accumulates secondary metabolites (LEGRAND et al., 2010), including essential oil in inflorescences, flowers. In folk medicine, sage flowers are used externally to cure wounds, to bathe, to make laundry, to take care of skin and ulceration and edema of hair (KINTZIOS, 2000). Inflorescences have anticatarrhal and antispasmodic action (SOLDATCENCO et al., 1999; LATTOO et al., 2006; GONCEARIUC, 2008), in addition to carminative and oestrogenic one (LATTOO et al., 2006). The antioxidant, antimicrobial (GÜLÇIN, 2004; HYO JUNG YANG et al., 2014), antibacterial and cytotoxic activity (HAYET et al., 2007); sedative, emenagogous and anticonvulsive action (PĂUN, 1995; GONCEARIUC, 2000, 2013) are also mentioned for this species. The essential oil of *S. sclarea* is used in osteoarthrosis and rheumatic arthritis treatment (RUSU & CALININA, 1999; RUSU & CAMINSCHI, 2006). It is also employed in the treatment of arterial hypertension (SEOL et al., 2013), catarrhal inflammation, tonsillitis, and inflammation of teguments (PĂUN, 1995; GONCEARIUC, 2002), in aromatherapy (SETZER, 2009; HYO JUNG YANG et al., 2014). The utilization of this oil is beneficial due to the analgesic, anti-inflammatory (MORETTI et al., 1997), antioxidant (PITAROKILI, 2002; GÜLÇIN, 2004; LATTOO et al., 2006; SETZER, 2009), antifungal (PITAROKILI, 2002; SIMIĆ et al., 2004; LATTOO et al, 2006; JIROVETZ et al., 2007; DZAMIC et al.,2008) antimicrobial actions (PEANA et al., 1999; PITAROKILI et al., 2002; GONCEARIUC, 2002; GÜLÇIN, 2004; LATTOO et al, 2006; JIROVETZ et al., 2007;). As a nice nervous tonic, the essential oil of Clary Sage is used to treat depression (SEOL, 2010). The essential oil and inflorescences of *S. sclarea* appeared to have a beneficial action in the treatment of cancer (SIMON et al., 1984). Not only the essential oil but each of its components has a certain beneficial action in different treatments. For example, some researchers found antibacterial, antifungal and growth regulating activity of the essential oil of *S. sclarea* due to sclareol (SYBILLE VAN DEN BRÛLE et al., 2002; CANIARD et al., 2012), others attribute the antibacterial action to linalool (SIMIĆ et al., 2004), etc. As a nice nervous tonic, the essential oil of Clary Sage is used to treat depression (SEOL et al., 2010). The essential oil and inflorescences of *S. sclarea* appeared to have a beneficial action in the treatment of cancer and extracts from inflorescences are beneficial in the treatment of Alzheimer's disease (FILIPA et al., 2013).

The carbon bonds of terpenes in the essential oil of Clary Sage are known to constitute intermediate products in the biosynthesis of steroid hormones, enzymes, antioxidants, and vitamins, etc., the terpenes demonstrating analgesic, anti-inflammatory, antimicrobial, antiviral, diuretic, hypotensive, sedative, spasmolytic, expectorant, antirheumatic actions listed above. The therapeutic properties depend on the combinations of natural compounds that occur in the form of monoterpenes to polyterpenes (KRYLOV et al., 1992).

The essential oil of *S. sclarea* is also used in food industry to produce beer, tonic beverages, liqueurs, as well as Muscat and Vermouth type wines. In addition, this essential oil is widely employed in perfumery (VOITKEVICI, 1999; KINTZIOS, 2000; CLEBSCH, 2013), where it is greatly appreciated for both odorous qualities and as an excellent fixer (PĂUN, 1995; GONCEARIUC, 2002; 2008).

Processing of fresh material or wastes from the distillation of the essential oil through extraction with organic solvents affords a product named *concret* which, along with other components, contains sclareol, a labdanein type diterpene alcohol (DIMAS et al., 2007; CAISSARD et al., 2012). It is a minor component in the essential oil, while it is a major one in concret. Sclareol is considered a refined odorant in perfumery (LAVILLE et al., 2013) and especially a fixer. In perfume industry, sclareol is a principal bioactive component that may be also used to produce Ambrox (DECORZANT et al., 1987; GÜNNEWICH et al., 2013), a chemical compound in the class of tetralabdanoxides considered as one of the most valuable perfumes of animal origin Zibet and Moschus. Previously, the source of Ambrox was Ambra, a waxy substance from the digestive tract of the whale.

The importance of the species, its multiple utilizations and the fact that *S. sclarea* has been cultivated and processed for over 65 years in the Republic of Moldova, and essential oil and concret are designed for export impel a number of researchers to develop new genotypes, lines, hybrids, cultivars that would synthesize and accumulate essential oil in the contents as high as possible. A number of the cultivars of hybrid origin have been already developed, registered, patented and employed (GONCEARIUC, 2013; 2014), and the development of the initial material for prospective improvement is in process. This work is dedicated to the findings of these studies.

MATERIAL AND METHODS

The biological material was represented by 124 inbred S2 lines in the second year of vegetation not affected by inbreeding degeneration. They have been derived from two cultivars of hybrid origin: Ambra Plus, early-ripening, which is a complex backcross hybrid and the cultivar Nataly Clary, a triple late-ripening hybrid. Both cultivars (hybrids) are characterized by the presence of fixed heterosis. The plants selected as genotypes, the genitors of future inbred lines, were subject to forced self-pollination at the beginning of the flowering stage after the inflorescences had a special toilet treatment. The phenotypic differences were studied in each line through evaluation of the phenological development stages and of the morphological characters (qualitative) that directly influence the productivity. The essential oil content was measured in the samples of fresh inflorescences (100 g) through hydrodistillation for 60 min in the Ginsberg apparatus, the results recalculated for standard humidity (70%) and dry matter (d.m.). Ambra Plus and Nataly Clary from which the lines were derived were used as witnesses for the inbred lines produced.

RESULTS AND DISCUSSION

The revaluation of aromatic and medicinal plants including the species *S. sclarea* through improvement of the genetic pool of the initial breeding material necessary for the development of new productive hybrids and varieties envisages research aimed at the production of new genotypes with an enhanced content of essential oil supported by the valuable quantitative traits, all these ensuring excellent qualitative and quantitative composition of the essential oil (GONCEARIUC, 2002). We have employed two methods: inbreeding and hybridizations of different types to diversify the genetic basis for *S. sclarea* quality through rising the capacity of essential oil accumulation.

These studies have been initiated because the hybrids we have developed earlier are not simple androsterile hybrids and may be used only in the F_1 generation when the heterotic effect is the highest. In *S. sclarea*, the utilization of F_1 hybrids is not feasible due to the complications at producing F_1 seeds caused by the morphological traits of the cloggy pollen of relatively large size and of the flowers adapted to entomophilic pollination (GONCEARIUC, 2000; 2002; 2008; 2013). Taking into consideration the importance of heterosis employment (KIRPICHNIKOV, 1967; FU et al., 2014) and to take advantage of this phenomenon, we have developed a large number of hybrids of different types, including very complex and heterotic ones. Among them, we have selected hybrids in which the heterotic effect in a number of quantitative traits, especially in the essential oil content, is manifested not only in the F1 but in the F2-Fn generations (GONCEARIUC, 2000; 2002; 2002a; 2008; 2013). It is well known that unstable heterosis is manifested only in F_1 and loses its amplitude in further generations. It is also known that transmissible, fixed heterosis (MAC KEY, 1976; ABEL et al., 2005; WESPEL & BECKER, 2008; WESPEL et al., 2009; PAYAL BANSAL et al., 2012) consolidates in the genetic systems of the organism becoming a value of evolution (MAC KEY, 1976).

Our varieties developed using the hybrids in which the fixed heterosis is attested in the important quantitative traits (GONCEARIUC, 2000, 2002, 2002a, 2008, 2013), including those of Ambra Plus and Nataly Cary, are distinct, uniform, stable and highly-productive (GONCEARIUC, 2014; 2014a), while the production of seeds poses no difficulties. All these are explained by the fact that cross-fertilized heterozygous hybrid populations reduce the variability to a mean value necessary for adaptation of the population to the conditions of local cultivation (LEWIS, 1953; LEWIS, 1954). By ensuring the lability retention, the heterozygous population (in our case, the varieties Ambra Plus and Nataly Clary) is the cause of the fact that the phenotypic traits of a large number of genotypes are optimally adapted to a specific environment of the population existence (MATHER, 1955; MATHER, 1955a), which results in a balanced situation where natural selection occurs, that is genetic homeostasis takes place (LERNER, 1954; TURBIN, 1967). It is also known that inbreeding allows identification of forms with recessive traits and selection of desired and promising ones (GONCEARIUC, 2002, 2008, 2013, with the reference to N. I. Vavilov). Thus, inbreeding is also well known as a method of development of the initial material for breeding of alogamic species, such as *S. sclarea*. Therefore, diversification of the genetic basis for the quality of *Salvia sclarea* was achieved using this method. Genitors, the donors of the quality – the varieties Ambra Plus (early-ripening) and Nataly Clary (late-ripening) are the most productive, both of them representing complex hybrids with a fixed heterotic effect. Both varieties meet the requirements of the International Union for the Protection of New Varieties of Plants for the DUS factors: distinct, uniform, stable.

It is known that inbreeding leads to phenological, phenotypic and genetic changes in the selected and inbred genotypes.

The assessment of the inbred lines derived from both Ambra Plus and Nataly Clary shows that they are divided into three groups, early-, mid, and late-ripening (Figs. 1 and 2; Tables 1, 2).

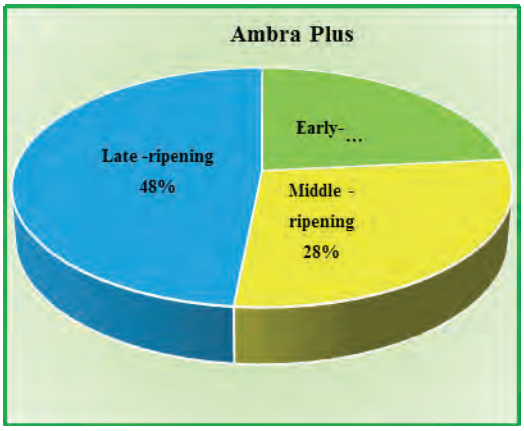

Figure 1. Inbred lines of *S. sclarea* derived from the early-ripening variety Ambra Plus with a different period of vegetation.

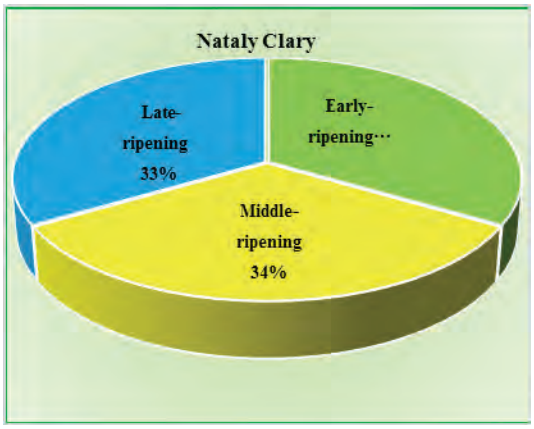

Figure 2. Inbred lines of *S. sclarea* derived from the early-ripening variety Ambra Plus with a different period of vegetation.

It should be mentioned that the number of late-ripening inbred lines originated from the early-ripening variety Ambra Plus is the highest (48%), while early- and mid-ripening varieties constitute 24 and 28%, respectively (Fig. 1). The inbred lines derived from the variety Nataly Clary are divided into early-, mid-, and late ripening equally for the duration of vegetation (Fig. 2).

Our studies have demonstrated that the quantitative traits that determine the phenotype in inbred lines differ from those of the original variety. The Tables present only the inbred lines derived from the same variety, that exhibit pronounced phenotypic diversity. For example, the height of the variety Ambra Plus is 120.6 cm (Table 1). The values for plant height in the early-ripening lines derived from this variety range from 105.3 (AP114-11S2) to 137.7 cm (AP37-11S2). The inbred lines with the lowest values for height were recorded in the set of lines with a medium period of maturation (AP103-11S2) – 98.1 cm and late-ripening ones (AP30-11S2) – 98.2 cm (Table 1).

The other important quantitative trait is inflorescence length. It makes 63 cm in Ambra Plus, while the lines derived from this variety have developed inflorescences with a length ranging from 47.9 cm to 74.3 cm. These lines also differ for the number of inflorescence ramifications, especially of the second degree. The number of oleiferous glands placed on their epidermis is known to be more significant and their density reaches that of the oleiferous glands on the flower calyx.

All these traits influence the synthesis and accumulation of essential oil, the content of which in the inbred lines derived from Ambra Plus makes 0.180-0.404% at a standard humidity (70%). Recalculated for dry matter, it varies between 0.458 in the early-ripening AP62-11S2 and 1.665% (d.m.) in the line with a medium period of vegetation

AP49-11S2 (Table 1). The oil content in Ambra Plus is 0.424% (70% of humidity) and 1.194% (d.m.). Thus, inbreeding allowed us to also obtain variability for the content of essential oil; thus, it was much higher in some lines, and much lower in others in comparison with the initial Ambra Plus variety. We conventionally divided the lines in four groups for the essential oil content: with a low (0.458-0.700% (d.m.), medium (0.701-1.000% (d.m.), high (1.001-1.350% (d.m.), and very high (1.351-1.958% (d.m.) content of oil.

It should be mentioned that in *Salvia sclarea*, the content of essential oil of more than 1% (dry matter) is considered high. The lines with the content of essential oil of over 1% derived from the variety Ambra Plus constitute 31% (Fig. 3a). The variety Ambra Plus belongs to this group with 1.192% (dry matter). Thus, by using inbreeding we have obtained lines with a very high content of essential oil, making 11% of the total lines derived from this variety.

Table 1. Diversification of quantitative trait values in the inbred lines of *Salvia sclarea* L. derived from early-ripening Ambra Plus, the 2nd year of vegetation, 2015.

Inbred line	Plant height, cm	Inflorescence length, cm	Number of ramifications:		Essential oil content, %	
			primary	secondary		
	X sX	X sX	X sX	X sX	Humidity 70%	Dry matter
Early-ripening 58-64 days						
AP 10-11 S_2	121.3±2.9	59.4±12.4	13.8±1.7	19.2±4.5	0.336	**1.367**
AP 37-11 S_2	137.7 ±11.4	74.3 ±1.3	14.8 ±3.5	24.7 ±8.6	0.359	1.142
AP 62-11 S_2	115.0 ±5.6	47.9 ±5.0	12.2 ±1.4	19.9 ±6.2	0.224	0.458
AP 77-11 S_2	118.2 ±12.0	67.8 ±11.5	15.1 ±2.3	22.2 ±6.1	0.180	0.547
AP 97-11 S_2	120.5 ±8.0	57.7 ±7.6	12.2 ±1.9	16.8 ±4.3	0.337	**1.224**
AP 114-11S_2	105.3 ±6.8	56.0 ±6.2	13.2 ±2.1	20.6 ±7.3	0.336	1.032
AP 115-11S_2	106.8 ±7.1	53.3 ±4.3	12.6 ±1.8	23.4 ±8.5	0.404	**1.333**
middle-ripening 65-70 days						
AP 34-11 S_2	118.6± 11.7	63.9± 0.7	14.2± 2.5	18.4 ±8.0	0.404	1.398
AP 49-11 S_2	102.1 ±5.2	51.0 ±6.3	12.0 ±1.8	16.0 ±5.5	0.494	**1.665**
AP 54-11 S_2	116.4 ±5.5	57.3 ±4.0	13.6 ±1.5	21.9 ±5.4	0.589	**1.367**
AP 66-11 S_2	99.4 ±5.3	51.7 ±4.2	13.6 ±0.8	19.7 ±3.1	0.404	1.158
AP103-11 S_2	98.1 ±4.7	49.3± 3.0	12.2 ±1.1	16.2 ±4.9	0.449	**1.351**
late-ripening, 71-82 days						
AP 28-11 S_2	**138.**1±9.2	73.4±5.3	16.0±2.1	29.8±10.1	0.359	1.279
AP 30-11 S_2	98.2± 5.3	52.8 ±5.0	11.8 ±1.4	14.4± 3.8	0.449	1.958
AP 52-11 S_2	105.7 ±6.5	50.1 ±4.2	12.6 ±1.3	17.8 ±3.8	0.426	1.397
AP 60-11 S_2	111.8± 6.5	51.7 ±4.7	11.4 ±1.3	18.2 ±4.1	0.359	1.310
AP 115-11S_2	106.8 ±7.1	53.3 ±4.3	12.6 ±1.8	23.4 ±8.5	0.404	1.333
Ambra Plus, st.	120.6±6.9	63.0±6.5	14.8.±2.2	20.8±6.3	0.424	**1.192**

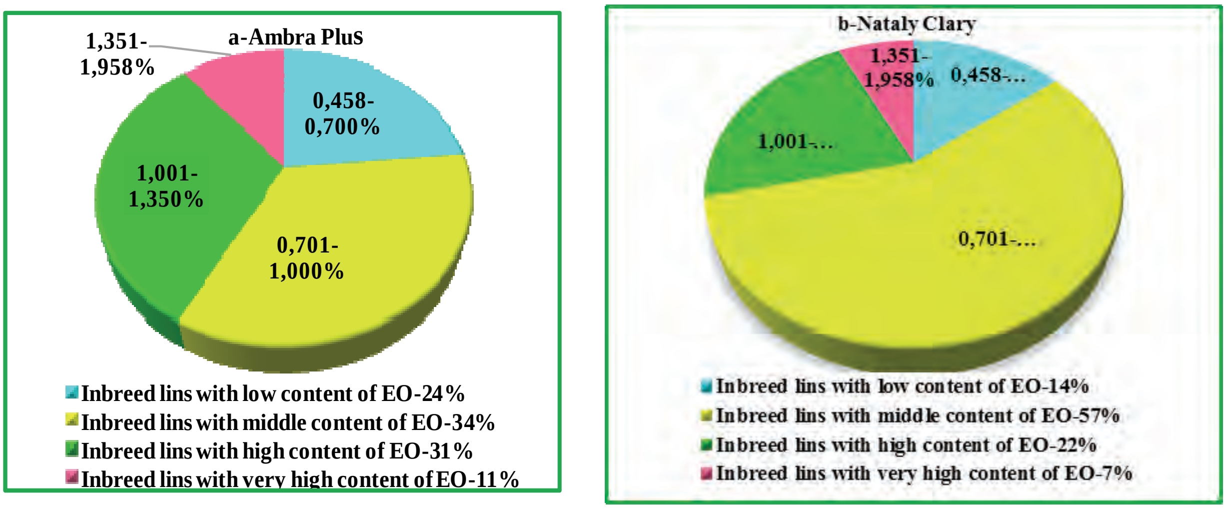

Figure 3. The content of essential oil in the inbred lines of *Salvia sclarea* derived from (a) Ambra Plus and (b) Nataly Clary.

As shown above, phenological and phenotypic traits, including quantitative traits that determine the line phenotype are subject to diversification.

The inbred lines derived from the late-ripening variety Nataly Clary, as well as those derived from early-ripening Ambra Plus differ phenologically providing early- and mid-ripening genotypes, the other lines remain late-ripening as the initial variety (Table 2, Fig. 2). The segregation of the quantitative trait indices is obvious. Only two early-ripening lines (NC4-11S2 and NC26-11S2) and two late-ripening ones (NC99-11S2 and NC21011S2) have provided plants with a higher length than that of the variety Nataly Clary from which they come from, this trait varying between 93.3 cm (NC60-11S2) and 132.6 cm in the line NC26-11S2 (Table 2). Among the early- and mid-ripening lines, seven developed longer inflorescences (58.6-60.8 cm) than those of the original variety. More marked differences were observed in the number of ramifications (the 2nd degree) of inflorescences, eight lines exceeding (22.0-28.3 cm) the indices recorded in the variety Nataly Clary (14.6-21.5 cm) for this trait.

Table 2. Diversity of the quantitative traits in S_2 inbred lines of *Salvia sclarea* L. derived from the late-ripening variety Nataly Clary, the 2nd year of vegetation, 2015.

Inbred line	Plant height, cm	Inflorescence length, cm	Number of ramifications: primary	Number of ramifications: secondary	Essential oil content, %	
	X ±sX	X± sX	X ±sX	X± sX	Humidity, 70%	Dry matter
Early, 59-64 days						
NC 6-11 S_2	116.2±6.8	**60.8**±6.7	14.8±2.5	23.6±11.0	0.359	**1.605**
NC 8 -11 S_2	95.5±5.3	**47.4**±5.3	10.4±0.8	11.4±2.5	0.359	1.069
NC 4 -11 S_2	125.8±2.9	62.4±6.7	15.0±2.3	28.3±9.3	0.359	**1.161**
NC 10 -11 S_2	110.8±7.3	54.0±6.2	13.6±1.8	22.3±7.6	0.337	1.075
NC 20 -11 S_2	110.8±5.6	58.7±4.5	14.0±1.8	22.6±4.3	**0.449**	1.064
NC 19 -11 S_2	110.4±6.2	52.4±3.8	14.0±1.6	24.4±5.3	0.584	**1.767**
NC 26 -11 S_2	**132.6**±6.3	**60.3**±4.5	13.4±1.6	19.6±5.1	0.269	0.696
NC 104-11 S_2	115.4±4.2	55.9±4.3	13.0±1.4	18.6±5.3	0.314	1.027
Middle, 65-70 days						
NC 61-11 S_2	106.1±7.5	49.8±3.1	10.8±1.3	14.2±2.8	**0.494**	**1.428**
NC 34 -11 S_2	118.7±5.4	56.1±6.3	14.4±2.2	30.6±9.5	0.224	0.567
NC 55-11 S_2	109.4±7.3	56.3±6.3	14.2±1.9	22.0±8.3	0.404	**1.162**
NC 96-11 S_2	116.4±5.3	54.9±3.9	13.2±1.9	23.0±8.3	0.337	1.198
Late, 71-80 days						
NC 13-11 S_2	108.0±5.5	53.9±7.5	14.6±1.6	21.4±7.1	0.202	0.725
NC 21 -11 S_2	124.8±3.6	61.4±6.6	15.0±1.9	20.0±6.6	**0.180**	0.619
NC 60-11 S_2	93.3±6.6	**47.1**±4.4	12.2±1.4	16.0±4.1	0.337	**1.188**
NC 75-11 S_2	117.7±5.3	57.1±5.3	13.6±2.0	22.6±7.3	0.404	**1.153**
NC 77-11 S_2	93.8±6.0	49.0±4.6	12.4±1.5	16.4±6.5	0.224	0.555
NC 99-11 S_2	**131.7**±3.9	52.2±3.1	12.6±2.3	26.6±10.0	0.359	0.978
NC 100-11S_2	126.4±7.1	**62.5**±8.5	16.4±2.9	**29.2**±8.5	0.292	0.779
Nataly-Clary, st	**118.6±4.0**	**57.7±6.9**	**14.6±6.4**	**21.6±6.4**	**0.402**	**1.154**

Inbred lines with enhanced and very enhanced content (1.161-1.767%, dry matter) of essential oil in comparison with 1.154% (dry matter) of the initial variety Nataly Clary have been obtained and attested from the variety Nataly Clary as in the case of the lines derived from early-ripening Ambra Plus (Table 2; Fig. 3b). As for the late-ripening inbred lines, the differences from the initial variety are less spectacular (Table 2). Differences were recorded in the trait of plant height, two inbred lines being higher (125.8 cm and 132.6 cm), the other lines developing lower plants. Other two inbred lines (NC21011S2 and NC100-11S2) formed longer inflorescences by 2.7 and 4.8 cm, respectively, than the variety Nataly Clary. Though the plants developed a higher number of ramification of the second degree of inflorescences, all these differences failed to provide a higher content of essential oil, on the contrary, the majority of the late-ripening inbred lines have a lower content of essential oil (0.555-0.725%, dry matter) with the exception of the lines NC75-11S2 (1.153%, dry matter) and NC60-11S2 (1.188%, dry matter). The inbred lines derived from the late-ripening variety Nataly Clary, similarly with those derived from early-ripening Ambra Plus, are conventionally divided into four groups for the content of essential oil: 14% of lines with low content, 57% of medium content, 22% of high content, and 7% of very high content (Fig. 3b).

Taking into consideration that the studies were carried out and the findings were obtained in the conditions of drought and intense heat of the year 2015, the value of the genetic material is indisputable.

The studies carried out to appreciate the uniformity/variability of the inbred lines derived from the early-ripening variety Ambra Plus have demonstrated that the variability of the trait plant length becomes low as early as in S_2 making V=2.3-10.1%, while the uniformity of inflorescence length varies from low values (V = 1.7-8.8) to medium ones (V = 10.4-19.6%).

All the inbred lines with increased and very high content of essential oil derived from both varieties are promising and will be included in the programs and plans of hybridization in different hybrid combinations as donors of genes to produce hybrid genotypes with enhanced content of essential oil, which ensures a superior quality of raw material of *Salvia sclarea* and a higher producing capacity of future hybrids and varieties.

CONCLUSION

1. The improvement of the quality and the development of new genotypes with valuable quantitative traits, including a very high content of essential oil through inbreeding of *Salvia sclarea* are efficient, the inbreeding provoking

phenotypic and genotypic segregation of complex hybrid populations into a wide range of genotypes, some of them promising.

2. The phenotype of the inbred lines derived from the varieties Ambra Plus and Nataly Clary is diversified, attesting genetic segregations expressed in diversity of the values of quantitative trait indices such as plant height, inflorescences length and their structure and in the content of essential oil supported by these traits.
3. Along with the inbred lines in which the content of essential oil is lower than that in the lines they originate from, lines have been produced with enhanced (1.001-1.350% d.m.) and very enhanced (1.351-1.958% d.m.) content, the latter ones making 11% of the lines derived from the variety Ambra Plus and 7% of the total number of the lines derived from Nataly Clary.
4. Inbreeding results in phenological changes: the S_2 inbred lines fall into three groups of maturation; early-, mid- and late-ripening that constitute 24, 48, and 28% respectively, in the lines derived from Ambra Plus and 33% in each group of the lines derived from Nataly Clary.
5. The inbred lines with enhanced and very enhanced content of essential oil are valuable genotypes in view of the improvement of raw material quality and the increase of the productivity of *Salvia sclarea* L. cultivars.

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Received: March 30, 2016
Accepted: June 12, 2016

POTENTIATION OF ANTIBIOTICS BY THE HYDROETHANOLIC EXTRACT OF *Juglans nigra* L.

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Abstract. Antibacterial resistance in the case of nosocomial infections, especially in immunocompromised patients is becoming a serious concern worldwide. Moreover, the harmful effects of antibiotics in some cases can endanger the patient's life. Herbs associated with common antibiotics may not only reduce the harmful effect but would have an effect in the potentiating the antibacterial properties. Another advantage of this combination would be the avoidance of the abuse of antibiotics that may develop uncontrolled adverse reactions. *Juglans nigra*, a less studied plant, has been shown to have *in vitro* the capacity to potentiate the antibiotics with bacterial resistance.

Keywords: *Juglans nigra*, antibiotics, potentiation, MDR bacteria.

Rezumat. Potențarea antibioticelor de către extractul hidroetanolic de *Juglans nigra* L. Rezistența antibacteriană în cazul infecțiilor nosocomiale, în special care vizează pacienții imunocompromişi, este o problemă serioasă la nivel mondial. De asemenea efectele nocive ale antibioticelor pot periclita în unele situații viața pacientului. Plantele medicinale asociate cu antibioticele uzuale ar putea nu numai să reducă efectul nociv al acestora, dar ar avea un efect și în potențarea proprietăților antibacteriene. Un alt avantaj al acestei asocieri ar fi evitarea abuzului de antibiotice care pot dezvolta reacții adverse necontrolate. *J. nigra*, o plantă mai puțin studiată s-a dovedit a avea capacitatea de a potența antibiotice cu rezistență bacteriană.

Cuvinte cheie: *Juglans nigra*, antibiotics, potențare, bacterii MDR.

INTRODUCTION

Obtaining drugs from herbal extracts has started to become an area that arouses an increasing interest in conditions in which the usual antibiotics do not seem to have the targeted effect. About 25% of the drugs prescribed worldwide come from plants. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from secondary metabolism of *Juglans nigra* (RATES, 2011). Juglone occurs naturally in the leaves, roots, husks, fruit (epicarp) and bark of plants in the Juglandaceae family (STUGSTAD & DESPOTOVSKI, 2012). MIC values for juglone showed it to have moderate antifungal activity and to be as effective as certain commercially available antifungal agents (CLARK et al., 2006). The mechanism for the toxic effects of juglone is still not fully understood. The antimicrobial, antidiarrheal, anthelmintic, depurative and tonic, antihemorrhagic, hypoglycaemic, diuretic, blood purifying, and detoxifying, vascular protective, inhibitory to tumours effects have been reported as being the expected effects of juglone (STUGSTAD & DESPOTOVSKI, 2012). After we took into consideration the wide spectrum of action of the juglone against a wide pathogenic microorganisms, we have analysed *in vitro* the activity of potentiating the hydro-ethanolic extract of *Juglans nigra* of antibiotics against Gram negative bacteria isolated from nosocomial infections**.**

MATERIAL AND METHODS

1. Preparation of the hydro-ethanolic extract of *J. nigra*

Raw fruit endocarp of *J. nigra* was collected in September-October and allowed to macerate in ethyl alcohol with distilled water in a ratio of 30 g *J. nigra* / 20 ml water / 50 ml ethyl alcohol for 10 days in the dark at 4°C. After that it was purified by Whatman filter paper no. 1 and was introduced into a rotary evaporator for 10-15 minutes. This was the stock solution and was stored in an amber glass container at 4°C.

2. Phenotypic analysis of resistance MDR strains from nosocomial infections by disc-diffusion method of Kirby and Bauer

The strains isolated from urogenital infections were from patients hospitalized at Theodor Burghele Hospital, Bucharest.

On solid media Mueller-Hinton (MH) agar, they were inoculated in a cloth with a suspension (0.5 McFarland standard) of the pathogen bacterial culture. Then, they were placed on filter paper disks (6 mm) impregnated with a known concentration of an antimicrobial compound (in the vicinity of the disc producing a higher concentration of antibiotic that decreases as the distance increases. The plates were incubated at 37°C for 16-18 hours. There occur the simultaneous growth of the bacteria and diffusion of the antimicrobial compounds. The point at which critical mass is reached is demonstrated by a sharply marginated circle of bacterial growth around the disk. The concentration of antimicrobial compound at this margin is called the critical concentration and is approximately equal to the minimum inhibitory concentration obtained in broth dilution susceptibility tests. The current interpretation standards can be found in the CLSI (Clinical Laboratory Standards Institute) 2009 (HUDZIKI, 2013).

3. Analysis of interactions between the extract of *J. nigra* and the antibiotics

The interactions between the extract of *J. nigra* and the antibiotics were determined using disc-diffusion method. Thus, on the solid media Mueller-Hinton agar seeded with the bacterial suspension, the disks impregnated with antibiotics was pipetted with 10 mg extract of *J. nigra*. The fractional inhibitory concentration was derived from the concentrations of the *J. nigra* extract and the antibiotics in combination permitting no visible growth of the test microorganisms in the Mueller-Hinton agar after incubation for 24 h at 37°C. Minimum inhibitory concentration (MIC) was made by the method of serial microdilution in a liquid medium BHI (Hearth Infusion Broth) in deepwell plates 96 (Eppendorf tubes of 550 ml) following the protocol published in a recent article (ROMAN et. al., 2015).

RESULTS AND DISCUSSION

Following the analysis of the antibiotic and extract of *J. nigra* resistance via disk-diffusion method resulted in many situations of synergism that can be observed in Fig. 1.

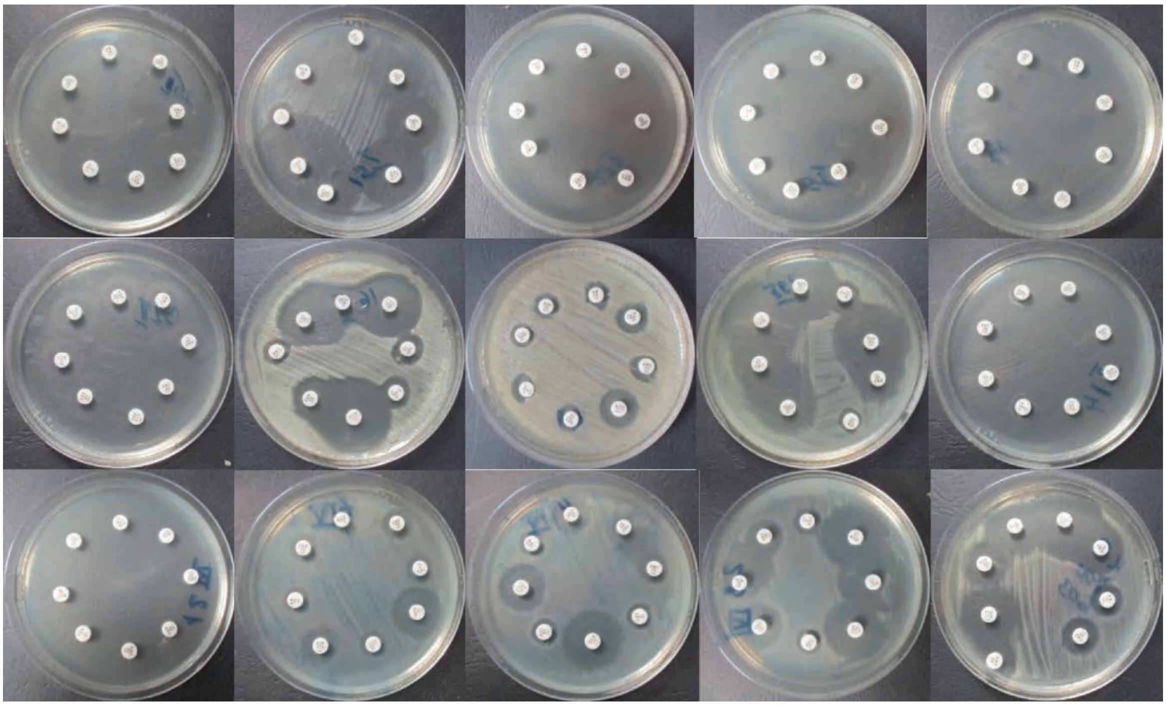

Figure 1. The potentiation of antibiotics by *J. nigra* extract. Inhibition zones of bacterial growth are marked by clear areas that appear around the paper disks impregnated with antibiotic and extract of *J. nigra* (*A. faecalis*$_{II25}$, *K. pneumoniae*$_{I15}$, *P. aeruginosa*$_{II27}$, *A. baumannii*$_{I5}$, *P. mirabilis*$_{I1}$, *K. pneumoniae*$_{I6}$, *K. pneumoniae*$_{III8}$, *K. pneumoniae*$_{II29}$, *E. coli*$_{III16}$, *P. aeruginosa*$_{I4}$, *K. pneumoniae*$_{I12}$, *E. coli*$_{IV7}$, *K. pneumoniae*$_{IV4}$, *E. coli*$_{IV23}$, *K. pneumoniae*$_{ATCC\ 700603}$. (original).

The antibiogram by disc diffusion method was performed by measuring inhibition zones around the antibiotic impregnated disks and the results were summarized in Table 1.

Table 1. Antibiogram by disc diffusion method.

Strain	Average zones of inhibition (±0.5 mm) of antibiotics							
	CN$_{10}$	**FEP$_{30}$**	**SXT$_{25}$**	**LEV$_{5}$**	**TZP$_{110}$**	**AMC$_{30}$**	**CAZ$_{30}$**	**MEM$_{10}$**
K. pneumoniae$_{II29}$	0	12	25	10	20	20	14	25
K. pneumoniae$_{III8}$	0	10	0	0	14	10	10	14
E. coli$_{IV23}$	0	20	0	0	25	14	25	25
E. coli$_{III16}$	0	0	0	14	25	0	20	25
A. baumannii$_{I5}$	0	0	10	12	12	10	0	0
P. mirabilis$_{II32}$	0	0	0	0	12	14	0	0
P. mirabilis$_{I1}$	0	0	14	0	14	12	0	0
P. aeruginosa$_{II27}$	0	0	12	10	0	0	0	10
A. faecalis$_{II25}$	0	0	14	12	10	14	0	0
K. pneumoniae$_{I12}$	14	14	20	0	0	0	0	0
P. aeruginosa$_{I4}$	0	0	14	0	12	14	0	0
K. pneumoniae$_{II30}$	0	0	0	0	25	32	0	0
K. pneumoniae$_{I6}$	20	30	14	18	25	25	20	30
K. pneumoniae$_{ATCC\ 700603}$	12	20	19	25	25	20	18	32
E. coli$_{IV28}$	0	14	14	12	18	12	12	14
K. pneumoniae$_{IV4}$	0	12	0	0	14	0	20	14
E. coli$_{IV7}$	0	10	0	0	12	12	25	20
E. coli$_{I\ ATCC\ 25922}$	25	30	30	30	30	30	30	30

The synergism between the antibiotic and *J. nigra* extract has been calculated by measuring the inhibition zone with graded ruler and centralized in Table 2.

Table 2. Synergism between the antibiotic and *J. nigra* extract.

Strain	**Average zones of inhibition (±0.5 mm) of hydro-ethanolic extract of *J. nigra* (JN) alone and MIC(µg/ml), combination of antibiotics and JN and the degree of potentiation of the antibiotic (A, S, I)**								
	JN area and MIC (g/ml)	**CN$_{10}$ +JN**	**FEP$_{30}$ +JN**	**SXT$_{25}$ +JN**	**LEV$_{5}$ +JN**	**TZP$_{110}$ +JN**	**AMC$_{30}$ +JN**	**CAZ$_{30}$ +JN**	**MEM$_{10}$ +JN**
K. pneumoniae$_{II29}$	14 (250)	10 (S)	22 (S)	32 (S)	12 (S)	32 (S)	22 (S)	28 (S)	32 (S)
K. pneumoniae$_{III8}$	17 (125)	12 (S)	14 (S)	14 (S)	14 (S)	18 (S)	13 (S)	14 (S)	18 (S)
E. coli$_{IV23}$	16 (125)	14 (S)	25 (S)	32 (S)	14 (S)	32 (S)	25 (S)	28 (S)	32 (S)
E. coli$_{III16}$	20 (15.625)	12 (S)	18 (S)	14 (S)	13 (S)	32 (S)	20 (S)	25 (S)	32 (S)
A. baumannii$_{I5}$	12 (250)	14 (S)	20 (S)	30 (S)	25 (S)	32 (S)	32 (S)	12 (S)	14 (S)
P. mirabilis$_{II32}$	16 (125)	12 (S)	12 (S)	14 (S)	14 (S)	32 (S)	32 (S)	12 (S)	14 (S)
P. mirabilis$_{I1}$	18 (31.25)	7 (S)	12 (S)	20 (S)	12 (S)	30 (S)	30 (S)	12 (S)	12 (S)
P. aeruginosa$_{II27}$	17 (31.25)	12 (S)	12 (S)	32 (S)	32 (S)	32 (S)	32 (S)	12 (S)	14 (S)
A. faecalis$_{II25}$	12 (250)	14 (S)	14 (S)	25 (S)	25 (S)	32 (S)	32 (S)	14 (S)	12 (S)
K. pneumoniae$_{I12}$	16 (125)	14 (I)	14 (S)	20 (S)	30 (S)	25 (S)	20 (S)	18 (S)	14 (S)
P. aeruginosa$_{I4}$	20 (15.625)	14 (S)	0 (I)	32 (S)	0 (I)	32 (S)	30 (S)	0 (I)	14 (S)
K. pneumoniae$_{II30}$	18 (31.25)	0 (I)	0 (I)	0 (I)	0 (I)	30 (S)	32 (S)	0 (I)	0 (I)
K. pneumoniae$_{I6}$	14 (250)	12 (A)	32 (S)	30 (S)	32 (S)	32 (S)	32 (S)	20 (I)	30 (I)
K. pn$_{ATCC\ 700603}$	17 (125)	14 (S)	20 (I)	25 (S)	30 (S)	25 (S)	20 (I)	18 (I)	32 (I)
E. coli$_{IV28}$	21 (15.625)	7 (S)	20 (S)	25 (S)	20 (S)	25 (S)	18 (S)	18 (S)	21 (S)
K. pneumoniae$_{IV4}$	12 (250)	7 (S)	12 (I)	7 (S)	7 (S)	22 (S)	10 (S)	20 (I)	18 (S)
E. coli$_{IV7}$	16 (125)	7 (S)	10 (I)	10 (S)	12 (S)	12 (I)	12 (I)	10 (A)	20 (I)
E. coli$_{I\ ATCC\ 25922}$	14 (250)	30 (S)	30 (I)	30 (S)	30 (I)	30 (I)	30 (I)	30 (I)	30 (I)

Legend: Abbreviations: S=Synergy; I=Indifference; A=Antagonism

The minimum inhibitory concentration (MCI) of the hydro-ethanolic extract of *J. nigra* ranged between 250 and 15,625 µg / ml. Inhibition zones of *J. nigra* extract ranged between 12 and 21 ± 0.5 mm. In combination with the TZP (piperacillin-tazobactam) these zones of inhibition were significantly increased; in 89% of the cases, it is observed the synergism in the interaction of the antibiotic and the extract of *J. nigra* and only 11% of the cases are characterized by indifference, having no significance in combination with the antibiotic (*E. coli*$_{IV7\ and}$ *E. coli*$_{I\ ATCC\ 25922}$). A remarkable interaction is also observed in the combination with AMC (amoxicillin-clavulanic acid); in 83% of the cases it is observed the synergism while 17% are characterized by indifference, without having any influence on the antibiotic. In combination with the CN (gentamicin), it has been observed one case of antagonism in the interaction between the antibiotic and extract (*K. pneumoniae*$_{I6}$) and in the combination with the CAZ (ceftazidime) and the extract of *J. nigra*, in the presence of a single strain (*E. coli*$_{IV7}$). It is noteworthy that in 9 cases where the antibiotic did not show any growth of the inhibition zone, the interaction of the antibiotic with the extract did not have any influence on the growth of the inhibition zone. In 49 cases, in which the antibiotic has not submitted any growth of the inhibition zone, in combination with the extract, the inhibition zone had a growth with values between 7 and 18 mm; the greatest interaction by potentiating the antibiotic was registered in combination with CAZ (*K. pneumoniae*$_{I12}$), LEV (levofloxacin) in case of *K. pneumoniae*$_{I12}$, SXT (sulfamethoxazole) in case of *E. coli*$_{IV23}$ and FEP (cefepime) in *A. baumannii*$_{I5}$. MEM (meropenem) belongs to the class carbapenems, antibiotics with large spectrum, being administered in circumstances where bacterial infections do not respond to other antibiotics. In interaction of the MEM and *J. nigra* extract, in 72% of cases it was observed the synergism and in 28% of the cases the interaction between the extract and the MEM did not have any influence on the inhibition zone. The action mechanisms of compounds of plants against bacterial growth are less understood. Their synergism with some antibiotics in certain situations, it may suggest their compatibility with the mode of action of antibiotics. The vegetal extracts have multiple action targets in the microbial cell, which represents an advantage of their use as antimicrobial agents with synergistic activity (BENNETTE & WALLSGROVE, 1994).

CONCLUSIONS

Hydro-ethanolic extracts of *J. nigra* in combination with antibiotics had an enhanced effect against resistant bacteria, by potentiating the antibiotics. This study has demonstrated the *in vitro* antibacterial activity-of the extract *J. nigra* against antibiotic resistant strains. The association of the antibiotic and *J. nigra* extract had a greater effect against the most studied bacteria. *J. nigra* is a plant with multiple therapeutic properties that deserve to be studied and used in the pharmaceutical industry in order to obtain a new antibacterial synthetic substance with large spectrum of action.

ACKNOWLEDGEMENS

We express our gratitude to Professor Coordinator, Mihăescu Grigore, Professor Carmen Mariana Chifiriuc director of the Research Institute of the Faculty of Biology, Dr. Camelia Tudor, head of the laboratory of "Theodor Burghele" Hospital, Bucharest.

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Received: March 31, 2016
Accepted: August 12, 2016

THE REACTION OF SOME VALUABLE TOMATO SORTS (VARIETIES) TO FILTRATES OF *Fusarium* spp. AND *Alternaria alternata* CULTURES

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Abstract. There are presented the data regarding the reaction of some valuable tomato genotypes to culture filtrates of pathogenic fungi, which frequently have been isolated from plants with symptoms of disease. The significant influence of *Fusarium* spp. and *A. alternata* pathogens on the early stages of ontogeny of the tomato genotypes by the suppression of seed germination and growth of radicles and stemlets (sometimes by their stimulation) has been found. The varieties Tomis and Mihaela are less sensitive to the action of fungi and can be successfully used as donors of resistance to fusariosis and alternariosis. The significant ponderosity of the role of fungus species (*Fusarium* spp., *A. alternata*) and of 'tomato genotype x species of fungus' interaction, as the sources of variation of plant organs of growth, reveals the need for constant monitoring of the composition and virulence of fungi species causing root rot on tomato.

Keywords: tomato, varieties, culture filtrate, *Fusarium* spp., *Alternaria alternate.*

Rezumat. Reacţia unor soiuri valoroase de tomate la filtratele de cultură *Fusarium* spp. şi *Alternaria alternata*. Sunt prezentate date despre reacţia unor genotipuri valoroase de tomate la filtratele de cultură a patogenilor fungici *Fusarium* spp şi *Alternaria alternata,* frecvent izolați din plante cu simptome de boală. S-a constatat că patogenii *Fusarium* spp. și *A. alternata* influențează semnificativ ontogeneza timpurie a genotiputilor de tomate prin reprimarea germinației semințelor și creșterii rădăciniței și tulpiniței (uneori - prin stimularea acestora). Soiurile Tomiş şi Mihaela sunt mai puţin sensibile la acţiunea fungilor şi pot fi cu succes utilizate ca donatori de rezistenţă la fuzarioză şi alternarioză. Ponderea semnificativă a rolului speciei fungului (*Fusarium* spp., *A. alternata*) și interacțiunii genotip de tomate x specie de fung în sursa de variație a organelor de creștere a plantelor, relevă necesitatea monitorizării constante a componenței și virulenței speciilor de fungi care cauzează putregaiul de rădăcină la tomate.

Cuvinte cheie: tomate, soiuri, filtrate de cultură, *Fusarium* spp., *Alternaria alternata.*

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) occupy an important place in the global (BARONE et al., 2008) and national (BOTNARI & CEBOTARI, 2003) economy due to the high nutritional value of fruits. Different ways and modes of their consumption are possible: as fresh fruits or mixed with other vegetables, as sauces, stews, stuffed tomatoes, and as many other kinds of processed products.

Among biotic unfavourable factors affecting growth and development of tomato plants in the Republic of Moldova fungal pathogens *Fusarium* spp. that cause the rot of root and basal stem, plants wilt (LUPAȘCU, 2004; ROTARU, 2011), as well as *Alternaria* spp. that cause brown / black and dried spots on leaves, followed by early yellowing of leaves and premature drying (GRIGORCEA, 2014), must be considered.

In order to create valuable varieties, sustainable in terms of productivity and quality, genotypes need to include, as mandatory support, the resistance to biotic and abiotic unfavourable factors. That is why screening of perspective forms, concerning their reaction to these factors, is required. Moreover, the elucidation of complex resistance of already created varieties obligates to identify valuable genotypes with broad opportunities, which are involved in breeding programs as possible donors of resistance to known pathogens with high frequency and virulence (LUPAȘCU et al., 2008; MIHNEA et al., 2016).

Fungal diseases are usually controlled by chemical fungicides that cause undesired consequences for the human health and environment (GAVRILESCU & CHISTI, 2005). For these reasons, long-term use of resistant varieties is economically and environmentally advantageous (GUIMARAES et al., 2007; TSUTOMU et al., 2007).

The aim of this research was to identify tomato varieties with complex resistance to the *Fusarium* spp. and *Alternaria alternata* pathogens based on the reaction to the treatment with the pathogens culture filtrates.

MATERIAL AND METHOD

Six tomato varieties with high indexes of productivity and quality, created in the Institute of Genetics, Physiology and Plant Protection (IGPPP), were used as a material for research.

Culture filtrates (CF) of the fungi *F. oxysporum*, *F. solani*, *F. redolens,* and *Alternaria alternata* (isolated from infected tomato plants) were applied. They were prepared by the inoculation of mycelium into the Czapek-Dox liquid medium and subsequent cultivation at the temperature 22-24ºC for 21 days.

Tomato seeds were treated with CF of the fungi for 18 hours. Seeds, which were kept in distilled water, were used as a control. The cultivation of seedlings took place in Petri dishes on the filter paper, moistened by distilled water, at room temperature 22-24ºC for 6 days. The important characteristics like growth and development of tomato at the early stages of ontogeny (germination, rootlet and stemlet length) were taken as index-test of the reaction of plants.

Bifactorial analysis of the data ANOVA (package STATISTICA 7) was applied to assess the role of genotype, fungus species and their interaction as a source of variation in quantitative traits.

RESULTS AND DISCUSSIONS

The response of the varieties to the treatment of seeds with CF *F. oxysporum*, *F. solani*, *F. redolens*, *A. alternata* demonstrated strong differences in dependence on the analysed trait: germination, rootlet and stemlet length. The response to the infection fits in categories: lack of reaction, inhibition, stimulation (Fig. 1).

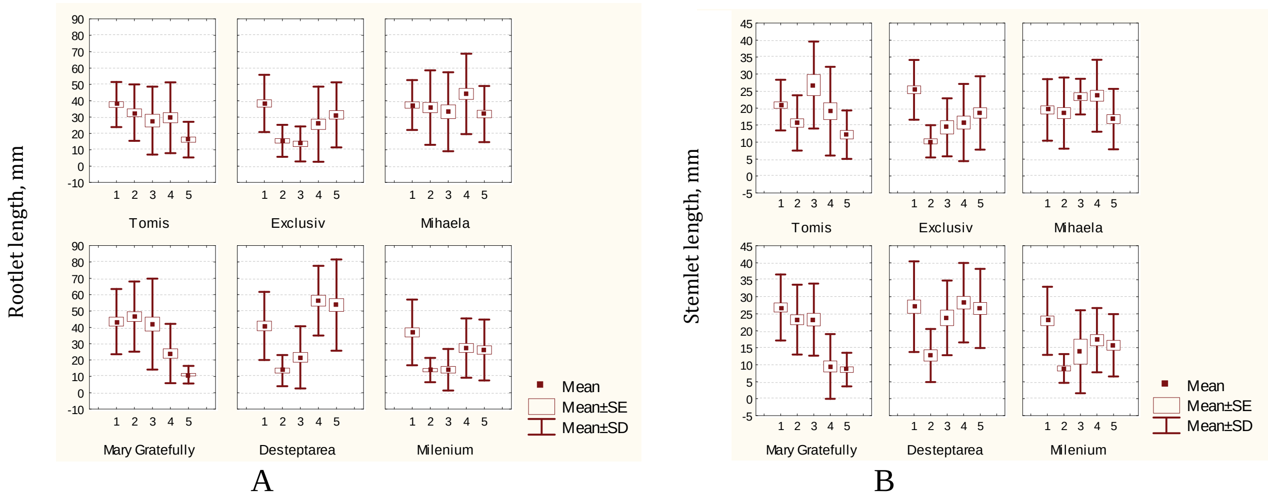

Figure 1. The influence of the filtrates of *Fusarium* spp. and *Alternaria alternata* cultures to the radicles and stemlets growth of tomato seedlings.
Horizontally: 1 – control (H_2O), 2 – *F. oxysporum*, 3 – *F. solani*, 4 – *F. redolens*, 5 – *A. alternata*.

Germination. In the control, seed germination ranged within 85.0 ... 100.0%, so it was quite high. These values show the high quality of seeds and allow the opportunity to test reaction of plants. In most cases CF influence decreased germination. The most pronounced decrease was manifested in Tomis and Mihaela varieties in case of *F. solani* fungus where germination declined to 48.3 % and 55.0 %, respectively. The cases of the germination raise to 13.3 % ... 15.0 % were registered in Milenium and Desteptarea varieties with the influence of *A. alternata* CF.

Rootlet. In the control, the rootlet length was registered in the ranges of 36.9-43.5 mm in the studied varieties (Table 1). In variants with CF it has been shown repression in Tomis, Exclusive, Desteptarea, Milenium varieties (*F. oxysporum*); Tomis, Exclusive, Mihaela, Desteptarea, Milenium (*F. solani*); Tomis, Exclusive, Mary Gratefully, Milenium (*F. redolens*); Tomis, Exclusive, Mihaela, Mary Gratefully, Milenium (*A. alternata*), and significant stimulation in Mihaela (*F. redolens*), Mary Gratefully (*F. oxysporum*), Desteptarea (*F. redolens*, *A. alternata*).

Table 1. The influence of *Fusarium* spp. and *Alternaria alternata* culture filtrates on some characteristics of tomato growth and development.

No.	Variant	Germination, %	Rootlet length, mm		Stemlet length, mm	
			$x \pm m_x$	S	$x \pm m_x$	S
		Tomis				
1	H_2O (control)	100	37. 7±1. 8	189. 1	20. 8±1. 0	55. 7
2	FC *F. oxysporum*	88. 3	32. 6±2. 4	297. 2	15. 6±1. 2*	66. 2
3	FC *F. solani*	48. 3	27. 9±3. 9*	431. 9	26. 8±3. 1	164. 1
4	FC *F. redolens*	81. 7	29. 6±3. 1*	468. 7	19. 1±2. 5	170. 1
5	FC *A. alternata*	78. 3	16. 2±1. 6*	119. 2	12. 2±1. 2*	50. 6
		Exclusiv				
1	H_2O (control)	98. 3	38. 3±2. 3	305. 8	25. 4±1. 1	77. 4
2	FC *F. oxysporum*	91. 5	15. 5±1. 3*	95. 2	10. 2±0. 8*	22. 7
3	FC *F. solani*	71. 2	13. 6±1. 6*	113. 9	14. 3±2. 0*	72. 9
4	FC *F. redolens*	89. 8	25. 6±3. 1*	527. 1	15. 8±1. 9*	128. 7
5	FC *A. alternata*	100	31. 3±2. 6	395. 2	18. 6±1. 5*	116. 4
		Mihaela				
1	H_2O (control)	100	37. 4±2. 0	230. 9	19. 5±1. 2	81. 8
2	FC *F. oxysporum*	90. 0	35. 8±3. 1	517. 6	18. 5±1. 6	109. 5
3	FC *F. solani*	55. 0	33. 3±4. 2	583. 3	23. 3±1. 2*	27. 5
4	FC *F. redolens*	88. 3	44. 2±3. 4	604. 2	23. 6±1. 6*	112. 0
5	FC *A. alternata*	90. 0	31. 8±2. 3	292. 3	16. 7±1. 3	78. 7
		Mary Gratefully				
1	H_2O (control)	91. 7	43. 5±2. 7	399. 2	26. 8±1. 3	94. 6
2	FC *F. oxysporum*	92. 7	46. 6±92. 7	460. 7	23. 3±1. 4	105. 6
3	FC *F. solani*	78. 2	42. 0±4. 2	770. 9	23. 3±1. 8	112. 1
4	FC *F. redolens*	70. 9	24. 0±2. 9*	328. 0	9. 5±1. 6*	90. 9
5	FC *A. alternata*	81. 8	11. 0±0. 8*	28. 8	8. 6±0. 8*	24. 2

Desteptarea						
1	H_2O (control)	85	40. 9±2. 9	433. 8	27. 1±1. 9	178. 2
2	FC *F. oxysporum*	76. 5	13. 6±1. 5*	90. 4	12. 7±1. 7*	61. 0
3	FC *F. solani*	80. 4	21. 6±3. 0*	360. 8	23. 8±2. 3	120. 4
4	FC *F. redolens*	80. 4	56. 3±3. 3*	453. 2	28. 2±1. 8	136. 5
5	FC *A. alternata*	100	53. 6±3. 9*	776. 9	26. 6±1. 7	136. 8
Milenium						
1	H_2O (control)	86. 7	36. 9±2. 8	403. 3	22. 9±1. 4	100. 7
2	FC *F. oxysporum*	98. 1	13. 9±1. 0*	55. 1	8. 9±0. 8*	18. 0
3	FC *F. solani*	73. 1	14. 0±2. 1*	161. 2	13. 8±3. 7*	149. 0
4	FC *F. redolens*	84. 6	27. 3±2. 7*	329. 1	17. 2±1. 6*	89. 4
5	FC *A. alternata*	100. 0	26. 1±2. 5*	346. 0	15. 7±1. 5*	84. 3

Legend: * – distinction from the control at $p \leq 0.05$.

It should be mentioned that the reaction of different varieties to the same isolate was quite different. For example, the effect of *F. oxysporum* CF and *F. solani* CF on Exclusive, Desteptarea, Milenium varieties was strong, inhibition, on Mihaela variety - insignificant deviation from the control. Mary Gratefully variety demonstrated some stimulative effect of *F. oxysporum* CF on radicle growth, as well as *F. solani* CF that caused minor deviations. The culture filtrate of *F. redolens* influenced as stimulator Mihaela and Desteptarea varieties and as strong inhibitor Mary Gratefully variety. The analysis of 24 variants of CF treatment showed that the rootlet growth was inhibited in 20 cases and stimulated in 4 cases. Plants responded to the CF action not only by the decrease or increase of the growth but also by changing their heterogeneity. The presented data demonstrate that the value of variance (S) increased in 14 cases and decreased in 10 cases.

Stemlet. In the control, the variation of stemlet length was found within 19.5 ... 27.1 mm. In CF variants, different genotypes demonstrated sufficiently differential sensitivity. The influence of CF inhibited stemlet growth in 20 cases, and stimulated it significantly in 4 cases. The values of variation demonstrate that the CF influence increased plant heterogeneity in 10 cases, however it was less pronounced than in the case of radicle. Strong inhibition was shown under the influence of CF *F. oxysporum* at Exclusive, Desteptarea, Milenium varieties, *F. solani* CF inhibited Exclusive and Milenium, stimulated – Tomis and Mihaela. The culture filtrates of *F. redolens* influenced stimulatively the growth of Mihaela and Desteptarea stemlet, and inhibitively – Exclusive, Mary Gratefully, Milenium. Tomis showed lack of reaction. It should be mentioned that CF of *A. alternata* inhibited the growth of stemlet in all analysed varieties.

The bifactorial analysis of variance (Table 2) allowed finding that a species of fungus was the main source of rootlet and stemlet length variation, its contribution was 41.07 % and 58.58 %, respectively.

Table 2. Factorial analysis of the *genotype x fungal pathogen* relationships in tomato.

Source of variation	**Degree of freedom**	**Mean sum of squares**	**Contribution in the source of variation,%**
Length of radicle			
Tomato	5	8,100*	32. 49
Species of fungus	4	10,241*	41. 07
Tomato genotype x species of fungus	20	6,242*	25. 03
Random effects	1,424	351	1. 41
Length of stemlet			
Tomato genotype	5	1,210.2*	22. 00
Species of fungus	4	3,222.3*	58. 58
Tomato genotype x species of fungus	20	975. 1*	17. 73
Random effects	1,103	93. 5	1. 70

Legend: *-$p \leq 0.05$.

It should be mentioned that the genotype played an important role, too; its factorial weighting consisted of 32.49 % for the rootlet length and 22.00 % for the stemlet length. The interaction *genotype x species of fungus* was 25.03 % and 17.73 %, respectively, for both traits. The significant ponderosity of the role of fungus species (*Fusarium* spp., *A. alternata*) and interaction *genotype of tomato x species of fungus*, as sources of variation of plant organs of growth, reveals the need for constant monitoring of the composition of species of pathogens and their virulence.

CONCLUSIONS

1. It has been found that the *Fusarium* spp. and *A. alternata* pathogens influence significantly the early ontogeny of tomato genotypes by the repression of seed germination, growth of rootlets and stemlets (sometimes by their stimulation).
2. Mihaela and Tomis varieties are less sensitive to applied CF and can be successfully used as potential donors of resistance to fusariosis and alternariosis.

3. The significant ponderosity of the role of fungus species (*Fusarium* spp., *A. alternata*) and of *genotype of tomato x species of fungus* interaction, as the sources of variation of plant organs of growth, reveals the need for constant monitoring of the composition and virulence of fungi species causing root rot on tomato.

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Received: March 29, 2016
Accepted: July 20, 2016

Muzeul Olteniei Craiova. *Oltenia. Studii şi comunicări. Ştiinţele Naturii.* Tom. 32, No. 1/2016 ISSN 1454-6914

CAUSATIVE AGENTS OF BROWN STAINING OF LEAVES AND ROOT ROT OF TOMATOES IN CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract. On the basis of macro- and microscopic characteristics of fungi isolated from tomato plants with symptoms of the disease, it was found that the aerial part of the plant was affected by fungi Alternaria – *A. alternata, A. consortiale,* and Phytophthora – *P. infestans*; the subterranean part (root) was attacked by fungi *Fusarium – F. oxysporum* var. *orthoceras* and *F. solani.*. The research of the grade of attack on the foliage of plants in the process of growth has demonstrated its increase from the beginning of fruiting to the mass fruit ripening. This indicates the intensification of the disease state. The correlation coefficients of the grade of attacks of blight and fusariosis were low (with one exception); it can suggest that the resistance of tomato to the causative agents is under the individual genetic control.

Keywords: tomato, fungi, *Alternaria* spp., *Phytophthora* spp., *Fusarium* spp.

Rezumat. Agenţii cauzali ai pătării brune a frunzelor şi putrezirii rădăcinii la tomate în condiţiile Republicii Moldova. În baza caracteristicilor macro- şi microscopice ale fungilor izolaţi din plante de tomate cu simptome de boală, s-a constatat că partea aeriană a plantelor a fost afectată de fungii Alternaria – *A. alternata, A. consortiale* şi Phytophthora – *P. infestans*, iar partea subterană (rădăcina) a plantelor – de fungii Fusarium – *F. oxysporum* var. *orthoceras* şi *F. solani*. Cercetarea gradului de atac al aparatului foliar al plantelor în evoluţia creşterii – de la începutul fructificării până la coacerea în masă a fructelor a demonstrat creşterea acestuia, ceea ce denotă intensificarea stării de boală. Coeficientul de corelaţie a gradului de atac al alternariozei cu cel al fuzariozei a înregistrat (cu o singură excepţie) valori joase, ceea ce denotă că rezistenţa tomatelor la agenţii cauzali se află sub control genetic individual.

Cuvinte cheie: tomate, fungi, *Alternaria* spp., *Phytophthora* spp., *Fusarium* spp.

INTRODUCTION

Although tomatoes are cultivated in different climatic conditions, growth and development of this crop are affected by the strong influence of the limiting environmental factors, including fungal diseases and low temperatures at early stages of development (FOOLAD, 2007), so demonstrating that genetic resistance are diminished by the above-mentioned factors. Tomatoes are susceptible to more than 200 pathogens - fungi, bacteria, viruses, nematodes (FOOLAD, 2007; YANG et al., 2005).

At present, fungal pathogens *Fusarium* spp. and *Alternaria* spp. are remarked in the Republic of Moldova as biotic unfavourable factors supressing growth and development of cultural plants and being significantly extended. They provoke different diseases in a wide circle of species of agricultural and technical plants: tomato, wheat, sorghum, barley, sunflower, rapeseed, cotton, etc., being responsible for huge economic losses (ROTEM, 1994; XU et al., 2008). Diverse relationships are established between plant and pathogen, which are defined by resistance of the genotype, virulence of the fungus, environmental conditions, etc. (LUPAŞCU et al., 2015).

Fungal pathogen *Fusarium* spp. causes root and stem rot, and *Alternaria* spp., together with that, causes brown staining of leaves and fruits on tomato (ROTARU, 2011; GRIGORCEA et al., 2011).

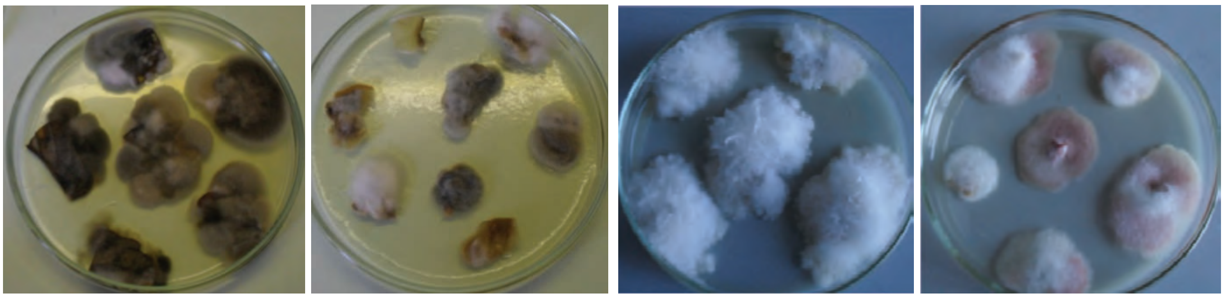
In accordance with modern literature data it is known that the species *Alternaria alternata* manifests high frequency and aggressiveness, occupying ecological niches or decreasing the extension of the species *A. solani* (Ell. et Mart.), which is the basic species on tomatoes in many regions (KUSABA & TSUGE, 1995).

The aim of our research is to identify the fungal pathogens that cause leaf brown staining and root rot in tomatoes and to determine the degree of attacks of the causative agents to plants.

MATERIAL AND METHODS

Four parental forms – Gloria, Jubiliar, Atlasnii, Zastava, and reciprocal hybrids F_4 – Gloria x Jubiliar (oblong fruit – o.f.); Gloria x Jubiliar (spherical fruit – s.f.); Jubiliar x Gloria (o.f); Jubiliar x Gloria (s.f.); Gloria x Atlasnii; Atlasnii x Gloria (I); Atlasnii x Gloria (II); Gloria x Zastava; Zastava x Gloria –were taken as the material for research.

Small fragments of leaves, fruits, and roots with symptoms of the disease were used for the isolation of fungi and identification of species that provoked the disease in tomato plants. The fragments were sterilized in the 2 % solution of lime hypochlorite for 1-2 minutes, then rinsed 2-3 times in bidistilled water, pressed between two sheets of filter paper and placed on the medium with PDA (*Potatoes Dextrosis Agar*) in aseptic conditions using gas flame (Fig. 1).



A B C D

Figure 1. The isolation of pathogens from fragments of leaves (A), fruits (B, C), and roots (D) of diseased tomato (original).

The species of causative agents have been identified on the basis of macro- (Fig. 1) and microscopic characteristics (Fig. 2) using fungal guides (ELLIS, 1971; PIDOPLICICO, 1977).

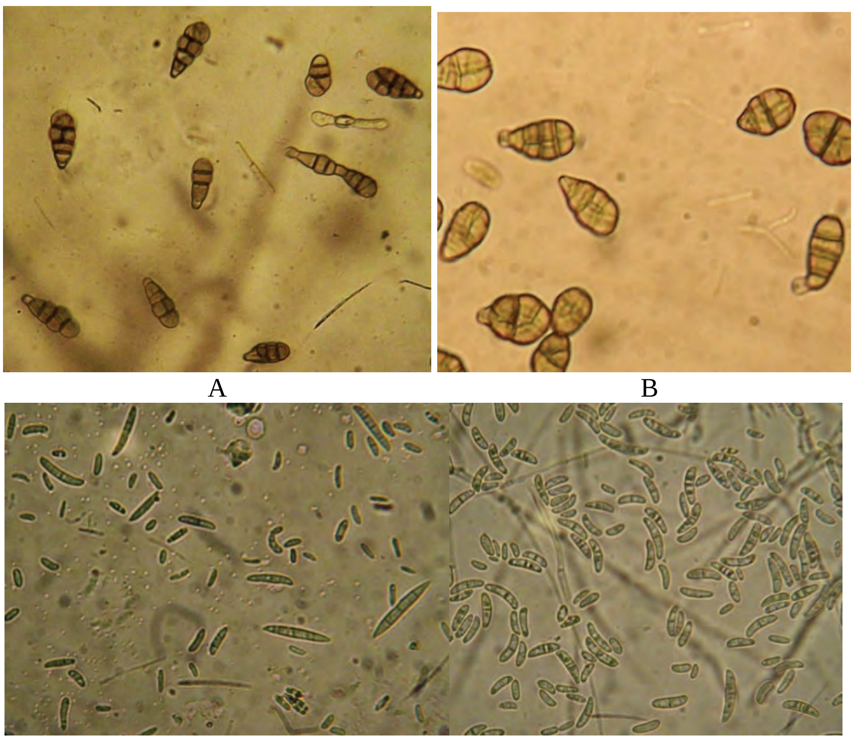
Evaluation of the degree of disease attacks was carried out in the field conditions based on symptoms of disease (brown stains, ulcers, necrosis) ranged in accordance with the scale of 6 grades, developed by the authors: 0 – healthy, immune (disease free), 1– highly resistant – HR (10 %), 2 – resistant – R (20 %), 3 – middle resistant – MR (30 %), 4 – sensitive – S (40 %), 5 – highly sensitive – HS (≥ 50 % of surface with symptoms of disease).

The obtained data obtained statistically processed using software package STATISTICA 7.

RESULTS AND DISCUSSION

Composition of the species involved in the developing of one or another disease is often different (XU et al., 2008). Moreover, high polymorphism of pathogenic agents, as a result of the liability of genetic and epigenetic systems, causes high adaptability of these agents to environmental conditions (YLI-MATTILA & GAGKAEVA, 2010). Taking this into account, one can understand that the studies of composition of fungi species, which cause diseases in tomato in the Republic of Moldova, are of interest.

The results of microscopic analysis of fungi showed that the aerial part of the tomato plant was affected by the fungi Alternaria – *A. alternata*, *A. consortiale,* and Phytophthora – *P. infestans*. The underground part of the plant (root) was affected by Fusarium fungi – *F. oxysporum* var. *orthoceras* and *F. solani* (Fig. 2).



A B

C D

Figure 2. Microscopic aspect of the fungi *Alternaria. alternata* (A), *A. consortiale* (B), *Fusarium oxysporum* var. *orthoceras* (C), *F. solani* (D) (500^x) (original).

The data demonstrated that the highest percentage of the manifestation of brown leaf spots is achieved by the species *Alternaria alternata*, coming up to 39.6%, and of root rot – by *F. oxysporum* var. *orthoceras*, coming up to 40.7%, respectively (Table 1).

Table 1. Composition of the fungi species that provoked diseases on tomato in the field conditions (2015).

Species	**Isolate, num.**	**Frequency,%**
Brown leaf spots		
Altemaria alternata	36	39.6
A. consortiale	11	12.1
Phytophtora infestans	6	6.6
Root rot		
Fusarium oxysporum var. orthoceras	37	40.7
F. solani	1	1.0
Total	91	100.0

The fungus *Phytophtora infestans* was registered with a frequency of 6.6 %.

The research of the degree of attack on the aerial surface of plants demonstrated its increase from the beginning stage of fruiting to the stage of mass fruit ripening. This indicates the intensification of the disease state.

Parental varieties as well as hybrids F_4 manifested different types of reaction: resistant (Jubiliar, Zastava, F_4 Gloria x Jubiliar (I), F_4 Jubiliar x Gloria (I), F_4 Jubiliar x Gloria (II), F_4 Jubiliar x Gloria (III), F_4 Gloria x Atlasnii, F_4 Gloria x Zastava, F_4 Zastava x Gloria), middle resistant (Gloria, F_4 Gloria x Jubiliar (II)), sensitive (Atlasnii, F_4 Atlasnii x Gloria (I), F_4 Atlasnii x Gloria (II)). Immune, highly resistant or highly sensitive genotypes were not found. Hybrids F_4 had values of the attack nearest to the parent with smaller value (i.e. more resistant) except of the combination F_4 Atlasnii x Gloria (I) (Fig. 3).

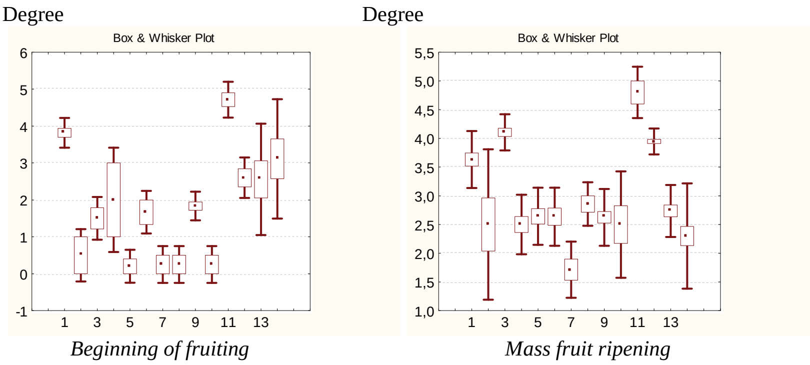


Figure 3. Brown leaf spots on tomato plants in the field conditions (2015).

Legend:
1 – Gloria; 2 – Jubiliar; 3 – Atlasnii; 4 – Zastava; 5 – F_4 Gloria x Jubiliar (I); 6 – F_4 Jubiliar x Gloria (I); 7 – F_4 Jubiliar x Gloria (II); 8 – F_4 Gloria x Jubiliar (II); 9 – F_4 Jubiliar x Gloria (III); 10 – F_4 Gloria x Atlasnii; 11 – F_4 Atlasnii x Gloria (I); 12 – F_4 Atlasnii x Gloria (II); 13 – F_4 Gloria x Zastava; 14 – F_4 Zastava x Gloria

The degree of fusariosis attack ranged within 2.58 ... 3.53 in parental forms and 1.26 ... 3.94 in reciprocal hybrids F_4, respectively. The following types of reactions were registered: resistant (F_4 Gloria x Jubiliar (I), F_4 Jubiliar x Gloria (I), F_4 Jubiliar x Gloria (II), F_4 Gloria x Jubiliar (II), F_4 Atlasnii x Gloria (I)), middle resistant (Jubiliar, Atlasnii, Zastava, F_4 Atlasnii x Gloria (II), F_4 Gloria x Zastava), sensitive (Gloria, F_4 Jubiliar x Gloria (III), F_4 Gloria x Atlasnii, F_4 Zastava x Gloria) (Fig. 4).

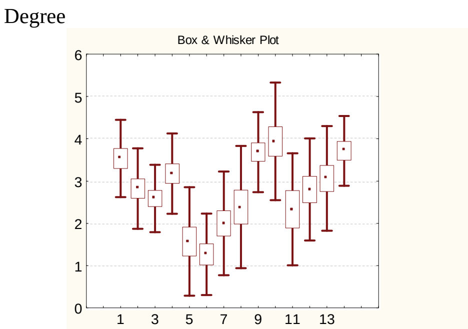


Figure 4. Root rot on tomato plants in the field conditions (2015).

Legend:
1 – Gloria; 2 – Jubiliar; 3 – Atlasnii; 4 – Zastava; 5 – F_4 Gloria x Jubiliar (I); 6 – F_4 Jubiliar x Gloria (I); 7 – F_4 Jubiliar x Gloria (II); 8 – F_4 Gloria x Jubiliar (II); 9 – F_4 Jubiliar x Gloria (III); 10 – F_4 Gloria x Atlasnii; 11 – F_4 Atlasnii x Gloria (I); 12 – F_4 Atlasnii x Gloria (II); 13 – F_4 Gloria x Zastava; 14 – F_4 Zastava x Gloria

The calculation of correlations between the degree of attack of the blight and of the fusariosis showed decreased levels of these coefficients except of the hybrid population F_4 Atlasnii x Gloria (I) (-1.0 *) (Table 2).

Table 2. Correlations between the degree of attack of the diseases on tomato plants.

Genotype/reciprocal hybrid F_4	**r**
	Brown leaf spots – fusariosis root rot
Gloria	0.11
Jubiliar	-0.22
Atlasnii	-0.04
Zastava	0.36
F_4 Gloria x Jubiliar (I)	-0.26
F_4 Jubiliar x Gloria (I)	0.45
F_4 Jubiliar x Gloria (II)	-0.00
F_4 Gloria x Jubiliar (II)	-0.54
F_4 Jubiliar x Gloria (III)	0.13
F_4 Gloria x Atlasnii	-0.40
F_4 Atlasnii x Gloria (I)	-1.0*
F_4 Atlasnii x Gloria (II)	0.18
F_4 Gloria x Zastava	0.37
F_4 Zastava x Gloria	-0.45

*-$p \leq 0.05$.

The results of the correlation analysis show that the resistance of tomato to fungi that produce brown leaf spots and root rot is under individual genetic control.

CONCLUSIONS

1. It was found that the diseases on tomato plants in the field conditions of the 2015 year were provoked by fungi Alternaria: *A. alternata*, *A. consortiale*, Phytophthora: *P. infestans*, and Fusarium: *F. oxysporum* var. *orthoceras*, *F. solani*; the highest frequency was registered by *A. alternata* (39.7%) and *F. oxysporum* var. *orthoceras* (40.7%).
2. Diseases on the foliage of tomato multiplied in the process of plants growth from the stage of beginning of fruiting to the stage of mass fruit ripening that indicated intensification of disease state.
3. The degree of infection of tomato plants ranged within 1.71 ... 4.8 and 1.2 ... 3.7 for brown leaf spots and fusariosis, respectively.
4. Correlation analysis has not demonstrated any dependencies between the degree of attacks of brown leaf spots and fusariosis; it can suggest that the resistance of tomato to the respective causative agents is under the individual genetic control.

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Received: March 12, 2016
Accepted: July 20, 2016

FLOWERING OF *Asimina triloba* (L.) DUNAL IN THE CONDITIONS OF TRANSYLVANIA

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DĂNĂILĂ-GUIDEA Silvana Mihaela

Abstract. The *Asimina triloba* plant, of exotic origin, is the only species belonging to the arboreal fam. Annonaceae that is prevalent in temperate climates. Currently, *Asimina triloba* L. is appreciated both in North America and Europe primarily as a fruit tree species. Wishing to approach it as an ornamental plant, this paper includes phenological and morphological results of observations conducted on flowers of the *Asimina triloba* tree in Transylvania. In this regard, we determined the characteristics of decorative flowers, the stages of flowering and the time period for this process. We also established the number of flowers formed on the tree, as well as their morphology.

Keywords: asimina, phenology, floral morphology, ornamental species.

Rezumat. Înflorirea la *Asimina triloba* (L.) Dunal în condiţiile din Transilvania. Planta *Asimina triloba*, de provenienţă exotică, este singura specie arboricolă aparținând fam. Annonaceae, cu răspândire în climatul temperat. La ora actuală, specia *Asimina triloba* L., este apreciată atât în America de Nord, cât și în Europa cu precădere ca specie pomicolă. În dorinţa abordării plantei din perspectiva ornamentală, lucrarea de faţă, reprezintă ansamblul rezultatelor din observațiile fenologice şi morfologice întreprinse asupra florilor arborelui de *Asimina triloba* din Transilvania. În acest sens, s-au determinat caracterele decorative ale florilor, fazele înfloritului şi perioada desfăşurării acesteia. De asemenea s-a stabilit numărul florilor care s-au format pe arbore, precum şi caracterele morfologice ale acestora.

Cuvinte cheie: asimina, fenologie, morfologia florală, specie ornamentală.

INTRODUCTION

Green areas represent the environment in which human life patterns correlate with natural or artificial elements thereof (SIMONDS, 1967), which fosters a sentiment for social, intellectual, emotional and emotive living. The nature of the urban environment, considered the widest and complex sphere of landscape architecture, comprises an inextricable intertwining between science and the art of green spaces (SZILAGYI et al., 2015).

Ornamental plants, a fundamental element of landscaping, form the main component of green spaces. In our country, the management of green spaces requires continuous improvement. In this context, the introduction of new decorative species is envisaged. We acknowledge that in our country, the interest for *A. triloba* is becoming more pronounced, making studies for its expansion to be increasingly deepened (SZILAGYI, 2015).

At present, *A. triloba* (L.) Dunal can be found in numerous botanical and private gardens in Italy and other European countries (SZILAGYI et al., 2015).

In Romania, *A. triloba* was first encountered as scattered fruit trees almost 50 years ago in the commune of Pianu de Sus (Alba County) (CEPOIU et al., 2004). Currently, in Romania, much research into the acclimatization of this tree species is ongoing, thanks to its landscaping and fruit potential (MIHĂILĂ et al., 2010; STĂNICĂ, 2012). A collection of several varieties of *Asimina* sp. is held by the Faculty of Horticulture at U.S.A.M.V. Bucureşti. Two other collections are in private gardens in the county of Argeş and Ilfov (STĂNICĂ, 2012).

The *Asimina* tree is deciduous, growing from 5 m to 10 m in height, but it also can develop as an ornamental plant bush. The value of *A. triloba*, lies in its shape (SZILAGYI, 2015): it has a natural tendency to create a crown-shaped pyramid in sunny places with dense foliage, with a straight trunk full of sap. It can also be globular, in which case it develops many lateral branches, which gives it the aspect of a typical chandelier [ARITON (DĂNĂILĂ-GUIDEA), 2005]. In the forests of different areas of the US, *Asimina* trees often grow in clusters or thickets that can result in root suckers or as a result of germination of seeds from the fruit fallen from the tree planted previously. The crown is dense, ornamental in type, and trees form a pyramidal crown. In the shade, pawpaw form a larger crown with few low branches and leaves arranged horizontally. The trunk is smooth, generally straight, with dark brown bark. The decor is enhanced by the coppery colour of the plant flowers that evolves into shades of dark purple, appearing in early spring before the leaves (SZILAGYI, 2015).

According to the assessments made by LAYNE (1996) and POMPER & LAYNE (2005), the appearance of this ornamental tree recommends *A. triloba* as an "integral component of the landscape aesthetic." Thus, *A. triloba* is a tree with high commercial potential highlighted by its ornamental qualities (SARGENT, 1890).

Taking into account the assessments of the researchers mentioned above, we believe that efforts to introduce *A. triloba* into the green areas of Romania are justified due to its ornamental characteristics and its relatively easy adaptation to the environmental conditions in our country, as well as the strength of its resistance to diseases and pests (SZILAGYI, 2015). This study takes into account the phenological and morphological observations taken on flowers of the *A. triloba* tree on specimens in Transylvania.

MATERIAL AND METHODS

In the spring of 2016, we made a series of observations and measurements on the flowers of 15 plants, which are 5-year-old *A. triloba* specimens in Transylvania. On every tree, 30 flowers were monitored in order to establish the stages of flowering and their morphological characteristics in the conditions of the northern part of Romania. To establish criteria for assessing the decorative characteristics of the anthesis, we established an arrangement of petals inside and outside of the flowers, the form of the androecium and the number of carpels that form the 15 plants analysed. Using digital calipers, we determined the length of the petals and the diameter of the androecium.

In order to obtain morphometric measurements of the number of branches of the plant, the number of flowers formed thereon and to determine their diameter and height, we took observations and measurements based on tree exposure to direct sunlight, diffused sunlight and shade. To establish the influence of light on *A. triloba* plants, we organized a single-factor experiment involving five plants with one variation, namely: V_1-plants exposed to direct sunlight (Mt.); V_2-plants exposed to diffuse sunlight, V_3-shade plants. The results are shown in table form.

RESULTS

In the spring of 2016, we made a series of observations and measurements on the flowers of 15 plants, which are *Asimina triloba* specimens aged 5 years in Transylvania. We monitored 30 flowers / *A. triloba* tree in order to establish the stages of flowering and morphological characteristics of their conditions in the north of the country. The results presented in Table 1 show the assessment criteria for anthesis in 2016 in Transylvania.

Table 1. Evaluation criteria for *A. triloba* by flowers in the conditions of Transylvania.

No.	Decorative character of the anthesis		Evaluation criteria	*Asimina triloba* flower
1	Calyx (sepal)		Double sepal with free member sepals stuck to the corolla	
2	Corolla (petal)	Layout of inner petals	Female: petals oriented erect	
			Male: stamen petals are removed	
		Layout of outer petals	They are free, equal in size and decorative shade: carmine red	
		Length inner petals	Size between 5.95 mm and 11.21 mm average value of 8.50 mm	
		Length outer petals	Size between 12-14 mm and 24.29 mm with a mean of 18.27 mm	
3	Androecium (stamen)		Globular-shaped stamen with a diameter ranging between 6.27 mm and 8.60 mm, and a mean of 7.74 mm	
4	Gynoecium (number rags)		3-9 in pieces	

Analyzing the results in Table 1 reveals that the average value is 8.50 mm for the inner petals, and for the exterior 18.27 mm, the androecium has an average value of 7.74 mm, while the gynoecium has between 3 and 9 carpels formed.

The flowers of *Asimina triloba* are the chief element of this ornamental plant. The ornamental effect of its flowers is enhanced by their coppery hue, decorating the beginning of spring, before leaf formation (Fig. 1).

a b c

Figure 1. Different phases of flowering and branch positioning on *Asimina triloba*
(Original photo-copies from a private collection-Baia Mare).

The following are the flowering stages for *Asimina triloba* in Transylvania, we present the unfolding period and its duration in days (Table 2).

Table 2. Dynamics of flowering phases of *A. triloba* in the conditions in Transylvania.

No.	Phase	The cumulative time in days (1-28)	Period (April 7 -May 5)
1	Phase floral button	X X X	April 7-9
2	Opening of flower	X X X X X X X X X X X X X X X X	April 10-25
3	Pistil formation (gynoecium)	X X X X X X X X X X	April 18-27
4	Opening stamen (androecium)	X X X X X X X X X X X X X X X X	April 18, May 2
5	Fall of corolla	X X X	May 3-5

Following the observations made, it was noted that the beginning of the vegetation period of the flowers is marked by the button phase and lasts three days (April 7). Regarding the ornamental potential of the flowers, it is given by floral decoration that displays itself until the fall of the corolla, which in 2016 lasted 24 days. The end of the floral decoration is marked by the fall of the corolla (Table 2).

To determine the size of the plant branches on *A. triloba*, we measured the same number of branches (75) for each variant (see Table 3).

Table 3. Morphometric determinations on *A. triloba* plant branches existing in temperate climate (Baia Mare).

No.	Variant	Number of analysed branches	Dimensions of branches (cm)		
			Minimum	Maximum	Average
1	V_1 - plants exposed to direct sunlight (Mt.)	75	9,8	116,0	61,9
2	V_2 - plants exposed to diffuse sunlight	75	12,5	138,0	57,3
3	V_3 - plants with shade exposure	75	6,7	90,0	52,3

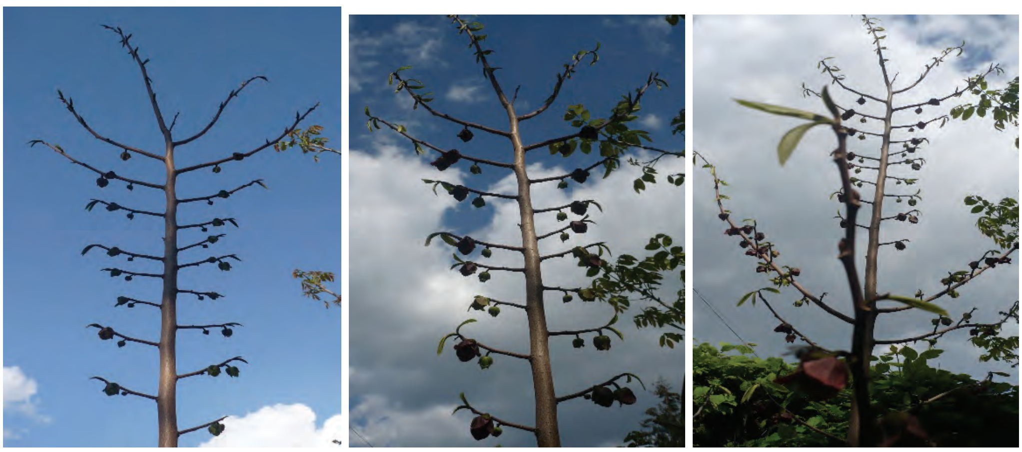
Analyzing the results shown in Table 3, we found that the size of the smallest branches, in plants exposed to shade, is between 6.7 and 90 cm. More developed branches were recorded for *A. triloba* plants exposed to diffuse sunlight, with values between 12.5 and 138 cm.

In order to introduce *A. triloba* among the ornamental plants of Transylvania, we determined the number of flowers per branch formed under different conditions of light exposure (Table 4).

Table 4. Morphometric determinations of the number of flowers formed on *A. triloba* plant branches.

No.	Variant	Number of analysed branches		
		Minimum	Maximum	Total flowers formed on tree
1	V_1 - plants exposed to direct sunlight (Mt.)	1	22	116
2	V_2 - plants exposed to diffuse sunlight	2	34	191
3	V_3 - plants with shade exposure	1	15	101

The number of flowers formed on the tree in the analysed period (spring 2016) varied between 101 and 191, while the maximum recorded number of flowers on a branch was 34.



a b c

Figure 2. Stages of growth, development and formation of *A. triloba* flowers on branches (Original photo-copies from a private collection-Baia Mare).

Likewise, in 2016, we determined diameter and length of *Asimina triloba* flowers on trees grown in different light intensities (Table 5).

Table 5. Morphological characteristics of *A. triloba* flowers.

No.	Variant	Analysed number of tree flowers	Analysed decorative characteristic (mm)			
			Min.	Max.	Med.	Flower *Asimina triloba*
1	V_1- plants exposed to direct sunlight (Mt.)	30	24.37	38.36	30.51	Diameter of flower
2	V_2- plants exposed to diffuse sunlight	30	27.42	33.47	30.52	
3	V_3- plants with shade exposure	30	18.58	32.23	25.62	
4	V_1- plants exposed to direct sunlight (Mt.)	30	15.95	22.71	19.64	Length of flower
5	V_2- plants exposed to diffuse sunlight	30	18.31	22.93	20.90	
6	V_3- plants with shade exposure	30	15.24	22.54	19.30	

The results showed that in the north of Romania *A. triloba* flower diameter is between 18.58 mm (V_3 - plants with shade exposure) and 38.36 mm (V_1 - plants with exposure to direct sunlight) and that the length of flowers varies between 15.24 and 22.93 mm.

DISCUSSIONS

The studied *Asimina triloba* plants were exposed to natural conditions in Baia Mare. Table 6 shows the climate conditions in Baia Mare under which the flowering of *A. triloba* took place this year.

Table 6. Climatic conditions recorded at Baia Mare during blooming period (April 7,-May 5).

Period	Average temperature (^{0}C)	Maximum temperature (^{0}C)	Minimum temperature (^{0}C)	Atmospheric pressure (hPa)	Air relative humidity (%)	Rainfall amount (mm)	Wind speed (km/h)
7.04-5.05	13.5	19.34	7.54	1,012.32	71.58	1.58	33.12

Also In the period under review (April 7-May 5), it was also determined sunrise and sunset time and azimuth (where the sun rises and where the sun sets) thereof (Table 7). The mean value recorded in Table 7 corresponds to *A. triloba* flowering period for which research reports were made in this paper.

Table 7. Moment of sunrise and sunset during the blooming period of *A. triloba* (April 7, May 5).

Location	Geographic coordinates	Period	Sunrise		Sunset	
			Hours	Azimuth (^{o})	Hours	Azimuth (^{o})
Baia Mare	Latitude $47^0 40^I$ N Longitude $23^0 35^I$ E	April 7-May 5	6.25	71.20	20.24	289.08

CONCLUSIONS

Our studies have shown that the *Asimina triloba* plant lends itself to partial sun exposure, so we recommend planting it in semi-shade in green spaces in Transylvania. The results obtained from the morphological measurements of the variations highlighted the growth in length of branches (52.3 cm and, respectively, 61.9 cm for plants in V_3 and plants in V_1-Mt) and of the number of flowers formed on trees due to their positioning in light of different intensities. Differences were found in the values of length and floral diameter. Based on the results, we recommend introducing *A. triloba* into the range of ornamental plants in Transylvania.

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Received: March 31, 2016
Accepted: August 12, 2016

PAST INDUSTRY VS. NATURE: WHICH ONE INFLUENCES MORE THE TERRESTRIAL ISOPOD ASSEMBLAGES FROM A TOWN IN WESTERN ROMANIA?

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Abstract. Despite the strong industrialization and the complex urban development, in Ștei town from western Romania there is a diverse terrestrial isopod fauna, consisting of more species than in other towns from the region. In Ștei, we have identified 16 terrestrial isopod species, the most common being *Hyloniscus riparius*, *Porcellio scaber* and *Trachelipus nodulosus*. The isopod fauna from Ștei does not present zoogeographic or ecological surprises, being formed of common species, which are native in the country and well distributed in the natural areas of western Romania. Species linked to natural wetlands were present even in the ruins of some abandoned buildings situated in severely affected areas in the past. They colonized probably these habitats after the cessation of the anthropogenic damage, coming from the neighbouring natural wetlands. The composition of the terrestrial isopod fauna from Ștei is a consequence of the town position in a depression area between mountains, wetlands and forests. For the terrestrial isopods, the existence of the natural habitats neighbouring Ștei is more important that the strong industrialization of the past.

Keywords: terrestrial isopods, small town, industrialization, wetlands, species, diversity.

Rezumat. Industria din trecut sau natura: cine influenţează mai mult comunităţile de izopode terestre dintr-un oraş mic din vestul României? În ciuda industrializării puternice şi a dezvoltării urbane complexe, în orașul Ștei din vestul României există o faună diversă de izopode terestre, conţinând mai multe specii decât în alte oraşe din regiune. În Ștei am identificat 16 specii de izopode terestre, cele mai comune fiind *Hyloniscus riparius*, *Porcellio scaber* şi *Trachelipus nodulosus*. Fauna de izopode din Ștei nu prezintă surprize zoogeografice sau ecologice, fiind formată din specii comune, care sunt native în ţară şi bine răspândite în zonele naturale în vestul României. Speciile legate de zone umede au fost prezente inclusiv în ruinele unor clădiri puternic afectate antropic în trecut. Aceste specii au colonizat probabil aceste habitate după încetarea activității antropice, venind din zonele umede naturale din apropiere. Compoziția faunei de izopode terestre din Ștei este o consecință a poziției orașului intr-o zonă depresionară, fiind înconjurat de munți, zone umede și păduri. Pentru izopodele terestre existența habitatelor naturale învecinate orașului Ștei este mai importantă decât industrializarea puternică din trecut.

Cuvinte cheie: izopode terestre, oraş mic, industrializare, zone umede, specii, diversitate.

INTRODUCTION

A recent research indicates that urbanization influences only negatively or neutrally the species abundance and richness (see in: SAARI et al., 2016). The town, Ștei, has recently obtained this statute, as a result of the industrialization from 1950s, when it became a development centre for the uranium mining of the region (e.g. FILIMON et al., 2012; PRASCA & OLĂU, 2013). The very quick development, subordinated to a single industrial branch, generated a very diverse urban settlement (FILIMON et al., 2012; PRASCA & OLĂU, 2013), grafted over an old agricultural rural zone (PRASCA & OLĂU, 2013). In the locality, beside the old buildings, there are block resident areas of different ages, industrial zones, etc. (FILIMON et al., 2012; PRASCA & OLĂU, 2013). In the last 25 years, the town went into a decline, the industry being almost totally closed, and the population decreased (FILIMON et al., 2012). In this context, we have proposed to study how much the biodiversity of this town was influenced by these changes on a small time scale, choosing terrestrial isopods, a useful group in this kind of studies. Terrestrial isopods are detritivorous (RADU, 1983), being important in the indication of heavy metal pollution; they are considered bioindicators (e.g. DALLINGER et al., 1992; DROBNE, 1997; PAOLETTI & HASSALL, 1999; MAZZEI et al., 2014; NANNONI et al., 2015). In western Romania, there are two studies upon isopods from towns (BODIN et al., 2013; FERENȚI et al., 2015), the first one being from the vicinity of Ștei (BODIN et al., 2013). Also, there is information on isopods from natural habitats of the region (TOMESCU et al., 2008; IANC & FERENȚI, 2014). We hypothesized that the quick development of the town modified the native terrestrial isopod assemblages. We also supposed that the block resident areas would have low species richness, as well as the entire town, compared to the natural areas or other towns.

MATERIAL AND METHODS

The research upon the terrestrial isopod fauna from Ștei took place in the year 2015. We realized two field trips on May 14 and July 4. We collected 34 samples from 27 collecting points from Ștei. The isopods were collected directly with hand under different shelters. The time spent for each sample was approximately 20 minutes, like in other studies (FERENȚI et al., 2015). In only one case, in a forested area, we used the litter sieve. Isopods were preserved in tubes with alcohol and determined in the laboratory at stereomicroscope, using the keys (e.g. RADU 1983, 1985; FARKAS & VILISICS, 2013; TOMESCU et al., 2015).

Ștei town is situated in the southern part of Bihor County in western Romania, in Beiuș Depression, along the Crișul Negru River (MÂNDRUȚ, 2006). Even if it is surrounded by mountains (MÂNDRUȚ, 2006), the town is situated in a relatively flat area, in the south of the depression. In the east, the town is surrounded by generally abandoned and ruined industrial zones, but also by small watercourses, the park of a hospital and a forest crossed by a stream, on which a small pond was furbished. The terrestrial isopods were collected from diverse areas: wetlands, forest, urban parks, residential block areas, abandoned or used buildings, ruins, abandoned industrial zones, etc. (Table 1). After the species identification, we calculated the percentage abundance and the frequency of occurrence. The data were analysed both by collecting points and habitat types. For species diversity we used Shannon - Wiever index (SHANNON & WIEVER, 1949). The similarity between the terrestrial isopod assemblages between the investigated habitats and the significance of these differences was calculated using the Past.3x software (HAMMER et al., 2001).

RESULTS

In Ștei, we identified 16 terrestrial isopod species (*Ligidium hypnorum*, *Trichoniscus* sp., *Hyloniscus riparius*, *H. transsilvanicus*, *Haplophthalmus danicus*, *Cylisticus convexus*, *Porcellium collicola*, *Protracheoniscus politus*, *Trachelipus nodulosus*, *T. rathkii*, *T. arcuatus*, *Porcellionides pruinosus*, *Porcellio spinicornis*, *P. scaber*, *Armadillidium vulgare*, *A. versicolor*). Totally, we collected 204 individuals. Of these 42 were males, 155 females and seven juveniles. The species from the *Trichoniscus* genus could not be identified, because we collected only one female. The species with the highest percentage abundance in Ștei was *H. riparius*, followed by *P. scaber* and then by *T. nodulosus* (Table 1). The same species occupy the top in the case of frequency of occurrence too, *T. nodulosus* being on the second place. There were also species with very low percentage abundance (*Trichoniscus* sp., *H. transsilvanicus*, *P. spinicornis*). The species spectrum is different between the collecting points, the number of species / collecting point being low (Table 1).

Table 1. The terrestrial isopod species identified in Ștei town (Lh – *L. hypnorum*, Trich – *Trichoniscus sp.*, Hr – *H. riparius*, Ht – *H. transsilvanicus*, Hd – *H. danicus*, Cc – *C. convexus*, Pc – *P. collicola*, Ppo – *P. politus*, Tn – *T. nodulosus*, Tr – *T. rathkii*, Ta – *T. arcuatus*, Ppr – *P. pruinosus*, Psp – *P. spinicornis*, Psc – *P. scaber*, Avu – *A. vulgare*, Ave – *A. versicolor*, Ns – species number, P% - percentage abundance, f% - frequency of occurrence).

Sampling points	**Lh**	**Trich**	**Hr**	**Ht**	**Hd**	**Cc**	**Pc**	**Ppo**	**Tn**	**Tr**	**Ta**	**Ppr**	**Psp**	**Psc**	**Avu**	**Ave**	**Ns**
Beltway, wet area	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	X	2
Debris, market	-	-	-	-	-	-	-	-	X	-	-	-	-	X	-	X	3
Abandoned sports hall interior	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	1
Abandoned sports hall exterior	-	-	X	-	-	-	X	-	-	-	X	-	-	-	-	-	3
Debris parking sports hall	-	-	X	-	-	-	-	-	X	-	-	-	X	-	X	-	4
Railway station pond store	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	X	2
New block resident zone downtown	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	1
Stream beltway industrial area	-	-	X	-	X	X	-	-	X	-	-	-	-	-	-	-	4
Debris old houses main road	-	-	-	-	-	-	-	-	X	-	-	-	-	X	-	-	2
Old block residents downtown I	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	1
Old block residents downtown II	-	-	-	-	-	-	-	-	-	-	X	-	-	X	-	-	2
Old block residents downtown III	-	-	-	-	-	-	-	-	-	X	X	-	-	X	-	-	3
Railway district ruins beltway	-	-	-	-	-	X	-	-	-	-	-	X	-	-	X	-	3
Railway station pond	-	-	X	-	-	-	-	-	X	-	-	-	-	-	-	X	3
Heating station new block residents	-	-	X	-	X	-	-	-	-	-	-	-	-	X	-	-	3
New block resident area bus station	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	1
Stream forest pond	X	X	-	X	-	-	-	-	-	-	-	-	-	-	-	-	3
Northern old block residential zone	-	-	X	-	-	-	-	-	X	-	-	-	-	X	-	-	3
Railway station repository	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	1
The Crisul Baitei river bank, near blocks	-	-	X	-	-	-	-	-	X	-	-	-	-	-	-	X	3
Northern old block residential zone - small park	-	-	X	-	X	-	-	-	-	-	-	-	-	X	-	-	3
Beltway debris industrial area	-	-	X	-	-	X	X	-	X	-	-	-	-	-	X	-	5
Forest to the pond	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	1
Northern old block residential zone garages	-	-	X	-	X	-	-	-	-	-	-	-	-	X	-	-	3
Debris hospital	-	-	X	-	-	X	-	-	-	-	-	-	-	-	X	-	3
Railway station	-	-	X	-	X	-	-	-	-	-	-	-	-	X	X	-	4
Repositories to the railway station	-	-	-	-	-	-	-	-	X	-	-	-	-	X	-	X	3
P %	**2.94**	**0.49**	**30.88**	**0.49**	**8.33**	**6.37**	**1.96**	**1.96**	**14.71**	**0.98**	**4.41**	**0.98**	**0.49**	**15.69**	**5.39**	**3.92**	
f %	**2.94**	**2.94**	**47.06**	**2.94**	**17.65**	**14.71**	**5.88**	**2.94**	**35.29**	**5.88**	**8.82**	**2.94**	**2.94**	**32.35**	**14.71**	**17.65**	

The diversity of the terrestrial isopod assemblages from Ștei town was H=2.16. The diversity differed according to the habitats, the highest being in wetlands (Table 2). The highest species richness and individual number was registered in ruins with debris and wetlands. Of the 16 species, seven were identified in only one habitat type (Table 2). *H. riparius* was found in seven of the eight habitat types (Table 2). In the case of the similarity between the habitat types (Fig. 1), the closest assemblages were found on the one hand between small urban green areas (park and railway station, which also has a small park) and on the other hand between constructed areas (old and new buildings). The most distinguished from the rest of the habitats is the forest, where only one species was present. The differences between the assemblages from different habitat types are significant (Anova: $p<0.05$).

DISCUSSIONS

The study hypotheses were verified only partially. The terrestrial isopod fauna from Ştei town does not seem to be diminished by the quick development and strong industrialization of the town. Both by species richness and diversity, Ştei is situated at the top between the western Romanian towns, where this group was studied (BODIN et al., 2013; FERENȚI et al., 2015). Even in Beiuş, a larger and older town without mining industry (FILIMON et al., 2011) only 15 species were identified (BODIN et al., 2013). The resemblance between the fauna from Ştei and Beiuş towns (BODIN et al., 2013) is a consequence of the small distance between them (only 16 km) and their location in the same geographic unit, namely Beiuş depression (MÂNDRUȚ, 2006). The difference is even greater compared with Salonta town, where only 11 species were present (FERENȚI et al., 2015). These differences are not determined directly by the town but by its surrounding areas. Thus, Salonta is situated in a flat plain without forests (FERENȚI et al., 2015), but Ştei is situated in a well forested zone, being in contact with forests. Some of the species identified in Ştei are sylvan species (*P. politus*) or wetland species characteristic to high altitudes (*L. hypnorum, H. transsilvanicus*) (see in: TOMESCU et al., 2011).

Table 2. The percentage abundance of the terrestrial isopods from Ştei in different habitat types ((Lh – *L. hypnorum*, Trich – *Trichoniscus*sp., Hr – *H. riparius*, Ht – *H. transsilvanicus*, Hd – *H. danicus*, Cc – *C. convexus*, Pc – *P. collicola*, Ppo – *P. politus*, Tn – *T. nodulosus*, Tr – *T. rathkii*, Ta – *T. arcuatus*, Ppr – *P. pruinosus*, Psp – *P. spinicornis*, Psc – *P. scaber*, Avu – *A. vulgare*, Ave – *A. versicolor*, Ns – species number, P% - percentage abundance, f% - frequency of occurrence, H – Shannon-Wiever diversity).

	Lh	**Tri**	**Hr**	**Ht**	**Hd**	**Cc**	**Pc**	**Ppo**	**Tn**	**Tr**	**Ta**	**Ppr**	**Psp**	**Psc**	**Avu**	**Ave**	**P%**	**Ns**	**H**
Wetlands	12.50	2.08	31.25	2.08	12.50	2.08	-	-	25.00	-	-	-	-	-	-	12.50	23.53	8	1.73
Debris, ruins	-	-	41.07	-	-	17.86	1.79	-	10.71	-	-	3.57	1.79	8.93	12.50	1.79	27.45	9	1.72
New buildings	-	-	33.33	-	4.76	9.52	14.29	-	9.52	-	4.76	-	-	23.81	-	-	10.29	7	1.72
Old buildings	-	-	21.62	-	8.11	-	-	-	18.92	5.41	21.62	-	-	24.32	-	-	18.14	6	1.68
Repositories	-	-	28.57	-	-	-	-	-	42.86	-	-	-	-	14.29	-	14.29	3.43	4	1.28
Parks	-	-	9.09	-	27.27	-	-	-	-	-	-	-	-	63.64	-	-	5.39	3	0.86
Forest	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	1.96	1	0
Railway station	-	-	35.00	-	20.00	-	-	-	-	-	-	-	-	25.00	20.00	-	9.80	4	1.36
f%	**12.5**	**12.5**	**87.5**	**12.5**	**62.5**	**37.5**	**25**	**12.5**	**62.5**	**12.5**	**25**	**12.5**	**12.5**	**75**	**25**	**37.5**			

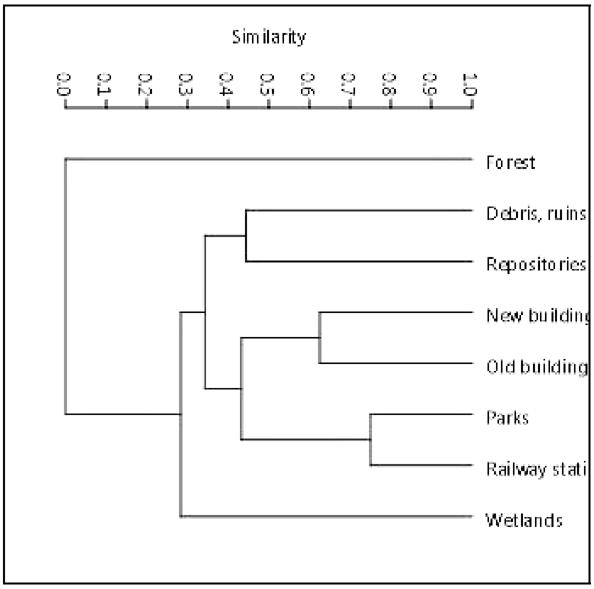

Figure 1. The similarity of terrestrial isopods assemblages between habitat types (Jaccard index).

The terrestrial isopod species from Ştei are well represented in Romania, generally native and characteristic for the region and habitats they were identified in (e.g. RADU, 1983, 1985; TOMESCU et al., 2011, 2015). Also, in other zones, the species identified in urban areas were widespread (VILISICS et al., 2012). The terrestrial isopod fauna from Ştei resembles the one from its vicinity, like Pădurea Craiului Mountains (IANC & FERENȚI, 2014). Thus, the relatively rich and diverse terrestrial isopod fauna from Ştei is mainly the consequence of the existence of some natural habitats from the vicinity of the town. The industrialization from the past did not manage to eliminate the species linked to natural areas from the locality, they being eventually eliminated from some constructed area during the constructions. After abandoning the industrial zones, the terrestrial isopods from the natural areas re-occupied those zones, including the most anthropogenically affected ones. Thus, the species linked to natural wetlands, like *P. collicola* are present in the ruins of the abandoned sports hall. At some hundreds of meters distance of it there is a stream, *P. collicola* being a wetland species (e.g. FERENȚI et al., 2013).

Even if many terrestrial isopod species from Ștei town are specific to natural zones, in the locality there are also other species linked to artificial habitats, like *T. nodulosus, P. scaber* and *A. vulgare*. These species were frequently reported in urban areas (e.g. VILISICS & HORNUNG, 2009; FERENȚI et al., 2015). However, unlike Salonta (FERENȚI et al., 2015), the non-native species are fewer. However, some of these synanthropic species were also reported in western Romania in natural and semi-natural habitats (e.g. FERENȚI et al., 2012; FERENȚI & COVACIU-MARCOV, 2015). Thus, even if Ștei developed strongly in the last decades (e.g. FILIMON et al., 2012), the non-native isopods did not arrive there or their establishment in the town was very difficult.

The best represented genus from Ștei is *Trachelipus*. This genus has numerous species in Romania, the three species from Ștei being common in the country (TOMESCU et al., 2015). In the case of Trichoniscus, there is a possibility that the unidentified female would belong to *T. carpathicus*, a species which was recently identified in Pădurea Craiului Mountains from the region (IANC & FERENȚI, 2014), or in other zones in western Romania (FERENȚI & COVACIU-MARCOV, 2015). Also, in other cases, from the genus Trichoniscus only females were captured, which could not be determined (e.g. BODIN et al., 2013; FERENȚI & COVACIU-MARCOV, 2016). Totally, the isopod species from Ștei town are the expected ones, without any zoogeographic or ecological surprises.

The highest diversity was registered in wetlands, habitats which repeatedly proved to be important for isopods including western Romania (e.g. FERENȚI & COVACIU-MARCOV, 2015; FERENȚI et al., 2013, 2015). The highest species richness and individual number was registered in ruins with debris, followed by wetlands, just like in other towns (FERENȚI et al., 2015). As wetlands are situated near ruins, wetland species are frequently collected from the ruins. The presence of a single species in the forest is a consequence of the sampling with the litter sieve. With this method we managed to capture only *P. politus*, a sylvan species (see in: TOMESCU et al., 2011). Generally, the forest shelters a higher number of species. Thus, wetland species (*H. transsilvanicus, L. hypnorum*) identified near streams, were also present in the forest, because that stream crosses the forest.

However, one of the hypotheses was partially verified. Thus, in the block residential areas less species were present, being usually synanthropic, frequent in localities (e.g. JĘDRYCZKOWSKI, 1981; FERENȚI et al., 2015). The presence of these species indicates the anthropogenic pressure affecting of a region, their advance in natural zones being also in other cases considered a result of anthropization (FERENȚI & COVACIU-MARCOV, 2016). The block residential areas have proven to be difficult for terrestrial isopods (VILISICS & HORNUNG, 2009). Also, in the case of new parks there are less species than in old parks (JĘDRYCZKOWSKI, 1981). In Ștei, the parks have a low species richness and diversity, like in other towns (FERENȚI et al., 2015).

For terrestrial isopods from Ștei the most important fact is the town location in a zone with many natural habitats, forests, wetlands, etc. Their presence has determined the composition of the present isopod fauna more than the past industrialization. Thus, for terrestrial isopods maintaining the natural zones near the anthropogenically affected ones is important. Also, in other cases the maintenance of the habitat heterogeneity was considered vital for the diversity and distribution of isopods (PURSE et al., 2012). As long as the regress of the industry from Ștei continues, the isopod fauna will regain new zones and will be organized closer to its initial aspect, these animals colonizing even abandoned and then rehabilitated mining zones (e.g. TAJOVSKÝ, 2001; PURGER et al., 2007). Thus, isopods succeed to survive in urban industrial zones too and colonize towns after the cessation of the industry pressure. Actually, Ștei town can be considered an experiment on the evolution of an urban industrialized locality grafted over a semi-natural rural zone, which after some decades of functioning seems to be back at the square. At least for terrestrial isopods this fact does not seem too difficult.

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Received: March 21, 2016
Accepted: May 12, 2016

THE VARIABILITY OF SOME PHENOTYPIC FEATURES AND LIFE CYCLE IN TWO *Aphis pomi* POPULATIONS FROM WESTERN ROMANIA

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Abstract. This paper presents data referring to the external morphological characteristics, the biometrical measurements and the life cycle of *Aphis pomi*. The researches have been carried out on the orchards from the experimental fields of the Didactic Station Timişoara (Timiş County) and Vârfurile, (Arad County), 2003-2008. At the *Aphis pomi* species the smallest length of the body established for aphids captured in the western zone of Romania was 1.80 mm, while the biggest was 2.00 mm. Regarding the length of the head and thorax, it may be noticed that the maximum length of these parts was 1 mm, and the minimum length was 0.65 mm. The minimum width of the head was 0.20 mm and the maximum width of the head was 0.35 mm. The thorax width had a minimum of 0.60 mm and a maximum of 0.80 mm. The minimum length of the abdomen was 0.90 mm and the minimum width was 0.70 mm, the maximum length of the abdomen was 1.20 mm and the maximum width was 0.90 mm. In the climatic conditions of Romania, *Aphis pomi* winters in the stage of egg in October usually on the stems of apple tree. In the second decade of March, the eggs hatch out and there appears the fundatrix giving birth to one or more generations of fundatrices, which are apterous parthenogenetic viviparous females. The winged aphid migrates to other nearby host plants like pear, peach trees and other herbaceous plants growing in the vicinity of apple trees. The reproductive process continues rapidly, the populations of aphids are developing quickly in a very short time. Green apple aphids usually remain on apple plants throughout the summer and gave birth parthenogenetically to 9 -12 generations. In October, Gynetypes (gynopara) give birth to wingless sexual, oviparous females, that mate with males and lay winter eggs.

Keywords: aphid, external morphology, biometrical observations, biology.

Rezumat. Variabilitatea unor caractere fenotipice şi ciclul de viaţă în două populaţii de *Aphis pomi* din vestul României. Lucrarea prezintă date referitoare la morfologia externă, observațiile biometrice și ciclul de viață al speciei *Aphis pomi*. Cercetările au fost efectuate în livezile Stațiunii Didactice Timișoara (Timiş) şi Vârfurile (Arad). La specia *Aphis pomi* cea mai mică lungime a corpului stabilită pentru afidele capturate în zona de Vest a României a fost de 1,80 mm, în timp ce cea mai mare a fost de 2,00 mm. În ceea ce privește lungimea capului şi a toracelui, se poate observa că lungimea maximă a acestor părti a fost de 1 mm, iar lungimea minimă a fost de 0,65 mm. Lățimea minimă a capului a fost de 0,20 mm, iar lățimea maximă a fost de 0,35 mm. Lățimea toracelui a avut un minim de 0,60 mm și un maxim de 0,80 mm. Lungimea minimă a abdomenului a fost de 0,90 mm, iar lățimea minimă a fost de 0,70 mm, lungimea maximă a abdomenului a fost 1,20 mm, iar lățimea maximă a fost de 0,90 mm. În condițiile climatice din România, *Aphis pomi* iernează în stadiul de ou depus, în octombrie, pe tulpinile de măr. În a doua decadă a lunii martie apare fundatrixul care dă naștere la una sau mai multe generații de fundatrigene care sunt femele aptere vivipare partenogenetice. *Aphis pomi* migrează spre alte plante gazdă din apropiere, cum ar fi părul, piersicul și alte plante erbacee care cresc în vecinătate. Procesul reproductiv continuă rapid, populațiile de afide se dezvoltă rapid într-un timp foarte scurt. Păduchele verde al mărului rămâne de obicei pe măr pe tot parcursul verii și dă naștere partenogenetic de la 9 la 12 generații. Gynopara în octombrie dă naștere la formele sexuate, ovipare și masculi, care depun ouăle de iarnă.

Cuvinte cheie: afide, morfologia externă, măsurători biometrice, biologie.

INTRODUCTION

The green apple aphids (*Aphis pomi* De Geer, 1773) are economically significant pests of apple trees (FOOTTIT et al., 2006; LOWERY et al., 2006). Aphids affect many agricultural and horticultural crops (BLACKMAN & EASTOP, 2007) and cause considerable loss by sucking their sap and transmitting many diseases. Although sometimes called Apple aphid, *Aphis pomi* is a member of the order Hemiptera, suborder Aphidinea, superfamily Aphidoidea, family Aphididae) and they can be found worldwide (BUCZAKI, 2002). This species plays a role in disease transmission (BLACKMAN & EASTOP, 2006) and affects healthy growth of the hosts (KAAKEH et al., 1993).

Aphids have adapted their life cycle to different geographical regions depending upon the environmental conditions prevailing in the area of study. Though sufficient work with regard to the biology of *Aphis pomi* has been done by earlier workers like BAKER & TURNER (1916) in North America and GAUTAM & KUMARI (2004). In Romania, in the consulted, specialized literature, we could not find information about the biology of this pest. Chemical control of the *Aphis pomi* attck in Romania was studied by different groups of researchers (BOLBOSE, 2009; ŢUCĂ et al., 2010), while others studies have been focused on the sensitivity of different traditional apple-trees cultivars to the attack of this pest (BÁLINT et al., 2013).

The feeding of *Aphis pomi* causes downwards curling of young apple leaves, flower and immature fruit (http://www.agri.huji.ac.il/mepests/pest/Aphispomi/). Indirect injury results from honey-dew deposits on both fruit and leaves. A black fungus grows on this honeydew causing considerable sootiness, especially of early apples. This sooty mould on leaves can also affect photosynthesis and may reduce fruit yield (KAAKEH et al., 1993; LOWERY et al., 2006).

Pale-green tubercles may arise on the young fruit. Its meager honeydew output is usually collected by ants, with little attendant sooty mould (BASKY et al., 2001).

MATERIAL AND METHODS

The research was carried out for a period of five years. The biology of *Aphis pomi* was observed for five years: 2005 – 2008 and 2015.

The biometrical observations of *Aphis pomi* were made for a total of 30 individuals/year, species captured from the orchards of the Didactic Station Timişoara (belonging to BUASVM Timişoara, Timiş County) and Vârfurile (Arad County). The data were statistically analysed with STATISTICA 10.0, Basic Statistics. The aphids were collected with yellow vessel traps on a three day time period. Specimens were preserved in ethanol 80% just after they were collected in the field.

To determine accurately the species, there were consulted the works made by: BLACKMAN & EASTOP (2000a, b), FERICEAN et al. (2006), FERICEAN et al. (2012), PERJU (2004), REMAUDIERE & REMAUDIÈRE (1997), WILLIAMS & DIXON (2007).

RESULTS AND DISCUSSION

Morphology. Apterous adults have yellow-green color, and measure between 1.5 and 2 mm. The ovoid body has little antennal tubercles. The antennae have six segments and are shorter than the body, siphunculi are cylindrical, long and black. The cauda is short and sharp (Figs. 1, 2).

The cauda has 10 - 19 hairs (rarely less than 13). Marginal tubercles are present on abdominal tergites 2 - 4, and the fused last two rostral segments are more than 120 um in length. The latter two characters are the best features to distinguish *Aphis pomi* from the very similar invasive *A. spiraecola* (FOOTTIT, 2009).

The alata individuals have lengths of 1.70 – 2.10 mm and they are pale green to pale yellow on the back and on each side. The head and thorax are black. The abdomen is green with black side circular spots with 3 pairs of black lateral circular spots on the anterior abdominal segments and a semicircular spot in front and behind each siphunculus (Fig. 2).

It can be observed that, out of a total of 30 individuals of the species *Aphis pomi* (Table 1), the smallest length of the body established for aphids captured in the western zone of Romania was 1.70 mm, while the biggest was 2.10 mm.

Table 1. The variability of the phenotypic charcters in *Aphis pomi*.

No. art.	Body length (mm)	Head+thorax length (mm)	Head width (mm)	Thorax width (mm)	Abdomen (mm)	
					Length	Width
Average	1.86 (1.86)	0.82	0.30	0.64	1.05	0.81
Average deviation	0.11	0.08	0.01	0.05	0.06	0.04
Standard deviation (s)	0.13	0.10	0.03	0.06	0.08	0.05
(m) Min	1.70	0.65	0.20	0.60	0.90	0.70
(M) Max	2.10	1	0.35	0.80	1.20	0.90

The average body length was 1.86 ± 0.11 mm (Fig. 3). The results are similar to those in the literature (DUBNIK, 1991), which states that the body length of *Aphis pomi* ranges from 1.80 to 2.00 mm.

By analyzing the data presented in Table 1 regarding the length of the head and thorax, it can be noticed that the maximum length of these parts was 1 mm and the minimum length was 0.65 mm. The average value calculated for the length of these parts was 0.82 ± 0.08 mm. The minimum width of the head was 0.20 mm and the maximum width of the head was 0.35 mm. The average value calculated for the head width was 0.30 ± 0.01 mm. As far as the thorax width is concerned, the minimum was 0.60 mm and the maximum 0.80 mm. The mean thorax width was 0.64 ± 0.05 mm

Analyzing the data on the length and width of the abdomen, it can be seen that the minimum length of the abdomen was 0.90 mm and minimum width was 0.70 mm, the maximum length of the abdomen was 1.20 mm and the maximum width was 0.90 mm. The mean length of the abdomen was 1.05 ± 0.06 mm, while the average value calculated for the abdomen width was 0.81 ± 0.04 mm.

Comparing the measurements related to the length of the head and thorax, the width of the head and thorax, abdomen width and length, with data from the literature cannot be carried out because we could not find their correspondent in the consulted specialized literature.

Aphids generally overwinter in the egg stage which hatches in spring into the females that reproduce parthenogenetically and give birth to young ones. Several generations of the pest are produced during the season in this way. BAKER & TURNER (1916) and GAUTAM & KUMARI (2004) during their studies reported that the aphids passed through a complex life cycle involving polymorphism, viviparity and telescoping of generations, stem mothers, wingless viviparous females, winged viviparous females, males and oviparous females in the study area.

In the conditions of the western part of Romania, (Fig. 4) *Aphis pomi* winters in the egg stage in October usually on the stems of apple-trees. In the second decade of March, the eggs hatch out and there appears the fundatrix giving birth to one or more generations of fundatrices, which are apterous parthenogenetic viviparous females. After a few days, they become mature and give birth to daughter aphids by parthenogenesis. The daughter aphids develop wings. Winged green apple aphids start appearing in the second period of May in Timişoara and in early June in Vârfurile.

Figure 1. *Aphis pomi* aptera (original)

Figure 2. *Aphis pomi* alata.(original).

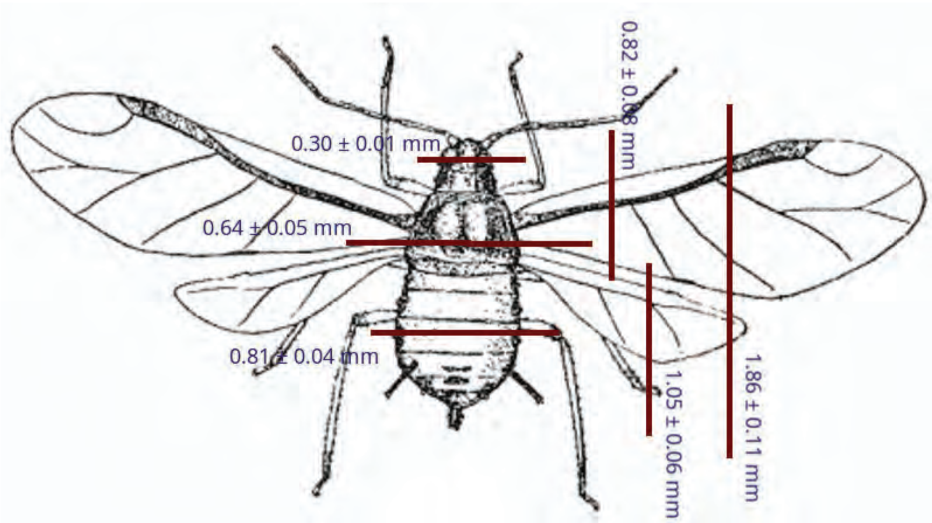

Figure 3. The body length of *Aphis pomi* (original).

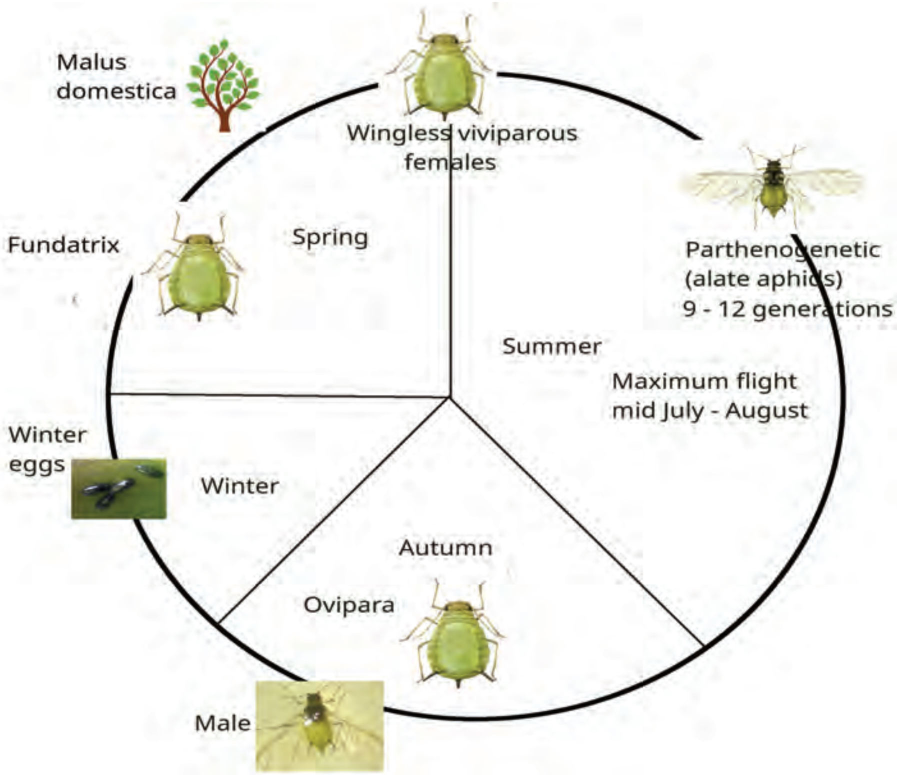

Figure 4. Life cycle of *Aphis pomi* (original).

Green apple aphids usually remain on apple plants throughout the summer and gave birth parthenogenetically to 10 to 12 generations in the conditions of Timişoara and to 9 to 10 generations in the conditions of the mountain area from Vârfurile. Although *Aphis pomi* is a species whose host plant is the apple tree, the winged aphid migrates to other nearby host plants like peach, pear trees and other herbaceous plant growing in the vicinity of apple trees. The reproductive process continues rapidly and the populations of aphids are developing quickly in a very short time.

The infestation of green apple aphid was found to be more intense in the apical parts of the plants (Fig. 5). Both nymphs and adults suck the sap from leaves, twigs, branches and young fruits, as a result of which the affected leaves curl up, blossoms shed and the young fruits drop prematurely and the quality of fruits is greatly impaired. Severely infected plants show stunted growth.

The average nymphal duration of this species is 13.50 ± 0.38 days which ranged from a minimum of 10.0 days to a maximum of 14.0 days. The average pre - reproductive, reproductive and post - reproductive periods of this aphid are 12.95 ± 0.27, 17.50 ± 0.47 and 1.45 ± 0.17 days respectively. The reproductive period starts with the laying of young ones and the progeny produced by a female varied from 19 to 79 aphids and averaged 47.80 ± 4.39 aphids (STROYAN, 1984; FERICEAN et al., 2006).

In the studied orchard, in 2015, the maximum flight was recorded in the second part of July and the first part of August, depending on weather conditions.


a


b

Figure 5. *Aphis pomi* attack in apple - a; colony detail - b. (original).

In October, Gynetypes (Gynopara) give birth to wingless oviparous sexual females that mate with males and lay winter eggs.

Egg laying occurs in October until mid-November on vegetative buds and bark of trees. The egg is attached to the support through a sticky substance, which is eliminated with the egg and hardens quickly (DIXON & THIEME, 2007).

CONCLUSIONS

In the *Aphis pomi* species, the smallest length of the body established for aphids captured in the western zone of Romania was 1.80 mm, while the biggest was 2.00 mm.

The average value calculated for the length of the head and thorax was 0.82 ± 0.08 mm. The average value calculated for the head width was 0.30 ± 0.01 mm. The mean thorax width was 0.64 ± 0.05 mm. The mean length of the abdomen was 1.05 ± 0.06 mm, while the average value calculated for the abdomen width was 0.81 ± 0.04 mm.

In the orchard, in 2015, the maximum flight was recorded in the second part of July and the first part of August.

Green apple aphids usually remain on apple plants throughout the summer and gave birth parthenogenetically to 10 to 12 generations in the conditions of the Western Plain (Timişoara) and to 9 to 10 generations in mountain conditions (Vârfurile).

ACKNOWLEDGMENTS

This research was supported by the grant number 2753/30.04.2015, awarded in the internal grant competition of the Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania".

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Received: May 06, 2016
Accepted: August 20, 2016

HIBERNATION OF THE PREDATORY STINK BUG *Perillus bioculatus* F. (HEMIPTERA, PENTATOMIDAE) UNDER LABORATORY CONDITIONS

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Abstract. Observations of the laboratory population of predatory bug *Perillus bioculatus* allowed us to establish the time periods and duration of the winter diapause. The imagoes of the 2nd and 3rd generations of the predator proved to be more adapted, while the 4th generation adults of *P. bioculatus* were most vulnerable during hibernation. The mortality rate of *P. bioculatus* individuals in the period from December 15 to March 15 was 12.2; 28.6 and 30.1% for the 2nd, 3rd and 4th generations, respectively. At the same time, the sex ratio (♀ : ♂) was distributed as follows: before the wintering of the 2nd generation – 1:2.9, 3rd – 1:1.3, and 4th – 1:1.4; after coming out of hibernation – for the 2nd generation – 1:2.1, 3rd – 1:1.3 - 1:1.5 and 4th – 1:0.8. It was found that the length of daylight is not the only determining index.

Keywords: predatory stink bug *Perillus bioculatus*, hibernation, overwintering, sex-ratio, generation.

Rezumat. Hibernarea ploşniţei răpitoare *Perillus bioculatus* F. (Hemiptera, Pentatomidae) în condiţii de laborator. Observațiile asupra populației ploșniței răpitoare *Perillus bioculatus* au permis stabilirea termenilor și durata diapauzei de iarnă. În perioada hibernării, adulții generației a doua și a treia ale răpitorului *Perillus bioculatus* sunt cei mai adaptați, în timp ce, cei din generația a patra au fost mai vulnerabili. Mortalitatea indivizilor de *P. bioculatus* în perioada de la 15 decembrie până la 15 martie a constituit 12,2; 28,6 și 30,1% pentru generația a doua, a treia și a patra, respectiv. Raportul dintre sexe (♀ : ♂) s-a distribuit în felul următor: până la hibernare, pentru generația a doua– 1:2,9, a treia– 1:1,3 și a patra – 1:1,4, după ieşire din hibernare pentru generația a doua – 1:2,1, a treia – 1:1,3-1:1,5 şi a patra – 1:0,8. S-a stabilit, că durata luminei zilei nu este unicul factor determinant.

Cuvinte cheie: ploşniţa răpitoare *Perillus bioculatus*, hibernare, iernare, raportul sexelor, generaţie.

INTRODUCTION

Adapting a predator of the Colorado potato beetle (*Leptinotarsa decemlineata* Say, Coleoptera, Chrysomelidae) – *Perillus bioculatus* F. (Hemiptera, Pentatomidae) on the European continent holds great promise for the biological protection of potato against phytophages. Currently, *P. bioculatus* is found in countries such as Bulgaria (SIMOV et al., 2012), Greece (PERICART, 2010), Serbia (PROTIC & NEBOJSA, 2012), Moldova (DERJANSCHI & ELISOVEŢCAIA, 2013, 2014), in the European part of Turkey (KIVAN, 2004; FENT & AKTAC, 2007), as well as in Crimean, Seversk, Slavyansk, Dinsk and Krasnoarmeysk districts of the Krasnodar region, Aksai district of the Rostov region (ARTOHIN et al., 2012), and in the Republic of Adygea (ISMAILOV et al., 2014). Moreover, according to the latest data in 2015, populations of the predator were found in Meerut (UP) North India (PRASAD & RISHI, 2015).

Researchers have gained experience in the study of the predator in laboratory and field conditions and conducted investigations on the selection of artificial diet; the trophic relations of *P. bioculatus*, as well as the influence of abiotic and biotic factors on the population density of the entomophage have been subject to scrutiny. In some countries (Russia, Turkey, Moldova), where the predatory bug adapted to the natural biocoenosis, it was introduced in the laboratory culture and used for mass rearing and release against the Colorado potato beetle (KIVAN & AYSAL, 2014; ISMAILOV et al., 2014; DERJANSCHI & ELISOVEŢCAIA, 2014). However, it is known that a huge role in the preservation of the population is played by the diapause stage when the entomophage is very vulnerable, both in laboratory and in natural conditions. Since the adaptation of the *Perillus bioculatus* species on the European continent took place relatively recently, the predator population is unstable and vulnerable in the new habitat. Maintenance of laboratory culture and mass releases allow to keep the genetic potential of the useful species in the agroecosystems.

Therefore, the aim of our paper was to study the effects of wintering conditions on the numbers and biological indicators of laboratory populations of the predatory bug *Perillus bioculatus*.

MATERIAL AND METHODS

The experiments were carried out during the years 2014-2016 on laboratory population of *P. bioculatus*, introduced into the culture in 2014. The insects were kept in cages, the air humidity was maintained through vessels with salt solution. Nutrition was mixed: *Leptinotarsa decemlineata* and *Galleria mellonella* L. (Lepidoptera: Pyralidae). We took into account the mortality of adult bugs during the preparation for winter diapause, in the torpor period (late autumn - early winter) and after coming out of diapause. We also monitored nutrition, mating, and some other behavioural reactions of *P. bioculatus*.

RESULTS AND DISCUSSIONS

As a result of a laboratory breeding in the conditions of 2014, we prepared for hibernation one population of the predatory bug *Perillus bioculatus* of the 3rd generation. Under the conditions of 2015 there were obtained, for overwintering, already 5 individual populations of the entomophage (three populations of the 2nd generation, and one population of the 3rd and 4th generation). Under the separate keeping of bugs of different generations it was found that not only adults of the 3rd generation were preparing for hibernation, but also the 2nd (which molted into imagoes in late July - early August). The bugs ate intensely, chose bigger victims (Fig. 1), during the day gathered in large groups, and hid in a shelter, where they spent most of the time, even during daylight hours (Fig. 3). The activity of adults resumed only on sunny days or hours. At the same time, despite the decrease in activity, females of both generations (the 2nd and 3rd) continued to lay eggs. Thus, we had prepared for wintering the fourth generation of the predator – larvae began molting in the imagoes of the 4th generation on September 30 and ended on November 15. The average development duration of the 4th generation larvae, as well as of the 1st, was 24 days (larvae of the 2nd and 3rd generation – 22 and 17 days, respectively). It should be noted that the death of the 4th generation larvae reached 50-80%, despite the abundance of food and constant temperature (+26±1°C) and humidity of 70-75% maintained in the room. Also, in the process of molting, the imagoes of the 4th generation revealed a significant proportion of individuals with defective elytra (Fig. 2) or undeveloped forelimbs - from 10 to 20%. Thus, the duration of the larval stage of the entomophage depended mainly on the temperature and length of daylight, whereas the survival rate of *P. bioculatus* individuals and their biological indicators were greatly influenced, most likely, by the quality of the food provided.

Earlier researchers (STRADIMOVA, 1973) determined that the decisive factor for the survival of the beetle during wintering is its physiological condition. Therefore, for the good preparation of the bugs for the winter diapause, special attention was paid to the quantity and quality of food. The voracity of the adults in late November – early December with decreasing photoperiod decreased slightly, compared to the month of October. It was noted that the bugs selectively treated the offer of food: in abundance of larvae of *Galleria mellonella* (in excess, based on the average daily demand of the bug in feed) the imagoes of *P. bioculatus* ate one caterpillar in groups of 2-5 (Fig. 2). Such behaviour is typical for the bugs, both adults and larvae, throughout the season. However, at the end of autumn, we noted an increase in cases of group nutrition. The ratio of separately feeding individuals and "groups" was distributed in such a way that among 5-6 bugs, and sometimes 7-8, only one fed separately from the group.

A comparative analysis of the bugs' behaviour before hibernation, with naturally decreasing photoperiod, throughout the seasons 2014 and 2015, showed that the decisive role in the formation of diapause of *P. bioculatus* adults was played by factors such as feeding on diapausing hosts, lowering of temperature, as well as the quality and quantity of food. Observations revealed a high mortality rate (till 51.7%) among *P. bioculatus* imagoes of the 4th generation in the last months of autumn (late October) and prior to their dormancy (the first decade of December). Among the imagoes of the 2nd and 3rd generations in the year 2014-2015 the mortality rate was significantly lower – 12.1-28.8% (Table 1). The period from August 9, 2014 to September 17, 2015 was conditionally accepted by us as settlement dates for the calculation of imago deaths before hibernation, as at this time the composition of populations of the 2nd and 3rd generations are no longer replenished with new individuals.

Table 1. Mortality rates in the laboratory populations of *Perillus bioculatus* in hibernation.

Generation of the *Perillus bioculatus*	**Percent mortalities (%)**			**Survival rate of adults (%)**
	Before hibernation	During hibernation	Total	
Year (period)	2014-2015			
Data (period)	September 9, 2014 – December 14, 2014	December 14, 2014 – March 31, 2015	September 17, 2014 – March 31, 2015	March 31, 2015
3rd	18.2	22.2	36.4	77.8
Year (period)	2015-2016			
Data (period)	September 17, 2015 – December 10, 2015	December 10, 2015 – March 15, 2016	September 17, 2015 – March 15, 2016	March 15, 2016
2nd	12.1	12.2	27.3	87.8
3rd	28.8	28.6	50.0	71.4
4th	51.7	30.1	75.9	69.9
		$HSD_{0.05}$=6.4		$HSD_{0.05}$=6.4

As it can be seen from Table 1, the most adapted to wintering were the second generation imagoes, their death rate during the period of hibernation (December 10, 2015 – March 15, 2016) being only 12.2%, while for the third and fourth generations it reached 28.6 and 30.1%. It was established that the duration of the winter diapause of *P. bioculatus* in 2014 was 3.5 months, and about 3 months in the conditions of 2015. The air temperature in the winter months and in the first half of March 2015 was higher than in 2014. This explains the different dates of waking up from hibernation of the imagoes. The bugs emerged when the average daily air temperature reached +12°C. Once the cages were transferred into a warm room, the imagoes proceeded to mating, feeding only after 2-3 days (Fig. 4).

As a result of the analysis of the data, it was determined that at the time the populations woke up from hibernation, the ratio of survivors ranged from 69.9 to 87.8%, depending on the generation (Table 1). The mathematical analysis of the data revealed a significant difference ($HSD_{0.05}$=6.4, χ= from 10.0 to 17.9) between the viability of adult *P. bioculatus* of the 2nd, 3rd and 4th generations during winter. At the same time, between the viability of the 3rd generation imagoes during the wintering period 2014-2015, there was not observed a significant difference ($HSD_{0.05}$=6.4, χ= 6.4).

We also determined the sex ratio of *P. bioculatus* adults before and after hibernation (Table 2).

Table 2. Sex ratio in the laboratory populations of *Perillus bioculatus* in hibernation.

Generation of the *Perillus bioculatus*	**Sex ratio (♀:♂)**	
	Before hibernation	**After hibernation**
Year (period)	2014-2015	
3rd	1:1.3	1:1.3
Year (period)	2015-2016	
2nd	1:2.9	1:2.1
3rd	1:1.3	1:1.5
4th	1:1.4	1:0.8

Figure 1. Imago of *P. bioculatus*, eating a larvae of *Galleria mellonella* before hibernation (original).

Figure 2. Imagoes of the 4th generation *P. bioculatus* – defective development of the elytrum on the right down (original).

Figure 3. Aggregation of the imagoes of *P. bioculatus* during hibernation (original).

Figure 4. Coupling of the imagoes of *P. bioculatus* on the third day after hibernation (original).

After the molting of *P. bioculatus* larvae into imago, the sex ratio is approximately 1:1, with minor deviations. However, later in the laboratory breeding, the sex ratio often varied towards male dominance. During hibernation and in the first two weeks after hibernation, the mortality rate among males is on an average higher than among females. Clearly, the viability of diapausing females is higher than that of males, which explains the cause of male dominance in a quantitative sense before hibernation.

CONCLUSIONS

Our data on the survival rate of adults of predatory bug *P. bioculatus* of the second – fourth generations during winter diapause allows to take into account factors that significantly affect the formation of diapause, as well as the viability of the species. The targeted selection of the best prepared for wintering populations in the conditions of laboratory breeding will help to optimize the process and reduce direct costs.

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Received: March 23, 2016
Accepted: April 26, 2016

INTERSPECIFIC COMPETITION OF *Trichogramma* sp. AT ITS MASS REARING

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Abstract. As a result of the laboratory experiments, it has been established that along the 4 generations in mixed populations of *T. evanescens* Westw. and *T. pintoi* Voeg., gradual substitution of *T. evanescens* by *T. pintoi* occurs. The mechanism of interspecific competition on grain moth eggs was established. In the field of cereal, technical and vegetable crops, *T. evanescens* is dominant species (up to 95-99%). *T. pintoi* represents a laboratory population, the preferred host of which is *Siotroga cerealella* Ol. In the presence of the two species of *Trichogramma* in the biotope, their quantitative ratio depends on the specific climatic conditions and resource of eggs of the preferred host Biological indices for *T. pintoi* are higher than those of *T. evanescens*. Therefore, in the laboratory conditions we substitute *T. evanescens* by *T. pintoi,* but in the field it is the opposite.

Keywords: biological indices, prolificacy, pests, *Trichogramma*, competition, biological efficacy.

Rezumat. Concurenţa interspecifică la *Trichogramma* sp. la înmulţirea în masă. În urma experimentelor de laborator s-a stabilit că, pe parcursul a 4 generaţii, în populaţiile mixte de *T. evanescens* Westw. şi *T. pintoi* Voeg., are loc substituirea treptată a *T. evanescens* de către *T. pintoi*. Totodată, s-a determinat mecanismul concurenţei interspecifice pe ouă de molia cerealelor (*Sitotroga cerealella* Ol.). În câmp *T. evanescens* reprezintă o specie dominantă în agrocenozele culturilor cerealiere, tehnice, legumicole şi leguminoase (până la 95-99%), iar *T. pintoi* reprezintă o populaţie de laborator, pentru care *S. cerealella* este o gazdă preferată. În prezenţa a două specii de *Trichogramma* în biotop, raportul lor cantitativ depinde de condiţiile concrete climatice şi resursa de ouă de gazdă preferată. Pentru *T. pintoi* indicii biologici sunt mai mari decât la *T. evanescens*. Din această cauză, are loc substituirea *T. evanescens* de către *T. pintoi* în condiţii de laborator, iar în câmp – invers.

Cuvinte cheie: indicii biologici, prolificitatea, gazdă, *Trichogramma,* concurenţa, eficacitatea biologică.

INTRODUCTION

Biological control plays an important role in integrated plant protection. Beneficial insects are rather important in modern biological control practice for reducing the density of pests.

The Institute of Genetics, Physiology and the Plant Protection of the Academy of Sciences of Moldova conducts fundamental and applied research with *Trichogramma* to settle technical issues, improve the quality of entomophage-and effectiveness in plant protection to obtain ecological safe products. To improve parasitoid quality and efficacy, it is necessary to select the right species best adapted to natural conditions of certain zones with certain species.

At the initiative of the academician Ion Popusoi, there was set up within the Institute of Genetics, Physiology and Plant Protection, the laboratory of *Trichogramma,* initially headed by Dr. Ala P. Adaskevici (1976-1977), then by Dr. Şoil M. Greenberg (1977-1992), later on by Dr. Vladimir A. Sleahtici (1995-1999) and finally by Dr. Lidia T. Gavriliţa (1999-2003).

In 2003, the Laboratory of *Trichogramma* was united with another laboratory and named „Phytopharmacy and Ecotoxicology" that has continued functioning till nowadays (2016). Thus the Laboratory of *Trichogramma* existed as a research unit in the Institute of Department Plant Protection within the Academy of Sciences of Moldova from year 1976 up to year 2013, i.e. 27 years and thus, *Trichogramma* has continued being studied for 40 years (1976 till present, 2016). Since its setting up, the Laboratory of *Trichogramma* has dealt with big and important issues. Along the period, *Trichogramma* studies in laboratory conditions led to important data, which were described in national and international scientific publications. These results have been presented at various conferences, symposia, round tables and exhibitions.

In mass rearing of *Trichogramma*, the numerical density of the initial colonies grows by tents, even hundreds of times leading to a depression of the number of inherited crossings and thus leading to sexual deregulation of the population, and to a lowering of the quality of *Trichogramma.*

Such factor as interspecific competition becomes particularly important at *Trichogramma* mass rearing and releasing into the field. At relative trophic specialization of *Trichogramma* competitive capacity impacts its efficacy (GAVRILIŢA et al., 1984; SOROKINA, 1984; MENCHER et al., 1980).

MATERIALS AND METHODS

During the rearing of the laboratory host, biological indices were determined for *T. evanescens* (prolificacy, hatching, rate of the females-reared on these eggs. Experiments were carried under laboratory and field conditions.

Experiments have been made in three climatic chambers SKP-1 at average daily temperatures of 15, 20 and 25°C and relative humidity of 50, 65 and 80%, the photoperiod lasting for 16 hours. Experiments have been effectuated according to Box-3 plan.

Collecting, identification, storage and accumulation of *Trichogramma* species were done according to DIURICI (2008). Rearing of the laboratory host – grain moth (*Sitotroga cerealella* Ol.), for *Trichogramma* production was done by ABAŞCHIN et al. (1979) authors' methods. Mathematical data processing has been done using variance analysis method after MENCER & ZEMSHMAN (1986).

RESULTS AND DISCUSSIONS

Five trial variants in five repetitions were conducted in the Institute of Genetics, Physiology and the Plant Protection of the Academy of Sciences of Moldova, using various reports on *Trichogramma* species under laboratory and field conditions: 1. *T. evanescens* – 100%; 2. *T. pintoi* – 100%; 3. *T. evanescens + T. pintoi* – 50% +50%; 4. *T. evanescens + T. pintoi* – 10% +90%; 5. *T. evanescens + T. pintoi* – 90% +10%. Experiments conditions are shown in Table 1.

Table 1. Box-3 (planned conditions).

Levels of factors	T, °C	W, %	*T. evanescens* : *T. pintoi*
-1	15	50	1 : 9
0	20	65	1 : 1
1	25	80	9 : 1

Laboratory experiments demonstrated that during 4 generations of *Trichogramma* development the *T. evanescens* species was gradually substituted by *T. pintoi* (Table 2, Fig. 1).

When the share ratio of *T. evanescens* and *T. pintoi* equals to 1:1 passed through Angoumois grain moth eggs (*Sitotroga cerealella* Ol.) after the fourth generation it was noticed that at T=15°C combined with different humidity values, *T. pintoi* share increased from 68.3 to 83.95% and *T. evanescens* share – from 16.1 to 31.7%; at T=20°C, these parameters ranged, from 76.0 to 95.0% and 5.0 to 24.0%, while at T=25°C – they ranged from 76.1 to 100% and from 0 to 23. 9%.

When shares of *T. evanescens* and *T. pintoi* equalled to 9:1, the substitution rate of *T. evanescens* was more reduced. However, at T=15°C in mixed batch, there remained from 42.9 to 45.0% of *T. evanescens* at T=20°C, respectively, from 38.9 to 45.0%, at T=25°C – from 31.5 to 38.35%. At the end of all experiment variants no *T. evanescens* were found, while at T=25°C, a similar situation was observed for the 3rd generation.

The mechanism of substituting one species by the other was explained by different responses of *T. evanescens* and *T. pintoi* to temperature and humidity regimes at mass rearing.

Lower prolificacy and sex index as well as longer duration of one generation of *T. evanescens* and *T. pintoi* was observed in all experiment variants. The obtained results demonstrated that the Angoumois grain moth is a more preferable laboratory host for *T. pintoi* and the introduction of even a small share of this species may lead to the substitution of *T. evanescens*. Experiments were made with 4 generations due to the fact that commercial biological laboratories recommended to rear no more than 4 generations of *Trichogramma* on the eggs of the Angoumois grain moth after passage on cabbage moth (*Mamestra brassicae*) eggs followed by field release. Substitution phenomenon of one species by the other may be explained by different reasons. Under the conditions of South-West region of the former USSR, *T. evanescens* was a dominant species (up to 95-99%) in the agrocoenoses of cereal, technical and leguminous crops. *T. pintoi* was the laboratory population for which the Angoumois grain moth became a preferable host.

Hence, the number of *T. pintoi* females that refused parasitizing the eggs of the Angoumois grain moth equalled to 2%, while that of *T. evanescens* – 38%. *T. pintoi* reared on the eggs of the Angoumois grain moth has higher prolificacy and sex index and a new generation develops faster than that of *T. evanescens*. It has been found that the intensity of substituting of *T. evanescens* by *T. pintoi* was regulated by the response to temperature and humidity regimes at mass rearing (Tables 3, 4). Ecologically, *T. pintoi* is a more plastic species, while *T. evanescens* stronger responds to humidity fluctuations under the same temperature. Field experiments showed that cabbage moth eggs were more parasitized by *T. evanescens* – 54 to 60% than by *T. pintoi* – from 13 to 14%.

Table 2. Influence of temperature and humidity on the substitution process of *T. evanescens* by *T. pintoi* (after the 4th generation).

Temperature, °C	**15°C**						**20°C**						**25°C**					
Species ratio	1:1		1:9		9:1		1:1		1:9		9:1		1:1		1:9		9:1	
Species	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*
Humidity, %	Humidity 80%																	
No. of individuals, %	16.1 ±0.5	83.9 ±3.2	7.0 ±0.5	93.0 ±3.9	45 ±2.0	55 ±2.2	5.0 ±2.2	95 ±3.8	0	100	45±2.0	55 ±2.5	0	100	0	100	38.5 ±1.5	61.5±2.0
Humidity, %	Humidity 65%																	
No. of individuals, %	29.5 ±0.8	70.5 ±3.7	9.0 ±0.3	91.0 ±4.2	44.0 ±2.2	56.0 ±2.3	7.0 ±2.3	93.0 ±4.8	0	100	43,0 ±2.0	57.0 ±2.6	5.0	95.0 ±3.5	0	100	35.0 ±1.5	65.0 ±2.2
Humidity, %	Humidity 50%																	
No. of individuals, %	31.7 ±1.8	68.3 ±3.0	22 ±0.9	78.0 ±3.8	42.9 ±3.1	57.1 ±2.6	24.0 ±2.8	76.0 ±3.3	6.0 ±0,5	94.0 ±4.9	38.9 ±2.4	61.1 ±2.8	23.9 ±1.8	76.1 ±3.8	8.0 ±0.8	92.0 ±4.9	31.5 ±0.8	68.5±2.8

Figure 1. Influence of temperature and humidity on the substitution of *T. evanescens* by *T. pintoi* (after the 4th generation).

Table 3. Influence of temperature and humidity on prolificacy and ratio of *T. evanescens* and *T. pintoi* species.

Temperature, °C	**Humidity, %**	**Prolificacy/female**		**Number of females, %**		**Period of generation development (days)**	
		T. evanescens	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*
15	80	17.6±1.0	19.7±1.8	58.4±2.8	60.2±2.9	27-28 ±1.3-1.9	25-26 ±1.2-1.6
	65	14.2±0.9	15.8±1.5	53.6±2.7	60.3±2.8		
	50	12.9±0.7	17.2±1.0	50.2±2.3	59.8±2.3		
20	80	19.7±1.4	25.7±1.9	60.2±2.9	62.9±2.9	19-20 ±1.2-1.7	16-17 ±1.1-1.5
	65	15.6±1.3	18.9±1.4	56.4±2.0	64.2±2.3		
	50	14.1±1.0	15.4±1.0	53.4±2.3	60.7±2.6		
25	80	29.0±1.8	33.5±2.4	63.0±2.9	62±2.3	12-13 ±1.1-1.5	10-11 ±1.0-1.4
	65	19.8±1.2	23.2±2.0	59.1±2.3	61.5±2.7		
	50	16.7±1.0	20.2±1.8	55.4±2.2	59.8±2.3		
DEM		2.54	3.40	4.3	4.55	1.40-1.8	2.20-2.6

Field experiments

Experiments were conducted in one farm from Grătieşti on cabbage to control *Mamestra brassicae* at a density of 10 eggs/m^2. Temperature and humidity were taken into accountduring the research experiments. After field experiments *Trichogramma* species were identified by their morphological structure, male genitalia and share of *Trichogramma* species that hatched from parasitized pest eggs. No releases were made in the check.

After the first release of mixed populations of *T. evanescens* and *T. pintoi* (50 : 50%), the share was 91.05% for *T. evanescens* and 8.95% for *T. pintoi* (Table 5). After the second release, the respective shares was as follows – 94.5% and 5.5%. After the first release of the mixture of *T. evanescens* and *T. pintoi* (10:90%) egg laying of the cabbage moth was parasitized at the level of 85.6% by *T. evanescens* and at the level of 14.4% by *T. pintoi*, after the second release – 88.8 and 11.2%, respectively. After the first and second releases of *T. evanescens* and *T. pintoi* mixed populations (90:10%) analyses showed that it is *T. evanescens* that actually controlled the pest in the field while *T. pintoi* was not found.

It was established that under natural conditions species competition was lower. Fluctuations of temperature and humidity, localization of host eggs in time and space, as well as a more intensive accumulation of species that are not specific for this biocoenosis (for example *T. pintoi*) contribute to the aforementioned situation. However, if further *Trichogramma* release is not made, the dominant species is restored. In case of the presence of two species in the biotope, the quantitative share will depend on the specific climatic conditions and the number of preferable host individuals.

Table 4. Ratio of *Trichogramma* species in *Mamestra brassicae* parasitized egg laying (%) after field release.

Experiment variant	Percentage of *Mamestra brassicae* eggs parasitized by *Trichogramma*.		Ratio of *Trichogramma* species in *Mamestra brassicae* parasitized egg-laying (%), after field release			
	Released on 23.06	Released on 19.08	Released on 3.06		Released on 9.08	
			T. evanescens	*T. pintoi*	*T. evanescens*	*T. pintoi*
T. evanescens (100 %)	54.3±2.7	60.4±3.7	100	0	100	0
T. pintoi (100 %)	13.8±1.4	12.6±0.8	0	100	0	100
T. evanescens + *T. pintoi* (50:50%)	40.8±1.9	39.0±1.8	91.1±2.4	8.9±1.0	94.5±3.4	5.5±0.5
T. evanescens + *T. pintoi* (10:90%)	14.2±1.0	23.3±1.1	85.6±3.9	14.4±1.8	88.8±3.3	11.2±0.7
T. evanescens + *T. pintoi* (90:10%)	42.4±3.5	49.9±3.0	100	0	100	0
DEM	2.33	2.45	3.67	3.56	4.32	3.78

CONCLUSIONS

In the laboratory experiments, it was established that along the 4 development generations of mixed populations of *Trichogramma*, gradual substitution of *T. evanescens* by *T. pintoi* occurs.

In the field of cereal, technical and vegetable crops, *T. evanescens* is a dominant species (up to 95-99%). *T. pintoi* represents a laboratory population, the preferred host of which is *Siotroga cerealella*. In the presence of the two species of *Trichogramma* in the biotope, their quantitative ratio depends on the specific climatic conditions and resource of eggs of the preferred host. Biological indices for *T. pintoi* are higher than the ones of *T. evanescens*, therefore, in the laboratory conditions we substitute *T. evanescens* by *T. pintoi*, but in the field it is the opposite.

It was established that under natural conditions, species competition was lower. Fluctuations of temperature and humidity, localization of host eggs in time and space, as well as a more intensive accumulation of species that are not specific for this agrocoenosis (for example *T. pintoi*) contribute to the aforementioned situation.

However, if further *Trichogramma* release is not made, the dominant species is restored. In case of the presence of two species in the biotope, the quantitative share will depend on the specific climatic conditions and the number of preferable host individuals.

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Received: March 31, 2016
Accepted: May 18, 2016

NOTES ON CERTAIN NEW GENERA AND SPECIES OF PTEROMALIDAE (HYMENOPTERA, CHALCIDOIDEA, PTEROMALIDAE) IDENTIFIED WITHIN PLAIUL FAGULUI NATURE RESERVE

MANIC Gheorghe

Abstract. As result of performed studies in various types of biotopes on the reserve territory and adjacent areas there were identified 118 species of pteromalid (Hymenoptera, Pteromalidae) belonging to 9 subfamilies and 64 genera, of which 3 genera (*Rhicnocoelia* Graham, *Merismus* Walker, *Callprymna* Graham), 10 species (*Rhicnocoelia impar* Walker, *Merismus splendens* Graham, *Gastrancistrus acutus* Walker, *Gastrancistrus campressus* Walker, *Trichomalopsis laticeps* Graham, *Trichomalopsis acuminatus* Graham, *Trichomalus rufinus* Walker, *Calliprymna bisetosa* Graham, *Pteromalus papaveris* Forster, *Pteromalus chlorogaster* Thomson) are new for the fauna of the Republic of Moldova.

Keywords: Hymenoptera, Pteromalidae, "Plaiul Fagului" natural Reserve, new genera and species, Republic of Moldova.

Rezumat. Note privind unele genuri si specii noi de Pteromalidae (Hymenoptera, Chalcidoidea, Pteromalidae) semnalate în Rezervaţia naturală Plaiul Fagului. Ca rezultat al investigaţiilor efectuate în diverse biotopuri pe teritoriul rezervaţiei şi zonele linitrofe au fost identificate 118 specii de pteromalide (Hymenoptera, Pteromalidae), incluse în 9 subfamilii şi 64 genuri, dintre care 3 genuri (*Rhicnocoelia* Graham, *Merismus* Walker, *Callprymna* Graham), 10 specii (*Rhicnocoelia impar* Walker, *Merismus splendens* Graham, *Gastrancistrus acutus* Walker, *Gastrancistrus campressus* Walker, *Trichomalopsis laticeps* Graham, *Trichomalopsis acuminatus* Graham, *Trichomalus rufinus* Walker, *Calliprymna bisetosa* Graham, *Pteromalus papaveris* Forster, *Pteromalus chlorogaster* Thomson) sunt noi pentru fauna Republicii Moldova.

Cuvinte cheie: Hymenoptera, Pteromalidae, Rezervaţia naturală „Plaiul Fagului", genuri şi specii noi pentru Republica Moldova.

INTRODUCTION

Scientific Reserve Plaiul fagului was organized in March 1992, based on hunting household Rădenii Vechi. It is located in the north-eastern part of the Central Plateau of R. Moldova and occupies an area of 5558.7 ha, including 5375.5 ha of forest. Within the reserve is highly fragmented landscape, sometimes with mountain features, the altitudes ranging between 410 and 140 m above sea level. Rădeni rivulet with cascading lakes divide the reserve in two equal massives. The vegetation includes about 680 vascular plants, of which 27 plants species are included in the Red Book of Moldova. Plant associations are represented by woods of central European type, where natural stands of beech, oak, linden, hornbeam, pedunculate oak with beech reaching the age of 150 years are preserved. The animal world is represented by 211 species, 142 bird, 49 mammal, 8 reptiles, 12 amphibian and 65 species of soil invertebrates. In prospect is expected the biodiversity conservation insurance and reserve regime; developing of measures and ecological reconstruction of stands in relation to growth conditions; restoration of native stands; implementation of measures to contribute to natural regeneration from seeds; regulation of animal number in relation to the material basis; increasing of environmental education efficiency.

MATERIAL AND METHODS

All the individuals were collected using the entomological net, between 2003 and 2013, mostly by the author. The majority of identified species were collected from different biotopes situated in the reserve's territory.

RESULTS AND DISCUSSION

Family PTEROMALIDAE
Subfamily Miscogarterinae
Genus *Rhicnocoelia* Graham, 1956

***Rhicnocoelia impar* Walker, 1836**

Material examined: Reserve "Plaiul Fagului", Rădenii Vechi (District Ungheni): 1♀ collected 10.VII. 2007, meadow; (Manic leg.).

Geographical distribution: Great Britain, Ireland, Whole Europe [4,5] (BOUCEK & RASPLUS, 1991; DZHANOKMEN, 1987).

Biology: unknown, but probably parasites of Diptera [4,5] (BOUCEK & RASPLUS, 1991; DZHANOKMEN, 1987).

The genus and the species are new for fauna of the Republic of Moldova. [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

Genus *Merismus*, 1833

***Merismus splendens* Graham, 1969**

Material examined: Reserve "Plaiul Fagului", Rădenii Vechi (District Ungheni): 1♀ collected 5.VII. 2006, orchard; (Manic leg.).

Geographical distribution: Mainly N.W. and C. Europe, [4,5] (BOUCEK & RASPLUS, 1991; DZHANOKMEN, 1987).

Biology: it is a parasitoid of *Agromyza albipennis* Mg. (Agromyzidae) [5] (DZHANOKMEN, 1987)

The genus and the species are new to fauna of the Republic of Moldova. [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

Genus *Gastrancistrus* Westwood, 1833

***Gastrancistrus acutus* Walker, 1834**

Material examined: Reserve "Plaiul Fagului": 1♀, 14.VI.2012, glade; (Manic leg.).

Geographical distribution: Sweden, Great Britain [5] (DZHANOKMEN, 1987)

Biology: parasite of cecidomyiids in their galls on various plants. [4,5] (BOUCEK & RASPLUS, 1991; DZHANOKMEN, 1987)

The species is new to fauna of the Republic of Moldova [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

***Gastrancistrus campressus* Walker, 1834**

Material examined: Rădenii Vechi (District Ungheni): 1♀, 11.VII.2008, orchard; (Manic leg.).

Geographical distribution: Sweden, Great Britain [5] (DZHANOKMEN, 1987)

Biology: unknown. [5]. (DZHANOKMEN, 1987)

The species is new to fauna of the Republic of Moldova [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

Subfamily Pteromalinae

Genus *Trihomalipsis* Crawford, 1913

***Trichomalopsis laticeps* Graham, 1969**

Material examined: Reserve "Plaiul Fagului": 1♀, 12.VIII.2003, skirt of a forest; (Manic leg.).

Geographical distribution: Great Britain [5] (DZHANOKMEN, 1987)

Biology: unknown, but probably parasitic in pupae, mainly Lepidoptera [4,5] (BOUCEK & RASPLUS, 1991, DZHANOKMEN, 1987).

The species is new to fauna of the Republic of Moldova [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

***Trichomalopsis acuminatus* Graham, 1969**

Material examined: Rădenii Vechi(District Ungheni): 1♀, 4.VII.2013, orchard; (Manic leg.).

Geographical distribution: Sweden, Irelan [5] (DZHANOKMEN, 1987)

Biology: unknown, but probably parasitic in pupae, mainly Lepidoptera [4,5] (BOUCEK & RASPLUS, 1991, DZHANOKMEN, 1987).

The species is new to fauna of the Republic of Moldova [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

Genus *Trichomalus* Thomson, 1878

***Trichomalus rufinus* Walker, 1835**

Material examined: Reserve "Plaiul Fagului": 2♀♀, 14.VI.2012, glade; (Manic leg.).

Geographical distribution: Austria, Belgium, Bosnia Herzegovina, Canary Islands, Cyech Respublic, Franse, Gernany, Greece, Hungary, Ireland, Lithuania, Montenegro, Norway, Romania, Slovakia, Spain, Sweden, Turkey, Ucraine, Uniteg Kingdom [1,5] (ANDRIESCU & MITROIU, 2004; DZHANOKMEN, 1987)

Biology: primary parasitoid of Apionidae (Coleoptera). [1,5] (ANDRIESCU & MITROIU, 2004; DZHANOKMEN, 1987).

The species is new to fauna of the Republic of Moldova [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

Genus *Calliprymna* Graham, 1966

***Calliprymna bisetosa* Graham, 1966**

Material examined: Reserve "Plaiul Fagului", Rădenii Vechi (District Ungheni): 1♀ collected 10.VIII. 2011, skirt of a forest; (Manic leg.).

Geographical distribution: Great Britain [5] (DZHANOKMEN, 1987).

Biology: unknown. [4,5] (BOUCEK & RASPLUS, 1991; DZHANOKMEN, 1987).

The genus and the species are new to fauna of the Republic of Moldova. [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

Genus *Pteromalus* Swederus, 1795

Pteromalus papaveris **Forster, 1841**

Material examined: Reserve "Plaiul Fagului", Rădenii Vechi (District Ungheni): 1♀ collected 16.VII. 2006, glade; (Manic leg.).

Geographical distribution: Mainly and C. Europe[5] (DZHANOKMEN, 1987).

Biology: parasite of Aylax papaveris Perris (Cynipidae) on the Papaver rhoeas. [5]. (DZHANOKMEN, 1987).

The species is new to fauna of the Republic of Moldova. [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

Pteromalus chlorogaster **Thomson, 1878**

Material examined: Reserve "Plaiul Fagului", Rădenii Vechi (District Ungheni): 1♀ collected 18.VI. 2005, orchard; (Manic leg.).

Geographical distribution: Sweden [5] (DZHANOKMEN, 1987).

Biology: unknown. [5]. (DZHANOKMEN, 1987).

The species is new to fauna of the Republic of Moldova. [2,3,6,7] (BOUCEK 1961, 1966; MANIC, 2010; TALICKIJ & KUSLICKIJ, 1990).

CONCLUSIONS

After identifying that 9 species (*Rhicnocoelia impar* Walker, *Merismus splendens* Graham, *Gastrancistrus acutus* Walker, *Gastrancistrus campressus* Walker, *Trichomalopsis laticeps* Graham, *Trichomalopsis acuminatus* Graham, *Trichomalus rufinus* Walker, *Calliprymna bisetosa* Graham, *Pteromalus papaveris* Forster, *Pteromalus chlorogaster* Thomson) and 3 genres (*Rhicnocoelia* Graham, *Merismus* Walker, *Callprymna* Graham) new for fauna of Republic of Moldova, number of known species and genres of fauna Natural Reserve Plaiul Fagului and adjacent areas increases from 109 to 118 species and respectively from 61 to 64 genres.

Thus our research contributes to a better acquaintance of diversity and area of spreading of the Pteromalidae in fauna of Republic of Moldova.

The species: *Merismus splendens* Graham, *Gastrancistrus acutus* Walker parasite of Cecidomyiidae and Agromyzidae. *Trichomalus rufinus* Walker primary parasitoid of Apionidae (Coleoptera). *Pteromalus papaveris* forster parasite of *Aylax papaveris* Perris (Cynipidae) on the *Papaver rhoeas*. The biology of six species (*Rhicnocoelia impar* Walker, *Gastrancistrus campressus* Walker, *Trichomalopsis laticeps* Graham, *Trichomalopsis acuminatus* Graham, *Calliprymna bisetosa* Graham, *Pteromalus chlorogaster* Thomson) is unknown.

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Received: April 29, 2016
Accepted: May 2, 2016

RELATIVE ABUNDANCE AND DOMINANCE OF FAMILIES OF EPIGEIC COLEOPTERA (ORDER COLEOPTERA) IN MAIZE FIELDS, BRĂILA (BRĂILA COUNTY) AND MOLDOVA (ROMANIA) 1978-2010 (12 seasons)

VARVARA Mircea

Abstract. The paper is a statistical, relevant synthesis of the original collecting data on the epigeic coleopterans in the maize crops from Walachia (Brăila County) and Moldova (5 counties), in the time interval 1978 -2010, 12 seasons actually, 17 localities (six in Brăila county, Walachia and 11 in Moldova). The material of coleopterans was collected using the ecological method of Barber pitfalls with preservative liquid formalin solution of 4%, protected against rainfalls. In total, 22,231 individuals of Coleoptera were collected belonging to 22 families. In the order of their abundances, they are: 1. Carabidae, 15,638 (70.34%) present in all the crops investigated (100%) 2. Tenebrionidae, 1,687 (7.59%), 12 localities (70.59%); 3. Elateridae 1,188 (5.34 %), 15 localities (88.24%); 4. Curculionidae, 832 (3.74 %), 14 localities (88.24 %); 5. Dermestidae, 650 (2.92%.), 8 localities (47.06%); 6. Coccinelidae, 534 (2.40%), 16 localities (94.12 %); 7. Staphylinidae, 509 (2.29%), 12 localities (70.59%); 8. Chrysomelidae, 403 (1.81%), 16 localities (94.12%); 9. Anthicidae, 240 (1.08%), 10 localities (58.82%); 10. Scarabaeidae, 185 (0.83%); 13 localities (76,47%); 11. Histeridae, 111 (0.50 %), 8 localities (47.06 %); 12. Silphidae, 78 (0.35 %), 7 localities (41.18%); 13. Chryptophagidae, 75 (0.34%), 6 localities (35.29%); 14. Cantharidae, 46 (0.21 %), 8 localities (47.06%); 15. Cerambycidae, 20 (0.094) localities (23.53%); 16. Nitidulidae, 11 (0.05%), 2 places (11.78%); 17. Lathridiidae, 8 (0.04%), 3 localities (17.65%). Five other families, Melyridae, Catopidae, Ptinidae, Phalacrididae, Meloidae have a total of 16 individuals, between 1 and 5 individuals.

Keywords: Maize crops, Brăila, Moldova, Coleoptera, families, distribution, abundance, dominance.

Rezumat. Abundenţa relativă şi dominanţa familiilor de coleoptere (Ord. Coleoptera) în culturile de porumb, Brăila (Judeţul Brăila) şi Moldova (România) 1978-2010 (12 sezoane). Lucrarea este o sinteză statistică, relevantă a datelor originale de colectare a coleopterelor din culturile de porumb Muntenia, (județul Brăila) si Moldova (5 județe) în intervalul de timp1978-2010, 12 sezoane, efectiv, 17 localități (șase în județul Brăila, Muntenia și 11 în Moldova). Materialul de coleoptere a fost colectat, folosind metoda ecologică a capcanelor Barber, cu lichid conservant, soluție de formol 4 %, protejate împotriva precipitațiilor. În total, s-au colectat 22.231 indivizi de coleoptere, aparținând la 22 de familii. In ordinea abundenții lor totale, ele sunt: 1. Carabidae, 15.638 (70,34%) prezente în toate culturile cercetate (100%); 2. Tenebrionidae, 1.687 (7,59%), 12 localități (70,59 %); 3. Elateridae, 1.188 (5,34 %), 15 localități (88,24 %); 4. Curculionidae, 832 (3,74%), 14 localități (88,24%); 5. Dermestidae 650 (2,92 %), 8 localități (47,06%); 6. Coccinelidae, 534 (2,40%), 16 localități (94,12%); 7. Staphylinidae, 509 (2,29%), 12 localități (70,59%); 8. Chrysomelidae, 403 (1,81%), 16 localități; (94.12%); 9. Anthicidae, 240 (1,08%), 10 localități (58,82%); 10. Scarabaeidae, 185 (0,83%); 13 localități (76,47%); 11. Histeridae, 111 (0,50%), 8 localități (47,06%); 12. Silphidae, 78 (0,35%), 7 localități (41,18%); 13. Chryptophagidae, 75 (0,34%), 6 localităti (35,29%); 14. Cantharidae, 46 (0,21%), 8 localități (47,06 %); 15. Cerambycidae, 20 (0,09%), 4 localități, (23,53%); 16. Nitidulidae, 11 (0,05%), 2 localitati (11,78%); 17. Lathridiidae, 8 (0,04%), 3 localități (17,65%). Alte cinci familii, Melyridae, Catopidae, Ptinidae, Phalacrididae, Meloidae au în total 16 indivizi între 1 și 5 indivizi.

Cuvinte cheie: cultura de porumb, Brăila, Moldova, Coleoptera, familii, răspândire, abundența, dominanța.

INTRODUCTION

The maize (*Zea mays* L.) is one of the most important crop plants for the food of people (21%), animals (72%), use in industry (7%). It is resistant to drought and heat (NET). Most part of Moldova (Romania) belongs to the favourable maize crop area. The maize is a monoculture, which occupies the cultivated soil from April-May until September-October. Crops (clover, alfalfa, sugar beet, wheat, maize, sunflower, sugar beet, grape vines, fruit trees, etc.) by their characteristics during the vegetation period (density of plants, degree of shading of the soil surface, vegetation period, etc.) influence the soil surface moisture and microcurrents, factors that influence the number of epigeic arthropods in their feeding activity, defense and reproduction in dependence on their ecological valences referring to humidity. There were published some papers about the species of carabids on the maize crops in the Brăila County and Moldova: ANDRIESCU et. al., 1983; BODESCU, 2013; VARVARA et. al., 1981; VARVARA et. al., 1985; VARVARA, et. al., 1987; VARVARA & BRUDEA, 1999; VARVARA & ZAMFIRESCU, 2008.

MATERIAL AND METHODS

The material of the paper is original, collected from two regions of Romania, Walachia (Brăila County) and Moldavia (the counties of Vaslui, Iași, Bacău, Neamț, Botoșani), in the period of the years 1978- 2010, 12 seasons.

For the collecting of the material there was used the method of pitfall traps, a classical, standard, ecological and continuous method. In total, 268 Barber pitfalls were used, between 5 and 24 traps in each research locality (Table 1). We used tin cans, 800 ml capacity, with a height of 11 cm and a diameter of 8 cm. Each pitfall was protected against precipitations by a cover sheet with four pillars stuck in the ground. The distance between the ground surface and lid was 5 cm. As the preservative liquid it was used a solution of 4% formaldehyde, about 350 ml in each trap. In 10 localities, there were placed 12 traps, covering an area of capturing of 400 square metres. The purpose of this paper is to present, synthetically, the diversity of the families of the order Coleoptera, their comparative abundance and dominance on the basis of original collectings, 12 seasons, actually.

Objectives of the paper:

Collecting and preserving of the material;

Taxonomic determination of the individuals, classification in families and their analysis; Drawing up tables and figures. Each family is concretized through a general collecting table (locality, year, abundance and dominance of the family, a synthetic table with the presence and absence of families in localities and the distribution of families in the structure of dominance); Synthesis of data, establishing the hierarchy of families based on the number of individuals, and the drafting of the text.

RESULTS

In this chapter, the work materializes the effort of displacement in the field, the effort of collecting, preservation of samples, the time consumed for determining of the individuals, analysis, the work of synthesis. Thus, there were accomplished 152 collectings during 12 seasons (1978-2010), meaning the taxonomic determination of the individuals from 40,736 samples (152 collectings x 268 pitfalls), 17 localities (Table 1).

Table 1. Localities, years and parameters of collecting of the epigeic Coleoptera in maize crops, 1978-2010.

No.	Crop, locality, county	Year	Exposition of traps	Days	Traps	Coll.	Sam.
1	Brăila (Brăila County)	1978	May 7-October 10	150	24	5	1,322
2	Brăila	1979	May 25-Sept.20	118	24	5	1,367
3	NegruVoda	1979	May 20-Sept.20	123	24	5	724
4	Brăila	1980	May 24- Sept 11	110	56	6	6,213
5	Braila.Terasă	1984	May 10-Sept.30	144	12	15	651
6	Brăila, Lacul Sărat	1984	May 10-Sept.30	144	12	15	567
7	Pogana (Vaslui C.)	1989	April 24- July 30	97	10	6	462
8	Negrești	1992	May 10-Aug.15	97	12	7	1,706
9	Negrești	1997	May 5- July 16	72	12	5	1,157
10	Osoi, (Iasi County)	1986	April 15-Sept.23	162	5	11	1,455
11	Osoi	1989	May 15-Sept.18	126	5	10	517
12	Breazu	2001	June 15-Sept.13	90	12	6	55
13	Hemeiusi(Bacău C.)	1981	June 10-Oct.18	130	12	14	933
14	Târgu Neamț (Neamț C.)	1998	May 10-Sept.1	114	12	8	1,498
15	Săbăoani,	1999	May 10-Sept.9	122	12	9	1,551
16	Cherchejeni, (Botoșani C.)	2000	May 5-Sept.12	130	12	9	676
17	Trușești.	2010	May 10-Sept.15	128	12	10	1,376
				2,057	268	152	22,230

Those 22,230 collected individuals belong, taxonomically, to 22 families of Coleoptera (Table 2). In the same table, it is mentioned the numerical and percentage presence and absence of the families in localities.

Table 2. Numerical and percentage variation of the individuals of coleopteran families in maize fields, from Brăila and Moldova, 1978- 2010.

				Present		Absent	
No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	Carabidae	15,638	70.34	17	100	0	0
2	Tenebrionidae	1,687	7.59	12	70.59	5	29.41
3	Elateridae	1,188	5.34	15	88.24	2	11.76
4	Curculionidae	832	3.74	14	88.24	2	11.76
5	Dermestidae	650	2.92	8	47.06	9	52.94
6	Coccinelidae	534	2.40	16	94.12	1	5.88
7	Staphylinidae	509	2.29	12	70.59	5	29.41
8	Chrysomelidae	403	1.81	16	94.12	1	5.88
9	Anthicidae	240	1.08	10	58.82	7	41.18
10	Scarabaeidae	185	0.83	13	76.47	4	23.53
11	Histeridae	111	0.50	8	47.06	9	52.94
12	Silphidae	78	0.35	7	41.18	10	58.82
13	Chryptophagidae	75	0.34	6	35.29	11	64.71
14	Cantharidae	46	0.21	8	47.06	9	52.94
15	Cerambycidae	20	0.09	4	23.53	13	76.47
16	Nitidulidae	11	0.05	2	11.76	15	88.24
17	Lathridiidae	8	0.04	3	17.65	14	82.35
18	Melyridae	5	0.02	3	17.65	14	82.35
19	Catopidae	5	0.02	1	5.88	16	94.12
20	Ptinidae	4	0.02	1	5.88	16	94.12
21	Phalacrididae	1	0.00	1	5.88	16	94.12
22	Meloidae	1	0.0	1	5.88	16	94.12
	Total	22,231	99.98				

Legend. **1**. Name of families, **2**. No. of individuals, **3**. %, **4**. No. of localities, 5. %, **6.** No. of localities, 7. %.

Carabidae Family occupies the first position in the number of individuals 15,638 (70.34%) captured on the soil surface in those 17 maize crops. The family Carabidae is eudominant in each locality (Table 3).

Table 3. Distribution, variation of activity abundance (A) and dominance (D) of Carabidae family in the investigated maize crops, Brăila and Moldova, 1978-2010.

No.	Locality and Year	A	D	No.	Locality, Year	A	D (%)
1	Brăila, 1978	1,122	84.87	9	Negreşti, 1997	821	70.96
2	Brăila, 1979	1,266	92.61	10	Osoi, 1986	404	27.77
3	Negru Vodă, 1979	644	71.96	11	Osoi, 1989	409	79.11
4	Brăila, 1980	4,910	79.03	12	Breazu, 2001	16	29.09
5	Brăila, Terrace, 1984	334	51.31	13	Hemeiuşi, 1981	826	88.53
6	Brăila, Sărat Lake, 1984	480	84.66	14	Târgu Neamt, 1998	1,138	75.72
7	Pogana, 1989	122	26.41	15	Săbăoani, 1999	1,131	72.87
8	Negreşti, 1992	384	25.53	16	Cherchejeni, 2000	641	94.82
				17	Truşeşti, 2010	990	71.94
					Total individuals	**15,638**	

Table 4 and Figure 1. Numerical and percentage variation of the presence and absence of Carabidae family and their dominance classes in maize fields, Brăila and Moldova, 1978-2010.

	Specification	No.	%
1	Presence in localities	17	100
2	Absence	0	0
1	Subrecedent below 1%	0	0
2	Recedent 1.1 - 2%	0	0
3	Subdominant 2.1 -5%	0	0
4	Dominant 5.1 -10%	0	0
5	Eudominant over 10.1%	17	100
	Total	17	100

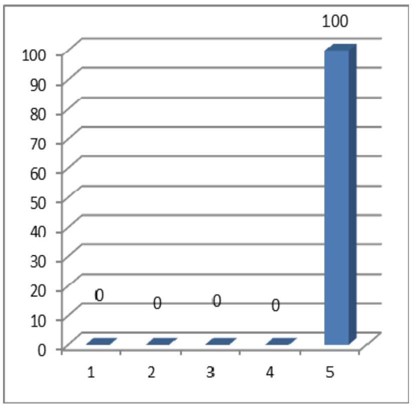

Figure 1.

Tenebrionidae Family occupies the second position in the order of the number of individuals, 1,687 (7.59 %) (i.e. nine times less than the family Carabidae). It was present only in 12 localities (70.59 %) and absent in five maize crops (29.41 %) (Table 5, Table 6, Fig. 2).

Table. 5. Distribution, variation of activity abundance (A) and dominance (D) of Tenebrionidae family in the investigated maize crops, Brăila and Moldova, 1979-2010.

No.	Locality and Year	A	D	No.	Locality, Year	A	D (%)
1	Brăila, 1979	20	1.46	7	Negreşti, 1997	122	10.54
2	Negru Vodă, 1979	33	3.69	8	Osoi, 1986	1	0.07
3	Brăila, 1980	22	0.35	9	Breazu, 2001	3	5.45
4	Brăila, Terrace, 1984	1	0.15	10	Săbăoani, 1999	180	11.60
5	Pogana, 1989	231	50.0	11	Cherchejeni, 2000	5	0.74
6	Negreşti, 1992	906	60.24	12	Truşeşti, 2010	163	11.84
					Total individuals	**1,687**	

Table 6 and Figure 2. Numerical and percentage variation of the presence and absence of Tenebrionidae family and their dominance classes in maize fields, Brăila and Moldova, 1979-2010.

	Specification	No.	%
1	Presence in localities	12	70.59
2	Absence in sites	5	29.41
1	Subrecedent below 1%	4	23.53
2	Recedent 1.1 - 2%	1	5.88
3	Subdominant 2.1 -5%	1	5.88
4	Dominant 5.1 -10%	1	5.88
5	Eudominant over 10.1%	5	29.41
	Total	12	70.59

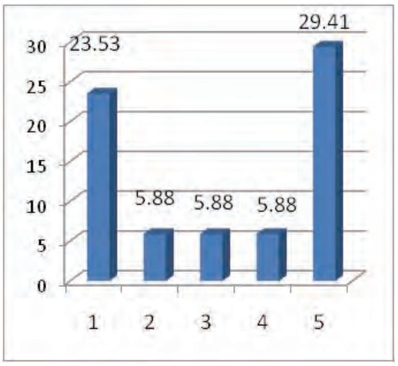

Figure 2.

Elateridae Family takes the third position in the maize crops, with a total number of 1,188, individuals, i.e. 5.34%, being present in 15 localities (88.24%) and absent in two localities (11.76 %) (Table 7, Table 8, Fig. 3).

Table. 7. Distribution, variation of activity abundance (A) and dominance (D) of Elateridae Family in the investigated maize crops, Brăila and Moldova, 1978-2000.

No.	**Locality and Year**	**A**	**D**	**No.**	**Locality, Year**	**A**	**D (%)**
1	Brăila, 1978	67	5.07	9	Osoi, 1986	911	62.01
2	Negru Vodă, 1979	28	3.13	10	Osoi, 1989	7	1.35
3	Brăila,1980	41	0.66	11	Breazu, 2001	3	5.45
4	Brăila, Terrace, 1984	27	4.15	12	Hemeiuşi, 1981	25	2.68
5	Brăila, Sărat Lake, 1984	9	1.59	13	Săbăoani, 1999	1	0.06
6	Pogana, 1989	1	0.22	14	Târgu Neamț, 1998	4	0.27
7	Negreşti, 1992	45	2.99	15	Cherchejeni, 2000	13	1.92
8	Negreşti, 1997	6	0.52		**Tot. individuals**	**1.188**	

Table 8 and Figure 3. Numerical and percentage variation of the presence and absence of Elateridae family and their dominance classes in maize fields, Brăila and Moldova, 1978-2000.

	Specification	**No.**	**%**
1	Presence in localities	15	88.24
2	Absence in sites	2	11.76
1	Subrecedent below 1%	5	29.41
2	Recedent 1.1 - 2%	3	17.65
3	Subdominant 2.1 -5%	4	23.53
4	Dominant 5.1 -10%	2	11.76
5	Eudominant over 10.1%	1	5.88
	Total	15	88.23

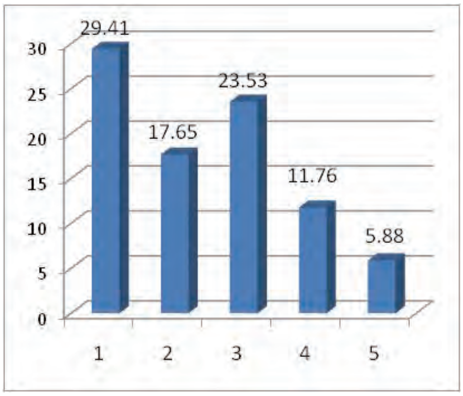

Figure 3.

The next family in the order of abundance is Curculionidae family with 832 individuals (3.74%), present in 15 localities (88.24%) and absent in two places (11.76 %) (Table 9, Table 10, Fig. 4).

Table 9. Distribution, variation of activity abundance (A) and dominance (D) of Curculionidae Family in the investigated Maize crops, Brăila and Moldova, 1979-2010.

No.	**Locality and Year**	**A**	**D**	**No.**	**Locality, Year**	**A**	**D (%)**
1	Brăila, 1979	36	2.63	**8**	Osoi, 1989	27	5.22
2	Brăila, 1980	524	8.43	9	Breazu, 2001	3	5.45
3	Brăila, Terrace, 1984	106	16.28	10	Hemeiuşi, 1981	3	0.32
4	Brăila, Sărat Lake, 1984	3	0.53	11	TârguNeamt, 1998	20	1.33
5	Negreşti, 1992	21	1.40	12	Săbăoani, 1999	44	2.84
6	Negreşti, 1997	11	0.95	13	Cherchejeni, 2000	1	0.15
7	Osoi, 1986	15	1.03	14	Truşeşti, 2010	18	1.30
					Tot. individuals	**832**	

Table 10 and Figure 4. Numerical and percentage variation of the presence and absence of Curculionidae family and their dominance classes in maize fields, Brăila and Moldova, 1979-2010.

	Specification	**No.**	**%**
1	Presence in localities	14	82.35
2	Absence in sites	3	17.65
1	Subrecedent below 1%	4	23.53
2	Recedent 1.1 - 2%	4	23.53
3	Subdominant 2.1 -5%	2	11.76
4	Dominant 5.1 -10%	3	17.65
5	Eudominant over 10.1%	1	5.88
	Total	14	88.24

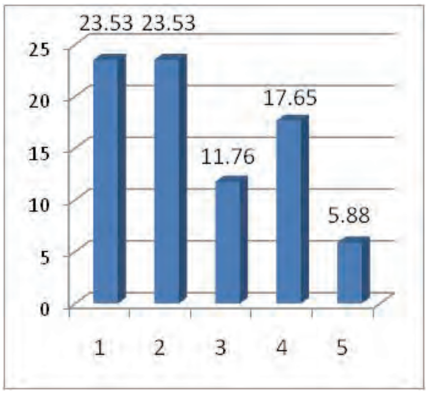

Figure 4.

Dermestidae Family totalized 650 individuals (2.92 %). The individuals of this family were collected from 8 localities (47.06 %) and they were not found in 9 places (52.94 %) (Table 11, Table 12, Fig. 5).

Table 11. Distribution, variation of activity abundance (A) and dominance (D) of Dermestidae Family in the investigated Maize crops, Brăila and Moldova, 1980-2010.

No.	Locality and Year	A	D	No.	Locality, Year	A	D (%)
1	Brăila, 1980	109	1.75	5	Breazu, 2001	2	3.64
2	Pogana, 1989	46	9.96	6	TârguNeamt, 1998	23	1.53
3	Negreşti, 1992	238	33.66	7	Săbăoani, 1999	14	0.90
4	Negreşti, 1997	165	14.26	8	Truşeşti, 2010	53	3.85
					Total individuals	**650**	

Table 12 and Figure 5. Numerical and percentage variation of the presence and absence of Dermestidae family and their dominance classes in maize fields, Brăila and Moldova, 1980-2010.

	Specification	No.	%
1	Presence in maize fields	8	47.06
2	Absence in sites	9	52.94
1	Subrecedent below 1%	1	5.88
2	Recedent 1.1 - 2%	2	11.76
3	Subdominant 2.1 -5%	2	11.76
4	Dominant 5.1 -10%	1	5.88
5	Eudominant over 10.1%	2	11.76
	Total	8	47.0

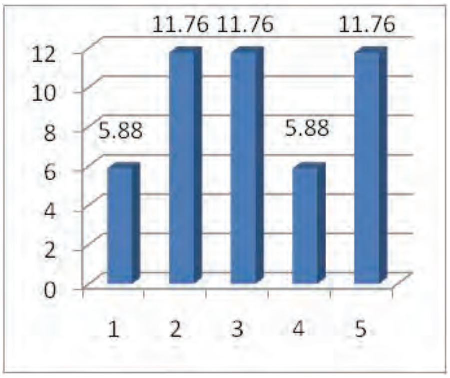

Figure 5.

Coccinelidae Family totalized 534 (2.40 %), occupying the 6th position in the numerical hierarchy. The individuals of this family were collected from 16 localities (94.12 %) (Table 13, Table 14, Fig. 6).

Table 13. Distribution, variation of activity abundance (A) and dominance (D) of Coccinelidae Family in the investigated Maize crops, Brăila and Moldova, 1978-2010.

No.	Locality and Year	A	D	No.	Locality, Year	A	D (%)
1	Brăila, 1978	106	0.02	9	Negreşti, 1997	3	0.26
2	Brăila, 1979	40	2.93	10	Osoi, 1989	1	0.19
3	Negru Vodă, 1979	15	1.68	11	Breazu, 2001	8	14.55
4	Brăila, 1980	176	2.83	12	Hemeiuşi, 1981	16	1.71
5	Brăila, Terrace, 1984	22	3.38	13	TârguNeamt, 1998	18	1.20
6	Brăila, Sărat Lake, 1984	13	2.29	14	Săbăoani, 1999	1	0.06
7	Pogana, 1989	4	0.87	15	Cherchejeni, 2000	5	0.74
8	Negreşti, 1992	72	10.18	16	Truşeşti, 2010	34	2.47
					Total individuals	**534**	

Table 14 and Figure 6. Numerical and percentage variation of the presence and absence of Coccinelidae Family and their dominance classes in maize fields, Brăila and Moldova, 1978-2010.

	Specification	No.	%
1	Presence in localities	16	94.12
2	Absence in sites	1	5.88
1	Subrecedent below 1%	6	35.29
2	Recedent 1.1 - 2%	3	17.65
3	Subdominant 2.1 -5%	5	29.41
4	Dominant 5.1 -10%	-	-
5	Eudominant over 10.1%	2	11.76
	Total	16	94.11

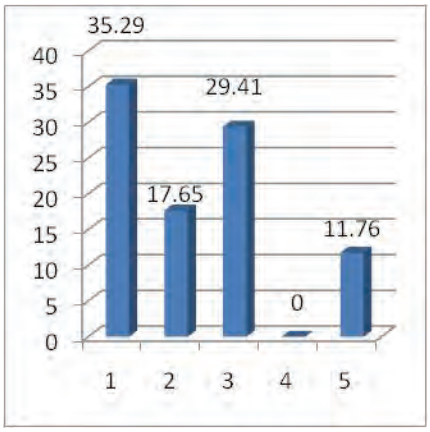

Figure 6.

Staphylinidae Family totalized 509 individuals (2.29%), present in 13 localities (76.47%) and absent in 5 localities 29.41%) (Table 15, Table 16, Fig. 7).

Table. 15. Distribution, variation of activity abundance (A) and dominance (D) of Staphylinidae Family in the investigated Maize crops, Brăila and Moldova, 1980-2010.

No.	**Locality and Year**	**A**	**D**	**No.**	**Locality, Year**	**A**	**D (%)**
1	Brăila, 1980	106	1.71	8	Osoi, 1989	5	0.97
2	Brăila, Terrace, 1984	26	3.99	9	Breazu, 2001	1	1.82
3	Brăila, Sărat Lake, 1984	3	0.53	10	Hemeiusi, 1981	6	0.64
4	Pogana, 1989	5	1.08	11	Tîrgu Neamț, 1998	180	11.98
5	Negrești, 1992	13	1.84	12	Săbăoani, 1999	104	6.70
6	Negresti, 1997	3	0.26	13	Trușești, 2010	8	0.58
7	Osoi, 1986	49	3.37		Total individuals	**509**	

Table 16 and Figure 7. Numerical and percentage variation of the presence and absence of Staphylinidae and their dominance classes in maize fields, Brăila and Moldova, 1978-2010.

	Specification	**No.**	**%**
1	Presence in localities	13	76.47
2	Absence in sites	4	23.53
1	Subrecedent below 1%	5	29.41
2	Recedent 1.1 - 2%	4	23.53
3	Subdominant 2.1 -5%	2	11.76
4	Dominant 5.1 -10%	1	5.88
5	Eudominant over 10.1%	1	5.88
	Total	13	76.46

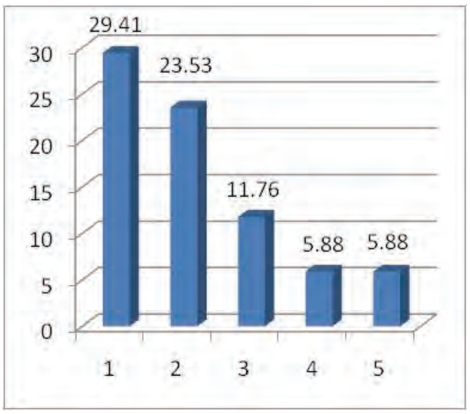

Figure 7.

Chrysomelidae registered a total number of individuals 403 (1.81%), present in 16 localities (94.12%), absent in one locality (5.88%) (Table 17, Table 18, Fig. 8).

Table 17. Distribution, variation of activity abundance (A) and dominance (D) of Chrysomelidae Family in the investigated maize crops, Brăila and Moldova, 1978-2010.

No.	**Locality and Year**	**A**	**D**	**No.**	**Locality, Year**	**A**	**D (%)**
1	Brăila, 1978	27	2.04	9	Osoi, 1986	42	2.89
2	Brăila, 1979	2	0.15	10	Osoi, 1989	34	6.58
3	Negru Vodă, 1979	1	0.11	11	Breazu, 2001	6	10.91
4	Brăila, 1980	122	1.96	12	Hemeiuși, 1981	21	2.25
5	Brăila, Terrace, 1984	44	6.76	13	Târgu Neamț, 1998	21	1.40
6	Brăila,Sărat Lake, 1984	7	1.23	14	Săbăoani, 1999	51	3.29
7	Negrești, 1992	8	1.13	15	Cherchejeni, 2000	**4**	**0.59**
8	Negrești, 1997	7	0.61	16	Trușești, 2010	**6**	**0.43**
					Tot. individuals	**403**	

Table 18 and Figure 8. Numerical and percentage variation of the presence and absence of Chrysomelidae Family and their dominance classes in maize fields, Brăila and Moldova, 1978-2010.

	Specification	**No.**	**%**
1	Presence in localities	16	94.12
2	Absence in sites	1	5.88
1	Subrecedent below 1%	5	29.41
2	Recedent 1.1 - 2%	4	23.53
3	Subdominant 2.1 -5%	4	23.53
4	Dominant 5.1 -10%	2	11.76
5	Eudominant over 10.1%	1	5.88
	Total	16	94.11

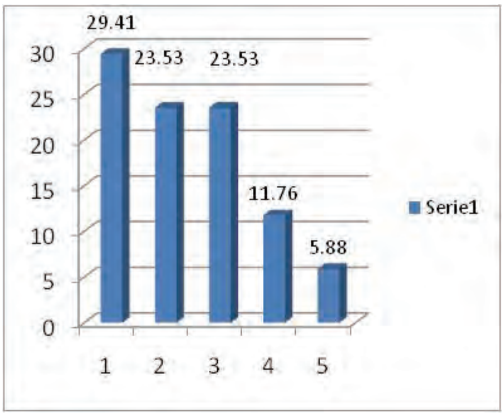

Figure 8.

Anthicidae Family is the last family that has a percentage of 1.08%, 240 individuals present in 10 localities (58.82%), 7 localities absent (41.18%) (Table 19, Table 20, Fig. 9).

Table 19. Distribution, variation of activity abundance (A) and dominance (D) of Anthicidae Family in the investigated Maize crops, Brăila and Moldova, 1980-2001.

No.	**Locality and Year**	**A**	**D**	**No.**	**Locality, Year**	**A**	**D (%)**
1	Brăila, 1980	110	1.77	7	Osoi, 1989	4	0.77
2	Brăila, Terrace, 1984	47	7.22	8	Breazu, 2001	2	3.64
3	Brăila, Sărat Lake, 1984	25	4.41	9	Săbăoani, 1999	13	0.84
4	Pogana, 1989	13	2.81	10	Cherchejeni, 2000	6	0.89
5	Negreşti, 1997	6	0.52		Tot. individuals	**240**	
6	Osoi, 1986	14	0.96				

Table 20 and Figure 9. Numerical and percentage variation of the presence and absence of Anthicidae family and their dominance classes in maize fields, Brăila and Moldova, 1980-2001.

	Specification	**No.**	**%**
1	Presence localities	10	58.82
2	Absence in sites	7	41,18
1	Subrecedent below 1%	5	29.41
2	Recedent 1.1 - 2%	1	5.88
3	Subdominant 2.1 -5%	3	17.65
4	Dominant 5.1 -10%	1	5.88
5	Eudominant over 10.1%	0	0
	Total	10	58.82

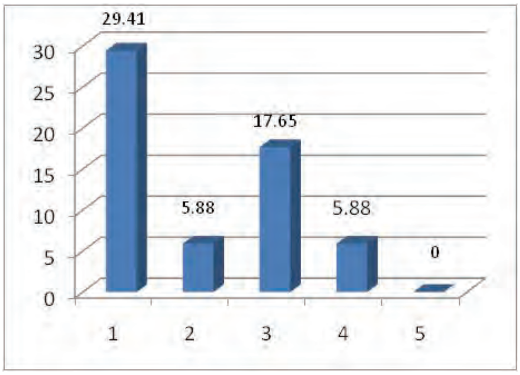

Figure 9.

Scarabaeidae Family is the first family of Coleoptera in maize crops with a percentage of individuals below 1%. The total number of individuals collected was 185 (0.83 %), present in 13 localities (76.47%) and absent in 4 places (23.53 %). The number of individuals collected from localities varied between 1 (Negreşti, 1992, Săbăoani, 1999 - Neamț County) and 51 (Truşeşti, 2010 - Botosani County) (Tabel 21, Table 22, Fig. 10).

Table 21. Distribution, variation of activity abundance (A) and dominance (D) of Scarabaeidae Family in the investigated Maize crops, Brăila and Moldova, 1979-2010.

No.	**Locality and Year**	**A**	**D**	**No.**	**Locality, Year**	**A**	**D (%)**
1	Brăila, 1979	3	0.22	7	Negreşti, 1992	1	0.07
2	Brăila, 1980	30	0.48	8	Negreşti, 1997	7	0.61
3	Brăila, Terrace, 1984	20	3.07	9	Osoi, 1989	11	2.93
4	Brăila, Sărat Lake, 1984	9	1.59	10	Săbăoani, 1999	1	0.06
5	Negru Vodă, 1979	3	0.34	11	Târgu Neamț, 1998	12	0.60
6	Pogana, 1989	26	5.63	12	Cherchejeni, 2000	1	0.15.
				13	Truşeşti, 2010	61	4.43
					Tot. individuals	**185**	

Table 22 and Figure 10. Numerical and percentage variation of the presence and absence of Scarabaeidae family and their dominance classes in maize fields, Brăila and Moldova, 1979-2010.

	Specification	**No.**	**%**
1	Presence in localities	13	76.47
2	Absence in sites	4	23.53
1	Subrecedent below 1%	8	47.06
2	Recedent 1.1 - 2%	1	5.88
3	Subdominant 2.1 -5%	3	17.65
4	Dominant 5.1 -10%	1	5.88
5	Eudominant over 10.1%	-	-
	Total	13	76.47

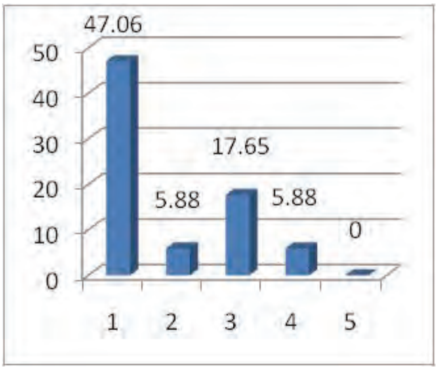

Figure 10.

Histeridae Family registered 111 individuals (0.50%), present in 8 localities (47.06%), absent in 9 localities (52.94 %) (Table 23, Table 24, Fig. 11).

Table 23. Distribution, variation of activity abundance (A) and dominance (D) of Histeridae Family in the investigated maize crops, Brăila and Moldova, 1980-2010.

No.	Locality and Year	A	D	No	Locality, Year	A	D (%)
1	Brăila, 1980	1	0.02	5	Osoi, 1986	1	0.07
2	Pogana, 1989	1	0.22	6	Hemeiusi, 1981	23	2.47
3	Negreşti, 1992	11	0.73	7	Târgu Neamţ, 1998	60	3.99
4	Negreşti, 1997	1	0.09	8	Truşeşti, 2010	13	0.94
					Tot. individuals	**111**	

Table 24 and Figure 11. Numerical and percentage variation of the presence and absence of Histeridae family and their dominance classes in maize fields, Brăila and Moldova, 1980-2010.

	Specification	No.	%
1	Presence in maize fields	8	47.06
2	Absence in sites	9	52.94
1	Subrecedent below 1%	6	35.29
2	Recedent 1.1 - 2%	0	0
3	Subdominant 2.1 -5%	2	11.76
4	Dominant 5.1 -10%	0	0
5	Eudominant over 10.1%	0	0
	Total	8	47.05

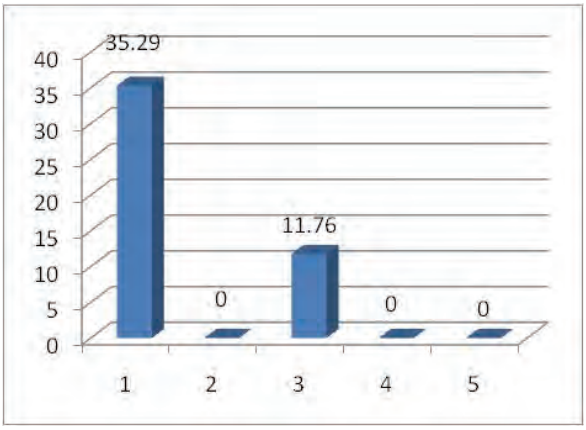

Figure 11.

Silphidae Family had 78 individuals (0.35%), present 7 localities (41.18%), absent in 10 localities (58.82%) (Table 25, Table 26, Fig. 12).

Table 25. Distribution, variation of activity abundance (A) and dominance (D) of Silphidae Family in the investigated Maize crops, Brăila and Moldova, 1980-2010.

No.	Locality and Year	A	D	No.	Locality, Year	A	D (%)
1	Brăila, 1980	1	0.02	4	Breazu, 2001	11	20
2	Pogana, 1989	13	2.81	5	Hemeiusi, 1981	12	1.29
3	Osoi, 1989	7	1.35	6	Săbăoani, 1999	7	0.45
				7	Truşeşti, 2010	27	1.96
					Tot. individuals	**78**	

Table 26 and Figure 12. Numerical and percentage variation of the presence and absence of Silphidae family and their dominance classes in maize fields, Brăila and Moldova, 1980-2010.

	Specification	No.	%
1	Presence in localities	7	41.18
2	Absence in sites	10	58.82
1	Subrecedent below 1%	2	11.76
2	Recedent 1.1 - 2%	3	17.65
3	Subdominant 2.1 -5%	1	5.88
4	Dominant 5.1 -10%	0	0
5	Eudominant over 10.1%	1	5.88
	Total	7	41.18

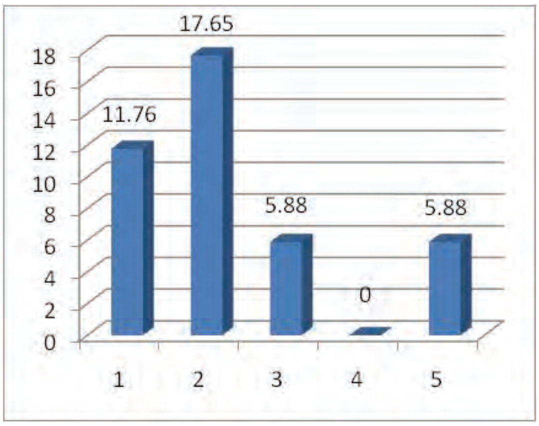

Figure 12.

Chryptophagidae Family registered 75 individuals (0.34 %), present in 6 crops (35.29%), absent 11 localities (64.71%) (Table 27).

Table 27. Distribution, variation of activity abundance (A) and dominance (D) of Chryptophagidae Family in the investigated maize crops, Brăila and Moldova, 1980-1999.

No.	Locality and Year	A	D	No.	Locality and, Year	A	D (%)
1	Brăila, 1980	35	0.56	4	Osoi, 1986	8	0.55
2	Terrace, 1984	13	2.00	5	Târgu Neamț, 1998	6	0.40
3	Sărat Lake, 1984	9	1.59	6	Săbăoani, 1999	4	0.26
					Tot. individuals	**75**	

Cantharidae Family had 46 individuals (0.21%), present in 8 maize crops (47.06%), absent in 9 localities (52.94 %) (Table 28, Table 29, Fig. 13).

Table 28. Distribution, variation of activity abundance (A) and dominance (D) of Cantharidae family in the investigated Maize crops, Brăila and Moldova, 1980-1998.

No.	Locality, Year	A	D	No.	Locality, Year	A	D (%)
1	Brăila, 1980	14	0.23	5	Negresti, 1997	3	0.26
2	Brăila, Terrace, 1984	7	1.08	6	Osoi, 1989	7	1.35
3	Brăila, Sărat Lake, 1984	6	1.06	7	Hemeiuși, 1981	1	0.11
4	Negrești, 1992	2	0.13	8	Târgu Neamț, 1998	6	0.40
					Tot individuals	**46**	

Table 29 and Figure 13. Numerical and percentage variation of the presence and absence of Family Cantharidae and their dominance classes in maize fields, Brăila and Moldova, 1980-1998.

	Specification	No.	%
1	Presence in localities	8	47.06
2	Absence in sites	9	52.94
1	Subrecedent below 1%	5	29.41
2	Recedent 1.1 - 2%	3	17.65
3	Subdominant 2.1 -5%	0	0
4	Dominant 5.1 -10%	0	0
5	Eudominant over 10.1%	0	0
	Total	8	47.06

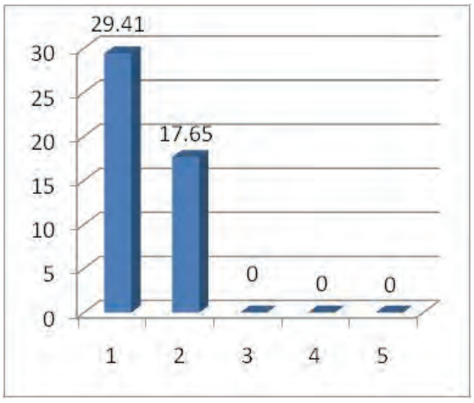

Figure 13.

Cerambycidae Family had 20 individuals (0.09%), present in 4 localities (23.53 %), absent in 13 localities (76.47 %) (Table 30, Table 31, Fig. 14).

Table 30. Distribution, variation of activity abundance (A) and dominance (D) of Cerambycidae Family in the investigated maize crops, Brăila and Moldova, 1986-2010.

No.	Locality and Year	A	D
1	Negrești, 1997	**2**	**0.17**
2	Osoi, 1986	**10**	**0.69**
3	**Osoi, 1989**	**5**	**0.97**
4	Trușești, 2010	**3**	**0.21**
	Tot. individuals	**20**	

Table 31 and Figure 14. Numerical and percentage variation of the presence and absence of Cerambycidae Family and their dominance classes in maize fields, Brăila and Moldova, 1986- 2010.

	Specification	No.	%
1	Presence in localities	4	23.53
2	Absence in sites	13	76.47
1	Subrecedent below 1%	4	23.53
2	Recedent 1.1 - 2%	0	0
3	Subdominant 2.1 -5%	0	0
4	Dominant 5.1 -10%	0	0
5	Eudominant over 10.1%	0	0
	Total	4	23.53

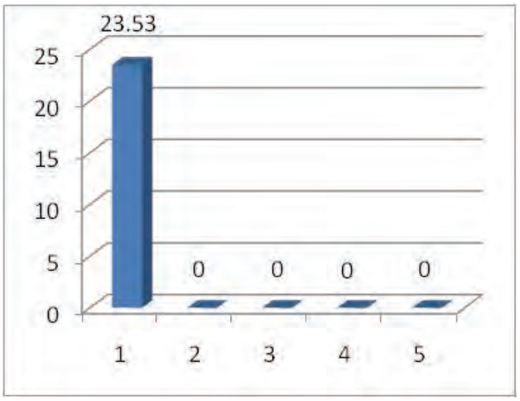

Figure 14.

The following 7 families (31.18 %) (Nitidulidae, Lathridiidae, Melyridae, Catopidae, Ptinidae, Phalacrididae, Meloidae) had between 1 (Meloidae and Phalacrididae) and 11 individuals (Nitidulidae).

DISCUSSIONS

Discussions have to point out some generalizations and interpretations of the results. The fundamental and unique form of existence of life is the individual that has all the general and specific characteristics (from the taxa of kingdom to that of species).

The numerical characteristics of the species are the number of individuals, a result of evolution and interactions with the structures of environment. There are eudominant species with bigger effective than 10.1 % and subrecedent species with effective less than 1 %.

The environment of a species is concretized by three components: ecological, biological and physical factors. The average number of individuals of Coleoptera in maize crops was 1,010 with limits extremely variable, between 1 individual (Phalacrididae, Meloidae) and 15,638 (Carabidae Family).

In the entomological analysed material in 17 maize crops, Walachia (one county) and Moldova (five counties), 12 seasons, from 1978 to 2010, the order Coleoptera is represented by 22 families (Table 2). The presence of the families of Coleoptera in maize crops is very variable. Only Carabidae Family was present in all the crops. The total effective was 15,638 individuals (70.34 %), other 21 families were present between one crop (5.88%) (Catopidae, Ptinidae, Phalacrididae, Meloidae) and 16 crops (94.12%) (Coccinelidae, Staphylinidae).

Due to the unity and interaction of organisms with the environment, the spectrum variations in local conditions in the maize crops, Brăila and Moldova (South, Central, North) determines the number of individuals of Coleoptera, causing the same family to have a subrecedent, recedent or subdominant dominance in some localities or dominant, eudominant position, in other favourable places.

The numerical limits of recedent families were between one crop (5.88%) (Tenebrionidae, Brăila, Terrace, 1984, Brăila county, Osoi, 1986, Iași) and four crops (23.52%), Staphylinidae (Brăila 1980, Brăila County, Pogana, 1989, Vaslui County, Negreşti, 1992, Breazu 2001, Iaşi County).

The limits of subdominant families were between one crop (5.88%), Tenebrionidae, Negru Vodă, 1979; Brăila County; Silphidae, Pogana, 1989; Vaslui County, and five crops (29.41%) (Coccinellidae, Brăila 1979, Brăila County, Brăila 1980, Brăila Terrace, 1984; Brăila, 1984; Salt Lake, 1984.)

Four families (18%) were dominant in one crop each (Tenebrionidae, Breazu 2001, Iași County; Dermestidae, Pogana, 1989, Vaslui County; Staphylinidae, Săbăoani, 1999, Neamț County; Anthicidae, Brăila, 1984, Brăila County).

Five families, Tenebrionidae, Elateridae, Curculionidae, Staphylinidae, Chrysomelidae were eudominant in one maize crop each.

Comparing the overall results from the maize crops, 12 seasons, actually, 1978 - 2010, with those obtained from the wheat crops, 12 seasons, actually, 1977 - 2002, during their vegetation period the wheat crops favour the presence of several individuals from five families (Carabidae, Staphylinidae, Anthicidae, Silphidae, Lathridiidae), the soil moisture in the wheat crops being one of those factors that favours the number of individuals. For example, in Staphylinidae family, mesophilous one, there were collected four times more individuals in wheat crops compared to maize crops. The total number of Staphylinidae was 2,366 individuals in wheat crops, compared to 509 in maize crops.

CONCLUSIONS

The collecting effort of the material, the taxonomic identification of those 22,230 individuals from 40,736 samples collected from 17 maize crops, during a period of 12 seasons, actually (1978-2010) in Brăila County (Wallachia) and Vaslui, Iași, Bacău, Neamț, Botoșani counties (Moldova) give us the opportunity to affirm the following:

The epigenous coleopterans belong to 22 families. In the order of their total abundance, they are: Carabidae, Tenebrionidae, Elateridae, Curculionidae, Dermestidae, Coccinelidae, Staphylinidae, Chrysomelidae, Anthicidae, Scarabaeidae, Histeridae, Silphidae, Chryptophagidae, Cantharidae, Cerambycidae, Nitidulidae, Lathridiidae, Melyridae, Catopidae, Ptinidae, Phalacrididae, Meloidae.

The numerical and percentage presence of the families varied from one locality (5.88%), families (Catopidae, Ptinidae, Phalacrididae and Meloidae) to 17 localities (100 %), only Carabidae family. Carabidae Family is present in all the maize crops as eudominant.

Six families (Tenebrionidae, Elateridae, Curculionidae, Dermestidae, Staphylinidae, Chrysomelidae) are present in crops. Thus, 1. **subrecedent**, one crop (Dermestidae) and five crops (Elateridae, Staphylinidae); 2. **recedent**, one crop (Tenebrionidae) and four crops (Curculionidae, Staphylinidae, Chrysomelidae); 3. **subdominant**, one crop (Tenebrionidae) and four crops (Elateridae); 4. **dominant**, one crop (Tenebrionidae, Dermestidae) and three crops (Curculionidae); 5. **eudominant**, one crop (Elateridae, Curculionidae) and five crops (Tenebrionidae, Chrysomelidae).

Carabidae family is eudominant in each crop of maize, the percentage of individuals varying between 26.41% (Pogana, Vaslui County), 1989 and 94.82% (Cherchejeni, 2000, Botoșani County).

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Received: May 06, 2016
Accepted: May 19, 2016

QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE AVIFAUNA WITHIN THE AREA OF INTERNATIONAL AIRPORT CRAIOVA, ROMANIA

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Abstract. The study renders the results of the ornithological research achieved in 2014-2015 within the perimeter and the proximity of International Airport Craiova (I. A. C.) on a range of 3 km, thus covering all the ecological aspects of the year. The list resulted after the research includes 58 bird species belonging to 13 systematic orders. Forest and shrub species are predominant (37). From the phenological point of view, 25 species are sedentary, 7 species are partially migratory, 22 species are summer guests, 3 species are winter guests and 1 species is a passage species. Within the perimeter of I. A. C., we identified 21 species that flew over the airport at different heights or were stationary in various places. Of the total number of identified species, 29 species may trigger certain risks for the safety of airplanes and, implicitly, for air traffic, either due to their large size and high flight or their gregarious behaviour. Among these, the most frequently met are: *Columba livia domestica, Streptopelia decaocto, Corvus monedula, C. frugilegus, Pica pica, Passer montanus,* followed by *Falco tinnunculus, Phasianus colchicus, Perdix perdix, Columba palumbus, Hirundo rustica, Sturnus vulgaris, Passer domesticus, Emberiza calandra*. Other potentially dangerous species were rarely (*Ardea cinerea, Anas platyrhynchos, Buteo buteo, Larus ridibundus,* etc.) or very rarely/accidentally observed (*Egretta garzetta, Ciconia ciconia, Vanellus vanellus, Larus cachinnans, Turdus pilaris, Fringilla* sp., *Carduelis* sp.). The most numerous number of individuals was registered for the species *Corvus* sp., *Sturnus vulgaris, Columba* sp., *Passer* sp. Most of the birds present maximum activity and dynamism during the first part of the day (7 a.m. to 12 p.m.) and towards the evening (2 to 5 p.m.), in the interval February – March and September – October (RIDICHE, 2016).

Keywords: ornithological analysis, International Airport Craiova (I. A. C.).

Rezumat. Analiza calitativă și cantitativă a avifaunei din zona Aeroportului Internaţional Craiova, România. Studiul prezintă rezultatele cercetărilor ornitologice realizate pe parcursul anilor 2014-2015 în perimetrul şi împrejurimile Aeroportului Internaţional din Craiova pe o rază de 3 km, în toate aspectele ecologice ale anului. Lista rezultată în urma inventarierii cuprinde 58 de specii de păsări, încadrate la 13 ordine sistematice. Preponderente sunt speciile de pădure şi tufărişuri (37). Din punct de vedere fenologic, 25 de specii sunt sedentare, 7 specii sunt parţial migratoare 22 de specii sunt oaspeţi de vară, 3 specii sunt oaspeţi de iarnă și 1 specie este de pasaj. Din totalul speciilor consemnate, 29 de specii pot genera anumite riscuri pentru siguranţa aeronavelor şi implicit pentru traficul aerian, fie datorită taliei mari şi zborului la înălţime, fie datorită comportamenelor gregare pe care le manifestă. Dintre acestea, cele mai frecvente sunt: *Columba livia domestica, Streptopelia decaocto, Corvus monedula, C. frugilegus, Pica pica, Passer montanus* urmate de *Falco tinnunculus, Phasianus colchicus, Perdix perdix, Columba palumbus, Hirundo rustica, Sturnus vulgaris, Passer domesticus, Emberiza calandra*. Alte păsări generatoare de potențiale pericole au fost rar semnalate (*Ardea cinerea, Anas platyrhynchos, Buteo buteo, Larus ridibundus,* etc.) sau foarte rar/accidental (*Egretta garzetta, Ciconia ciconia, Vanellus vanellus, Larus cachinnans, Turdus pilaris, Fringilla* sp., *Carduelis* sp.). Efectivele cele mai mari le-am înregistrat la speciile: *Corvus* sp., *Sturnus vulgaris, Columba* sp., *Passer* sp. Marea majoritate a păsărilor au un maxim de activitate şi dinamism în prima parte a zilei (orele 7.00-12.00) şi spre seară (14.00-17.00), în lunile februarie-martie şi septembrie-octombrie (RIDICHE, 2016).

Cuvinte cheie: analiza ornitologică, Aeroportul Internațional Craiova.

INTRODUCTION

Craiova Municipality represents the largest and the most important urban settlement in southwestern Romania; it is located on the left bank of the Jiu River, at a distance of 68 km from the Danube River and 227 km from the capital of the country, Bucharest (http://ro.wikipedia.org/wiki/Craiova#cite).

International Airport Craiova is located east of Craiova, about 7 km from the city centre; from the geomorphological point of view, it is located in Oltenia Plain (subunit of the Romanian Plain) (BADEA et al., 2011). The climate of the city and, implicitly, of the airport, is temperate continental with slight sub-Mediterranean influences (CETĂŢEANU et al., 1981).

The area adjacent to I. A. C. (on a range of 3 km) is part of the Teslui hydrographical basin in the north-east and east and of the Jiu basin in the west and south-west.

The ecosystems present within the perimeter of the I. A. C. and its adjacent area are generally subject to anthropization as they are developed in open fields with grass vegetation (meadows) or cultivated vegetation (agroecosystem) or on surfaces covered by trees and shrubs belonging to spontaneous or ornamental flora. The meadows are xerophilous and/or xeromezophilous with shrubs of *Rosa canina, Prunus spinoasa, Rubus caesius*, etc. and they cover the largest part of I. A. C.; at the same time, they also predominate within the neighbouring area, together with cultivated plots. The surfaces covered by tree vegetation are located in the immediate proximity of the airport track and the recreation area Hanul Doctorului (The Doctor's Inn). The vegetal associations present here (shrubs: *Rosa canina, Crataegus monogyna, Ligustrum vulgare, Thuja orientalis*, etc.; trees: *Robinia pseudoacacia, Populus* sp., *Malus domestica, Fraxinus excelsior, Pinus* sp.; unmown grass) developed and extended and, thus, attract a quite varied specific avifauna. Aquatic ecosystems are insignificant in terms of extension and stability and they have only a temporary (streams, swampy or liable to floods fields) or permanent character (Gherceşti Pool supplied by the springs located north-west of I. A. C.).

Birds are an important component of the ecosystems developed within I. A. C. and their study became a necessity as there may occur collisions with aircraft that land or take off, thus, endangering the safety of air traffic. The factors that attract birds in the area of the airport, the distribution of bird species within the biotopes located on a range of 3 km, as well as the risk degree birds may represent for air traffic are some of the goals of the studies previously achieved by RIDICHE et al., 2015; RIDICHE & MUNTEANU, 2015.

MATERIAL AND METHODS

The present study is the result of the ornithological research achieved in 2014 and the first half of 2015 within the perimeter and the proximity of International Airport Craiova (I. A. C.) on a range of 3 km, thus covering all the ecological aspects of the year. As part of our field activities (identification and monitoring of bird species) we made trips to all biotopes (meadows and agricultural fields, forests or tree clumps, pools, settlements) located within the studied area. Observations were made from fixed points or in motion, with naked eye or by means of binoculars (Zeiss Jena 10x50, Bushnell 12x40), using different acoustic devices (CD-player, recorder) to record and play the singing of the males of small songbirds; for capturing some aspects of nature and certain bird species, we used a Sony 15 x camera. The most frequent observations were made in the months with the greatest dynamism of birds, respectively February-March and September-October, which are characterized by ample seasonal movements or movements determined by food search.

The scientific data processing (systematic, ecological, phenological category) was based on the specialized literature (SZABÓ-SZELEY & BACZÓ, 2006; CĂTUNEANU et al., 1978; MUNTEANU, 2012). The frequency of bird species was calculated according to the formula $F\% = p \times 100/P$, where p = number of observations when the bird species was noticed and P = total number of observations (GOMOIU & SKOLKA, 2001).

The data referring to the flying speeds are mostly based on the specialized literature (CIOCHIA, 1984), while those regarding the size/length and weight of the species were taken from the register of the bird collection.

RESULTS AND DISCUSSIONS

According to the investigations made within the biotopes located within the perimeter of I. A. C. and its neighbouring area (0-3 km), we identified 58 species, belonging to 13 systematic orders. 21 species were observed in various points of I. A. C., either stationary for rest and food or flying above the airport at different heights. For all the species recorded during the study we noted the typical biotope (biotope suitable for breeding), the phenological status and the potential risk for air traffic (Table 1).

Table 1. Systematic list of bird species observed within the perimeter of I. A. C. and its proximity (0-3 km).

No.	Species	Records		Typical biotope	Phenological status	Risk for air traffic
		Perimeter of I. A. C.	Proximity of I. A. C.			
ORD. CICONIIFORMES						
1	*Ardea cinerea*	√	√	We	SV, P	●
2	*Egretta garzetta*	-	√	We	SV, P	●
3	*Ciconia ciconia*	-	√	We	SV, P	●
ORD. ANSERIFORMES-						
4	*Anas platyrhynchos*	-	√	We	PM, WV	●
ORD. ACCIPITRIFORMES						
5	*Accipiter nisus*	-	√	Wo	WV, P	-
6	*Buteo buteo*	√	√	Wo	R, P, WV	●
7	*Falco tinnunculus*	√	√	Wo	PM, P, WV	●
ORD. GALLIFORMES						
8	*Perdix perdix*	√	√	AG	R	●
9	*Coturnix coturnix*	-	√	AG	SV, P, RI	-
10	*Phasianus colchicus*	√	√	Wo	R	●
ORD. GRUIFORMES						
11	*Fulica atra*	-	√	We	PM, P, WV	-
ORD. CHARADRIIFORMES						
12	*Vanellus vanellus*	-	√	We	SV, P	●
13	*Chidonias hybrida*	-	√	We	SV, P	-
14	*Larus ridibundus*	-	√	We	PM, P, WV	●
15	*Larus cachinnans*	-	√	We	R	●
ORD. COLUMBIFORMES						
16	*Columba livia domestica*	√	√	Hs	R	●
17	*Columba palumbus*	-	√	Wo	PM	●
18	*Streptopelia decaocto*	√	√	Hs	R	●
ORD. CUCULIFORMES						
19	*Cuculus canorus*	-	√	Wo	SV, P	-

ORD. STRIGIFORMES						
20	*Athene noctua*	-	√	Wo	R	-
ORD. CORACIIFORMES						
21	*Coracias garrulus*	-	√	Wo	SV, P	-
22	*Upupa epops*	√	√	Wo	SV, P	-
ORD. PICIFORMES						
23	*Dendrocopos major*	-	√	Wo	R	-
24	*Dendrocopos syriacus*	-	√	Wo	R	-
ORD. PASSERIFORMES						
25	*Galerida cristata*	-	√	AG	R	-
26	*Alauda arvensis*	-	√	AG	PM	-
27	*Hirundo rustica*	√	√	Hs	SV	●
28	*Delichon urbicum*	√	√	Hs	SV	●
29	*Anthus campestris*	√	√	AG	SV	-
30	*Motacilla flava*	-	√	AG	SV	-
31	*Motacilla alba*	-	√	Eur.	SV	-
32	*Troglodytes troglodytes*	-	√	Wo	R / PM, WV	-
33	*Luscinia luscinia*	-	√	Wo	SV	-
34	*Turdus merula*	-	√	Wo	PM	-
35	*Turdus pilaris*	-	√	Wo	WV	●
36	*Hippolais icterina*	-	√	Wo	SV	-
37	*Sylvia communis*	-	√	Wo	SV	-
38	*Phylloscopus collybita*	-	√	Wo	SV	-
39	*Phylloscopus trochilus*	-	√	Wo	P, SV	-
40	*Parus caeruleus*	-	√	Wo	R	-
41	*Parus major*	√	√	Wo	R	-
42	*Oriolus oriolus*	-	√	Wo	SV	-
43	*Lanius collurio*	√	√	Wo	SV	-
44	*Lanius minor*	√	√	Wo	SV	-
45	*Garrulus glandarius*	-	√	Wo	R	-
46	*Pica pica*	√	√	Wo	R	●
47	*Corvus monedula*	√	√	Wo	R	●
48	*Corvus frugilegus*	√	√	Wo	R	●
49	*Corvus cornix*	-	√	Wo	R	●
50	*Sturnus vulgaris*	√	√	Wo	PM	●
51	*Passer domesticus*	√	√	Wo	R	●
52	*Passer montanus*	√	√	Wo	R	●
54	*Fringilla montifringilla*	-	√	Wo	WV	●
55	*Fringilla coelebs*	-	√	Wo	R, SV	●
56	*Carduelis chloris*	-	√	Wo	R	●
57	*Carduelis carduelis*	-	√	Wo	R	●
58	*Emberiza calandra*	√	√	AG	R	●

Legend:
Records: √ – stationary and/or in transit (in flight).
Phenological type: R – resident; PM – partially migratory; SV – summer visitors; WV – winter visitors; P – passage visitors.
Typical biotope: AG – agroecosystems and grasslands; Wo – woodlands (forests and shrubs); We – wetlands; Hs – human settlements.
Risk for air traffic: ● – birds that may trigger risks.

According to the typical biotope, forest and shrub species (37) predominate both within the airport and its adjacent area, on a range of 3 km. Aquatic species (9) were mainly observed in the proximity of the airport. There was only one exception, the grey heron (*Ardea cinerea*) that was observed near the track, in the north-eastern part, attracted here by the marshes resulted from rains and grassy meadows that favour the presence of food sources (batrachians, small mammals, etc.). The species characteristic to meadows and agroecosystems (7), as well as the anthropophilic species (4) are frequent both within the perimeter of the airport and its adjacent area (0-3 km). One species is eurytopic (*Motacilla alba*) and it was noticed just outside the perimeter of I. A. C.

According to the phenological type, there was registered the following situation: 25 species are sedentary, 8 species are partially migratory, 21 species are summer visitors, 3 species are winter visitors and 1 species is only in transit.

Many of the identified bird species present a high degree of adaptability to the environmental conditions greatly influenced by human activity (phonic pollution, vehicles, people activity, etc.). By analysing the temporary or longer relationship birds have with the frequented habitat, we found out that there are several natural and anthropogenic factors (e.g. high tree vegetation, shrubs, tall grasses, crops, bodies of water, different constructions, waste) that attract them in the area and facilitate their living within I. A. C. (RIDICHE et al., 2015).

Among the 58 identified species, 29 species may trigger certain risks for the safety of airplanes because of possible collisions with airplanes. This is the case of the species that fly actively during the day, in numerous groups, near the area airplanes take off or land (*Columba livia domestica*, *Streptopelia decaocto*, *Corvus monedula*, *C. frugilegus*, *Passer* sp.), the species that fly together in flocks during migration periods (e.g. *Sturnus vulgaris*), as well as the large size species that reach great height taking advantage of the air currents above I. A. C. (e.g. *Buteo buteo*).

Birds that may trigger risks for air traffic do not have the same degree of presence within I. A. C., some species having a constant relationship with this specific biotope, while other species are just in passage or occasionally stationary within the perimeter of I. A. C. and its proximity. Taking into account the values resulting from the calculation of frequency (F) of each species, we can talk about four categories of birds generating potential risks, numerically distributed as follows (Table 2): 6 euconstant species / very common (frequency between 75.1% and 100% of the total number of observations); 8 constant species (frequency between 50.1% and 75% of total observations); 6 rare species (frequency between 25.1% and 50% of all observations); 9 accidental species (frequency between 1% and 25% of the total number of observations). Their percentage distribution is rendered in Fig. 1.

Table 2. Categories of bird species that may trigger potential risks compared to the frequency of their observation in the area of Craiova Airport (0-3 km).

Euconstant species (very frequent)	Constant species	Rare species	Accidental species
Columba livia domestica	*Falco tinnunculus*	*Ardea cinerea*	*Egretta garzetta*
Streptopelia decaocto	*Phasianus colchicus*	*Anas platyrhynchos*	*Ciconia ciconia*
Pica pica	*Perdix perdix*	*Buteo buteo*	*Vanellus vanellus*
Corvus monedula	*Columba palumbus*	*Larus ridibundus*	*Larus cachinnans*
Corvus frugilegus	*Hirundo rustica*	*Delichon urbicum*	*Turdus pilaris*
Passer montanus	*Sturnus vulgaris*	*Corvus cornix*	*Fringilla montifringilla*
	Passer domesticus		*Fringilla coelebs*
	Emberiza calandra		*Carduelis chloris*
			Carduelis carduelis

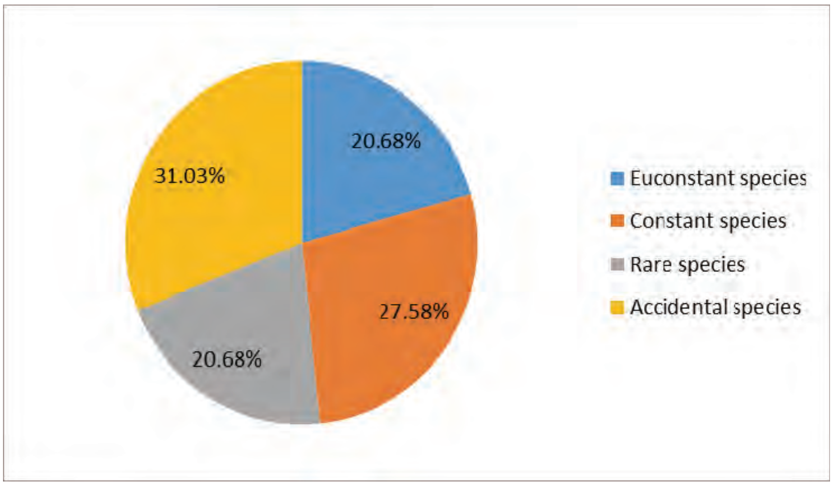


Figure 1. Percentage distribution of frequency groups of the bird species that trigger potential risks in the area of I. A. C.

The intense frequency of different species of birds within the perimeter of the airport or in its immediate proximity increases the risk of collisions with aircraft. The risks of accidents and their severity also depend on other parameters such as abundance, bird size, their flying speeds, their behaviour during the flight on different distances, etc. In Table 3, there are rendered the weight, size and flying speeds of the species generating potential risks to aircraft / air traffic.

The species that cause the most serious accidents by colliding with airplanes are the birds flying in large flocks or solitary at great height (*Anas platyrhynchos*, *Ciconia ciconia*, *Buteo buteo*, etc.), followed by crows and western jackdaw (*Corvus* sp.), black-headed gull (*Larus ridibundus)*, pigeons (especially *Columba livia domestica*) and, finally, by flocks of small birds (the most frequent *Sturnus vulgaris*).

Table 3. Weight, size and flying speeds of the bird species susceptible to collisions with aircraft.

No.	SPECIES	Weight (g)	Size (cm)	Flying speed (Km/h)	Risk for traffic
			Eucontant species		
1.	*Columba livia domestica*	250-300	32-35	60-75	X, XX
2.	*Streptopelia decaocto*	150-250	31-33	62-72	X, XX
3.	*Pica pica*	200-230	45	45-55	X, XX
4.	*Corvus monedula*	200-280	33	40-60	X, XX
5.	*Corvus frugilegus*	400-600	46	46-67	XXX
6.	*Passer montanus*	18-29	14	40-60	XX
			Constant species		
7.	*Falco tinnunculus*	180-210	31-37	38-42	X
8.	*Phasianus colchicus*	1000-1200	65-85	40	X
9.	*Perdix perdix*	430-500	30	40	XX
10.	*Columba palumbus*	370-600	40-43	86	X, XX
11.	*Hirundo rustica*	15-20	17-19	50-77	X X

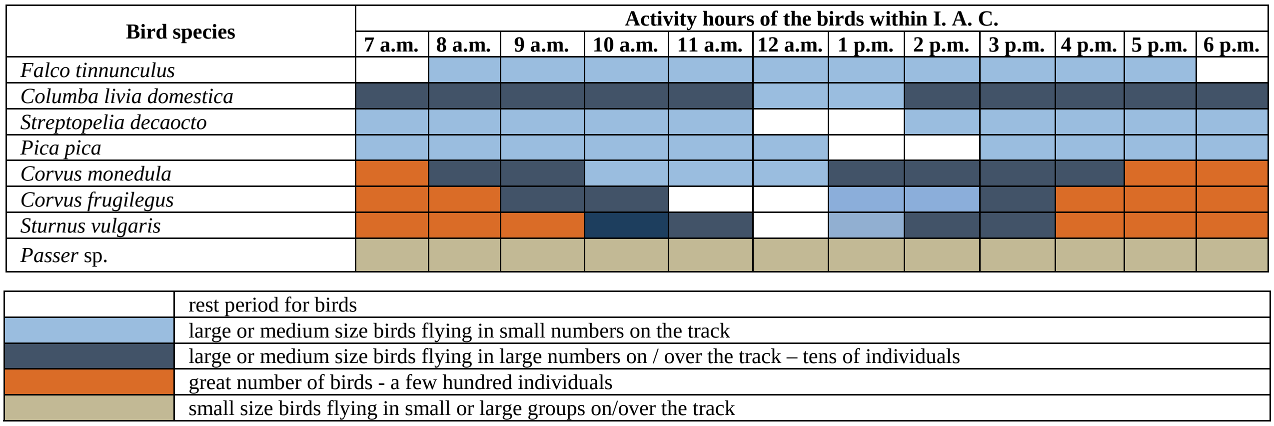
12.	*Sturnus vulgaris*	50-100	21	40-82	XX
13.	*Passer domesticus*	23-40	14,5-15	38-56	XX
14.	*Emberiza calandra*	40-65	15-1	38-55	XX
Rare species					
15.	*Ardea cinerea*	1000-1900	84 - 102	38-40	X, XXX
16.	*Anas platyrhynchos*	760-1400	50-60	74-96	XXX
17.	*Buteo buteo*	750-1400	48-58	42-47	X
18.	*Larus ridibundus*	200-400	35-38	60-80	XXX
19.	*Delichon urbicum*	12-16	12-14	47-50	XX
20.	*Corvus cornix*	445-520	41-46	30-50	X
Accidental species					
21.	*Egretta garzetta*	350-600	55 - 65	35-40	X, XXX
22.	*Ciconia ciconia*	3500-4000	110	50-60	X, XXX
23.	*Vanellus vanellus*	200-320	28-31	60	XX
24.	*Larus cachinnans*	600-1300	56-78	60-80	XXX
25.	*Turdus pilaris*	70-140	25	28-50	XX
26.	*Fringilla montifringilla*	20-30	15	35-55	XX
27.	*Fringilla coelebs*	20-40	15	33-56	XX
28.	*Carduelis chloris*	20-30	14,5-15	35-50	XX
29.	*Carduelis carduelis*	15-20	14	40-45	XX

Legend:
Risk for traffic**:** X – large size and/or medium size birds that fly solitary or in small groups (1 – 10 individuals); XX – small or medium size birds that fly in flocks (tens / hundreds of individuals); XXX – medium or large size birds that fly in flocks.

The flying speeds rendered in Table 3 are relative as they were evaluated on short distances thus, certain errors being possible (CIOCHIA, 1984). In certain situations (alerts of danger or prey attack), the flying speed may increase. In case of frontal collision, the collision between bird / birds and aircraft may have a force that increases with speed increase of both parts involved.

The most abundant and active species identified within I. A. C. belong to the euconstant and constant categories. They have a daily diurnal program, most of the time carefully looking for food within the specific biotopes, usually in mixed groups (Table 4).

Table 4. Bird species frequent within the airport and their activity hours during a spring or autumn day.



The data rendered in Table 4 are the result of the observations achieved between the 28th of September 2014 and the 28th of October, between 7 a.m. and 6 p.m., in the days when air temperature at noon was between 15 and 20ºC and there were not rainfalls or strong winds.

The common starling (*Sturnus vulgaris*), the rook (*Corvus frugilegus*) and the western jackdaw (*C. monedula*) are the most numerous and dynamic species from the perimeter of I. A. C. During spring and autumn days they feed together in the fields around the airport, mainly those located in the northern part (more than 500 individuals). Often, they were seen on the meadows near the track, also in mixed populations (more than 100-200 individuals). During the seasonal migrations (spring and autumn), before dusk, there can be observed numerous flocks of starlings (hundreds / thousands of individuals) transiting the track and the surroundings of I. A. C. In summer, the area is dominated by corvides, which placed their colonies in the trees located in the western vicinity of I. A. C. or along the national-European road located at the southern edge of I. A. C., and species of *Pica pica, Passer* sp., *Columba livia domestica, Streptopelia decaocto* that fly above the airport area countless times, in more or less numerous groups, so that they seem to be present at all times.

The peak of activity in the vast majority of birds occurs early in the morning (7 a.m.-10 a.m.), gradually decreasing at noon, when birds rest; then, they resume their activity gradually, reaching another peak between 4 p.m. and 6 p.m.

Falco tinnunculus begins hunting later, after 8 a.m., and continues until 4-5 p.m., taking advantage of higher temperatures and currents of warm air for its flight.

CONCLUSIONS

During the research achieved in 2014-2015 upon the avifauna within the perimeter of International Airport Craiova and its adjacent area on a range of 3 km, we identified 58 bird species belonging to 13 systematic orders. From the point of view of the typical biotope, forest and shrub species (37) predominate both within the airport and its adjacent area, on a range of 3 km; aquatic species (9) were mainly observed in the proximity of the airport (an exception represented by the species *Ardea cinerea* that was observed stationing near the track); the species characteristic to meadows and agroecosystems (7), as well as the anthropophilic species (4) are frequent both within the perimeter of the airport and its adjacent area (0-3 km). One species is eurytopic (*Motacilla alba*) and it was noticed just outside the perimeter of I. A. C.

According to the phenological type of the bird species, it results the following situation: 25 species are sedentary, 8 species are partially migratory, 21 species are summer visitors, 3 species are winter visitors and 1 species is only in transit.

Half of the identified species (namely 29) may trigger risks for air traffic either due to their large size and high flight or their gregarious behaviour.

The birds that may represent a risk for the safety of air traffic are distributed according to the frequency (F) values calculated for each species as follows: 9 accidental species; 6 rare species; 8 constant species; 6 euconstant species / very frequent.

The most abundant and active bird species are those belonging to euconstant category (*Columba livia domestica, Streptopelia decaocto, Pica pica, Corvus monedula, C. frugilegus, Passer montanus*) and constant category (*Falco tinnunculus, Phasianus colchicus, Perdix perdix, Columba palumbus, Hirundo rustica, Sturnus vulgaris, Passer domesticus).* Other potentially dangerous species were noticed more rarely (*Ardea cinerea, Anas platyrhynchos, Buteo buteo, Larus ridibundus, Delichon urbicum, Corvus cornix*) or very rarely/accidentally (*Egretta garzetta, Ciconia ciconia, Vanellus vanellus, Larus cachinnans, Turdus pilaris, Fringilla* sp. *Carduelis* sp.).

The greatest dynamism of birds is registered in February-March and September-October when migration takes place or they are in search for food and the peak activity in most species occur early in the morning (7 to 10 a.m.) and between 4 and 6 p.m.

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Received: March 31, 2016
Accepted: June 22, 2016

A YEAR OF ORNITHOLOGICAL OBSERVATIONS ON VÂLCELE, BUDEASA, BASCOV, PITEŞTI, AND GOLEŞTI RESERVOIRS FROM ROSPA0062 LACURILE DE ACUMULARE DE PE ARGEŞ

MESTECĂNEANU Adrian, GAVA Radu

Abstract. In this paper, there are rendered the results of the research performed during February 2013 – January 2014 on the avifauna from Vâlcele, Budeasa, Bascov, Piteşti, and Goleşti reservoirs from the Argeş River, included in ROSPA0062 Lacurile de acumulare de pe Argeş. The 129 observed species belong to 16 orders, Passeriformes being the richest (with 56 species). Goleşti reservoir sheltered the biggest number of species and the biggest number of individuals, a reason for which it creates the general state of the avifauna development from the researched area. The vernal season is highlighted due to the biggest number of species and the hiemal season because of the biggest number of individuals. Broadly, the number of species and the number of individuals increased from upstream to downstream. The biggest similarity, by the Jaccard index, was established between the avicoenoses from Piteşti and Budeasa reservoirs and by the Bray–Curtis index, between the avicoenoses from Vâlcele and Budeasa reservoirs. The diversity values for every reservoir and for every ecological season are medium and there is a little equitability between the strengths of the species, regardless the ecological season. The power regression applied on the specific richness suggests a low rate of the species strength accumulation. 18 species (13.95% of all) were euconstant and 4 species – 3.10%, *Anas platyrhynchos* Linnaeus, 1758, *Aythya ferina* (Linnaeus, 1758), *Fulica atra* Linnaeus, 1758 and *Larus ridibundus* Linnaeus, 1766 – were eudominant as emphasized by both dominancy and Dzuba ecological index of significance. At general level, Anseriformes and Charadriiformes orders were overdominant; Anseriformes order was overdominant every month, except May, Charadriiformes order only in January, July, August, September and October.

Keywords: ROSPA0062, birds, reservoirs, protection.

Rezumat. Un an de observaţii ornitologice pe lacurile de acumulare Vâlcele, Budeasa, Bascov, Piteşti şi Goleşti din ROSPA0062 Lacurile de acumulare de pe Argeş. În această lucrare sunt prezentate rezultatele cercetărilor efectuate, în perioada februarie 2013 – ianuarie 2014, asupra avifaunei lacurilor de acumulare Vâlcele, Budeasa, Bascov, Piteşti şi Goleşti de pe râul Argeş, cuprinse în ROSPA0062 Lacurile de acumulare de pe Argeş. Cele 129 de specii observate aparţin la 16 ordine, dintre care Passeriformes a fost cel mai bine reprezentat (cu 56 de specii). Lacul de acumulare Goleşti se evidenţiază prin cel mai mare număr de specii şi cel mai mare număr de exemplare, acesta imprimând tabloul general al evoluţiei avifaunei din zona studiată. Prin cel mai mare număr de specii se remarcă sezonul vernal, iar prin cel mai mare număr de exemplare, sezonul hiemal. S-a observat că, în general, numărul de specii şi numărul de exemplare cresc din amonte spre aval. Cea mai mare similaritate, după indicele Jaccard, a fost stabilită între avicenozele lacurilor Piteşti şi Budeasa, iar după indicele Bray–Curtis, între avicenozele lacurilor Vâlcele şi Budeasa. Valorile diversităţii pentru fiecare lac în parte şi pentru fiecare sezon ecologic sunt medii şi există o mare inechitabilitate între efectivele speciilor, indiferent de sezon. Regresia putere aplicată asupra bogăţiei specifice sugerează o rată scăzută de creştere a efectivelor speciilor. 18 specii (13,95% din total) au fost euconstante, iar 4 specii – 3,10% din total, *Anas platyrhynchos* Linnaeus, 1758, *Aythya ferina* (Linnaeus, 1758), *Fulica atra* Linnaeus, 1758 şi *Larus ridibundus* Linnaeus, 1766 – au fost eudominante, atât din punctul de vedere al dominanţei, cât şi al indicelui de semnificaţie ecologică Dzuba. La nivel general, ordinele Anseriformes şi Charadriiformes au fost supradominante. Ordinul Anseriformes a fost supradominant în fiecare lună, cu excepţia lui mai, iar ordinul Charadriiformes numai în ianuarie, iulie, august, septembrie şi decembrie.

Cuvinte cheie: ROSPA0062, păsări, lacuri de acumulare, protecţie.

INTRODUCTION

The study of the fauna from reservoirs is very important because it offers an interesting image of the anthropogenic pressure on the natural environment. Regarding the avifauna, on the one hand, these dam basins destroy the former habitats that were on the respective segments of the rivers and, on the other hand, they create other habitats. In this manner, these new spaces change the qualitative and quantitative characteristics of the bird coenoses and, if they are correctly administrated, they can finally have a positive effect on the birdfauna.

Such research studies were developed on the reservoirs from the upper and middle course of the Argeş River immediately after the ending of their construction (MĂTIEŞ, 1969; MUNTEANU & MĂTIEŞ, 1983) and until now (CONETE et al., 2008, 2011; CONETE, 2011; GAVA, 1997; GAVA et al., 2004a,b, 2007, 2011; MESTECĂNEANU et al., 2004, 2010, 2013, etc.).

MATERIAL AND METHOD

The avifauna of Vâlcele, Budeasa, Bascov, Piteşti, and Goleşti reservoirs was the subject of this work. These dam basins appertain to a series of reservoirs that were built on the course of the Argeş River beginning with 1965 (Fig. 1). Recently, they have been included in the protected area ROSPA0062 Lacurile de acumulare de pe Argeş, component of the Natura 2000 network. From upstream to downstream, their surface is: 640 ha – Vâlcele, 643 ha – Budeasa, 140 ha – Bascov, 150 ha – Piteşti and 680 ha – Goleşti (cf. http://www.baraje.ro). They are situated in the south of the Făgăraş and Iezer – Păpuşa Mountains. Argeş Platform is in the North, Cotmeana Platform is in the West, Cândeşti Platform is in the East, and Piteşti High Plain is in the South.

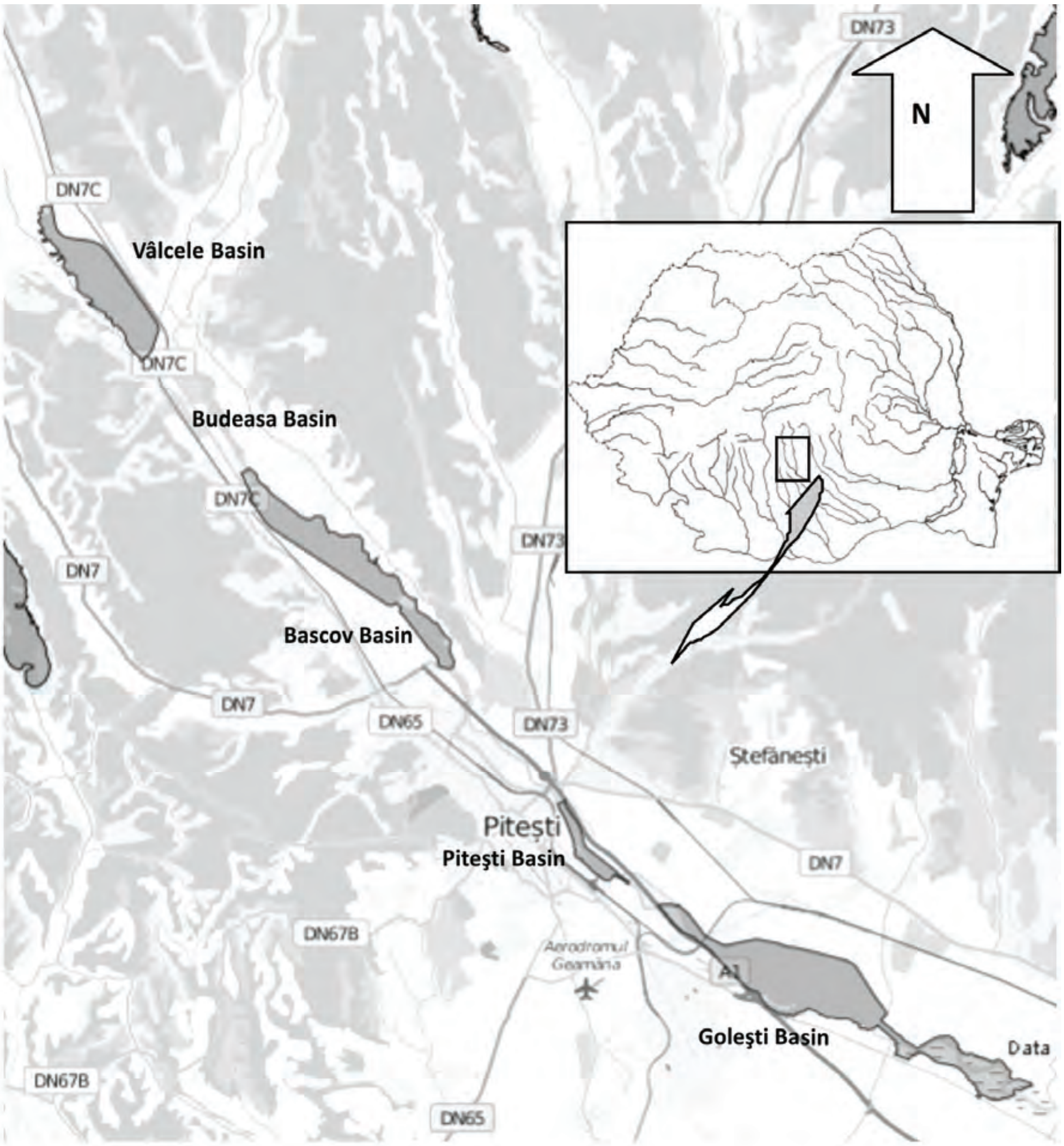

Figure 1. The map of the area.
(by http://biodiversitate.mmediu.ro, modified).

The vegetation is typical of wetland areas, chiefly with reed beds, bulrush, alder, and willow. Depending on the process of silting, it occupies variable surfaces and generally, it is disposed upstream and in the median parts of each reservoir, along the banks. The terrain from the vicinity has different roles. The hills are covered with orchards, broad leaf forests (beech, hornbeam, diverse species of oak, etc.), sparse artificial coniferous forests and grasslands. The meadows are cultivated with cereals, fodder, green goods, etc. The settlements are placed mainly at the foothills or in the meadows and, generally, close to the reservoirs.

The continental climate with hilly characteristics is specific to the area. The average annual temperature of the water is 9^0C at Piteşti, few degrees colder upstream and about 1^0C warmer downstream. In winters, when temperature decreases for a longer period of time below 0 ^{0}C, mainly in January, a bridge of ice is formed (BARCO & NEDELCU, 1974).

For the bird surveillance, it was used the itinerary method, combined to one of the fixed point of observations (where the field conditions were inadequate). Between 10 and 20 of each month, from the period February 2013 – January 2014, one day field trip was performed at all reservoirs. The same track (the most favourable for the observation of water birds from the banks) was traversed every time. Binoculars (10x50), a spotting scope (14–45x50) and a photo device (42x optical zoom) were used.

The scientific nomenclature and classification of the birds are compatible with the Hamlin Guide (BRUUN et al., 1999) and the ecological indices are the ones used in the scientific literature (GACHE, 2002; GOMOIU & SKOLKA, 2001).

RESULTS AND DISCUSSIONS

Between February 2013 and January 2014, 129 bird species and 85,318 individuals were observed on Vâlcele, Budeasa, Bascov, Piteşti, and Goleşti reservoirs (Table 1). They belong to 16 orders: Gaviiformes (1 species), Podicipediformes (4 species), Pelecaniformes (3 species), Ciconiiformes (8 species), Anseriformes (15 species), Falconiformes (5 species), Galliformes (2 species), Gruiformes (3 species), Charadriiformes (20 species), Columbiformes (3 species), Cuculiformes (1 species), Strigiformes (1 species), Apodiformes (1 species), Coraciiformes (3 species), Piciformes (3 species) and Passeriformes, the best represented (56 species). The number of species varied between 36 (in December) and 66 (in August) but also in April it was registered a big number (61), and, for many Charadriiformes and other wetland birds, this corresponds to the period of autumn, respectively spring migration. The number of individuals was the highest in February (19,017 individuals) and November (11,568 individuals), largely reflecting the migratory dynamics of the birds from the Anseriformes and Charadriiformes orders, and the smallest from March to July, with the minimum in May (808 individuals). These suggest a relative small number of breeding birds in the area. Analysing the evolution of these parameters in relation with the average temperature of the air recorded at Pitești Weather Station every month (cf. rp5.ru) we notice that there is a better correlation between the number of the species and the temperature of the air (0.68, positive and good correlation) than between the strengths of species and the temperature of the air (–0.46, negative and acceptable correlation), fact that was evidenced for the hiemal season (MESTECĂNEANU & GAVA, 2015), too. Also, we observe that, generally, while the number of species increases as the air temperature increases and inversely, the number of species decreases as the air temperature decreases, the strength of species increases as the air temperature decreases and inversely, the strength of species decreases as the air temperature increases.

As against the status of the birds of all the dam basins from the upper and middle course of the Argeş River, registered in the second half of the last century, when there were over 10,000 individuals in the optimal season, we observe now a better situation (with almost 20,000 individuals in February, only on the dam basins between Vâlcele and Goleşti) that indicates that the reservoirs are into a stage of advanced stability of the avifauna (which means a constant periodicity of the species and of their strengths), condition determined by the phenomena of eutrophication of water, initially oligotrophic (MUNTEANU & MĂTIEȘ, 1983).

Table 1. The occurrence of the birds along the year and some ecological indexes.

No.	Species	January	February	March	April	May	June	July	August	September	October	November	December	Absolute abundance	Class of constancy	Class of dominancy	Class of Dzuba index of ecological significance
	I. Order **Gaviiformes**																
1	*Gavia arctica* (Linnaeus, 1758)*												+	3	C1	D1	W1
	II. Order **Podicipediformes**																
2	*Podiceps cristatus* (Linnaeus, 1758)*	+	+	+	+	+	+	+	+	+	+	+	+	1352	C4	D2	W3
3	*Podiceps grisegena* Boddaert, 1783								+					2	C1	D1	W1
4	*Podiceps nigricollis* Brehm, 1831*				+			+	+					14	C1	D1	W1
5	*Tachybaptus ruficollis* (Pallas, 1764)*	+	+	+				+	+	+	+	+	+	185	C3	D1	W2
	III. Order **Pelecaniformes**																
6	*Phalacrocorax carbo* (Linnaeus, 1758)*	+	+	+	+	+	+	+	+	+	+	+	+	1163	C4	D2	W3
7	*Phalacrocorax pygmeus* (Pallas, 1773)*	+	+	+								+	+	109	C2	D1	W1
8	*Pelecanus crispus* Bruch, 1832*						+		+					13	C1	D1	W1
	IV. Order **Ciconiiformes**																
9	*Ixobrychus minutus* (Linnaeus, 1766)*					+		+	+					4	C1	D1	W1
10	*Egretta garzetta* (Linnaeus, 1766)*				+	+	+	+	+					138	C2	D1	W1
11	*Egretta alba* (Linnaeus, 1758)*	+	+	+	+					+		+	+	60	C3	D1	W1
12	*Ardeola ralloides* (Scopoli, 1769)*					+								1	C1	D1	W1
13	*Ardea cinerea* Linnaeus, 1758*	+	+	+	+	+	+	+	+	+	+	+	+	148	C4	D1	W2
14	*Ardea purpurea* (Linnaeus, 1766)*			+										1	C1	D1	W1
15	*Nycticorax nycticorax* (Linnaeus, 1758)*					+	+	+	+					22	C2	D1	W1
16	*Ciconia ciconia* (Linnaeus, 1758)*					+	+	+						14	C1	D1	W1

	V. Order **Anseriformes**																
17	*Cygnus olor* (Gmelin, 1789)*	+	+	+	+	+	+	+	+	+	+	+	+	1202	C4	D2	W3
18	*Cygnus cygnus* (Linnaeus, 1758)*	+	+											21	C1	D1	W1
19	*Anser albifrons* (Scopoli, 1769)*		+										+	660	C1	D1	W2
20	*Anas platyrhynchos* Linnaeus, 1758*	+	+	+	+	+	+	+	+	+	+	+	+	22599	C4	D5	W5
21	*Anas strepera* Linnaeus, 1758*	+	+											22	C1	D1	W1
22	*Anas penelope* Linnaeus, 1758*	+	+	+	+							+	+	208	C2	D1	W2
23	*Anas querquedula* Linnaeus, 1758*			+	+				+	+				291	C2	D1	W2
24	*Anas crecca* Linnaeus, 1758*	+	+	+	+			+	+	+	+	+	+	3639	C4	D3	W3
25	*Anas clypeata* Linnaeus, 1758*	+			+		+		+	+	+			55	C2	D1	W1
26	*Tadorna tadorna* (Linnaeus, 1758)*	+	+						+			+		52	C2	D1	W1
27	*Aythya fuligula* (Linnaeus, 1758)*	+	+	+	+		+	+	+	+	+	+	+	5942	C4	D4	W4
28	*Aythya ferina* (Linnaeus, 1758)*	+	+	+	+	+	+	+	+	+	+	+	+	14140	C4	D5	W5
29	*Aythya nyroca* Güldenstädt, 1770*							+	+					6	C1	D1	W1
30	*Bucephala clangula* (Linnaeus, 1758)*	+	+	+								+	+	490	C2	D1	W2
31	*Mergus albellus* (Linnaeus, 1758)*	+	+								+			8	C1	D1	W1
	VI. Order **Falconiformes**																
32	*Buteo buteo* (Linnaeus, 1758)	+	+	+	+				+	+	+	+	+	19	C3	D1	W1
33	*Circus aeruginosus* (Linnaeus, 1758)*				+				+					3	C1	D1	W1
34	*Falco subbuteo* Linnaeus, 1758						+							1	C1	D1	W1
35	*Falco vespertinus* Linnaeus, 1766									+				1	C1	D1	W1
36	*Falco tinnunculus* Linnaeus, 1758		+	+	+	+	+	+	+			+		11	C3	D1	W1
	VII. Order **Galliformes**																
37	*Phasianus colchicus* Linnaeus, 1758	+	+	+	+	+					+		+	14	C3	D1	W1
38	*Coturnix coturnix* (Linnaeus, 1758)					+								1	C1	D1	W1
	VIII. Order **Gruiformes**																
39	*Porzana porzana* Linnaeus, 1766*							+						1	C1	D1	W1
40	*Gallinula chloropus* (Linnaeus, 1758)*				+	+	+	+	+	+	+			22	C3	D1	W1
41	*Fulica atra* Linnaeus, 1758*	+	+	+	+	+	+	+	+	+	+	+	+	9831	C4	D5	W5
	IX. Order **Charadriiformes**																
42	*Vanellus vanellus* (Linnaeus, 1758)*			+		+	+		+	+				39	C2	D1	W1
43	*Charadrius dubius* Scopoli, 1786*			+	+	+			+					8	C2	D1	W1
44	*Gallinago gallinago* (Linnaeus, 1758)*								+					1	C1	D1	W1
45	*Limosa limosa* (Linnaeus, 1758)*								+					1	C1	D1	W1
46	*Calidris alpina* (Linnaeus, 1758)*								+					1	C1	D1	W1
47	*Calidris minuta* (Leisler, 1812)*								+					2	C1	D1	W1
48	*Actitis hypoleucos* (Linnaeus, 1758)*				+			+	+					25	C1	D1	W1
49	*Tringa ochropus* Linnaeus, 1758*	+	+		+				+				+	10	C2	D1	W1
50	*Tringa glareola* Linnaeus, 1758*								+					22	C1	D1	W1
51	*Tringa nebularia* (Gunnerus, 1767) *					+			+					4	C1	D1	W1
52	*Philomachus pugnax* (Linnaeus, 1758)*								+					4	C1	D1	W1
53	*Recurvirostra avosetta* Linnaeus, 1758*				+									7	C1	D1	W1
54	*Himantopus himantopus* (Linnaeus, 1758)*							+	+					30	C1	D1	W1
55	*Larus argentatus* Pontoppidan, 1763*	+	+	+	+	+	+	+	+	+	+	+	+	2236	C4	D3	W3
56	*Larus canus* Linnaeus, 1758*	+	+							+	+	+	+	2025	C2	D3	W3
57	*Larus ridibundus* Linnaeus, 1766*	+	+	+	+	+	+	+	+	+	+	+	+	13163	C4	D5	W5
58	*Larus minutus* Pallas, 1776*								+					4	C1	D1	W1
59	*Chlidonias niger* (Linnaeus, 1758)*								+					9	C1	D1	W1
60	*Chlidonias hybridus* (Pallas, 1811)*				+	+	+	+	+					24	C2	D1	W1
61	*Sterna hirundo* Linnaeus, 1758*				+	+	+	+						30	C2	D1	W1
	X. Order **Columbiformes**																
62	*Columba palumbus* Linnaeus, 1758					+		+	+	+				10	C2	D1	W1
63	*Streptopelia turtur* (Linnaeus, 1758)					+	+		+					9	C1	D1	W1

64	*Streptopelia decaocto* (Frivaldszky, 1838)	+		+	+	+	+	+	+	+	+	+		30	C4	D1	W1
	XI. Order **Cuculiformes**																
65	*Cuculus canorus* Linnaeus, 1758					+								1	C1	D1	W1
	XII. Order **Strigiformes**																
66	*Athene noctua* (Scopoli, 1769)											+		1	C1	D1	W1
	XIII. Order **Apodiformes**																
67	*Apus apus* (Linnaeus, 1758)				+	+	+	+	+					32	C2	D1	W1
	XIV. Order **Coraciiformes**																
68	*Alcedo atthis* (Linnaeus, 1758)*								+	+	+	+		6	C2	D1	W1
69	*Merops apiaster* Linnaeus, 1758					+								4	C1	D1	W1
70	*Upupa epops* Linnaeus, 1758			+	+			+						5	C1	D1	W1
	XV. Order **Piciformes**																
71	*Picus canus* Gmelin, 1788	+												1	C1	D1	W1
72	*Dendrocopos major* (Linnaeus, 1758)										+	+		4	C1	D1	W1
73	*Jynx torquilla* Linnaeus, 1758				+									1	C1	D1	W1
	XVI. Order **Passeriformes**																
74	*Galerida cristata* (Linnaeus, 1758)		+							+	+	+		6	C2	D1	W1
75	*Alauda arvensis* Linnaeus, 1758			+	+	+	+							10	C2	D1	W1
76	*Riparia riparia* (Linnaeus, 1758)					+	+		+					251	C1	D1	W1
77	*Hirundo rustica* Linnaeus, 1758				+	+	+	+	+	+				204	C2	D1	W2
78	*Delichon urbica* (Linnaeus, 1758)					+	+	+	+					90	C2	D1	W1
79	*Anthus trivialis* (Linnaeus, 1758)				+					+				9	C1	D1	W1
80	*Anthus spinoletta* (Linnaeus, 1758)	+		+							+	+	+	40	C2	D1	W1
81	*Anthus pratensis* (Linnaeus, 1758)										+	+		9	C1	D1	W1
82	*Motacilla flava* Linnaeus, 1758				+	+	+	+	+					32	C2	D1	W1
83	*Motacilla cinerea* Tunstall, 1771*		+											1	C1	D1	W1
84	*Motacilla alba* Linnaeus, 1758		+	+	+	+	+	+	+	+	+			136	C3	D1	W2
85	*Lanius collurio* Linnaeus, 1758					+	+	+	+					8	C2	D1	W1
86	*Lanius excubitor* Linnaeus, 1758				+							+		3	C1	D1	W1
87	*Oriolus oriolus* (Linnaeus, 1758)						+							2	C1	D1	W1
88	*Sturnus vulgaris* Linnaeus, 1758			+	+	+	+	+		+	+	+		371	C3	D1	W2
89	*Garrulus glandarius* (Linnaeus, 1758)	+										+		2	C1	D1	W1
90	*Pica pica* (Linnaeus, 1758)	+	+	+	+	+	+	+	+	+	+	+	+	391	C4	D1	W2
91	*Corvus monedula* Linnaeus, 1758	+	+	+	+	+	+	+	+	+	+	+	+	1260	C4	D2	W3
92	*Corvus frugilegus* Linnaeus, 1758	+	+	+	+	+	+		+	+	+	+	+	1104	C4	D2	W3
93	*Corvus corone cornix* Linnaeus, 1758	+	+	+	+	+			+	+		+	+	21	C3	D1	W1
94	*Corvus corax* Linnaeus, 1758	+	+	+	+	+				+	+	+	+	41	C3	D1	W1
95	*Troglodytes troglodytes* (Linnaeus, 1758)	+	+											2	C1	D1	W1
96	*Prunella modularis* (Linnaeus, 1758)										+	+		5	C1	D1	W1
97	*Locustella luscinioides* Savi, 1824*				+									2	C1	D1	W1
98	*Acrocephalus schoenobaenus* (Linnaeus, 1758)*					+	+							2	C1	D1	W1
99	*Acrocephalus palustris* Bechstein, 1798*				+	+	+	+	+					30	C2	D1	W1
100	*Acrocephalus scirpaceus* Hermann, 1804*				+		+							2	C1	D1	W1
101	*Acrocephalus arundinaceus* (Linnaeus, 1758)*					+	+	+						20	C1	D1	W1
102	*Sylvia borin* Boddaert, 1783						+							2	C1	D1	W1
103	*Sylvia atricapilla* (Linnaeus, 1758)				+		+	+	+					7	C2	D1	W1
104	*Sylvia communis* Latham, 1787					+	+							4	C1	D1	W1
105	*Sylvia curruca* (Linnaeus, 1758)				+									17	C1	D1	W1
106	*Phylloscopus collybita* Vieillot, 1817							+	+	+	+			17	C2	D1	W1
107	*Ficedula albicollis* Temminck, 1815				+									1	C1	D1	W1
108	*Oenanthe oenanthe* (Linnaeus, 1758)				+					+				6	C1	D1	W1
109	*Saxicola rubetra* (Linnaeus, 1758)								+	+				13	C1	D1	W1
110	*Saxicola torquata* (Linnaeus, 1766)				+									1	C1	D1	W1

111	*Erithacus rubecula* (Linnaeus, 1758)			+							+	+	+	5	C2	D1	W1
112	*Luscinia megarhynchos* Brehm C.L., 1831				+	+	+							10	C1	D1	W1
113	*Luscinia luscinia* (Linnaeus, 1758)				+				+					2	C1	D1	W1
114	*Turdus merula* Linnaeus, 1758	+		+			+						+	4	C2	D1	W1
115	*Turdus philomelos* Brehm C.L., 1831			+							+			3	C1	D1	W1
116	*Parus caeruleus* Linnaeus, 1758	+	+				+	+	+	+	+	+		52	C3	D1	W1
117	*Parus major* Linnaeus, 1758	+	+	+		+	+	+	+		+	+		27	C3	D1	W1
118	*Remiz pendulinus* (Linnaeus, 1758)*						+	+				+		8	C1	D1	W1
119	*Passer domesticus* (Linnaeus, 1758)		+	+	+	+	+	+	+	+	+	+		245	C4	D1	W2
120	*Passer montanus* (Linnaeus, 1758)	+	+		+	+	+	+	+	+	+	+	+	211	C4	D1	W2
121	*Fringilla coelebs* Linnaeus, 1758	+									+	+	+	47	C2	D1	W1
122	*Coccothraustes coccothraustes* (Linnaeus, 1758)		+											1	C1	D1	W1
123	*Carduelis chloris* (Linnaeus, 1758)				+	+	+	+	+		+	+		35	C3	D1	W1
124	*Carduelis spinus* (Linnaeus, 1758)											+		7	C1	D1	W1
125	*Carduelis carduelis* (Linnaeus, 1758)	+	+	+	+	+	+	+		+	+	+	+	65	C4	D1	W1
126	*Carduelis cannabina* (Linnaeus, 1758)		+		+	+						+	+	24	C2	D1	W1
127	*Emberiza schoeniclus* (Linnaeus, 1758)*		+		+						+	+	+	11	C2	D1	W1
128	*Miliaria calandra* (Linnaeus, 1758)		+	+	+	+	+				+	+		34	C3	D1	W1
129	*Emberiza citrinella* Linnaeus, 1758	+	+	+						+	+	+	+	188	C3	D1	W2

Legend:
* – birds dependent on wetlands; + – presence; C1 – occasional species, C2 – accessory species, C3 – constant species, C4 – euconstant species; D1, W1 – subrecedent species, D2, W2 – recedent species, D3, W3 – subdominant species, D4, W4 – dominant species, D5, W5 – eudominant species; AI, AII, AIII – annexes of the Birds Directive, Bern Convention and, respectively, Bonn Convention, A, B – parts of the annexes.

Referring to the birds that are dependent on wetlands, these totalized 64 species (Table 1) and 80,148 individuals. In the case of the individuals, the graphic is almost identical with the one of individuals from all species (with maximum in February – 18,441 individuals and minimum in May – 382 individuals). Few decades ago, until the construction of the dam basins, there was almost an identical situation, with the richest populations of aquatic birds present during passages and in winter time (MUNTEANU & MĂTIEȘ, 1983). As regards the number of species, more substantial differences emerged: the maximum is clearly in August (41 species), when many Charadriiformes appeared, and the minimum is in October (18 species). Also, in September, March, and, even, in November and December, the number of species was relatively low (Fig. 2). These show that there is also a variation of the species that are not dependent on water, as they come out in bigger numbers from April (32 species) to June and in October and November. In December, they were the worst represented (15 species). From the individuals' point of view, there is a fluctuation of these species, too, the maximum of the curve being in November (1,019) and the minimum in April (229) and July. Compared to the situation registered here 30–35 years ago (MUNTEANU & MĂTIEȘ, 1983), 14 extra species appeared (*Phalacrocorax carbo*, *Pelecanus crispus*, *Circus aeruginosus*, *Recurvirostra avosetta*, *Himantopus himantopus*, *Alcedo atthis*, *Motacilla cinerea*, *Locustella luscinioides*, *Acrocephalus schoenobaenus*, *A. palustris*, *A. scirpaceus*, *A. arundinaceus*, *Remiz pendulinus*, and *Emberiza schoeniclus*), but only some of them are veritable aquatic birds. Most of them are rare. There are some interesting aspects, such as *Phalacrocorax carbo*, that currently is constant (100% frequency) and recedent species (Table 1), *Pelecanus crispus*, vagrant from the Danube Delta and present here many times in the recent years, and the occurrence of the birds from the Passeriformes order, linked mainly by the swamp vegetation appearance. A series of species – *Gavia stellata* (Pontoppidan, 1763), *Botaurus stellaris* (Linnaeus, 1758), *Platalea leucorodia* Linnaeus, 1758, *Plegadis falcinellus* (Linnaeus, 1766), *Ciconia nigra* (Linnaeus, 1758), *Anser anser* (Linnaeus, 1758), *A. erythropus* (Linnaeus, 1758), *A. fabalis* (Latham, 1787), *Anas acuta* Linnaeus, 1758, *Tadorna ferruginea* (Pallas, 1764), *Netta rufina* (Pallas, 1773), *Aythya marila* (Linnaeus, 1761), *Mergus merganser* Linnaeus, 1758, *M. serrator* Linnaeus, 1758, *Pandion haliaetus* (Linnaeus, 1758), *Rallus aquaticus* Linnaeus, 1758, *Porzana parva* (Scopoli, 1769), *P. pusilla* (Pallas, 1776), *Charadrius hiaticula* Linnaeus, 1758, *C. alexandrinus* Linnaeus, 1758, *C. morinellus Linnaeus*, 1758, *Pluvialis apricaria* (Linnaeus, 1758), *P. squatarola* (Linnaeus, 1758), *Lymnocryptes minimus* (Brünnich, 1764), *Numenius arquata* (Linnaeus, 1758), *Calidris ferruginea* (Pontoppidan, 1763), *C. temminkii* (Leisler, 1812), *Tringa totanus* (Linnaeus, 1758), *T. erythropus* (Pallas, 1764), *Limicola falcinellus* (Pontoppidan, 1763), *Haematopus ostralegus* Linnaeus, 1758, *Larus fuscus* Linnaeus, 1758, *Sterna caspia* Pallas, 1770, *S. albifrons* Pallas, 1764) were not observed, because of the short period of research, their low profile or scarcity all over the area.

Regarding the distribution of the species and strengths on reservoirs and periods (months, whole year) we state that Golești dam basin held the majority of the species and strength every month. It was followed by Pitești dam basin, which often overpasses other reservoirs with bigger surface (Budeasa and Vâlcele). As a result, the situation of Golești reservoir creates the general situation of the dam basins. Bascov dam basin was generally the last in these hierarchies (Table 2). Very suggestive in this sense are the percentage distributions on whole year: Golești had 70.5% of all number of species, respectively 53.9% of all number of individuals, Pitești – 66.7%, respectively 21.1%, Vâlcele – 50.4%, respectively 10.6%, Budeasa – 46.5%, respectively 12.5% and Bascov – 29.5%, respectively 1.9%.

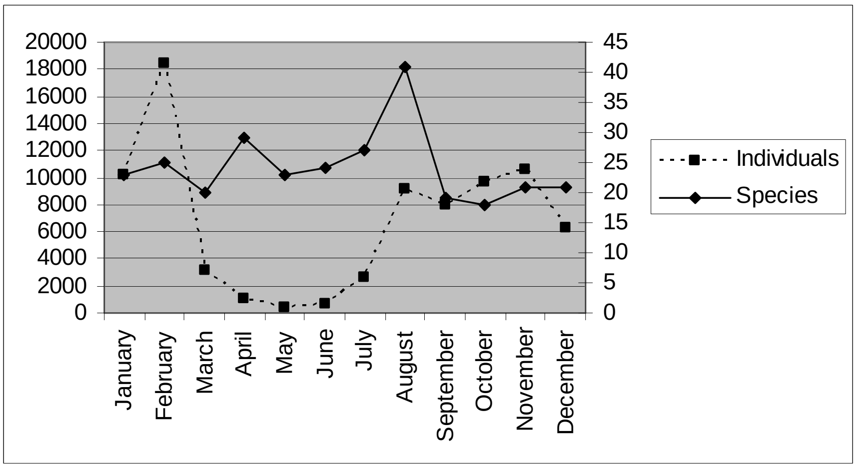

Figure 2. The monthly variation of the number of the individuals and of the number of species dependent on the wetlands.

Regarding the distribution of the species and strengths on reservoirs and ecological seasons, we observe that Goleşti and Piteşti were the first in top (alternatively for the number of species and in this succession for the number of individuals) every considered period and, again, Bascov dam basin was the last, in both situations. The number of species from all the dam basins is the smallest in vernal and autumnal seasons; the biggest number was recorded in prevernal season, but this does not reflect only the dynamics of the birds but the different number of field work from each ecological season, that varied between 1 (in vernal and serotinal) and 4 (in hiemal). The strengths varied by a concave curve: in the hiemal season, it was noted the biggest strength; it decreases to the minimum from the vernal season and increases again to the end of the interval (Table 3). The surface of the dam basins influences partially the facts. So, the correlation between the number of species and the surface was 0.29 and the correlation between the number of individuals and the surface was a 0.43. Both are positive and acceptable correlations (by COLTON, 1974) and this means that the number of species and, mainly, the number of the bird individuals can grow with the increase of the reservoirs surface.

Table 2. The distribution of the species and strengths on reservoirs and intervals of time.

Reservoir	Parameter	January	February	March	April	May	June	July	August	September	October	November	December	All period
Vâlcele	**Species**	15	18	17	23	17	19	15	20	20	22	25	18	65
	Individuals	990	1,098	351	277	298	101	121	394	1,926	1,028	1,239	1,218	9,041
Budeasa	**Species**	13	24	12	18	17	15	17	14	15	21	25	16	60
	Individuals	717	4,989	191	94	90	132	387	200	565	1226	1,189	872	10,652
Bascov	**Species**	10	7	10	8	10	8	9	6	9	8	8	10	38
	Individuals	136	219	229	88	91	63	139	204	104	95	37	215	1,620
Piteşti	**Species**	30	24	21	30	29	37	32	24	19	30	23	17	86
	Individuals	2,523	4,339	407	153	145	260	630	1,205	2,698	778	1,894	3,012	18,044
Goleşti	**Species**	24	28	27	35	28	30	26	42	24	26	25	22	91
	Individuals	6,163	8,372	2,316	607	184	542	1,582	7,505	3,278	6,949	7,209	1,254	45,961

Table 3. The distribution of the species and strengths on reservoirs and ecological seasons.

Reservoir	Parameter	Hiemal	Prevernal	Vernal	Aestival	Serotinal	Autumnal
Vâlcele	**Species**	33	31	17	26	20	31
	Individuals	4,545	628	298	222	394	2,954
Budeasa	**Species**	36	21	17	25	14	25
	Individuals	7,767	285	90	519	200	1,791
Bascov	**Species**	19	14	10	14	6	14
	Individuals	607	317	91	202	204	199
Piteşti	**Species**	41	39	29	47	24	34
	Individuals	11,768	560	145	890	1205	3,476
Goleşti	**Species**	39	44	28	38	42	35
	Individuals	22,998	2,923	184	2124	7505	10,227
All dam basins	**Species**	64	72	54	64	66	54
	Individuals	47,685	4,713	808	3,957	9,508	18,647

As regards the density – the ratio number of species/surface of the reservoir and ratio number of individuals/surface of the reservoir we state that, generally, they grow from upstream to downstream, the exception being Goleşti reservoir (Table 4). So, despite the vicinity of Piteşti, Piteşti reservoir is one of the most preferred places from the area by birds, as we saw with other occasions, too (MESTECĂNEANU & GAVA, 2015; CONETE et al., 2012a). The multiple habitats from here, the lower degree of direct anthropogenic pressure, the position of the reservoir on the course of the Argeş River in the continuation of Rucăr – Bran pass of migration over the Carpathians and near other wetlands from the South of Romania can be the explanation (MĂTIEŞ, 1969; CONETE et al., 2012b; MESTECĂNEANU & GAVA, 2013). Some of the general conditions were stated by other authors on diverse reservoirs from Romania, too (MUNTEANU, 2000; MITRULY, 2002).

Table 4. The ratio number of species/ha and number of individuals/ha for every reservoir.

Reservoir	Number of species	Number of individuals	Number of species/ha	Number of individuals/ha
Vâlcele	65	9,041	0.10	14.12
Budeasa	60	10,652	0.09	16.56
Bascov	38	1,620	0.27	11.57
Piteşti	86	18,044	0.57	120.29
Goleşti	91	45,961	0.13	67.58
Total	129	85,318	0.05	37.86

On the subject of the similarity between the avicoenoses from the reservoirs, by Jaccard index (Table 5, Fig. 3) the biggest similarity is between Piteşti and Budeasa (55.31%) and the lowest between Vâlcele and Bascov (28.75%). By Bray–Curtis index (Table 6, Fig. 4), the highest similarity was between Vâlcele and Budeasa (63.57%) and the lowest between Goleşti and Bascov (6.71%), a situation similar to the one observed in the same area, in the hiemal period, as we showed in a previous work (MESTECĂNEANU & GAVA, 2013). The differences between the values represent the consequences of the detail that the Jaccard index is based only on the presence/absence of the respective species in the samples and the Bray–Curtis index is based on the presence/absence of the species in the samples and on their number of individuals.

Table 5. The similarity matrix (by Jaccard) between the avicoenoses of the reservoirs.

Similarity	Vâlcele	Budeasa	Bascov	Piteşti	Goleşti
Vâlcele	*	47.05	28.75	43.80	45.79
Budeasa	*	*	46.26	55.31	42.45
Bascov	*	*	*	34.78	30.30
Piteşti	*	*	*	*	50.00
Goleşti	*	*	*	*	*

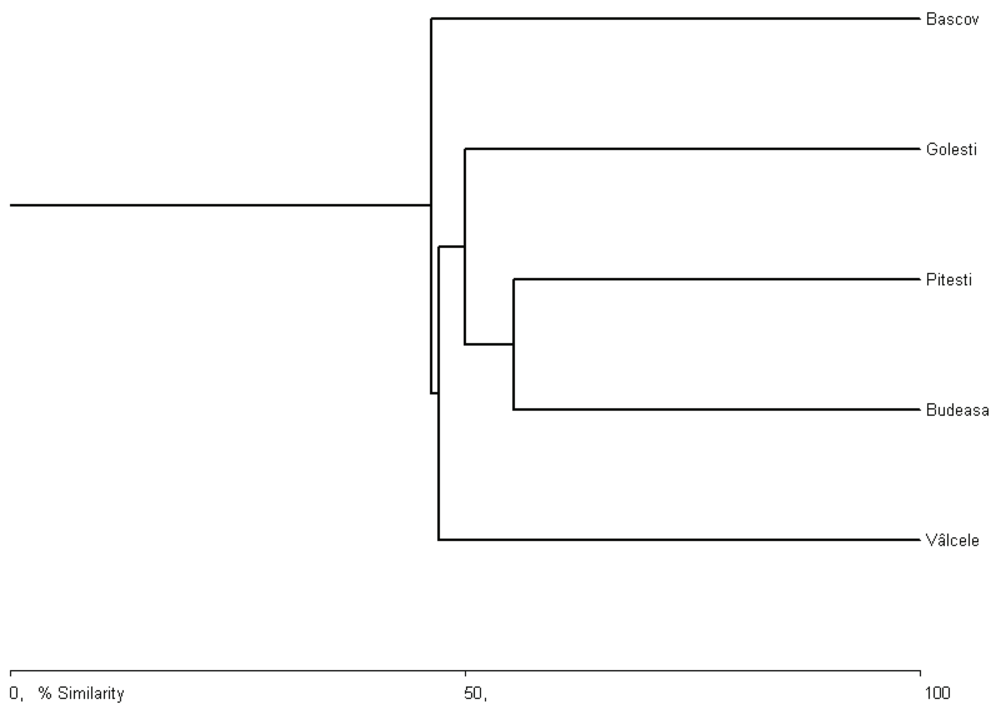

Figure 3. The Jaccard Cluster Analysis (Single Link).

Table 6. The similarity matrix (by Bray–Curtis) between the avicoenoses of the reservoirs.

Similarity	Vâlcele	Budeasa	Bascov	Piteşti	Goleşti
Vâlcele	*	63.57	23.54	44.98	28.19
Budeasa	*	*	25.19	48.58	35.94
Bascov	*	*	*	16.33	6.71
Piteşti	*	*	*	*	47.06
Goleşti	*	*	*	*	*

The Shanon–Wiener ecological diversity was between 2.11 (for Vâlcele) and 2.29 (for Piteşti) and the Simpson ecological diversity was between 4.38 (for Vâlcele) and 5.70 (for Piteşti). For all the dam basins, it was 2.41, respectively 6.95. From the evenness point of view, the smallest evenness was at Goleşti dam basin (0.48, respectively 0.06) and the biggest was at Bascov dam basin (0.60, respectively 0.13). For the whole researched area, it was 0.50 (in case of the Shanon–Wiener index), respectively 0.05 (in case of the Simpson index), (Table 7).

Regarding the ecological seasons, the diversity varied between 2.11 (in the serotinal season) and 2.99 (in the vernal season) for the Shanon–Wiener index and between 4.80 (in the serotinal season) and 12.95 (in the vernal season) for the Simpson index. The corresponding evenness varied, also, between 0.50, respectively 0.07 (in the serotinal season) and 0.75, respectively 0.22 (in the vernal season), (Table 8).

The results reflect the natural spatial and temporal dynamics of the birds, the complexity of the habitats from every dam basin, and the anthropogenic pressure upon each of them. The food and the shelters availability are factors that influence the bird occurrence in the area, too.

It is obvious that, generally, the values of diversity for each coenosis of the reservoirs and for each ecological season are medium. That means that the dam basins do not provide the best ecological area necessary for many bird species, from this point of view the avicoenoses being moderately stable. On the one hand, we observe that the avicoenosis of Vâlcele dam basin is the least diverse and the avicoenosis of Piteşti dam basin is the most diverse and, on the other hand, that the serotinal season is characterised by a moderate stability in the avicoenosis and the vernal season by a better stability. Also, it results that at Goleşti there are few dominant species and at Bascov the strengths of the species are relatively more uniformly distributed. Also, the ecosystems are characterised by a great inequitability regarding the strengths of the species, whatever the ecological seasons. This fact is more obvious in the hiemal and in the passage seasons and a better distribution is in the vernal season.

The differences of the values consist in the fact that the Shannon–Wiener index takes into account both the number of species and the number of individuals of the each species and the Simpson index takes into account the number of individuals of the each species in relationship with the number of individuals of all observed species.

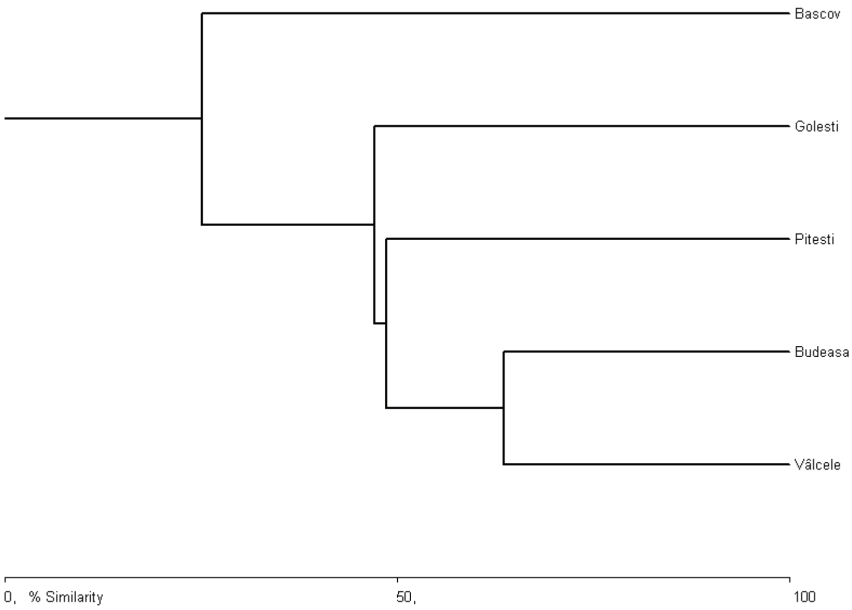

Figure 4. The Bray–Curtis Cluster Analysis (Single Link).

Table 7. The ecological diversity and the evenness of the avifauna from the dam basins.

Dam basin	Shanon Wiener index	Hsmax	Shanon Wiener evenness	Simpson index (1/λ)	S	Simpson evenness
Vâlcele	2.11	4.17	0.50	4.38	65.46346	0.07
Budeasa	2.23	4.09	0.55	5.23	60.33421	0.09
Bascov	2.20	3.64	0.60	4.98	38.88875	0.13
Piteşti	2.29	4.45	0.51	5.70	86.40706	0.07
Goleşti	2.17	4.51	0.48	5.57	91.17855	0.06
Overall	2.41	4.86	0.50	6.95	129.1938	0.05

Table 8. The seasonal ecological diversity and evenness of the avifauna.

Season	Shanon Wiener index	Hsmax	Shanon Wiener evenness	Simpson index (1/λ)	S	Simpson evenness
Hiemal	2.21	4.16	0.53	5.66	64.08467	0.09
Prevernal	2.54	4.28	0.59	6.86	73.10149	0.09
Vernal	2.99	3.99	0.75	12.95	57.79576	0.22
Aestival	2.64	4.16	0.63	7.25	65.03571	0.11
Serotinal	2.11	4.19	0.50	4.80	66.45435	0.07
Autumnal	2.12	3.99	0.53	6.06	54.15393	0.11

If we apply the power regression on the relation between the surface of the every reservoir and their species richness, we state that the slope is positive and quite small and this means that the species rate of accumulation grows slowly as the reservoirs surface increases (Fig. 5). The assertion was observed with other occasions, too (CONETE, 2011) but, in our case, the slope is more accentuated because of the shorter period of observations.

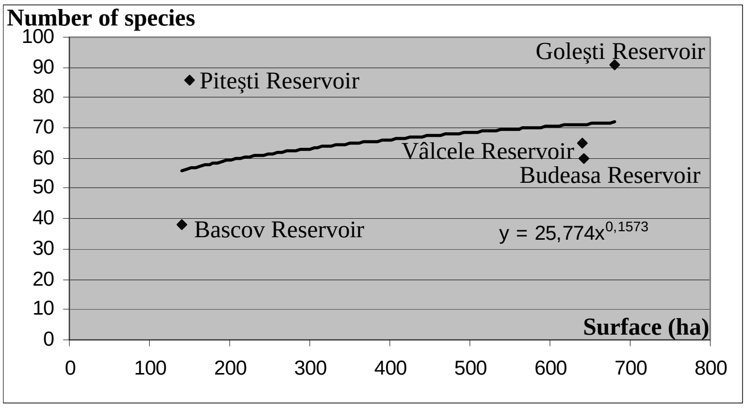

Figure 5. The relation between the surfaces of the reservoirs and their species richness.

According to the constancy (Table 1), 64 species (49.61% of all) were occasional species, 32 species (24.81% of all) were accessory species, 15 species (11.63% of all) were constant species and 18 species (13.95% of all) were euconstant species (*Podiceps cristatus*, *Phalacrocorax carbo*, *Ardea cinerea*, *Cygnus olor*, *Anas platyrhynchos*, *A. crecca*, *Aythya fuligula*, *A. ferina*, *Fulica atra*, *Larus argentatus*, *L. ridibundus*, *Streptopelia decaocto*, *Pica pica*, *Corvus monedula*, *C. frugilegus*, *Passer domesticus*, *P. montanus*, *Carduelis carduelis*). 11 species of the later class are dependent on wetlands (1 from the Podicipediformes order, 1 from the Pelecaniformes order, 1 from the Ciconiiformes order, 5 from the Anseriformes order, 1 from the Gruiformes order and 2 from the Charadriiformes order) and only 6 are Passeriformes; 1 is from the Columbiformes order. Therefore, these were seen most frequently in the observations. 11 species (*Podiceps cristatus*, *Phalacrocorax carbo*, *Ardea cinerea*, *Cygnus olor*, *Anas platyrhynchos*, *Aythya ferina*, *Fulica atra*, *Larus argentatus*, *L. ridibundus*, *Pica pica* and *Corvus monedula*) had even a frequency of 100%, unlike the circumstances from the 1970s–1980s, when without doubt *Anas platyrhynchos* was the most frequent species (MUNTEANU & MĂTIEŞ, 1983).

Depending on the dominancy (Table 1), 116 species (89.92% of all) were subrecedent species, 5 species (3.88% of all) were recedent species, 3 species (2.33% of all) were subdominant species, 1 species (0.78% of all) was dominant species (*Aythya fuligula*) and 4 species (3.10% of all) were eudominant species (*Anas platyrhynchos*, *Aythya ferina*, *Fulica atra*, *Larus ridibundus*). The later species belong to the Anseriformes, Gruiformes and Charadriiformes orders and, thus, they are the most important species as strength in the avicoenosis. The situation is rather dissimilar to the one from the past, when *Anas plathyrhynchos* was the most abundant species; it was distantly followed by *A. querquedula*, *A. crecca*, *Vanellus vanellus* and *Larus ridibundus*; *Fulica atra* was represented by few individuals and *Aythya ferina* and *A. fuligula* were absent (MUNTEANU & MĂTIEŞ, 1983). More recently (MUNTEANU et al., 1989), on diverse types of water bodies from Muntenia and Oltenia, *Anas platyrhynchos* was followed by *A. crecca* (at a little difference in January 1988 and at significant one in January 1989); *Fulica atra* was absent in 1988 and present with important strength in 1989 and *Aythya ferina* and *A. fuligula* were absent at both evaluations. In our case, in the hiemal season, *Aythya fuligula* and *Fulica atra* were the dominant species and *Anas platyrhynchos*, *Aythya ferina*, and *Larus ridibundus* were the eudominant species (see also MESTECĂNEANU & GAVA, 2015). At the census from January 2013 (MESTECĂNEANU et al., 2013), *Anas platyrhynchos* and *Fulica atra* were the eudominant species, *Cygnus olor*, *Anas crecca*, *Aythya ferina*, *Larus argentatus*, and *L. ridibundus* were the dominant species and *Phalacrocorax carbo*, *Aythya fuligula* and *Larus canus* were subdominant species. From these and by comparison to the current situation with the one from the Danube Delta in 1988, 1989, where different species from the genera *Aythya*, *Anas*, *Netta*, *Mergus*, *Cygnus*, etc. were present in relevant number, we can say that over time, the structure of the avifauna from the dam basins from the Argeş River tends to become increasingly more similar to that of a natural ecosystem.

By Dzuba ecological significance index (Table 1), 103 species (79.84% of all) were subrecedent species, 13 species (10.08% of all) were recedent species, 8 species (6.20% of all) were subdominant species, 1 species (0.78% of all) was dominant species (*Aythya fuligula*) and 4 species (3.10%) were eudominant species (*Anas platyrhynchos*, *Aythya* *ferina*, *Fulica atra*, *Larus ridibundus*). It is obvious that there are the same dominant and eudominant species as in the case of the dominancy index and they have the biggest ecological importance in the local bird fauna.

Taking into account the index of relation, as we expected, owing to the dominant and overdominant species, Anseriformes and Charadriiformes were the overdominant orders over the whole period. They totalised 66,980 individuals. Gruiformes was the only dominant order: 9,854 individuals. Every other order was complementary. The static axis (As) is 6.25 and the dominance axis (Ad) is 12.5 (Fig. 6).

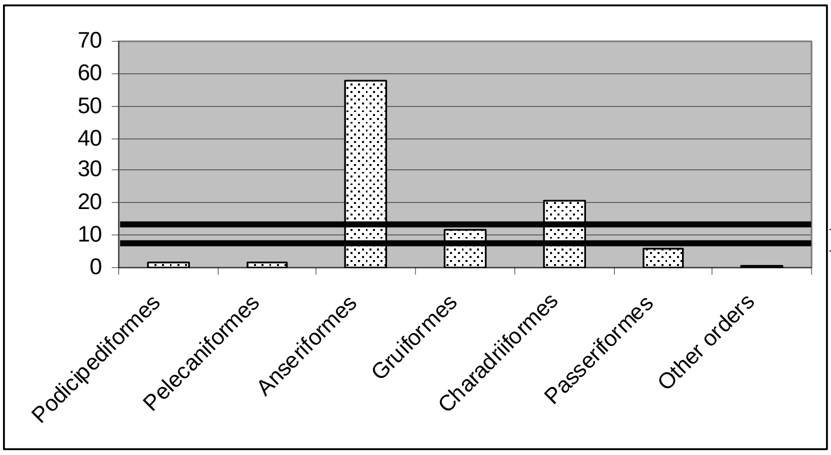

Figure 6. The participation of the orders to the formation of the avicoenosis.

Regarding the monthly dynamics of the orders, it is noticeable that no order was every month overdominant. Anseriformes was overdominant, except May, when it was dominant. Charadriiformes was overdominant in January, July, August, September, and December. From February to May, and in November it was dominant and in June and October it was complementary. Gruiformes was overdominant in March, September, October, and December, dominant in February, April – July, and December, and subdominant in January, August and November. From April to June, Passeriformes became overdominant, in March, July, September and November it was dominant and in the rest of the year it was complementary. Podicipediformes was dominant in April, Pelecaniformes was overdominant in May, dominant in June and July; in the rest of the year these were subdominant. The other orders were complementary every month. The static axis (As) and the dominance axis (Ad) had the same values as in the precedent discussion (Fig. 7).

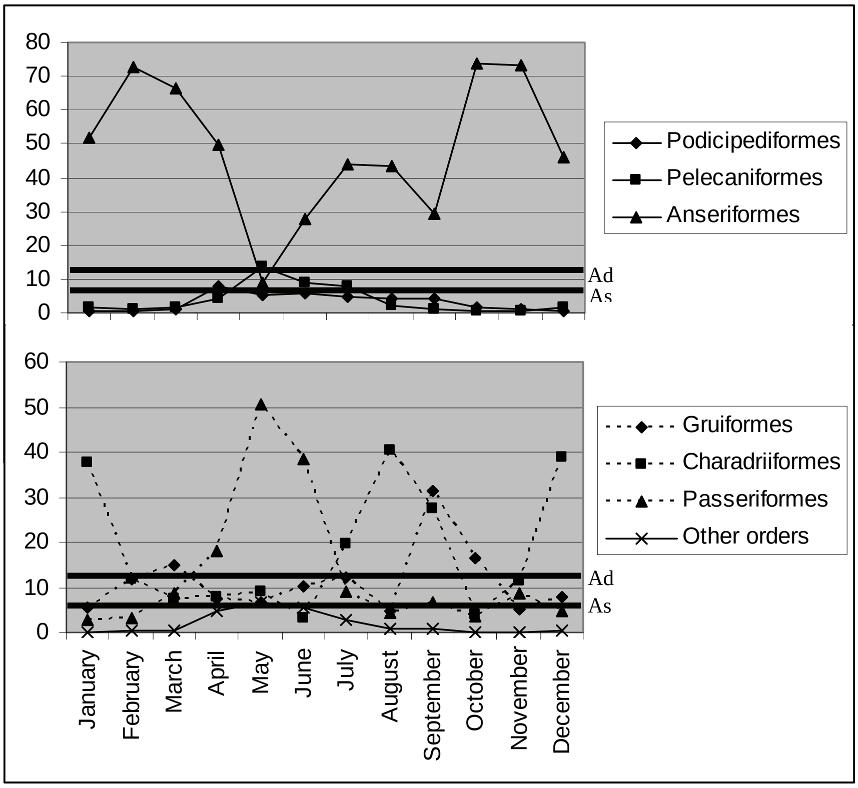

Figure 7. The monthly dynamics of the orders.

CONCLUSIONS

Between February 2013 and January 2014, on the reservoirs Goleşti, Piteşti, Bascov, Budeasa and Vâlcele, from the Argeş River, 129 bird-species and 85,318 individuals were registered.

The identified species belong to 16 orders, Passeriformes being the richest (with 56 species).

In August, it was registered the biggest number of species (66 species) and in February, the highest number of individuals (19,017 individuals); the same thing could be said about the species that depend on wetlands, too (41 species in August, respectively 18,441 individuals in February).

The variation in the number of species and strengths reflects mainly the migratory dynamics of the birds from the Anseriformes and Charadriiformes orders and suggests a relatively small number of breeding birds in the area.

The migratory dynamics of the birds is partially influenced by the air temperature and the occurrence of the birds on reservoirs is also somehow determined by their surface, the habitats from here, the degree of direct anthropogenic pressure, the position of the reservoir on the course of the Argeş River.

Goleşti dam basin detained the majority of the species and strength regardless the period and Bascov dam basin was generally the last.

The species and individuals density, generally, grows from upstream to downstream.

Despite its position next to settlements, Piteşti reservoir is a place that birds prefer.

By Jaccard index the biggest similarity was between Piteşti and Budeasa and the smallest between Vâlcele and Bascov and by Bray–Curtis index, the highest similarity was between Vâlcele and Budeasa and the lowest between Goleşti and Bascov.

Generally, the values of diversity for each coenosis of the reservoirs and for each ecological season are medium, from this point of view the avicoenoses being moderately stable: the avicoenosis of Vâlcele dam basin is the least diverse and the avicoenosis of Piteşti dam basin is the most diverse; the serotinal season is characterised by a moderate stability in the avicoenosis and the vernal season by a better stability.

On Goleşti dam basin there are few dominant species and on Bascov dam basin the strengths of the species are relatively more uniformly distributed.

The ecosystems are characterised by a great inequitability regarding the strengths of the species, more obvious in the hiemal and in the passage seasons.

The species rate of accumulation grows slowly as the reservoirs surface increases.

According to the constancy, 64 species (49.61% of all) were occasional species, 32 species (24.81% of all) were accessory species, 15 species (11.63% of all) were constant species and 18 species (13.95% of all) were euconstant.

Depending on the dominancy, 116 species (89.92% of all) were subrecedent species, 5 species (3.88% of all) were recedent species, 3 species (2.33% of all) were subdominant species, 1 species (0.78% of all) was dominant species (*Aythya fuligula*), and 4 species (3.10% of all) were eudominant species.

By Dzuba ecological significance index, 103 species (79.84% of all) were subrecedent species, 13 species (10.08% of all) were recedent species, 8 species (6.20% of all) were subdominant species, 1 species (0.78% of all) was dominant species and 4 species (3.10%) were eudominant species.

Anseriformes and Charadriiformes were the overdominant orders over the whole period.

Regarding the monthly dynamics of the orders, Anseriformes was overdominant all months, except May, when it was dominant; Charadriiformes was overdominant in January, July, August, September, and December.

As the time went by, the structure of the avifauna from the dam basins from the Argeş River has become increasingly more similar to that of a natural ecosystem.

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Received: March 30, 2016
Accepted: June 22, 2016

SEASONAL DYNAMICS OF BAT FAUNA IN COMARNIC CAVE, ROMANIA

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Abstract. Our study aimed to provide information about the population of bats present in Comarnic Cave, Romania about which little information was previously known. We found an important hibernating colony of *Rhinolophus ferrumequinum*, which is the dominating species in terms of numbers, as well as other five species, of which three were accidental and two were constant in the population (*Myotis myotis/oxygnathus* and *Rhinolophus hipposideros*). We measured microclimate parameters (air temperature, rock temperature and relative humidity) and evaluated site preferences in relation to them. For an easier analysis we used GIS, which allowed us to graphically interpret population data.

Keywords: bats, Comarnic Cave, hibernacula, Romania.

Rezumat. Dinamica sezonieră a faunei de lilieci din Peștera Comarnic, România. Studiul nostru a intenționat să furnizeze informații privind populația de chiroptere prezentă în Peștera Comarnic, România, despre care se cunoșteau foarte puține informații. Am identificat o colonie importantă de *Rhinolophus ferrumequinum*, specie dominantă din punct de vedere al efectivelor, precum și alte cinci specii din care trei au fost întâlnite accidental, iar două în mod constant (*Myotis myotis/oxygnathus* și *Rhinolophus hipposideros*). Am măsurat parametrii microclimatului și am evaluat preferințele chiropterelor în relație cu aceștia. Pentru o analiză mai simplă am folosit GIS, care ne-a permis să evaluăm grafic datele privind populația.

Cuvinte cheie: lilieci, Peștera Comarnic, hibernacul, România.

INTRODUCTION

The present study has been conducted in Comarnic Cave, situated in southwestern Romania, Caraș – Severin County, Banat area. The cave is part of The Semenic – Cheile Carașului National Park and is a natural reservation, IUCN category IV, class B. It is one of the largest natural cavities in southwestern Romania, with a total length of 6,201m and an unevenness of -101m. The cave has seven entrances and is structured on three levels: fossil, subfossil and active. Tourist activity is known to be happening for at least 100 years. Presently, the cave is open to guided tours, for a length of 1,750m (BLEAHU et al., 1976; GORAN et. al., 1982) (Fig. 1). The touristic impact on the cave and the fauna is minimal due to the very small number of tourists every year and the minimum modifications brought to the cave, which are limited to a few ropes for tourists to grab onto and stairs which are dug into clay.

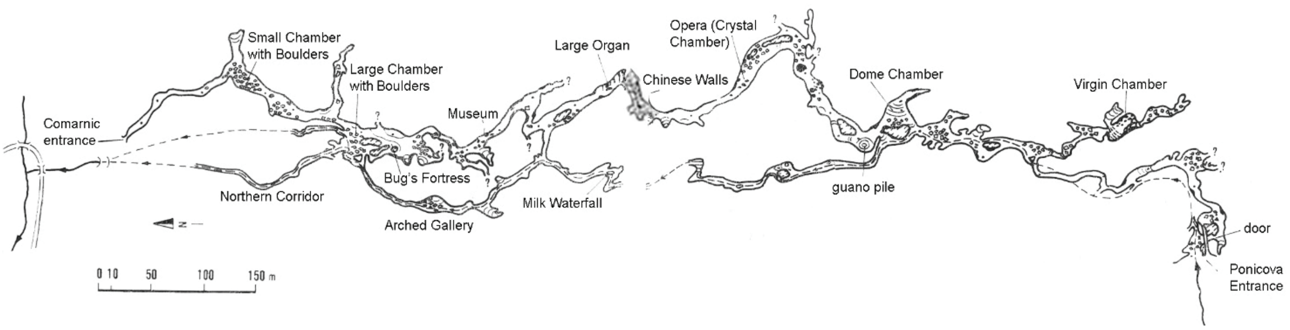

Figure 1. Comarnic Cave map (after Bleahu et al., 1976, modified).

The climate in the area is a moderate, temperate-continental, Banat subtype with sub-Mediterranean influences. This type of climate is characterized by annual precipitations which are higher than the national average, frequent warm periods during winter and early springs. The cave climate is characterized by a unidirectional ventilation, with air temperatures of 3 to 10°C, water temperatures of 2 to 9°C and soil temperatures of 0.2 to 9°C. Relative humidity varies between 75 and 100%, depending on the level of rainfall and the functioning of the hydrogeological system.

Even though it is one of the largest caves in southwestern Romania and certainly the most accessible large cave, there is scarce data regarding bat fauna and no study dedicated to the way the cave is being occupied by bats. DUMITRESCU et al. (1963) mentions the presence of isolated *Rhinolophus hipposideros* in June 1952, without giving information about the number of individuals. NEGREA & NEGREA (1971) have a study on the guano synusia in the Banat area, which includes Comarnic Cave and provide us with multiple observations over a period of eight years. In July 1961, they mention individuals of *Rhinolophus ferrumequinum* and in October 1961, a colony of 500 *R. ferrumequinum,* isolated individuals of the same species and also *R. hipposideros* and *Myotis oxygnathus*. Observations in July 1963 showed isolated individuals of *Myotis myotis*. No numeric data is presented for any of these observations. In September 1965, a group of 20 *R. ferrumequinum* is recorded and in June 1968 no bats are found. Data for October 1969 show the presence of a colony of 200 *R. ferrumequinum.*

NEGREA & NEGREA (1983) give us the location for the colony observed in 1961 and 1969, which is Sala Domului (Dome Chamber). The presence of colonies in the past in the Dome Chamber was confirmed by cavers. The four species are also referenced in a review upon bat fauna of the Romanian Carpathians (GHEORGHIU et al., 2001). A study upon the mammals from the south-western part of Romania (including Semenic – Carașului Gorges National Park) identifies 17 bat species (MURARIU, 2002), of which 4 species were found by us in Comarnic Cave. The size and multiannual stability of *R. ferrumequinum* colonies offer this cave the possibility of introduction in EUROBATS management and protection programs (HUTSON et al., 2015; MITCHELL-JONES et al., 2007).

MATERIAL AND METHODS

The study was conducted from December 2006 to December 2007, period over which six observation visits were made to the cave, in order to evaluate the status as a bat shelter, species and individual numbers, and also microhabitat conditions, which determine inhabitancy of the cave. We chose to include two hibernation periods because from the previous data it was clear that Comarnic Cave is primarily a hibernacula for *R. ferrumequinum.* In the case of the two sibling species, *M. myotis* and *M. oxygnathus*, when observations were made without capturing the individuals, they were treated as a group in order to avoid confusions, which may arise from their similar morphological characters (we will generically name *M. myotis*). Individuals in colonies were counted directly and not estimated, because the topology of the cave and the disposition of the colonies permitted it. The identification key used was SCHOBER & GRIMMBERGER (1993) and DIETZ & HELVERSEN (2004).

An anemometer was used in order to measure air currents, but no air currents were found to pass 1m/s, which was the minimum measurable value. Air temperature, rock temperature and relative humidity measurements were made in 17 station points chosen inside the cave and one outside the cave entrance for reference. During the first two visits, air and rock temperatures were taken using a digital Cole Parmer probe thermometer (Digi-Sense). Air temperatures and relative humidity measurements were taken during the next four visits, using a digital thermohygrometer (TFA-HygroLogger). During two visits, minimum-maximum thermometers were place in the vicinity of the identified colonies, in order to investigate temperature variations over a longer period of time.

In order to evaluate the microclimate parameters and correlations with the occupancy observations made we developed a method of graphical data interpretation by using a Geographical Information System. We created a georeferenced digital map of the cave starting from the map published in GORAN (1982). The advantage of this method is that data can be introduced graphically as points, but each point has a database behind it and based on that it can be illustrated in different shapes, sizes and colours. By clicking on a data point the information behind it can be quickly accessed. We created separate layers for each observation period and for bat observations and measurements.

RESULTS

During the study period we have identified a total of six species (Table 1), of which only three had a constant presence. The dominating species in terms of individual numbers is *R. ferrumequinum*, which forms large hibernating colonies. The other two species with a constant presence in Comarnic Cave are *M. myotis* and *R. hipposideros.*

Table 1. Bat species in Comarnic Cave (only during winter period).

No.	Species	Mean (min. - max.) number of individuals	Period of counting
1	*Rhinolophus ferrumequinum* Schreber	264 (123 – 477)	01.12.2006 – 27.12.2007
2	*R. hipposideros* Bechstein	10 (2 – 23)	01.12.2006 – 27.12.2007
3	*Myotis myotis* Borkhausen	44 (13 – 86)	01.12.2006 – 27.12.2007
4	*M. dasycneme* Boie	2	31.12.2006
5	*M. daubentonii* Kuhl	1	21.02.2007
6	*Plecotus austriacus* Fischer	1	21.02.2007

The first observations (December 1, 2006) were done in the context of higher than average outside temperatures for this time of the year (4.9°C – Fig. 2). Therefore, the bats were still found in the period of pre-hibernation cave colonization. Individuals were still active with the presence of isolated individuals, small groups and also a colony of 194 *R. ferrumequinum* found at the “Bug’s Fortress” area (about 480m from the main entrance to the cave) in the fossil area of the cave. The distribution was not compact, with individuals forming small groups of 10-20 bats, situated 30-40cm from each other. A minimum-maximum thermometer was placed in the vicinity of the colony and was retrieved on February 21, 2007. The temperature variation for this entire period was only 0.2°C.

The next observations (December 31, 2006) found the hibernating colony of *R. ferrumequinum* in a different place, at the intersection of the Arched Gallery and the Northern Corridor, just at the start of the active sector of the cave. Outside temperatures had fallen below 0°C (Table 2) and there was a layer of snow. The colony contained only 174 *R. ferrumequinum* individuals, which formed a single group, most of which had entered torpor and were wrapped in their patagium. The overall number of bats found in the cave was higher, but we found a smaller number of *R. ferrumequinum* than at the previous visit, but a higher number of *M. myotis* and *R. hipposideros* (Fig. 3), as well as two *M. dasycneme* individuals. A minimum-maximum thermometer was placed close to the hibernating colony and was later picked up on February 21, 2007. The temperature variation recorded over this short period was 1.1°C, higher than the one recorded at the previous colony site. This could show that temperature stability may not be as important in choosing a hibernation site as other factors, such as relative humidity, which should be higher in this active part of the cave.

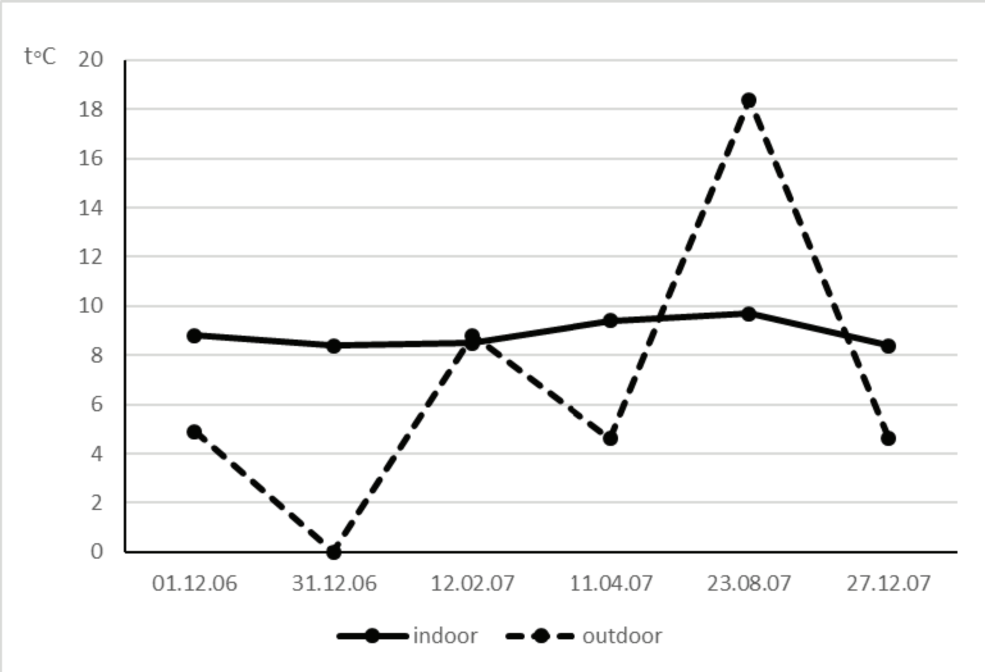

Figure 2. Temperature dynamics indoor and outdoor at Comarnic Cave.

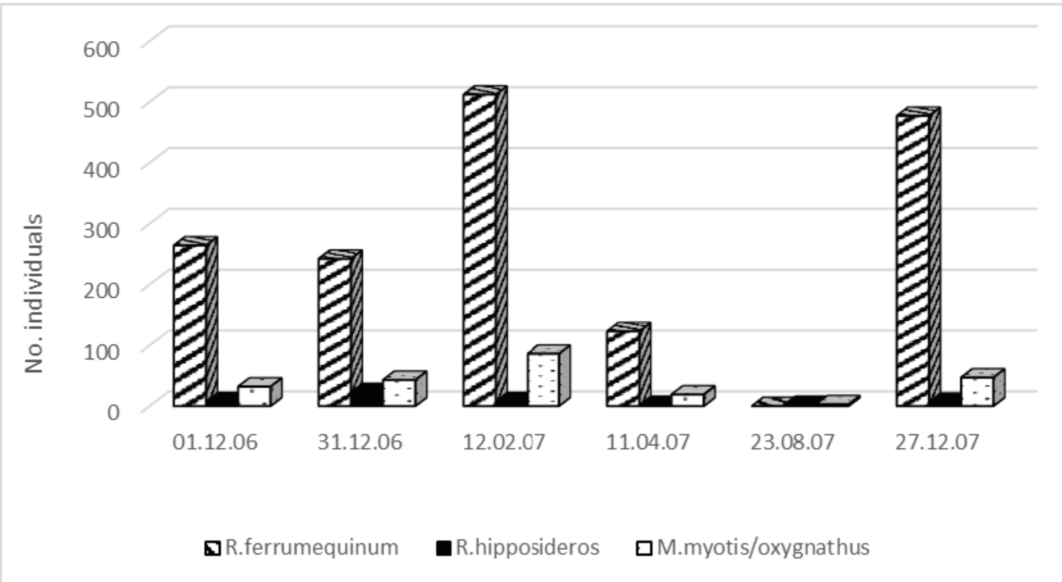

Figure 3. The number of bat individuals for the main three species identified in the colonies.

The third observations, on February 12, 2007, were done in the conditions of an early spring, with an outside temperature of 8.8°C and visible insect activity. We found a much larger hibernating colony of *R. ferrumequinum*, with a total number of 482 individuals. The colony was loosely distributed in small groups and had moved about 20m compared to the site they were found before. They had exited torpor and were slightly active. Most other bat species were found in the northern quarter of the cave, closer to the main entrance than previously, suggesting they were preparing to leave the cave. The number of *M. myotis* was higher than previously and *R. hipposideros* was lower (Fig. 3).

A very small number of bats that had not left the cave yet was found on April 11, 2007 (Fig. 3), but all signs pointed that they were preparing to leave. Outside temperatures were lower than the average for this period (Fig. 2). Most bats were found 150-200m to the main entrance of the cave. At this point it is safe to say that this is the preferred access way into the cave. We found a colony of 114 active *R. ferrumequinum* in the Small Chamber with Boulders, 150m from the entrance.

In the summer observations (August 23, 2007), there were identified only three individuals, one *M. myotis* and two *R. hipposideros*. We also had an unplanned trip to the cave on July 27, 2007, time at which we did not identify any individuals present. Even though the cave is not used as a nursery, it is possibly used as an autumn temporary shelter.

Observations on December 27, 2007 were done in the conditions of a much earlier winter than the previous year, with ice formations present for the first 50m of the cave. Due to this, the total number of observed individuals was a lot higher than the previous year, with a total of 531 recorded individuals (Table 3). What is most interesting is that there was a colony of 461 *R. ferrumequinum* and 23 *M. myotis* in the same point they were found in April 2007; The Small Chamber with Boulders, in an area strongly influenced by outside temperatures. The air temperature measured only a few meters away from the colony was only 4.6°C, very unusual for hibernating *R. ferrumequinum.* It is possible that due to collective thermoregulation the temperature inside the colony was higher. The bats had entered torpor and no individuals were active. The distribution of the colony was in four larger compact groups, surrounded by smaller compact groups and groups of 2-5 individuals of *M. myotis.* Until this time there is no record (written or oral) of a hibernating colony found so close to the main cave entrance. Most of the individuals were located in this area with only 30 other individuals scattered throughout the cave.

DISCUSSIONS

We have observed a lack of fidelity when it comes to the site of the hibernating colonies, with a tendency over the years for the colony to be sited in the more northern areas. NEGREA & NEGREA (1971) place the colony at the Dome Chamber, in the southern sector of the cave. In the same place, a hibernating colony was observed in 2004, while in 2005, there was no hibernating colony present (personal, unpublished observations). It is possible that changes in microclimate have taken place inside the cave, since in 2004 a new gate was placed, which reduced airflow, due to a solid area, with bars only on top, to allow bat access, while the previous gate was completely made of bars. CURRIE (2000) showed that placing gates, even bat-friendly ones can lead to the abandonment of shelters, because of the way they can drastically change microclimate.

By graphically overlapping all layers of information available for the study period, using ArcView, we have observed a clear preference for bat inhabitation of the first northern quarter of the cave, with most observations made in this area and also most of the population concentrated in it. This area is also most influenced by outside temperature. It is possible that this area offers the most advantageous microclimate conditions for hibernation, while also providing waking bats to evaluate outside conditions. Since we believe there has been a reduction in airflow in this area, it is possible that due to lower evaporation, good hibernating conditions have been created here (RANSOME, 1990).

Using the data collected on December 1, 2006 and December 31, 2006, we tried to see if there is a linear correlation between air temperature, rock temperature and the number of *R. ferrumequinum* by using the Pearson product-moment correlation coefficient (*r*). All obtained values were close to 0, which translated into no linear correlation. It is possible that there is a non-linear correlation between these variables. Data analysis has shown that 99% of total bats were found in areas where rock temperature was between 7 and 9°C and 95% of total bats were found in area where air temperature is between 7.8 and 9°C. Most bats were found in area where air temperature is close to rock temperature or higher. This leads to the conclusions that air temperature is a more important microclimate parameter because of the higher contact area.

CONCLUSIONS

Taking into consideration available data as well as observations made in this study we can say that Comarnic Cave is only used as a hibernacula and is also holds the largest known hibernating colony of *R. ferrumequinum* in the Semenic – Cheile Carașului National Park. The bat species dynamics is consistent with known data from the past, which takes us to the conclusion that the colonies are stable in terms of intraseasonal and multiannual dynamics. We believe that the winter dynamics of bats is determined, at least in part, by the sub-Mediterranean climate in which Comarnic Cave is located. It is possible that there are changes in the way that the cave is being used a shelter, but also due to lack of data it may be well within the normal limits. Because more data is required and also because it is one of the most important bat roosts in the area, further studies should be conducted. Using GIS has made it a lot easier to interpret data from several visits and also the relation between microclimate and bats.

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Received: March 31, 2016
Accepted: June 25, 2016

THERMAL COMFORT WITHIN OLTENIA PLAIN

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Abstract. Oltenia Plain, the western of the sector of the Romanian Plain, is characterized by a thermal regime marked by increased temperature during summer, when frequent heat waves occur, while in winter, temperature values may also decrease a lot, even if usually they maintain close to 0°C. In this context, the assessment of thermal comfort is very important, especially for the summer half of the year. Thus, there were calculated four indexes, namely thermohygrometric index (THI), DI ARAKAWA discomfort index (DIa), windchill equivalent temperature (WCET), relative strain index (RSI), in order to emphasize the bioclimatic comfort or discomfort. In spite of obvious similarities, the obtained values also indicate significant differences of the type of bioclimate specific to certain months. Cold stress is characteristic to winter, January in particular, as indicated by THI, DIa and WCET, while heat stress was confirmed by THI and DIa for July, August and, partially, June. RSI indicated bioclimatic comfort for average temperature and vapour pressure values, but when applied on some particular values, registered after 2000, discomfort for both more sensitive and all the people, clearly emerged. Even if the results do not indicate the same months characterized by thermal comfort, taking into account the values, the most balanced months are April, May, October and November. Spatially, it was noticed that cold stress is higher in the northern part of the plain, but there are not important differences in terms of heat stress.

Keywords: Oltenia Plain, thermohygrometric index (THI), DI ARAKAWA discomfort index (DIa), windchill equivalent temperature (WCET), relative strain index (RSI).

Rezumat. Confortul termic în Câmpia Olteniei. Câmpia Olteniei, care reprezintă sectorul vestic al Câmpiei Române, se caracterizează printr-un regim termic marcat de temperaturi ridicate pe perioada verii, când se produc frecvent şi valuri de căldură, în timp ce iarna, deşi temperaturile se menţin apropiate de pragul de 0°C, acestea pot să scadă destul de mult. În acest context, evaluarea stării de confort termic este foarte importantă, mai ales pentru semestrul cald. Astfel, pentru a reda confortul sau disconfortul termic au fost calculaţi patru indici, indicele termohigrometric (THI), indicele de disconfort DI ARAKAWA (DIa), temperatura echivalentă a puterii de răcire a vântului (WCET), indicele de tensiune relativă (RSI). În ciuda asemănărilor evidente, valorile obținute indică diferenţe semnificative în ceea ce priveşte tipul de bioclimat specific anumitor luni. Stresul termic indus de expunerea la frig este caracteristic iernii, mai ales lunii ianuarie, fapt confirmat de trei dintre indici – THI, DIa şi WCET, în timp ce stresul cauzat de căldură a fost confirmat de THI şi DIa pentru lunile iulie şi august şi, parțial, pentru iunie. RSI a indicat confort bioclimatic pentru valorile medii multianuale de temperatură şi tensiune a vaporilor de apă, dar disconfortul termic, atât pentru persoane mai sensibile, cât şi pentru întreaga populație, a fost în mod clar evidențiat pentru anumite valori înregistrate la nivel lunar după anul 2000. Chiar dacă rezultatele obținute nu indică aceleași luni ca fiind caracterizate de confort termic, plecând de la valorile medii, lunile cele mai echilibrate sunt aprilie, mai, octombrie şi noiembrie. Din punct de vedere teritorial, s-a constatat că stresul termic indus de expunerea la frig este mai ridicat în partea nordică a câmpiei, dar nu s-au înregistrat diferenţe semnificative în privinţa stresului indus de căldură.

Cuvinte cheie: Câmpia Olteniei, indicele termohigrometric (THI), indicele de disconfort DI ARAKAWA (DIa), temperatura echivalentă a puterii de răcire a vântului (WCET), indicele de tensiune relativă (RSI).

INTRODUCTION

At international level, thermal comfort is defined by ISO 7330-2005 as “that state of mind that expresses satisfaction with the thermal environment” (www.iso.org/obp/ui/#iso:std:iso:7730:en). Taking into account the definition, it is quite difficult to render thermal comfort as it depends on a wide range of factors, both environment-related and person-related factors. Environmental factors refer to air temperature (dry bulb temperature), air velocity, relative humidity, vapour pressure and radiant temperature (the temperature of the surfaces that usually surround a person), each of these factors conditioning the state of thermal comfort or discomfort. Personal factors are related to clothing, metabolic heat, the state of well-being or sickness, age group, etc. LEE & HENSCHEL (1966) defined comfort as thermal neutrality, general satisfaction, no anxiety, while discomfort refers to the sensations of heat and cold perceived by a person at a certain moment, thus being more difficult to globally quantify.

There were developed different biometeorological indexes and various models to render thermal comfort from different points of view, such as its influence on the general health state, work productivity, tourism, etc. Among more complex approaches, there can be mentioned MEMI, Munich Energy Balance Model for Individuals (HÖPPE, 1993), Man-Environment heat Exchange model MENEX_2005 (BLAZEJCZYK, 1994; 2005), Universal Thermal Climate Index – UTCI (initially developed by Commission 6 of the International Society of Biometeorology, http://www.utci.org/isb.php). Besides these models, there are more than 100 simple or complex indices (BLAZEJCZYK et al., 2012). Certain indexes highlight better thermal comfort all over the year (such as thermohygrometric index, DI ARAKAWA discomfort index, Physiological Equivalent Temperature, etc.), while others are more adequate for extreme seasons– summer (Summer Scharlau Index, Relative Strain Index, Summer SIMMER index, Heat index, Predicted Heat Strain, Temperature-Humidity Index, etc.) and winter (Winter Scharlau Index, Wind Chill Index, Windchill Equivalent Temperature, etc.). The purpose of the present study was to assess thermal comfort by using different indexes to emphasize if the obtain results highlight the same state of comfort or discomfort during the year, taking into account that the latest studies (DOBRINESCU et al., 2015) indicate a significant upward trend for certain indexes (THI, WCT).

MATERIAL AND METHODS

Oltenia Plain represents the western sector of the Romanian Plain, the largest plain unit of the country, located between the Getic Piedmont in the north and the Danube Valley in the south (Fig. 1). This relief unit does not present notable differences in terms of altitude or exposure to certain predominant air masses and thus, the main climatic parameters, such as temperature, humidity, wind speed and direction, relative humidity, vapour pressure, etc. register relatively reduced territorial differences. However, there emerge certain distinct features imposed by location and predominance of influence factors.

The considered bioclimatic indexes – thermohygrometric index (THI), DI ARAKAWA discomfort index (DIa), windchill equivalent temperature (WCET), relative strain index (RSI) were calculated based on mean monthly values of air temperature, vapour pressure, wind speed and relative humidity. These indexes highlight the effects of temperature combined with air humidity or wind speed upon the human organism for both extreme seasons – winter and summer. The data cover a mean period of forty years (1971-2010). The six stations are located in different sectors of Oltenia Plain (Fig. 1, Table 1).

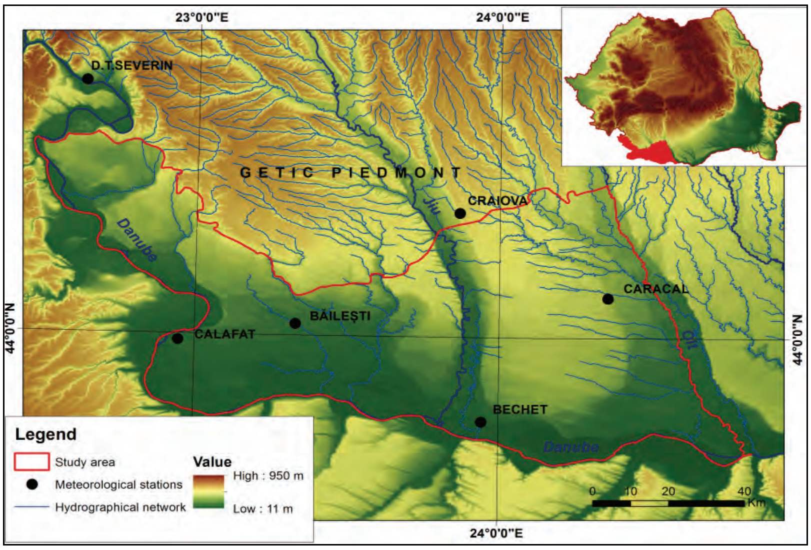

Figure 1. Location of the meteorological stations within Oltenia Plain.
Source: VLĂDUȚ & ONȚEL, 2013.

Table 1. Geographical coordinates of the considered meteorological stations.

No.	Station	Altitude (m)	Latitude	Longitude
1.	**Calafat**	61	43°59`	22°57`
2.	**Bechet**	36	43°47`	23°57`
3.	**Băilești**	57	44°01`	23°20`
4.	**Caracal**	106	44°06`	24°22`
5.	**Craiova**	192	44°19`	23°52`
6.	**Dr. T. Severin**	77	44°38`	22°38`

Thermohygrometric index (THI) (ºC) was developed by KYLE (1994). The formula used for the calculation of this index is:

$$THI\ (ºC) = T_d - (0.55 - 0.0055 \times R)\ (T_d - 14.5),\ \text{where:}$$

T_d = air temperature (ºC) (dry-bulb temperature);

R = relative humidity (%)

The comfort conditions are established according to the temperature perceived by the human body (Table 2). THI is one of the widely used indexes for rendering thermal comfort (UNGER, 1999; EMMANUEL, 2005; GRIGORE, 2013; IONAC & CIULACHE, 2008; TOY et al., 2007; TOY & YILMAZ, 2010).

DI ARAKAWA discomfort index (AGOSTINI et al., 2005) renders the combined effect of temperature and relative humidity upon the human body. The advantage of using this index consists in the fact that it emphasizes the discomfort state induced by both high and low temperatures (Table 3). In Romania, the index was applied by MIHALCA & ALEXE (2014) for Dornelor Depression. The formula used for the calculation of the index is:

$$DI_A = 0.81\ T_d + [0.01 \times R\ (0.99\ T_d + 14.3)] + 46.3\ \text{where:}$$

T_d = air temperature (ºC) (dry-bulb temperature);

R = relative humidity (%)

Table 2. THI values and the corresponding bioclimate type.

THI (°C)	Bioclimate type	THI (°C)	Bioclimate type
THI < – 40	Hyper-glacial	**13 < THI < 15**	Cool
–40 < THI < –20	Glacial	**15 < THI < 20**	Comfortable
–20 < THI ≤–10	Extremely cold	**20 < THI < 26.5**	Hot
–10 < THI ≤–1.8	Very cold	**26.5 < THI < 30**	Very hot
–1.8 < THI < 13	Cold	**THI > 30**	Torrid

Source: KYLE, 1994

Table 3. Bioclimatic discomfort according to DI ARAKAWA index.

DI ARAKAWA (units)	Bioclimatic discomfort
DIA < 55	Unbearably cold bioclimate
DIA = 55-60	Discomfort caused by cooling
DIA = 60-75	Bioclimatic comfort
DIA = 75-80	Discomfort caused by warming
DIA > 80	Unbearably hot bioclimate

Source: MIHALCA & ALEXE, 2014

Windchill equivalent temperature (WCET) represents the effective temperature air would reach at certain wind speeds. Initially, this index was proposed by SIPLE (1945) and developed by SIPLE & PASSEL (1945). The formula is:

$$T\ pr = [33 + (Td – 33) \times (0.474 + 0.454\sqrt{v} – 0.0454v)],$$ where

Td = air temperature (dry bulb temperature) (°C)
v = wind speed in m/s

The physiological effects, depending on the intensity of the caloric losses experienced by a human body, are rendered in Table 4.

Table 4. Windchill equivalent temperature (WCET) and its physiological effects.

WCET – T (°C)	Physiological effects
Twt > +10	Comfort
+10 ≥ Twt >-1	Slight discomfort
-1 ≥ Twt > -10	Increased discomfort
-10 ≥ Twt >-18	Very cold
-18 ≥ Twt >-29	Hypocaloric stress
-29 ≥ Twt >-50	Risk of frostbite in case of prolonged exposure
Tpr ≤-50	Risk of instantaneous frostbite

Source: TEODOREANU & MIHĂILĂ, 2012 apud. IONAC & CIULACHE, 2008

Relative strain index (RSI) was developed by SHAPIRO et al. (1982) taking into account a healthy person dressed with business suit at a place not acclimatized to direct heat in order to consider the clothing insulation. This index was calculated according to the formula:

$$RSI = (T_a - 21) / (58 - e) \quad (3),$$ where

T_a = air temperature
e = the water vapour pressure

RSI renders the effects of heat on the human organism, thus, being relevant for the summer months. In Romania, it was calculated for Moldova (IONAC, 2006), Dobrogea (IONAC & CIULACHE, 2007), Parâng Mountains (MERCIU, 2010). The bioclimatic discomfort based on the values of the RSI is rendered in Table 5.

Table 5. The Relative Strain Index- RSI (units).

Relative Strain Index	Bioclimatic discomfort
RSI ≤0.15	Bioclimatic comfort
0.15 ≤RSI ≤0.25	Bioclimatic discomfort for more sensitive persons*
0.25 ≤RSI ≤0.35	Bioclimatic discomfort for all persons
0.35 ≤RSI ≤0.45	Overheating risk for more than 50% of the population
RSI ≥0.45	Heat-stroke risk for all population
* In case of older and sick people, the RSI values equalling to 0.20 actually represent the maximum limit of tolerance, above which heat stroke is evident	

Source: IONAC, 2006

RESULTS

Physiologically, the human body permanently adapts to the outdoor conditions aiming at reaching the state of thermal comfort and wellbeing. However, in certain situations, heat or cold impose a higher stress on the human body than usually and thermal adaptation becomes difficult especially for certain age categories, such as children and elderly, or people that have health problems. In order to assess heat or cold stress, there were elaborated certain simple or complex indexes.

According to the *thermohygrometric index (THI)* values, comfort is registered between 15 and 20°C. Any values below 15°C indicate a certain state of discomfort induced by cold, while values above 20°C indicate discomfort produced by heat. The analysed region displays a similar bioclimate type during the year taking into account that there are not notable differences in terms of temperature and relative humidity, the two parameters considered in THI case. Thus, based on the obtained values, it results that there are only two months – May, respectively September characterized by thermal comfort within the entire Oltenia Plain. In June, the bioclimate is considered hot only in the south of the plain, where temperature and relative humidity are higher, while in the other sectors, the bioclimate is comfortable, but close to the hot threshold. The intervals January-April and October-December present a cold bioclimate, even if the values are mainly positive (the only negative values are registered in January) (Table 6).

Table 6. THI values within Oltenia Plain and the bioclimate type.

Station	1	2	3	4	5	6	7	8	9	10	11	12
D.T. Severin	1.1	3.3	7.6	12.5	16.9	19.8	21.4	21.1	17.5	12.4	7.2	2.7
Calafat	0.7	2.7	7.3	12.6	17.2	20.1	21.7	21.3	17.5	12.2	6.7	2.1
Bechet	-0.7	1.8	6.9	12.6	17.2	20.2	21.7	21.1	17.2	11.6	5.9	1.1
Băileşti	-0.4	1.9	6.9	12.4	17.2	20.0	21.5	21.0	17.1	11.6	6.0	1.1
Caracal	-0.9	1.5	6.5	12.1	16.9	20.0	21.6	21.1	17.2	11.8	6.0	0.7
Craiova	-0.8	1.7	6.4	11.8	16.5	19.5	21.1	20.7	16.9	11.7	6.0	0.8
Legend	Cold				Comfort				Hot			

DI ARAKAWA discomfort index (DIa) (units) can be used for rendering bioclimatic comfort for the entire year, the values ranging from < 55 units, which means unbearable cold bioclimate to > 80 units which means unbearably hot bioclimate. This index illustrates a higher variation compared to the THI. Thus, discomfort caused by cooling is characteristic to the winter months – December, January and February (except for December in the southwestern extremity, which is characterized by bioclimatic comfort). In fact, February and December are very close the 60 units threshold, displaying value above 59. Thermal comfort is characteristic to spring months – March and April and to November, while October is a month characterized by discomfort caused by warming (Table 7). The interval May – September registers more than 80 units and the bioclimate is unbearably hot, especially in July and August, when the 90 unit threshold is exceeded within the entire plain, except for the southwestern extremity and the central sector.

Table 7. DI ARAKAWA discomfort index values within Oltenia Plain and the bioclimate type.

Station	1	2	3	4	5	6	7	8	9	10	11	12
D.T. Severin	57.3	59.6	65.8	74.0	82.0	86.5	88.7	88.3	83.5	76.2	67.6	60.0
Calafat	56.8	59.9	66.4	74.5	83.0	87.6	90.0	90.1	84.5	76.5	67.6	59.7
Bechet	56.4	59.9	66.8	75.1	83.4	88.3	90.9	90.9	85.3	76.7	67.7	59.4
Băileşti	56.3	59.8	66.3	75.3	83.3	87.8	89.9	89.9	84.7	76.4	67.5	59.2
Caracal	56.0	59.9	66.4	75.1	83.6	88.6	90.8	90.3	84.1	76.5	68.1	59.4
Craiova	56.2	59.7	65.5	74.1	81.9	87.2	90.0	89.5	84.2	76.1	67.5	59.0
Legend	Discomfort caused by cooling			Bioclimatic comfort			Unbearably hot bioclimate			Discomfort caused by warming		

Windchill equivalent temperature (WCET) is a useful index in assessing thermal comfort during the cold season, based on air temperature and wind speed. Values above 10°C indicate comfort, while values below this threshold, different states of discomfort caused by cold exposure. Thus, within the analysed region, comfort characterizes the interval April-October, except for the northern sector, where the first and the last month display a slight discomfort. Increased discomfort is characteristic to January for most of the plain; in December and February, only the northern and northeastern sectors register such values, while in the rest of the region there is a slight discomfort. In March and November, the values oscillate between -0.2°C and 9.5°C, namely the physiological effects indicate a slight discomfort (Fig. 2).

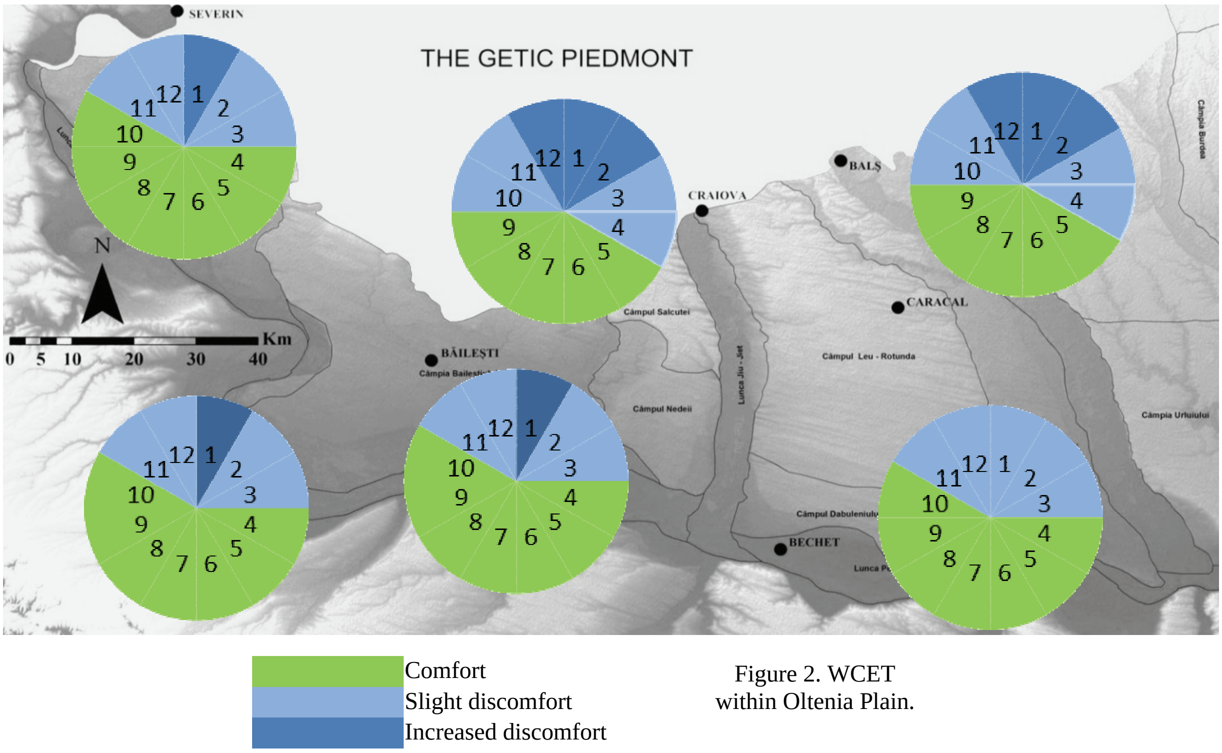

Figure 2. WCET within Oltenia Plain.

Relative strain index (RSI) is widely used for rendering the thermal comfort in summer months. Applied on monthly average values of temperature and vapour pressure, RSI emphasized values below 0.15 during the entire year, which means a comfortable bioclimate. Taking into account that after 2000, Oltenia region confronted with increasing temperatures and longer dry periods in summer, RSI was calculated for the months with average temperatures higher than 23°C. Thus, in the last ten years of the analysed period, it resulted that July and August in particular, registered values indicating bioclimatic discomfort for more sensitive persons (0.15 ≤RSI ≤0.25). In June, RSI values maintain within the accepted limits of bioclimatic comfort (RSI≤0.15) in most of the cases, the most exposed area being the southwestern one, where temperatures are usually higher. There should be noticed that in July, in the interval 2000-2004, RSI was higher than 0.15 within the entire plain, except for the central and northern part, while in August, the threshold was exceeded in 2000, 2001, 2003 and 2007 at all the stations. July 2007 registered the highest values indicating a bioclimatic discomfort for all persons (0.25 ≤RSI ≤0.35) (Table 8). This situation was confirmed by previous studies (VLĂDUŢ, 2011). Consequently, in summer, Oltenia Plain has been exposed to a quite increased risk of overheating.

CONCLUSIONS

The main purpose of the present study was to render certain aspects related to thermal comfort within Oltenia Plain and to compare the type of bioclimate indicated by different indexes.

According to the obtained results, there emerged some common characteristics, but also notable differences. Thus, both THI and DIa, indicate cold stress during all winter months, while heat stress is characteristic to July and August, and only partially to June (in the south of the plain, along the Danube). In case of DIa, heat stress is also registered in May and September, while THI indicates these two months as comfortable months. Bioclimatic comfort is characteristic to March, April, November and we may consider even October (as the values are quite close to 75 units) according to DIa, but the same months present a cold bioclimate according to THI. In order to clarify the differences between these two indexes, there were also used another two indexes, namely WCET and RSI, which better emphasize thermal stress during extreme seasons, winter and summer.

Table 8 Bioclimatic discomfort rendered by RSI.

D.T. Severin	0.16/2000; 0.16/2002 0.22/2003; 0.18/2007	0.18/2000; 0.22/2001 0.22/2002; 0.18/2003 0.17/2004; **0.26/2007***	0.22/2000; 0.24/2001 0.23/2003; 0.19/2007
Calafat	0.17/2000; 0.17/2002 0.20/2003; 0.21/2007	0.22/2000; 0.22/2001 0.22/2002; 0.19/2003 0.18/2004; **0.28/2007***	0.24/2000; 0.23/2001 0.23/2003; 0.17/2004 0.19/2007
Bechet	0.16/2002; 0.19/2003 0.19/2007	0.22/2000; 0.23/2001 0.22/2002; 0.18/2003 0.17/2004; **0.25/2007***	0.22/2000; 0.24/2001 0.22/2003; 0.17/2007

Băileşti	0.17/2002; 0.19/2003 0.23/2007	0.18/2000; 0.20/2001 0.18/2003; **0.28/2007***	0.19/2000; 0.22/2001 0.26/2003; 0.19/2007
Caracal	0.16/2000; 0.19/2003 0.19/2007	0.21/2000; 0.23/2001 0.21/2002; 0.18/2003 0.17/2004; **0.28/2007***	0.21/2000; 0.24/2001 0.22/2003; 0.18/2007
Craiova	0.16/2003; 0.17/2007	0.18/2000; 0.19/2001 0.19/2002; **0.26/2007***	0.21/2000; 0.22/2001 0.20/2003; 0.17/2007
*** Bioclimatic discomfort for all persons**			

Thus, cold stress is confirmed for winter months, but only January is characterized by increased discomfort within the entire analysed region, while February and December only in the northern and northeastern sectors, at Craiova and Caracal. In this sector, a slight discomfort is also registered in April and October, which are comfortable months in the other sectors. Heat stress was not confirmed by RSI when applied on monthly average values of temperature and vapour pressure. However, in the last ten years of the analysed interval, there were numerous cases (2000, 2002, 2003), when summer months were characterized by bioclimatic discomfort for sensitive persons or for all the persons (2007), confirming in this way the increased occurrence of heat stress.

Spatially, the results for the used indexes do not indicate notable differences within the analysed region, taking into account that the average monthly values of temperature, air velocity, relative humidity and vapour pressure display close values. However, cold stress or thermal discomfort induced by cooling affects the northern part of the plain more intensely and on longer periods (from December to February compared to January in the rest of the plain). Heat exposure induces discomfort during all the summer months within the entire plain and on longer periods (July and August and partially June). Thus, on the whole, the analysed region is more exposed to heat stress than to cold stress.

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Received: March 19, 2016

Accepted: May 12, 2016

STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF BIOCOENOSES IN THE FLOODED AREA OF THE DANUBE FOR THE BIOTOPE RECONSTRUCTION FROM INDUSTRIAL CONTAMINATED HABITATS

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Abstract. The floodplain of the Danube (811 – 661 km), commonly known as the Oltenian sector, is the benchmark that conserves the biocoenotic structures specific to wet areas. Even though the damming work of the river caused fundamental changes in the structure and functioning of the floodplain, the mentioned sector is characterized both by the new structural elements that are the result of the river embankment and especially by the existence of old specific ecosystem formations. In this regard, it is relevant to mention the specific terrestrial ecosystems: dunes, interdunes, meadows, forests, hayfields and the aquatic ecosystems represented by lakes, ponds, streams, swamps. Corresponding to this ecosystem diversity there are characteristic structures of flora and fauna communities. Research carried out on the extreme habitats has revealed the presence of some physiological groups of organisms that acquired a perfect adaptation to the physico-chemical characteristics of the substrates. The growth and multiplication of bacteria in the extreme biotopes, as well as the intensity of their activity depend to a fullest extent on the ecological conditions of the environment. The wealth of the species of microorganisms, invertebrates and plants represents a natural patrimony that must be protected taking into account its importance in ecogenetic and social development.

Keywords: microorganisms**,** invertebrates, plants, terrestrial ecosystems**,** the Danube, Romania.

Rezumat. Caracteristicile structurale şi funcţionale ale biocenozelor din zona inundabilă a Dunării pentru reconstrucţia biotopului din habitate contaminate industrial. Zona inundabilă a Dunării (Km 811 – 661), cunoscută sub denumirea de sectorul oltean, reprezintă un etalon care conservă structurile biocenotice specifice zonelor umede. Cu toate că lucrările de îndiguire a fluviului au determinat modificări fundamentale în structura şi funcţionarea zonei inundabile, sectorul menţionat se caracterizează atât prin noile elemente structurale apărute în urma îndiguirii fluviului cât mai ales prin existenţa vechilor formaţiuni ecosistemice specifice. În acest sens sunt relevante ecosistemele terestre specifice: dune, interdune, pajişti, păduri, fâneţe şi ecosistemele acvatice reprezentate prin lacuri, bălţi, gârle, mlaştini. Corespunzător acestei diversităţi ecosistemice îi sunt caracteristice structuri floristice şi faunistice. Cercetările efectuate asupra biotopurilor extreme au evidenţiat prezenţa cu pregnanţă a anumitor grupe fiziologice de organisme, care au dobândit o perfectă adaptare la caracteristicile fizico-chimice ale substratelor. Creşterea şi multiplicarea bacteriilor în biotopurile extreme, cât şi intensitatea activităţii lor, depind în cea mai mare măsură de condiţiile ecologice ale mediului. Bogăţia de specii de microorganisme, nevertebrate şi plante reprezintă un patrimoniu natural ce trebuie protejat având în vedere importanţa sa in dezvoltarea ecologică şi socială.

Cuvinte cheie: microorganisme, nevertebrate, plante, ecosisteme terestre, Dunăre, România.

INTRODUCTION

The floodplain of the Danube (811 – 661 Km) known as the Oltenian sector sums up an area of 104,543 ha and includes a great diversity of physico-geographical, hydrological and hydrobiological elements present along the lower sector of the Danube (Fig. 1) (BREZEANU, 1967; TOMESCU, 1998; CIOBOIU & BREZEANU, 2008).

In the context of works carried out during 1965 – 1971, on 90 % of the total area of the floodplain of the Oltenian sector, there were performed complex works for land improvement that led to the disappearance of lacustrine ecosystems, deforestation and setting up fishing ponds. In this circumstance, due to these changes, the old morphology of the floodplain transformed so as to correspond with the needs of agricultural development. In this regard, there appeared anthropogenic forms of relief. Million m^3 of excavated lands used to build the longitudinal dyke and transversal dykes delimited the dammed areas of the agricultural lands. The negative forms of relief represented by the network of drainage channels or depression cuvettes of the former lacustrine basins: Potelu, Rast, Nedeia – Nasta – Sclavogul Complex became real fields of lacustrine origin transformed into agricultural lands (BREZEANU & MARINESCU, 1965).

The current situation demonstrates that, due to the special physical and geographical conditions of the area, the dam and transformation works of the floodplain in the agricultural area, at least in the sector between 811 – 661 km, did not give the expected results. In other words, the agricultural function of the floodplain, which excluded all other uses, proved to be inefficient from the economic viewpoint.

It is known that the areas liable to flooding represent depositories of a large biodiversity. The areas liable to flooding of the Danube are ones of the most important wetlands in Europe.

The studied sector includes all characteristics that make this part of the floodplain be considered a benchmark for the reconstruction of the ecosystems in the southern part of Romania (Fig. 2). This fact is reflected by the structures and functions of specific ecosystems in the area liable to flooding. There are integrated specific terrestrial ecosystems: dunes, interdunes, meadows, forests, hayfields and aquatic ecosystems represented by lakes, ponds, streams, swamps. Floristic and faunistic structures are characteristic to this ecosystemic diversity.

Figure 1. The Oltenian Sector of the Danube Floodplain from the Territory of Dolj County (Google Earth, accessed: March 21, 2016).

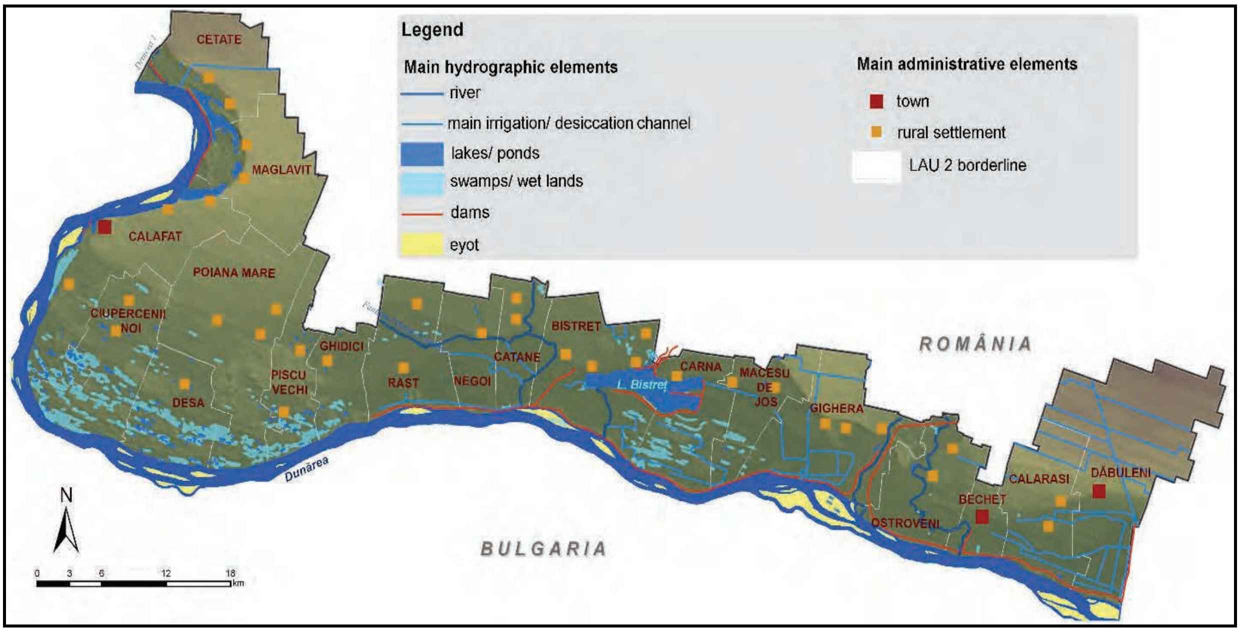

Figure 2. Main hydrographical characteristics of the Danube Floodplain in the Oltenian Sector (after POPESCU et al., 2015).

Microorganisms were isolated from all natural environments where water is present regardless of its temperature and pH values. The isolation and characterization of mesophilic, thermophilic and hyperthermophilic microorganisms generated several studies, mainly focused on highlighting the ways of their adaptation to high temperatures. The natural communities of microorganisms formed by a large variety of species that live together, frequently as dense populations, are relatively stable and hard to disturb. The indigenous microorganisms oppose to the disequilibrium produced by temporary ecological changes (for example, discharge of waste waters through soil or into natural waters). On the basis of knowledge regarding the nature of the interactions between acidophilic microorganisms, it is likely to design the microbial consortia for the biotope reconstruction of contaminated industrial areas.

The evolution of the technology, deepening knowledge of the microbial diversity and the action of microorganisms on different natural substrates allowed the implementation of economic biotechnological processes with low polluting effects. The populations of microorganisms from a particular habitat establish a series of interpopulation relationships leading to the formation of a definite structure of a stable community at a certain level of diversity, which ensures the optimum use of energy flow from the ecosystems and a certain homeostasis, namely a degree of invariance and resistance to the environmental disturbing factors. In this way, the diversity of a community reflects the state of the population interactions from the community in dynamic terms (AXINI, 2012; CISMAŞIU et al., 2002; CISMAŞIU, 2003, 2004, 2009).

MATERIALS AND METHODS

1. Technology selection

In view of assessing the hydrobiological characteristics, there has been performed an extensive research program. Based on the existing data in the specialized literature and on our own research, it was made a summary which allowed the monitoring of the lacustrine ecosystem biodiversity from the studied sector (ANTIPA, 1910; BREZEANU & MARINESCU P., 1965; BREZEANU, 1967; NEGREA & NEGREA, 1975; GROSSU, 1993; TIŢĂ & NĂSTASE, 1997; TOMESCU, 1998; NICOLESCU et al., 1999; RÂŞNOVEANU & VĂDINEANU, 2000; PARPALĂ et al., 2002; PLENICEANU, 2003; BREZEANU et al., 2011; CIOBOIU, 2002, 2003, 2004, 2008, 2015; IONICĂ, 2007; GAVRILESCU, 2008; CIOBOIU & BREZEANU, 2008, 2014).

2. Mechanisms for metal removal

The cooperating interactions between microorganisms, invertebrates and plants in extreme conditions (acidic pH and higher concentrations of metallic ions) have a great ecological importance, particularly in achieving the circulation of biogeochemical elements in the biotope reconstruction from the Oltenian sector of the Danube. The formation of microorganism colonies is probably an adaptation based on cooperative interactions in the population. The production of extracellular enzymes by some members of colonies makes substrates available to all members of the population. The formation of colonies ensures not only aggregating individual organisms, but also to the efficient use of available resources. Thus, the positive interactions are especially important in nature, where the recalcitrant substances or the production of metabolites solubilizes the various compounds of the habitat, by making them available for other microorganisms (JOHNSON & RANG, 1993; ZARNEA, 1994; LAZĂR et al., 1997; JOHNSON, 1999; OBASOHAN et al., 2010; JUNG et al., 2014).

RESULTS AND DISCUSSION

1. Site and technology description

The previous researches and those carried at present emphasize the structural and functional characteristics of the area between 811 and 661 km, which is the premises of the ecological reconstruction (TOMESCU, 1998; PLENICEANU, 2003; BREZEANU et al., 2011).

According to the evaluation of the biodiversity of this area, it may be determined which are the plant and animal species specific to each type of ecosystem. In regard to terrestrial ecosystems (dunes, interdunes, meadows, forests, grassland) the characteristic species are: plants *Salix* sp., *Populus* sp., *Ulmus minor* Mill., *Fraxinus excelsior* L., *Acer campestre* L., *Rosa canina* L. *Chrysopogon gryllus* (L.) Trin., *Salsoa kali* L., *Plantago scabra* Moench., *Centaurea arenaria* Bieb., *Secale sylvestre* Host., *Festuca vaginata* Waldst et Kit., *Euphorbia cyparissias* L., *Silene conica* L., *S. trinervia* L., *Trifolium arvense* L., *Dianthus kladovanus* Degen., *Bassia laniflora* (S. G. Gmelin) A. J. Scott., *Corispermum nitidum* Kit., *Polygonum arenarium* Waldst et Kit., *Puccinelia distans* (Jacq.) Parl., *Salicornia europaea* L., *Juncus gerrardi* Lois. (TIŢĂ & NĂSTASE, 1997), animals *Helicella candicans* L. Pfeiffer, *Cepaea vindobonensis* (Fer.), *Helix pomatia* L., *Cerambyx cerdo* L., *Lucanus cervus* L., *Nimphalis vaualbum* L., *Bombina variega* (L.), *Bufo bufo* (L.), *Natrix natrix* L., *Testudo hermani* Gmelin, *Accipiter nisus* (L.), *Buteo buteo* (L.), *Coturnix coturnix* (L.), *Phasianus colchicus* (L.), *Cuculus canorus* (L.), *Asio flammeus* (Pontopp.), *Upupa epops* (L.), *Picus viridis* (L.), *Galerida cristata* (L.), *Anthus campestris* (L.), *Lanius excubitor* (L.), *Garrulus glandarius* (L.), *Pica pica* (L.), *Corvus monedula* (L.), *Citellus citellus* Pal., *Cricetus cricetus* L., *Vulpes vulpes* L., *Canis aureus* L. (TOMESCU, 1998; CIOBOIU, 2004).

The floristic and faunistic structure of the lacustrine ecosystems is particularly diverse. The biggest diversity belongs to algae represented by the groups Cyanophyceae, Euglenophyceae, Pyrrophyceae, Heterokontae, Bacillaryophyceae and Chlorophyceae; the largest number of species is registered by Cyanophyceae, Bacillariophyceae and Chlorophyceae (NICOLESCU et al., 1999; MOLDOVEANU & FLORESCU, 2013).

Marsh and aquatic macrophytes occupy an important place in the bioeconomy of ecosystems, the dominant species being *Phragmites communis*, *Typha angustifolia*, *Nuphar luteum*, *Nymphaea alba*, *Rorippa amphibia*, *Poygonium amphibium*, *Iris pseudacorus*, *Equisetum arvense*, *Euphorbia palustris*, *Scirpus lacustris*, *Carex riparia*, *Ranunculus aquatilis*, *Salvinia natans*, *Stratiotes alloides*, *Myriophylum spicatum*, *Hydrocharis morsus-ranae* (DIHORU & ARDELEAN, 2009) (Table 1, Fig. 3).

The fauna includes 24 groups of invertebrates, the dominating ones being protozoa (*Vorticella* sp.), rotifers (*Filinia longiseta* Ehrbg., *Brachionus angularis* Gosse, *Keratella quadrata* Muller), copepods (*Acanthocyclops vernalis* L., *Cyclops leuckarti* Claus), cladocerans (*Bosmina longirostris* Fish., *Ceriodaphnia pulchella* Muller, *Moina brachiata* Jurine), polychaeta (*Hypania invalida* Grube, *Hypaniola kowalewskii* Grimm.), oligochaete (*Eiseniella tetraedra* Sav., *Nais simplex* Pig., *Stylaria lacustris* Mull.), gastropods, bivalves (*Unio tumidus* Philip., *U. pictorum* L., *Anodonta cygnaea* L., *A. c. piscinalis* Nils., *Sphaerium riviculum* Lam., *S. corneum* L., *Dreissena polymorpha* Pall.), isopoda (*Jaera sarsi sarsi* Valk.), amphipods (*Corophium curvispinum* Sars, *Chaetogammarus tunellus* Mart., *Dikerogammarus* *haemobaphes fluviatilis* Mart., *Pontogammarus obesus* Mart.), dragonflies (*Ghamphus flavipes* Charp.), chironomida (*Pelopia punctipennis* Mg., *Cricotopus silvestris* F., *Diamesa campestris* Edw., *Prodiamesa olivacea* Mg., *Cryptochironomus demeijeri* Krus., *Tanytarsus exiguus* Joh.) (BREZEANU & MARINESCU P., 1965; NEGREA & NEGREA, 1975; RÂŞNOVEANU & VĂDINEANU, 2000; PARPALĂ et al., 2002; CIOBOIU, 2004, 2015).

Table 1. The species of marsh and aquatic macrophytes.

SPECIES	
MARSH	**AQUATIC**
Phragmites communis Trin.	*Lemna minor* L.
Typha angustifolia L.	*Nimphaea alba* L.
Typha latifolia L.	*Nuphar luteum* L.
Scirpus lacustris L.	*Polygonium amphibium* L.
Heleocharis palustris L.	*Potamogeton natans* L.
Juncus effusus L.	*Potamogeton crispus* L.
Mentha aquatica L.	*Potamogeton perfoliatus* L.
Mentha longifolia L.	*Potamogeton pectinatus* L.
Iris pseudacorus L.	*Salvinia natans* L.
Carex riparia L.	*Stratiodes aloides* L.
Carex hirta L.	*Schoenoplectus mucronatus* L.
Ranunculus aquatilis L.	*Myriophyllum spicatum* L.
Ranunculus repens L.	*Ceratophyllum submersum* L.
Polygonium hydropiper L.	*Hydrocharis morsus-ranae* L.
Pastinaca sativa L.	*Glyceria maxima* L.
Vicia peregrina L.	*Rorippa amphibia* (L.) Besser
Equisetum arvense L.	
Euphorbia palustris L.	

Figure 3. The area of the island invaded by macrophytes (original).

In the scientific literature it is well known that one of the fundamental factors of floodplain life is the Danube, the interdependence of which contributes to the structuring and functioning of planktonic and benthic populations. The river water entering periodically into the lakes is the engine of the productivity and the organic production in the aquatic ecosystems, leading to the development of primary producers and further through these to the consumers.

A major component of the biological production from the lacustrine ecosystems in the area is represented by the populations of gastropods, preponderantly the benthic organisms that respond actively to the heterogeneity of the microhabitats they inhabit. There was identified a number of 25 species (Table 2), among which *Viviparus acerosus*, *Radix balthica*, *Physella* (*Costatella*) *acuta*, *Lymnaea stagnalis*, *Planorbarius coneus* are characteristic to the eutrophic lacustrine ecosystems from the area liable to flooding (GROSSU, 1993; CIOBOIU, 2002, 2008, 2015).

The gastropods spread in the lakes and marshes of the area liable to flooding taken in the study is determined by the nature of the benthal facies as rendered in table 3; thus, in the muddy facies, there were 18 species, 15 species in the detritus and 9 species in the sandy facies. It is noted that the largest number of species populates the muddy – detritus bottom near the shore in shallow areas (Fig. 4). These areas present the best possible conditions for food. Gastropods find abundant food on the coarse detritus, on the leaves fallen in the water but still unaffected by putrefaction on which a rich develops and on the silt rich in organic substances (NEGREA & NEGREA, 1975; CIOBOIU & BREZEANU, 2014).

The smallest diversity of species was determined in the areas where the substrate is predominantly sandy. Certainly, their distribution depending on the facies is relative, because the species from a certain category may be present (in low number) in other types of facies as well considering that there exists some degree of interference between the categories of the typical facies.

Table 2. The taxonomic diversity of gastropods.

CLASS GASTROPODA Cuvier 1798	
SUBCLASS PROSOBRANCHIA Milne Edward 1848	
ORDER ARCHAEOGASTROPODA (Thiele 1952)	
Family Neritidae Rafinesque 1815	*Theodoxus danubialis* C. Pfeiffer 1828 *Theodoxus fluviatilis* Linnaeus 1758
ORDER MESOGASTROPODA (Thiele 1925)	
Family Viviparidae Gray 1847	*Viviparus acerosus* Bourguignat 1870 *Viviparus viviparus* Linnaeus 1758
Family Valvatidae Thomson 1840	*Valvata (Cincina) piscinalis* O. F. Muller 1774
Family Lithoglyphidae Troschel 1857	*Lithoglyphus naticoides* C. Pfeiffer 1828
Family Bithyniidae Gray 1849	*Bithynia tentaculata* Linnaeus 1758
Family Thiaridae Troschel 1857	*Esperiana esperi* (Ferussac 1829) *Esperiana (Microcolpia) daudebardii acicularis* Ferussac 1823
SUBCLASS PULMONATA Cuvier 1817	
ORDER BASOMMATOPHORA A. Schmidt 1855	
Family Physidae Fitzinger 1833	*Physa fontinalis* (Linnaeus 1758) *Physella (Costatella) acuta* (Draparnaud 1805) *Aplexa hypnorum* (Linnaeus, 1758)
Family Lymnaeidae Rafinesque 1815	*Lymnaea stagnalis* (Linnaeus 1758) *Stagnicola palustris* (O. F. Muller 1774) *Stagnicola corvus* Gmelin 1788 *Radix auricularia* (Linnaeus 1758) *Radix ampla* (Draparnaud 1805) *Radix balthica* (Linnaeus 1758) *Galba truncatula* (O. F. Muller 1774)
Family Ancylidae Rafinesque 1815	*Ancylus fluviatilis* O. F. Muller 1774
Family Acroloxidae Thiele 1931	*Acroloxus lacustris* (Linnaeus 1758)
Family Planorbidae Rafinesque 1815	*Planorbis planorbis* (Linnaeus 1758) *Anisus (Anisus) spirorbis* (Linnaeus 1758) *Segmentina nitida* (O. F. Muller 1774) *Planorbarius corneus* (Linnaeus 1758)

Table 3. The taxonomic composition according to the benthal facies.

SPECIES	**BENTHAL FACIES**		
	SANDY	**MUDDY**	**DETRITUS**
Theodoxus danubialis	+	+	
Theodoxus fluviatilis	+		
Viviparus acerosus		+	+
Viviparus viviparus	+	+	
Valvata (Cincina) piscinalis		+	+
Lithoglyphus naticoides		+	+
Bithynia tentaculata		+	
Esperiana esperi	+	+	
Esperiana (Microcolpia) daudebardii acicularis	+	+	+
Physa fontinalis			+
Physella (Costatella) acuta		+	+
Aplexa hypnorum			+
Lymnaea stagnalis		+	+
Stagnicola palustris	+	+	
Stagnicola corvus			+
Radix auricularia		+	+
Radix ampla		+	+
Radix balthica	+		+
Galba truncatula	+	+	
Ancylus fluviatilis	+		
Acroloxus lacustris		+	
Planorbis planorbis		+	+
Anisus (A.) spirorbis		+	
Segmentina nitida			+
Planorbarius corneus		+	+

Figure 4. The preferred areas of gastropods (original).

The fish populations are characteristic to the lakes and ponds in this region: *Esox lucius* L., *Rutilus rutilus* Raf., *Scardinius erythrophthalmus* L., *Aspius aspius* L., *Chondrostoma nasus* L., *Leucaspius delineatus* Heckel, *Alburnus alburnus* Raf.., *Cobitis taenia* L., *Pelecus cultratus* L., *Lepomis gibbosus* L., *Carassius gibelio* Bloch, *Cyprinus carpio* L., *Silurus glanis* L., *Perca fluviatilis* L., *Stizostedion lucioperca* L. (BREZEANU et al., 2011).

2. Mechanisms for metal reduction potential

The exploitation of microorganism diversity in the biotechnology depends on the efficient way of detecting new microorganisms and their useful activities in natural environments. The industrial and agro-zootechnical development leads to the generation of impressive volumes of waste waters contaminated with metallic ions, sulphates, nitrates/nitrites and other pollutants that affect the environment. As a result of the extraction and advanced processing of ores there result significant quantities of solid, semisolid or liquids waste, which contains high concentrations of toxic elements. These, under the influence of physical, chemical and biological agents (solubilisation) are mobilized and migrate around horizontally, contaminating the soil, lakes and rivers from the vicinity or, vertically, contaminating the aquifers.

In the last decade, the discovery of the microorganism capacity to degrade a variety of synthetic organic compounds into inorganic products contributed to the development of bioremediation technologies. The plasticity of microbial metabolism represents a higher adaptive character, with ecological significance. The accumulated data demonstrate that acidophilic chemosynthetic bacteria underlie a food chain, which allows the abundant development of some populations by organisms specifically adapted for growth in the vicinity of industrial contamination sources from the Oltenia Plain. From the ecological point of view, it is an absolutely original phenomenon due to its total independence by energy from the sun. Although they are concentrated on limited area, these communities develop very efficiently benefiting from the position of primary producers of the bacteria, which ensures the transformation of the geothermal energy into chemical energy required to generate the organic carbon.

According to their behaviour to molecular oxygen, acidophilic microorganisms can be grouped into 4 respiratory types: (1) strictly aerobic microorganisms, which require for their development atmospheric molecular oxygen that they use as a final acceptor of electrons in the course of cellular respiration; (2) strictly anaerobic microorganisms which cannot grow in the presence of molecular oxygen; (3) anaerobic microorganisms, aerobic optionally, capable to orient their metabolism according to the availability of oxygen; (4) microaerophilic microorganisms that require a lower quantity of O_2 than the one of the atmospheric air. In this context, acidophilic heterotrophic bacteria performed incomplete oxidation of organic substances resulting from the fermentation of alcohols, aldehydes and ketones. In this process, there are produced organic acids such as: pyruvic acid, fumaric acid, citric acid, glycolic acid, oxalic acid.

Acidophilic chemolithotrophic bacteria have the capacity to produce energy through the oxidation of inorganic compounds, such as: hydrogen, iron, the reduced compounds of sulfur and nitrogen. Acidophilic microorganisms have the ability to metabolize organic compounds through the oxidation-reduction reaction with the formation of less complex organic substances. In these metabolic reactions, both the donors as well the acceptors of electrons are organic compounds.

Acidophilic heterotrophic and chemolithotrophic bacteria act as biochemical agents by two mechanisms which complement one another: (1) secretion of acids which dissociate-mineral elements (silicates of aluminium, potassium, iron, magnesium) that make up the rocks; (2) introduction of organic acids containing carboxyl group, hydroxyl group or amino group, resulting from the catabolism of organic residues or secreted by microorganisms into the environment (PETRIŞOR et al., 1997, 2000, 2002; ZLĂGNEAN et al., 2007; TOMUŞ & CISMAŞIU, 2014).

Certain global ecological processes can concentrate different toxic substances, through the complex biological processes. In these processes, microorganisms isolated from extreme environments due to their metabolic activity and the higher report of surface/volume can act as important vectors for the introduction of heavy metals in the trophic networking (AL-AZKI, 2014).

The discovery of biological detoxification mechanisms has suggested the possibility of using them to control the pollution with heavy metals. Thus, heavy metals may have important environmental effects on the growth of biological species, but in a certain concentration range. Nevertheless, there are several heavy metals with noxious effect on aquatic ecosystems (VOICU et al., 1999a, b; 2000; 2009; STANCU, 2015).

CONCLUSIONS

The area liable to flooding of the Danube between 811 and 661 Km is characterized by a diversity of ecosystem types specific to wet areas. Due to this diversity of ecosystems, there are characteristic flora and fauna structures. One of the fundamental factors of the floodplain life is the Danube, the interdependence of which contributes to the structuring and functioning of planktonic and benthic populations.

The fauna includes 24 groups of invertebrates, protozoa, rotifers, copepods, cladocerans, oligochaete, gastropods, bivalves, amphipods, dragonflies, chironomida being predominant. The populations of gastropods constitute an important part of the biological production of the eutrophic lacustrine ecosystems in the area. The ichtyofauna characterizes and particularizes the functionality of such ecosystems pre-existing the impoundment of the river.

The compounds of the heavy metals have an obvious influence on plants, especially near the metallurgical companies; the limits of the heavy metal toxicity are established according to their influence effects on plants. The toxicity of heavy metals depends on the component size of the soil, the soil acidity and soil moisture, the ratio between the metals and nutritional elements from the environment as well as on individual plant species. Thus, it has been found that their maximum tolerance to different metal ion concentrations is achieved only under certain conditions of temperature and pH.

ACKNOWLEDGEMENT

The study is the result of the collaboration between the Institute of Biology Bucharest, Department of Microbiology and the Oltenia Museum Craiova, respectively the collaboration agreements 1797 / 20.05.2015 and 1402 / 21.05.2015 with the theme: ***The biodiversity of the microbiota from areas with the industrial contamination of Oltenia and potential biotechnological applications in order to reduce it.***

The paper is dedicated the anniversary of 150 years since the establishment of the Romanian Academy. The presented study is part of the project nr. RO1567-IBB05/2016 developed at the Institute of Biology Bucharest of Romanian Academy.

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Received: March 31, 2016
Accepted: June 19, 2016

THE INFLUENCE OF NICKEL AND CADMIUM COMPOUNDS ON GAMETOPHYTE DIFFERENTIATION IN *Dryopteris affinis* (Lowe) Fraser-Jenkins AND *Dryopteris filix-mas* (L.) Schott

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Abstract. The aim of this research was to study the influence of compounds containing Ni and Cd on gametophyte differentiation in two native ferns, *Dryopteris affinis* (Lowe) Fraser-Jenkins and *D. filix-mas* (L.) Schott. The variants tested were V_1Cd: 3 mg·L^{-1} Cd acetate, V_2Cd: 15 mg·L^{-1} Cd acetate, V_3Cd: 30 mg·L^{-1} Cd acetate, V_1Ni: 75 mg·L^{-1} Ni sulphate, V_2Ni: 375 mg·L^{-1} Ni sulphate, V_3Ni: 750 mg·L^{-1} Ni sulphate and Control. The Pearson correlation index showed a significant negative correlation between the percentage of germinated spores and the heavy metal concentration in the culture medium, in both species. The main changes observed in the gametophyte were: a longer period needed to reach the characteristic stages, damage to rhizoid elongation, the formation of three-dimensional cell masses, chlorosis and necrosis of the gametophyte cells, and accumulation of crystal structures on the surface of the gametophyte.

Keywords: spore germination, chlorosis, necrosis, metal accumulation.

Rezumat. Influența compușilor cu nichel și cadmiu asupra diferențierii gametofitului la *Dryopteris affinis* (Lowe) Fraser-Jenkins și *Dryopteris filix-mas* (L.) Schott. Scopul acestei lucrări a fost acela de a studia influența compușilor ce conțin nichel și cadmiu asupra diferențierii gametofitului la două specii native de ferigi, *Dryopteris affinis* (Lowe) Fraser-Jenkins și *D. filix-mas* (L.) Schott. Variantele experimentale testate au fost V_1Cd: 3 mg·L^{-1} acetate de Cd, V_2Cd: 15 mg·L^{-1} acetate de Cd, V_3Cd: 30 mg·L^{-1} acetate de Cd, V_1Ni: 75 mg·L^{-1} sulfat de Ni, V_2Ni: 375mg·L^{-1} sulfat de Ni, V_3Ni: 750 mg·L^{-1} sulfat de Ni și varianta Martor. Indicele de corelație Pearson arată o corelație negativă între procentul de spori germinați și concentrația metalului greu din mediul de cultură, în cazul ambelor specii. Principalele modificări observate la nivelul gametofitului au fost: o perioadă mai lungă de timp necesară pentru atingerea stadiilor caracteristice, alterarea alungirii rizoizilor, formarea de mase celulare tridimensionale, cloroze și necroze ale celulelor gametofitului, acumularea de structuri cristaline pe suprafața gametofitului.

Cuvinte cheie: germinația sporilor, cloroze, necroze, acumulare de metal.

INTRODUCTION

Nickel (Ni) and cadmium (Cd) are heavy metals found in the Earth's crust, occupying, in point of their relative abundance, in 24th and, 64th place, respectively. The natural sources of pollution are volcanic emissions, vegetation fires, dust or powder resulting from weather conditions affecting rocks and soil. The anthropogenic sources are varied: Cd results from mining and ore processing, from chemical plants, fertilizers, paints, metal plating, dyes, and oil refining, and Ni - from the pulp and paper industry, dyeing and textile printing, chemical fertilizer industry, and waste incineration (AGARWAL, 2009), as well as from other common sources: metallurgy, coal burning, and Ni-Cd batteries. Sewage sludge and the effluents used to irrigate crops lead to increased concentrations of heavy metals in the soil: thus, CHAUDRI et al. (2001) and AHMAD et al. (2015) concluded that the relationship between the concentration of soluble Cd in the soil and grain crops became more linear after the application of sludge, and SMOLEN et al. (2010) and AHMAD et al. (2015) found that there are moderate to strong positive correlations between the concentrations of Fe, Cd, Co and Pb in the crops (spinach and lettuce) and the soil.

According to the United States Geological Survey (USGS), the countries that produce the largest amount of Cd are in Asia: China, Korea, and Japan. It is again Asia that dominates with respect to the primary production of Ni in 2013, with 921 tonnes, followed by Europe with 495.4 tonnes, and America with 268.6 tonnes (according to the International Nickel Study Group - INSG).

Heavy metals can be transmitted along the food chain, affecting both producers and consumers. Cd inhibits seed germination (HEIDARI & SARANI, 2011; ZIAR-UR-REHMAN et al., 2015) and also affects root growth (HAOUARI et al., 2012; ZIAR-UR-REHMAN et al., 2015) and absorption of nutrients; it induces oxidative stress by releasing free radicals (KHAN et al., 2007; ZIAR-UR-REHMAN et al., 2015). Cd is "the only metal that can affect human or animal health even if concentrations in plant tissues are not phytotoxic" (PEIJNENBURG et al., 2000; PERALTA-VIDEA et al., 2009).

Nickel is a heavy metal essential for higher plants, indeed less important than Zn or Cu, yet having a special role as a constituent of urease (SEREGIN & KOZHEVNIKOVA, 2006). Excess Ni inhibits seed germination, photosynthesis and transpiration, affecting plant metabolism, nutrient absorption, and causing ultrastructural changes (AHMAD & ASHRAF, 2011).

In order to study the influence of heavy metals on superior plants one can perform phytotoxicity tests that use fern spores (CATALÁ et al., 2011). Ferns are excellent models for study in several ways: 1. biodiversity - there are over 250 genera and 10,000 species of ferns (RATHINASABAPATHI, 2006); 2. adjustment to extreme environments - resistance to aridity, such as certain species of the families Actiniopteridaceae, Sinopteridaceae, Pteridaceae and

Selaginellaceae (POREMBSKI & BARTHLOTT, 2000; RATHINASABAPATHI, 2006), tolerance to salinity - *Acrostichum aureum* is a halophilous species (MEDINA et al., 1990; RATHINASABAPATHI, 2006); 3. invasive and competitive capacity: *Lygodium microphyllum* in North and South America, and the species of the genus *Salvinia* threaten all water bodies in the world (ABBASI & NIPANEY, 1986; RATHINASABAPATHI, 2006); 4. capacity of forming numerous spores, which are easy to collect and preserve; 5. the gametophyte is small and grows rapidly; 6. acute and chronic toxicity tests are not expensive and require common laboratory equipment (CATALÁ et al., 2011).

The aim of this research was to study the influence of a number of heavy metals on gametophyte differentiation in two native species of the genus *Dryopteris*: *D. affinis* (Lowe) Fraser-Jenkins (*Da*) and *D. filix-mas* (L.) Schott (*Dfm*).

MATERIALS AND METHODS

The biological material was collected from individuals located in the Vâlsan Valley, a protected area of national interest (Argeş County, Romania). Mature leaves of both species were collected from individuals in different sites, then wrapped in paper and carried in plastic bags. In the laboratory, the leaves were maintained at room temperature with the lower part down on paper, in order to release the spores from the sporangia.

In the experiment we used compounds containing heavy metals, namely cadmium acetate and nickel sulphate, compounds that are carcinogenic (as indicated by the International Agency for Research on Cancer - Group I: cadmium and cadmium compounds, and nickel compounds). According to Directive 76/464/EEC and daughter directives, these metals are on the list of priority substances/hazardous, i.e.: Cd and its compounds are comprised in List I (Directive 83/513 of 26 September 1983), and Ni and its compounds in List II. Under Directive 86/278/EEC, which regulates the use of sewage sludge in agriculture, the limit values for heavy metal concentrations in the soil are established (Cd: 1-3 $mg \cdot Kg^{-1}$, Ni 30-75 $mg \cdot Kg^{-1}$ of dry matter in a representative soil sample). For each species we prepared the following variants (Table 1).

Table 1. Experimental variants.

Variants	Concentration
Control (C)	Knop solution
DaV_1Cd/*Dfm* V_1Cd	3 mg cadmium acetate$\cdot L^{-1}$ Knop solution
DaV_2Cd/*Dfm*V_2Cd	15 mg cadmium acetate$\cdot L^{-1}$ Knop solution
DaV_3Cd/*Dfm*V_3Cd	30 mg cadmium acetate$\cdot L^{-1}$ Knop solution
DaV_1Ni/*Dfm*V_1Ni	75 mg nickel sulfate$\cdot L^{-1}$ Knop solution
DaV_2Ni/*Dfm*V_2Ni	375 mg nickel sulfate$\cdot L^{-1}$ Knop solution
DaV_3Ni/*Dfm*V_3Ni	750 mg nickel sulfate$\cdot L^{-1}$ Knop solution

The concentrations were determined by taking account of Order 756 of 3 November 1997 approving the Rules on the assessment of environmental pollution, where alert soil thresholds for Cd and Ni are established at 3 $mg \cdot Kg^{-1}$, and 75 $mg \cdot Kg^{-1}$ dry matter.

The spores were grown on the surface of the liquid culture medium. The culture dishes, covered and sealed with Parafilm, were kept in a POL EKO 350 growth chamber. The temperature values were: 25°C during the day, and 15°C at night, and the humidity and lighting conditions were controlled (photoperiod: 16 hours of light, and 8 hours of dark). After one week from the initiation of the study, the percentage of germinating spores was determined. It is safe to consider that the spores have germinated if the rhizoid cell can be identified microscopically. Three repetitions were performed for each variant, from which spores were randomly selected, for the microscopic spore preparations to determine the percentage of germination. The statistical interpretation was performed using SPSS (version 16 for Windows). We calculated: the mean value, the standard deviation, and the P (Pearson) correlation factor. We also performed the comparisons between the mean values using the Duncan test. Gametophyte differentiation was monitored periodically. The biological material was microphotographed under a B275 OPTIKA microscope with an A630 Canon Power Shoot camera.

RESULTS AND DISCUSSION

The data obtained one week after the initiation of the experiment showed that spore germination was influenced by the presence of heavy metal compounds in the culture medium. There were significant differences in the germination percentages in the Control and the other experimental variants (Table 2). For *Da*, the differences between germination percentages ranged from -11 (C-V_1Cd) to -76 (C-V_3Cd), and for the species *Dfm* the differences ranged from -13.40 (C-V_1Cd) to -54 (C-V_2Ni). By comparing the results obtained using the Duncan test, we found that for *Da* the only variants between which there are no significant differences are the Control and V_1Cd, and for *Dfm* the differences are between the variants V_1Cd and V_2Cd, and V_2Ni and V_3Ni. The Pearson correlation index showed a significant negative correlation between the percentage of spores germinated and the heavy metal concentration in the culture medium, in both species (*Da*: R= -0.958 for the Cd acetate variants, and R= -0.976 for the nickel sulphate variants, and for *Dfm*: R= -0.958 for Cd acetate variants, and R= -0.790 for the Ni sulfate ones; $p<0.01$).

The literature mentions the fact that the gametophyte is the most sensitive stage in the life cycle of the pteridophytes (GUPTA et al., 1992), as numerous exogenous factors affect spore germination. Among these there are pollutants such as pesticides (KEARY et al., 2000; SHEFFIELD, 2002; LUO & IKEDA, 2007; CASSANEGO et al. 2010; DROSTE et al., 2010), metals (NISHIZONO et al., 1987; SELA et al., 1989; GUPTA & DEVI, 1992; GUPTA et al., 1992; MA et al., 2001; KAMACHI et al., 2005; MUCCIFORA, 2008; KIELING-RUBIO et al., 2011; SOARE et al., 2013c), etc. Lead acetate (10 mg/100ml, respectively 50 mg/100ml Knop solution) affected spore germination and gametophyte differentiation in the species *Athyrium filix-femina*, *Dryopteris affinis* and *D. carthusiana* (SOARE et al., 2014). Nickel in a concentration of 0.05 to 100 mg L^{-1} affected spore germination in *Regnellidium diphyllum* (KIELING-RUBIO et al., 2012). The spore germination of *Ceratopteris thalictroides*, *Drynaria quercifolia*, *Cristella parasitica*, *Pteris ensiformis*, *Amelopters prolifera* and *Adiantum lunulatum* was affected by different Cadmium concentrations (GUPTA & DEVI, 1992).

Table 2. The influence of heavy metals on the germination of spores.

Experimental variants							
Species	C	V_1Cd	V_2Cd	V_3Cd	V_1Ni	V_2Ni	V_3Ni
Germination percent (mean±SD)							
Da	82±3.6[a]	71±3[a]	59±10.5[b]	6±2.6[c]	66.6±2.5[b]	45±5.1[c]	19±6.5[d]
Dfm	69±1.7[a]	55.6±4.7[b]	48.3±5.5[b]	15.3±3.5[c]	42.3±9.2[b]	15±2[c]	18±10.1[c]

Legend: The values are the means of 3 repetitions ± standard deviation; a, b, c, d - Duncan test results: the comparisons were made between Control and $V_{1\text{-}3}$Cd, and Control and $V_{1\text{-}3}$ Ni for each species.

Throughout the monitoring period in terms of gametophyte differentiation, differences were observed both between the two species, and between the experimental variants set for the same species. Thus, one month after the initiation of the experiment, in both species, the Control was at the stage of prothallium blade, and the other variants were at less advanced stages (Table 3). We found that there was a tendency of prothallium filaments to branch (*Da*: V_1Cd and V_1Ni). In some variants, we also observed the tendency of the gametophyte to form three-dimensional cell masses (*Da*: V_1Cd, V_2Cd, V_1Ni, *Dfm*: V_2Cd and V_1Ni). The V_3Cd variants of *Da* and the V_2Ni and V_3Ni variants of both species were at the stage of germinated spores. Another change observed in the *Da* gametophyte, in the V_1Ni variant, was that the rhizoids were fewer and short in comparison with the other variants.

Table 3. Stages of gametophyte differentiation one month after the initiation of the experiment.

Variants	Species	
	Dryopteris affinis	***Dryopteris filix-mas***
C	prothallium blade	prothallium blade
V_1Cd	prothallium filament, blade differentiation, branching prothallium filaments, three-dimensional cell masses	blades differentiation
V_2Cd	short filament, three-dimensional cell masses	prothallium filament, three-dimensional cell masses
V_3Cd	germinated spores	filaments differentiation
V_1Ni	branched filaments (short rhizoid), three-dimensional cell masses	filaments differentiation, three-dimensional cell masses
V_2Ni	germinated spores	germinated spores
V_3Ni	germinated spores	germinated spores

Two months after the start of the experiment, the differences in the stages of gametophyte differentiation maintained in both species (Table 4). As far as *Da* was concerned, C, V_1Cd and V_2Cd were at the stage of chordate prothallia, while V_1Ni was at the stage of prothallium filaments and blades, whereas the other variants were at the stage of germinated spores. In *Dfm*, the stages of differentiation were the prothallium blades for C, V_1Cd, prothallium filaments for V_2Cd, V_3Cd, V_1Ni, and germinated spores for V_2Ni and V_3Ni. As far as the V_2Cd and V_1Ni variants are concerned, we observed a tendency to form three-dimensional cell masses, a phenomenon reported by SOARE et al. (2013a) in *Athyrium filix-femina* and *Polypodium vulgare* gametophytes treated with bifenthrin.

Also, three-dimensional cellular masses were found in abnormal gametophytes that developed from aged spores (BALLESTEROS et al., 2011, 2012; SMITH & ROBINSON, 1975). Similarly, the chlorophyll pigments in *Da*V_1Ni were also affected, the filaments and prothallium blades were discoloured. The variants that had a higher concentration of the metal compound (V_2Ni, V_3Ni) resulted in the necrosis of the germinated spores. WAHID et al. (2008) showed that Cd stress produces chlorosis, necrosis and loss of pigments in *Vigna radiata* leaves. Chlorosis of leaves was reported by PERVEEN et al. (2011) as Cd toxicity symptoms in *Zea mays*. Also, the decrease of photosynthetic pigments in *Pisum sativum* leaves was noticed by SINGH (2014) in response to Cd toxicity.

When the last monitoring of the gametophyte differentiation was conducted after four months, the differences in the development stages were maintained both between the species and the experimental variants (Table 5). In both species, variants containing Ni had a stronger negative influence on the gametophyte, while the control variants were at the stage of chordate prothallium. For V_3Cd, V_2Ni and V_3Ni, the germinated spores became necrotic, thus ceasing the development cycle.

Table 4. Stages of gametophyte differentiation after two months from the start of the experiment.

Variants	**Species**	
	Dryopteris affinis	***Dryopteris filix-mas***
C	prothallium blade, chordate prothallia, antheridia	prothallium blade, chordate prothallia
V_1Cd	chordate prothallia	prothallium blade, young chordate prothallia
V_2Cd	prothallium blade, chordate prothallia, three-dimensional cell masses	prothallium blade, young chordate prothallia
V_3Cd	germinated spores (necrotic)	prothallium blade, chordate prothallia, antheridia
V_1Ni	prothallium filament and discoloured prothallium blade (necrotic)	prothallium filament and prothallium blade (necrotic)
V_2Ni	germinated spores (necrotic)	germinated spores (necrotic)
V_3Ni	germinated spores (necrotic)	germinated spores (necrotic)

Table 5. Stages of gametophyte differentiation after four months from the start of the experiment.

Variants	**Species**	
	Dryopteris affinis	***Dryopteris filix-mas***
C	prothallium blade, branched chordate prothallia, antheridia	chordate prothallia
V_1Cd	branched chordate prothallia	chordate prothallia (necrotic)
V_2Cd	chordate prothallia	chordate prothallia
V_3Cd	germinated spores (necrotic)	prothallium blade, chordate prothallia
V_1Ni	prothallium blade (discoloured)	prothallium filament and prothallium blade (discoloured)
V_2Ni	germinated spores (necrotic)	germinated spores (necrotic)
V_3Ni	germinated spores (necrotic)	germinated spores (necrotic)

After two months from the start of the experiment we observed an accumulation of metal on the surface of the gametophyte in some Cd variants (*Da*V_1Cd, *Dfm*V_3Cd) (Fig. 1) and the precipitation of metals. The latter was due to biosorption to cell walls, or precipitation of metal compounds (GADD, 2010).

We believe that, in the particular case of the gametophyte of the leptosporangiate pteridophytes the deposit could be mostly favoured by their small size and the single-layered structure, as almost all cells are exposed directly to the environment. Precipitation of crystalline structures around the rhizoids of the prothallia of *Athyrium filix-femina* was noticed in gametophytes treated with a copper fungicide (SOARE et al., 2013a), as well as at the tip of the papillae in the gametophytes of *Asplenium scolopendrium*, the latter with a fungicide containing 20% metallic copper (SOARE et al., 2013b). This property may have a particular importance in the bioremediation of environments contaminated with metals.

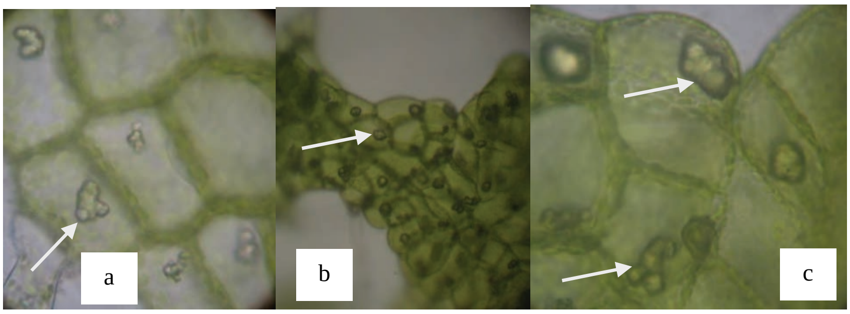

Figure 1. Gametophyte of *Dryopteris affinis* (Lowe) Fraser-Jenkins, V_1Cd experimental variant: two months (a) and four months after sowing the spores (b, c). Accumulation of metal (arrow) on the gametophyte cells (a, c x400; b x100; original).

CONCLUSIONS

In conclusion, cadmium acetate and nickel sulphate affect both the germination of spores and the differentiation of the gametophyte in the ferns *Dryopteris affinis* and *D. filix-mas*. The main changes noticed in the process of gametophyte differentiation were as follows: a longer period of time necessary to reach the characteristic stages, damage to rhizoid elongation, the formation of three-dimensional cell masses, chlorosis and necrosis of the gametophyte cells and an accumulation of crystal structures on the surface of the gametophyte. This accumulation may have a particular importance in the bioremediation of environments contaminated with metals.

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Received: March 31, 2016
Accepted: May 29, 2016

THE PRODUCTION OF LIPASES AND DECARBOXYLASES BY HALOPHILIC BACTERIA ABLE TO GROW IN THE PRESENCE OF WASTE GLYCEROL

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Abstract. Chemically, biodiesel is obtained from the trans-esterification of vegetable oil or animal fat, in the presence of alcohol, such as methanol or ethanol, generating glycerol as by-product. The aim of this work was to test the capacity of several halophilic microorganisms to grow on culture media supplemented with waste glycerol, derived from different sources (Mediaș and Slobozia), and to produced lipases and decarboxylases used as biocatalysts to convert residual glycerol to valuable compounds. From a total of 30 halophilic bacterial strains, isolated from Balta Albă, Buzău County, and Techirghiol salt lakes, a number of eight halophilic/halotolerant strains showed lipolytic activity. *Marinococcus halophilus* strain JCM 2472, a moderately halophilic bacteria, was used in this study for its capacity to synthesized extracellular lipases and decarboxylases.

Keywords: waste glycerol, halophiles, lipases, decarboxylases.

Rezumat. Producerea de lipaze și decarboxilaze de către bacterii halofile capabile să se dezvolte în prezența glicerolului rezidual. Biodiesel se obține prin reacția de trans-esterificare a uleiurilor vegetale sau a grăsimilor de origine animală, în prezența unui alcool, metanol sau etanol, cu generare de glicerol ca produs secundar. Scopul acestui studiu a fost de a testa capacitatea microorganismelor halofile de a se dezvolta în prezența glicerolului rezidual și de a produce lipaze și decarboxilaze utilizate ca biocatalizatori în conversia glicerolului rezidual la compuși valoroși. Dintr-un număr de 30 de tulpini de bacterii halofile izolate din probe de apă, prelevate din lacul sărat Balta Albă, județul Buzău și din lacul Techirghiol, au prezentat activitate lipolitică un număr de opt tulpini de bacterii halofile/halotolerante. *Marinococcus halophilus* JCM 2472, a fost selectată și utilizată în studiu datorită capacității acesteia de a sintetiza extracelular, lipaze și decarboxilaze.

Cuvinte cheie: glicerol rezidual, halofile, lipaze, decarboxilaze.

INTRODUCTION

The obtaining process of biodiesel consists in transforming triglycerides into fatty acid alkyl esters in the presence of alcohol, such as methanol or ethanol, and an acid or alkali catalyst, generating glycerol as a major by-product (PALLIGARNAI et al., 2007). The biodiesel, regarded as the fuel of the future, and an alternative of the fossil fuel, represents a nontoxic, biodegradable, and renewable source of combustible. It is estimated that the production of biodiesel will generate about 10% (w/w) glycerol as the main by-product (FANGXIA et al., 2012). The development of the biodiesel industry entails a surplus of waste glycerol considered as waste stream, being an inconvenience for the efficiency/costs ratio of biodiesel process. Taking this into account, it is necessary to convert waste glycerol to useful products. The studies reported in the literature revealed the possible valorisation of raw glycerol by involving different processes, such as direct application, chemical transformation, or microbial conversion (CHENG et al., 2013). Making a comparison between direct applications, which implies utilization of waste glycerol as a simple carbon source, chemical transformation with processes, which involve expensive metal catalysts, toxic intermediates, and low conversion rates, the microbial conversion of glycerol became a valuable and attractive application (CHENG et al., 2013).

Several microbial strains belonging to Enterobacteriaceae Family were involved in the fermentation of glycerol. *Escherichia coli,* considered as bacterial platform for the production of useful metabolites (CHENG et al., 2007), has the capacity to transform glycerol into ethanol, lactic, succinic or acetic acid, fatty acids omega-3 polyunsaturated, 1,2-propanediol (1,2-PDO), 1,3-propanediol (1,3-PDO) (YANG et al., 2012). Also, *Klebsiella*, *Citrobacter,* and *Clostridium* species present the ability to convert glycerol to 1,3-propanediol (1,3-PDO) (CHENG et al., 2007; METSOVITI et al., 2013; BIEBL et al., 1992). Some yeast and fungi species, such as *Yarrowia lipolytica*, or *A. niger* showed the potential to transform waste glycerol into citric acid (NICOL R.W. et al, 2012). The biocatalytic conversion of waste glycerol to valuable compounds, such as glycidol, or glycerol carbonate, using an enzymatic cocktail formed by lipases and decarboxylases, represents an attractive and environmentally friendly alternative (NEAGU et al., 2015).

The main goal of this work was to identify moderately halophilic microorganisms able to grow in the presence of waste glycerol as a carbon source and to select the strains able to synthesized extracellular lipases and decarboxylases involved in the conversion of substrate (waste glycerol) to value added product like glycerol carbonate and glycidol.

MATERIAL AND METHODS

Cultures and media

The halophilic bacterial strains used in this study were isolated from the saline lake Balta Albă, located in Romania, in Buzău-Brăila counties approximately 170 km East of Bucharest, and Techirghiol Lake, located near the Black Sea coast. A set of serial dilutions was made into sterile saline solution, and one millilitre from each water sample and dilution was distributed in drops onto Petri dishes. The molten agar media (around 50 – 55^{0}C) was poured and the plates were incubated at 28^0C, for 7-10 days. The resulted colonies were counted and after that were purified by repeated streaking on 10% MH agar medium. The isolation media (MH) contained (g/L): NaCl - 100, $MgCl_2x6H_2O$ - 7, $MgSO_4x7H_2O$ - 9.6, $CaCl_2x2H_2O$ - 0.36, KCl - 2, $NaHCO_3$ - 0.06, NaBr - 0.026, glucose - 1, proteose peptone - 5, yeast extract - 10 (VENTOSA et al.,1989). The pH of the culture medium was adjusted to 7.0 – 7.2 before autoclaving. It was established the affiliation of halophilic strains to *Bacteria* or *Archaea* domain. The growth of halophilic strains on MH solidified medium containing deoxycholic acid sodium salt, a bile acid salt, at a concentration of 0.004%, or chloramphenicol 0.002% allowed the differentiation between halophilic bacteria and halophilic archaea. All tested strains grew on media with deoxycholic acid sodium salt and, thus, were considered halophilic bacteria. *Marinococcus halophilic* strain JCM 2472 (courtesy of dr. Takashi Itoh – Japan Collection of Microorganisms) was used in further experiments, and growth medium for the strain was R-AGAR, and contained (g/L): NaCl - 50, peptone - 10, casamino acids - 5, malt extract - 5, yeast extract - 5, beef extract - 2, $MgSO_4x7H_2O$ – 1, and Tween 80 – 0.05.

Waste glycerol source

The types of residual glycerol used in the experiments were represented by manufacturer suppliers: Mediaș and Slobozia sources. The growth ability of strains in the presence of waste glycerol was monitored at 660 nm with a UV/VIS spectrophotometer (BMG LABTECH FLUOStar Omega microplate reader).

Determination of extracellular lipase activity

Lipase activity of the bacterial cultures was screened qualitatively following the method described by BAHTNAGAR et al. (2005) with some modifications. The sterilized basal medium was supplemented with 1.25% olive oil (w/v) and the mixture was homogenized. The 0.001% rhodamine B (w/v) was added in medium at 50^0C, before pouring into plates. The lipase activity was tested at 0, 1.7, and 3M NaCl. The similar sized wells were cut in the solidified medium and a volume of 200 µl of bacterial culture was placed in each well, and incubated at 28^0C, for 48 hours. The colonies with an orange-red halo under UV light were considered positive.

Determination of decarboxylase activity

The production of decarboxylases by halophilic investigated strains was performed on Moeller's decarboxylase media with some modifications, based on the formula established by MOELLER (1955). The basal medium used for tests contained (g/L): peptic digest of animal tissue 5, yeast extract 3, dextrose 1, bromocresol purple 0.02, NaCl 100, supplemented with lysine, arginine, or ornithine in percent of 0.5%. 4.5 mL of sterile liquid medium distributed into tubes were inoculated with 0.5 mL liquid bacterial culture. Each inoculated tube was covered with 1 mL sterile mineral oil, and incubated at 35^0C for 24 hours. Fermentation of glucose by bacteria with fermentative metabolism leads to acidification of media, and the pH indicator (bromocresol purple) converts the colour of media from purple to yellow. Tubes were incubated for an additional 24 hours to allow microorganisms to use amino acid. Alkaline conditions created by the enzyme which used amino acid indicated a positive reaction, and were observed when the colour of the medium changed from yellow to purple. If the test organism ferments glucose, but does not produce decarboxylase, the medium remains yellow in colour, and the result is considered negative.

RESULTS AND DISCUSSIONS

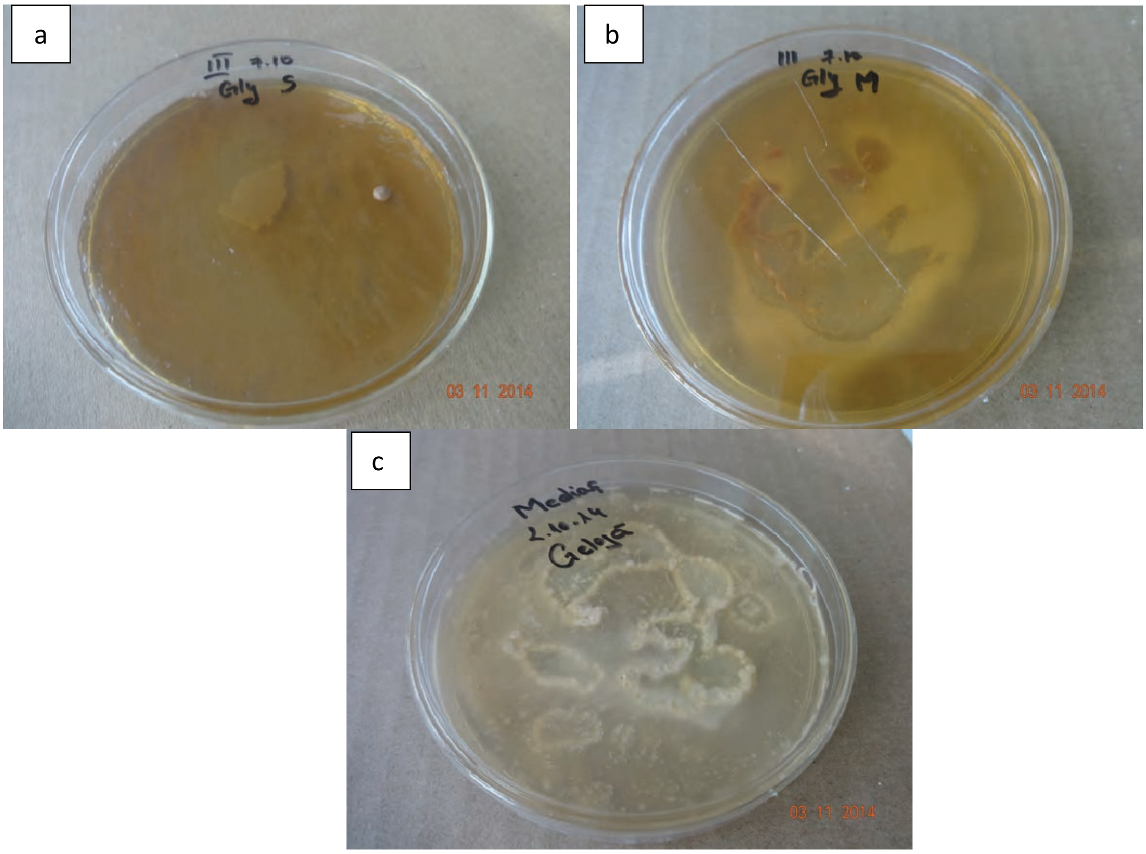
The growth ability of microorganisms on agar media in the presence of waste glycerol

In the first step of the study we try to isolate some putative microorganisms present on the tested types of waste glycerol. In this way it was tested the potential of any type of microorganisms to grow on the agar medium using the waste glycerol as source of microbial strains. The composition of agar medium was changed, using three work variants. The first (I) agar medium variant, contained (g/L): meat extract – 3, peptone – 1, yeast extract – 3, NaCl – 5, agar – 20. The second (II) medium contained (g/L): meat extract – 6, peptone -20, yeast extract – 6, NaCl – 5, agar – 20. The third variant (III) of medium contained (g/L): meat extract – 9, peptone – 20, yeast extract – 9, NaCl – 5, agar – 20. 1 mL of residual glycerol from Mediaș and Slobozia sources was distributed in drops into Petri dishes and the liquefied agar media was poured. After solidification, plates were incubated at 37^0C. In the result, we found that in the third medium variant (III) it was observed the development of two types of bacterial colonies, after 7 days of incubation. The colony which grew on the surface of medium culture was circular, umbonate, opaque, entire, smooth, mauve pigmented with a diameter of 4.7 mm. Also, a large cream colony was observed in depth of the media (Photos 1a, b, c). The bacterial colonies were observed only on variant III of the media with waste glycerol resulted from biodiesel from Slobozia source.

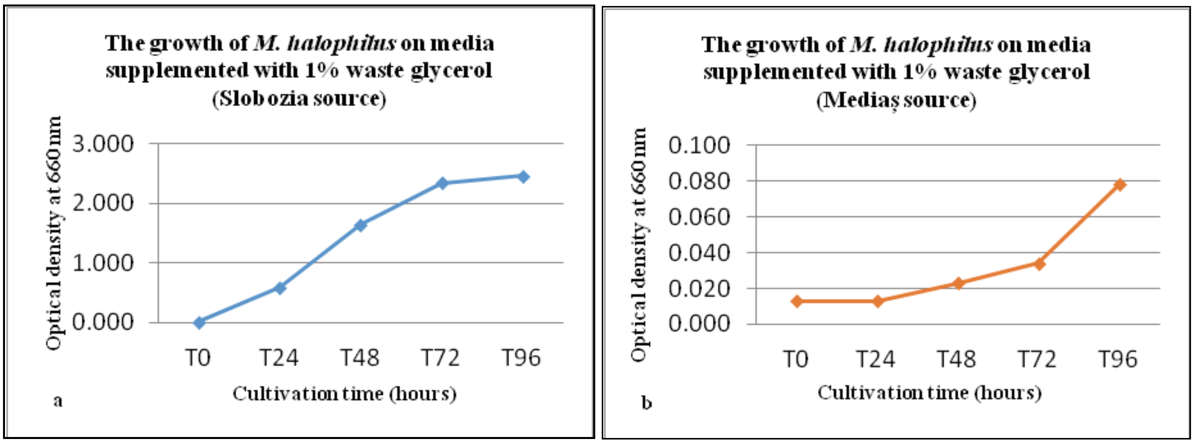
The growth ability of M. halophilus JCM 2472 on R-AGAR media in the presence of 1% waste glycerol

Marinococcus halophilus JCM 2472 strain was selected in our experiments for their ability to produce lipases and decarboxylases. The strain was cultivated on a moderately halophilic (R-AGAR 5% NaCl) medium at 37^0C, pH 7.0-7.2, for 48 hours, and stirring at 130 rpm. Also, it was tested their potential to grow on liquid media with 1% waste glycerol from Mediaș and Slobozia. Growth was monitored by turbidity at OD_{660} using a spectroscopic method (BMG LABTECH FLUOStar Omega – microplate reader) and followed to within 24 hours.

The results revealed that the halophilic strain showed no growth in the presence of residual glycerol from Mediaș (Fig. 1b). On the other hand, the bacterial strain disclosed the ability to grow in the presence of 1% residual glycerol resulted from the biodiesel obtained from Slobozia (Fig. 1a). The differences which appeared in the results may be due to the residual glycerol composition, which consists of some organic matter, phosphate salts and potassium salts, according to the manufacturer data (NEAGU et al., 2015). The residual matters could be a factor which affected the growth of the bacterial strain.



Photos 1a, b, c. (original). The bacterial strains growth on the III variant of agar media, using waste glycerol as inoculum from Slobozia (a), and Mediaș sources (b), and I variant of agar media using waste glycerol from Mediaș (c).



Figures 1a, b. The growth of *M. halophilus* in the presence of 1% waste glycerol from Slobozia (left) and Mediaș (right) sources.

The production of lipases and decarboxylases from halophilic bacterial strains

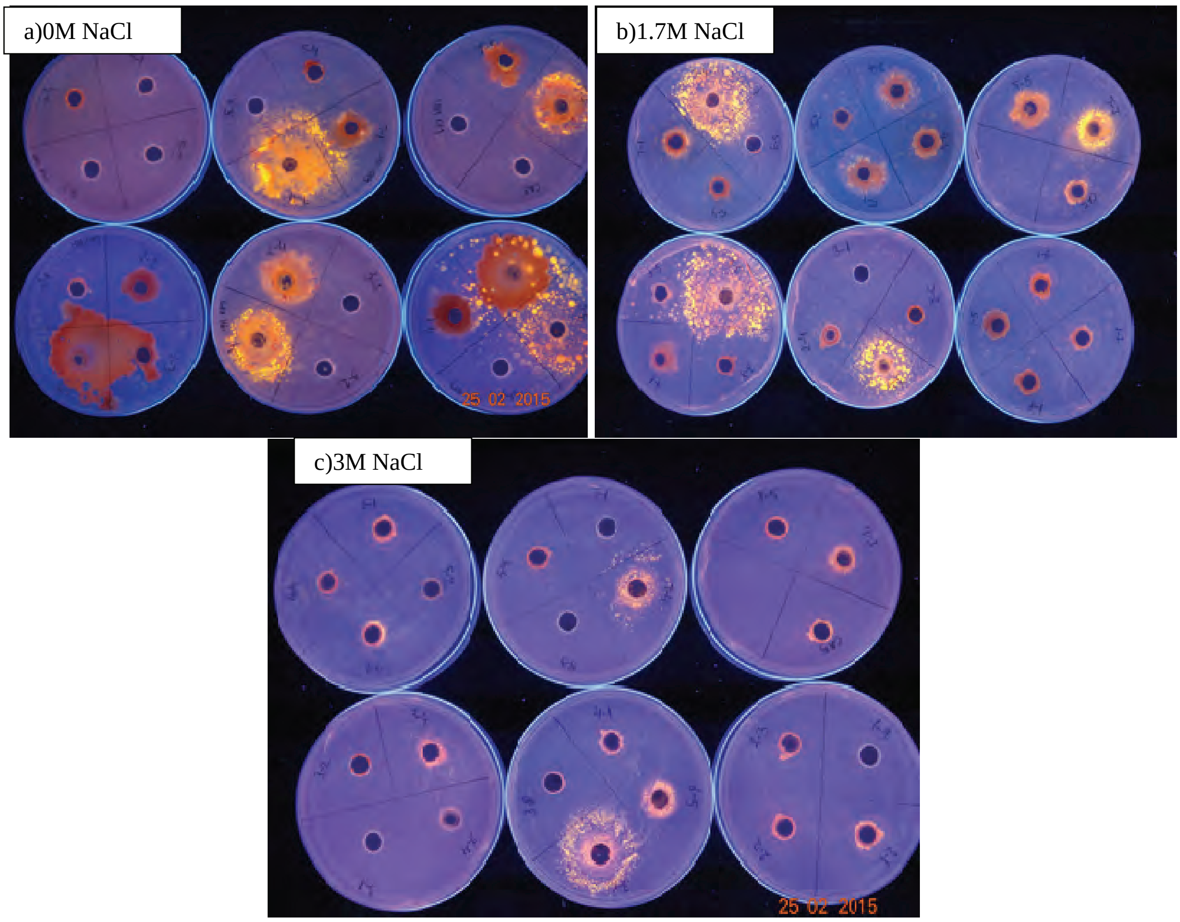
The bacterial strains investigated for their capacity to produce lipases and decarboxylases were isolated from Balta Albă and Techirghiol Lake. The chloride content of the water sample from Balta Albă ranged from 3.5 g/L in a rain period, until to 16 g/L in the absence of rain, with a bacteria density of $3.5x10^5$ colonies forming units per mL (NEAGU et al., 2015). In case of the water sample of Techirghiol Lake, the chloride content was 64 g/L, with $2.1x10^3$

colonies forming units per mL. The number of moderately halophilic bacteria was remarkably higher than that of non-halophilic bacteria. The extreme halophilic bacteria were not recorded. The optimum concentration of NaCl for moderately halophilic bacteria was around 2M NaCl. A number of 76 bacterial strains were isolated from these lakes, but after successive passages on MH media only 30 strains remained cultivable in the laboratory, and used for further experiments. The production of lipases was tested on MH media, as described previously, at different concentration of NaCl (0, 1.7, and 3M). Table 1 shows the lipase and decarboxylase activity of the investigated strains.

Table 1. Lipases and decarboxylases produced by halophilic bacteria isolated from Balta Albă and Techirghiol Lake.

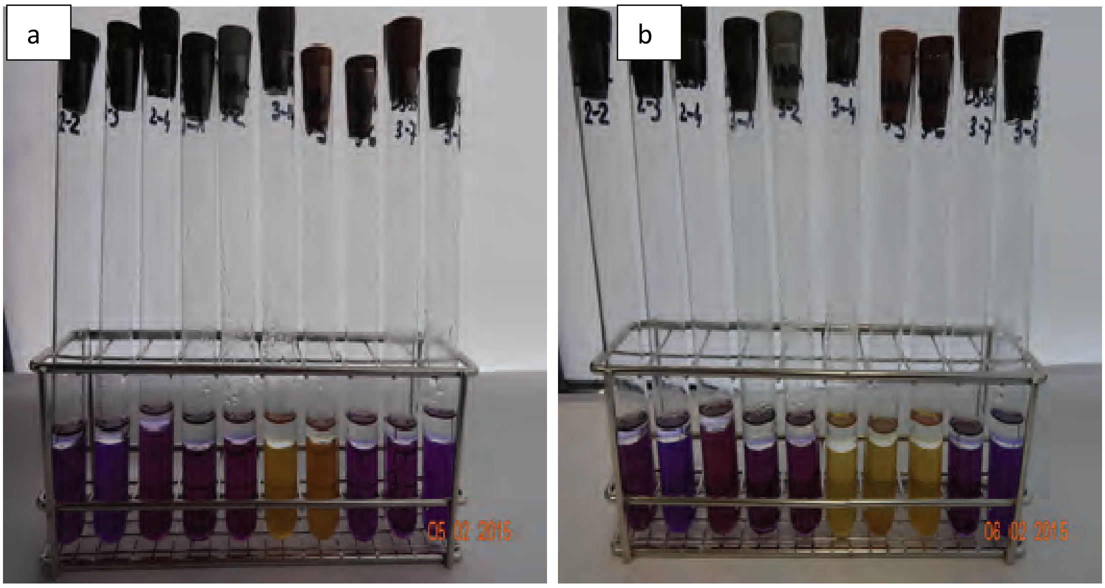
Halophilic strains **Enzyme**	**Gram positive rods**	**Gram negative rods**	**Gram positive cocci**	**Gram negative cocci**
Lipase	0/30	1/30	4/30	3/30
Ornithine-decarboxylase	0/30	0/30	0/30	0/30
Arginine-decarboxylase	0/30	0/30	0/30	0/30
Lysine-decarboxylase	0/30	0/30	0/30	0/30

The results showed that eight halophilic bacterial strains presented in their enzymatic equipment lipases. Also, it was observed the influence of NaCl concentration on lipase activity, which was more intensely at 0M NaCl and decreased with the increasing of NaCl concentration (Photos 2a, b, c). Other strains did not produce lipases, accumulated rhodamine B, formed pink coloured colonies, and did not show orange fluorescence upon UV irradiation.



Photos 2a, b, c (original). The lipase activity of some halophilic bacterial strains tested at 0M (a), 1.7M (b), and 3M(c) NaCl.

The results obtained for the identification of decarboxylase activity tests suggested that the halophilic bacterial strains did not respond positively, even if the acidic conditions were created, and the decarboxylase activity was stimulated, which would have allowed decarboxylation of lysine, arginine, or ornithine amino acids. Figs. 2a, b show the capacity of some investigated strains to ferment dextrose, but the organisms do not produce decarboxylase, and the medium colour remains yellow. Non-utilizers of dextrose did not show any change of the medium colour, which remained purple.



Figures 2a, b. (original). Testing the lysine-decarboxylase activity of some bacterial strains: after 24 hours (a); after 48 hours (b).

CONCLUSIONS

It is already known the potential of halophilic bacteria to produce a variety of extracellular enzymes, and more than that, to tolerate a wide range of salinity, pH, temperature, being valuable organisms for some biotechnological applications (MARGESIN et al., 2001). Some of the investigated bacterial strain isolated from the saline and hypersaline environment from Romania exhibited the ability to synthesize lipases and to use the residual glycerol. Waste glycerol resulted from biodiesel process represents a carbon source that is available at relatively low-cost, and convenient for many applications. Halophilic microorganisms can use glycerol as simple carbon source, and much more than that, to convert it to valuable compounds, such as glycidol, or glycerol carbonate, using an enzymatic cocktail formed by lipases and decarboxylases (NEAGU et al., 2015). Microbial conversion represents an attractive and environmentally friendly alternative for industrial processes.

ACKNOWLEDGMENTS

This work was supported by Program Partnerships in Priority Domains – PN II developed with the support of MEN-UEFSCDI, Project no. 273/2014.

This paper is dedicated to the 150th anniversary of the Romanian Academy.

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Received: March 31, 2016
Accepted: May 25, 2016

HOW DO THE STAGNANT AND FLOWING SYSTEMS INFLUENCE THE PRODUCTION OF ROTIFERS IN THE DANUBE DELTA?

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Abstract. The studies about the ecology of rotifers highlight their cosmopolitan character, being found among the most numerous zooplankton organisms. Due to their features of simple organisms, rotifers respond to different changes in the environment in a very short time. Consequently, the physiology and morphology of rotifers are into a permanent change due to the need for adaptation. Our results showed that the type of ecosystem influenced the dynamics of rotifers. Loricate species were better adapted to the flowing water conditions, while illoricate species preferred shallow waters (especially lakes). The depth and pH determined the spatial variations of the production of rotifers, while the temperature influenced the seasonal dynamics.

Keywords: lentic and lotic ecosystems, loricate and illoricate rotifers, Roşu Lake, secondary production.

Rezumat. Cum influențează apele stagnante și curgătoare producția rotiferelor în Delta Dunării? Studiile care urmăresc ecologia rotiferelor evidențiază caracterul lor cosmopolit, regăsindu-se printre cele mai numeroase organisme zooplanctonice. Totuși, prin trăsăturile lor de organisme simple, rotiferele sunt într-un timp foarte scurt afectate de modificările mediului, astfel fiziologia și morfologia acestora este într-o permanentă schimbare în nevoia de adaptare. Rezultatele noastre au evidențiat că tipul de ecosistem a influențat dinamica rotiferelor. Speciile loricate au avut o adaptare mai bună în condițiile de apă curgătoare, în timp ce speciile neloricate au preferat apele cu adâncimi mici (în special lacurile). Adâncimea și pH-ul au fost parametrii de mediu care au determinat variațiile spațiale ale rotiferelor, în timp ce temperatura a influențat variațiile sezoniere ale producției.

Cuvinte cheie: ecosisteme lentice și lotice, specii loricate și neloricate, Lacul Roșu, producție secundară.

INTRODUCTION

The assessment of the zooplankton biomass and production represents an important scientific concern due to their important role in biogeochemical and energy cycles, as well in aquatic ecosystem food web. Thus, the biological production highlights the importance of a population into ecosystems and their contribution in providing the necessary nutritive base to higher levels (MAKAREWICZ & LIKENS, 1979; GUTELMAKHER & MAKARTSEVA, 1990). The turnover (the production/biomass ratio) completes the production knowledge with indices of the replacement rate of a population, in terms of the existing conditions of an ecosystem. Zooplankton productivity is strongly influenced by physicochemical factors, food resources and consumer pressures (ARNOT et al., 1999).

Zooplankton communities constantly change under the influence of seasonal and spatial variations. Even if, rotifers are considered ubiquist species and are found among the most numerous planktonic organisms, nevertheless they have affinities for stagnant waters with a high coverage of macrophytes (KOSTE et al., 1982; BIELAŃSKA-GRAJNER & GŁADYSZ, 2010; NOVA et al., 2014). Their presence in lotic ecosystems is much weaker being determined mainly by the low food availability and also by the characteristic unstable conditions (CASANOVA et al., 2009). Thus, the rivers diversity is much lower compared with lake ecosystems, with few dominant species (especially with lorica) adapted to the running waters. In the Danube Delta, there are about 400 lakes, with different sizes, which communicate with each other through a complex hydrological network consisting of various channels (COOPS et al., 2008). Aquatic ecosystems are characterized by a variety of hydrogeomorphological features. These traits are reflected in the diversity and biological production.

The aim of our study was to assess the differences in terms of rotifer production depending on the lotic or lentic traits of the ecosystem. Mândra Lake is a small lake (1.47 km^2) with high vegetation, presenting the most favourable conditions for the development of rotifers. The second one, Roșu Lake represents the same type of the ecosystems, but with different features compared to the previous one (14.31 km^2). To highlight the lotic conditions, there were chosen Roșu-Împuțita channel (approx. 5.80 km) and Roșu-Puiu channel (approx. 2.70 km). The advantage of the study in the Danube Delta was represented by the opportunity to have all these types of ecosystems under similar climatic conditions.

MATERIAL AND METHODS

Study site – The study was conducted between 2011 and 2013 with three sampling campaigns each year during the productive seasons (spring, summer and autumn). There were established one sampling point for Mândra Lake and 2 for the channels. In Roșu Lake, there were established five different sampling points in order to determine the variation of the hydrological regime (Fig. 1).

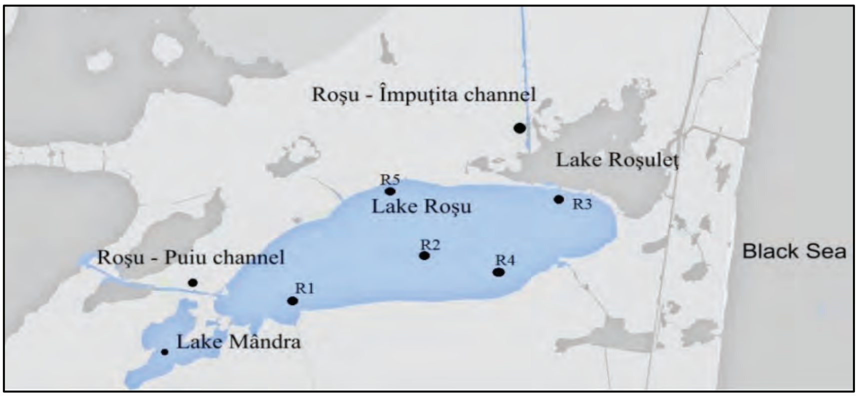

Figure 1. Study site with the sampling points (modified after Google Map).

Environmental parameters – The monitoring program includes measurements of depth, transparency, temperature and pH. The phytoplankton samples were collected without filtering, in 0.5 L bottles, preserved with 4% formaldehyde solution. The phytoplankton and zooplankton biomass was assessed by volumetric and gravimetric measurements.

Zooplankton - The zooplankton samples were taken by filtering maximum 50 L of water using Patalas-Schindler plankton trap (5 L), plankton nets (65 µm mesh size) and fixed with formaldehyde solution 4%. The rotifer species were identified and counted using a Zeiss inverted microscope and following keys: VOIGHT, 1956; RUDESCU, 1960. In order to evaluate the productivity, it was necessary to calculate de density (ind L^{-1}) (UTERMÖHL, 1958) and biomass (µg wet weight L^{-1}) (DUMONT et al., 1975; MCCAULEY, 1984). The secondary production was assessed as µg wet weight L^{-1}/24h, according to EDMONDSON & WINBERG, 1971.

Statistical analyses were performed using XLSTAT 2014 and PAST software (HAMMER et al., 2001).

RESULTS AND DISCUSSIONS

The depth of the studied ecosystems showed generally a downward trend from spring to autumn. The highest depth was recorded in Roşu-Puiu channel, about 4 m in spring, due to the flooding characteristic to this time of the year. Mândra is considered a very shallow lake, ranging from 1.87 to 1.17 m. Roşu-Impuţita channel differs from the other channel having much lower depths (1.70 - 1.57 m) (Table 1).

The delta waters generally have a slightly alkaline pH, varying in narrow limits in our ecosystems, being independent of the season. The minimum value was 8.01, in Roşu-Impuţita channel and the maximum value 8.85, in Roşu Lake. In fact, the highest pH values were found in Roşu Lake, in all three seasons (Table 1).

The water temperature was dependent on season, with high values in spring (22.35 in Roşu-Impuţita channel) and summer (26.67) in the same channel. The lowest temperatures were in autumn, about 16º C in most ecosystems (Table 1).

Table 1. The averages of the environmental variables of the study sites.

		Lentic systems		Lotic systems	
		Roşu	**Mândra**	**Roşu -Împuţita**	**Roşu-Puiu**
Depth	Spring	2.49	1.87	1.70	3.90
	Summer	2.29	1.47	1.73	3.07
	Autumn	2.20	1.17	1.57	2.67
Transparency	Spring	1.44	1.43	1.70	1.67
	Summer	0.47	0.67	0.63	0.63
	Autumn	0.84	0.43	1.17	0.48
Temperature	Spring	21.75	20.90	22.35	21.30
	Summer	26.01	25.47	26.67	24.83
	Autumn	16.31	16.07	17.03	16.03
pH	Spring	8.36	8.08	8.03	8.15
	Summer	8.85	8.63	8.07	8.16
	Autumn	8.53	8.52	8.01	8.49

The phytoplankton biomass recorded high values, especially in summer, in all the studied ecosystems. The values exceeded up to 8 times (in Roşu Lake and Roşu-Puiu channel) the algal bloom threshold of 5 mg L^{-1} established by OLTEAN, 1985. In fact, the maximum values were registered in these systems and the lowest was found in Mândra and Roşu-Impuţita channel (Table 2). It was noticed a variation dependent on the season because in autumn, when temperatures have minimum values, the phytoplankton biomass values were the smallest.

Table 2. The seasonal averages of phytoplankton biomass (mg wet weight L^{-l}) in the two different systems.

	Lentic systems		Lotic systems	
	Roşu	**Mândra**	**Roşu -Împuţita**	**Roşu-Puiu**
Spring	40.88	11.07	1.11	45.50
Summer	33.03	22.07	15.04	43.85
Autumn	33.66	10.10	6.44	10.21

The seasonal averages of rotifer production showed higher values in Mândra, 59.21 µg wet weight L^{-1}/24 h (Table 3), compared to Roşu, while Roşu - Împuțita (the long channel) presented a much higher production than the other ecosystems. In most ecosystems, the lowest values of rotifer production were registered in autumn, while the summer was the season with high values in most systems.

Table 3. The seasonal averages of rotifer production (µg wet weight L^{-1}/24h) of the study period.

	Lentic systems		Lotic systems	
	Roşu	**Mândra**	**Roşu -Împuțita**	**Roşu-Puiu**
Spring	26.91	59.21	12.02	34.21
Summer	56.91	50.53	87.37	33.51
Autumn	12.46	32.94	38.09	9.69

During the 3 years of the study a number of 106 species were registered, varying in the two types of ecosystems as follows: Roşu Lake (80 sp.), Mândra Lake (63 sp.), Roşu-Impuțita channel (71 sp.) and Roşu-Puiu channel (62 sp.). Of this sum, 76 species were loricate and 30 soft-bodied species (illoricate). The high percentage of loricate species was reflected by the secondary production of rotifers (Fig. 2).

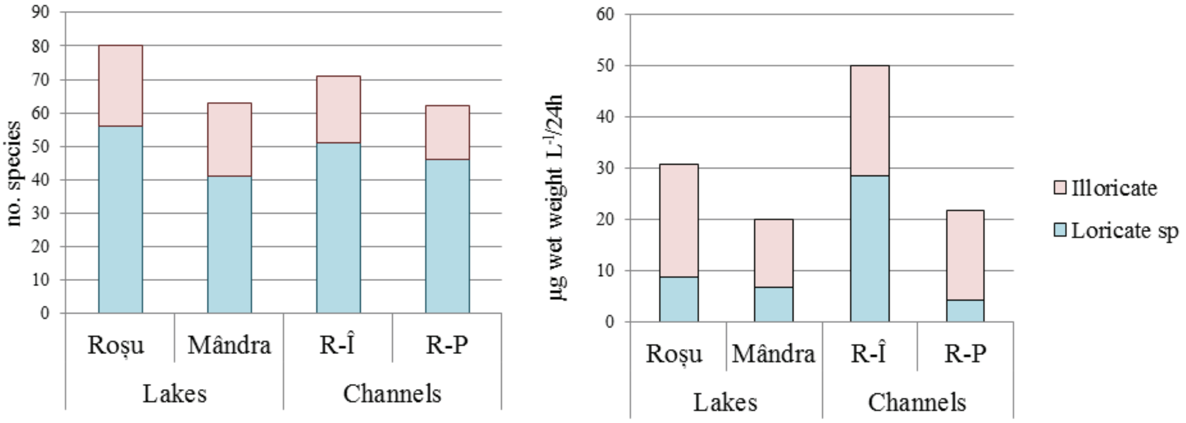

Figure 2. The variation of the two types of rotifer species in the study sites (number of species and production).

The distribution of loricate and soft-bodied species in the two types of ecosystems was explored using Detrended Correspondence Analysis (DCA). Most of the loricate species showed no preference for a particular type of ecosystem and highlighted a distribution due to the connectivity of ecosystems (Fig. 3). With regard to the soft-bodied species, it can be observed differences in distribution between the lentic and lotic ecosystems.

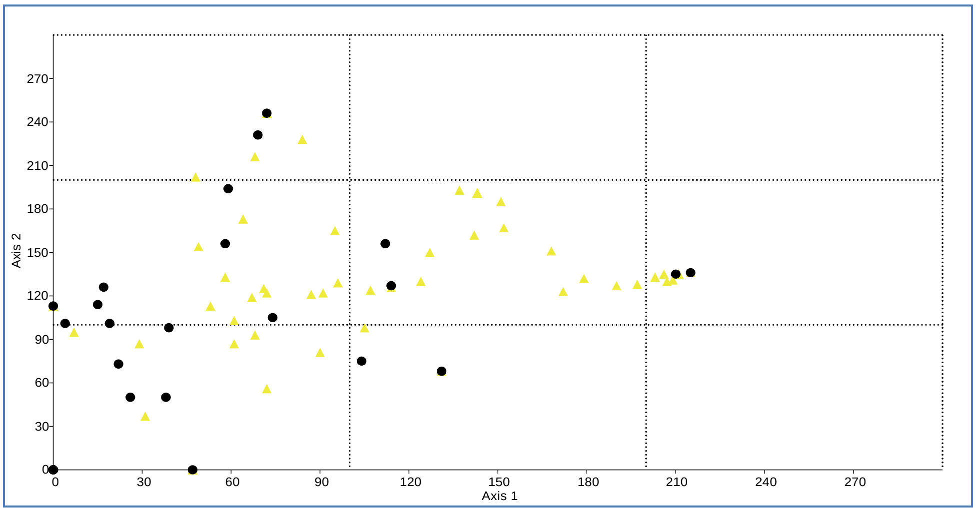

Figure 3. DCA analysis of the rotifer community distributions in the four studied ecosystems (triangles – loricate species, points – soft-bodied species).

The highest similarity was found between Roşu Lake and the short channel, Roşu-Puiu (Fig. 4). This reflects the communication between the two ecosystems and loricate species adaptability to conditions of rivers. In the case of soft-bodied species, the highest resemblance was observed between lentic ecosystems, while between the lotic ones, there were found clear differences.

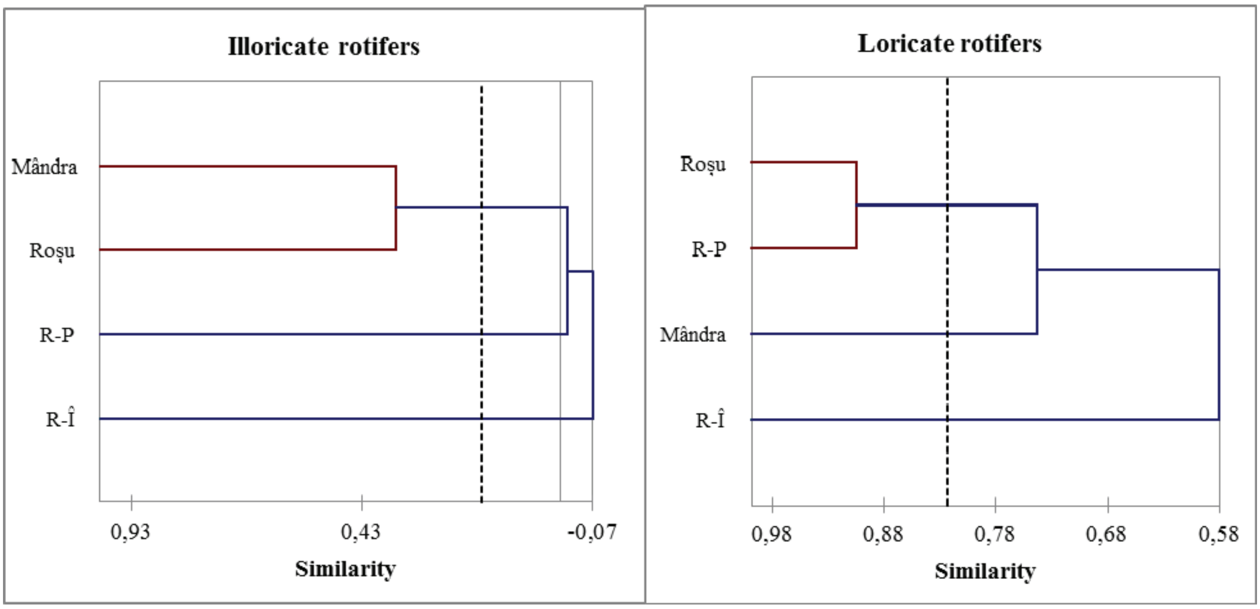

Figure 4. Agglomerative hierarchical clustering (AHC) based on similarity matrix.

An analysis of the influence of environmental parameters on the species in the 2 types of ecosystems, revealed a different pattern of responses. In the lentic ecosystems, pH and transparency were the significant parameters, while in case of channels, depth influenced the 12 species of rotifers (Table 4).

Table 4. The species number influenced by environmental parameters ($p<0.05$) depending on ecosystem type.

	Depth	**Transparency**	**Temperature**	**pH**
Lentic systems	0	6	4	14
Lotic systems	12	2	1	5

The organisms respond differently to environmental conditions, depending on the type of ecosystem. For instance, 7 species of rotifers that were found in both categories were influenced by depth in lotic systems and pH in the lentic ones (Table 5).

Table 5. The R values of the significant correlations between rotifers and environmental parameters ($p<0.005$).

	Rotifer species	**Depth**	**Transparency**	**Temperature**	**Ph**
Lakes					
	Anuraeopsis fissa (Gosse 1851)		-0.614		0.537
	Epiphanes clavulata (Ehrenberg 1832)		0.514		
	Filinia maior (Carlin 1943)			0.499	0.784
	Trichocerca dixton- nuttali (Jennings 1903)				0.494
	Trichocerca rattus (Müller 1776)				0.762
	Trichocerca ruttneri (Donner 1953)				0.855
	Trichocerca similis (Wierzejski, 1893)				0.468
Channels					
	Anuraeopsis fissa (Gosse 1851)	0.530			
	Epiphanes clavulata (Ehrenberg 1832)				0.985
	Filinia maior (Carlin 1943)	0.563			
	Trichocerca dixton- nuttali (Jennings 1903)	0.583			
	Trichocerca rattus (Müller 1776)	0.534			
	Trichocerca ruttneri (Donner 1953)	0.525			
	Trichocerca similis (Wierzejski, 1893)	0.617			

The transparency is an important factor influencing indirectly the zooplankton components through phytoplankton distribution (their main food source) (BERNER-FRANKHAUSER, 1987). In our study, the transparency had a negative influence on most species of rotifers. The rotifers do not show a pronounced positive phototaxis, as some species are independent of it (WILLIAMSON et al., 1996). However, rotifer studies mention a negative reaction to sunlight of these populations explaining the avoidance of intense lit superficial layer. Also, in the case of phytoplankton, there were recorded negative effects on Cyanobacteria ($R = -0.38$, $p = 0.02$) and Chlorophyceae ($R = -0.48$, $p = 0.005$). In fact, seasonal variations of light and temperature are important factors influencing life cycles of organisms, their production and productivity (VOPEL, 1999).

The use of rotifers as bioindicators was often regarded with scepticism but the opinions have changed with the accumulation of data over time (KARABIN, 1983). Regarding the effect of pH on rotifers it was found that most are neutrophils but there are some species adapted to alkaline environment (DHURU et al., 2015, CASANOVA et al., 2009).

Temperature was the significant factor determining the total production of rotifers in lakes (R = 0.533; p = 0.023). Temperature is an important limiting factor, affecting the abundance, age structure, the rate of development, the longevity of rotifers (GALKOVSKAJA, 1987). Although rotifers are well adapted to low temperatures, they are more productive in warm seasons.

In order to detect the influence of the environmental factors on plankton groups, a Canonical Correlation Analysis (CCorA) was performed. The tested environmental parameters explained significantly the variation of plankton (82.24 %). Temperature affected directly the rotifer productions and some phytoplankton groups, like Chlorophyceae, Euglenophyceae, Pyrrophyceae, while transparency had a negative influence. For Cyanobacteria and Bacillariophyceae, the most important factor was depth (Fig. 5).

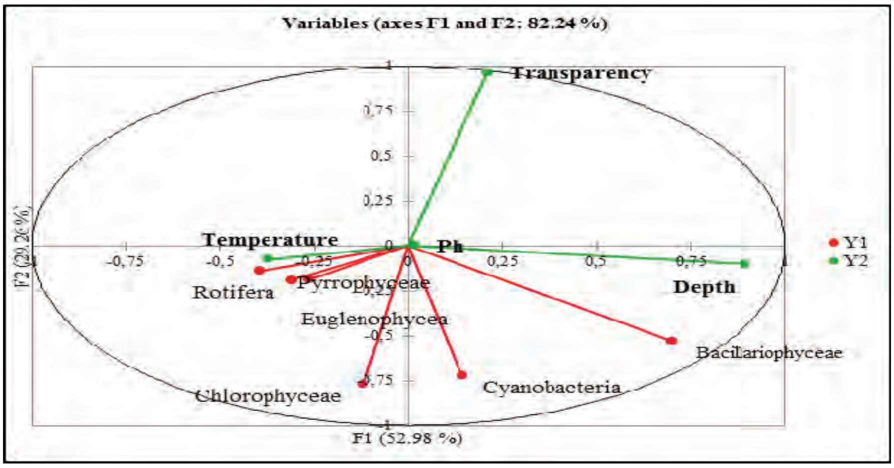

Figure 5. The influence of the environmental factors on plankton groups.

Even if rotifers are considered easily adaptable organisms, they are among the most sensitive to environmental changes in the zooplankton communities. Thus, in our study we found differences between areas in terms of rotifer production using Anosim test (R = 0.07; p = 0.057). The results have also shown the most significant differences between Roşu Lake and Roşu-Impuţita channel (Table 6).

Table 6. The Anosim test (R values in grey; p- values in white) for ecosystem differences in rotifers distribution.

	Roşu	**Mândra**	**Roşu -Împuţita**	**Roşu-Puiu**
Roşu		-0.08	**0.21**	0.12
Mândra	0.87		0.07	0.05
Roşu -Îmuţita	**0.02**	0.18		0.07
Roşu-Puiu	0.06	0.20	0.18	

CONCLUSIONS

The type of ecosystems determines the level of rotifer production in the Danube Delta

Most of the loricate species show no preference for a particular type of ecosystem and highlight a distribution due to the connectivity of ecosystems.

The soft-bodied species have differences in distribution between the lentic and lotic ecosystems preferring the lentic ones.

Temperature affects directly the rotifers productions and some phytoplankton groups, while transparency has a negative influence.

ACKNOWLEDGEMENTS

This study was funded by the project no. RO1567-IBB02/2013 from the Institute of Biology of the Romanian Academy, Bucharest.

The authors thank to Stela Sofa for technical support.

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Received: March 29, 2016
Accepted: May 25, 2016

THE IMPACT OF ANTHROPOGENIC FACTORS ON THE BIOCENOTIC RECONSTRUCTION OF INDUSTRIAL ECOSYSTEMS FROM OLTENIA PLAIN

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Abstract. The researches performed within an ample national program for the recognition of the representative ecosystems in Romania, make a significant contribution to defining the place and role of a lowland watershed system – the Preajba Valley basin of Oltenia Plain. Indigenous microorganisms are present in anthropogenic environments as mixed populations and interact with each other both positively and negatively. Their presence is evidenced by the products of metabolism and not by the accumulation of biomass. The accumulation of products with inhibitory activity and the antagonistic phenomena contribute in conjunction with quantitative changes of nutrients to the emergence of new microorganisms that form succession populations in an ecosystem. In this ensemble ecosystem, gastropod populations have an important role among consumers, representing a factor of accumulation as well as transfer of mass and energy by consumers of higher order such as fish. Also, the populations of *Viviparus acerosus* and *Radix balthica* are one of the reference factors regarding the heavy metals accumulation of the Cu^{2+} and Cd^{2+} type. The clogging of lakes lead to the appearance of these marshlands covered with paludous vegetation, reduction of the depth and surface of lakes, algal blooms that harm human health and fish welfare.

Keywords: microorganisms, gastropods, Preajba Valley hydrographical basin, Oltenia Plain.

Rezumat. Impactul factorilor antropici asupra reconstrucției biocenotice a ecosistemelor industriale din Câmpia Olteniei. Cercetările efectuate în cadrul unui amplu program național de cunoaştere a unor ecosisteme reprezentative pentru teritoriul României aduc o contribuție importantă la definirea locului şi rolului unui sistem bazinal de câmpie – bazinul hidrografic Valea Preajba din Câmpia Olteniei. În mediile antropice, microorganismele autohtone există sub formă de populații mixte, interacționând unele cu celelalte atât pozitiv, cât şi negativ. Prezența lor este evidențiată prin produșii de metabolism şi nu prin acumularea de biomasă. Acumularea produșilor cu activitate inhibitoare şi fenomenele de antagonism contribuie împreună cu modificările cantitative ale nutrienților la apariția noilor comunitățíi de microorganisme care stau la baza succesiunii populațiilor într-un ecosistem. În acest ansamblu ecosistemic populațiile de gastropode ocupă un rol important între consumatori, ele reprezentând un factor de acumulare, precum şi transfer de masă şi energie către consumatorii de ordin superior – peştii. De asemenea, populațiile de *Viviparus acerosus* și *Radix balthica* reprezintă unul din factorii de referință în ceea ce priveşte acumularea de metale grele de tip Cu^{2+} și Cd^{2+}. Colmatarea lacurilor duce la apariția terenurilor mlăştinoase acoperite de vegetație palustră, reducerea adâncimii şi suprafeței lacurilor, dezvoltarea algelor care dăunează vieții peştilor şi sănătății oamenilor.

Cuvinte cheie: microorganisme, gastropode, sistem bazinal Valea Preajba, Câmpia Olteniei.

INTRODUCTION

The research conducted within a broad national program for the recognition of the representative ecosystems to the Land Shaft Romania, makes an important contribution to defining the place and role of a lowland basin system – Preajba Valley basin from Oltenia Plain. The system, placed in the hydrographical basin of the Jiu, includes a network of terrestrial and aquatic ecosystems covering an area of 30 km^2. This space groups a great diversity of ecosystems, low hills covered by pastures and meadows, farmland and a complex river system – springs, streams, rivers, marshes, small reservoirs.

The two human activities that seem fundamental for the biological evolution of continental aquatic ecosystems, but also for the evolution of human society, are pollution (physical, chemical, biological) and hydro-technical facilities of water courses. In such a case, there were determined changes in the structure and functioning of natural ecosystems (BREZEANU et al., 1968; BREZEANU & GRUIȚĂ, 2002; CISMAȘIU, 2015; PĂCEȘILĂ, 2015).

The construction of dams and reservoirs can have a double impact on the river – positive, by amplifying the diversity of ecosystem types, biodiversity and negative, by imbalances of the biocoenotic structure and disruption of the physiology and behaviour of populations, especially fish (blocking migrations, reproduction or feeding of fish).

The same situation occurs in case of the Preajba Valley River, on the one hand, it is the enrichment of the natural environment with new elements of landscape and appearance of the lacustrine ecosystem with structures of specific population, and on the other, the degradation of the reophilous ecosystem downstream the dam, due to the reduced water flow, below the minimum required for keeping and functioning of reophilous biocoenotic structures (BREZEANU et al., 2011; CIOBOIU, 2011).

The primary factors of pollution consist of discharges of the untreated wastewater and poorly organized infrastructure. The activities practiced in the area are sports fishing, grazing, water for domestic use, irrigation. In addition to industrial pollution, the lacustrine ecosystems on the Preajba River shall be subject to the anthropogenic process of eutrophication. In this context, by intensifying the eutrophication process, the reservoirs are in an advanced process of biological clogging. The clogging of these reservoirs lead to the appearance of marshlands covered with paludous vegetation, reduction of their depth and surface algal blooms that harm on fish welfare and human health (CIOBOIU & BREZEANU, 2002; CISMAȘIU et al., 2015b).

In this context a domain much discussed internationally, with immediate practical applications, is the use of acidophilic microorganisms in the recovery of metals by means of microbiological processes. This method can be applied to ores and poor concentrates and mining waste that accumulate over time and cannot be processed by conventional hydrometallurgical methods (CHEN & LIN, 2000; DEAK et al., 2005; CISMAȘIU, 2011).

The bacteria in the sulfur cycle can be used to remove heavy metals. The sulphates produced by the reduction of the sulphides using the sulfate-reducing bacteria can be a way of bonding metal ions. The formation of iron sulphide is a biogeochemical important reaction because, at low temperatures, it leads to the formation of the pyrite. In addition, the presence of iron sulfide and pyrite may alter the solubility of other dissolved metals (Cu^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+}) by reactions of absorption and co-precipitation. H_2S produced by the sulphate-reducing bacteria reacts to the free or absorbed metallic ions, which it precipitates as insoluble metal sulphides.

The process can be used for the concentration of heavy metals, followed by their secondary recovering, as well as to treat industrial effluents for the purpose of bioremediation (DOPSON et al., 2003; FAUR & GEORGESCU, 2009; FURTUNA et al., 2009; CISMAȘIU et al., 2015a).

MATERIALS AND METHODS

1. Surface water, substrate chemistry and biomass measurements

Located 6 Km south of Craiova municipality, the hydrographical network composed of springs, streams, the main course of the Preajba River and small reservoirs, is under the pressure of local anthropogenic factors (Fig. 1).

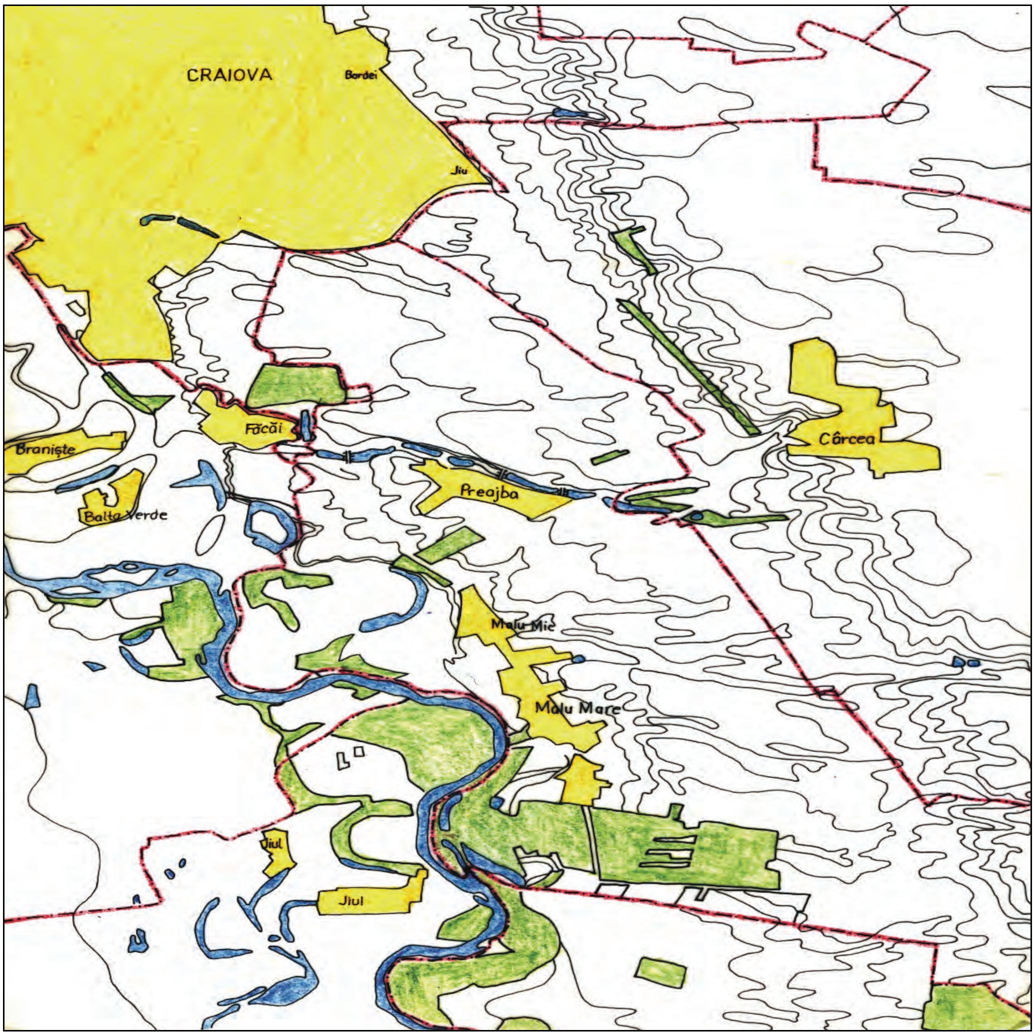

Figure 1. The location of the hydrographical basin in the lower area of the Jiu River (after CIOBOIU, 2011).

There were taken water samples (250ml) collected in clean sterile bottles for determining the chemistry with the spectrophotometer DR 2000 and sediment samples from the habitat of the reservoirs located on the Preajba Valley, based on sediment samples, the levels of chromium, nickel, zinc, lead, cadmium, copper, manganese and iron were analysed using Avanta atomic absorption spectrophotometer, SN 5378. By the same method, it was also determined the concentrations of heavy metals in the shells of *Radix balthica*, the dominant species in the reservoirs. Samples are taken in clean containers made of inert material (glass, plastic, paper); after sampling, the organic material crushed in the mortar, is dried at about 70^0C.

Mineralization made in order to shift the metals in solution is achieved in a microwave mineralizer, Ethos type D, power 1000W, equipped with Teflon tubes, programmable and it occurs as follows:

- weigh approximately 0.5g-1g dry crushed organic material and placed it in Teflon tube;
- add 3 ml of nitric acid 65%, 2 ml of HCl 37% and 1ml of hydrogen peroxide into the tube and then leave it for a few minutes;
- mineralized samples are filtered through quantitative filter paper; each pure acid extract is collected in a 25 ml flask; there are added 5 ml of standard etalon of 5 ppm and then distilled water up to the mark;
- aspirate the standard solution in the ascending order of concentration and the witness sample (zero) to construct the calibration curve, for the following wavelengths of Avante (GBB) atomic absorption spectrometer equipped with a burner for flame air / acetylene and hollow cathode lamps corresponding to the determined metals (Table 1).

Table 1. Synoptic data of wavelengths for some heavy metals determined.

Determined metal	**Wavelength (nm)**
Pb	217
Cd	228.8
Cu	324.7
Ni	232

2. *Environmental control and survival growth*

Among the microorganisms capable of removing metal ions we mention the heterotrophic bacteria from the *Acidiphilium* genus, anaerobic bacteria, which have the ability to form a gelatin polysaccharide capsule at the cell surface. The mechanism for attracting the metal ions by these bacteria is biosorption, as they retain relatively large amounts of dissolved metals through attraction facilitated by an electrical charge (GEORGESCU, 2005; CISMAȘIU et al., 2010).

The use of the heterotrophic bacteria isolated from industrial sites contaminated with metal ions to reduce heavy metal content is substantiated by numerous publications; consequently, the development of a profitable biotechnology with this group of microorganisms is very important (KISHIMOTO et al., 1993; JOHNSON et al., 1994; JOHNSON, 2001).

RESULTS AND DISCUSSION

1. *Biotechnology and metals extraction*

Biotechnology as a science for the concentration of metals is based on studies of biology, chemistry and physical studies of the microorganisms involved in natural processes taking place in mines and it is aimed at developing a technology applicable to ores, concentrates, especially those poorer, in order to obtain useful metals on an industrial scale. These processes refer, on the one hand, to the direct effect of the bacteria on the minerals (oxidation or reduction processes) and, on the other hand, to the action of their metabolites (KARAVAIKO et al., 1994; 1997; PETRIȘOR, 2010).

In this regard it is important to know the three recovery processes of metals from ores and mining effluents: (1) the oxidation of mineral sulphides, elementary sulfur, ferrous iron and other metals by chemoautotrophic microorganisms; (2) the formation by heterotrophic microorganisms of certain organic compounds capable to disintegrate minerals from rocks and dissolve them forming organometallic complexes; (3) the accumulation or precipitation of metals in the solution with biomass or organic compounds produced by microorganisms (BROWN & LESTER, 1982; KOMNITSAS et al., 1998; GEORGESCU et al., 1999).

The main bacteria involved in leaching ores are *Thiobacillus ferrooxidans, T. thiooxidans, T. thioparus* present in acid mine waters. These bacteria are present where metal sulphides and oxygen occur under acidic conditions. From the physiological point of view, thiobacilli are the chemoautotrophic bacteria that have the ability to oxidize inorganic iron or sulphur and its compounds to obtain the necessary energy for growth; they use atmospheric CO_2 and inorganic nitrogen as a source of carbon and nitrogen. These bacteria are aerobic, mesophilic, able to develop in strongly acidic environments and in the presence of high metal concentrations, which are usually very toxic to other life forms: 10,000 – 15,000 ppm copper; 40,000 ppm zinc, 40,000 ppm iron (ZLIGNIEW, 1988; BRIDGE & JOHNSON, 1998; JOZSA et al., 1999).

Water chemistry is characterized by a high concentration of nitrogen and phosphorous compounds (CIOBOIU & CHICIUDEAN, 2003). The amount of nitrates varies between 0.288 and 18.56 mg/l, phosphates between 3.504 and 7.964 mg/l and nitrate between 0.025 and 0.244 mg/l (Table 2).

Table 2. Concentration of nitrogen and phosphorous compounds from the reservoir water (V – XII).

	V	**VI**	**VII**	**VIII**	**IX**	**XI**	**XII**
NO3	0.288	0.288	0.96	4.278	18.56	0.288	1.848
NO2	SLD	0.025	0.053	0.155	0.244	0.045	0.091
PO4	3.504	7.946	4.684	SLD	SLD	3.539	4.892

Overall, water chemistry varies in relation with both natural factors, such as springs and streams, and anthropogenic factors mainly represented by nutrient loads carried by stormwater that washes the neighbouring agricultural areas (CIOBOIU & PLENICEANU, 2005). In terms of the quality requirements for surface water, the small reservoirs located on the Preajba River fall into the category II (bicarbonato – sulfate – calcium – magnesium) and they can be used for fish farming (except for salmoniculture), as well as for tourism and leisure activities (CIOBOIU & BREZEANU, 2009) (Table 3).

Table 3. Physico-chemical composition of water in the downstream reservoirs (average values).

Nr. crt.	Indicators	Measured values		Permissible values	Method of analysis	The used equipment
		The VII lake	The IX lake	Order no. 161/2006- The Cl. II quality		
1	Hydrogen ions conc. (pH), unit. pH	8.29	7.94	6.5 – 8.5	STAS 6325-75	pH-meter WTW 330, series 08090178
2	Electrical conductivity µS/cm, max.	664	695	-	STAS 7722-84	Cond WTW 340, series 08082507
3	Total hardness, German degrees, max.	16.28	18,04	-	STAS 3026-76	-
4	Fixed residue, mg/dm^3, min./max.	332	348	750	STAS 3638-76	Analytical balance KERN 770 series 17308244
5	Ammonia (NH_4), mg/dm^3, max.	0.25	0,69	1.0	STAS 6328-85	Spectrophotometer DR 2000, no. series 930700025411
6	Calcium (Ca^{2+}), mg/dm^3, max.	73	78	100	STAS 3662-62	-
7	Magnesium (Mg), mg/dm^3, max.	27	31	50	STAS 6674-77	-
8	Nitrite (NO_2), mg/dm^3, max.	<0.01	<0.01	0.1	Method 571	Spectrophotometer Lovibond PC spectro series 100510
9	Nitrate (NO_3), mg/dm^3, max.	13.2	9.7	13	Method 355	Spectrophotometer DR 2000, no. series 930700025411
10	Chloride (Cl), mg/dm^3, max.	49	44	50	STAS 3049-86	-
11	Oxidizable organic substances CCOCr (O_2) mgO_2/dm^3, max.	5.8	4.2	25	STAS 3002-85	-
12	Pb^{2+} (mg/dm^3, max.)	< 0.1	< 0.1	0.01	Method of operating in flame using atomic absorption spectrometer	Spectrophotometer GBC AVANTA PM, s. n. A5378
13	Zn^{2+} (mg/dm^3, max.)	0.0052	0.0056	0.2		
14	Ni^{2+} (mg/dm^3, max.)	< 0.09	< 0.09	0.025		
15	Cu^{2+} (mg/dm^3, max.)	< 0.1	< 0.1	0.03		
16	Cr^{2+} (mg/dm^3, max.)	< 0.03	< 0.03	0.05		
17	Fe^{2+} (mg/dm^3, max.)	< 0.05	< 0.05	0.5		

An important component of these industrial ecosystems is represented by microorganisms. The microbiological analysis of the affected ecosystems was focused on establishing the presence of the main physiological groups of chemoautotrophic and heterotrophic microorganisms (ESCOBAR & GODOY, 1999).

Chemoautotrophic microorganisms are primary producers, able to use chemical energy to produce complex organic substances from inorganic substances (nitrification bacteria, colourless sulfur bacteria) (GOMEZ et al., 1999). Heterotrophic microorganisms are involved in the detritic chain by the decomposition of organic waste of primary producers (Table 4).

Table 4. The physico-chemical composition of the sediment from the reservoirs (average values).

No.	Indicators	Measured values	Method of analysis	Used equipment
	Aqueous leachate analysis 1/10 (solid/lichid)			
1.	Hydrogen ions conc. (pH), unit. pH	7.8	STAS 6325-75	pH-meter WTW 330, series 08090178
2.	Electrical conductivity µS/cm	122	STAS 7722-84	Cond WTW 340, series 08082507
3.	Ammonia (NH_4), mg/dm^3	1.136	STAS 6328-85	Spectrophotometer DR 2000, series 930700025411
4.	Calcium (Ca^{2+}), mg/dm^3	19.2	STAS 3662-62	-
5.	Magnesium (Mg), mg/dm^3	3.8	STAS 6674-77	-
6.	Nitrite (NO_2), mg/dm^3	<0.01	Method 571	Spectrophotometer Lovibond PC spectro series 100510
7.	Nitrate (NO_3), mg/dm^3	1.3	Method 355	Spectrophotometer DR 2000, series 930700025411
8.	Chloride (Cl), mg/dm^3	7.1	STAS 3049-86	-
9.	DOC ($mg/dm^{3)}$)	4.3	STAS 3002-85	-

All these transformations confirm the crucial role of microbiological communities in the food web as primary producers, consumers as well as primary and secondary decomposers. The energy released by nitrification bacteria from oxidation reactions of NH_3 to nitrite and nitrite to nitrate is used in reducing CO_2. There was made a preliminary study regarding the presence of heavy metals in water, sediment and freshwater snails, which can accumulate higher levels of Cu^{2+} and Cd^{2+} than the average environmental concentration (Tables 5, 6).

Table 5. Concentrations of heavy metals in the water of studied reservoirs compared with the maximum permissible levels stipulated in Order no. 161 / 2006.

	Preajba Valley	**MPL ac. Ord. 161/2006**
Cr	<0.003	0.1
Ni	0.016	0.1
Zn	<0.005	0.05
Pb	<0.01	0.01
Cd	0.001	0.005
Cu	<0.01	0.01
Mn	<0.001	0.01
Fe	<0.005	0.1

Parameter	**The unit of measure**	**Measured values**		**Used equipment**
		Preajba Valley	**Method of analysis**	
Chromium	mg/ l	< 0.003	Working method specified in the user manual atomic absorption spectrometer GBS-Avanta	Avanta GBC atomic absorption spectrometer SN A 5378
Nickel	mg/ l	0.016		
Zinc	mg/ l	< 0.005		
Lead	mg/ l	< 0.01		
Cadmium	mg/ l	<0.001		
Copper	mg/ l	< 0.01		
Manganese	mg/ l	<0.001		
Iron	mg/ l	<0.005		

Table 6. Concentrations of metals from the soil and shells of the pulmonate snail *Radix balthica*.

No.	**METALS**	**PREAJBA Valley (sol)**	**SNAIL SHELLS**
1	Fe	0.148	0.124
2	Mn	0.0075	0.014
3	Ni	0.0045	< SLD
4	Cr	< SLD	< SLD
5	Cu	< SLD	< SLD
6	Zn	0.0005	0.006
7	Cd	0.0015	< SLD

Note: SLD – below detection limit

The performed analyses illustrate the ability of pulmonate snails species such as *Radix balthica* to accumulate metal ions of the type Fe^{2+}, Mn^{2+} and Zn^{2+} in direct correlation with the concentration of the respective ions from the soil. Also, studies have shown increased tolerance of these snail species (e.g. species of branchial snails *Viviparus acerosus)* to the presence of metal ions derived from industrial activities of solid tailings processing (CIOBOIU, 2002; CISMAȘIU et al., 2015b). These snail species are bio-indicators of contaminated industrial environments from Oltenia Plain because they indicate the emergence of negative changes in lake ecosystems earlier.

2. Biotechnology and environmental control

The effect of eutrophication process is manifested by excessive growth of phytoplankton (78 species) and paludous / aquatic macrophytes (34 species) (Figs. 2; 3). Bacillariophyceae and Chlorophyceae are the dominant groups of the phytoplankton and, during the summer, the cyanobacteria also have an intense development (Table 7).

In this context, considering the values of density and biomass and comparing, from this point of view, the studied reservoirs with other lacustrine ecosystems (BREZEANU & GÂŞTESCU, 1996), it results that they are within the category of the eutrophic ecosystems, taking into account that the reservoirs are mostly invaded by paludous and aquatic macrophytes, with specific character of highly eutrophic ecosystems.

Periphyton has a special place in the structure of the ecological communities in its composition there were identified 72 taxa. In this case, the highest number of species belongs to Bacillariophyceae and Chlorophyceae (CIOBOIU & NICOLESCU, 1999).

Abundant, especially in shore areas covered with concrete slabs and the stems of the paludous macrophytes, periphyton, where besides algae there also live bacterial populations and numerous micro-invertebrates (particularly protozoa), represents the favourite food of gastropods.

Along with periphytic and phytoplankton primary producers, macrophytes represent an important part of the organic production of the studied ecosystems.

Table 7. Numerical (specimen/l) and biomass (mg /L) density on taxonomic groups of the phytoplankton (average values).

TAXONOMIC GROUPS	NUMERICAL DENSITY THOUSAND SPECIMENS/L	WET BIOMASS MG/L
Cyanobacteria	76	0.335
Euglenophyceae	51	0.144
Pyrrophyceae	1	0.003
Heterokontae	7	0.045
Bacillaryophyceae	543.5	1.142
Chlorophyceae	394.5	0.841
TOTAL	1,073	2.510

Figure 2. Reservoir VII - Strong cyanobacterial blooms (original).

Figure 3. Large surfaces of the reservoirs covered with macrophytes (original).

In the surrounding springs, basins and small streams, there were identified the following macrophytes: *Mentha aquatica, Heleocharex palustris, Polygonium amphibium, Carex riparia*. On the surface of stems and plant leaves and on the substrate around them, it was noticed the almost exclusive presence of the diatoms loving lower temperatures, clean water, rich in silicates.

20-30% of the surface of the reservoirs is covered by paludous macrophytes, developing especially in shallow areas (5-20 cm) located at their *tail*. It also has to be added that, in the water and on the bottom, set in the substrate, aquatic plants form large populations. There were identified 34 species, including: *Phragmites communis, Typha angustifolia, Scirpus lacustris, Mentha aquatica, Carex riparia, Lemna minor, Nuphar luteum, Potamogeton crispus, P. natans, Myriophyllum spicatum* (DIHORU & ARDELEAN, 2009).

An overall assessment of macrophyte biomass production demonstrated that it can be obtained an amount of 85,200 kg/ha/year of wet biomass. This is a proof about the trophic capacity of the ecosystems.

It is known that by damming rivers and streams and formation of reservoirs there take place profound hydrological, geomorphological, hydrochemical modifications of the reophilous ecosystem and, in accordance with these, modifications of the structures and functions of animal and plant populations (BREZEANU & GÂȘTESCU, 1996).

Such modifications are also evident in the case of the Preajba Valley hydrographic basin, especially within its limits, along the unharnessed segment of the river (the upper sector) that can be considered a witness segment. Due to the diversity of the benthic biota and the structure of the zoobenthos, it acquires an outstanding diversity demonstrated by the presence of the main groups of invertebrates: ostracods, Gammarida, gastropods, bivalves, Chironomidae, Ephemeroptera, Heteroptera (Table 8).

On the muddy typical facies as well as on the sandy soil, diversity of the groups is the lower. The largest diversity was found in shore areas with paludous macrophytes, all the 13 groups being present here, some with a large number of individuals (4,600 Chironomida ind./m^2, 1,750 ostracods ind./m^2, 213 Ephemeroptera ind./m^2, 226 Plecoptera ind./m^2) (CIOBOIU, 2014).

The invertebrates in the areas with macrophytes make up populations with great numerical density and biomass. Phytophilous fauna is dominated by larvae of Chironomidae, Coleoptera, Ephemeroptera, Heteroptera, Gastropoda. In the areas from the vicinity of the shores, where the facies is made up of vegetable detritus and silt, it is present a large number of Chironomida, Ephemeroptera, Plecoptera, Oligochaeta. These areas are also populated with the highest number of gastropod individuals/m^2.

A smaller diversity of benthic invertebrates was observed in the deep areas where the facies is sandy-oozy. In the areas with macrophytes and vegetable detritus, there was identified a large number of protozoa, gastropods, larvae of Tendipedidae, Trichoptera, Odonata, Chironomida and Gammarida.

Table 8. The structure of zoobenthos (average values).

Taxonomic group	Lake VI			Lake XII		
	Nr. ex. / m^2	**Ab. %**	**F %**	**Nr. ex. / m^2**	**Ab. %**	**F %**
Chironomidae	4,693	50.3	100	800	26.4	100
Gammarida	3,506	37.6	33	-	-	-
Ostracoda	267	2.8	66	440	14.5	66
Heteroptera	213	2.2	100	160	5.2	100
Gastropoda	386	4.1	66	200	6.6	66
Bivalvia	53	0.5	33	-	-	-
Cladocera	-	-	-	227	7.4	33
Copepoda	-	-	-	320	10.5	33
Ephemeroptera	160	1.7	66	267	8.8	66
Plecoptera	-	-	-	453	14.9	33
Isopoda	26	0.3	33	80	2.6	66
Oligochaeta	-	-	-	53	1.7	33
Hirudinea	13	0.1	33	27	0.9	33
TOTAL	9,317	100.00		3,027	100.00	

3. Metal removal by active sulfate oxidation

In this context, microbial degradation is defined as a biological catalyst reduction in the chemical complexity of the sectional elements. The final product of this reaction depends on the biological transformation faced by the compound - mineralization, bioaccumulation, polymerization or co-metabolism.

The solubilisation of metals from ores or bacterial leaching is achieved by dissolving minerals or impurities using acidophilic bacteria of the *Acidithiobacillus* genus and their metabolic products. According to the available data from the scientific literature, it can be noticed that chemical and bacterial solubilisation of minerals does not correspond to a single mechanism, but to different mechanisms depending on the nature of the metal, the nature of microorganisms and physico-chemical conditions in which the bacterial culture acts (MALLK et al., 2002; ZHANG et al., 2009; HEDRICH et al., 2011).

From the point of view of the speed of the chemical and bacterial solubilisation process of useful minerals, it has been found out that, in the presence of the bacteria of the type *Acidithiobacillus ferrooxidans* and *A. thiooxidans*, the oxidation reaction of elementary sulphur under the action of oxygen and the oxidation action of the ferrous sulphate under the action of sulphuric acid and oxygen is developed faster as compared to other chemical reactions.

On the hand, the iron-oxidizing chemolithotrophic bacteria are intended to catalyse the oxidation reactions of the sulphides in the presence of oxygen, carbon dioxide and water and on the other hand, they are involved indirectly in the solubilisation process of the sulphides by the regeneration of the ferric sulphate, which is a leaching agent with increased capacity of oxidation.

The activity as primary producers of the mentioned chemosynthetic bacteria has a limited significance, because most are present in specific environmental conditions and their contribution to the synthesis of organic matter is insignificant compared to photosynthesizing organisms. These have a special importance in sulphur and nitrogen cycles (BRIDGE & JOHNSON, 1998; BACELAR-NICOLAU & JOHNSON, 1999; PASPALIARIS et al., 1999; VOICU et al., 1999; LAZĂR et al., 2004).

CONCLUSIONS

The microbiota of the rivers is so much different, mainly due to local conditions, so that there cannot be synthesized in a list of the main species present constantly. The situation is complicated by the large number and diversity of allochthonous microorganisms from soil, rainfall and tributary streams, in the case of clean rivers, and from different contamination sources in case of polluted rivers.

In anthropogenic environments, indigenous microorganisms develop as mixed populations, interacting with each other both positively and negatively. Their presence is evidenced by the products of metabolism and not by the accumulation of biomass.

The microbiota research on the aquatic biodiversity represents a complex study, which includes knowledge of the structure and life of microbial communities and the interaction between the component microorganisms with other organisms such as invertebrates and plants.

The knowledge of the existing microorganisms in the industrial areas contaminated with metallic ions and the interaction between them leads to the establishment of the role that they can play in the matter and energy flow of those ecosystems. The accumulation of the products with inhibitory activity and antagonistic phenomena contributes, together with the quantitative changes of the nutrients, to the appearance of new microorganism communities underlying the succession of populations into an ecosystems.

In this ecosystem, gastropod population have an important role among consumers, representing a factor of accumulation, as well as of mass and energy transfer to the consumers of higher order – fish. The populations of *Viviparus acerosus* and *Radix balthica* represent one reference factor with regard to the accumulation of heavy metals of the type Cu^{2+} and Cd^{2+}.

ACKNOWLEDGEMENT

The study is the result of the collaboration between the Institute of Biology Bucharest, Department of Microbiology and the Oltenia Museum Craiova, respectively the collaboration agreements 1797 / 20.05.2015 and 1402 / 21.05.2015 with the theme: *The biodiversity of the microbiota from areas with industrial contamination of Oltenia and potential biotechnological applications in order to reduce it.*

The paper is dedicated the anniversary of 150 years since the establishment of the Romanian Academy. The presented study is the part of the project nr. RO1567-IBB05/2016 developed at the Institute of Biology, Bucharest of the Romanian Academy.

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Received: March 23, 2016
Accepted: May 25, 2016

STUDIES UPON THE DYNAMICS OF THE MACROZOOBENTHIC INVERTEBRATE COMMUNITIES FROM VALEA ROȘIA RIVER (BIHOR COUNTY)

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Abstract. During the present study we examined the monthly dynamics of the macrozoobenthic invertebrate communities from Valea Roșia River, for three months (July-September) 2013. The sampling sites were chosen both in the spring area and along the river course in different point allowing us to investigate a great number of habitats. We followed the modifications of the macrozoobenthic invertebrate communities due to the hydrological characteristics of the river sectors, the monthly dynamics and those produced by the human impact. We calculated Shannon-Wienner diversity index and Hilsenhoff index to determine the pollution level in the analysed sectors.

Keywords: macrozoobenthos, Valea Roșia, dynamics, diversity, anthropic impact.

Rezumat. Studii privind dinamica asociațiilor de nevertebrate macrozoobentice din râul Valea Roșia (Județul Bihor). În cursul prezentului studiu am cercetat dinamica lunară a comunităților de nevertebrate macrozoobentice din râul Valea Roșia, pe o perioadă de trei luni (iulie-septembrie) 2013. Stațiile de prelevare a probelor au fost fixate în apropierea zonei de izvor, respectiv de-a lungul râului, astfel încât să surprindă o diversitate cât mai mare de habitate. Au fost urmărite modificările comunităților de nevertebrate macrozoobentice datorate caracteristicilor hidrologice ale sectoarelor de râu, cele lunare și cele datorate impactului antropic. S-a calculat indicele de diversitate Shannon-Wienner și indicele Hilsenhoff pentru a determina gradul de poluare a apei în sectoarele analizate.

Cuvinte cheie: macrozoobentos, Valea Roșia, dinamică, diversitate, impact antropic.

INTRODUCTION

Macrozoobenthic invertebrates are present at the bottom of every natural body of water. This community represents an important part of the trophic web of the aquatic environment and very a useful bioindicator for characterising the pollution of natural waters **(**ROSENBERG & RESH, 1993). The structure of the macrozoobenthic community differs depending on the permanent or temporary character of the water, the flowing regime, chemical parameters, substratum type and aquatic vegetation.

Macrozoobenthic invertebrates are adapted to certain values of these parameters (CELIK, 2002), some of them to flowing waters (COOKSEY & HYLAND, 2007; CUPȘA et al., 2002a; CUPȘA et al., 2003a, b; CUPȘA & BANYAI, 2006; PRINCIPE et al., 2007) and cannot stand the profound modifications of the physico-chemical parameters (DOBRE & TATOLE, 2002; FLOREA & GRIGORAȘ, 2000; TULLOS & NEUMANN, 2006). Some of them are adapted to still waters, or clean and with high oxygen content (CUPȘA & BANYAI, 2006**)** or with great organic content and oxygen deficiency (CUPȘA et al., 2002b; CUPȘA et al., 2003b).

However, the use of macrozoobenthic invertebrates has some disadvantages and limits (DE PAUW et al., 2006). For example the flowing speed and the nature of the substratum can vary between wide limits along the watercourse, inducing modifications in the structure and functions of the macrozoobenthic communities (GILLER & MALMQVIST, 1998). Some of the invertebrate groups cannot be found in the benthos only in some periods of the year, because their adult stage is terrestrial (TACHET et al., 2002)

The achieved investigations were carried out along the Valea Roșia River, an important tributary of the Crișul Negru. This is a 38 km long river with a catchment surface of 308 km^2. Its spring is situated in Pădurea Craiului Mountains at Izbucul Roșia. This spring has the greatest debit from all karst springs in Roșia area. The river crosses Beiușului Depression and flows into the Crișul Negru near Petrani locality. The most important localities crossed by the river are: Roșia, Căbești, Josani, Gurbești, Remetea, Șoimuș, Pocola.

The aim of our study was to identify the monthly modifications of the macrozoobenthic invertebrate communities along the river and to identify the changes induced by human impact as the river crosses certain localities.

MATERIALS AND METODS

The macrozoobenthic invertebrates were sampled from the Valea Roșia River monthly during July-September 2013. The five sampling sites were chosen close to the spring where human influence is minimal and downstream the villages along the river (Table 1).

The macrozoobenthos was sampled by quantitative methods, using a Surber dredge with the dimensions of the mesh of 250 μm. The samples were preserved in the field in 4% formalin, sorted in the lab under a 40 magnifying stereomicroscope and transferred in 70% ethylic alcohol. The organisms were identified using specific keys (TACHET et al., 2002; BAUERNFEIND & HUMPESCH, 2001; WARINGER & GRAF, 2013; AUBERT, 1959; GIBBONS, 1986). For statistical analysis we used the following indexes: abundance, Shannon-Wienner index, equitability, Hilsenhoff index (DE PAUW et al., 1993; HILSENHOFF, 1987; LUDWIG & REYNOLDS, 1988; WASHINGTON, 1984).

Table 1. Sampling sites and their characteristics.

Sampling site	Latitude N	Longitude E	Riverbed width (m)	Riverbed depth (m)	Substratum	Riverbank vegetation
Roșia spring area (R_1)	46.83891^0	22.36986^0	2	0.25	Pebbles and boulders covered by phytobenthos and allochthonous organic matter	Beech forest
Roșia downstream village (R_2)	46.79330^0	22.39433^0	15	0.40	Pebbles and sand, with sandy beaches near the shore and places with mud accumulations	Willows on the river banks and in the fields nearby
Josani (R_3)	46.75700^0	22.35672^0	15	0.35-0.50	Pebbles and sandy regions	Willows and poplars on the river banks
Remetea (R_4)	46.73775^0	22.34215^0	10-15	0.50-1.00	Mostly sandy, in some places pebbles	Willows and poplars on the river banks
Pocola (R_5)	46.69545^0	22.29356^0	15–20	0.30-0.50	Sandy with pebble regions	Willows on the river banks, very close to human settlement

RESULTS AND DISCUSSIONS

We have identified a number of 12 taxonomic groups in the macrozoobenthic samples (Table 2). The greatest number of specimens were collected in July in sample site R_2 (1,438 specimens), and the smallest number in site R_5 (240 specimens) (Fig. 1) Site R_2 is situated in the upper sector of the river downstream Roșia village. Here, the substratum is covered by pebbles and sand, the river crosses a region shadowed by trees. The river receives organic substances intake from the locality situated upstream. These nutrients nourish the aquatic communities and allow the existence of a richer macrozoobenthic community compared to the site situated near the river spring (Fig 1).

The organic substances intake is low, so it does not affect fundamentally the quality of the water as shown by the presence of the Ephemeroptera, Plecoptera and Trichoptera larvae, which are very sensitive to pollution (Table 2). The organic content is however showed by the presence of Gammarida, Oligochaeta and Chironomida larvae.

In August, we have observed a decrease of the number of specimens from each sampling site. The greatest number of specimens in August were found at site R_4 (372 specimens) and the smallest at R_5 (4 specimens) (Fig. 1). The decrease of the number of specimens can be caused by the emergence of the adults of some insects, which have aquatic larvae (CORBET, 2003; FRICK, 1998; ALHEJOJ et al., 2014) and also by the low water level and the high water temperature. In the period between samplings (July and August) the weather was very hot and the rainfall almost absent, so the water level dropped drastically and the high water temperature induced an oxygen deficit near the substratum (CELIK, 2002; COOKSEY & HYLAND, 2007). The drastic water level decrease left some of the macrozoobenthic invertebrates on the dried shore.

The high temperatures stimulate also the decomposing processes, which consume more oxygen from the water and eliminate toxic substances that can cause mortality of the macrozoobenthic invertebrates (CELIK, 2002; COOKSEY & HYLAND, 2007).

In September, the number of specimens is lower than in August. The greatest number of specimens was registered at site R_1 (40 specimens) and the smallest at site R_2 (5 specimens) (Fig. 1). Between August and September, it fell a large amount of rainfall. This large water amount washed heavily the riverbed and dislocated the macrozoobenthic invertebrates already affected by the drought period from the previous months.

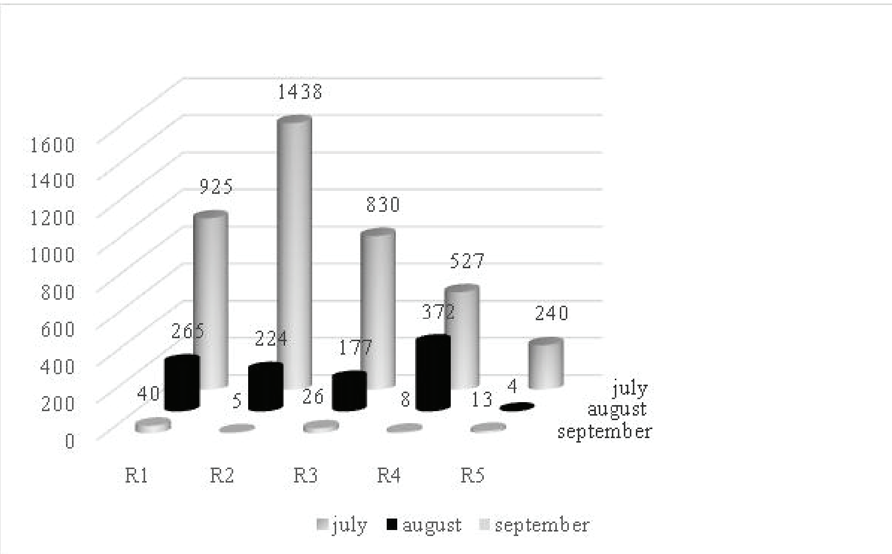

Figure 1. Number of individuals by sampling sites and months.

Abundances of macrozoobenthic invertebrates have great variations from site to site. At R_1 the community is dominated by Ephemeroptera larvae in July, by Gammarida in August and by Chironomida larvae in September. At R_2 the Ephemeroptera larvae are dominant in July, Coleoptera in August and Diptera and Trichoptera larvae in September. At R_3, in July and August, Gammarida are dominant and in September, Coleoptera. At R_4 Gammarida are dominant in July, Chironomida larvae in August and Ephemeroptera larvae in September. At R_5 Ephemeroptera larvae are dominant in July, Diptera larvae in August and Chironomida larvae in September.

The great abundance of Ephemeroptera larvae (50.05%) at R_1 in July (Table 2) is characteristic for this river sector because the substratum is covered by stones and pebbles, favourable for this group and the water flows fast. This insect group is found in clean, unpolluted waters and they are very sensitive to pollution (ALHEJOJ et al., 2014).

Table 2. Abundance of macrozoobenthic groups by sampling sites and months.

		R1			R2			R3			R4			R5	
	July	Aug	Sept	July	Aug	Sept	July	Aug	Sept	July	Aug	Sept	July	Aug	Sept
Turbelaria	0 .97	0	0	0 .07	0	0	0 .24	0	0	0 .00	0	0	0 .00	0	0
Oligochaeta	0 .11	0	0	1 .18	0	0	0 .00	0	0	0 .19	0	0	0 .00	0	0
Hirudinea	0 .00	0	0	0 .00	0	0	0 .24	0	0	0 .00	0	0	0 .00	0	0
Gastropoda	0 .54	0	0	0 .21	0	0	0 .00	0	0	0 .00	0	0	0 .00	0	0
Hydrachnidia	0 .00	0	0	0 .00	0 .44	0	0 .00	0 .56	0	0 .00	0	0	0 .00	20	0
Gammaridae	23 .14	49 .43	20	23 .57	22 .22	0	52 .77	49 .44	3 .85	43 .64	7 .80	0	4 .58	0	7 .69
Ephemeroptera larvae	50 .05	4 .53	25	51 .95	4 .89	0	12 .89	20 .22	0	36 .62	30 .65	75	59 .17	0	7 .69
Odonata larvae	0 .00	0	7 .5	0 .00	0	20	0 .00	0	3 .85	0 .38	0 .81	0	0 .83	0	0
Plecoptera larvae	7 .35	6 .04	0	0 .49	0	0	3 .73	0 .56	50	2 .85	0	0	1 .25	0	0
Coleoptera	11 .14	30 .94	2 .5	0 .97	66 .67	0	0 .60	2 .81	26 .92	0 .19	0	0	7 .08	20	7 .69
Trichoptera larvae	3 .24	5 .66	5	0 .90	1 .78	40	1 .93	1 .12	11 .54	4 .36	0 .27	0	0 .42	0	0
Diptera larvae*	2 .70	3 .40	2 .5	0 .70	4	40	0 .12	1 .12	3 .85	0 .95	0 .81	0	0 .00	40	0
Chironomida larvae	0 .76	0	37 .5	19 .96	0	0	27 .47	24 .16	0	10 .82	59 .68	25	26 .67	20	76 .92
H	1 .69	1 .29	1 .76	1 .4	0 .86	1 .05	1 .22	1 .24	1 .32	1 .28	0 .96	0 .56	1 .1	1 .01	0 .79
E	0 .74	0 .72	0 .91	0 .61	0 .48	0 .96	0 .56	0 .6	0 .74	0 .58	0 .6	0 .81	0 .57	0 .73	0 .57
* other than Chironomida															

In July, the smallest abundance at this sample site (Table 2) is reached by Gastropoda (0.54%). We have identified Ancylidae from Class Gastropoda, which are characteristic to the upper sector of the rivers but do not have great abundances in their habitats (MIENIS & RITTNER, 2013).

An important proportion is represented also by Gammarida because this sector despite the fact it is situated near the spring is very rich in submerged aquatic vegetation, especially mosses; this habitat is very favourable for Gammarida (YEMELYANOVA et al., 2002). Trichoptera and Plecoptera larvae are also abundant in this sector with fast running water, low temperature and high oxygen content.

At sample site R_2 the greatest abundance is registered by Ephemeroptera larvae (51.95%) in July and the lowest by Triclads - Turbelariata (0.07%) (Table 2). At this sample site, we can observe an increase of Chironomida larvae abundance as a consequence of detritus accumulation in the substratum. This detritus is a good trophic base for Chironomidae larvae (HENRIQUES-OLIVIERA et al., 2003; GOMEZ MACHADO et al., 2015). Trichoptera and Plecoptera larvae are also present in the sample but with a smaller abundance. This situation is according to the hydrology of this sector, characterised by a slower current, the substratum covered by pebbles and sand, the decrease of the oxygen content, the increase of the temperature and the organic content. At this sample site we can find also Oligochaeta in the sandy and muddy portions of the substratum.

At R_3, in July, Gammarida have a great abundance (52.77%), followed by Chironomida larvae (27.47%) and the lowest values are reached by Diptera larvae (0.12%) (Table 2). The great abundance of Gammarida is due to the abundance of the organic substances and the presence of the well-developed phytobenthos. The characteristic groups for the upper sector - Trichoptera, Plecoptera and Ephemeroptera have a reduced abundance compared to the upstream sites. This is a consequence of the hydrological modifications in the hilly sector of the river and of the different substratum (STOYANOVA et al., 2014; FRICK, 1998).

At this sample site we have found Hirudineea specimens, which indicate a greater organic content compared to the upstream sites (GRANTHAM & HANN, 1994; KAZANCI et al., 2015). The increase of the organic content is due to the fact that the river flows through several localities from were a certain quantity of household waste is discharge.

These accumulate more and more downstream because the mentioned localities do not have sewage plants and some of the household waste are evacuated directly in the river.

At R_4 the greatest abundances are reached by Gammarida (43.64%) followed by Ephemeroptera larvae (36.62%) and the smallest by Oligochaeta and Coleoptera (0.19%) (Table 2). At this sample site the structure of the macrozoobenthic community shows an improved water quality due to the decrease of the organic content. This fact is certified by the increase of the abundance of Ephemeroptera, Plecoptera and Trichoptera larvae. Their greater abundance is the result of the increase of the quantity of dissolved oxygen in the water and lower temperature (STOYANOVA et al., 2014; FRICK 1998). The river in this sector crosses a forest area and the shade of the trees do not allow the water to warm as much as at upstream sites, so the oxygen content of the water due to temperature will be higher.

In this sector we have identified Oligochaeta, which have sandy and muddy patches where they can settle. We have also identified Odonata larvae, which are not very sensitive to the water quality and find enough food to develop in this sector. Another sign of the improvement of the water quality is the lack of Hirudineea in this sector. The greater water volume of the river dilutes the organic substances and also some of them settle in the riverbed upstream before reaching this sector.

At R_5 the greatest abundance is reached by the Ephemeroptera larvae (59.17%) in July, followed by Chironomida larvae (26.67%). Trichoptera larvae have the smallest abundance (0.42%) at this site in July (Table 2). Gammarida abundance decreases because of the lack of the submerged vegetation and phytobenthos in this sector (YEMELYANOVA et al., 2002).

Oligochaeta are absent despite the existence of the sandy regions where they can settle, but probably if they exist they are very rare and they escaped from sampling. Chironomida larvae are very abundant and this fact proves a high organic content in the riverbed (HENRIQUES-OLIVIERA et al., 2003; GOMEZ MACHADO et al., 2015). The abundance of Odonata increases in this river sector with a slow flow.

Gammarida (49.43%) and Coleoptera (30.94%) register the greatest abundances at R_1 in August and the smallest Ephemeroptera larvae (4.53%) and Diptera larvae (3.40%) (Table 2). The decrease of Ephemeroptera abundance may be the result of the emergence of a generation of adults. Gammarida are more abundant than in July probably due to the reproduction and emergence of a new generation. There can be observed an important increase of the abundance in case of Coleoptera also, probably due to the reproduction.

At R_2, in August, the greatest abundance was reached by Coleoptera (66.67%), followed by Gammarida (22.22%). Trichoptera larvae have the smallest abundance (0.44%) (Table 2). Chironomida and Ephemeroptera larvae decrease significantly their abundance. This fact may be caused by the reduced water volume and the high temperature in this month affecting the sensitive macrozoobenthic taxa.

At R_3, in August, the highest abundance is reached by Gammarida (49.44%), followed by Chironomida larvae (24.16%) and Ephemeroptera larvae (20.22%). Plecoptera larvae have the smallest abundance (0.56%) (Table 2). At this sampling site, the abundance of the first three groups remains almost the same as in the previous month. During the three months of study this site showed the greatest stability in the composition of the macrozoobenthic community along the whole river.

At R_4, in August, the most abundant group is Chironomida larvae (59.68%) and the smallest abundance was reached by Trichoptera larvae (0.27%) (Table 2). Chironomida probably increase as a result of the accumulation of organic substances and water warming which stimulates the riverbed microbiota decomposing activity. As a result of the organic substratum decay the oxygen content decrease, toxic substances are released and inhibit the development of most macrozoobenthic taxa, so Chironomida larvae have no competitors in the substratum (HENRIQUES-OLIVIERA et al., 2003; GOMEZ MACHADO et al., 2015).

At R_5, in August, the most abundant are Diptera larvae (40%) and the other two identified groups, Chironomida larvae and Coleoptera, have the same abundance (20%) (Table 2) At this site, the macrozoobenthic community is profoundly modified so we suppose there were other influences not just the temperature and oxygen which affected the benthic community.

We suppose that an pollution phenomena occurred, which affected the most macrozoobenthic groups. The small number of taxa represented by small number of specimens is typical for an environment affected by anthropogenic pollution.

In September, at R_1 the greatest abundance is reached by Chironomida larvae (37.50%) and the smallest by Diptera larvae and Coleoptera (2.50%) (Table 2). The great increase of Chironomida larvae abundance is due to the decomposing activity of the bacteria from the substratum stimulated by the high water temperatures during the summer period. Plecoptera larvae are absent from the site in September and Gammarida and Ephemeroptera larvae have great abundances. An interesting fact is the presence of Odonata larvae at this site, probably resulted from the eggs laid by the adults during summer. Odonata adults are good flyers and they can fly to long distances from the place they have emerged. As a result they can lay their eggs far from their emergence place (CORBET, 2003).

At R_2, in September, the greatest abundance is reached by Diptera and Trichoptera larvae (40%) and the smallest by Odonata larvae (20%) (Table 2). The structure of the macrozoobenthic community at this site is very different from the previous months. This can be caused by the heavy rains fallen at the beginning of the month, which washed the substratum at this site, where the water is shallow and the submerged vegetation is absent, so the macrozoobenthic invertebrates were probably moved downstream.

At R_3, in September, the greatest abundance was reached by Plecoptera larvae (50%) and the smallest by Gammarida and Diptera larvae (3.84%) (Table 2). The increase of the Plecoptera is probably due to the reproduction and the specimens which were washed away from upstream. We also identified Odonata larvae resulted from the reproduction in summer.

At R_4, in September, the greatest abundance is reached by Ephemeroptera larvae (75%) followed by Chironomida larvae (25%) (Table 2). The changing of the abundance of the groups in this month in behalf of the Ephemeroptera larvae is a consequence of the heavy rains, which washed out from the substratum the organic debris which favoured the settlement of Chironomida larvae, so their abundance decreased compared to the previous months.

At R_5 the greatest abundance is reached by Chironomida larvae (76,92%) and the smallest by Gammarida, Ephemeroptera larvae and Coleoptera (7,69%) (Table 2). Chironomida larvae are very abundant compared to the previous months because this site is the most affected by anthropogenic pollution. In the substratum, during the summer period, a great amount of organic debris accumulated, which favoured the settlement of Chironomida larvae (HENRIQUES-OLIVIERA et al., 2003; GOMEZ MACHADO et al., 2015).

The values of the Shannon-Wiener diversity index were the greatest at R_1 in September (1.76) and July (1.70). At the rest of the sample sites the value of this index decreases reaching the smallest values at R_4 (0.56) an R_5 (0.79) (Table 2).

The greatest value of the index is near the spring because this sector is the less affected by anthropogenic impact. During the study period, the minimum value of the index was reached in August when the community is affected probably by high temperature values.

In the next two sample sites, the values of the index are smaller with the same decrease in August. From these two sites at R_3 the variations of the index values between the months is smaller showing a more stable community due to the low human impact and greater water volume.

At the last two sites, the values of the index are smaller than upstream; during the study period there was also a progressive decrease of the diversity. This is due to the raised temperature of the water, decomposing processes and the increase of the oxygen deficiency.

The equitability index has the greatest values at R_2 (0.96) and R_1 (0.91). The smallest values are at R_2 in August (0.48) and R_3 in July (0.56) (Table 2). The values of the equitability are relatively great especially at the two sites upstream. At the next three sample sites the values decrease but have close values between the three study months.

The Hilsenhoff index shows that the water quality is between excellent and good and the degree of pollution is from inexistent to poor (Fig. 2).

In July, the quality of the water in all sites is excellent (HBI< 3.75) with site R_5 situated exactly at the limit value. In August, the water quality is very good at R_3 (HBI 3.76 – 4.25) with a very small degree of organic pollution and in the rest of the sites is excellent (HBI < 3.75) with no pollution.

The pollution downstream R_4 had anthropogenic origins. At this site, the river has passed through several localities, which increased the organic content of the water and probably have polluted it with different chemical substances, which eliminate the most sensitive taxa from the macrozobenthic community.

In September, at R_5 the quality of the water is good (HBI 4.26 – 5.0) moderately polluted, at R_4 the water quality is very good (HBI 3.76 – 4.25) and at the rest of the sample sites is excellent (HBI < 3.75) (Fig. 2).

The increase of the pollution level at R_5 in September is probably caused by anthropogenic influences, which are not permanent and are accidental.

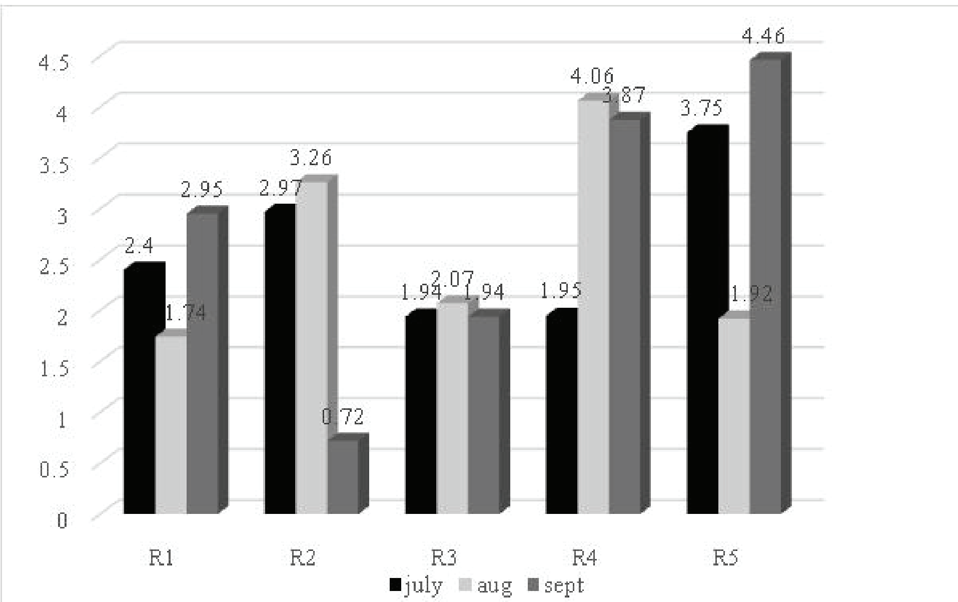

Figure 2. Hilsenhoff index by sampling sites and months.

CONCLUSIONS

As a result of our study in the Valea Roșia River, we can affirm the following: at R_1 the macrozoobenthic community is typical for the mountainous region and has a pronounced dynamics depending on the environmental conditions in each month. The variations of the community structure are due to the fluctuations of the water level, dissolved oxygen, water temperature and intensity of debris decay.

At R_2 the community is modified as a result of the hydrological modification of the river, the human impact from Roșia village and the fish pond from this locality.

The next three sites are typical for the hilly region and are under anthropogenic influence because the river passes through several localities. Between the localities, there is possible that the river undergoes through a self-purification process which is in favour of macrozoobenthic invertebrate communities. The human impact is not very pronounced as emphasized by the Hilsenhoff index values.

In order to maintain or even to improve the water quality the periodically monitoring is recommended. The river sectors where the shading of the water is pronounced it will be necessary to prevent the raising of the water temperature during summer period, especially in the portions where the water is shallow and flows slowly. Here during the summer period the decomposing processes consume the oxygen from the water and the raised temperature will deplete more the oxygen content, so the macrozoobenthic invertebrate development will be limited.

It will be important to control the pollution with domestic residues from the localities along the river. In the households a great amount of washing powders and other chemicals are used and because of the lack of the sewerage system they reach directly or indirectly the river, generating eutrophication. This phenomenon also modifies the structure of the benthic community.

We can state that the investigated river has clean, unpolluted water in the spring sector, in the rest of the course the human impact is still low, but it is necessary to monitor its evolution permanently in order to be able to act as soon as the water quality will be affected by anthropogenic activities.

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Received: March 31, 2016
Accepted: June 3, 2016

BALANCE OF PRODUCED WASTE IN LAGOONS OF THE FORMER OIL REFINERY IN KUÇOVA TOWN, ALBANIA

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Abstract. The District of Kucova, is a part of a higher administrative unit, Berat. The premises of the former refinery are located on a total area of about 25 ha. Currently, this site is without any industrial use; from the geomorphological point of view, the wider vicinity of the site can be characterized as a flat region with local elevations not exceeding 25 m. The former refinery lies in the floodplain of the Driza valley. The highest point of the premises is located on a Neogene elevation, which runs through the western part of the premises and reaches an altitude of about 53 m. The lowest-lying part of the premises reaches an altitude of about 36 m. The oil refineries in Kucova had a great impact on the environment. Currently, they do not work and are sold for scrap. However, the environment is still polluted by petroleum products remained from the former refinery. In this paper, we will discuss only about the former oil refinery in Kuçova town. The article is the result of research work carried out by different institutions. The results refer to the year 2011. The surveys were performed during subsequent years. All the technological facilities of the former refinery caused environmental pollution. The main hot spots of contamination are the lagoons. Lagoons caused the greatest impact on the environment. Technological wastes were discharged into them and are stored for many years. The authors' goal is to determine the amount of contaminated waste in the lagoons. In the area of the former refinery, there are 6 lagoons. Two lagoons (No. 1, 2) have the technological waste of distillation units, referred to as asphalt storage lagoons; in two lagoons (No. 3, 4), there are deposited acid wastes; in the lagoons no. 5 and no. 6, there are deposited coke technological waste. Lagoons are separated into two products: Waste volume (13,500 m^3) and produced waste volume (> 2.0831 million m^3). This is a unique case study for the pollution level in Albania, highlighting contamination and the impact on the environment.

Keywords: former oil refinery, lagoons, tars, produced waste.

Rezumat. Balanța deșeurilor produse în lagunele fostelor rafinării petroliere din orașul Kuçova, Albania. Districtul Kucova este o parte a unei unități administrative mai mari, Berat. Incinta rafinăriei se extinde pe o suprafață totală de aproximativ 25 ha. În prezent, aceasta nu are nici o utilizare industrială; din punct de vedere geomorfologic, cea mai mare parte a arealului limitrof poate fi caracterizat ca o regiune plană, cu ușoare înălţări locale ce nu depășeșc 25 m. Fosta rafinărie se află în lunca văii Driza. Cel mai înalt punct este situat pe o zonă neogenă care trece prin partea de vest a incintei și ajunge la o altitudine de aproximativ 53 m. Partea cea mai joasă are o altitudine de aproximativ 36 m. Rafinăriile de petrol din Kucova au avut un impact mare asupra mediului. În prezent, acestea nu funcționează și sunt vândute la fier vechi. Cu toate acestea, mediul este în continuare poluat cu produse petroliere ce au rămas de la fosta rafinărie. În această lucrare, vom discuta doar despre fostele rafinării de petrol din orașul Kucova. Articolul este rezultatul unei cercetări efectuate de către diferite instituții. Rezultatele aparțin anului 2011. Anchetele s-au desfășurat pe parcursul anilor următori. Toate facilitățile tehnologice ale fostei rafinării au cauzat poluarea mediului. Principalele puncte fierbinți de contaminare sunt lagune. Lagunele au provocat cel mai mare impact asupra mediului. Deșeuri tehnologice au fost evacuate în ele și sunt păstrate timp de mulți ani. Scopul autorilor este de a determina cantitatea de deșeuri contaminate din lagune. În zona fostei rafinării sunt 6 lagune. Două lagune (nr. 1, 2) au deșeuri tehnologice provenite de la unitățile de distilare, denumite iazuri de depozitare de asfalt; două lagune (nr. 3, 4) au deșeuri acide; în lagunele nr. 5 și nr. 6 sunt depozitate deșeuri tehnologice de cocs. Lagunele sunt separate în două produse: Volumul de deșeuri (13.500 m^3) și volumul de deșeuri produse (> 2,0831 milioane m^3). Acesta este un studiu de caz unic pentru nivelul de poluare din Albania, care subliniază contaminarea și impactul asupra mediului.

Cuvinte cheie: fosta rafinărie de petrol, lagune, gudron, deșeuri produse.

INTRODUCTION

In 1934, the refinery in Kucova started its operation with lamp-oil production; it belonged to the Italian company AIPA. In 1944, the refinery passed into the property of the state enterprise. Since 2008 to the present time, the owner of the refinery and land has been the Town of Kucova (Municipality of Kuçovë, 2004; 2005; 2008).

The site concerned has been used for industrial purposes since the mid-20th century. In 1934, at the site, the operation of an oil refinery was started in the old plant. This plant was gradually extended; new technological equipment was installed in order to increase production and the effectiveness of the production process. In 1963, the operation was launched in a new, separated plant, which was located in the close proximity to the old plant. The refinery was definitively closed down in 1992.

Currently, the former oil refinery is without any industrial use. Up to this day, 2016, about forty smaller buildings and structures have been preserved from the era of the refinery and other buildings illegally constructed. At present, the premises are not safeguarded against the entry of unauthorized persons and therefore it is inhabited by 100 – 150 persons without authorization. Residents created small gardens between the lagoons (dykes). They plant vegetables and use water from the lagoons. This is an extreme case of how to live in a polluted area. The former refinery area lies within the residential areas and increases the impact on the environment.

In area of the former oil refinery and outside it, particularly in the proximity of the northern and eastern margins, there are several functional oil wells with operating equipment. Conducted studies have assessed a large volume of waste produced in the lagoons. This is done to precede subsequent projects for environmental rehabilitation.

MATERIAL AND METHODS

The amount of Waste and Waste products are calculated on the basis of three methods:

Topographic survey; Geophysical survey; Digging holes and drilling of wells without rallied.

The topographic survey is conducted by licensed companies. The results of the topographic survey are presented in the archive of the Municipality of Kucova. On the basis of this report there is estimated the surface of lagoons. In this area, there are not included the dykes between the lagoons (Fig. 1).

Figure 1. Topographic map of the lagoons and east are of former petroleum refinery (original).

Environmental study. This is performed to estimate the thickness of the waste produced in the lagoons. These works are carried out according to the approved methods. There are also digging pits and drilled wells (no casing) to measure the thickness of the produced waste. On the basis of these methods, there are defined the sizes of the lagoons and the corresponding volumes are calculated.

RESULT AND DISCCUSION

Based on the above methods there are calculated the surface, the thickness of free phase of waste and their volume.

Balance of waste of the lagoons no. 1 and 2. The maximum verified thickness of asphalts deposited in the lagoons no. 1 and 2 was 0.5 m. The total area of the lagoons and the general balance of the vacuum residues (asphalts) are shown in the following table.

In case of lagoon no. 1, the presence of ps (petroleum substances) contamination was sensorially and analytically verified at a depth interval of 0.2 – 2.7 m of underlying soils in the area adjacent to the foot of the Neogene elevation. It is not certain whether the contamination is connected with wastes deposited above the soils or whether it is rather transport contamination originated from another area. In a part of the lagoon no. 1, which lies closer to the axis of the valley, the presence of contamination of underlying rocks was not detected (Fig. 2).

Legend
Lagoons 1, 2 - Lagoons of asphalts
Lagoons 3, 4 - Lagoons of tars.
Lagoons 5, 6 - Lagons of coking residues

Figure 2. Topographic map of the Lagoons 1 - 6 (original).

Table 1. General balance of wastes (asphalts) deposited in the lagoons no. 1 and 2.

Lagoons	Area (m^2)	Average thickness of asphalts (m)	Volume of vacuum residues deposited in the lagoon (m^3)
1	3,500	0.4	1,400
2	6,000	0.3	1,800
TOTAL			3,200

Beneath the lagoon no. 2, we mention the presence of a contaminated layer of sandy clays sometimes of about 0.5 m in thickness. Theoretically, a very limited amount of potential contaminated leachates from wastes can penetrate this layer and reach the underlying layer of water-saturated highly sandy clays (Table 1).

Some diggings located in the southern dyke of the lagoon no. 2 verified the presence of a contaminated layer of slightly sandy clays at a depth interval of about 1.0 – 2.0 or 2.0 – 3.0 m, respectively. A layer of fills with waste was detected in other digging at a depth interval of about 1 to 2 m. Contamination of slightly sandy clays can originate from overlying layers containing waste, from where it can spread farther down to the underlying layer of water-saturated highly sandy clays. It may also be caused by the accumulation of contaminants in the zone of groundwater fluctuation; groundwater transports contaminants from other parts of the former oil refinery.

Balance of waste of the lagoons no. 3 and 4. Tars (acid substances) were deposited in these lagoons in the past. The survey verified their presence in both the lagoons and they have a maximum thickness of 0.8 m. The following table shows a general calculation of the balance of tars deposited in these lagoons.

The presence of contamination, besides wastes, was detected by sensorial evaluation in all dug holes, practically in its entire thickness, which ranges from about 1.5 m. to 3.5 m. and is predominantly represented by clays to sandy clays. The total area of contaminated soils underlying the wastes can be estimated on the basis of the known surface area of the lagoons at about 12,000 m^2, including dykes (Table 2).

Table 2. General balance of wastes (tars) deposited in the lagoons no. 3 and 4.

Lagoons	Area (m^2)	Average thickness of tars (m)	Volume of tars in the lagoons (m^3)
3	7, 700	0.5	3,850
4	4, 400	0.5	2,200
TOTAL			6,050

Balance of waste of the lagoons no. 5 and 6. Wastes from the coking operation were deposited in these lagoons in the past. When the survey was conducted, the ground in this hot spot was so inaccessible that the planned survey work could not be carried out. Based on consultations with the representatives of the former management of the refinery, the maximum thickness of the deposited waste could be about 0.5 – 1.0 m. The surveyed surface area of the lagoon no. 5 is of about 2,900 m^2 and of the lagoon no. 6 of about 1,200 m^2, altogether about 4,100 m^2. Due to the absence of data from the field survey and details on the character of operation, no balance was prepared for this type of waste. An estimate of the volume of waste deposited in the lagoons nos. 5 and 6 is 2,050 to 4,100 m^2.

Evaluation of the balance of wastes. The above-given general balances of the lagoons of wastes occurring at the site of concern, i.e. vacuum residues (asphalts) and acid residues on oil refining (tars). The vacuum residues were primarily dispatched outside the premises for further processing.

Due to the fact that we could not manage to find out the length and frequency of technological shutdowns and periods of falling-off demands for vacuum residues through the study of archive materials, the calculation of the volume of vacuum residues in the lagoons is only for orientation. It is necessary to emphasize that since the end of operation of the refinery, the material from the lagoons nos. 1 and 2 has been manually excavated by people for secondary use, thereby reducing the volume of the dumped waste. By comparing the volume of vacuum residues deposited in the lagoons to their total production it arises that the remaining dumped waste is the mere fracture of the total volume of the production of this type of waste.

When calculating the balance of tars, we considered the situation that all the produced volume of tars was deposited in the lagoons inside the premises of the refinery from the beginning of the production of lubricating oils. As compared to the calculation of the volume of tars dumped in the lagoons no. 3 and 4, based on a field survey, the balance can be considered as even.

The following estimate of the balance of volumes is prepared for wastes dumped in the lagoons nos. 1 to 6. The balance of contaminated soils could not be made because of the low degree of exploration (Table 3).

Table 3. Estimate of balance of wastes deposited in the lagoons.

Lagoons	Area (m^2)	Average thickness of asphalts (m)	Volume of vacuum residues deposited in the lagoon (m^3)
1	3,500	0.4	1,400
2	6,000	0.3	1,800
3	7,700	0.5	3,850
4	4,400	0.5	2,200
5	2,900	1.0	2,900
6	1,200	1.0	1,200
TOTAL	25, 700	-	13, 350

CONCLUSIONS

Contamination of the area of the former oil refinery has been identified in a larger part of the premises of the former refinery, which is, together with associated plots of land, currently in possession of the Town of Kuçovë (NISHANI, 2004; URBÁNEK & BARTOŇ, 2009; URBÁNEK & POLENKOVA, 2010).

The main hot spot of contamination is represented by the lagoons nos. 1 to 6 containing wastes from oil processing, i.e. asphalts, tars and coking residues. The amount of Waste and Waste products are (area and volume) estimated on the basis of three methods: topographic survey; geophysical survey and digging holes and drilling of wells without rallied.

The total amount of waste dumped in the lagoons no. 1 to 6 on a surface of about 25,700 m ^{2} can be estimated at 13,350 m^3, while the total volume of produced waste is estimated >2 083 100 m^3 (Table 3).

This is a unique case study for the pollution level in Albania, emphasizing contamination and the impact on the environment as mentioned above (Plates I, II).

Table 3. Balance of the total volume of produced waste.

Lagoon no.	Type of deposited waste	Area of the lagoon (m^2)	Average thickness of deposited waste (m)	Volume of waste deposited in the lagoons (m^3)	Total volume of waste deposited in lagoons (m^3)	Estimate of the volume of produced waste (m^3)
1	Asphalts	3,500	0.4	1,400	3,200	2077000
2		6,000	0.3	1,800		
3	Tars	7,700	0.5	3,850	6,050	6100
4		4,400	0.5	2,200		
5	Coke-oven residues	2,900	1.0	2,900	4,100	It is not estimate
6		1,200	1.0	1,200		
TOTAL		25 ,700	-	13, 350	13, 350	>2 083 100

ACKNOWLEDGEMENTS

The authors thank to the Municipality of Kuçovë for their support. Papers and scientific reports were made available to us for the article. We also thank to the local residents for their understanding shown during the field surveys.

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Received: March 22, 2016
Accepted: May 12, 2016

PLATE I

Photo 1. Oil refinery during 1965 (original).

Photo 2. Lagoon no. 3 (original).

PLATE II

Photo 3. Lagoon no. 2 (original).

Photo 4. High level of toxicity on lagoon no. 2 (urban waste) (original).

Photo 5. Lagoons no. 4, 5, 6 (original).

Photo 6. An urban trash container in a polluted area (original).

COMPARATIVE ECOLOGICAL CHARACTERIZATION OF THE SOIL MITE POPULATIONS (ACARI: MESOSTIGMATA) FROM SOME ANTHROPOGENIC ECOSYSTEMS, ROMANIA

MANU Minodora, ONETE Marilena

Abstract. The paper presents the taxonomical structure and diversity of soil mite populations (Acari: Mesostigmata) from some anthropogenic ecosystems: arable fields from Insula Mare a Brăilei; urban parks from Bucharest and spoil dump areas from Retezat Mountains. Forty three species were identified, with 197 individuals. Each investigated anthropogenic ecosystem was characterized by the specific acarological structure. The Shanon index of diversity demonstrated that the urban ecosystems were the most favourable habitats for these arthropods (invertebrates), in opposition with arable fields. The dominant species for the studied ecosystems were *Hypoaspis aculeifer* (Canestrini 1884) and *Asca bicornis* (Canestrini & Fanzago 1887). The dominance and equitability index revealed that the ecosystem with a lower diversity was characterized by a few species with a highest number of individuals (arable field with *Zea mays* L.). In urban parks and spoil dump areas, these indexes showed us that the numerical abundances of the identified species had an equitable distribution. The Bray-Curtis similarity index between soil mite populations revealed the affinity between invertebrates from the three categories from the anthropogenic ecosystems.

Keywords: agroecosystems, diversity, indexes, dominance, dump, mites, urban.

Rezumat. Caracterizarea ecologică comparativă a populațiilor de acarieni edafici (Acari: Mesostigmata) din câteva ecosisteme antropice, România. Lucrarea prezintă structura taxonomică şi diversitatea faunei de acarieni edafici (Acari-Mesostigmata) din câteva ecosisteme antropice: culturi agricole din Insula Mare a Brăilei, parcuri urbane din Bucureşti şi halde de steril din munţii Retezat. Au fost identificate 43 de specii, cu 197 indivizi. Fiecare ecosistem antropic investigat a fost caracterizat de o structură acarologică specifică. Indicele de diversitate Shannon a demonstrat faptul că ecosistemele urbane au oferit cele mai bune condiții de dezvoltare pentru aceste nevertebrate, în opoziție cu agroecosistemele. Speciile de acarieni dominante pentru ecosistemele studiate au fost *Hypoaspis aculeifer* (Canestrini 1884) şi *Asca bicornis* (Canestrini & Fanzago 1887). Indicele de dominanţă şi de echitabilitate au evidențiat că ecosistemele agricole sunt caracterizate de câteva specii dominante din punct de vedere numeric (în special terenul arabil cu porumb, *Zea mays* L.). În parcurile urbane și haldele de steril, indivizii de acarieni au fost distribuiți în mod echitabil. Indicele de similaritate Bray-Curtis a indicat o afinitate semnificativă între populațiile de acarieni din cele trei categorii de ecosisteme antropice studiate.

Cuvinte cheie: agroecosistem, diversitate, indici ecologici, dominanţa, haldă, acarian, urban.

INTRODUCTION

It is known that soil mites - Mesostigmata were used as bioindicators, due to their low tolerance to changes in soil environment, being relatively easy to collect undamaged, reaching high densities in soil and recording high trophic and taxonomic diversity. Their presence or absence in the soil horizons may be a good base for describing changes of environmental conditions and ecosystem perturbations (GULVIK, 2007; KOEHLER & MELECIS, 2010; SKORUPSKI et al., 2013). SKORUPSKI et al., (2013), declared that "even common species may be used to evaluate the effects of human influence on the environment, not only at the species level but also the zoocoenosis level".

In Europe, studies concerning the structure and dynamics of Mesostigmata mites were intensively made in different anthropogenic ecosystems (arable fields, urban, industrial and post-industrial habitats) from Germany, Poland, Austria, Latvia, Ireland, Norway, Spain and Greece (NIEDBALA, 1982; 1990; RUF & BECK, 2005; SALMANE & BRUMELIS, 2010; KOEHLER & MELECIS, 2010; BADIERITAKIS et al., 2012; PÉREZ-BOTE & ROMERO, 2012; WISSUWA et al., 2012; ARROYO et al., 2013; SKORUPSKI et al., 2013; COULSON et al., 2015; TELNOV & SALMANE, 2015; MANU et al., 2015).

In Romania, the soil fauna (including the soil mites) from anthropogenic ecosystems was investigated and the results were published in few scientific papers (FIERA, 2009; VASILIU-OROMULU et al., 2009; MANU & HONCIUC, 2010; HONCIUC & MANU, 2010; MANU, 2010; FIERA et al., 2013). However, a comparative study is required, in order to highlight the importance on an extensive research program on national level, being significantly to demonstrate that soil fauna constitutes a valuable tool to characterize the anthropogenic impact on natural ecosystems, even if it will be investigated a microscopic edaphic group, as mites.

MATERIAL AND METHODS

The research was made in period 2005-2007, in three types of anthropogenic ecosystems from Romania: agroecosystems from Insula Mare a Brăilei – *Triticum aestivum* L. (TA); *Glycine max* L. (GM); *Zea mays* L. (ZM), urban parks from Bucharest city – Cişmigiu (CS); Unirea (UN); Izvor (IZ) and spoil dump areas from Retezat Mountains – Bârlii (BR); Râuşor (RS); Ciurila (CR). The geographical description of the investigated ecosystems is presented in Table 1 and Fig. 1.

Taking into account the vegetation, the agroecosystems were monocultures with *Triticum aestivum*, *Glycine max* and *Zea mays*. The urban parks are characterized by the presence of 42 species of trees, 23 species of shrubs, 9 species of lichens and 78 vascular plants. Common species for all urban parks were: *Aesculus hippocastanum* L., *Quercus rubra* L., *Parmelia saxatilis* L., *Geranium pusillum* L. and *Agrostis stolonifera* L. The natural vegetation had mainly disappeared, being replaced by planted species (especially trees brought from Europe, China, Japan, America, etc.) (MANU & HONCIUC, 2010).

Table 1. Characterization of the investigated anthropogenic ecosystems.

Ecosystems	Code	GIS coordinates	Altitude	Soil
Agroecosystems	TA	44°49’33.09” N 27°59’35.34” E	1. 5 m.	Alluvial
	GM	44°49’26.35” N 27°59’29.54” E	2 m.	Alluvial
	ZM	44°49’01.43” N 27°59’31.59” E	1. 75 m	Alluvial
Urban parks	CS	44°25’56.6” N 26°05’27.5” E	77 m.	Sandy
	UN	44°25.5’56.6”N 26°08’09.9” E	77 m.	Sandy
	IZ	44°25’56.4” N 26°05’27.8” E	78 m.	Sandy
Spoil dump areas	BR	45°23’29.13” N 22°47’58.87” E	1,090 m.	Partially covered with acidophilous rendzinic soil
	RS	45°25’13.57” N 22°50’48.80” E	1,100 m.	Partially covered with acidophilous rendzinic soil
	CR	45°25’11.06” N 22°51’33.62” E	1,155 m.	Stable acidophilous rendzinic soil stratum

In spoil dump areas, the vegetation is characterized by woody species (*Betula pendula* Roth.; *Picea abies* (L.) H. Karst.; *Rubus idaeus* L.), hemicryptophyte species, ruderals and grassy species. In Bârlii dump area (BR), there were identified ombrophilous species, as: *Myosotis sylvatica* Ehrh. ex. Hoffm.; *Chrysosplenium alternifollium* L., *Impatiens noli-tangere* L., *Laserpitium archangelica* Wulfen ex Jacq. Râuşor (RS) spoil dump area is defined by herbaceous species, characteristic for the forest cuttings, the wet weeds and for the meadows. In Ciurila dump area (CR), there were observed species with high biomass: *Deschampsia caespitosa* (L.) P.Beauv.; *Dryopteris filix-mas* (L.) Schott; *Petasites albus* (L.) Gaertn. and *Tussilago farfara* L. (MANU, 2010).

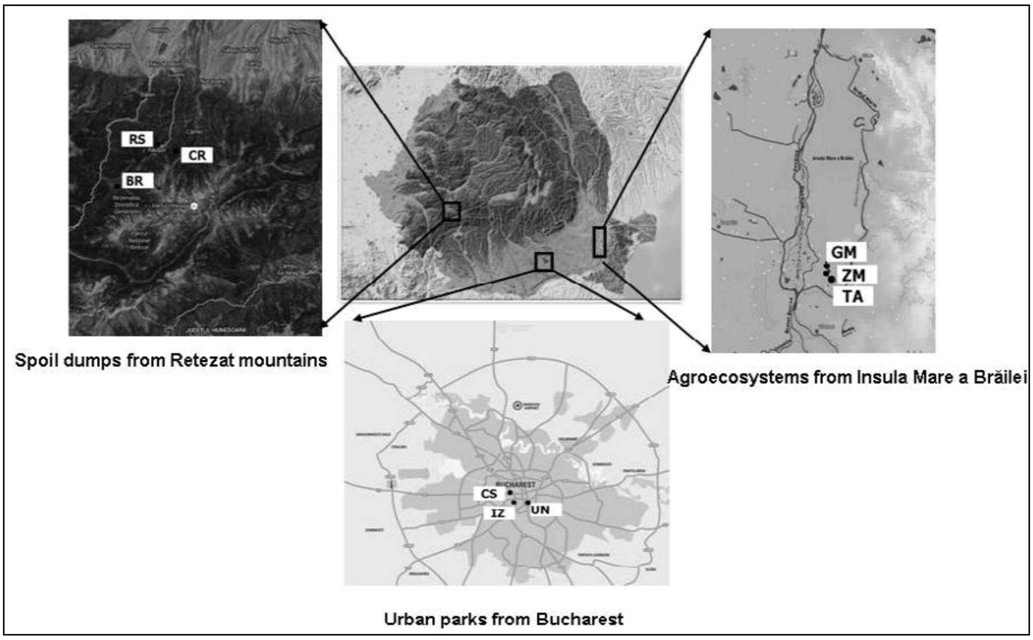

Figure 1. The geographical position of the anthropogenic investigated ecosystems from Romania (www.google earth.com).

The soil samplings were collected randomly, with MacFadyen soil core, by 5 cm diameter. The sampling was made till 10 cm depth. The extraction of the mites was made in 10-14 days by the Berlese-Tullgren method, modified by BALOGH (1972). The samples were kept in a refrigerator till the next extraction. In total, 270 samples (5 samples, 3 times/year, 2 years, 9 ecosystems), 43 species with 197 individuals were analysed. Data were collected during the vegetation period – June, August and September, for two years. Counting and identification of the mites were made under a Zeiss binocular and microscope, using the systematic keys (GHILIAROV & BREGETOVA, 1977; KARG,

1993; MAŠAN, 2003, 2007; MAŠAN & FENĎA, 2004; MAŠAN et al., 2008; MAŠAN & HALLIDAY, 2010). Preservation of the soil mites was made in an alcohol and glycerine mixture. All identified specimens are deposited in the mite collection of the Institute of Biology – Ecological Stationary from Posada.

Mite diversity (Shannon index), dominance (D), and equitability (J) were calculated using the PAST software (HAMMER et al., 2001). The similarity of mite presence was assessed using Bray-Curtis dendrogram.

RESULTS AND DISCUSSIONS

In all investigated ecosystems, 43 Mesostigmata mites were identified, with 197 individuals. The highest number of species was identified in urban ecosystems (25 species), in comparison with agroecosystems (11 species) and spoil dump habitats (16 species). The same situation was recorded for numerical abundance and Shannon diversity index. The highest values were recorded in the investigated urban parks, in comparison with the other two types of ecosystems (Table 1). It is possible that the irrigation from urban parks in summer and the highest cover of soil with vegetation (even if we identified sandy soil, which is not so reach in organic matter) constituted proper environmental factors for Mesostigmata mites (Fig. 2). On the other hand, in urban habitats the import of different types of allochthonous soil could influence the structure of mite communities. In the spoil dump areas, the coverage with vegetation does not exceed 15% and the soil layer with organic matter is missing or very thin (Figs. 2a-i).

a. *Triticum aestivum* (TA) agroecosystem

b. *Glycine max* (GM) agroecosystem

c. *Zea mays* (ZM) agroecosystem

d. Cişmigiu (CS) urban park

e. Unirea (UN) urban park

f. Izvor (IZ) urban park

g. Bârlii (BR) spoil dump area

h. Râuşor (RS) spoil dump area

i. Ciurila (CR) spoil dump area

Figures 2(a-i). Investigated anthropogenic ecosystems (original).

If we refer to these results as preliminary ones for Romania, they are comparable with those obtained in different countries from Europe for the three types of ecosystems (Table 3). It is one exception: in Poland, intensive studies from industrial and post-industrial sites revealed the presence of 80 Mesostigmata species (SKORUPSKI et al., 2013). Seventy-five percent of the identified species from Romanian spoil dump ecosystems are similar to those described by the Polish researchers.

The dominant species were: *Hypoaspis aculeifer* (Canestrini 1883) and *Rhodacarellus kreuzi* Karg 1965 for agroecosystems; *Ameroseius fimentorum* Karg 1971, *Asca bicornis* (Canestrini & Fanzago 1887), *Hypoaspis aculeifer* (Canestrini 1883), *Rhodacarellus perspicuous* Halaskova 1959 and *Pseudolaelaps doderoi* (Berlese 1910) for urban parks; *Pachyseius humeralis* Berlese 1910, *Asca bicornis* (Canestrini & Fanzago 1887), *Hypoaspis aculeifer* (Canestrini 1883) for spoil dump areas (Table 2). It is obvious that the most abundant species for the investigated ecosystems were the last two mentioned species. *Asca bicornis* was reported in moss, rotting wood, in industrially polluted areas and derelict industrial land, being characterized as an indicator species for the first stage of ecological succession (GWIAZDOWICZ, 2007).

Table 2. The soil mites (Acari: Mesostigmata) from the investigated anthropogenic ecosystems.

	Agroecosystems			**Urban parks**			**Spoil dump areas**		
Species	**TA**	**GM**	**ZM**	**CS**	**UN**	**IZ**	**BR**	**RS**	**CR**
1. *Alliphis siculus* (Oudemans 1905)					1				
2. *Alloparasitus oblonga* (Halbert 1915).									1
3. *Amblyseius meridionalis* Berlese 1914					1	2			
4. *Amblyseius obtusus* (C. L. Koch 1839)					2	3			
5. *Ameroseius fimentorum* Karg 1971				1	3	5			
6. *Ameroseius plumigerus* (Oudemans 1930)									1
7. *Arctoseius cetratus* (Sellnick 1940)								1	1
8. *Asca bicornis* (Canestrini & Fanzago 1887)					11	6			
9. *Cheiroseius borealis* (Berlese 1904)							1	2	3
10. *Crassicheles concentricus* (Oudemans 1904)					2				
11. *Dendrolaelaps* sp.					1				
12. *Geholaspis longispinosus* (Kramer 1876)			5						
13. *Hypoaspis aculeifer* (Canestrini 1883)	7	4		2	3	6	4	1	1
14. *Hypoaspis praesternalis* Willmann 1949					3				
15. *Leioseius magnanalis* (Evans 1958)								1	1
16. *Leptogamasus* sp.				1					
17. *Lysigamasus neoruncatellus* Schweizer 1961					1				
18. *Lysigamasus truncus* Schweizer 1961				1					
19. *Macrocheles decoloratus* (C. L. Koch 1839)							1	3	
20. *Macrocheles glaber* (Muller 1860)								5	
21. *Macrocheles montanus* Willmann 1951								5	
22. *Macrocheles* sp.				1					
23. *Olopachys vysotskajae* Koroleva 1976			3		1				
24. *Pachydellus furcifer* Oudemans 1904		3		1		1			
25. *Pachyseius humeralis* Berlese 1910				4			1		8
26. *Parasitus beta* Oudemans & Voigts 1904				2		1			
27. *Parasitus loricatus* (Wankel 1861)							2		
28. *Pergamasus barbarus* Berlese 1904		2						1	
29. *Pergamasus quisquiliarum* (Canestrini 1882)	5								
30. *Pergamasus* sp.	2					1			
31. *Prozercon fimbriatus* (C. L. Koch 1839)						1			
32. *Prozercon kochi* Sellnick 1943							1		
33. *Pseudolaelaps doderoi* (Berlese 1910)					9	2			
34. *Rhodacarellus kreuzi* Karg 1965		6							
35. *Rhodacarellus perspicuus* Halascova 1959				9		3			
36. *Rhodacarellus silesiacus* Willmann 1936				4	1	2			1
37. *Rhodacarus denticulatus* Berlese 1921					1				
38. *Veigaia exigua* (Berlese 1917)						1			

39. *Veigaia nemorensis* (C. L. Koch 1939)				2		1		2	
40. *Vulgarogamasus kraepelini* (Berlese 1905)							1		
41. *Vulgarogamasus oudemansi* (Berlese 1903)	2								
42. *Zercon peltatus* C. L. Koch 1836		1							
43. *Zercon romagniolus* Sellnick 1944	5								
Total no. of species	5	5	2	11	14	14	7	9	8
		11			**25**			**16**	
Total no. of individuals	21	16	8	28	40	35	11	21	17
		45			**103**			**49**	
Shannon (H)	1.49	1.46	0.66	2.08	2.22	2.40	1.77	1.99	1.66
		2.20			**2.75**			**2.49**	
Dominance (D)	0.24	0.25	0.53	0.16	0.15	0.10	0.20	0.16	0.27
		0.13			**0.08**			**0.10**	
Equitability (J)	0.93	0.90	0.95	0.87	0.84	0.91	0.91	0.90	0.79
		0.92			**0.86**			**0.90**	

The dominance and equitability index revealed that the ecosystems with a lower diversity were characterized by a few species with the highest number of individuals. This phenomenon was observed in agroecosystems, especially in *Zea mays* field, where there were identified only two species with 8 individuals (Table 2).

Table 3. The number of Mesostigmata species recorded in anthropogenic ecosystems from Europe.

Type of ecosystem	**Country**	**No. of species**	**Reference**
Arable field	Ireland	13	ARROYO et al., 2013.
	Germany	6-23	KARG & FREIER, 1995. KOEHLER, 1999; 2000. RUF & BECK, 2005. VAN CAPELLE et al., 2012.
	Greece	1-14	BADIERITAKIS et al., 2012.
	Austria	22-36	WISSUWA et al., 2012.
	Netherlands	4-20	POSTMA-BLAAUW et al., 2012
	Latvia	28-104	SALMANE, 2001. SALMANE & BRUMELIS, 2010.
Spoil dump area	Poland	28-32 6-19 80	MADEJ & SKUBALA, 2002 MADEJ & KOZUB, 2014. SKORUPSKI et al., 2013
	Norway-Svalbard	9	COULSON et al., 2015.
	Germany	4-33	CHRISTIAN, 1995; 2002. WANNER & DUNGER, 2002 KOEHLER & MELECIS, 2010.
Urban area	Poland	1-32	NIEDBALA, 1982; 1990.
	Latvia	25	TELNOV & SALMANE, 2015.
	Germany	37	HELDT, 1995.

Analyzing the Bray-Curtis similarity between mesostigmatids, we observed that the mite populations were divided in three main clusters: spoil dump areas, with higher similarity index recorded between communities from RS and CR (q_{CR-RS} = 0.31) and between RS and BR (q_{RS-BR} = 0.23); urban parks (q_{IZ-UN} = 0.33 and q_{IZ-CS} = 0.39) and arable fields, with the highest Bray-Curtis index between TA and GM (q_{TA-GM} = 0.11) (Fig. 3). On the opposite, the highest differences were obtained between populations from urban parks and agroecosystems on one hand and between spoil dump area and urban ecosystems, on the other hand, where the index of similarity recorded values lower than q = 0.55 (Fig. 3).

We suppose that these differences between soil mite populations from the three types of the studied ecosystems were due to the specific environment conditions, type of vegetation and geographical position, taking into account that in the present study there were analysed ecosystems from mountain and plain areas.

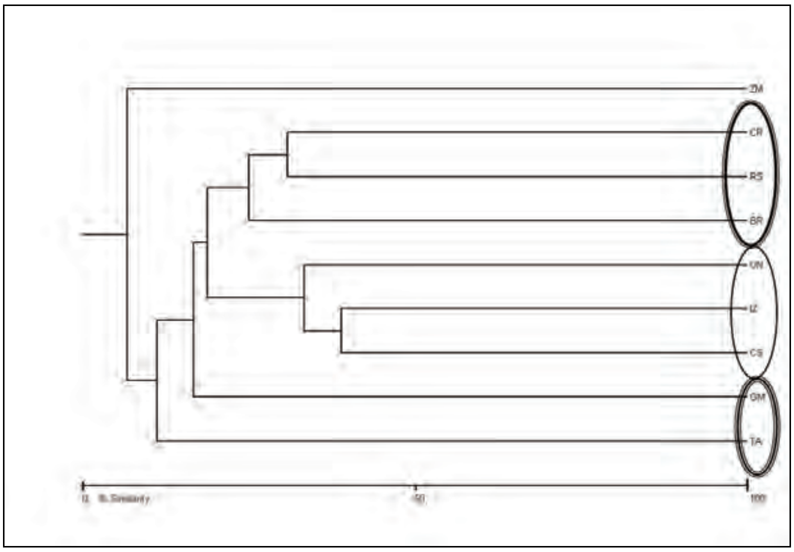

Figure 3. The similarity dendrogram (Bray-Curtis index) of the mite populations from the investigated anthropogenic ecosystems.

CONCLUSIONS

Taxonomical structure of the mite communities from anthropogenic ecosystems (arable fields, urban parks and spoil dump areas) led to the identification of 43 species, with 197 individuals. Each type of anthropogenic ecosystem was defined by a characteristic structure of the mite populations. Ecosystems with a lower diversity were characterized by a few species with the highest number of individuals. The highest species diversity was recorded in urban areas. On the opposite, there were agroecosystems, where the lowest species diversity was recorded. The dominant species in all studied ecosystems was *Hypoaspis aculeifer*. The species *Asca bicornis* was signalled in spoil dump areas and urban ecosystems. The similarities between the soil mite populations from the investigated areas revealed an affinity between invertebrates from the same type of anthropogenic ecosystems.

The present data could be considered as preliminary ones. Extensive studies concerning the soil fauna (especially mites) in correlation with environment variables, from anthropogenic ecosystems, must be developed in Romania, in order to obtain a richer database, which has to be used as an important tool for ecosystem characterization depending on the type of land use.

ACKNOWLEDGEMENTS

This study was carried out in the framework of RO1567-IBB01/2016 project, from the Institute of Biology-Bucharest of the Romanian Academy and was financed by UEFISCDI in the framework of Contract 50/2012 “Accounting for the service providing units of plants in the environmental assessment of plans and projects with biogeochemical impact at multiple scales in Rivers basins” (ASPABIR). We thank to Simona Plumb and Rodica Iosif for their assistance in the laboratory and in the field.

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Received: March 10, 2016
Accepted: April 16, 2016

POTATO RESISTANCE TO CYST NEMATODES - PECULIARITIES FOR ROMANIA

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Abstract. Potato cyst nematodes (PCN) are pests of phytosanitary quarantine with high economic implication on potato production and commercialization. Both nematode species *Globodera pallida* and *G. rostochiensis* have the origin in Peru and evolved with the potato dissemination all over the world during the last two centuries. *G. rostochiensis* was recorded in Romania for the first time in 1984 and starting with 2007 all the country is under phytosanitary quarantine for both species. The analysis of the official potato management programme reveals that there are no measures in place to address abiotic (dry wind, run off water) and biotic factors (i.e. sheep and goat transhumance and boars movement in arable land) as well as climate change impact. The interview of 122 city halls in Sibiu and Brașov counties revealed the constant presence of boars into arable land as well as of sheep and goat movement or transhumance that is positively correlated with the presence of both nematode species in soil. The article renders the need to integrate scientific achievements regarding potato resistance, environmental factors impact and new agricultural practices into the current potato management programme.

Keywords: genetic resistance, potato cyst nematodes, environmental factors, transhumance.

Rezumat. Rezistența cartofului la nematozii cu chiști - particularități pentru România. Nematozii cu chiști ai cartofului (PCN) sunt dăunători de carantină fitosanitară, cu importante implicații economice asupra producției și comercializării cartofului. Ambele specii de nematode *Globodera pallida* și *G. rostochiensis* își au originea în Peru și au evoluat odată cu răspândirea cartofului la nivel global în ultimele două secole. *G. rostochiensis* a fost înregistrată în România pentru prima dată în 1984 și începând cu anul 2007 toată țara se află în carantină fitosanitară pentru ambele specii. Analiza programului oficial de management al cartofului relevă faptul că nu există măsuri în vigoare pentru a aborda factorii abiotici (vânt uscat, apa de infiltrație) și biotici (transhumanța cu ovine și caprine și migrația porcului mistreț în terenurile arabile), precum și impactul schimbărilor climatice. Interviul a 122 primării din județele Sibiu și Brașov a relevat prezența constantă a porcilor mistreți în terenuri arabile precum și transhumanța ovinelor și caprinelor care este corelată pozitiv cu prezența în sol a ambelor specii de nematode. Acest articol discută necesitatea integrării în actualul program de management al cartofului realizările științifice în ceea ce privește rezistența la cartof, impactul factorilor de mediu și al noilor practici agricole.

Cuvinte cheie: rezistență genetică, nematozii cu chiști ai cartofului, factori de mediu, transhumanță.

INTRODUCTION

Potato is today one of the major crop species for the entire world according to the UN Food and Agriculture Organization (FAO) being ranked on the fifth place in 2015. The inheritance of resistance to pests in potato continues to be a major challenge in practice and research from the beginning of the last century (SALAMAN & LESLEY, 1923). After the Second World War, it was published one of the most impressive works on potato regarding geographical origin, genetic relatives, the dramatic infestation that took place in 1840 in Ireland due to *Phytophtora infestans*, the importance in breeding of the potato germplasm collection of the International Potato Centre of Lima in Peru, including historical, economic and social relevance for Europeans (REDCLIFFE, 1949). The first comprehensive compendium regarding potato diseases was published in 1981 and at least six nematodes have been described such as: potato cyst nematodes (PNC), root-knot nematodes, false root-knot nematodes, lesion nematodes, potato rot nematodes and stubby-root nematodes (HOOKER, 1981). According to this compendium *Globodera pallida* and *G. rostochiensis* were recorded up to 1980 in South America and in all Europe. Today it is a fact that *G. rostochiensis* is recorded all over Europe and that *G. pallida* is not present in only five European countries (i.e. Denmark, Estonia, Latvia, Lithuania and Slovakia) (ROSSI, 2012).

Once accepted the idea of centre of origin of crop species proposed in 1917 by VAVILOV, after 1920, there started scientific expeditions in South America that ended with an impressive germplasm collection comprising at least 200 wild and 8 tuber-producing cultivated species belonging to the genus *Solanum*. In this period of time potato became the subject for high standard scientific research all over the world especially due to its nutrition potential as it became one of the seven pillar crops in the world (HAWKES, 1990; GEBHARDT & VALKONEN, 2001).

Potato was the subject of Potato Genome Sequencing Consortium (PGSC) aiming to elucidate the complete genome sequence of potato that ended by 2011. As potato is a tetraploid species (2n=4x=48) the above mentioned authors consider that it is far from an ideal species for genetic analysis. Thus, at least 25 genetic models should be considered in inheritance studies only for four alleles, per locus, supporting the studies on inheritance and linkage of single genes for potato resistance made by COCKERHAM in 1970. Tremendous efforts have been made for the analysis of potato genome in direct relationship with the genetic basis of resistance to PCN. It is accepted today that PCN comprises 8 pathotypes (Ro1 to Ro5 of *G. rostochiensis* and Pa1 to Pa3 of *G. pallida*) making really difficult the research of testing and further breeding programme for improving resistance to PCN (ASANO et al., 2012).

In 1999, it was proved the presence of forty-five SNPs at six loci spanning 2 cM in the interval between markers *GP21* and *GP179* that were associated with resistance to *G. pallida* (ACHENBACH et al., 2009). QTL markers for resistance to PCN among other pests were identified on all 12 chromosomes of potato genome (GEBHARDT & VALKONEN, 2001). The authors revealed the complexity of potato resistance and proved that the most prominent resistance allele was associated with the linkage disequilibrium group C and positioned close to R genes on the V chromosome. After years of research, in 2008, it was proved for the first time that the origin of the Western European populations of *G. pallida* is in the Peruvian population showing a clear south to north phytogeographical pattern originating from Peru and reconfirming the results published by STONE in 1979 (PLANTARD et al., 2008). Thus, studying the phylogenetic tree of the mitochondrial haplotypes of *G. pallida* by using molecular markers it was proved that western European pathotypes (UK1-4; FR 1, 3, 5, 7 and 9; NL 1 and 2; SW and SP) have correspondence in the Arapa population in Peru.

The genetic variability of the Western European strains is very high and comparable with that of two clades of Peruvian origin. Genetic studies proved that the *Grp1* locus detected on chromosome V seems to confer resistance both against *G. rostochiensis* and *G. pallida* (FINKERS-TOMCZAK et al., 2009). *Ro1* is the only one pathotype found in Japan and researchers are working mainly on the resistance gene *H1* which confers almost full resistance against *G. rostochiensi* (ASANO et al., 2012). The same pathotype was recorded in Russia and resistant cultivars (i.e. cv. 'Hydra', 'Gelda' and 'Kardia') developed in Germany and placed on the market on 1977 have been used as genetic resources for new potato cultivar breeding (SIMAKOV et al., 2008). In Spain, it is relevant the presence of the pathotypes *Pa2/3* (RITTER et al 2008). In 2013, it was published the most complete result regarding the molecular basis of resistance to *G. pallida* and *G. rostochiensis*, different loci belonging to different chromosomes and contributing to the expression of this characteristic (GEBHARDT, 2013).

The rapid dissemination of new potato cultivars all over the world for the last century opened new challenges due to agricultural practices, the novelty of new agro-ecosystems and the interaction with other management practices or species (i.e. domesticated or feral native species) as well as new climatic conditions. There are at least two facets of the issue: the continuous scientific knowledge generation and on the other hand the transfer to the economic domain of this knowledge and to be integrated into issues related to agricultural practices, trade and commercialization. Thus, if science is looking for provable evidences in connection to the genetic basis of potato resistance to PCN and vectors for disseminating it related to strict conditions, agricultural practices and commercialization may further contribute to perturbing the consistency of scientific results due to added factors. One major problem recognized by Romania after 2007 is the continuous infestation of arable land with PCN (DG SANCO, 2010), including the potato seed area (GROZA et al., 2011). Moreover, from a country exporting seed potato after 2007, Romania is under phytosanitary quarantine, complying with a strict monitoring plan of phytosanitary measures (DG SANCO, 2010). The scope of this article is to discuss connections between the current potato monitoring programme and environmental factors contributing to the huge infestation of Romania arable land with PCN.

MATERIAL AND METHODS

This paper is developed based on an integrative approach regarding the SWOT analysis (strengths, weaknesses, opportunities, and threats) of the national Potato Programme. In this regard there have been used different data bases: the European Cultivated Data Base, the National Catalogue for Plant Varieties and screening literature for PCN resistance. All city halls from Brașov County (i.e. 58 city halls) and Sibiu County (i.e. 64 city halls) have been interviewed for the presence of PCN and boars in the arable land in the period 2009-2014.

RESULTS AND DISCUSSIONS

Potato cultivation in Romania. Potato as a crops species is mentioned to be cultivated in the gardens of Transylvania of the 18th century but not for commercial use, being considered as not important at that time for fiscal system of that region in Europe (GYÉMÁNT et al., 2009). In less than 200 years this gardening crop becomes more and more important in our country due to its remarkable adaptation to climatic conditions. After 1989, Romania passed a political change that is more and more addressed by scientists analysing the socio and economic factors evolution in connection with different subject including land use and crop production (FRASER & STRINGER, 2009). According to these authors the political climate installed after 1989 had major negative impact especially on land use change and crop production making from potato one of the most important crop of the country. Still, according to FAO database, the largest potato harvesting area was of 351,400.00 ha recorded in 1989 and followed by a dramatic decrease in 1992 (i.e. 218,695.00 ha). After 1992 the total harvesting area is about 250,000.00 ha up to 2011 and another decrease is registered in 2014 (i.e. 202,657.00 ha). In terms of productivity, Romania recorded the highest productivity in 1985 (6,631,200.00 t), followed by that of 1989 (i.e. 4,420,000.00 t). Starting with 1989 the potato productivity decreased continuously until 2014 even if it became one of the most important crops for the countryside (i.e. 3,519,329.00 t). It can be considered that after 2007, the year of accessing the European Union, Romania continued to lose potato in terms of harvesting area and productivity.

National Collections. Gene Bank of Suceava is in charge to preserve the germplasm collection of the country and currently there are 801 accessions. A single one accession is described as a landrace and collected from high altitude in 1986. In 2009, a complete study regarding potato germplasm collection, including landraces was published in Târgu Secuiesc (BACIU, 2009).

Potato Breeding Programme. The National potato breeding programme is implemented in four major research institutions namely the National Institute of Research and Development for Potato and Sugar Beet Brasov (NIRDPSBB), Research and Production Stations Târgu Secuiesc, Research and Production Stations Miercurea Ciuc and Agricultural

Research Station Suceava (STRAJERU & BODEA, 2000). Romania designated a national institute for running research on potato in 1977 based on resources taken from the older research stations dating from 1949. Today, the NIRDPSBB also supports the national potato phytosanitary system. *G. rostochiensis* was for the first time signalled by researchers of the NIRDPSBB in 1984 in Lăzarea, Harghita County, a place for virus free potato seed production (ROJANCOVSCHI & DEHELEANU, 1986; DONESCU & ENOHJ, 1987). In 1991, it was stated that 40-45% of potato production may be destroyed by the presence of these PCN in the soil when the concentration may be over 20 eggs/100 g of soil with a persistence of up to 28 years (BOȚOMAN, 1991). In 2005, the NIRDPSBB stated that it is compulsory that infested lands with *G. rostochiensis* and *G. pallida* to be excluded from the potato seed production (CHIRU, 2005).

Results regarding resistance testing for couple of potato cultivars (Astral, Victoria, Magic and Claudiu) to *G. rostochiensis* pathotypes *Ro1* have been published (BODEA, 2004). According to the Official Catalogue only three varieties have been placed on the market in Romania (i.e. cultivars 'Astral' in 2001, 'Magic' in 2001 and 'Claudiu' in 2003). After four years, there were officially promoted for commercial purpose new potatoes varieties such as Luiza and Ioana (MIKE et al., 2008) not yet accepted into the European Cultivated Potato Database. Still, cultivars such as 'Hydra', 'Gelda' and 'Kardia', with proved resistance against *G. rostochiensis* developed in Germany and placed on the market on 1977, have never been tested in Romania for placing on the market.

Official Catalogue Analysis. By investigating the Official Catalogue for plant varieties in Romania for registering years and varieties origin new gaps may be revealed for the current phytosanitary management system. Thus, based on this analysis it can be considered that Romania officially started importing seed potato cultivars for commercialization and cultivation in 1961, after becoming a Party to the International Convention for the Protection of New Varieties of Plants of the International Union for the Protection of New Varieties of Plants (UPOV Convention). Romania ratified the Convention in 2001 but the plant variety protection system is in place in Romania since 1955. The three cultivars: 'Bintje', 'Ostara' and Desiree are considered today as the more susceptible potatoes to the PTC attack (Table 1). 'Bintje' was erased from the Official Catalogue before 1986 but 'Ostara' and 'Desiree' continued to be promoted up to 2004, when they were erased from the Official Catalogue. It can be considered that there was a serious gap between science results and the policy for promoting new potato cultivars and responsible for the spreading of PCN. Cultivars such as 'Roxy', 'Hilta', 'Nicola', 'Gloria', 'Anosta', 'Concorde', 'Koretta' and 'Sante' entered the market place after 1988 (Table 1) and have been officially promoted for being cultivated as resistant to PCN after 1993 (DRAICA & CHIRU, 1994). In case of the cultivar 'Roxy' that was placed on the Romanian market in 1988, it was officially promoted after 5 years in the complex fight against PCN due to its proved resistance to *G rostochiensis* pathotypes *Ro1* and *Ro5*. The same delay is registered for all other cultivars.

Table 1. Potato cultivars imported before 1989 based on the survey of the Official Catalogue for Plant Varieties.

No.	Cultivar	Country of origin	Year for placing on the Romanian market	Year for placing on the market according to potato database*	Resistance testing to *G. pallida*	Resistance testing to *G. rostochiensis*
1.	Bintje	Netherlands	1961	1910	Low: Pa1 and Pa2	Low: Ro1 and Ro3
2.	Ostara	Netherlands	1971	1961	Very low to low: Pa2	Very low/low: Ro1
3.	Desiree	Netherlands	1965	1962	Low: Pa1, Pa2, Pa3	Low: Ro1, Ro3
4.	Procura	Netherlands	1976	1971	-*	High: Ro1
5.	Gloria	Germany	1988	1972	-	High: Ro1
6.	Manuela	Germany	1976	1972	-	Low: Ro1
7.	Nicola	Germany	1985	1973	Low: Pa1 and Pa2	High/very high: Ro1 Very high: Ro4
8.	Adretta	Germany	1978	1975	Low: Pa1 and Pa2	Low: Ro1; Very low to low: Ro5
9.	Anosta	Netherlands	1988	1975	-	High: Ro1
10.	Astarte	Netherlands	1989	1976	Low: Pa2 and Pa3	Moderate to high: Ro1 Moderate: Ro2 Low/moderate: Ro3
11.	Frezja	Poland	1984	1979	-	Low: Ro1
12.	Darwina	Netherlands	1989	1981	High: Pa2	High: Ro1, Ro2, Ro3, Ro4, Ro5
13.	Roxy	Germany	1988	1981	-	High: Ro1, Ro5
14.	Koretta	Germany	1989	1983	-	High: Ro1
15.	Hilta	Germany	1988	1983	-	High: Ro1, Ro2, Ro3, Ro5
16.	Sante	Netherlands	1989	1983	Low to moderate: Pa1, Pa2, Pa3	High: Ro1, Ro2, Ro3, Ro4
17.	Concorde	Netherlands	1989	1986	Low: Pa1 and Pa2	High: Ro1 Very high: Ro4

*- no data available

The Potato Monitoring Programme (PMP) is adopted by the National Phytosanitary Agency working within the Ministry of Agriculture and it is implemented with the support of County Phytosanitary Units, County Phytosanitary Inspectorates and four Phytosanitary Laboratories in close cooperation with customs. The national PMP between 2007 and 2015 proposed strict quarantine measures for PCN. Starting with 1991 the NIRDPSBB communicated clear specifications regarding PCN sampling period such as September 15 for start and July ending 15 (COJOCARU, 1991).

The subject of these measures includes: potatoes as commodity, seed potato, soil, producers and equipment (NAN, 2005). Thus, even in 2005, the NIRDPSBB stated that drastic measures should be put in place; however, the official monitoring system did not include potential activities that may further contribute to PCN spreading. Finally, the PMP for 2015-2016 proposed the following major measures: to be used only tested seed potatoes for commercial purpose, to keep a healthy environment on the arable land, to limit circulation between parcels, to maintain a constant surveillance on the plots and waste management. However, no indications regarding associated costs to such a programme were in place and no definition for limiting the circulation between potatoes parcels either. Furthermore, analysing the chapter II of the management programme, regarding the monitoring of native production and environment, there miss measures regarding abiotic and biotic factors as vectors in spreading PCN. In this regard it will be discussed environmental factors (i.e. dry wind, water runoff, wild herbivores) and agricultural practices (i.e. sheep and goats transhumance) that are not mentioned into the current PMP.

Environmental factors. White, in 1953, documented the spreading of PCN by hot dry winds and run off water that was supported by further studies (TURNER & EVANS, 1998; REPŠIENĖ & MINEIKIENE, 2006). Romania comprises a broad range of agricultural landscapes due to relief on one hand and to land management on the other hand (HARTEL et al., 2016). Still, Romania faces now the climate change effects all around the Carpathian Mountains (SPINONI et al., 2015). Thus, the diversity of agricultural landscapes provided conditions for implementing different agricultural practices that contributed further to the spreading of this pest.

It is accepted today that PCN can be spread by biotic factors such as contaminated livestock (HODDA & COOK, 2009). The process become too complex for Romania where livestock represented by sheep and goats or boars passing the fields are not taken into consideration into the national monitoring programme. Similar cases have been already described and the probabilities of PCN establishment in new areas beyond the current distribution were both studied in Australia in 2009 (HODDA & COOK, 2009).

Considering that the transhumance of sheep and goats facilitates the spread of PCN in the field it can be seen that in 1985 it was recorded the highest number of these species (i.e. 19,391,000.00) that continuously decreased up to 2002 (i.e. 7,776,300.00). This increase may be associated to the first official recording of PCN in 1984. Starting with 2002 the number of sheep and goats continuously increased up to 2014 (i.e. 10,448,645.00) as well as the covering area with this livestock, which also may be associated with the spread of the PCN all over the country up to potato seed production areas. It is relevant to underline that in Romania, even diminished, the transhumance is still in practice (STANCIU et al., 2012) and no phytosanitary management measures are in place to reduce the spreading of the PCN.

Moreover, in Romania agro-ecosystems are closely connected to wild biodiversity and mainly with large herbivores species that consume potatoes such as boars (i.e. *Sus scrofa*). Only in 2015 there have been recorded about 28,153.00 boars according to the data base of the Ministry of Environment and in Sibiu or Brașov County the arable land is constantly the subject of boars migrations. Between 2007 and 2014, Romania was under phytosanitary quarantine for exporting pork meat (i.e. in Romania the swine population comprises 6,815,000 individuals in 2007) due to swine fever and boars were accepted to be the most important pest reservoir (INDRIE & MÂNZAT, 2009) with major economic loss in the region (BELTRÁN-ALCRUDO et al., 2009). According to the results of the interview applied to all 122 city halls (i.e. in Sibiu and Brașov counties), boars are a constant presence in the arable land that can be associated with the presence of PCNs. Thus, it should be taken into account restriction measures for the cultivation of seed potatoes in the bordering areas with boars in order to obtain free PCN zones.

Agricultural practices in Europe had a major historical contribution to the spreading of PCNs, as well as to the increase of their genetic variability (GEBHARDT, 2013). Potato fields in Europe are ploughed to higher depth compared to Peru and further homogenising potato cyst densities in soil, supporting a hypothesis earlier published (MUGNIÉRY & ZAOUCHI, 1976). Although, there are couples of new agricultural practices promoting the cultivation of potato with pests' repellent plant species (SAND et al., 2013). It can be added that the current PMP should also include the need to promote new agricultural practices use.

CONCLUSIONS

Potato monitoring programme should put in place measures according to the latest scientific achievements. It should also follow the national and international breeding programmes in order to substantiate the promotion for placing on the market of the most resistant potato cultivars. On the other hand it should take into account all environmental peculiarities of the country including measures for adaptation and mitigation to climate change considering that the PCN may resists up to 28 years. Livestock transhumance and boars migration should be the subject of this potato monitoring programme in direct connection with the type of potato culture in re-establishing free PCN zones. New agricultural practices should be further developed in supporting the full implementation of quarantine phytosanitary measures.

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Received: March 12, 2016
Accepted: June 3, 2016

Gentiana lutea L. – CONSIDERATIONS FOR A SUCCESSFUL PROTOCOL ON MICROPROPAGATION

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Abstract. Lately, has been increasingly important to ensure the conservation of biodiversity and look for alternatives to support the survival of the species. Endangered species list grows from year to year and climate change emphasizes this phenomenon. Adoption of conservation strategies to appeal to the tools offered by biotechnology has been more frequent after 2000. The aim of this paper is to provide information on successful micropropagation of the species *Gentiana lutea* L., as an endangered species for our country.

Keywords: *Gentiana lutea*, inoculation, micropropagation, *in vitro* cultivation.

Rezumat. *Gențiana lutea* L. - Considerații privind un protocol de succes pentru micropropagare. În ultima perioadă este tot mai important pentru asigurarea conservării biodiversității, găsirea de alternative care să asigure perpetuarea speciilor. Lista speciilor pe cale de dispariție crește de la un an la altul, iar schimbările climatice accentuează acest fenomen. Adoptarea de strategii de conservare care să apeleze la instrumentele oferite de biotehnologii este tot mai frecventă după 2000. Scopul acestei lucrări este acela de a oferi informații cu privire la micropropagarea cu succes a speciei *Gentiana lutea* L., periclitată pentru țara noastră.

Cuvinte cheie: *Gentiana lutea*, inoculare, micropropagare, cultivare *in vitro*.

INTRODUCTION

Most of the known plant species have been accessed for millennia by humankind for useful products: food, textile, medicine, wood, perfumes, dyes, food; some have been known for thousands of years, others are still waiting to be discovered (SHAHID & MOHAMMAD, 2013). Inventory of valuable genotypes of endangered plant species and cloning them through *in vitro* culture support today the obtaining of superior active principles that are highly valuable (PATHAK & ABIDO, 2014). *In vitro* inoculation and micropropagation are safety tools for the environment in protecting medicinal plants at an advanced stage of endangerment, according to commitments taken under the Bern Convention (LEE & ACKERMAN, 1993).

Romania also needs to comply with these political commitments and therefore should finance projects to safeguard the heritage of flora, as these national priceless treasures are not irretrievably lost yet (COSTE et al., 2012). This technique allows the creation of models (lots) of experimental producers of medicinal plants, given the high requirements of medicinal plants both for internal and external market (JUMA et al., 2014). The idea of associating arable land on large areas to be profitable it is still rejected, forcing us to find solutions for farmers who do not have large areas of land, especially in hilly and foothill areas where they can cultivate their land with herbs (BARROWS et al., 2013). Currently there are two major trends of particular practical interest, but unfortunately antagonistic: replacing medicinal chemical synthesis with the natural world, particularly derived from plants, on the one hand, and preserving biodiversity and protecting vegetable species, on the other hand (MARIENHAGEN & BOTT, 2013).

The World Health Organization announced that 75-80% of the world population is treated with herbal remedies (PANDEY et al., 2015). This is another reason that the culture of medicinal plants should be done as effectively, both in terms of raw material quality and economic production at a price that would make it competitive as many markets. As wild medicinal plants present a high genetic variability it makes difficult the industrialization processes of extraction for the same quality in active principles leading to increased costs.

This technology, originating from the beginning of the last century allows the revival of culture of medicinal plants, especially those belonging to endangered species, addressing new technologies and farming practices both beneficial to environment. However, it supports the idea of sustainable agriculture practiced in Europe not only for the noble purpose of ensuring food for a growing population but also to sustainable exploitation of the Earth: respect for nature that is still so generous and desire you keep your balance. It is noteworthy that in countries of Western Europe there is no interest in the cultivation of medicinal plants on a large scale due to the high cost of labour for harvesting and therefore they prefer to import plants in less developed areas in eastern Europe, obviously criticizing them especially for destroying biodiversity and protected species.

Cultivation of medicinal plants widely obtained by *in vitro* culture is such an international priority (BERMAN et al., 2013). In Romania, the wild flora is represented today by 3,700 native species of which there have been identified 800 species of medicinal interest and 370 species of these have already been tested in terms of pharmaceutical effects (DELIU et al., 2013). Herbs processed are used from simple teas to aromatic mixed fruit, tinctures, oils, extracts, syrups, concentrates for soft drinks, condiments, cosmetics and medicines, homeopathic products, functional foods, products for aromatherapy, bioproducts for plant protection organization of the organic crops, etc. (PÂRVU, 2002).

In 1990, the largest area of a total surface of 9,350 ha planted with herbs was occupied by coriander (8,675 ha), followed by poppy (50 ha), cumin (12 ha) and fennel (5 ha), with a total production of approximately 5,390 tons (ISTUDOR, 2001). However, plants from spontaneous flora have at this time the largest share of the total processing and, worse, in some cases imported herbs, so increasing the number of cultivated species, while increasing the cultivated area should be a national priority as it is estimated that the global trade may come with 2.5 billion $/year (BARATA et al., 2016). Given that many species of medicinal plants are under law protection, many research conducted in Romania are geared towards protecting the country flora heritage by providing feedstock crops. In Romania, there have already been published protocols for *G. lutea* collected from the wild (ZĂPÂRAȚAN, 1996; HOLOBIUC & BLINDU, 2006).

The scope of this article is to introduce the *in vitro* conditions characteristic to this species as a selected population from the wild named 'Săcel' (TANASE et al., 2007) in order to set up a micropropagation protocol and to test new culture media. This species is a monument species for the natural heritage and protected by the national regulatory framework and therefore it would be suitable to contribute for a balanced *in situ* and *ex situ* conservation strategy for our country (ANTOFIE, 2011).

MATERIAL AND METHODS

Plant material. The plant material is represented by mature healthy plants of *Gentiana lutea* L, population 'Săcel' acclimated for field cultivation and originating from the experimental field in Brașov. Apical fragments of 10 cm each (i.e. including meristems, leaves and petioles) have been cut from the plants and prepared for sterilization.

Sterilization. The plant fragments have been maintained under cold running tap water for more than 15 min followed by a washing in jars of 800 ml with water and TWEEN (3 drops) for other 15 min followed by three repeated washing under the cold tap water 3 min for each. These jars are all prepared for the next steps in sterilization into the laminar flow hood. It is used Domestos (a commercial detergent containing 4.5% sodium hypochlorite and most environmental friendly compared to other sterilizing agents) for 1 ml at 100 ml sterilized water for 3 min and continuing stirring of the jar. The sterilization followed three rinsing of 5 min each using sterilized water. The sterilized plant material is maintained into the closed jars up to inoculation. Inoculation comprises shoot apexes, leaf fragments and petiole fragments and it was realized meristem inoculation under the stereomicroscope - using the same culture medium.

Culture media. Meristems, petiole and leaf fragments have been inoculated on MS62 culture medium in three variants according to table 1. Agar was added after pH correction at 5.8 and before sterilization. There have been used jars of 400 ml with 50 ml culture medium. The micropropagation medium consists of the same composition and for each variants there have been removed the cytokinins and added 0.4 mg/l of NAA (1-Naphthaleneacetic acid). All reagents are of Merck origin (Table 1).

Table 1. The variants of culture medium used for *Gentiana lutea* inoculation.

Composition	**V-I**	**V-II**	**V-III**
Macro-elements	50 ml	100 ml	100 ml
Micro-elements	10 ml	1 ml	1 ml
Fe EDTA	30 mg	30 mg	30 mg
Inositol	100 mg	100 mg	100 mg
NaH_2PO_4 x H_2O	85 mg	-	-
Adenine sulphate	80 mg	-	-
Tyrosine	100 mg	-	-
Thiamine	15 ml	0,1 ml	5 ml
Pyridoxine	1 ml	0,5 ml	0,1 ml
Nicotinic acid	10 ml	0,5 ml	1 ml
Kinetin	9 ml	0,2 ml	-
Indole-3-acetic acid	0,4 mg	-	-
1-Naphthaleneacetic acid	-	0,1 ml	0,1 ml
Sucrose	30 g	30	30 g
Agar	6 g	6 g	6 g

Growth room conditions. The temperature was 20°C during the day (16 h) and 18°C during the night (8 h). It was applied a light intensity of 2000 lux with cold light Philips.

RESULTS AND DISCUSSIONS

In this study, there have been used 120 explants collected from 10 donor plants, in a healthy status during June 2014. From these explants, there have been inoculated 90 meristems (i.e. 30 per variant) and 120 petiole and leaf fragments (i.e. 40 of each per variant) a total of 330 explants. Following the inoculation all meristems have been transferred on a new culture medium, with the same composition, after 10 days, according to our experience that was also applied by different authors (ZĂPÂRȚAN, 1996).

During the first passage it was registered the first removal due to infections with different microorganisms or necrosis (Table 2). Also, it was observed that the plant fragments tissues already evolved presenting hypertrophy at the basal part and also following the cut-edges of petiole and leaf fragments. It was recorded a good evolution for all explants cultivated on V-I (i.e. all explants presented a visible hypertrophy with a survival rate of 80%).

The explants cultivated on the variant V-2 presented a survival rate of 31.2% and mainly meristems presented a positive evolution for morphogenesis. Almost the same survival rate 31% was recorded for the variant V-3. In this case all leaf or petiole fragments ceased to develop presenting clear necrosis installation into the plant tissues. It is difficult to clear state that the survival rate was mainly due to the culture conditions and not to the manipulation of the excised material. Still, considering that the explants have been randomly inoculated it might be considered that the composition of the culture medium may have a clear influence on the development of the explants (i.e. the lack of cytokinins that supports cell division).

Table 2. The viability of explants of *G. lutea* 10 days after the inoculation.

Explant	**Culture medium Murashige-Skoog 1962 (MS62)**					
	Variant I (V-I) K 9 ml/l + 0.4 ml/l AIA		**Variant II (V-II) K 0.2 ml/l**		**Variant III (V-III) ANA 0.1 mg/l**	
	% inoculum	Significance	% inoculum	Significance	% inoculum	Significance
Foliar explants	63 %	A	21.2 %	A	25 %	A
Axillary meristems	69 %	A	25.8 %	B	31 %	B
Petiole fragments	10 %	D	10.7 %	D	0 %	-

Further after inoculation, for micropropagation it was used the same culture medium and 6 weeks (i.e. 8 weeks of cultivation) after the first transfer of plant material cytokinins have been removed and the culture media have been supplemented with NAA at 0.4 mg/l. After another 8 weeks from the second transfer of explants, morphometric analyses have been realized for the resulted plantlets and results are presented in table 3. It can be said that the same culture medium is suitable for the inoculation of this species for *in vitro* cultivation (Table 3).

Table 3. Morphometry of the plantlets of *G. lutea* 3 months after the first inoculation.

Explant	**Roots length (cm)**			**Roots no.**			**Leaf no.**			**Plantlets height cm)**		
Variants	V-I	V-II	V-III	V-I	V-II	V-III	V-I	V-II	V-III	V-I	V-II	V-III
Foliar fragments	2.3	1.6	2.5	3	3	4	4	3	4	6.5	7.3	7.5
Axillary meristems	1.8	1.4	2.2	2	3	3	4	3	5	6.1	7.0	7.3
Petiole fragments	1.1	0.9	1.8	3	2	2	3	2	4	6.3	6.8	7.0

Based on the analysis of the results presented in table 3 it can be said that it is easy to transfer into *in vitro* culture *G. lutea* and to apply biotechnology as a tool for supporting the conservation of the species (Fig. 1).

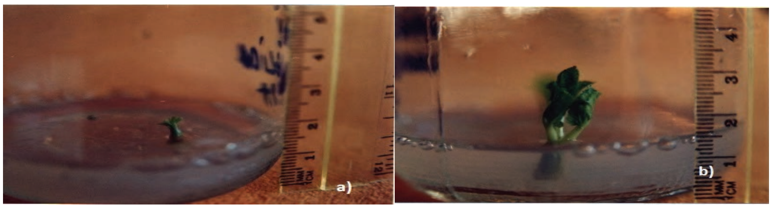

Figure 1. *Gentiana lutea* – in vitro inocula 4 weeks after inoculation of meristems (a) and micropropagated plantlets (b) (original).

Taking into account the results of HOLOBIUC & BLINDU (2006), we consider that the starting inoculum may have an important impact as they used as a starting material seeds collected from the wild. They also considered that MS62 was one the most appropriate culture medium for the inoculation of the species. Still, their experimental data stopped for that stage. In 1996, it was for the first time published a protocol (ZĂPÂRȚAN, 1996) for *G. lutea* using different culture media with comparable results.

CONCLUSIONS

The analysis of the results of this study revealed that *G. lutea* is a species that is reactive for *in vitro* culture by taking into account the results on inoculation from 1996 to 2006. All tested explants generated micro-propagules and all organs of the species may be obtained: stems, leaves, roots. The first variant, similar to the original MS62 provided the best results supporting previously studies on this species. It can be considered that the species may be successfully introduced into *in vitro* conditions using these protocols depending on the laboratories facilities and resources.

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Received: March 12, 2016
Accepted: June 3, 2016

ASSESSING THE EFFICIENCY OF THE ROMANIAN NATURAL PROTECTED AREAS IN CONSERVING PRIORITY HABITATS

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Abstract. In an attempt to safeguard its biodiversity, the European Union carried out more programs, including an inventory of its priority habitats (CORINE Biotopes) and the establishment of natural protected areas, including the Natura 2000 network. The Romanian national system of protected areas was developed in accordance with the guidelines of the International Union for the Conservation of Nature, although there are some variations concerning the definition of categories and stronger or weaker management guidelines. However, the process of establishing new areas was a true challenge, resulting into overlapping categories and even a lawsuit from the European Union. This study was aimed to use spatial metrics to examine the efficiency of the Romanian protected areas in the conservation of priority habitats. The results indicate that 81% of the habitats are included in the protected areas; the most efficient areas in preserving the key habitats are Ramsar sites and the Reserves of Biosphere, with 72, respectively 70% of their territory covering key habitats. Nevertheless, the efficiency of protection is debatable due to the land cover and changes affecting these areas.

Keywords: Natura 2000, Biosphere Reserves, Ramsar, IUCN, CORINE.

Rezumat. Evaluarea eficienţei ariilor protejate din România în conservarea habitatelor prioritare. Încercând să îşi conserve biodiversitatea, Uniunea Europeană a derulat mai multe programe, incluzând un inventar al habitatelor prioritare (Biotopuri CORINE) şi stabilirea ariilor naturale protejate, inclusiv reţeaua Natura 2000. Sistemul naţional românesc de arii naturale protejate a fost dezvoltat conform recomandărilor Uniunii Internaţionale pentru Conservarea Naturii, deşi există diferenţe în definirea unor categorii şi constrângeri de management mai puternice sau mai slabe. Cu toate acestea, declararea noilor arii naturale protejate s-a dovedit a fi o adevărată provocare, având ca rezultate categorii suprapuse şi chiar un proces din partea Uniunii Europene. Acest studiu şi-a propus să examineze eficienţa ariilor protejate din România în conservarea habitatelor prioritare folosind măsurători spaţiale. Rezultatele arată că 81% din aceste habitate se află în arii naturale protejate. Cele mai eficiente tipuri sunt siturile Ramsar şi rezervaţiile biosferei, al căror teritoriu este format în proporţie de 72, respectiv 70% din habitate prioritare. Cu toate acestea, eficienţa protecţiei este discutabilă datorită modificărilor acoperirii şi utilizării terenului din interiorul acestor arii.

Cuvinte cheie: Natura 2000, rezervaţii ale biosferei, Ramsar, IUCN, CORINE.

INTRODUCTION

Natural protected areas are crucial to sustainability by safeguarding a part of today's biodiversity (MÜCHER et al., 2009; KATI et al., 2015). In an attempt to safeguard its biodiversity, the European Union used two directives, Birds and Habitats, as a start point for assessing its biodiversity, and carried out more programs, including an inventory of the priority natural habitats, produced within the frame of CORINE Biotopes program, started in the mid 1980's (EVANS, 2012b), with a special focus on the priority ones (MOSS & WYATT, 1994; EVANS, 2006; MÜCHER et al., 2009; EVANS, 2012a). Based on the results, the Natura 2000 network was not designated for the strict preservation of biodiversity, but aimed at sustainability in partnership with local communities (STĂNCIOIU et al., 2010; SINGH et al., 2014), and consists of sites conserving priority habitats in the Habitat Directive (SCIs), areas for the protection of birds under the Birds Directive (SPAs) and sites connecting the first two categories (SACs) (MÜCHER et al., 2009; EVANS, 2012a; STRINGER & PAAVOLA, 2013).

The Romanian national system of protected areas was developed in accordance with the guidelines of the International Union for the Conservation of Nature, although there are some variations concerning the definition of categories and stronger or weaker management guidelines; for example, national parks have a more restrictive management, protected landscapes are more permissive, and natural monuments include species and individuals in addition to sites (MUNTEANU & SEVIANU, 2014). However, the process of establishing new areas was a true challenge (VANONCKELEN & VAN ROMPAEY, 2015), resulting into overlapping categories (IOJĂ et al., 2010) and even a lawsuit from the European Union (COJOCARIU et al., 2010). Currently, the national system of protected areas covers 18% of the national territory (STĂNCIOIU et al., 2010), a percentage similar to the European one (KATI et al., 2015). In particular, the Natura 2000 network was designated to cover the 90 types of natural habitats of a communitarian importance (PĂTROESCU et al., 2007).

Previous studies carried out in Romania aimed at assessing the efficiency of the national system of natural protected areas by looking at the overlap of categories (IOJĂ et al., 2010), coverage of biogeographical regions (PETRIŞOR, 2008, landform diversity PETRIŞOR, 2009) or coverage of wetlands (PETRIŞOR, 2010a). Most studies used Geographical Information Systems (GIS) in conjunction with spatial metrics or indicators, since the use of GIS alone is insufficient (PĂTROESCU et al., 2007).

This study was aimed to use GIS in conjunction with spatial metrics to examine the efficiency of the Romanian protected areas in the conservation of priority habitats.

DATA AND METHODS

The study used several datasets, freely available from the European Environment Agency and the Romanian Ministry of the Environment, Waters and Forests, presented in Table 1. Data were processed by re-projecting and sub-sampling subsets for Romania, clipping and dissolving contours based on sub-categories, and ultimately computing areas using the X-Tools extension of ArcView GIS 3.X. The analyses were aimed at assessing the coverage of habitats by the natural protected areas, comparing their share within and outside the natural protected areas, and the share of their area from the total area of each type of protected area.

Table 1. Specifications on the data used in the study: dataset, provider, URL, remarks and transformations.

Dataset	Provider	URL	Remarks	Transformation
CORINE biotopes in PHARE countries	Romanian Ministry of the Environment, Waters and Forests	http://www.eea.europa.eu/data-and-maps/data/corine-biotopes-in-phare-countries#tab-gis-data	2005 data	Project into Stereo 1970, sub-sample for Romania
Natural protected areas	Romanian Ministry of the Environment, Waters and Forests	http://mmediu.ro/new/?page_id=5178	Not all types of protected areas legally defined are available	None needed

RESULTS AND DISCUSSION

Table 2 and Figure 1 show the coverage of the priority habitats by the Romanian natural protected areas. The results indicate that 81% of the habitats are included in the protected areas; the most efficient areas in preserving the key habitats are Ramsar sites and the Reserves of Biosphere, with 72, respectively 70% of their territory covering key habitats.

Table 2. Coverage of the priority habitats by the Romanian natural protected areas.

	CORINE habitats within the protected surface	CORINE habitats		Protected surface	
	Area (km^2)	Area (km^2)	% CORINE Habitats	Area (km^2)	% protected surface
All categories	78.06	96.74	80.69	569.02	13.72
Natural parks, protected landscapes	53.36		55.16	166.39	32.07
Ramsar sites	45.25		46.77	62.72	72.15
Scientific and natural reserves, natural monuments	7.35		7.59	25.11	29.25
Biosphere reserves	46.30		47.86	66.17	69.97
Natura 2000 SCIs	73.10		75.57	415.22	17.61
Natura 2000 SPAs	63.77		65.92	369.36	17.26
Natura 2000 SACs	4.45		4.60	19.35	23.01

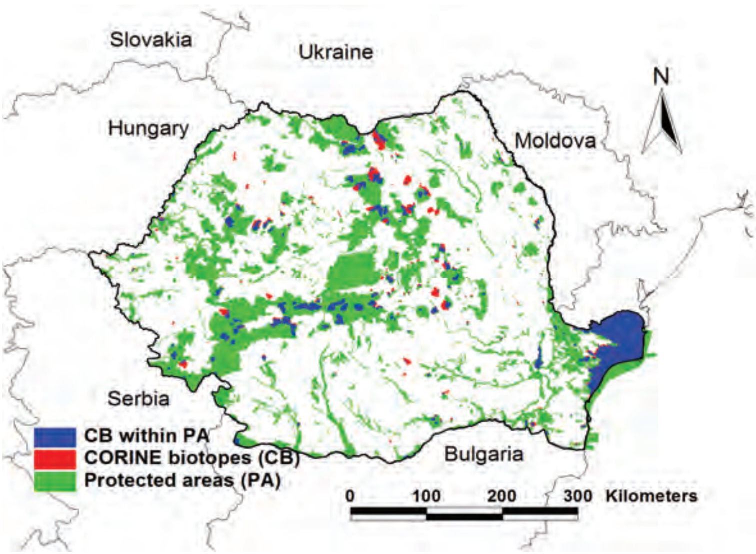

Figure 1. Coverage of the priority habitats by the Romanian natural protected areas (original).

Romanian protected areas face different threats despite the protection status; the main issues include land cover and use changes (PETRIŞOR, 2015), climate changes (PETRIŞOR, 2010b, 2011; SÂRBU et al., 2014), economic (STĂNCIOIU et al., 2010; KATI et al., 2015; WILFRED & MACCOLL, 2015) and social issues (STAN et al., 2013; ADETOLA & ADETORO, 2014). In addition to the efficiency of spatial coverage, an in-depth study should also look at the efficiency of enforcing the protection status (PETRIŞOR, 2010a; SINGH & BORTHAKUR, 2015) and of the management plans (POP et al., 2010).

The limitations of the study refer to the unavailability of data for some categories. Scientific and natural reserves and natural monuments are included only if their area exceeds 5 hectares; however, the missing categories account for a very limited portion of the national system of protected areas.

CONCLUSION

The results suggest a very good coverage of the priority habitats by the Romanian natural protected areas, especially by the Ramsar sites and Reserves of Biosphere. Nevertheless, the efficiency of protection is debatable due to the land cover and changes affecting these areas.

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Received: January 12, 2016
Accepted: May 12, 2016

A CITIZEN OF CRAIOVA, THE GEOLOGIST LUDOVIC MRAZEC

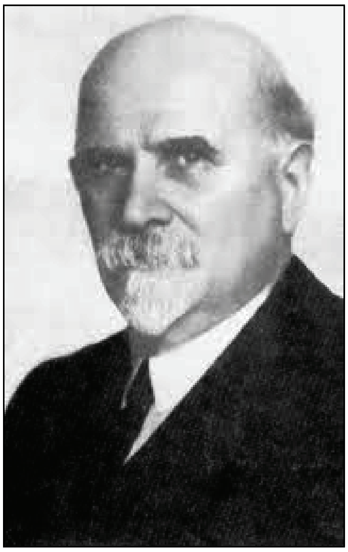
ENACHE Constantin

Abstract. Born in 1867 in Craiova, with basic studies at Carol I High School in same town, Ludovic Mrazec was the first director of the Romanian Geological Institute, professor at the University of Bucharest, the first president of the Romanian Society of Geology, academician and president of the Romanian Academy. Ludovic Mrazec identified two distinct groups in the crystalline schists of the South Carpathians, with different metamorphic history the diapiric folds. He authored over one hundred scientific papers, ten of them at least regarding the geology of Oltenia.

Keywords: Ludovic Mrazec, geologist, Oltenia, scientific works.

Rezumat. Un cetățean al Craiovei, geologul Ludovic Mrazec. Născut în 1867 la Craiova, cu studii la Liceul Carol I din același oraș, Ludovic Mrazec a fost primul director al Institutului Geologic al României, profesor la Univeresitatea din București, primul preşedinte al Societăţii Geologice a României, academician și președinte al Academiei Române. Prof. Ludovic Mrazec a identificat în Carpații Meridionali două grupuri distincte de şisturi cristaline, cu istorie metamorfică diferită şi a descoperit fenomenul de diapirism, elaborând teoria cutelor diapire. Este autorul a peste o sută de lucrări științifice publicate, din care cel puţin zece privesc geologia Olteniei.

Cuvinte cheie: Ludovic Mrazec, geolog, Oltenia, lucrări științifice.



The great scholar Ludovic Mrazec was born in Craiova on July 17, 1867, in a family of Czech-Austrian ethnic origin. His father, Ludovic Severin Francisc Mrazek, born in the town Ober Cerekwe - today Horní Cerekev, in southern Bohemia (Czech Republic), was a pharmacist, graduate of the Vienna University, who settled in Romania in 1861. Ludovic Mrazek jr. followed the primary school, then Carol I High School in Craiova.

Passionate since childhood of natural sciences, he devoted most of his life to the study of geology. But following his father's wish, he. attended the Faculty of Pharmacy in Bucharest, taking the bachelor's degree in 1889. Then he went to Geneva to get the a postgraduate diploma in physico-natural sciences in 1891, the doctor's degree in physico-natural sciences with the thesis: "La protogine du Mont-Blanc et les roches éruptives qui l'accompagnent" in 1892, and doctor of science's degree in 1893.

In the same year, he came back home and was appointed assistant at the Geology Laboratory of the Faculty of Sciences in Bucharest. On December 1, 1894, he was appointed professor of Crystallography, Mineralogy and Petrography at the University of Bucharest, being the head of the Department of Mineralogy in the period 1894-1937. He taught the course of "Crystallography, Mineralogy and Petrography" at the Faculty of Natural Sciences at the University of Bucharest, the Faculty of Pharmacy and the Faculty of Industrial Chemistry of the Polytechnic Institute.

Together with other Romanian geologist, Ludovic Mrazec founded the Geological Institute of Romania in 1906, the director of which was from 1906 to 1928. In 1907, he was elected president of the International Congress of Oil.

He was corresponding member (1901), member (1905) and president of the Romanian Academy (1932-1935), founding member and first president of the Romanian Geological Society in 1930, where he worked until the last year of his life. In 1927, Mrazec served as Minister of Trade and Industry.

He was member of the Imperial Society of Naturalists in Moscow, the Academy of Sciences and Letters in Cracow, the Society of Mineralogy and Geology in Prague, the Geological Society of Belgium, the International Commission of Oil in London, the Academy of Sciences of the Institute of France and Doctor honoris causa of the University of Strasbourg.

The research approached by the professor Mrazec cover a wide spectrum: mineralogy, metamorphic and magmatic petrology, oil and salt geology, structural geology, cartography and sedimentary petrography.

In a paper from 1904, Mrazec divided the metamorphic rocks of the South Carpathians in two distinct groups, i.e. the group I, of high-grade metamorphism, overlapping the group II, of low-grade metamorphism. Together with I. Popescu-Voiteşti, he extended the theory of nappe structure in the East Carpathians, subsequently confirmed by drillings. For these reasons, he is one of the greatest Romanian tectonicians.

Ludovic Mrazec was the first Romanian geologist who supported the organic origin of oil and highlighted the role of the bituminization processes in nature. At the International Exhibition in Paris (1900), the scientist presented the first map of the salt formations in Romania. He firstly discovered the phenomenon of diapirism developing the diapire theory and related folding (Mrazek's fold), which was exposed at the World Petroleum Congress in 1907. This theory opened new prospects in the exploration and exploitation of oil and gas, and strongly influenced the entire oilfield geological activity.

Ludovic Mrazec authored over one hundred papers, also giving conferences on a lot of topics concerning the geology and mining in Romania: oil, gold mines, flysch classification, sulfur deposits, origin of salty lakes, loess origin, etc.

He passed away on June 9, 1944 in Bucharest.

The main works regarding the geology of Oltenia:

- *Contribuţiune la studiul petrografic al rocelor din zona centrală a Carpaţilor de sud, şi anume: din judeţele Mehedinţi, Gorjiu şi Muscel.* An. Muz. Geol. Paleont., vol.I, București, 1895.
- Note *sur la géologie de la partie sud du haut plateau de Mehedinţi.* Bull. Soc. Sci. Phys., vol. V/12. București, 1896.
- *Essai d'une classification des roches cristallines de la zone centrale des Carpathes Roumaines.* Arh. Soc. Sci. Phys. Nat., tome III. Genéve, 1897.
- *Contribution à l'étude des roches de la zone centrale des Carpathes méridionales. II. Sur les gneiss à cordiérite des Montagnes du Lotru, III. La wehr~~h~~lite du Mont Ursu.* (with G. Munteanu-Murgoci). Bull. Soc. Sci., vol.VI/ 3, București, 1897.
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I presented the life and work of this great scientist not only because, as geologist I knew him, if not the man, his work, but also because as inhabitant of Oltenia and of Craiova I am glad that Ludovic Mrazec had a constant concern for deciphering the geology of Oltenia.

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Received: March 29, 2016
Accepted: June 30, 2016

ISSN 1454-6914