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STUDII ŞI CERCETĂRI

Biology

15

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THE HALOPHYTE VEGETATION WITH *PEUCEDANUM LATIFOLIUM* (Bieb) D.C. FROM THE TRANSYLVANIA

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Abstract: This paper presents the halophyte vegetal associations from Transylvania, in which the tall forb of the mesohaline grasslands – *Peucedanum latifolium* - is developing. These associations are affiliated with the coenotaxonomical system based on the dominant species and are analyzed from an ecological, phytogeographical and of bioforms point of view, based on phytocoenological tables. Meadows installed on salty soils make up six vegetal associations: *Bolboschoenetum maritimi, Alopecuretum pratensis, Festucetum pratensis, Agrostidetum stoloniferae, Scorzonero parviflorae - Juncetum gerardii* and *Caricetum distantis.* These associations belong to three vegetation classes - *Phragmitetea australis, Mollinio-Arrhenatheretea, Puccinellio-Salicornietea.*
Key words: *vegetal association, halophyte vegetation, ecological studies, phytosociological table, Transylvania.*

The halophyte vegetation is an important part of the steppe and forest-steppe landscape, where the dry – but not necessarily also warm – climate favours it extension in the large floodplains. The halophyte vegetation from the Transylvanian Plain is concentrated in its western part, because here there are large areas affected by diapiric folds. In the central and southern part, this vegetation is much rare and atypical, because here the soils with a high salt content are missing. In the northern and eastern part, the halophyte vegetation is rare and is present only in the perimeters where the Mireş Formation occurs. In the Transylvanian Basin, the halophyte vegetation occupies insular habitats, more or less large, that are not climatically but geologically determined. Halophyte habitats are linked with the inversion relief developed on diapiric folds and gas domes, when the valleys cut through the soft saline cores of these, and also with the salt rich marls of the Mireş Formation (that has a Badenian age) (Bădărău, 2005).

Peucedanum latifolium is mainly a ponto-balkan species, that lives in mesohygrophile slightly saline meadows (Prodan, 1922; Ţopa, 1954), which became rarer and rarer because of the tough and intense overgrazing from the saline areas. The main geographical area of species is Central and South-Eastern Europe (Tutin, 1968), in Italy and in Slovenia being a relict species (Bojňanský et Fargašová, 2007). In Transylvania, the species was reported long time ago, in 1853 F. Schur showing

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that *Peucedanum latifolium* from Lerchenfeld herbarium (ca. 1790-1800) comes from Cluj area. In Transylvanian Plain, the species was cited as being present in the wet and salted valleys surrounding Cluj, such as Băile Someşeni, Apahida, Valea Florilor, Sic and Gherla (Simonkai, 1886). In "Romanian Flora" (Todor, 1958), *Peucedanum latifolium* is cited as being present between Gherla and Sic localities, Dej, Someşeni, Dezmir, Apahida, Sînmiclăuş and Valea Florilor.

In our field studies in Transylvania, we found *Peucedanum latifolium* in five locations - Hăşdate, Sânicoară, Budaştău (near Apahida), South-West of Cojocna and Şesul Sibiului (fig. 1) (the last two populations - Cojocna and Şesul Sibiului - have not been quoted, in the scientific literature, before) - in six different vegetal associations, presented in the following coenotaxonomical system:

PHRAGMITETEA AUSTRALIS R. Tx. et Preising 1942
Bolboschoenetalia maritimi Hejny in Holub et al. 1967
Cirsio Brachycephali - Bolboschoenion (Passarge 1978) Mucina 1993
1. Bolboschoenetum maritimi Eggler 1933

MOLINIO-ARRHENATHERETEA Tx. 1937
Potentillo–Polygonetallia R. Tx. 1947
Potentillion anserinae R. Tx. 1937
2. Alopecuretum pratensis Regel 1925

Molinietalia Coerulae W. Koch 1926
Agrostion stoloniferae Soó (1933) 1971
3. Cirsio cani-Festucetum pratensis Májovsky & Ruzicková 1975
4. Agrostidetum stoloniferae (Ujvárosi 1941) Burduja et al. 1956

PUCCINELLIO-SALICORNIETEA ŢOPA 1939
Puccinelietalia Soo 1947 em. Vicherek 1973
Scorzonero-Juncion gerardi (Wendelbg. 1943) Vicherek 1973
5. Scorzonero parviflorae - Juncetum gerardii (Wenzl. 1933) Wendelbg. 1943
6. Caricetum distantis Rapaics 1927

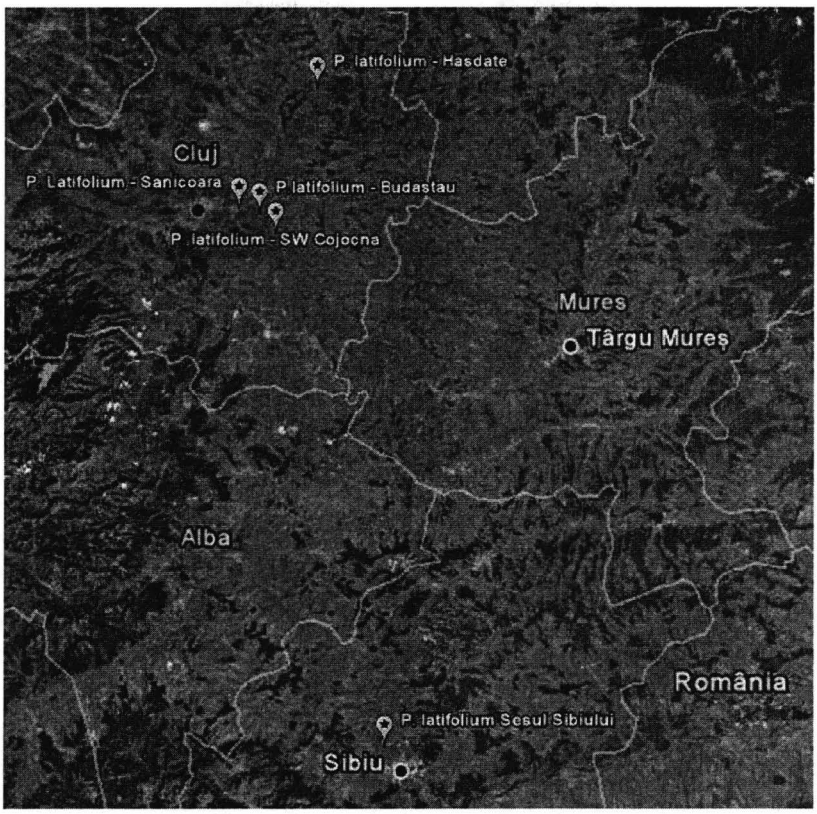

Fig. 1. A Google Earth image with the situation of the five location of *Peucedanum latifolium* in Transylvania

For plant association's identification, we used phytosociological research methods according to the Central-European school. The species are named after Ciocârlan (2000) and *Flora Europaea* (electronic edition). The establishment of the bioforms, floristic elements (geoelements) and ecological indices was made after Ciocârlan (2000) and Popescu et Sanda (1998).

1. Bolboschoenetum maritimi Soo (1927) 1957

This association is frequently found in marshy places, eutrophic, with excess water in spring and dry in summer and autumn. The association consists of a few species, with the dominance of characteristic species *Bolboschoenus maritimus* and *Phragmites australis.* Beside the associations included in class Puccinellio – Salicornietea (in which are included the halophile azonal grassy vegetation), other syntaxons should be added, like those from the order *Bolboschoenetalia maritimi* that live in aquatic more or less saline habitats (Bădărău, 2005).

Table 1

Bolboschoenetum maritimi Soó (1927) 1957

					No. of the survey	**1**
					Altitude (m)	**314**
					Slope inclination in degrees	**0**
					Covering (%)	**100**
U	**T**	**R**	**Geoelem.**	**Biof.**	**Surface (m^2)**	**40**
3	4	4	Eua(Cont)	H	*Althaea officinalis*	+
3	3	0	Eur(Med)	Th	*Anthemis arvensis*	+
4,5	3	5	Cosm	HH-G	*Bolboschoenus maritimus*	4
2,5	3,5	3,5	Cosm	H-G	*Convolvulus arvensis*	+
5	0	2	Circ	H	*Epilobium palustre*	+
5	3	4	Circ	HH-H	*Glyceria maxima*	+
4	4	4.5	Pont-Balc	H	*Peucedanum latifolium*	1
6	0	4	Cosm	HH	*Phragmites australis*	3
4	3	5	Eur(Med)	H	*Plantago cornuti*	1
4	0	0	Eua	H	*Poa trivialis*	2
3	0	0	Cosm	G	*Sonchus arvensis*	+
4,5	3,5	4	Eua	G	*Sonchus palustris*	+

Place and date of the surveys: 1 – Budaştău, near Apahida (Cluj county), 18.07.2008

The diagram of the ecological values shows the mesohydrophyte and hydrophyte, mesothermic to moderately thermophilic and low acid-neutrophylous to basophylous nature of the association's phytocoenoses. We also notice the large percentage of euriionic species.

Most of the geoelementes are Cosmopolites (33,33%), followed by Eurasiatics (25%), European (16,66%) and Circumpolar ones (16,66%).

The biologic forms are dominated by hemi-cryptophytes (50%), followed by hydrophytes (25%), geophytes (16,66%) and therophytes (8,33%).

2. Alopecuretum pratensis Regel 1925

The coenosis of this association are widespread in our country, from lowland to mountain floor and vegetate, generally, on humic-gleyic soil. The association consists of 38 species, with the dominance of *Alopecurus pratensis* (characteristic species for the association) and *Ranunculus acris* – which form the *ranunculetosum acris* Juhasz Nagy apud Soo 1957 (Syn.: *Ranunculo – Alopecuretum pratensis* Krisch 1973) subassociation (table 2 – survey no. 2)..

Table 2

Alopecuretum pratensis Regel 1925

					No. of the survey	**2**	**3**
					Altitude (m)	**339**	**313**
					Slope inclination in degrees	**20**	**10**
					Covering (%)	**90**	**100**
					Surface (m^2)	**35**	**20**
U	**T**	**R**	**El. fl.**	**Biof.**	**Orientation**	**E**	**E**
3	0	0	Eua	H	*Achillea millefolium*	-	+
2	3	4	Eua	H	*Agrimonia eupatoria*	-	+
2	3	4	Eua(Cont)	H	*Ajuga genevensis*	+	-
5	0	4	Cosm	H	*Alopecurus geniculatus*	-	+
4	3	0	Eua	H	*Alopecurus pratensis*	3	4
2,5	4	0	Eua(Cont)	Ch-H	*Artemisia santonicum*	+	-
2	3	4	Eua(Cont)	H	*Aster linosyris*	+	-
3	3	0	Eua(Med)	H	*Betonica officinalis*	+	1
3	2,5	3	Euc	H	*Centaurea phrygia*	-	+
2,5	0	3,5	Circ	Ch	*Cerastium arvense*	-	+
3	0	3	Eua	H-TH	*Cichorium intybus*	-	+
1,5	4	3	M	Ch-N	*Cytisus albus*	+	-
3	0	4	Eua(Med)	H	*Dactylis glomerata*	+	-
2	5	4	Euc(Med)	Ch-H	*Dorycnium herbaceum*	+	-
3,5	2	0	Eua	H	*Festuca pratensis*	+	-
3,5	3	3	Eua(Med)	H(G)	*Ficaria verna*	+	-
2,5	3	4,5	Eua	H	*Filipendula hexapetala*	1	-
2,5	2,5	0	Eua	H	*Galium verum*	+	+
2,5	3	2	Eua	Ch-N	*Genista tinctoria*	+	+
3	3	0	Eua(Med)	TH-H	*Inula britanica*	-	+
4	3,5	5	Euc	G	*Iris spuria*	-	+
4,5	3	5	Circ	G	*Juncus gerardi*	-	+
2,5	3,5	4	Pont-Pan-Balc	H(G)	*Lathyrus pallescens*	-	+
3	4	0	Eua(Cont)	Ch-H	*Ononis arvensis*	-	+
2	4	4	M	G	*Ornithogalum gussonei*	+	-
4	4	4,5	Pont-Balc	H	*Peucedanum latifolium*	1	1
2	3	3	Euc-Med	H	*Peucedanum officinale*	-	+
2,5	0	4,5	Eua	H	*Plantago media*	+	-
3	0	0	Eua	H	*Plantago lanceolata*	-	+
3	0	0	Cosm	H	*Poa pratensis*	-	3
3,5	0	0	Eua(Med)	H	*Ranunculus acris*	2	+
3,5	3	0	Eua(Med)	H	*Serratula tinctoria*	1	+
2,5	2	0	Eua	H	*Trifolium montanum*	+	-
3	0	0	Eua-Cosm	H	*Taraxacum officinale*	-	+

3	0	0	Eua	H-TH	*Trifolium pratense*	-	+
3,5	0	0	Eua	H	*Trifolium repens*	-	+
2	2	2	Eua	Ch	*Veronica officinalis*	-	+
1,5	4	4,5	Eua(Med)	H	*Veronica teucrium*	-	+

Place and date of the surveys: 2 – SW from Cojocna (Cluj county), 27.06.2009; 3 - Budaştău, 25.07.2008

In the phytocoenosis from Budaştău (survey no. 3) with *Alopecurus pratensis*, *Poa pratensis* species dominates, indicating the direction of evolution of this association to mesophilic phytocoenosis. From the ecological point of view, the values shows that the association is a mesophyte to meso-hydrophyte, mesothermic to moderate termophilic and low acid-neutrophylous to neutrobasiphile one. We also notice the great percentage of the euriionic species (42,10%).

In the geoelements' analysis the dominant are the Eurasiatic species (78,94%), followed by Mediterranean's (10,52%), while the European and Ponto-Balkan elements are totally subordinated (Euc-5,26%; Pont-Balk-5,26%).

The phytocoenosis' analysis by the bioforms shows the great share of the hemi cryptophytes (73,68%), followed by the chamaephytes (21,05%), geophytes being represented by a single species (5,26%).

3. Cirsio cani-Festucetum pratensis Májovsky & Ruzicková 1975

Meadows edified by *Festucetum pratensis* include transitional phytocoenosis from the meso-hydrophyte to mesophilic ones. From a structural point of view, these are composed of species such as: *Festuca pratensis, Achillea millefolium, Crepis biennis*, etc. In high soil moisture conditions, *Poa trivialis* become abundant and when the anthropogenic factors are having increased influence, the species *Bromus mollis* is becoming more abundant, each forming characteristic subassociations *poëtosum trivialis* Soo 1938 respectively *brometosum mollis* (Siroki ined.) apud Soo 1964 (Table 3).

The diagram of the ecological values shows the mesophylous to mesohydrophyte, mesothermic and low acid-neutrophylous nature of the association's phytocoenosis. The euriionic species are also in great percentage (27%).

The spectrum of geoelements reveals the dominance of Eurasian elements (74,07%), followed by European ones (7.40%), Ponto-Balkan (3.70%), Balkan – Pannonian (3.70%), Ponto - Pannonian (3,70%), Mediterranean (3,70%) and Cosmopolite (3,70%).

The biologic forms are dominated by hemi cryptophytes (74.07%), followed by terophytes (14,81), geophytes (7.40%) and camephytes (3,70%).

Table 3

Cirsio cani-Festucetum pratensis Májovsky & Ruzicková 1975

					Nr. releveului	**4**	**5**	**6**
					Altitudinea (m)	**349**	**314**	**314**
					Suprafața (m^2)	**20**	**35**	**25**
			El. fl	**Biof.**	**Grad de acoperire (%)**	**100**	**100**	**100**
3	0	0	Eua	H	*Achillea millefolium*	1	+	-
2	3	4	Eua(Cont)	H	*Ajuga genevensis*	+	-	-
3	3	0	Eur(Med)	Th	*Anthemis arvensis*	-	+	-
5	0	5	Eua	H	*Aster tripolium*	-	-	+
4,5	3	5	Cosm	HH-G	*Bolboschoenus maritimus*	-	3	-
0	3	0	Eua	Th	*Bromus mollis*	3	-	-
5	3	4	Eua	H	*Calystegia sepium*	-	+	-
2	4	4	Eua(Med)	H	*Cardaria draba*	+	-	-
4	3	4	Eua	H	*Carex vulpina*	-	-	+
3	0	0	Eur	H	*Centaurea jacea*	-	+	-
3	0	3	Eua	H-TH	*Cichorium intybus*	-	+	+
4,5	3	4,5	Eua(Cont)	G	*Cirsium canum*	-	+	-
3	3	0	Eua	H	*Cirsium vulgare*	-	+	-
3	3	4	Eur	TH	*Crepis biennis*	1	-	-
2,5	3	0	Eua(Med)	TH-H	*Daucus carota*	-	-	1
2	3	4	Eua	H-G	*Euphorbia cyparissias*	+	-	-
3,5	2	0	Eua	H	*Festuca pratensis*	4	4	4
1,5	4	4	Eua(Cont)	H	*Festuca rupicola*	+	-	-
2,5	3	4,5	Eua	H	*Filipendula hexapetala*	1	-	-
3	3	0	Eua(Med)	TH-H	*Inula britannica*	-	-	+
2	0	0	Pont- Pan	G	*Iris aphylla*	+	-	-
2	3	3	Eua	H	*Linaria vulgaris*	+	-	-
3,5	3	4	Eua(Med)	H	*Lotus tenuis*	-	-	+
4	2,5	0	Cosm	H-HH	*Lythrum salicaria*	-	+	+
5	3	0	Eur	H-HH	*Mentha aquatica*	-	+	-
2	4	4	M	G	*Ornithogalum gussonei*	+	-	-
4	4	4,5	Pont-Balc	H	*Peucedanum latifolium*	+	+	+
4	3	5	Eur(Med)	H	*Plantago cornuti*	-	1	+
3	0	0	Eua	H	*Plantago lanceolata*	+	-	-
4	0	0	Eua	H	*Poa trivialis*	-	+	3
2,5	3	3	Eua(Cont)	H	*Ranunculus polyanthemos*	+	-	-
4,5	3	0	Eur	H	*Ranunculus repens*	-	+	+
4	3,5	4	Eua(Cont)	TH-H	*Scorzonera parviflora*	-	-	+
3,5	3	0	Eua(Med)	H	*Serratula tinctoria*	1	-	-

2	3,5	4	Balc-Pan	H	*Seseli varium*	+	-	-
4,5	3,5	4	Eua	G	*Sonchus palustris*	-	+	-
3,5	3	4	Eur	H	*Trifolium hybridum*	-	+	-
2	2	2	Eua	Ch	*Veronica officinalis*	+	-	-
3,5	3	3	Eua(Med)	Th	*Vicia tetrasperma*	-	+	-

Place and date of the surveys: 4 – SW from Cojocna (Cluj county), 27.06.2009;

5, 6 – Budaştău, 25.07.2008

4. Agrostidetum stoloniferae (Ujvárosi 1941) Burduja et al. 1956

This association develops on low lands, periodically flooded especially during spring. It prefers heavy soils (clayey) and soils with groundwater near the surface. It's one of the most widespread meadows associations. Environmental conditions determine a mesohydrophyte character of association, which is reflected in the floral structure. The relatively high abundance of *Deschampsia caespitosa* forms the *deschampsietosum* Soo 1957 subassociation.

The analysis of the ecological values shows the mesohydrophyte, mesothermic and low acid-neutrophylous nature of the association's phytocoenosis.

Table 4

Agrostidetum stoloniferae (Ujvárosi 1941) Burduja et al. 1956

					No. of the survey	**7**
					Altitude (m)	**470**
					Slope inclination in degrees	**0**
					Covering (%)	**100**
U	**T**	**R**	**Geoelem.**	**Biof.**	**Surface (m^2)**	**20**
0	0	0	Circ	G	*Agropyron repens*	+
0	6	0	Circ	H	*Agrostis alba*	4
3	2,5	3	Euc	H	*Centaurea phrygia*	+
3	0	3	Eua	H-TH	*Cichorium intybus*	1
2,5	3	0	Eua(Med)	TH-H	*Daucus carota*	+
4	0	0	Cosm	H	*Deschampsia caespitosa*	2
2	3	4	Eua(Cont)	H	*Eryngium planum*	+
2	4	4	Eua(Cont)	H	*Festuca pseudovina*	+
2,5	3	2	Eua	Ch-N	*Genista tinctoria*	+
4	3,5	5	Euc	G	*Iris spuria*	1
3	3	0	Eua	Th	*Odontites serotina*	+
4	4	4,5	Pont-Balk	H	*Peucedanum latifolium*	+
2,5	3	3	Eua	H	*Senecio jacobaea*	+
4	3	4	Circ	H-G	*Stachys palustris*	+

Place and date of the survey: Şesul Sibiului, 19.08.2008

Most of the geoelements are Eurasiatics (50%), followed by Circumpolars (21,42) and Europeans (14,28%), the Ponto - Balkan and Cosmopolite ones being weakly represented (7,14%).

The biologic forms are dominated by hemi cryptophytes (64,28%), followed by terophytes and geophytes (14,28%), the camephytes being weakly represented (7,14%).

5. Scorzonero parviflorae – Juncetum gerardii (Wenzl. 1933) Wendelbg. 1943

The wet and salty grasslands are dominated by the phytocenosis of *Scorzonero parviflorae-Juncetum gerardii* associations, *Scorzonera parviflora* being one of the most typical plants of these grasslands. This association is concentrated mostly in the western and central-southern part of the Transylvanian Plain (frequently interspersed with *Triglochineto maritimi –Asteretum pannonici*), where some large surfaces of slightly saline floodplains at the bottom of some valley sectors that cross the Mireş Formation in the gas dome perimeters are occupied by this association.

The analysis of the main ecologic values shows the mesohydrophyte, mesothermic and low acid-neutrophylous to basophylous nature of this association.

The dominant bioforms are the hemi cryptophytes (68,75%), followed by therophytes (15,62%).

The geoelements are mostly Eurasiatics (75%), followed by Europeans (9,37 %). The other floral elements are present in low percentages: Circ – 6,25%, Pont-Pan – 3,12%, Pont-Balk – 3,12% and Cosm – 3,12%.

Table 5

Scorzonero parviflorae – Juncetum gerardii (Wenzl. 1933) Wendelbg. 1943

					No. of the survey	**8**
					Altitude (m)	**276**
					Slope inclination in degrees	**0**
					Covering (%)	**80**
U	**T**	**R**	**Geoelem.**	**Biof.**	**Surface (m^2)**	**15**
3	0	0	Eua	H	*Achillea millefolium*	+
2,5	3	4	Eua	H	*Agrimonia eupatoria*	+
4	3	0	Eua	H	*Alopecurus pratensis*	1
2,5	4	4,5	Eua(Cont)	H(Ch)	*Artemisia pontica*	+
2	3	4	Eua(Cont)	H	*Aster linosyris*	+
5	0	5	Eua	H	*Aster tripolium*	+
4	3	4	Eua(Med)	H	*Carex distans*	+
4	3	4	Eua	H	*Carex vulpina*	1
3,5	3	3	Eua	TH	*Carum carvi*	+
2	3	4	Pont-Pan	H	*Cirsium pannonicum*	+
2,5	3,5	3	Eur	M	*Crataegus monogyna*	+
4	3,5	4	Eua(Cont)	TH	*Dipsacus laciniatus*	+
2	3	3	Eua(Cont)	H	*Echinops sphaerocephalus*	+
2,5	2,5	0	Eua	H	*Galium verum*	+
4,5	3	5	Circ	G	*Juncus gerardi*	4
2	3	5	Eua	H	*Medicago falcata*	+
4	4	4,5	Pont-Balk	H	*Peucedanum latifolium*	+
4	3	5	Eur(Med)	H	*Plantago cornuti*	+
4	0	0	Eua	H	*Poa trivialis*	1
2	3	3	Eua(Med)	M	*Prunus spinosa*	+
4	3	0	Eua	H	*Rumex crispus*	+
4	3,5	4	Eua(Cont)	TH-H	*Scorzonera parviflora*	3
3	0	3	Eua	H	*Vicia cracca*	+

Place and date of the survey: Hăşdate, near Gherla (Cluj county) 12.07.2009

6. Caricetum distantis Rapaics 1927

Inhabit areas with excess moisture in a good period of vegetation season. Is an association that appear in small patches in the saline floodplains but also on the slopes in the slightly saline ponds from the slopes affected by landslides developed upon the marls of the Mireş Formation. The hydrophyte character of this association is marked by *Carex distans, Carex vulpina, Carum carvi, Ranunculus repens* species.

Table 6

Caricetum distantis Rapaics 1927

					No. of the survey	**9**
					Altitude (m)	**338**
					Slope inclination in degrees	**0**
					Covering (%)	**100**
U	**T**	**R**	**Geoelem.**	**Biof.**	**Surface (m^2)**	**20**
4	0	0	Circ	H	*Agrostis stolonifera*	+
4	3	4	Eua(Med)	H	*Carex distans*	4
4	3	4	Eua	H	*Carex vulpina*	+
3,5	3	3	Eua	TH	*Carum carvi*	+
4,5	3	4,5	Eua(Cont)	G	*Cirsium canum*	2
2,5	3	4,5	Eua	H	*Filipendula hexapetala*	+
4,5	3	5	Circ	G	*Juncus gerardi*	+
3	0	0	Eua	H	*Leontodon autumnalis*	+
3,5	3	4	Eua(Med)	H	*Lotus tenuis*	+
4	4	4,5	Pont-Balk	H	*Peucedanum latifolium*	1
2,5	3	3	Eur	H	*Ranunculus repens*	+
3,5	3	0	Eua(Med)	H	*Serratula tinctoria*	+
3,5	3	4	Eur	H	*Trifolium hybridum*	+

Place and date of the survey: SW from Cojocna (Cluj county), 27.06.2009

From the ecological point of view, the values shows that the association is a mesohydrophyte, mesothermic and low acid-neutrophylous to basophylous one.

In the geoelements' analysis the dominant are the eurasiatic species (61,53%), followed by circumpolar (15,38%) and European (15,38%) elements.

The phytocoenosis' analysis by the bioforms shows the great share of the hemicryptophytes (76,92%), followed by the geophytes (15,38%) and therophytes (7,69%).

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SAMENKEIMUNG UND QUANTITATIVE MORPHOLOGISCHE MERKMALE BEI EINIGEN *HYPERICUM*-ARTEN SIEBENBÜRGENS (RUMÄNIEN)

Martin KEUL*

Zusammenfassung: In vorliegender Arbeit wurden die Samenkeimung und das Keimlingswachstum mehrerer *Hypericum*-Arten (*H. perforatum*, *maculatum*, *H. hirsutum*, *H. richerii* ssp. *grisebachii* unter Laborbedingungen untersucht, sowie biometrische Messungen zur quantitativen Erfassung morphologischer Merkmale (Pflanzenhöhe, Verweigungsdichte und Blütenanzahl) bei einigen in Siebenbürgen identifizierten spontanen Populationen dieser Arten während ihrer Hochblüte durchgeführt. Die Samenkeimung ist artverschieden und je nach dem Substrat (Filterpapier, Gartenerde) bei *H. perforatum* auf Filterpapier am höchsten (86%) bzw. bei *H. richerii* ssp. *grisebachii* am niedrigsten (10%). Bei den aus Samen regenierierten Keimpflänzchen beträgt die Überlebungsrate nach Verpflanzung auf Gartenerde etwa 70%, während das Wachstum sehr langsam verläuft. Die durchgeführten biometrischen Messungen ergeben vergleichende quantitative Angaben hinsichtlich bedeutender morphologischer Merkmale zur Evaluierung ihrer Variabilität innerhalb und zwischen den Populationen.
***Stichwörter**: Hypericum, Samenkeimung, Biometrie, morphologische Merkmale.*

Einleitung

Die heute in die Fam. *Hypericaceae* (Ord. *Clusiales*) eingeordnete Gattung *Hypericum* umfasst über 400 krautige bis strauch- und baumartige, insbesondere in der gemäßigten Zone verbreitete Arten (Robson, 1968, 1977; Upton und Mitarb. 1997), unter denen die in Europa als Johanniskraut bekannte Art *Hypericum perforatum* L. seit uralten Zeiten bei der Behandlung der verschiedensten Beschwerden verwendet wurde. In der europäischen (aber auch in der japanischen und chinesischen) Volksheilkunde, wird das Johanniskraut seit uralten Zeiten dank seiner wundheilenden, entzündungshemmenden, blutstillenden, schmerzlindernden und beruhigenden Wirkungen traditionsgemäss als bewährtes Fiebermittel, Diuretikum, Sedativum, Wundheilmittel, regional aber auch bei Magen-, Leber-, und Gallenblasenleiden und vielen anderen Leiden, wie Rheuma und Gicht, verwendet (Dall'Agnol und Mitarb., 2003; Duke, 2002; Schempp, 2002; Dall'Agnol und Mitarb., 2003; Muntean, 2007).

Eingehendere neuere wissenschaftliche Untersuchungen haben die in der Volksheilkunde überlieferten heilkräftigen Eigenschaften des Johanniskrautes z.T. auch klinisch bestätigt und zur Klärung der Wirkungsmechanismen der Heilwirkung durch Identifizierung der dafür verantwortlichen Wirkstoffe beigetragen.

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Hypericum perforatum ist wohl die wissenschaftlich am meisten untersuchte Heilpflanze. Heute wird *Hypericum perforatum* insbesondere als klinisch getestetes wirksames Mittel bei der Behandlung milder bis mässiger Depressionserscheinungen verwendet (Hippius, 1998; Greeson und Mitarb., 2001; Schulz, 2006). Neben den oben erwähnten phytopharmazeutischen Eigenschaften (Dall'Agnol und Mitarb., 2003; Muntean, 2007) wurden in den letzten Jahren antivirale, antitumorale und anticanzerigene Wirkungen nachgewiesen, die u. a. auch mit den photosensibilisierenden Eigenschaften des wichtigsten Wirkstoffes (Hypericin) zusammenhängen (Bernd und Mitarb., 1999), so dass Rohpräparate dieser Pflanze potentiell bei der Krebs- und AIDS-Behandlung eingesetzt werden könnten (Schinazi und Mitarb., 1990).

Die vielfältigen heilkräftigen Wirkungen von *Hypericum perforatum* sind dem breiten Spektrum an biochemischen Wirkstoffen zu verdanken, die den verschiedensten Stoffklassen angehören und die in den letzten Jahren biochemisch und phytopharmezeutisch intensiv untersucht wurden (Maleš und Mitarb., 2006; Pavlovic und Mitarb., 2006; Gudžic und Mitarb., 2007; Bagdonaite und Mitarb., 2007). Nachgewiesen wurden u. a. Anthracenderivate (Hypericin und Pseudohypericin), Flavonoide (Quercitrin, Hyperosid, Rutin), Gerbstoffe, ätherische Öle usw. Andere Arten der Gattung *Hypericum* haben wahrscheinlich ein ähnliches Spektrum an wirksamen Stoffen und werden derzeit in verschiedenen Regionen der Welt intensiv hinsichtlich ihrer phytopharmazeutische Eigenschaften (Pérez-Garcia und Mitarb., 2006; Bagdonaite und Mitarb., 2007) untersucht.

Die Bedeutung des Johanniskrautes als Heilpflanze hat die Durchführung genauer biologischer, ökologischer und biochemischer Untersuchungen zur pharmazeutischen Verwertung der *Hypericum*-Arten stimuliert, aber auch das Problem der Beschaffung des Pflanzenmaterials gestellt, denn die Verfügbarkeit an Wildflanzen der Gattung *Hypericum* ist durch unkontrollierte Ernten streng begrenzt (Cirak und Mitarb., 2006). Deshalb muss das für pharmazeutische Zwecke in landwirtschaftlichen Kulturen erzielt werden. Die Vermehrung durch Samen ist die beste, einfachste und wirtschaftlichste Methode für die Konservierung der genetischen Ressourcen von Heilpflanzen und die wirtschaftliche Produktion, obwohl auch eine ungeschlechtliche Vermehrung der Pflanzen möglich ist (Pank, 2003 ; Pérez-Garcia und Mitarb., 2006) und es Ansätze für *in vitro*-Kulturen gibt (Ishimaru und Shimomura, 1992). Für den Erfolg der Kulturen sind genaue Kenntnisse hinsichtlich der Ansprüche der Samenkeimung und des Keimlingswachstum notwendig. Die Samenkeimung bei *Hypericum* ist artspezifisch und wird durch komplizierte Dormanz-Vorgänge erschwert (Campbell, 1985; Osinska und Mitarb., 2002; Cirak und Mitarb., 2004, 2007).

Im Rahmen eines Projektes zur Untersuchung der potentiellen offizinalen Eigenschaften siebenbürgischer *Hypericum*-Arten wurden in diesem Zusammenhang 1. Labor-Versuche über das Keimverhalten und das Keimlingswachstum durchgeführt und 2. biometrische Messungen an Pflanzen verschiedener *Hypericum*-

Arten und -Populationen während der Blütezeit zur Erfassung charakteristischer morphologischer Merkmale unternommen.

Material und Arbeitsmethoden

Als Pflanzenmaterial wurden verwendet:

1. Samen verschiedener *Hypericum*-Arten aus der Sammlung des Botanischen Gartens Cluj-Napoca (*Hypericum perforatum* L., *H. olympicum* L.) oder während der Jahre 2007 und 2008 aus der Wildflora geerntete Samen der Arten *H. perforatum* L.- Valea Morii-Cluj, 2007; *H. perforatum* L.- Colina Cluj, 2008;, *H. maculatum* Cr.- Băişoara, 2007; *H. hirsutum* L.–Zoreni, Cluj, 2008; *H. alpigenum* Kit. =Syn. *H. richerii* (Vill.) ssp. *grisebachii* (Boiss.) Nyman-Curcubăta, 2008) zur Bestimmung des Keimverhaltens der Samen und Regenerierung der Pflanzen aus Samen.

2. Zur Durchführung makroskopischer Beobachtungen und biometrischer Messungen wurden während der Blütezeit 2008 Pflanzenproben einiger in Siebenbürgen vorkommenden *Hypericum*-Arten (Flora României, 1956) in natürlichen Populationen eingesammelt: *H. perforatum* L.-Valea Drăganului (Kr. Cj), Chiribiş (Kr. BH), Botanisches Reservat Suatu II und Klausenburger Heuwiesen (Kr. Cj); *H. hirsutum*- Botanisches Reservat Suatu II (Kr. Cj) und Zoreni (Kr. BN); *H. maculatum* Cr. - Valea Drăganului, Floroiu (Kr. Cj) und Gârda de Sus (Kr. Alba); *H. alpigenum* Kit. (=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.) Nyman)- Căldarea Iezerului (Rodnaer Gebirge).

Zur Untersuchung der Keimfähigkeit wurden je 35 Samen von *Hypericum olympicum* L., *H. perforatum* L., *H. hirsutum* L., *H. maculatum* Cr. und şi *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman in Petri-Schalen auf befeuchtetem Filterpapier oder unmittelbar auf Garten-Erde in Blumentöpfe in 3 Wiederholungen ausgesät. Die Keimung erfolgte in einem klimatisierten Vegetationsraum bei 24-25°C, 30-32% Luftfeuchtigkeit und künstlicher Belichtung (Neonröhren).

Der Ablauf der Keimung wurde täglich über einen Zeitraum von 24 (1. Ansatz) bis 60 Tagen (2. Ansatz) nach der Aussaat verfolgt.

Nach Abschluss der Keimversuche wurden die Keimlinge zwecks Erfassung des Pflänzchenwachstums in Gartenerde in Blumentöpfe verpflanzt. Vorher wurden Messungen hinsichtlich Sprosslänge, Wurzellänge und Anzahl der Knoten (Beginn der Verzweigung) durchgeführt. Das weitere Pflänzchenwachstums erfolgte im Labor bei Zimmertemperatur (22-24 °C), unter diffusem natürlichem Licht/Dunkel-Wechsel und tasgüber (12 Std) mit künstlichem Zusatzlicht (Neonröhren).

Die biometrischen Messungen einiger in Siebenbürgen vorkommenden *Hypericum*-Arten wurden während der Blütezeit an den in verschiedenen Populationen gesammelten Exemplare durchgeführt und verfolgten die quantitative Erfassung morphologischer Merkmale (Gesamtlänge oberirdischer Teile, Anzahl Verzweigungen, mittlere Anzahl der Blüten/Zweig und Gesamtzahl der Blüten/Pflanze). Bei einigen Arten wurden auch Länge und Breite der Blätter erfasst.

Ergebnisse und Diskussion

Die Samenkeimung bei *Hypericum perforatum* und anderen Arten wurde oft untersucht, denn die Keimung ist ein kritisches Stadium im Lebensyzklus dieser Arten und ein entscheidender Faktor für die Kontrolle der Populationen in der relativ dürren gemäßigten Zone (Cirak und Mitarb., 2006) und daher von großer theoretischer und praktischer Bedeutung. Die bisher erzielten Ergebnisse zeigen, dass die Samenkeimung bei *Hypericum* in Abwesenheit von Licht und dank komplizierter Dormanz-Vorgänge ausbleibt oder nur niedrige Werte erreicht (Macchia und Mitarb., 1983; Zinati und Mitarb., 2000).

In vorliegender Arbeit wurden 1. Ansatz orientierende Keimversuche mit mehreren *Hypericum*-Arten zur Untersuchung des Einflusses der Unterlage (befeuchtetes Filterpapier bzw. Gartenerde verwendet). Die Ergebnisse dieser Keimversuche sind in Tabelle 1 für *H. olympicum* L. und *H. perforatum* L. (Erntejahr 2007) vergleichend dargestellt.

Tabelle 1. Die Samenkeimung bei *Hypericum perforatum* L. und *H. olympicum* L. (Erntejahr 2007) in Abhängigkeit von der Unterlage (A= Filterpapier, B= Gartenerde).

A. Filterpapier-Unterlage

Art	Tage nach der Aussaat											
	2	4	6	8	10	12	14	16	18	20	22	22
H. perf.	-	-	-	11,4	51,4	62.9	68,5	74,3	77,1	80,0	83,0	85,7
H.olymp.	-	-	-	2,9	5,7	8,6	10,0	11,0	14,0	17,1	20,7	20,7

B. Gartenerde

Art	Tage nach der Aussaat											
	2	4	6	8	10	12	14	16	18	20	22	22
H. perf.	-	-	-	-	26,7	28,3	43,3	50,0	56,7	63,3	68,3	75,0
H.olymp.	-	-	-	-	5,3	10,5	10,5	26,0	37,0	63,0	63,0	63,0

Die Ergebnisse zeigen, dass die Samenkeimung im Falle der beiden Arten 6-8 Tage nach der Aussaat beginnt und im weiteren Ablauf von der Art und der Unterlage abhängt. Bei *H. perforatum* nehmen die Keimprozente auf beiden Substraten (bis auf etwa 86% auf Filterpapier) zu, während die Keimung bei *H. olympicum* allgemein kleiner bleibt, die höchsten Werte (65%) aber auf Gartenerde gegenüber der Variante auf Filterpapier (21%) erreicht.

In einem anderen Versuchsansatz wurden Samen von *H. perforatum* L. (Valea Morii-Cluj, 2007 und Colina-Cluj, 2008, *H. maculatum* Crantz (Băişoara, 2007), *H. hirsutum* L. (Zoreni (Cluj, 2008) und *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman (Curcubăta, 2008) auf Filterpapier, unter Neon-Licht, bei 24-25 °C angesetzt.

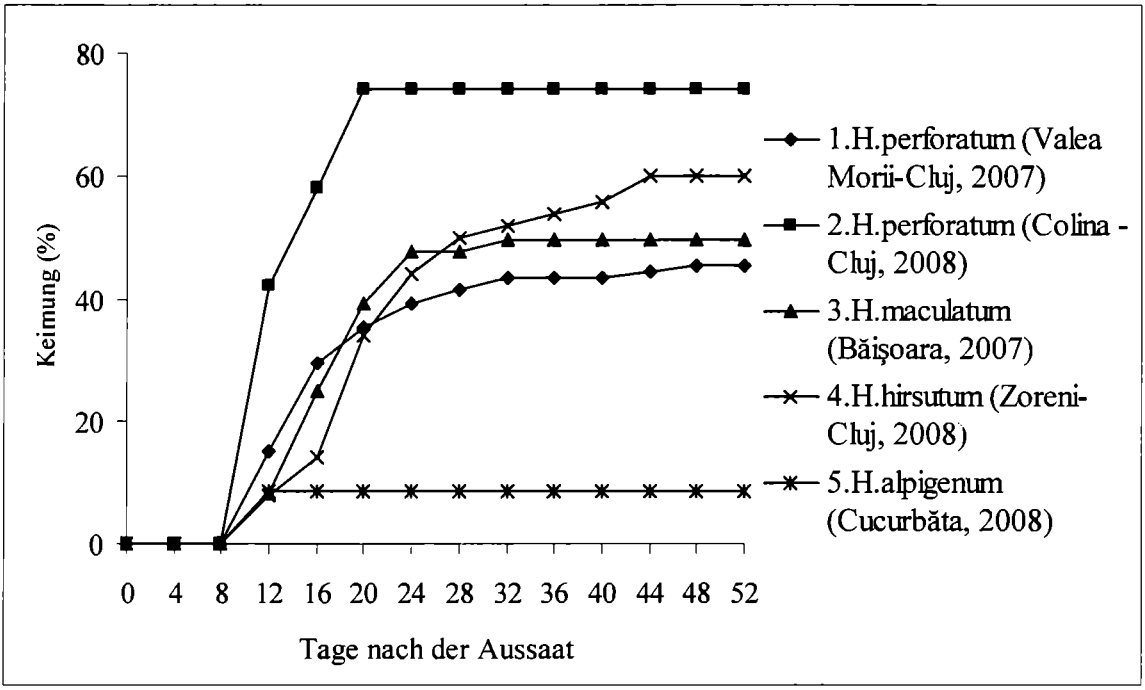

Abb.2. Keimung (%) der Samen von 1. *Hypericum perforatum* L. (Valea Morii-Cluj, 2007; 2. *H. perforatum* L. (Colina-Cluj, 2008; *H. maculatum* Crantz (Băişoara, 2007; *H. hirsutum* L.(Zoreni-Cluj, 2008) und *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman (Cucurbăta, 2008).

Die Keimwerte unterscheiden sich nach der Art signifikant voneinander, bei Proben derselben Art z. T. aber in Abhängigkeit von der Herkunft (Standort) und dem Erntejahr. Die höchsten Keimprozente (ca. 75%) wurden bei *H. perforatum* mit Samen von Colina-Cj (2008) erzielt, während die im Herbst 2007 in Valea Morii geerntete Samen erheblich kleinere Keimwerte (ca. 45%) zeigten. Die Keimfähigkeit bei *H. maculatum* (ca. 50%) und *H. hirsutum* (ca. 60%) ist ebenfalls zufriedenstellend, während sie bei *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman relativ sehr klein bleibt (ca. 10%).

Die festgestellten Differenzen im Keimverhalten der untersuchten Samenproben werden wohl insbesondere artspezifisch bestimmt, hängen jedoch wahrscheinlich auch von anderen inneren und äußeren Faktoren ab, die hier nicht näher präzisiert werden können. Neben komplizierten und artabhängigen Dormanzvorgängen ist in dieser Hinsicht sicher das Zusammenspiel der Aussenfaktoren währtend der Samenreife von Bedeutung, möglicherweise aber auch die meteorologischen Faktoren und der physiologische Reifezustand bei der Samenernte.

Hypericum perforatum zeigt exo- und endogene Dormanz und für die Induktion der Samenkeimung spielen insbesondere die Lichtbedingungen eine entscheidende Rolle (Çirak, und Mitarb., 2004).

Die unter Laborbedingungen aus Samen regenerierten Keimpflänzchen von *Hypericum perforatum* L., *H. olympicum* L., *H. maculatum* Cr., *H. hirsutum* L., und *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman wurden nach Abschluss der Keimversuche auf Gartenerde in Blumentöpfe verpflanzt und im Vegetationsraum weiter kultiviert (24-25°C, 30-32% relative Luftfeuchtigkeit, Neonröhren-Beleuchtung) gehalten. In zusätzlichen Ansätzen wurden die Samen direkt auf Gartenerde in Blumentöpfe zur Keimung ausgesät.

Die Überlebensrate der auf Gartenerde umgepflanzten Pflänzchen beträgt etwa 70-80 %, jedoch erfolgt das Wachstum in den ersten Entwicklungsphasen äußertst langsam. So zeigen biometrischen Messungen ca. 60 Tage nach der Aussaat auf Filterpapier bei *Hypericum olympicum* eine mittlere Wurzellänge von 4,02±0,60 cm (cv%=14,9), eine mittlere Sprosslänge von 1,85±0,39 (cv%=21,1) mit 3-6 Knoten, während die entsprechenden Daten bei *H. perforatum* 5,13±1,25 cm (cv%=24,4) für die Wurzel- und 1,08±0,15 cm (cv%=13,9) für die Sprosslänge betragen. Es ist zu beachten, dass die natürlichen *Hypericum*-Pflanzen und -Populationen jährlich durch schnelles Wachstum aus Rhizomen regenerieren.

Die Ergebnisse der biometrischen Messungen hinsichtlich einiger morphologischer Merkmale von Pflanzen der Arten *Hypericum hirsutum*, *H. perforatum*, *H. maculatum* Cr. Und H. *richerii* (Vill.) ssp. *grisebachii* (Boiss.) Nyman (=*H. alpigenum* Kit., = *H. alpinum* Waldst. &Kit.) aus Siebenbürgen sind nach den untersuchten Populationen und als Mittel zwischen den Populationen in Tab. 2. zusammengefasst.

Tabelle 2. Biometrische Angaben hinsichtlich Pflanzengröße (cm), Anzahl der Verzweigungen, Anzahl der Blüten/Zweig und Gesamtzahl der Blüten/Pflanzenexemplar der untersuchten *Hypericum*-Arten und Populationen Siebenbürgens (arith. Mittel mit Standardabweichung)

Art	Population	Merkmal			
		Pfl. Höhe cm±s	Anzahl Zweige	Anzahl Blüten/Zweig	Gesamtzahl Blüten/Pfl
Hypericum hirsutum	Suatu II	65,7 ± 23,8	12,4 ± 3,1	4,3 ± 1,6	55,9 ± 30,7
	Zoreni	88,8 ± 16,3	13,9 ± 1,5	5,2 ± 1,3	71,7 ± 18,4
	Mittel	**77,3 ± 16,3**	**13,2 ± 2,5**	**4,7 ± 0,6**	**63,8 ± 11,2**
Hypericum perforatum	V. Drăganului	50,8 ± 3,1	14,0 ± 4,5	9,3 ± 3,1	46,5±107,7
	Chiribiş 1	61,1 ± 9,4	7,0 ± 3,2	11,9 ± 8,7	62,0 ± 22,2
	Chiribiş 2	61,3 ± 9,7	11,1 ± 3,4	7,1 ± 4,4	76,9 ± 40,2
	Chiribiş 3	67,5 ± 8,5	8,8 ± 3,3	7,6 ± 6,6	62,8 ± 36,8
	Suatu II -1	67,4 ± 7,0	14,9 ± 3,5	5,3 ± 1,6	76,6 ± 25,0
	Suatu II-2	61,0 ± 9,7	12,8 ± 1,9	5,4 ± 1,4	68,2 ± 17,5
	Suatu II-3	39,5 ± 4,3	11,3 ± 2,4	3,8 ± 0,7	44,0 ± 14,7
	Fânaţe Cluj-1	55,3 ± 6,3	9,6 ± 2,4	9,6 ± 2,4	43,0 ± 19,5
	Fânaţe Cluj-2	63,0 ± 9,2	9,3 ± 2,5	5,3 ± 2,2	50,3 ± 32,0
	Mittel	**58,5 ± 8,9**	**10,2 ± 3,0**	**6,7 ± 2,6**	**69,9 ± 31,0**
Hypericum maculatum	V. Drăganului	54,5 ± 2,1	8,3 ± 2,6	1,7 ± 0,4	14,5 ± 7,3
	Floroiu Cluj	42,8 ± 7,2	9,5 ± 3,5	1,9 ± 0,4	18,8 ± 10,5
	Gârda de Sus	56,8 ± 7,2	9,5 ± 2,8	1,8 ± 0,4	17,5 ± 7,6
	Mittel	**51,4 ± 8,5**	**9,1 ± 2,9**	**1,8 ± 0,4**	**16,9 ± 8,5**
Hypericum richerii ssp. grisebachii	Căldarea (1)	28,4 ± 3,3	4,1 ± 1,6	Einzelblüten	4,1 ± 1,6
	Iezerului (2)	31,1 ± 3,0	3,2 ± 1,4		3,2 ± 1,4
	Mittel	**29,7 ± 3,5**	**3,7 ± 1,5**		**3,7 ± 1,5**

Es ist ersichtlich, dass sich die untersuchten Parameter quantitativ nicht nur von Art zu Art erheblich voneinander unterscheiden, sondern dass bei derselben Art je nach der Population mehr oder weniger ausgeprägte Differenzen bestehen. Die hohe morphologische Variabilität von *Hypericum perforatum* ist bekannt, wobei neue Untersuchungen auf die enge Beziehung zwischen morphologischer Variabilität und Wirkstoffgehalt der pharmazeutischen Präparate in Abhängigkeit von Genotyp, Umweltbedingungen und Entwicklungsphase hinweisen (Bagdonaitė und Mitarb., 2007). Aus diesem Grunde sind Untersuchungen über morphologische Parameter nicht nur für die Gattung *Hypericum*, sondern auch für Heilpflanzen im allgemeinen von Bedeutung.

Die morphologischen Merkmale variieren bei den untersuchten Exemplaren und Populationen zwischen weiten Grenzen. Die mittlere Pflanzenhöhe erreicht bei *Hypericum hirsutum* z. B. 77,3 cm, bei *H. perforatum* 58,5 cm, bei *Hypericum richerii* ssp. *grisebachii* aber nur 29,7 cm, während die Längen der Einzelexemplare zwischen 42-115 cm, 30-88 cm bzw. 24-39 cm schwanken. Ähnliche Variationen

werden auch betreffend die anderen Parameter festgestellt, wie z. B. hinsichtlich der phytopharmazeutisch wichtigen Gesamtzahl der Blüten (*H. perforatum* 69,92±31,03, zwischen 0-310 bzw. bei *H. richerii* ssp. *grisebachii* 3,65±1,53, zwischen 1-6). Ausserdem lässt die Auswertung der biometrischen Messungen vermuten, dass zwischen den paarweise miteinander in Beziehung gesetzten Parametern möglicherweise mehr oder weniger betonte Korrelationen bestehen.

Schlussfolgerungen

Die Samenkeimung der untersuchten *Hypericum*-Arten (*H.* olympicum, H. *perforatum*, *H. hirsutum*, *H. maculatum* und *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman wird 6-8 Tage nach der Aussaat induziert und verläuft auf Filterpapier oder Gartenerde unter kontinuierlicher Belichtung relativ langsam.

Die Samenkeimung verläuft artspezifisch und ist je nach der Herkunft der Samen und teilweise vom Samenalter abhängig.

Die höchsten Keimwerte (ca 75%-86) wurden bei *H. perforatum*-Samen (Herkunft Colina Cj, 2008), die niedrigsten bei (um 10%) bei *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman aus dem Rodnaer-Gebirge (2008). Die restlichen Samenproben erreichen Keimwerte von 45% (*H. perforatum*-Valea Morii, 2007), 50% (*H. maculatum*) und 60% (*H. hirsutum*).

Die Überlebensrate der Keimpflänzchen nach Transplantation in Blumentöpfe auf Gartenerde ist mit etwa 70% zufriedenstellend, die Zuwachsrate aber sehr langsam erfolgt. Unter natürlichen Bedingungen im Freiland erfolgt die Vermehrung und die jährliche Regeneration bei der *Hypericum*-Arten besonders vegetativ durch unterirdische Rhizome.

Die während der Blütezeit durchgeführten biometrischen Messungen hinsichtlich einiger morphologischer Merkmale (Pflanzenhöhe, Verzweigung und Blütenbildung) der Pflanzen im Rahmen der identifizierten Wildpopulationen der *Hypericum*-Arten geben quantitative Angaben hinsichtlich Pflanzenwachstum, Verzweigung, mittlere Blütenanzahl pro Zweig bzw die Gesamtzahl der Blüten pro Pflanze, die die Variabilität dieser Merkmale innerhalb und zwischen den Populationen der betreffenden widerspiegeln.

Danksagung: Herrn Dr. Gheorghe COLDEA sei auch an dieser Stelle für die Unterstützung bei der Bestimmung der *Hypericum*-Arten, die Identifizierung der Populationen und die Bereitstellung des Pflanzenmaterials gedankt.

Rezumat. Genul *Hypericum* (Fam. *Clusiaceae*) cuprinde cca. 400 de specii de plante ierboase sau arbustive cu distribuţie predominantă în zonele climatice temperate, dintre care specia *H. perforatum* (Iarba Sf. Ion), cunoscută încă din antichitate pentru proprietăţile sale medicinale, este utilizată în medicina modernă în special ca remediu clinic atestat în tratamentul formelor uşoare moderate de depresie, având însă şi numeroase alte însuşiri tămăduitoare certe (Dall' Agnol şi colab., 2003, Muntean, 2007). Deşi alte specii ale genului au probabil un spectru similar de principii active (ca derivatul antracenic hipericină,

flavonoizi, taninuri, uleiuri eterice), sugerând proprietăţi fitofarmaceutice asemănătoare, ele nu sunt aprofundat studiate în această privinţă, având însă importanţă fie ca plante ornamentale, fie ca buruieni în culturile agricole sau fiind responsabile, datorită conţinutului lor în hypericină (compus fotosensibilizator), pentru inducerea unor fotodermatoze şi altor afecţiuni la om şi animale.

În studiul de faţă s-au urmărit aspecte fiziologice şi morfologice ale diferitelor specii de *Hypericum* din Transilvania, şi anume: 1.Teste de germinaţie. 2. Creşterea plantulelor în condiţii de laborator. 3. Măsurători biometrice asupra unor specii de *Hypericum* din flora spontană pentru studii morfologice comparative.

Din date obţinute rezultă, că rata germinaţiei seminţelor diferitelor specii de *Hypericum* (*H. olympicum* L., *H. perforatum* L., *H. hirsutum* L., *H. maculatum* Cr. şi *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman este relativ înceată şi debutează la peste 6-8 zile de la punerea la germinat la lumină continuă, pe substrat de hârtie de filtru în cutii Petri sau după însămânţare în ghivece direct pe sol de grădină.

Rata germinaţiei diferă semnificativ de la o specie la alta, la aceeaşi specie şi în funcţie de locul şi anul de recoltare al materialului semincer (vechimea seminţelor). Rata cea mai înaltă de germinaţie (cca. 75%) s-a înregistrat la *H. perforatum* de la Colina (2008), mult mai redusă (cca. 45%) la seminţele recoltate din Valea Morii (2007), destul de bună la seminţele *H. maculatum* (cca. 50%) şi *H. hirsutum* (cca. 60%), dar mult mai slabă (cca. 10%) la *H. alpigenum* Kit. [=*H. richerii* (Vill.) ssp. *grisebachii* (Boiss.] Nyman.

Diferenţele constatate în rata de germinaţie a probelor de seminţe analizate se datorează, evident, în primul rând particularităţilor determinate de specie, dar depind desigur şi de alţi factori externi şi interni care trebuie precizaţi în cercetări ulterioare pentru fiecare specie în parte.

Creşterea plantulelor diferitelor specii de *Hypericum* obţinute prin germinarea seminţelor în condiţii de laborator şi transplantate succesiv din cutii Petri în ghivece pe suport de sol de grădină (cameră de vegetaţie, temperatura cca 24-25°C, umiditatea relativă cca. 30-32%, lumina fluorescentă), iar apoi în condiţii naturale în lotul experimental din grădină, este extrem de înceată, dar supravieţuirea plantulelor este satisfăcătoare. Trebuie precizat, că populaţiile spontane de *Hypericum* se regenerează anual prin creşterea rapidă a părţii aeriene din rizomii subteran cu formarea anuală de tulpini florifere, iar regenerarea din seminţe este înceată, formându-se în primul an o rozetă de frunze şi rizomul subteran, iar în anul următor tulpini florifere. Această particularitate este importantă pentru iniţierea unor culturi intensive de *Hypericum* pentru valorificare în industria farmaceutică.

Măsurătorile biometrice efectuate asupra unor indici morfologici la nivelul părţilor aeriene ale diferitelor specii de *Hypericum* recoltate din mai multe populaţii spontane din Transilvania furnizează date cantitative privind creşterea în lungime, ramificaţia, numărul mediu de flori pe ramuri şi numărul total de flori formate pe întreaga plantă sau, la unele specii, dimensiunile frunzelor, date utile pentru caracterizarea speciilor, evaluarea variabilităţii intra şi interpopulaţionale şi sugerează legăturile statistice existente între diferitele caractere morfologice.

Rezultatele experimentelor efectuate aduc o contribuţie teoretică şi de importanţă practică inedită la cunoaşterea particularităţilor biologice ale speciilor de *Hypericum* din Transilvania, datele fiind utile pentru evaluarea genofondului genului, pentru elaborarea unor recomandări privind realizarea de culturi intensive pentru specia *Hypericum perforatum* cu

importanţă medicinală atestată şi recunoscută, dar şi pentru includerea eventuală şi a altor specii în rândul plantelor cu potenţial fitofarmaceutic.

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THREE SPECIES OF PHANEROGAM NEW FOR THE COUNTY BISTRIŢA-NĂSĂUD (ROMANIA)

Constantin SVOBODA*

Abstract: In the year 2007 I have discovered Hepatica transsilvanica FUSS cultivated ornamentally in a garden on the Eminescu street no.2A in the city of Bistriţa. In the year 2008 I have discovered the species Eranthis hyemalis (L.) SALISB. cultivated ornamentally in a garden on the Petre Ispirescu street no.4 in the city of Bistriţa. In the year 2001 I have identified the spontaneous species Brachyactis ciliata (LEDEB.) LEDEB., on the railway in the former industrial area of municiple Bistriţa.
***Key words**: Brachyactis ciliata (LEDEB.) LEDEB. (Asteraceae), Eranthis hyemalis (L.)SALISB. (Ranunculaceae), Hepatica transsilvanica FUSS (Ranunculaceae), phanerogam plants, Bistriţa-Năsăud County, Romania (Europe).*

The Bistriţa-Năsăud County of Romania covers an area of 5355 km^2. 2200 species of phanerogam plants grow on this area. These are partly wild growing plants (about 85%) and partly cultivated plants (about 15%).

In the spring of the year 2007 I have discovered the endemic spontaneous for Transylvania Hepatica transsilvanica FUSS in Bistriţa, in the garden from the street Eminescu, no 2A, cultivated ornamentally.

At the beginning of March 2008 I have discovered the species Eranthis hyemalis (L.)SALISB. cultivated ornamentally in a garden at no.4 of the Petre Ispirescu street in Bistriţa. The species is subspontaneous or ornamental in the South of Romania.

In the summer of the year 2001 the spontaneous species of Brachyactis ciliata (LEDEB.)LEDEB., was identified on the railway West of the Bistriţa Glass Factory. I have collected the plant 10 years ago, but I determined it only in year 2001 at the Institute for Biology of the Romanian Academy (Institutul de Biologie, Academia Română, Bucureşti), with the aid of the botanist Gheorghe DIHORU, Ph.D. All the three plant species are new for County Bistriţa-Năsăud's phanerogam flora.

Rezumat. În trei ani diferiţi am descoperit Brachyactis ciliata (LEDEB.) LEDEB., Hepatica transsilvanica FUSS şi Eranthis hyemalis (L.)SALISB. Cele trei specii de fanerogame descoperite sunt noi pentru flora judeţului Bistriţa-Năsăud din Transilvania.

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MICROBIOLOGICAL, ENZYMOLOGICAL AND PHYSICO-CHEMICAL RESEARCH ON THE BISTRIŢA ARDELEANĂ RIVER

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Abstract: The Bistriţa River has its source in the Călimani Mountains and flows across the county from east to west, and at Sărăţel locality flows into the Şieu River. This study was carried out in the spring and summer of 2009. The water and sediment samples were collected from six sampling sites along a distance of about 60 km of the river, from the Colibiţa lake to the Sărata locality. The physico-chemical parameters of water analyzed were: temperature, pH, Eh, conductivity, salinity and O_2 concentration. The physico-chemical analyses were carried out *in situ*, using a portable multiparameter. The number of the aerobic mesophilic heterotrophs has been established, both in water and in sediment. The number of bacteria in sediments exceeds by approximately one order of magnitude the number recorded in water. All of the four enzymatic activities analyzed in sediments (phosphatase, catalase, actual and potential dehydrogenase) were registered at every sampling site. Enzymatic indicators of sediment quality were calculated, based on the values of each enzymatic activity.The values of all the analysed parameters were higher in summer than in spring. The presence of total coliform germs was also analysed. Results showed a fairly large number of coliform germs than acceptable limits in the sampling point situated downstream the Bistriţa city.
Key words: *polluted water, coliform germs, aerobic heterotrophs, physico-chemical parameters, enzymatic indicators.*

Introduction

The water pollution is a pressing matter of our times worldwide, still insufficiently studied in our country. There are even cases when upon studies conducted by official institutes or by independent researchers, the surface waters are classified correctly according to the classes of quality legally defined, but they have no impact on population nor on pollution agents, or anyway they can't persuade them to follow some rules and to fix the problem.

Dufour (1984) proposes couple of bacterial indicators for measuring quality of water used leisure activities. Grimes *et al.* (1984) study the microbiological effects caused by the dump of used water in the coastline waters of Puerto Rico, resulting in the increase in number of faecal indicator bacteria and in water pollution with pathogen microorganisms. Gerba and Speed (1997) show the effects of pollution indicator bacteria living in a small tropical river and their impact on the quality of water used for leisure activities. A surface water with faecal pollution is recorded by Venter *et al.* (1997) in South Africa.

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Anderson and Carpenter (1998) observe the pollution of Umpqua River, Oregon, leading to the eutrophic phenomenon. While investigating the water of Japanese rivers, Yamai *et al.* (1998) come out with a method for detecting the microorganisms within water filtering stations and within the above rivers.

Noble *et al.* (2003a) compare the bacterial indicators (total coliforms, faecal and enterococci) of water quality for ocean water used in leisure activities. Same year, Noble *et al.* (2003b) make another comparison of measurement methods for bacterial indicators of water quality in oceanic water from seashore zone.

To identify coliform germs, it seeks the presence of numerous morphological and biochemical features, using various tests. In the strictest sense, in accordance with morphological and biochemical characteristics, germ group includes coliforms bacteria belonging to genera *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter* (Campbell, 1977).

This study aimed to detect the presence in the Bistrița Ardeleană River water of microorganisms used as faecal pollution indicators, in order to offer information regarding the use of indicator organisms in predicting the presence of pathogen organism and human health risks.

Materials and Methods

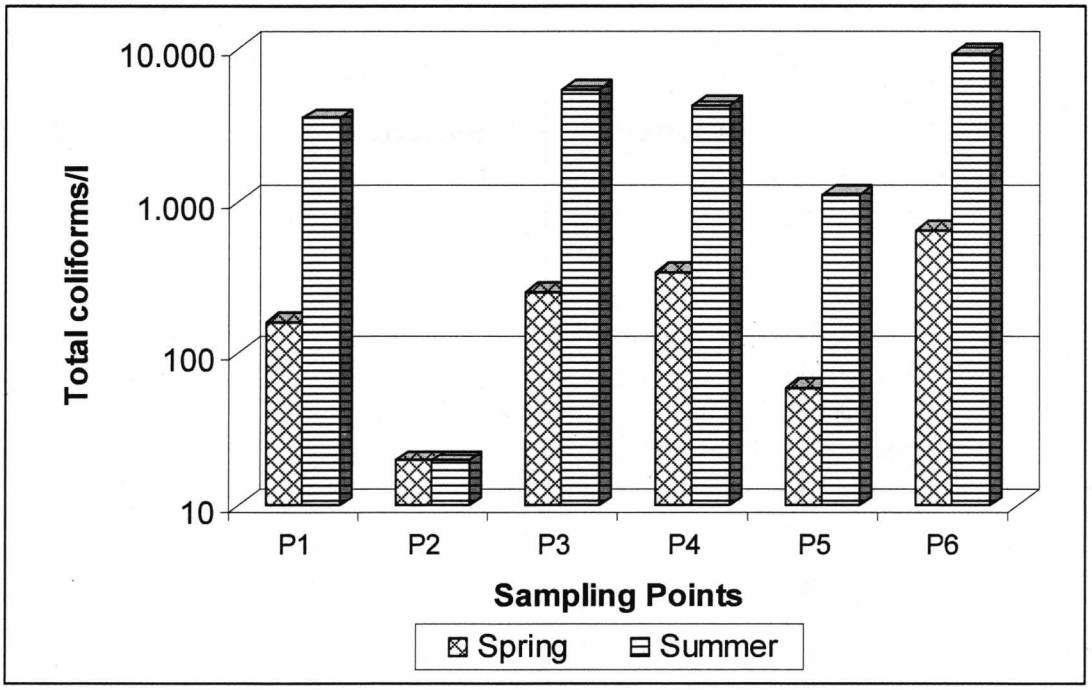
The analyses were carried out on water and sediments samples from six sites, as follows: P1 – Mița Village (the tail of Colibita Lake), P2 – 150 m upstream from the dam, P3 – Josenii Bârgăului Locality, P4 – Before Bistrița City, P5 – Bistrița city, at the junction with the creek on the valley Căstăilor, and P6 – Sărata Locality, downstream from the town waste water treatment plant.

The microbiological analyses have persueded the total coliform bacteria, according to the STAS 3001-91, and the number of aerobic heterotrophs, using cultures on plates. The following enzymatic activities were also carried out: catalase activity using Kappen method (1913), phosphatase activity using method proposed by Kramer and Erdei (1959) and actual and potential dehydrogenase activity using method proposed by Casida *et. al* (1964). We have also measured *in situ* the following phisico-chemical parameters: pH, Eh, conductivity, salinity, O_2 concentration and temperature, using a portable multiparameter.

Results and discussion

Fig. 1 presents the seasonal variation of the number of total coliform germs in the 6 sampling sites.

Fig. 1. The number of total coliforms (logarithmic expression).



The figure shows a pretty big difference between the six sampling points in the number of total coliform germs in each season. The lowest number of total coliform germs wass recorded in the P2 sampling site (<20 germs/l). In the points P1, P3 and P4 the higher number of germs is influenced by the discharge or deposit of waste and domestic sewage by the people.

The waste water treatment plant spilt the water in Bistrița upstream from the P6 sampling site. Along the river till the P6 point, the locals illegally discharged waste and domestic sewage, facilitating the development of total coliform germs so that the highest number of total coliform germs are found in P6 summer (9600 germs/l), when temperatures are high.

A clear seasonal variation of the number of total coliform germs is also recorded. Evolution is parallel in all sampling points: the minimum values are registered in spring and the maximum one in the summer, except for the P2, where the number of coliform germs was very low and stable, less than 20 germs/l.

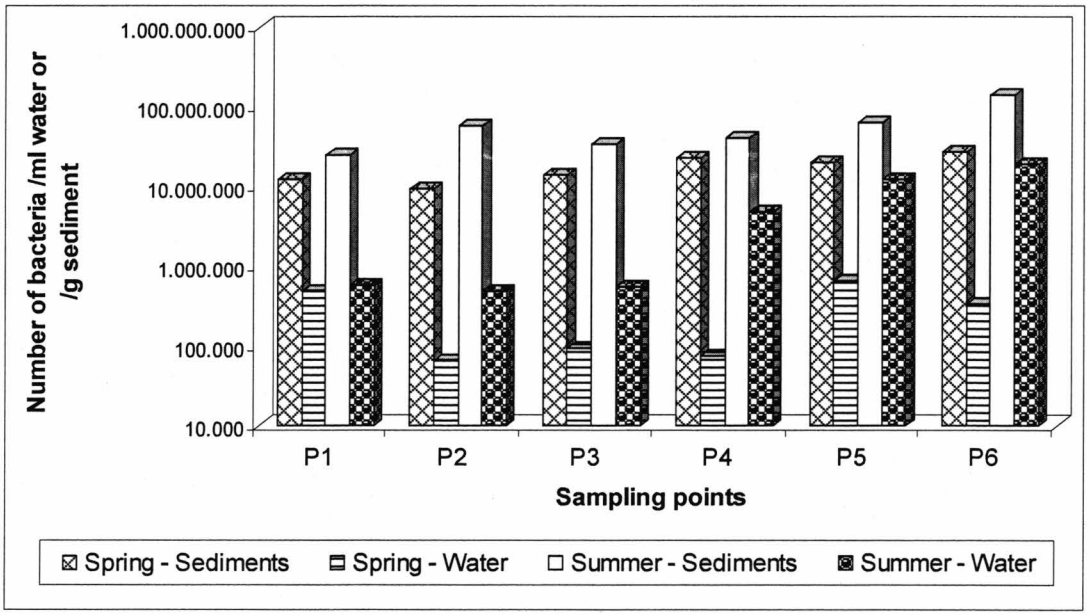
Persuing a presence in waste water treatment plants of faeces, Mureşan and Nistorescu-Avram (1984) isolated and identified bacteria belonging to the genera: *Escherichia, Enterobacter, Citrobacter* and *Salmonella*. The researchers concluded that the respective sewage station had a low efficiency, in terms of reducing the number of bacteria.

The presence of faecal enterococci in water indicates water pollution with faeces. *Enterococcus bovis*, *E. equinus* and *E. avium* are indicators of water pollution with mamalian and avian faeces. They don't resist much in the environment.

Enterococcus faecalis and *E. faecium* are predominantly presents in human faeces, and they rest longer in the environment (Muntean *et al.*, 2008).

According to this indicator, in the Bistriţa Ardeleană River the faecal pollution is predominantly animal. With only few exceptions, in the waters from the waste water treatment plant (P6), the human source of the faecal pollution was noticed, revealing the very low efficiency of the town waste water treatment plant.

Fig. 2. The number of aerobic heterotrophs (logarithmic expression).



The minimum number of aerobic heterotrophs in water samples was recorded in spring in P2, with value 67667/ml and maximum was recorded in summer in P6 with 19210000/ml value, and for sediment minimum number recorded was 12513333/g in P1 and the maximum was 142346667/g in P6, so you can see that the number of aerobic heterotrophs is clearly higher than in water and sediment is also higher in summer than spring. The number of bacteria remaining points are close, indicating different sources of pollution with organic substances which determines a large number of bacteria.

Based on absolute values of enzyme activity of each sample analyzed were calculated enzymatic indicators of sediment quality (EISQ) using the formula proposed by Muntean (1996).

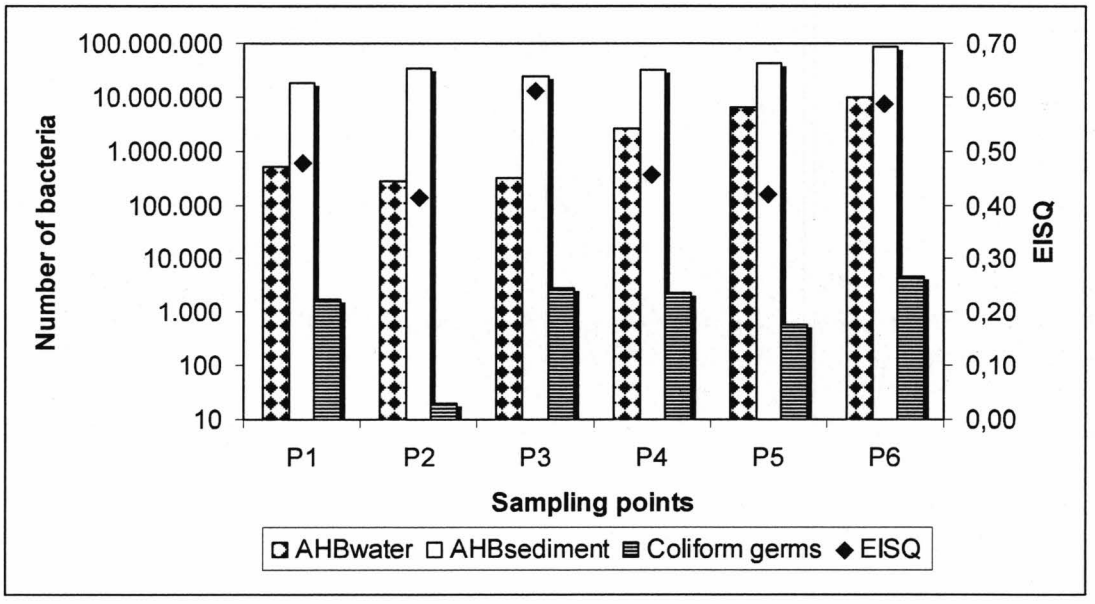
In all sediments analyzed were detected all the 4 activities studied with seasonal variations (**Table 1**).

Table 1. Results of sediment analysis durring spring and summer, 2009.

Point	Season	Enzyme activity /g dry sediment				EISQ
		Phosphatase (mg phenol)	Catalase (mg H_2O_2 split)	Present dehydrogenase	Potential dehydrogenase	
				(mg formasane)		
P1	Spring	10.640	41.155	0.484	3.480	0.369
	Summer	21.129	31.011	3.638	7.659	0.584
P2	Spring	5.744	51.596	0.375	0.403	0.296
	Summer	18.157	48.862	3.093	3.261	0.532
P3	Spring	6.574	80.664	0.733	3.204	0.486
	Summer	16.146	46.923	6.167	12.982	0.739
P4	Spring	2.569	63.736	0.152	1.230	0.321
	Summer	13.340	54.474	3.473	7.876	0.593
P5	Spring	0.785	45.047	0.991	1.207	0.238
	Summer	10.491	45.173	8.276	7.472	0.603
P6	Spring	2.063	65.368	1.143	3.799	0.388
	Summer	16.988	40.627	7.180	15.409	0.786

Intensity of phosphatase activity falls within fairly broad limits - 0.785 mg phenol/g dry sediment in the sampling point P5, spring and 21.129 mg phenol/g dry sediment in the sampling point P1, summer.

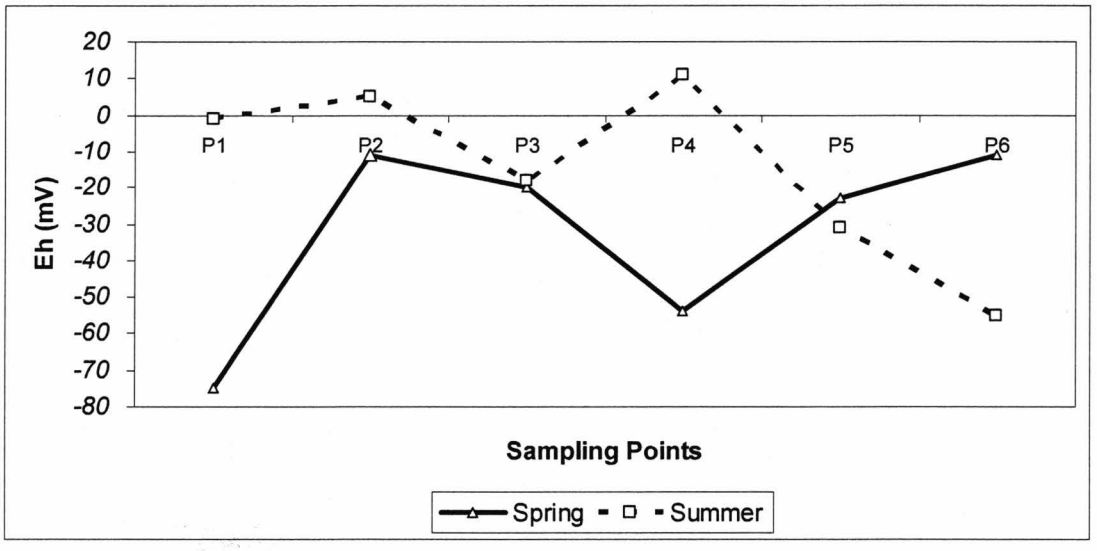
Fig. 3. Logarithmic expression of the number of aerobe heterotrophic bacteria (AHB) in water (bacteria/ml) and sediment (bacteria/g), coliform germs (bacteria/l) and enzymatic indicator of sediment quality (EISQ).



The highest enzymatic potential, as it is defined by the EISQ values, was registered in the P3 sampling site (EISQ = 0.612), and in the P6 sampling site (EISQ = 0.587), respectively. As one can see in Fig. 3, in the two sampling site was also registered a high number of each bacterial ecophysiological studied group. The high enzymatic and bacterial potential registered in the P6 sampling site could be correlated with a putative organic pollution originating in the Bistriţa town, and especially in the waste water treatment plant of the city.

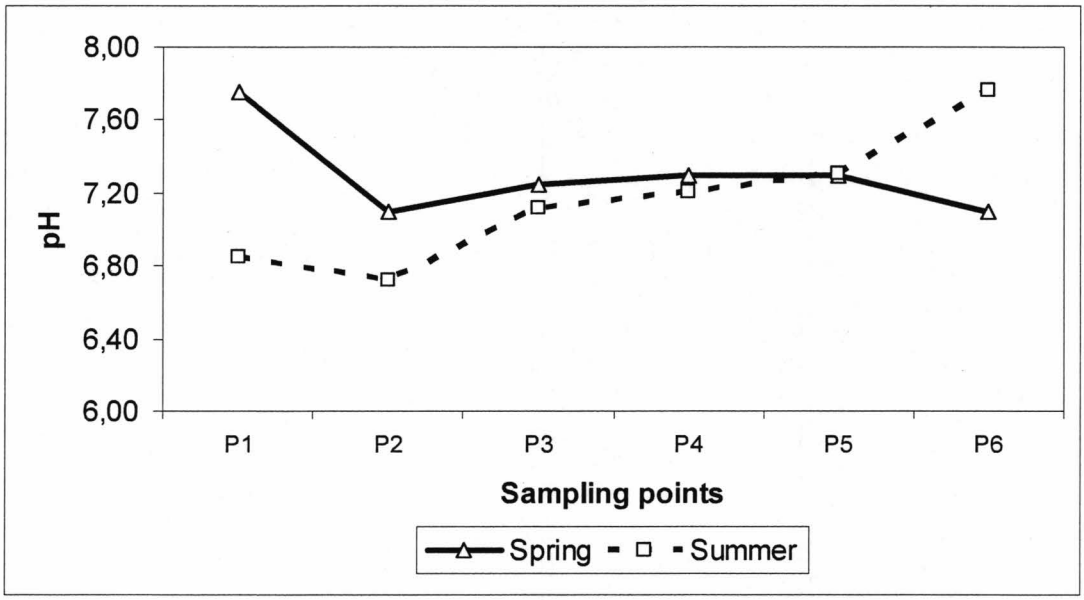
The lowest value of the EISQ was recorded in P2 (EISQ = 0.414). In the same sampling site we registered the lowest number of coliform germs, the lowest number of aerobic heterotrophs in water, as well as a low number of aerobic heterotrophs in sediment. The fact ilustrates the same correlation already signaled between the enzymatic and the bacterial potential of sediment.

Fig. 4. Seasonal variation of redox potential (Eh).



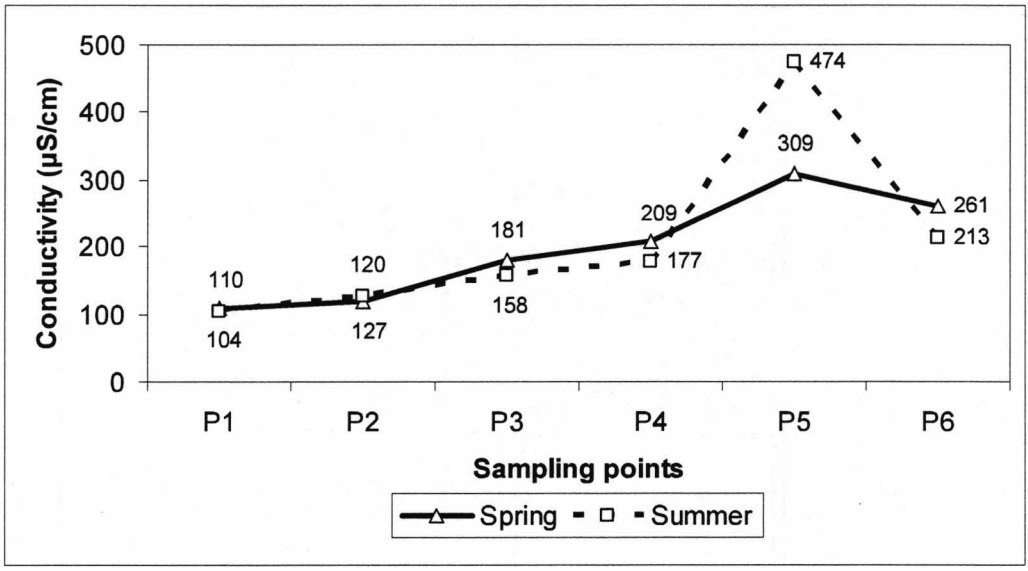
As shown in Fig. 4, an obvious variation of redox potential was noticed: the lowest value -75 mV in P1 in spring, ant the highest value +11 mV in P4 in summer. Only two slight positive values were recorded: +11 mV in P4 and +5 in P2, both in summer. The values from P2, P3 and P5 are very close in both seasons. Taking into account that the natural limits of redox potential are - 421 mV and + 816 mV, one can be stated that water has a slightly reducing capacity.

Fig. 5. Seasonal variation of pH.



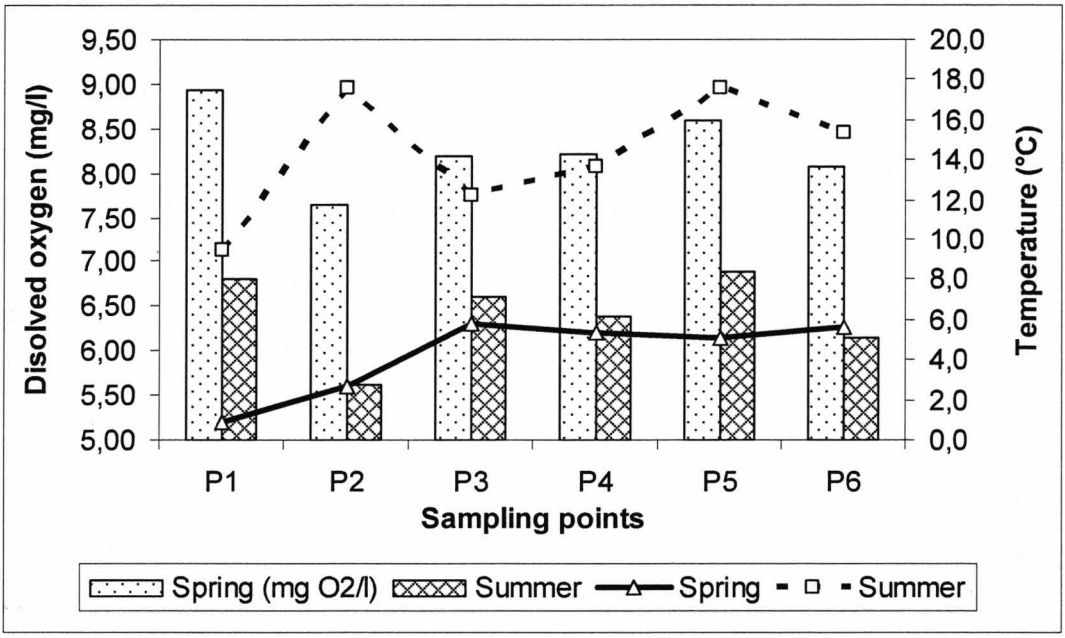
In the spring, water has an alkaline pH which tends to be almost neutral in the Şieu River. A cause for these results in spring is snowmelt, rains, and the large amount of water fallen on the ground can influence the pH value. In the summer, when the quantity of rain is poor, is clearly observe that the water have an acid pH. pH registered in P1 was 6.85 and 6.72 in P2.

Fig. 6. Seasonal variation of conductivity.



Seasonal variation of conductivity is almost constant during the two seasons in all points, except for the P5 sampling site, where the difference registered between the two seasons was higher then 150 µS/cm: 309 µS/cm in the spring and 474 µS/cm in the summer. Increased conductivity indicates a possible water pollution by various inorganic substances. Since data on conductivity can be conclude that the Bistrița River basin is located on specific geological substrate that can influence this indicator. In P5 conductivity was 474 µS/cm and salinity 0.12 g/l; this may indicates a possible pollution, knowing that Căstăilor Valley pass nearby industrial area of the town.

Fig. 7. Relationship between the amount of oxygen dissolved in water and water temperature.



In spring, water temperature is low and amount of oxygen is increased: at 0.9°C amount of dissolved oxygen is 8.93 mg/l and at 5.6°C is 8.08 mg dissolved oxygen/l. In the summer, when water temperature increases, the amount of dissolved oxygen decreases: at 17.6°C an amount of 5.62 mg dissolved oxygen/l was registered. The data confirm the negative correlationship between temperature and dissolved oxygen in water: if temperature increase the amount of dissolved oxygen will decreases and if water temperature decrease the amount of dissolved oxygen will increase.

Conclusions

Results of physico-chemical parameters of water revealed:

- a pH close to neutral;
- the redox potential with slightly reducing values;
- conductivity almost constant in all points (values between 104 and 309 μS/cm), except for the P5 samplind site; actualy, here it was recorded the only one measurable salinity – 0.12 g/l at a conductivity of 474 μS/cm;
- oxygen concentration in water enframes in normal values, affected primarily by environmental temperature.

The presence of total coliform germs was registred in each season in the water samples in all the six sampling sites. The large numger of germs is probably due to the low efficiency of waste water treatment plant of the Bistrița city, situated upstream.

The number of aerobic heterotrophs is influenced by the amount of nutrients in the water, season and pollution. The highest values were also registered in the sampling site P6, both in water and in sediment.

An appreciable enzymatic potential of sediments was registered in all the studied sediments. The highest values of the enzymatic indicator of sediment quality (EISQ) were registered in the P3 sampling site (EISQ = 0.612), and in the P6 sampling site (EISQ = 0.587).

The high enzymatic and bacterial potential registered in the P6 point, compared with all the other sampling sites, could be correlated with a putative organic pollution originating in the the city Bistrița.

Rezumat. Râul Bistrița își are izvoarele în Munții Călimani și traversează județul de la est la vest, până în localitatea Sărățel unde se varsă în Râul Șieu. Acest studiu a fost realizat în primăvara și vara anului 2009, colectându-se probe de apă și sediment din 6 stații diferite de pe traseul de aproximativ 60 km al râului, de la lacul Colibița până în localitatea Sărata. Parametrii fizico-chimici ai apei analizate au fost: temperatura, pH, Eh, conductivitatea, salinitatea și concentrația de O_2 dizolvat. Analizele au fost realizate la fața locului, cu ajutorul unui multiparametru portabil. Numărul de bacterii heterotrofe aerobe a fost evidențiat atât în probele de apă cât și în cele de sediment. Numărul de bacterii din sediment a fost mai mare cu un ordin de mărime comparativ cu numărul înregistrat în probele de apă. Toate cele patru activități enzimatice analizate în sedimente (fosfataza, catalaza, dehidrogenaza actuală și potențială) au fost evidențiate în toate punctele de prelevare. Pe baza activității enzimatice studiate a fost calculat indicatorul enzimatic al calității sedimentului. Toate valorile parametrilor analizați au fost mai ridicate vara decât primavara. De asemenea, în toate punctele de prelevare a fost evidențiată prezența germenilor coliformi totali. Rezultatele au evidențiat un număr mare de germeni coliformi, peste limitele acceptate, la punctul de prelevare situat în aval de orașul Bistrița.

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THE INFLUENCE OF AERATION ON GROWTH AND NITROGEN FIXATION AT BACTERIAL STRAINS ISOLATED FROM ALTITUDINAL VEGETATION ZONES OF PARÂNG MOUNTAINS

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Abstract: The study consisted in validating the effect of aeration on proliferation of *Azotobacter* species. Physiologic studies involving growing of the strains in different aeration conditions and on different culture media were performed. The nitrogen fixing strains isolated out of five vegetation zones of Parâng Massif were grown on mannitol medium and on sucrose medium at pH 7. The evolution of the bacteria was recorded in Erlenmeyer flask in different aeration conditions.

At all the bacterial strains and on both media it was observed that at the least aeration (the one in case 2) corresponds the least growth. This is due to *Azotobacter* being an aerobic bacteria genus. Although the growth took place on medium free of nitrogen and the nitrogenase is inactivated in the presence of oxygen, the growth is not inhibited at the two more aerated cases (3 and 1) than the case 2.

Concomitant with assessing the effect of oxygenation on growth an assessment of the products issued from nitrogen fixation (extracellular proteins and ammonia secretion).

Key words: *aeration, nitrogen fixing, Azotobacter, altitudinal vegetation zone.*

Introduction

Because the nitrogen biological fixation is an essential source of fixing this element in the biosphere it is imposed a further knowdlege of the physiology of microorganisms able to perform it.

Azotobacter are free, aerobes bacteria but it was proved that these can also grow at lower oxygen concentrations (Tejera *et al.,* 2005).

Although nitrogenase is inactivated in the presence of oxygen, nitrogen fixation by *Azotobacter sp.* continues also in aerobous conditions, because at these microorganisms nitrogenase is protected from inactivation either by O_2 removal

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through respiration, or by producing viscous layers which lead to the formation of a special cell type, the heterocyst (Wong *et al.,* 1995; Brock, 2000).

Because the aeration is different in the soils of the five studied altitudinal vegetation zones growth at different aeration degrees was tested.

In the specialty literature it is mentioned that together with the increase of agitation speed the bacteria growth would improve (Tauchert and Oelze, 1990; Pena *et al.,* 2000).

The purpose of this work was to evaluate the effect of oxygenation on bacterial consortia growth and also to assess the products resulted from nitrogen fixing (extracellular proteins and ammonia secretion).

Materials and Methods

In order to validate the efect of aeration on the proliferation of *A. chroococcum* in different culture media, mannitol and sucrose (Atlas, 2004), Erlenmeyer flasks of different volumes were used for every studied sample.

Thus, the optical density of all 1 ml of bacterial culture was measured by spectrophotometry at 600 nm wavelength (visible light), at different times. Besides assessing the effect of oxygenation on growth an evaluation of the products resulted from nitrogen fixation was also performed (extracellular proteins and ammonia secretion). The extracellular proteins concentration was measured by modyfied Lowry method (Hartree, 1972). The ammonia concentration was measured by Nessler methods following the procedures described in standard methods (Eric and Triplett, 2000). The working conditions were as follows:

- Flask 1 (250 ml Erlenmeyer) with 30 ml of inoculated medium were placed in the thermostatated chamber, at 35 °C, on continuous shaking at 150 rpm.
- Flask 2 (250 ml Erlenmeyer) with 30 ml of inoculated medium were kept at the same temperature but stationary.
- Flask 3 (500 ml Erlenmeyer) with 30 ml of inoculated medium were placed in the thermostatated chamber, at 35 °C, on continuous shaking at 150 rpm.

By applying the statistical analysis tests One-Way ANOVA and the Bonferroni post-test the statistical semnification was determined for every altitudinal zone and every culture medium studied. For a general comparison of the samples from the two culture media the One-Way ANOVA test was performed (Fisher, 1925). A $p<0.05$ value was considered statistically significant (Măruşteri, 2005; Măruşteri, 2006). In order to compare also the samples with different aeration degrees the Bonferroni post test was applied. The statistical computation was performed with free GraphPad Prism4 version. All data represent the media of three independent measurements.

Results and Discussions

Influence of aeration on the growth of isolated strains. It was noticed that in the flask 2 (the least aerated) at the samples from the alpine vegetation zone the growth on mannitol was larger than at those from the flood plain (Fig. 1). On sucrose instead, also in flask 2, growth was smaller at the samples from the alpine zone than on those from the flood plain. Therefore the altitude of the sample prelevation from the alpine zone is not high enough for the existence of strains specialized on less aerated environment. Probably in the alpine zone the lower atmospheric pressure is compensated by stronger winds so the oxygen is not less accesible.

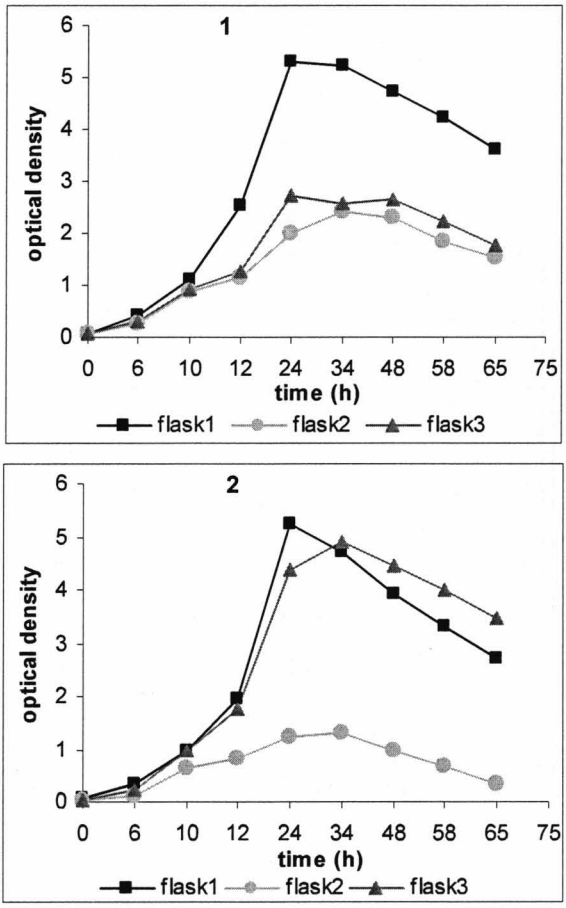

Fig. 1. Effect of oxygen on growth of nitrogen fixing consortium isolated from alpine zone (The carbon sources used were 1 = mannitol and 2 = sucrose).

By applying the statistical analysis tests, it was noticed that the bacterial consortia densities from the alpine vegetation zone presented a very statistically significant difference ($p<0.0001$) between the three cases studied.

At the samples from the subalpine vegetation zone, in the flasks 1 and 2, the bacterial consortia growth was stronger on the mannitol medium (Fig. 2). The bacterial consortia from the subalpine vegetation zone use efficiently mannitol as carbon source because it is present in the soil of this zone in significant quantities due to the conifers (juniper and mugo pine). At these samples a very statistically significant difference was obtained, the value of p being 0.0005.

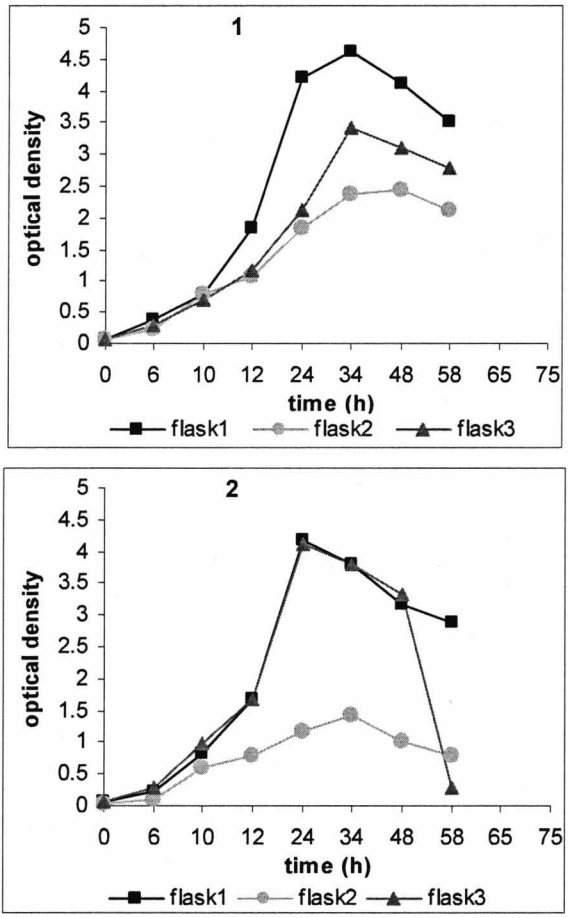

Fig. 2. Effect of oxygen on growth of nitrogen fixing consortium isolated from subalpine zone (The carbon sources used were 1 = mannitol and 2 = sucrose).

At the samples from the coniferous zone it is noticeble that the maximum values reached by the growth are considerably smaller then those of the samples from other altitudinal vegetation zones (Fig. 3). This is probably due to the small number

of nitrogen fixing bacteria found in this acid soil. A preference for mannitol is also visible at the bacteria from the coniferous vegetation zone.

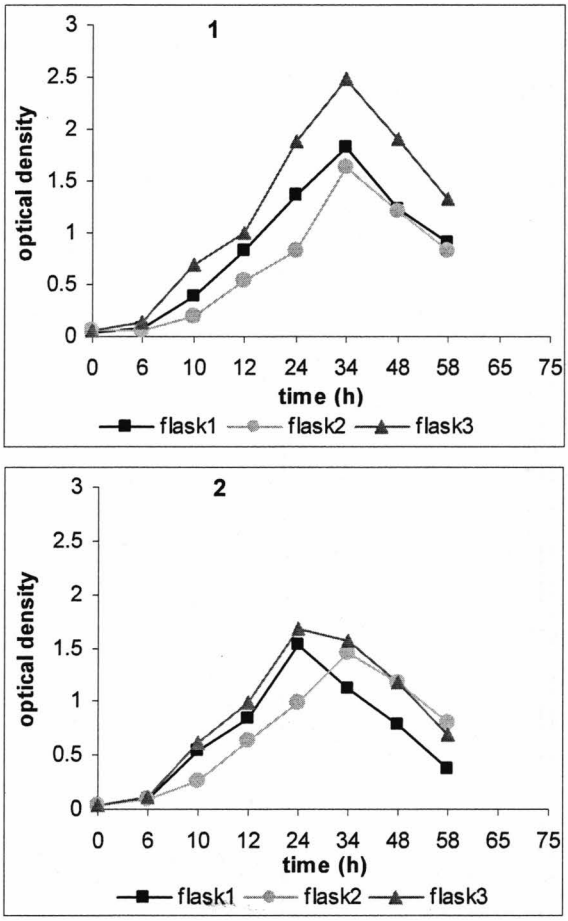

Fig. 3. Effect of oxygen on growth of nitrogen fixing consortium isolated from coniferous zone (The carbon sources used were 1 = mannitol and 2 = sucrose).

At the samples from coniferous zone a very statistically significant difference was obtained, the value of p being 0.0003.

As expected, at the samples from the beech zone the maximum growth is on sucrose (Fig. 4).

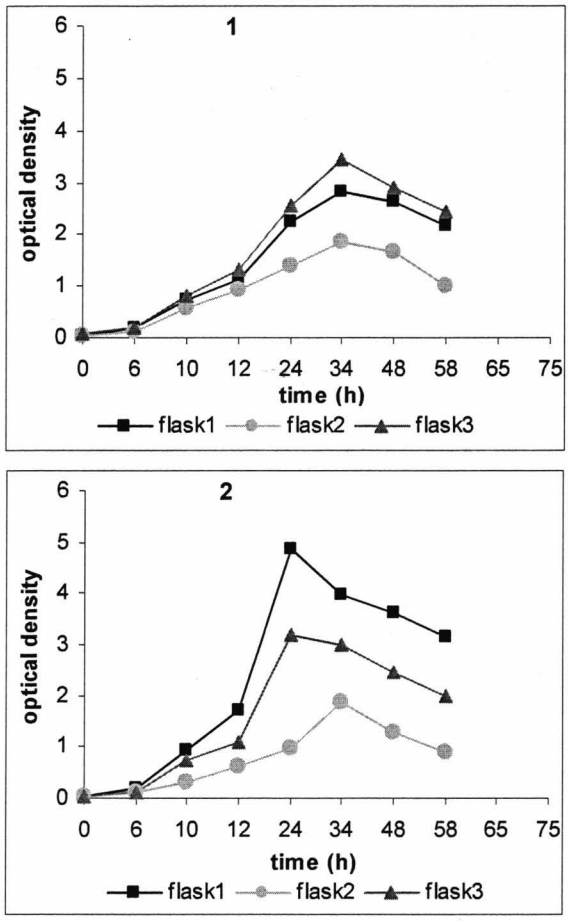

Fig. 4. Effect of oxygen on growth of nitrogen fixing consortium isolated from beech zone (The carbon sources used were 1 = mannitol and 2 = sucrose).

At all the altitudinal vegetation zones and on both media it turns out that at the most reduced aeration (in the flask 2) is the smallest growth. This is because *Azotobacter* is a genus of aerobe bacteria. Although the growth took place on nitrogen free medium and the nitrogenase is inactivated in the presence of oxygen the growth is not inhibated in the two most aerated flasks (3 and 1) compared to flask 2. The explanation is the efficiency of the mecanisms that protect the nitrogenase against oxygen. Is visible that in some cases growth is maximum in flask 1 and in other cases it is maximum in flask 3, althogh aeration is stronger in flask 3. This is because in flask 1 the oxygen saturation threshold is attained, so the supplementary oxygen from flask 3 does not increase the growth.

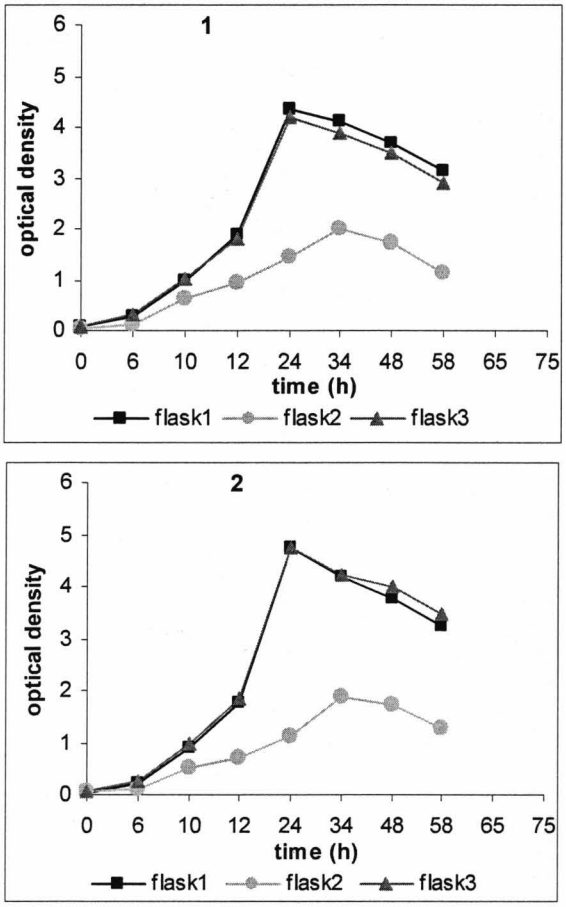

Fig. 5. Effect of oxygen on growth of nitrogen fixing consortium isolated from Maleia food plain (The carbon sources used were 1 = mannitol and 2 = sucrose).

Applying the statistical analysis tests it turned out that the densities of bacterial consortia from the beech zone and the Maleia flood plain presented a very significant statistical difference ($p<0.0001$) for the three studied cases.

The influence of aeration on the extracellular protein level. In the figures 6-10 a growing trend of the extracellular protein level can be observed during incubation at all three experimental cases. During incubation, even after 48 hours, when the cultures are already in the stationary phase, the extracellular protein levels appear to be growing.

At the strains from the alpine zone the maximum extracellular protein concentration on mannitol cultures was obtained on flask 2 (stationary conditions) after 58 hours of incubation, attaining 9.219 mg/l. The maximum value of extracellular protein concentration on sucrose (10.927 mg/l) was obtained in flask 3 (case with stronger aeration). As we can see in Fig. 6 the protein concentration is

growing in all the cases, only on sucrose after 65 incubation hours the values begin to decrease slowly.

These differences were observed also statistically. It was concluded that the extracellular protein levels from the culture media studied presented a very statistically significant difference ($p<0.0001$) for the threee studied cases.

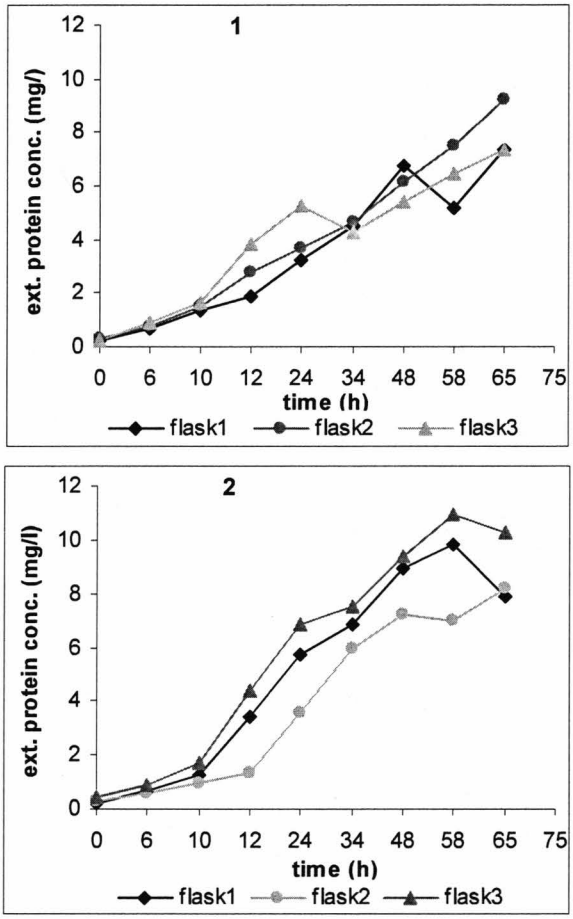

Fig. 6. Effect of oxygen on extracellular protein levels at strains isolated from alpine zone (1 = mannitol, 2 = sucrose).

At the strains from the subalpine zone the maximum value of extracellular protein concentration was 8.984 mg/l for sucrose cultures and 7.298 mg/l for the ones on mannitol. As visible in Fig. 7, protein concentration on the two culture media follow a parallel growth. In the case of sucrose medium a decreasing of the proteins level can be observed even after 34 incubation hours (flask 1 and 3).

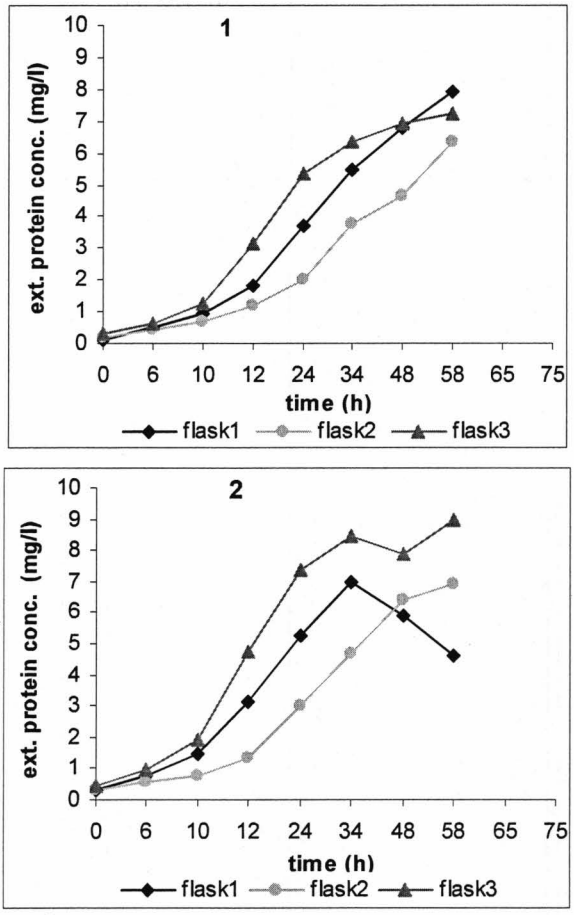

Fig. 7. Effect of oxygen on extracellular protein levels at strains isolated from subalpine zone (1 = mannitol, 2 = sucrose).

At the samples from coniferous zone the lowest protein values from the tested culture media were recorded (Fig. 8). However, a maximum of 2.763 mg/l extracellular proteins was recorded on sucrose medium in the flask 3 (case with stronger aeration). For this altitudinal vegetation zone a very statistically significant difference resulted, the value of p being 0.0002.

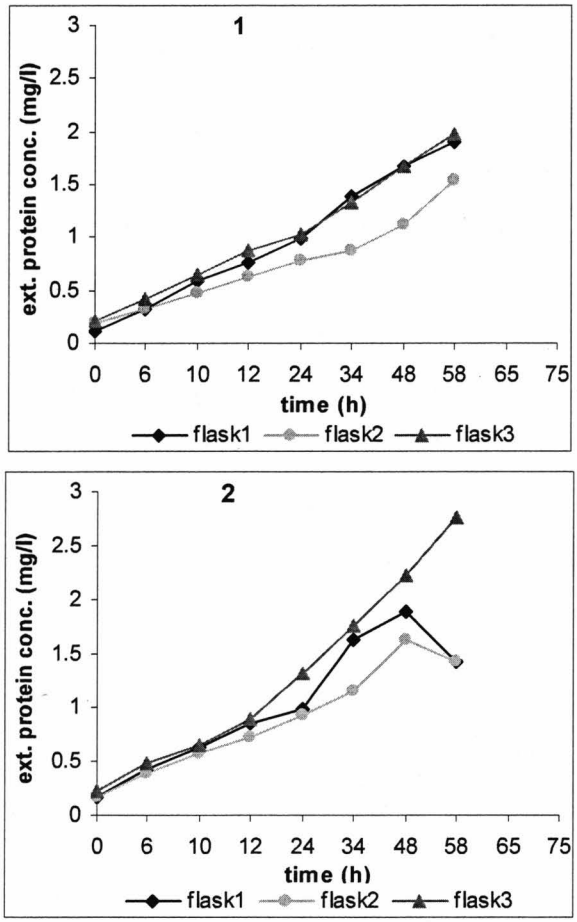

Fig. 8. Effect of oxygen on extracellular protein levels at strains isolated from coniferous zone (1 = mannitol, 2 = sucrose).

The aeration level influenced the extracellular protein concentration at the samples from the beech zone also. In Fig. 9 a growth of extracellular proteins can be observed, higher values of their concentration being recorded on sucrose medium (the maximum attaining 9.683 mg/l). In these cases a continous growth can be observed even after 58 incubation hours. The differences between the two culture media were also observed from statistically. It turned out that extracellular protein levels from the studied culture media presented a very statistically significant difference ($p<0.0001$) for the three cases studied.

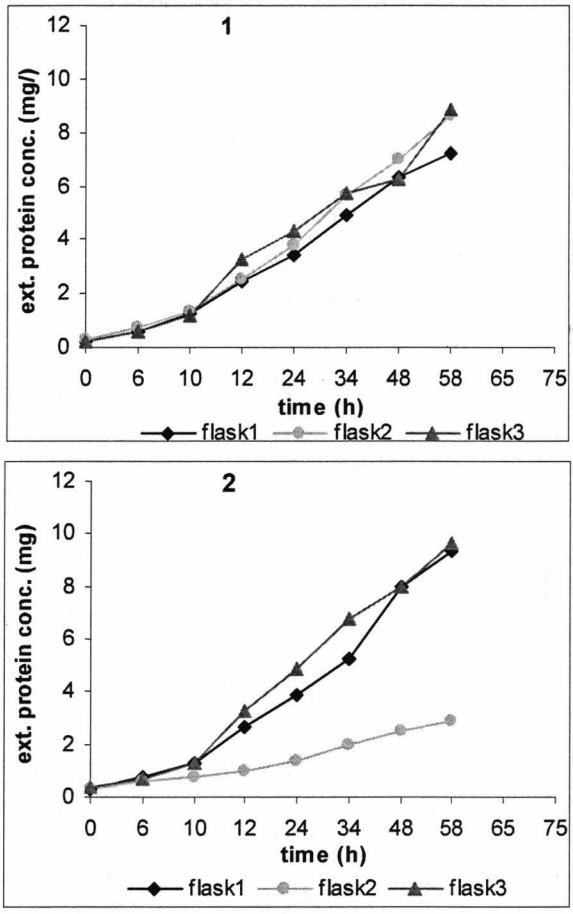

Fig. 9. Effect of oxygen on extracellular protein levels at strains isolated from beech zone (1 = mannitol, 2 = sucrose).

The highest extracellular protein level in the culture media was recorded at the samples from Maleia flood plain (Fig. 10). The maximum protein concentration level was obtained on sucose, attaining 11.673 mg/l. Even after 58 hours of incubation the growing trend on the two culture media is still present. Also at this zone the extracellular protein levels from the studied culture media presented a very statistically significant difference ($p<0.0001$) at the three studied cases.

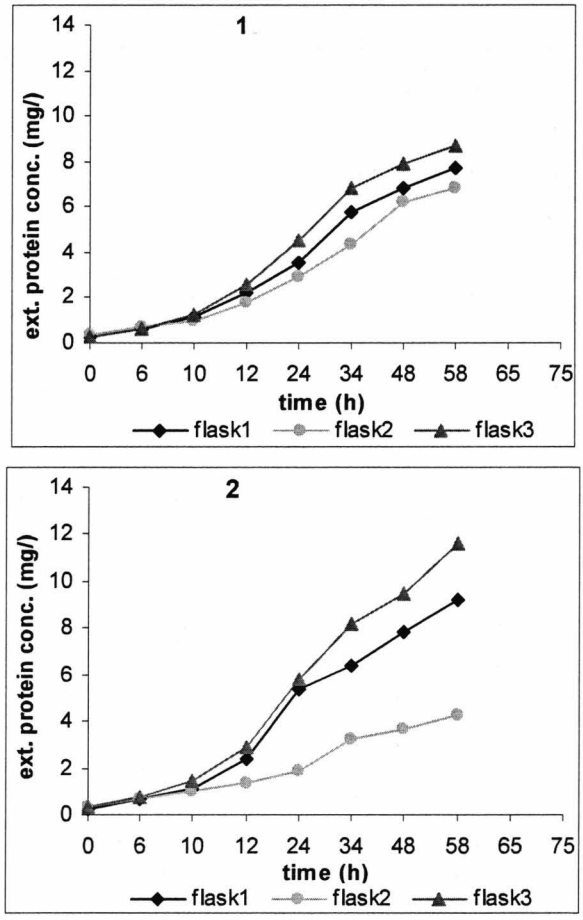

Fig. 10. Effect of oxygen on extracellular protein levels at strains isolated from Maleia food plain (1 = mannitol, 2 = sucrose).

The influence of aeration on produced ammonia. In Fig. 11 can be noticed that in the case of flask 1 the ammonia secretion attains a maximum of 2.227 mg/l on mannitol and 3.983 mg/l on sucrose after 34 incubation hours. In the case of flask 3 the maximum ammonia secretion concentration was reached in the 24-th incubation hour.

By applying statistical tests, resulted that the produced ammonia level in the culture media presented a very statistically significant difference ($p<0.0001$) at all the studied cases.

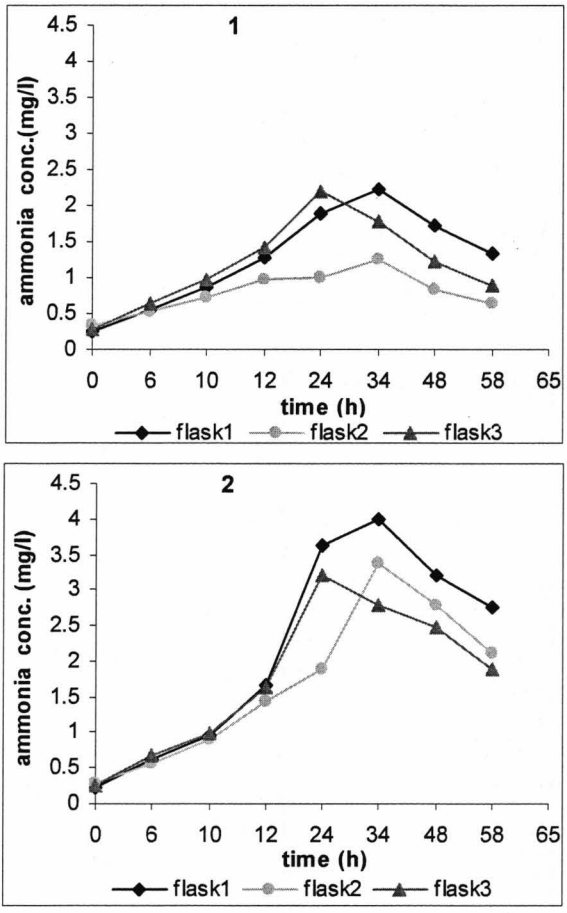

Fig. 11. Effect of oxygen on ammonia secretion at strains isolated from alpine zone (1 = mannitol, 2 = sucrose).

At the bacterial consortia from the subalpine zone larger values than at the alpine zone were obtained, the maximum values being attained after 24 incubation hours, in flask 3 (case with stronger aeration), for both culture media, 2.347 mg/l on mannitol and 5.236 mg/l on sucrose (Fig. 12). A very statistically significant difference ($p<0.0001$) rezulted also here, for the studied cases.

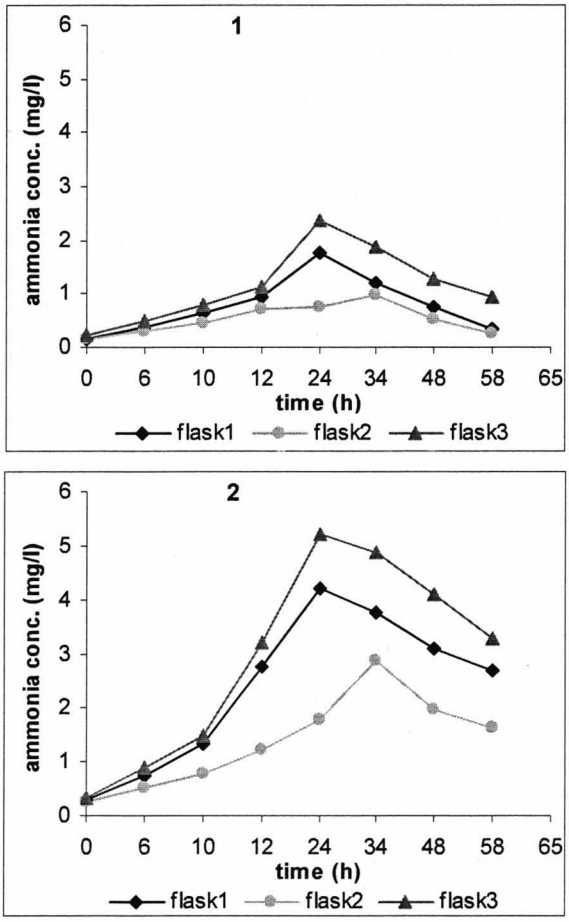

Fig. 12. Effect of oxygen on ammonia secretion at strains isolated from subalpine zone (1 = mannitol, 2 = sucrose).

The lowest level of ammonia concentration produced in the two growth media was recorded at the samples from coniferous zone (Fig. 13). Here ammonia concentration attains the maximum of 1.263 mg/l on mannitol and 1.728 mg/l on sucrose, after 24 incubation hours at the samples from flask 3, and then the ammonia level decreases abruptly.

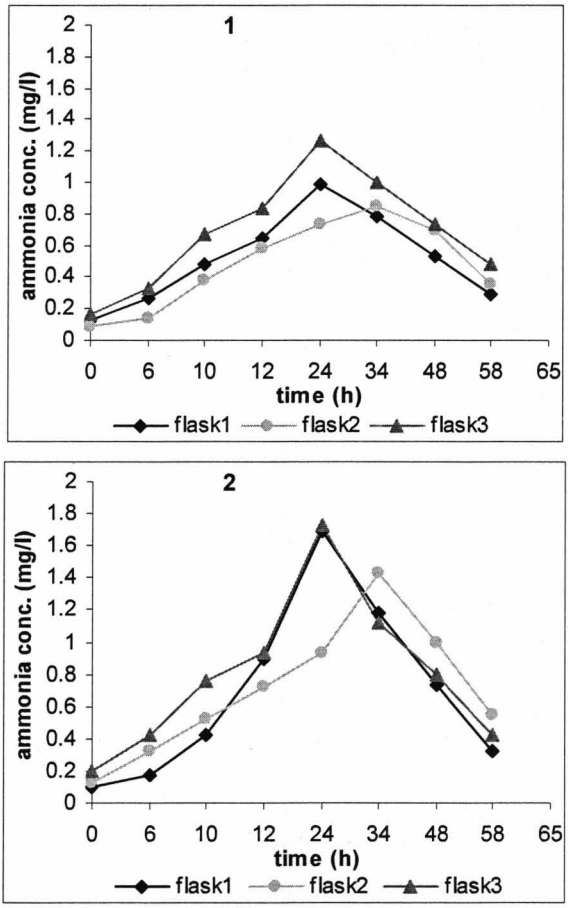

Fig. 13. Effect of oxygen on ammonia secretion at strains isolated from coniferous zone (1 = mannitol, 2 = sucrose).

For this zone the difference in ammonia secretion was very statistically significant at the two culture media, the p value being 0.0032.

At the samples from beech zone the maximum level of produced ammonia was also obtained at the ones grown in flask 3. It can be observed a parallel evolution between the three experimental cases at each of the two culture media (Fig. 14). Although the ammonia secretion is very similar at the two culture media analyzed, a very statistically significant difference was obtained, the p value being 0.0001.

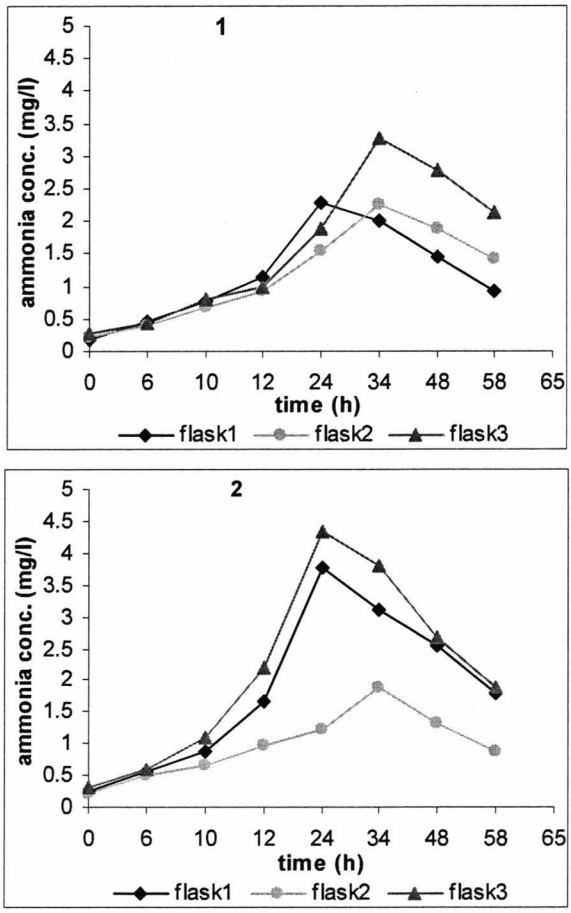

Fig. 14. Effect of oxygen on ammonia secretion at strains isolated from beech zone (1 = mannitol, 2 = sucrose).

High levels of ammonia secretion in the culture media were also recorded at the samples from Maleia flood plain (Fig. 15). The maximum value of ammonia concentration produced in the sucrose medium was 5.210 mg/l and in the mannitol medium it was 3.218 mg/l.

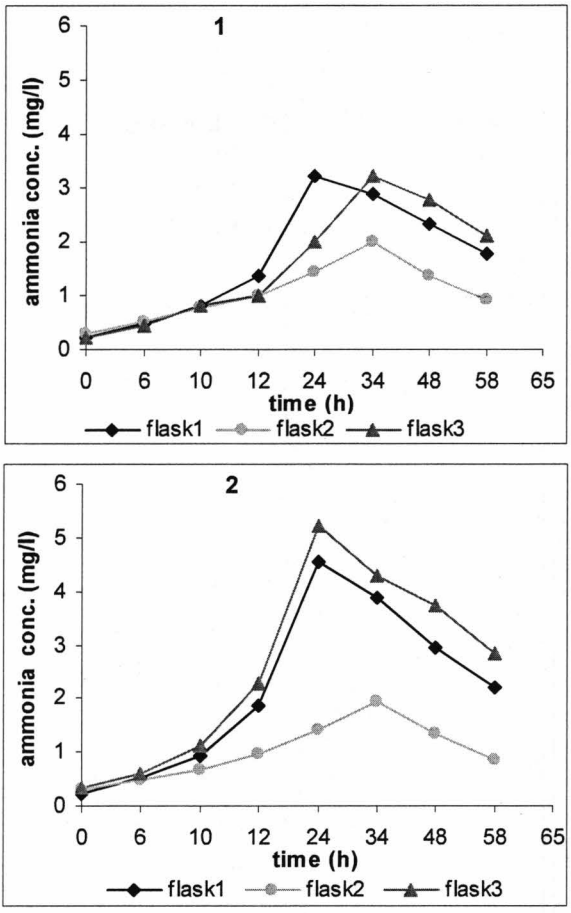

Fig. 15. Effect of oxygen on ammonia secretion at strains isolated from Maleia food plain (1 = mannitol, 2 = sucrose).

At the flood plain it was noticed that the level of ammonia produced in the culture media presented a very statistically significant difference ($p<0.0001$) for all the studied cases.

The minimum ammonia concentration level was recorded at the samples placed in stationary conditions (vasul 2). The maximum ammonia secretion level was attained between the 24-th and the 40-th incubation hour when the bacterial strains were grown in the conditions of flask 1 and 3. The maximum ammonia concentrations were attained in the section were growth attainsthe stationary phase, then it decreases abruptly even to exhaustion level. This is due to the fact that ammonia is volatile and in short time it loses in the atmosphere.

Conclusions

1. In these experiments the effect of aeration on the proliferation of bacteria from the diazotroph consortia specific to each altitudinal vegetation zone.

2. At the samples from all the altitudinal vegetation zones and on both culture media it was noticed that at the lowest aeration (stationary case, in flask 2) the growth is lower. This is due to *Azotobacter* being a genus of aerobe bacteria.

3. Although the growth took place on nitrogen free medium and the nitrogenase is inactivated in the presence of oxygen growth was not inhibited in the other two more aerated flasks (case 3 and case 1) compared to flask 2. This is due to the efficiency of the protection mechanisms of nitrogenase against oxygen.

4. It was noticed that in some situations the growth was maximum in flask 1 (less aerated) and in other situations in flask 3, although aeration was higher in flask 3. This is due to the fact that in flask 1 the oxygen saturation threshold was attained, so in the flask 3 the supplementary oxygen did not emphasize the growth.

5. For every strain from the different altitudinal vegetation zone at the three experimental cases, a growing trend of the extrecellular protein levels was noticed even after 48 hours, when the cultures were already in the stationary phase.

6. The aeration degree influenced the extracellular protein concentration in the two culture media. The levels of extracellular proteins from these culture media prezented a very statistically significant difference ($p<0.0001$) for each of the three studied cases.

7. The maximum ammonia concentrations were obtained in the section where growth attains the stationary phase, afterwards these decrease abruptly even to exhaustion level. This is due to the fact that ammonia is volatile and it soon releases in the atmosphere.

8. The minimum level of ammonia concentration was recorded in the case of stationary conditions (in flask 2).

9. The maximum level of ammonia secretion was attained between 24-th and 40-th incubation hour when the bacterial strains were grown in the cases of flask 1 and flask 3, first less aerated then the second, both placed in shaking conditions at 150 rpm.

10. In all the three experimental cases the ammonia secretion attained the maximum on sucrose. Between the two media there is also a very statistically significant difference ($p<0.0001$).

Rezumat. Studiul a constat in validarea efectului de aerație asupra proliferării speciilor de *Azotobacter*. S-au realizat studii fiziologice care au constat în creşterea tulpinilor în condiţii diferite de aerare şi pe diferite medii de cultură. Tulpinile fixatoare de azot izolate din cinci etaje de vegetaţie ale Masivului Parâng au fost cultivate pe mediu cu manitol şi pe mediu cu zaharoză la pH 7. Evoluţia bacteriilor a fost urmărită în vase Erlenmeyer în condiţii diferite de aerare. La toate tulpinile bacteriene şi pe ambele medii s-a constatat că la aeraţia cea mai redusă (cea din varianta 2) creşterea este cea mai mică. Aceasta deoarece *Azotobacter* este un gen de bacterii aerobe. Deşi creşterea

a avut loc pe mediu fără azot şi nitrogenaza este inactivată în prezenţa oxigenului creşterea nu este inhibată în cele două variante mai aerate (3 şi 1) faţă de varianta 2. Concomitent cu evaluarea efectului oxigenării asupra creşterii s-a realizat şi o evaluare asupra produşilor rezultaţi în urma fixării azotului (proteinele extracelulare şi secreţia de amoniac).

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THE ACTION OF *KOCH BACILLUS*, INCREASED BY THE SUBTERRANEOUS MEDIUM OF THE COAL MINES FROM JIU VALLEY

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Abstract: This study approaches on a 9 years period the diagnostic of silicosis by pulmonary radiography and of silicotuberculosis by bacteriological examination, which emphasizes the presence of Koch bacillus in sputum, being a certain indicator of bacillus infection. Once *Mycobacterium* sp. detected, antibiograms were performed testing at least 3 tuberculostatings, which were then indicated in treatment. In this period of study there were put on the spot both the existing cases and the cases newly appeared in the Jiu Valley. The highest incidence of cases is found at E.M. Petrila (163 cases), followed by E.M. Livezeni (61 cases), E.M. Lonea (30 cases). The least cases frequency is found at E.M. Aninoasa (5 cases), E.M. Uricani (2 cases), E.M. Valea de Brazi (2 cases). The location of the newly appeared silicosis cases in the Jiu Valley coalfield is the following: E.M. Petrila: 34 cases; E.M. Livezeni: 15 cases; E.M. Lonea: 10 cases.

A comparison between the incidences of the studied professional diseases in the East and in the West sides of Jiu coalfield was carried out. It was noticed that in the East side the silicosis frequency is higher, the most cases being recorded at M.E. Petrila, Livezeni and Lonea while in the West side, although there is a higer number of mines, the silicosis incidence is lower This situation can have as one explanation the fact that M.E. Petrila, situated in the East side, is the largest mine of Jiu Valley coal field both by exploatation surface and number of miners, thus explaining the high incidence of illness cases.
Key words: *Koch bacillus, tuberculosis, silicosis, mining exploitation, Jiu Valley.*

Introduction

Silicotuberculosis represents the most frequent tuberculosis form in the silicogen environment (underground mines with dry exploitation, sandblast workrooms, etc.). It is also called pulmonary tuberculosis and it is inflicted through infection with *Mycobacterium tuberculosis*, sometimes with atypical mycobacteria or even with bovine bacilli which are usually not pathogen for man. The cough, hemoptysis and general symptoms can suggest the search for the bacterium in: sputum, alveolar lavation, pulmonary biopsy, pleural liquid, urine (Becklarke, 1994).

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Tuberculosis is associated with silicosis quick progression, the 2 diseases influencing each other in a potentiating way. The tuberculosis lesions are established either on pre-existing silicotic lesions or they develop separately, making silicosis to reach quickly pseudotumoral forms. The risk of tuberculosis sickness is higher at silicotic patients then in general population (McConnochie,1990; Niţu, 1999).

It is hard to establish the diagnostic, especially at incipient forms, with small silicosis opacity. There are however some clinical criteria, radiologic and laboratory, which allow us to suspect the presence of an infection, to establish the active or inactive silicotuberculosis diagnostic. The evolution of the disease can be also highlighted by modifications which appear on **radiographs** (Petran and Cocârlă, 1999; Cocârlă, 2000). In the mixed conglomerates of silicotuberculosis, on **scintigraphic exam**, the cold zones are, in general, wider then at pure silicotic conglomerates of similar dimensions in the standard radiographs; therefore this discordance suggests the bacillar complication (Manu *et al.,* 1983). By laboratory examination the tuberculosis bacillus is highlighted in sputum, which is a certain indication of bacillary infection.

The diagnostic is also based on: a) assessing the sedimentation speed of erythrocytes, which is increased above 10/20 mm in nodular silicosis and above20/40 mm in pseudotumoral silicosis; b) intradermoreaction to tuberculin with 1 unit P.P.D. positive at a papule diagnostic above 10% (Manu, 1983).

The *Mycobacterium* genus includes a great number of bacilli with Gram positive wall structure, acid-alcohol resistant, immobile, unsporulating, which grow in aerobiosis without producing a pigment. The *Koch bacillus*, the causal agent of tuberculosis, is an obligated parasite, does not live free. One of the main infection sources is represented by the sick man, which eliminates bacilli through sputum while speaking, cough and seldom also through urine and feces.

In most of cases the entrance room is upper respiratory. Once arrived in the alveoli normally the bacilli replicate slowly, with a generation period of 15-20 hours. After about 3 weeks there will be 106 bacilli in the alveolar macrophages and the monocytes recruited from blood, from where, by lymphocytes way they arrive in the lymphatic ganglions. Except the situation when the Koch bacillus settles on the alveolar level, there is the eventuality that a small bacilli quantity, arrived in the regional lymphatic vessels, passes in the blood stream from where they will populate especially some organs: kidneys, brain, bone tissue etc. (White *et al.*, 2001).

The *Koch bacillus* manifests an accentuated parasitism. Due to this fact it grows slow and hard on artificial culture media. The growing media must contain a carbon source, a source of nitrogen and essential ions (Fe and Mg). The nitrogen source is represented by simple ammonium salts or amino acids (aspargine, glutamate). The carbon source is represented, in general, by carbohydrates: glucose, glycerol or pyruvic acid (Homorodeanu, 2002).

This paper aims to highlight the mycobacteria present in the pathologic offspring from silicosis patients from some mines in the Jiu Valley. The study is

based on using some bacteriologic diagnosis methods, namely microscopic examination, culture, identification and antibiogram.

Materials and Methods

The bacteriologic diagnosis was based on microscopic examination and culturing of mycobacteria from two or three sputum samples, gathered in successive days before beginning the antisilicosis treatment.

In order to achieve the bacteriologic examination of sputum in the lab the next activities were performed:

- microscopic examination of biologicals by Ziehl-Neelsen coloration (Drăgan-Bularda, 2000).
- culture on Lőwenstein Jensen solid medium, recommended through the National Program for Struggle against Tuberculosis for growing mycobacteria (Pneumology Specialty Commission, National Program for Struggle against Tuberculosis 2001-2005). The Lőwestein – Jensen medium is a solid medium, glycerinated with egg and potato starch. The glycerol ant the egg supplies the fat acids and the proteins needed for the metabolism of mycobacteria. The malachite green is added as partial inhibitor of other microorganisms (Drăgan-Bularda, 2000).
- identification of isolated bacteria.
- antibiogram.

Results and Discussions

Microscopic examination. *Smear achieving* – The smears wear made in special chambers in order to prevent contamination. *The colouring of smears* – was done by classic Ziehl-Neelsen coloration (Drăgan-Bularda, 2000). The smears prepared this way were than examined by optical microscope in order to highlight the tuberculosis bacilli (fig. 1).

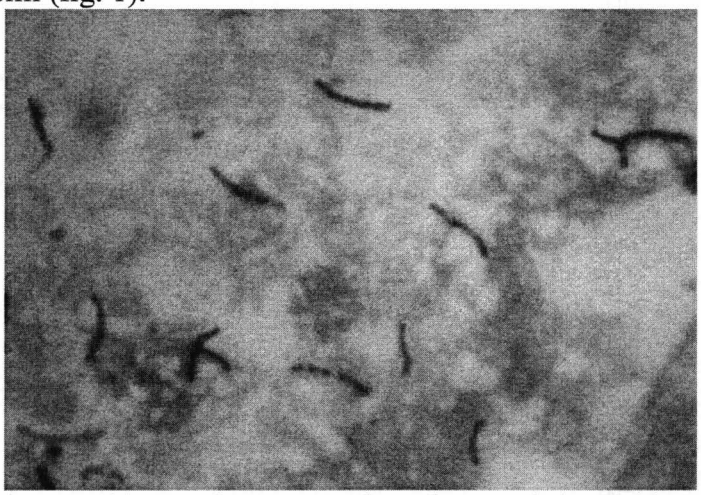

Fig. 1. Smear with *Mycobacterium tuberculosis* made by Ziehl-Neelsen coloration

A minimal set for identification of *M. tuberculosis* in the pathologic offspring from silicotuberculosis patients, which includes:
- macroscopic suggestive morphologic characters for *Mycobacterium* genus.
- proving the acid-alcohol resistance (by Ziehl – Neelsen stain).
- disposing of bacilli in "strings", in the smears (fig. 2).
- slow growing in subculture (over 9-14 days), at a temperature between 33 - 37 °C.
- positive test for nitrat-reductase and positive test for niacin.
- the catalase test positive at 20°C, negative after incubation for 20 minutes at 68 °C.
- the resistance of the strain at 5μg/ml tiophen-2-carboxylic acid hydrazide (TCH).

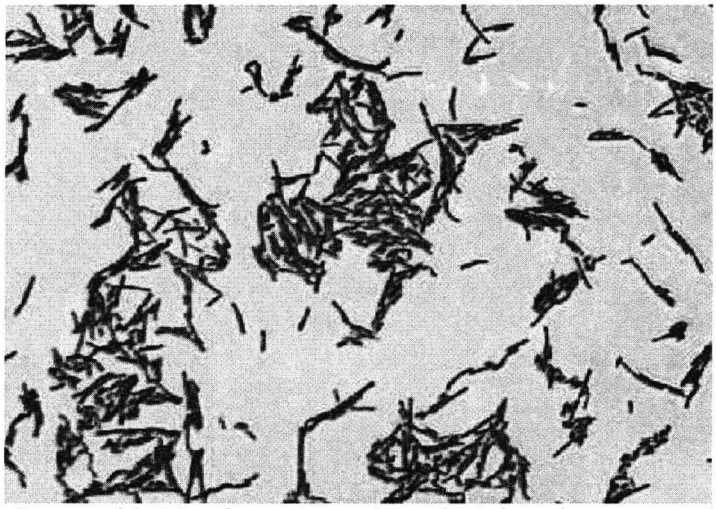

Fig. 2. Smear with *Mycobacterium tuberculosis* in strings accomplished by Ziehl-Neelsen classic coloration

After obtaining the primary culture the time needed for highlighting each characteristic vas different and it varied from a few minutes to 3-4 weeks.

The means of control of microscopic examination by rereading the smears is considered useful because it helps us getting a general impression on the routine activity from the lab and after analysing the results we can identify the deficiencies in order to correct them.

In order to be cautious regarding the possible errors the work technique was revised for the microscopic examination and the culture and the plausible cause of errors was the smear making method: direct smears were made out of sputum without insisting on choosing the purulent parts, the quantity exposed on the slide being around 0.02 ml, while for the culture a 1 – 3 ml quantity was processed.

The incidence of silicosis cases in the period 1999-2007 in the Jiu Valley. Exposure to silicogen dust for a long period produces the most frequent professional affections in the Jiu Valley, namely the pneumoconioses. Renouncing the method of exploiting by coal faces, in which the highest dust level is 22.8 mg/m^3 and laying stress on longwalls the dust level decreases to 13.6 mg/m^3. At working in coal and

sterile the dust levels change from one mine to another, smaller medium values being registered for coal than for sterile. The mine dust contains 81 – 83 % particles in the breathable fraction, presenting higher percentages at works in sterile (85- 86 %) compared to mix coal (75 – 80 %) (Todea and Ferencz, 2000).

A general situation of silicosis occurrence in the whole mine complex of Jiu Valley is presented in the tables 1 and 2 and also in the graphics 3 and 4 for an easier interpretation; data taken from the same lab of Petroşani SCSM.

Table 1. Silicosis situation – existing cases at the mining units from National Bituminous Coal Company in the last 9 years

Unit	1999	2000	2001	2002	2003	2004	2005	2006	2007	Total
Lonea	6	3	3	3	5	4	2	2	2	30
Petrila	14	24	17	19	30	20	14	13	12	163
Livezeni	3	8	11	11	10	6	4	4	4	61
Aninoasa	2	1	1	1						5
Vulcan	5	3	7	6	5	1	1			28
Paroşeni	1	2	3	4	5	5	3	2	2	27
Lupeni	2	2	2	3	3	2	2	2	2	20
Bărbăteni		2	2	1	1					6
Uricani	1			1						2
V. Brazi	1		1							2
Total	35	45	47	49	59	38	26	23	22	344

During the years taken in consideration it can be observed a relative decrease of silicosis sickness cases, especially in the period 2003-2007. A high incidence of the disease is recorded at Petrila Mining Exploitation (163 cases). A high occurrence of the disease is also present at Livezeni M. E., with 61 cases, and also at Lonea M. E., with 30 cases (table 1).

In fig. 3 it can be observed that in the studied period the most cases of silicosis sickness were recorded at Petrila M.E., 163 cases, and the least silicosis cases were recorded at Uricani M.E. (2 cases) and Valea de Brazi M.E. (2 cases).

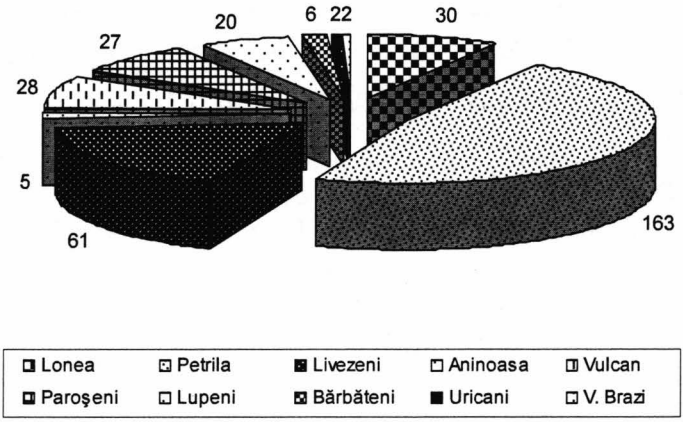

Fig. 3. Graphic representation of silicosis, existing cases at NBCC in the last 9 years

In the last 9 years 80 new cases were recorded, 9cases/year on average (table 2).

Table 2. Silicosis situation- new cases found in the last 9 years at NBCC

Unit	1999	2000	2001	2002	2003	2004	2005	2006	2007	Total
Lonea			3		4	3	-		-	10
Petrila	1	5	9	12	1	5	-	1	-	34
Livezeni	1	6	3		3	1	-	1	-	15
Aninoasa	1						-		-	1
Vulcan	1		4			1	-		-	6
Paroşeni	2		2		1		-		-	5
Lupeni		1	2				-		-	3
Bărbăteni	3						-		-	3
Uricani		2		1			-		-	3
V. Brazi							-		-	
Total	9	14	23	13	9	10	0	2	0	**80**

Out of these more new silicosis sickness cases were also found at Petrila M.E., counting 34 cases, followed by Livezeni M.E. (15 cases) and Lonea M. E. (10 cases). The medium sickness manifestation age for silicosis is 44.8 years, after an 17.9 years exposure.

Out of the whole declarated cases 24.9 % include people which have worked mostly in coal, 22.7 % in mix and 38.9 % in sterile. The prevalence is 1.8 % for those working in sterile and 0.6 % for those working in coal.

The highest percentage of new silicosis sickness cases was recorded at Petrila Mining Exploitation (fig. 4).

Out of all these cases the ones which progressed from a silicosis degree to another represent in average 17 cases, forming about 21.25% of the total. The feared complication of silicosis is pulmonary tuberculosis, out of 8 cases with old bacillar lesions, 2 cases have revived. At 2 cases of this lot the bronchial asthma was found as associated disease.

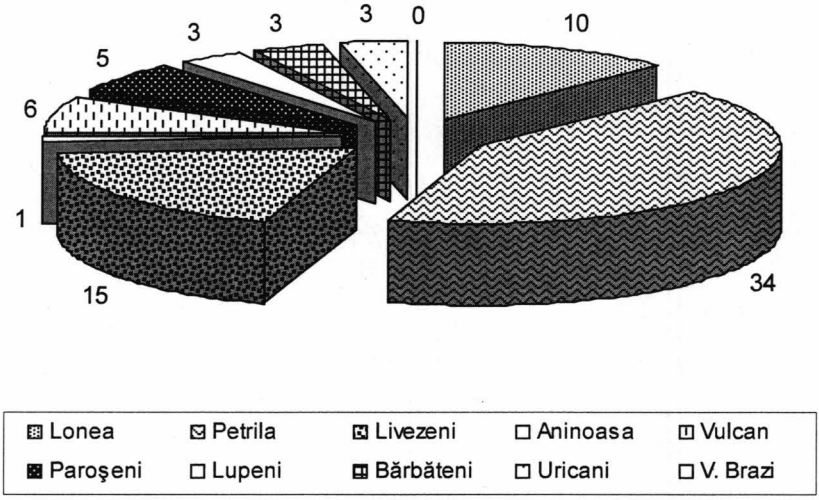

Fig. 4. Graphic representation of silicosis frequency, new cases found at NBCC in the last 9 years

Silicosis is a pure professional disease, her severeness consists in: exposure to sickness of a very large number of workers; it inflicts an important decrease of work capacity; diagnosis without a well set periodic control causes diagnostic only in a late phase, when the evolution cannot be stopped anymore; there is no satisfying specific treatment for silicosis so all the means needed to prevent dust formation must be taken, as close as possible to the forming source because once it gets in suspension it is hard to remove.

The advanced silicosis forms are eventuating in silicotuberculosis, respiratory insufficiency or cor pulmonale (Hope, 1995). Respiratory insufficiency appears as a combination of two or more respiratory syndromes, with hypoxemia, hypercapnia and right cardiac insufficiency (Marttila *et al.,* 1998).

The pulmonary hypertension is yet present at silicosis in a less advanced phase, but the diagnostic is hard to determine due to the specific symptoms and the insufficient methods in the current investigation.

Observing the diagnostic at the silicosis sick persons from The Jiu Valley Coalfield the situation is as follows (fig. 5): pure silicosis at whose working in sterile: 94%; anthracosilicosis: 4%; anthracosis: 2%.

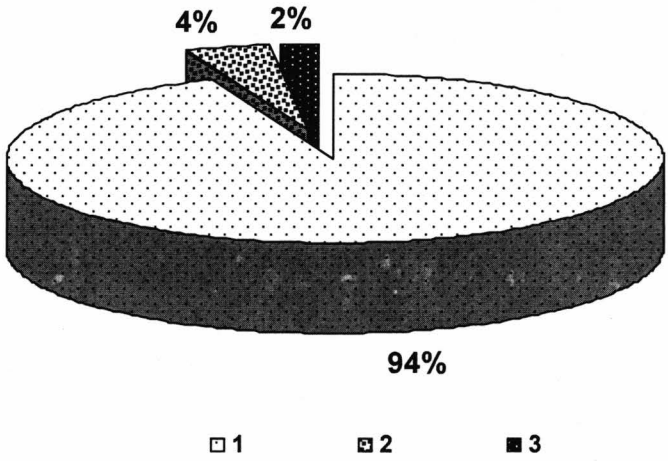

Fig. 5. Situation on diagnostic (1=pure silicosis 94%; 2=anthracosilicsis 4%; 3=anthracosis 2%).

The advanced form of cor pulmonale presents major symptoms of right cardiac insufficiency, with edema of legs, big liver, cyanosis, turgescence of jugular veins and increasing ***right cardiac shadow at radiologic examination*** (Pănuc, 2004).

Silicosis and cancer risk is also found at workers from foundings where more carcinogen factors act.

Calvert *et al.,* (2003) evaluated the health risk at those professionally exposed to silica by studying the death certificates, on the first position situating silicosis, followed by pulmonary cancer, COPD and pulmonary TB.

The comparative analysis of data from the east and the west of Jiu Valley Coalfield. Silicosis is a chronic pulmonary professional disease, incurable, produced by accumulation of particles of free crystalline silicon dioxide in the lungs and consecutive tissular reactions, with progressive evolution after cease of exposure.

By comparing the number of silicosis cases in the two subdivisions of Jiu Valley Coalfield it was noticed that in the eastern part there is a larger extent of silicosis cases (fig. 6). The highest number of silicosis sickness cases was recorded at Petrila M.E., especially in the year 2002, when 12 silicosis cases were recorded.

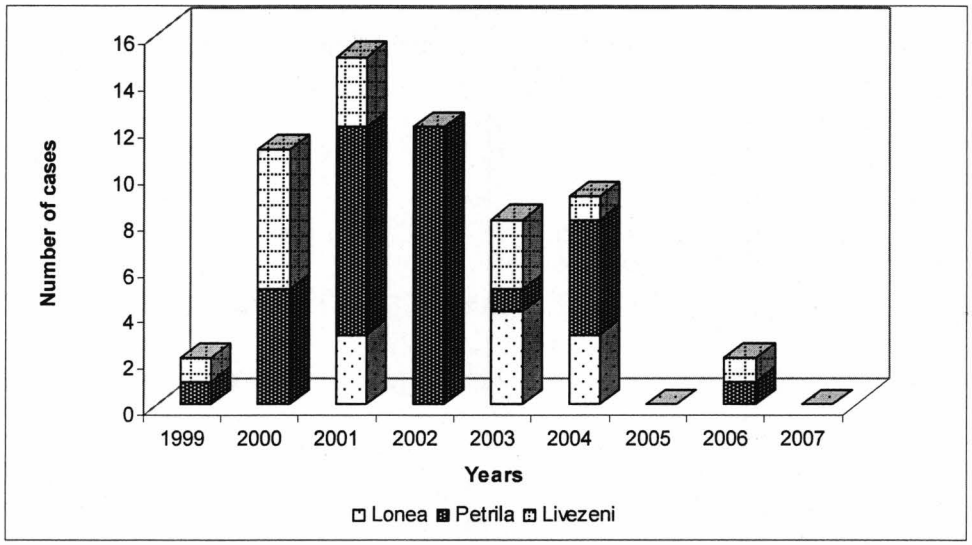

Fig. 6. The number of cases at the mines from the east of Jiu Valley

Although in the western part of the coalfield there are more mining exploitations the number of sickness cases is much lower (fig. 7). Due to cumulating of subterranean conditions with surface pollution, more pronounced in this western part (the presence of Paroşeni thermal power station) it was expected to be more cases of silicosis illness then in the eastern part. The high number of silicosis cases in the eastern part is also explained by the fact that Petrila Mining Exploitation is the biggest one in all the coalfield therefore the number of employee is the greatest.

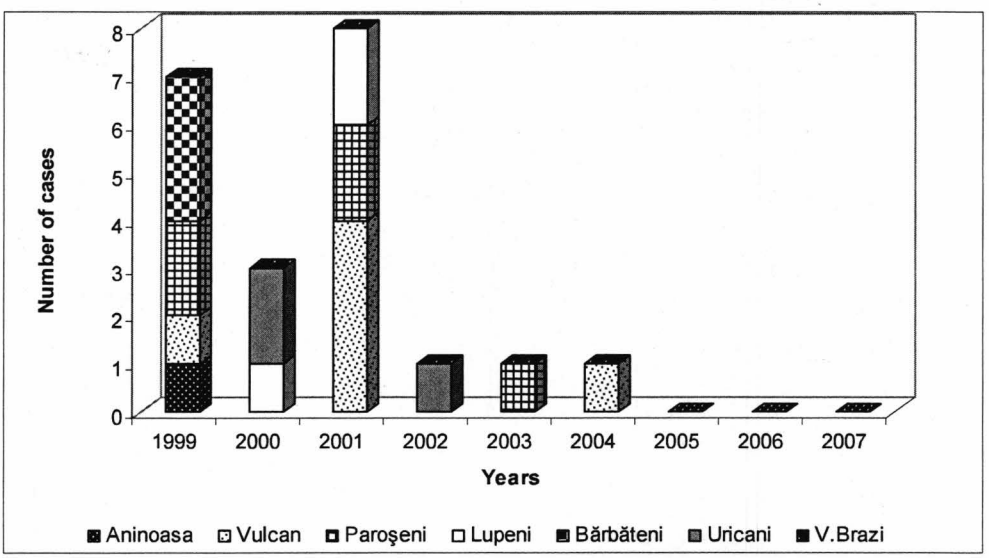

Fig. 7. The number of cases at the mines from the west of Jiu Valley

From the graphics it can be observed a diminishing of the silicosis cases during the years taken in consideration. The highest number of sicknesses was recorded in the years 2001 and 2002, and then a decreasing of the new sickness cases is noticeable, lowering to only 2 cases in the last 3 years (2005-2007).

Statistics regarding silicosis in the Jiu Valley and in the country. The new cases of silicosis with tuberculosis complications inclusively reported by Pâslaru in the span 1952-1982 in Romania represented 11.5% while the uncomplicated ones totalled 88.5%. The silicotuberculosis cases have diminished continuously from one year to another and they significantly dropped from 25% in the '50 to 4-5% of the total after 1980.

In the fig. 15 it can be observed that in Romania in the period 1996-2006, the frequency of the simple silicosis cases is higher then the one of silicosis associated with tuberculosis.

Silicosis is on the first place in the hierarchy of occupational diseases at national level, representing 29.34% (267 cases out of 910), a high level considering the severity of this affection.

The repartition on production branches was established hereby: extraction and preparation of metalliferous ore – 125 cases, machines and equipments industry – 52 cases, metallurgic industry – 22 cases.

According to profession the repartition is as follows: miner: 69 cases, founder: 38 cases, putter: 18 cases, sander: 11 cases, underground locksmith: 8 cases, crane operator: 8 cases, underground mechanic: 8 cases, locksmith: 7cases. There were found 8 cases of "acute silicosis", with a professional seniority below 4 years.

In the year 2006 in the Jiu Valley, regarding the stage of the disease, the next cases prevailed (fig.8): silicosis stage I - 50 cases, silicosis stage I/II -27 cases; silicosis stage II – 14 cases; silicosis stage II/III – 6 cases; silicosis stage III – 3 cases (retired before 1990 and declared subsequently).

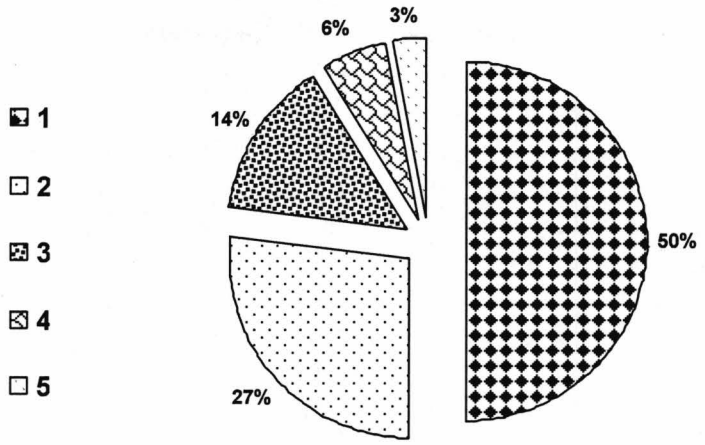

Fig. 8. Distribution of the disease on stages in the Jiu Valley (year 2006) (1. stage I=50 cases; 2. stage I/II=27 cases; 3. stage II=14 cases; 4. stage II/III=6 cases; 5. stage III=3 cases).

In the year 2006 (fig.9) in all the mining regions of Romania where silicosis sickness occurs regarding the stage of the disease the next cases prevailed in declaration:

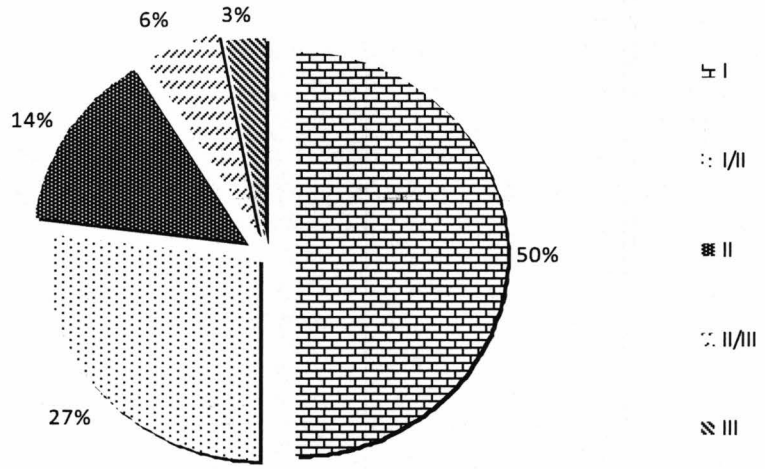

Fig. 9. Distribution of the disease by stages in Romania (year 2006).

It can be noticed that in the Jiu Valley (50%) but also in the other regions of the country (65%) the stage I of silicosis prevails.

Conclusions

From the study regarding the incidence of silicosis and silicotuberculosis in the Jiu valley Coalfield the next ideas emerged:

1. Koch bacilli responsible for tuberculosis burst were isolated out of the sputum samples from tuberculosis patients and microscopically examined. The results from the microscopic examination were related to the result of the culture which is considered standard for the bacteriological examination of tuberculosis.

2. The stages of silicosis were established by differential diagnostic at investigated sick persons. The actual radiologic pulmonary semblance was compared with previous radiographs to appreciate the progression of disease in time. The silicosis diagnostic was established at the average age of 44.8 years. The medium exposure duration before the silicosis diagnostic was 17.9 years.

3. It was noticed that in the eastern part of the coalfield the number of silicosis cases is higher than in the western part. One explanation could be the fact that Petrila M.E. is the biggest mine so it has the greatest number of employees, plus it is the deepest mine so the ventilation of the galleries is harder to accomplish.

4. The proportion of stage I silicosis patients is lower in the Jiu Valley than on the whole country (50% versus 65%). An explanation could be given by lack of silicon in the structure of pure coal, so in the Jiu Valley the sickness is due only to sterile.

5. Overall it is noticed a diminishing of silicosis cases in the Jiu Valley, during the studied years, lowering in the last 3 years (2005-2007) to only two new cases.

Rezumat. Acest studiu abordează pe o perioadă de 9 ani diagnosticarea silicozei prin radiografie pulmonară şi a silicotuberculozei prin examenul bacteriologic, care pune în evidenţă prezenţa bacilului Koch în spută, fiind un indiciu sigur al infecţiei bacilare. Odată depistată *Mycobacterium* sp. s-au efectuat antibiograme în care au fost testate cel puţin 3 tuberculostatice, care apoi au fost indicate în tratament. În perioada studiată au fost luate în evidenţă atât cazurile existente, cât şi cazurile noi de silicoză apărute în Valea Jiului. Cea mai mare frecvenţă a cazurilor se întâlneşte la E.M.Petrila-163 de cazuri, urmată de E.M.Livezeni (61 cazuri), E.M.Lonea(30 cazuri). Cea mai scăzută frecvenţă a cazurilor se întâlneşte la E.M. Aninoasa (5 cazuri), E.M.Uricani (2 cazuri), E.M.Valea de Brazi (2 cazuri). Situaţia cazurilor noi de silicoză apărute în bazinul carbonifer al Văii Jiului se prezintă astfel: E.M.Petrila: 34 cazuri; E.M.Livezeni: 15 cazuri; E.M.Lonea: 10 cazuri. S-a realizat o comparaţie între incidenţele bolilor profesionale studiate din Estul şi Vestul Bazinului carbonifer al Văii Jiului şi s-a constatat că în partea de Est frecvenţa apariţiei silicozei este mai mare, cele mai multe cazuri înregistrându-se la E.M.Petrila, Livezeni şi Lonea, iar în partea de Vest, deşi există un număr mai mare de mine, frecvenţa apariţiei silicozei este mai scăzută. Acest lucru poate fi explicat şi prin faptul că E.M.Petrila, situată în partea Estică este cea mai mare mină a bazinului carbonifer al Văii Jiului atât ca şi suprafaţă de exploatare, cât şi ca număr de angajaţi, justificându-se astfel incidenţa ridicată a îmbolnăvirilor.

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USE OF MICROORGANISMS IN SOIL QUALITY ASSESSMENT

Codruţa Violeta SIMULE*, Mihail DRĂGAN-BULARDA**

Abstract: Integrated Monitoring System for the Environment in Romania includes only physical and chemical indicators of air, soil and water quality. The soil monitoring system does not include biodiversity measurements, such as microbial biomass, enzymatic activity or the diversity of other soil fauna. The necessity of using microorganisms in ecological soil classification and assessment concepts follows from many obvious reasons. Soil bacteria and fungi are considered to be largely, but not totally, responsible for the cycle and transformation within the soil of carbon, nitrogen, phosphorus, sulphur and other plant nutrient. For this reason, microbial and biochemical properties are thought to be more sensitive than chemical and physical soil properties and more predictive of short-term effects. The following 7 ecophysiological groups were investigated in unpolluted (Cheile Turzii protected area) and polluted (Turda hexachlorocyclohexane deposit) soils: aerobic heterotrophic bacteria, ammonifying bacteria, nitrifying bacteria (ammonium and nitrite oxidizing bacteria), denitrifying bacteria, iron-reducing bacteria and sulphate reducing bacteria. The phosphatase, catalase, urease, actual and potential dehydrogenases activities have also been studied. Our measurements showed that high concentrations of pollutants have negative impact on soil microorganisms. The toxicity exerted by heavy metals and hexachlorocyclohexane pesticide decreased microbial biomass and enzyme activities of soil, perhaps as a result of the suppression of sensitive parts of the microbial community.
Key words: *polluted soil, soil monitoring system, bacterial communities, enzymatic activities.*

Introduction

Soil quality can be defined as the capacity of a specific soil to function as a vital living system, within natural or managed ecosystem boundaries, to sustain plant and animal health and productivity, maintain or enhance quality of air and water environments, and support human health and habitation (Winding et al., 2004). This article presents the importance of using microorganisms in soil quality assessment and monitoring programs.

Microorganisms manifest very different life forms from autotrophic, lithotrophic to heterotrophic life forms, with the heterotrophic life form turning over the largest amount of carbon in soil. Microorganisms serve as food for many other soil organisms and they make up the largest part of the total biomass in the soil (Brookes et al., 1982). Furthermore, they perform many steps in the degradation and

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mineralization of complex organic materials, thereby releasing minerals to the benefit of other organisms.

Microorganisms can also degrade a wide range of anthropogenic, including problematic organic compounds. Consequently, the heterotrophic action of microorganisms is a crucial basis for the soil ecosystem and can possibly be used to determine soil quality.

In addition to their effect on nutrient cycling, microorganisms also affect the physical properties of soil. Microorganisms affect soil structure, water-holding capacity, infiltration rate, crusting, erodibility and susceptibility to compaction (Elliot et al., 1996).

Microorganisms and microbial communities can provide an integrated measure of soil quality, an aspect that cannot always be obtained with physical and chemical measures and/or analyses of higher organisms. Microbial communities adapt sensitively to changing environmental conditions by varying individual activity, by increasing reproduction of species with favorable abilities, and by spreading new capabilities via horizontal gene transfer. Due to their high surface-to-volume ratio microorganisms respond sensitively to changes and environmental stress because they have intimate relations with their surroundings. In some instances, changes in microbial communities can precede detectable changes in soil properties or in plant and animal communities, thereby providing an early sign of soil improvement or an early warning of soil deterioration (Pankhurst et al., 1995).

The objectives of this article are to assess soil quality using microbial indicators and to support their inclusion in monitoring programs.

Materials and methods

Soil samples. Two composite soil samples were collected from the top layer (0-20 cm) of an polluted and unpolluted area in Turda. One sample was collected from industrial waste disposal (hexachlorocyclohexane waste deposit from Turda) and the other from the Cheile Turzii Nature Reserve, which is one of the most important protected wildlife sites in Cluj County. Individual soil cores were taken with a PVC core sampler (at a depth of 0-20 cm) from three different places and mixed together to prepare a composite sample for each site. The composite samples were used for all subsequent analyses. All operations connected to the bacteriological analyses were carried out under sterile conditions. The content of the soil in dry substance was established by drying parts of samples at 105°C for three days.

Physical and chemical analyses. The pH values were determined with ProfiLine 197 handheld pH meter; soil organic matter by sulfochromic oxidation method (ISO 14235:2000 Soil quality – Determination of organic carbon by sulfochromic oxidation) and organochlorine pesticides concentrations by gas chromatography-mass spectrometry. Gas chromatography was performed using a Hewlett Packard HP6800 series equipped with a Micromass AutoSpec Ultima mass spectrometer (Micromass Ltd., Manchester UK). Heavy metals concentrations were analyzed with atomic absorption spectrophotometer type Varian SpectrAA 880.

Microbiological analyses. The following 7 ecophysiological groups of bacteria have been analyzed: aerobic heterotrophic bacteria (bullion agarized medium) (Atlas, 2004), ammonifiers (peptone medium), nitrifiers (nitrite- and nitrate bacteria) (Drăgan-Bularda medium, 2000), denitrifiers (De Barjac culture medium) (Pochon, 1954), iron-reducing Bacteria (Ottow medium, 1968), and sulphate reducing bacteria (Van Delden medium) (Allen, 1957). Except for the aerobic heterotrophic bacteria (where the method of successive dilutions was used), the most probable number of bacteria was calculated according to the statistical table of Alexander (1965).

Enzymological analyses. The following five enzymes were studied: phosphatase – activity expressed in mg phenol/g dry matter soil (Drăgan-Bularda, 2000); catalase – activity expressed in mg splitted H_2O_2/g dry matter soil (Drăgan-Bularda, 2000); urease – activity expressed in mg NH_4/g dry matter soil (Drăgan-Bularda, 2000); actual and potential dehydrogenase – activities expressed in mg formazan/g dry matter soil (Drăgan-Bularda, 2000). The analytical data serves as the base for calculating the enzymatic indicator of the soil quality (EISQ) (Muntean et al., 1996; 2006).

Results and discussion

Physical and chemical analyses were carried out in order to establish the effects of the pollutants on the soil microorganisms. The physico-chemical characteristics of the analysed samples are presented in Table 1.

Table 1.

Physico-chemical characteristics of the soil samples

Sample location	pH	OM %	HCH ppm	Heavy metal (ppm)						
				Zn	Cr	Cu	Pb	Cd	Ni	Co
HCH deposit	7.77	5.71	473	94.0	10.4	59.1	64.6	0.4	13.8	46.8
Cheile Turzii	7.82	11.38	-	16.3	5.3	5.2	8.7	0.3	9.9	0.2
Normal limit	-	-	0.005	100	30	20	20	1	20	15

OM – organic matter, HCH - hexachlorocyclohexane, Normal limit – according to the Order number 756/1997 of the Ministry of Waters, Forests and Environmental Protection *Reglementation regarding evaluation of environment contamination*

The normal limits for Zn, Cr, Cu, Pb, Cd, Ni and Co are: 100 ppm (Zn), 30 ppm (Cr), 20 ppm (Cu, Pb and Ni), 15 ppm (Co) and 1 ppm (Cd), according to the Order number 756/1997 of the Ministry of Waters, Forests and Environmental Protection Reglementation regarding evaluation of environment contamination.

The concentrations of Cu, Pb and Co were higher than the normal limits in sample collected from Turda hexachlorocyclohexane deposit. Also, we have detected a high concentration of organochloride pesticide (hexachlorocyclohexane) in Turda hexachlorocyclohexane deposit, much more over the normal limit which represents an

alarm signal for human and environmental health. The sample collected from Cheile Turzii has presented the heavy metal concentrations below normal limits.

Table 2.

The bacterial density in the analyzed soils from Cluj County

Sample location	AHB	AM	AOB	NOB	DB	SRB	IRB	BISQ
HCH deposit	60,000	7,074	799	72	80	0	559	2.561
Cheile Turzii	9,600,000	82,620	50,450	1,836	4,200	0	420	3.708

AHB - Aerobic heterotrophic bacteria; AM - Ammonifying bacteria; AOB - Ammonium oxidizing bacteria; NOB - Nitrite oxidizing bacteria; DB - Denitrifying bacteria; SRB - Sulphate reducing bacteria; IRB – Iron reducing bacteria

Six ecophysiological groups were present in both samples analyzed: aerobic heterotrophic bacteria, ammonifying bacteria, ammonium oxidizing bacteria, nitrite oxidizing bacteria, denitrifying bacteria and iron-reducing bacteria. In the soil from Cheile Turzii was a large number of aerobic heterotrophic bacteria of order 10^6 colony-forming unit (CFU)/g dry matter soil. The number of bacteria belonging to the other groups was much smaller.

In the order of their abundance, the aerobic heterotrophic bacteria (10^4 -10^6 CFU/g dry matter soil) were followed by the ammonifying bacteria (10^3 - 10^4 cells/g dry matter soil), ammonium oxidizing bacteria (10^2 - 10^4 cells/g dry matter soil), denitrifying bacteria (10^1 - 10^3 cells/g dry matter soil) and nitrite oxidizing bacteria (10^1 - 10^3 cells/g dry matter soil). The smallest numbers were observed for the iron-reducing bacteria (10^2 cells/g dry matter soil).

The microbiological analyses showed lack of the sulphate reducing bacteria in the soils from hexachlorocyclohexane deposit and Cheile Turzii protected area.

The general bacterial potential of the soils was appreciated on the base of the bacterial indicators of soil quality (BISQ) values, taking into account the number of bacteria belonging to each ecophysiological group (Muntean, 1995-1996).

An obvious observation was the low level of the bacterial potential of the soil from the Turda hexachlorocyclohexane deposit area (2.561) compared to the soil samples from Cheile Turzii (3.708). In Turda area was also recorded the highest concentration of heavy metals and pesticides. BISQ had the maximum value in the control sample Cheile Turzii, area unaffected by pollution, where the content of heavy metals was the lowest, which reveals an active and balanced microbial community.

Enzymological analyses were carried out on soil samples taken from the same points that have been analyzed from physical and chemical point of view. The samples were taken from a depth of 20 cm.

Table 3.

Enzymatic activity of investigated soils from Cluj County

Sample location	Phosphatase activity (mg phenol/g dry matter soil)	Catalase activity (mg H_2O_2/g dry matter soil)	Urease activity (mg NH_4/g dry matter soil)	Actual dehydrogenase activity (mg formazan/g dry matter soil)	Potential dehydrogenase activity (mg formazan/g dry matter soil)	EISQ
HCH deposit	0.38	6.47	2.25	0.17	0.63	0.047
Cheile Turzii	7.82	37.82	25.56	1.77	3.45	0,336

In both samples analyzed were observed the presence of each of the five studied enzymes and the intensity of these activities varied within larger limits. Soil enzymatic activities were lowest in hexachlorocyclohexane deposit area as a result of high heavy metals and hexachlorocyclohexane concentrations.

As compared with the data in the literature (Drăgan-Bularda et al., 1995; Lee et al., 2002), we may consider that the phosphatase, urease and especially catalase activities are intense, while the dehydrogenase activities have lower values.

The enzymatic indicator of the soil quality (EISQ) was calculated based on the absolute values of each enzymatic studied activity (Muntean et al., 1996). The enzymatic indicator of the analyzed habitats quality offers an overall image on the intensity of the enzymatic activity and, implicitly, of the general biological activity in the analyzed soils. The enzymatic indicator may have values ranging between 0 (when no real activity of any of the studied enzymes is detected) and 1 (when all the activities have real individual values equal to the maximum theoretic values).

Based on the results and in comparison with the data in the speciality literature (Pasca et al., 1993; Drăgan-Bularda et al., 1995), can be considered that the analyzed soil from Cheile Turzii has an appreciable biological potential when compared to the polluted area.

Conclusions

Microbial communities are integral parts of soil and their activity is very important to the functioning of soil. Therefore, microorganisms should be included in soil quality classification and assessment concepts. The challenges of using microbial indicators are to identify the best choice among the many techniques available to

assess soil quality and to convert the information obtained from the microbial indicator into a form relevant for policy makers.

In this article, two possible microbial indicators were presented; each provides slightly different information on soil quality: measures of microbial biomass and enzyme activities.

The harmful effect of the pollutants was obvious, in all the eco-physiological groups the recorded values in polluted area being lower than in the control sample area, which is unpolluted. The presence of pollutants, even at low concentrations, had inhibitory effect on soil microorganisms. For this reason, the soil pollution will lead most probably to a reduction in the decomposition and nutrient cycling rates.

Soil enzyme activities are considered to be sensitive to pollution and have been proposed as indicators for measuring the degree of soil degradation. In this work concludes that high concentrations of pollutants have negative impact on soil enzyme activities. The most sensitive enzyme activity was the dehydrogenase. The toxicity exerted by heavy metals and hexachlorocyclohexane pesticide decreased enzyme activities of soil, perhaps as a result of the suppression of sensitive parts of the microbial community.

Rezumat. În România, Sistemul de monitorizare a stării mediului vizează numai caracteristicile fizice şi chimice ale factorilor de mediu, neluând în calcul modificările apărute în structura şi activităţile comunităţilor microbiene. Utilizarea parametrilor microbiologici şi biochimici în evaluarea riscurilor poluanţilor asupra solului este necesară din următoarele motive: bacteriile şi fungii din sol, datorită abundenţei lor, sunt responsabili în mare parte de circuitul în sol a carbonului, azotului, fosforului, sulfului şi a altor nutrienţi ai plantelor; analizele microbiologice şi biochimice sunt mai sensibile decât analizele chimice şi fizice ale solului şi mai adecvate pentru prognozarea efectelor poluanţilor pe termen scurt; metodele utilizate curent pentru determinarea proprietăţilor chimice şi fizice nu sunt întotdeauna îndeajuns de sensibile pentru detectarea variaţiilor mici ale acestora apărute ca răspuns la schimbările în managementul agricol sau ca urmare a prezenţei agenţilor poluanţi. Următoarele şase grupe ecofiziologice de bacterii au fost determinate într-un sol poluat (depozitul de dexaclorciclohexan Turda) şi nepoluat (aria protejată Cheile Turzii) din judeţul Cluj: heterotrofe aerobe, amonificatoare, nitrificatoare (nitritbacterii şi nitratbacterii), denitrificatoare, fier-reducătoare şi desulfoficatoare. De asemenea, au fost măsurate activităţile fosfatazică, catalazică, ureazică, dehidrogenazică actuală şi potenţială. Valorile înregistrate în zona poluată au fost mai mici decât cele înregistrate în zona nepoluată, ceea ce demonstrează impactul negativ al poluanţilor asupra microorganismelor din sol. Efectele toxice ale metalelor grele şi a pesticidului organoclorurat (hexaclorciclohexan) au condus la descreşterea biomasei microbiene şi la reducerea intensităţii activităţilor enzimatice din sol, consecinţă a menţinerii în ecosistemul modificat doar a speciilor rezistente la poluare. Factorii cheie care au controlat densitatea şi activitatea bacteriilor au fost: conţinutul de substanţe organice, pH-ul, concentraţia poluanţilor şi temperatura.

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POSITIVE ASPECTS OF URBANIZATION ON BIRD BEHAVIOR

László – Erno BERKESY*, Corina-Michaela BERKESY**

Summary: The evolution of modern world led to the increase of urban areas and, together with it, to the modification of many habitats which represented the natural environment for numerous species of birds. The modification of surfaces and of the structure of vegetation areas from the urban zones had negative effects on many species of birds which had a reduced adaptation capacity, while for birds with a better adaptation capacity, the effects were less relevant. Some bird species benefited from the conditions offered by the new habitat, the great majority of these bird populations living in urban areas. In this work we show some positive effects on birds' behavior caused by urbanization.
Key words: *urbanization, bird adaptation, natural habitat.*

Introduction

The evolution of human civilization determined profound changes in every day's life, influencing the life of numerous species which had their habitat in that area and because of the profound changes it suffered. Under these circumstances, those species who had the capacity of adapting to the newly created conditions survived, while those that were strictly adapted to their natural environment were in danger.

Although the urban environment is in a continuous expansion, here we find a big number of species which adapted to this new environment and with growing populations.

The changes in the structure and dimension of bird populations is also determined by the degree of urbanization. Therefore, these changes are less important in small rural towns, where the habitat's structure hasn't been essentially modified and they are more pronounced in cities (big cities), where there is a great number of buildings and a small quantity of green places. In these cases, many species of birds can be found in the peripheral areas of cities, where vegetation is more present or in parks and gardens, in these cases the habitat is fragmented. Under these circumstances of high urbanization, we notice a decrease of the number of species, the existing ones possessing a degree of adaptation to the new and high lifestyle.

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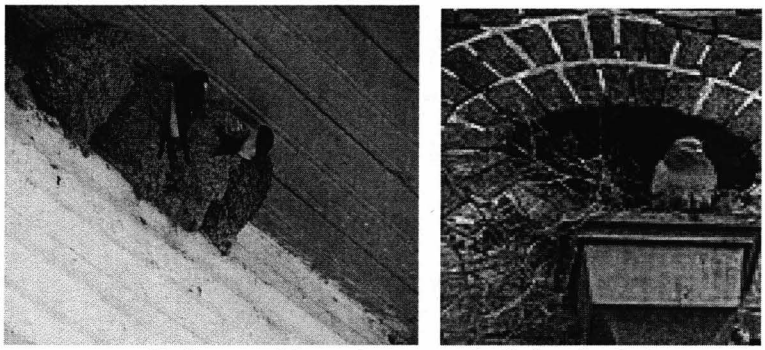
Habitat and nesting

Besides the negative effects of urbanization on birds, there are many positive effects too. Therefore, many bird species choose their biotope in the vicinity of humans in cities, villages, etc. (Fig.1, 2,). The adaptation to the habitat's changes, determined by urbanization, depends also on the possibilities of adaptation of the different species.

In urban areas, the surfaces with green places are more reduced, being divided into larger or smaller areas, depending on urban plans. The green areas are usually represented by clusters of trees, extended surfaces with vegetation; in the urban areas they are rare and are represented by extended parks or gardens.

Green areas are most of the times represented by native species, but there are cases when they are replaced with plant species which are not specific to that area. As a consequence, there are situations when bird species cannot find their nesting places.



a.*Delicon urbica* b.*Columba livia*

Fig. 1. Birds nest in town.

The urbanized species are adapted to very different forms of nesting places. Most of the times they are in the vicinity of intensely circulated areas (Fig.1). In these cases, baby birds which fall out of their nests or those which leave their nest before being able to fly, have minimum surviving changes. If they survive from being hit by a car, they are the victims of animals – dogs, cats, because they don't have a place to hide. Without the help of people, they are condemned to death.

a. *Columba livia*

b.*Passer domesticus*

c. *Parus major*
Parus caeruleus

Fig. 2. Birds live in town.

If this phenomenon takes place in the forest, the baby birds are able to find vegetation areas, bushes where they can hide and where they can be fed by their parents.

Many bird species nest in non-specific places, such as balconies, flower pots, gutters, etc. These species are able to adapt to human disturbance and live in close proximity to humans.

For some species, nesting in urban areas is advantageous. These are species which are not necessarily adapted to a particular nesting place.

The most frequent species which nest in cities are the house sparrows, the pigeons, the collared doves, but many of those which belong to the perching birds, such as great tit (*Parus major*), have adapted to this lifestyle. There were found nests even inside the letter boxes located on gates. Storks and swallows breeds exclusively in urban areas (Alderton, 2008).

The bird species which breed in urban areas build their nests from artificial materials, most of the times. This happens because natural materials of vegetal origin (dried leaves, branches, dried grass, moss, pieces of bark) and of animal origin (hair, feathers) are more and more rare. Among the materials which are more often encountered in nests, we notice – pieces of paper, mineral cotton, wires, plastic bags, etc. Most of the times these materials endanger the health and life of baby birds. There were cases when birds used wire wrapped in fiberglass cloth to build their nests. When it became moistened, the canvas deteriorated and baby birds died being stung by glass fibers.

Monitoring program

Monitoring programs outlined the way in which the loss of a habitat or its modification can determine changes in the structure of bird populations and in their number in a given area. These changes can differ very much taking into account the disturbing factor and their intensity in time and space (Newmark 2006).

Some species can also take advantage of the new environment created by human activities (MkKinney & Lockwood, 1999).

Many authors have turned their attention to the phenomenon of replacement of more generalist species (Owens & Bennett, 2000; Marvier et. al., 2004; Julliard et al., 2006). This phenomenon, named functional homogenization, is generally quantified as a spatial development of communities over time (Olden & Poff, 2004; Olden, 2006).

Also, in the case of habitats fragmented by human activities, it is very important to specify the changes regarding the diversity of species and their distribution not only in space but also over time (Blaier, 2004).

For example, the landscape's structural changes, such as the multiplication of urban areas, affected the ability of some organisms to spread. All these lead to the prediction that, communities consisting in more species with specific needs from the point of view of habitat, should have a greater local extinction rate, while species which are more spread and tolerant from the point of view of the habitat's conditions, benefit from the destruction of their habitat and show a greater stability.

We can examine these predictions from the data provided by the monitoring programs on a large scale. In order to analyze these predictions, with the help of the data provided by the monitoring programs on a large scale, we can state that these programs can be useful as long as the monitoring process does not allow us to account for potential trends.

Resistance to stressful factors

Bird species which live in urban areas tolerate the presence of people and are more resistant to stressful factors. Many species exploit the advantages of urbanization in a way or another. For example, with a milder microclimate, food can be easily found. Compared to their fellows which live in natural areas, these species have adapted to noise, light, chemical pollution, to the continuous presence of humans, dogs, cats, vehicles.

The Max Planck Institute in Germany performed an experiment on baby blackbirds which was based on measuring the corticosterone hormone which indicated bird stress. The baby blackbirds which were tested originated from two areas, namely the city and a natural area. They have been raised together for a year, then left in freedom. They continued to monitor their behavior and further measurements were made. The results of the experiment showed that, in adult birds originating from cities, the stress hormone was lower (www.biology-blog.com).

Food abundance

Urban areas provide enough food to bird species which live in these areas. Usually food is easily found, therefore birds save energy. Omnivorous birds are advantaged because there is plenty of household waste. Insectivorous birds search for specific areas. Blackbirds are attracted by green areas, artificially watered, where they find plenty of food everyday.

The food quantity depends also on vegetation and season. The indigenous flora is richer in insects than the exotic one. Some prey birds have to change their

usual menu. For example, the long-eared owl, which usually eats rodents, in big cities it has to eat small birds (Alderton, 2008).

Microclimate advantages

Urban microclimate is advantageous for some bird species which are able to exploit it especially during the cold season. In winter, urban areas offer shelter to many bird species. The urban microclimate and the abundance of food also attracts those species which have their habitat in the vicinity of cities. In these periods, artificial feeding provides a rich and easily obtainable meal to many species. Some species, even the migratory ones, don't leave the city because here they can find food and shelter. For example, the blackbird, the European robin, the greenfinch, etc.

In urban areas, the carnivorous and granivorous species are more frequent, the insectivorous ones being more rare. The latter migrate to warmer southern areas. In urban areas, spring arrives earlier, leaves begin to appear earlier and insects appear too. Because of this, the nesting period begins earlier and some species, such as the collared dove, nest even in November. Other species of perching birds which naturally nest 2 times per year, in urban areas they nest 3-4 times per year (Kelemen A., 1978).

Below there is a brief presentation of the birds which can be found in Cluj-Napoca city:

Like other large cities, Cluj is in a continuous expansion and development. The forests and pastures have been replaced by houses. The actual surface of the city is 179,5 sq.m. (www.kolozsvar.ro). In Cluj, the bird species which are specialized in different habitats, don't have chances to face these new changes. Only species which can adapt to the new changes can survive.

The most common species are the pigeons (*Columba livia*), the house sparrow (*Passer domesticus*), the collared dove (*Streptopelia decaocto*). Besides these, there are species which live inside the city and in its vicinity, such as the great tit (*Parus major*), some species of finches (Fringillidae), the common magpie (*Pica pica*), the blackbird (*Turdus merula*).

Besides these common species, the most frequent species are:

- Collared Dove (*Streptopelia decaocto*) etc. etc.
- Rock Dove (*Columba livia*)
- Wryneck (*Jynx torquilla*)
- Great Spotted Woodpecker (*Dendrocopos major*),
- Syrian Woodpecker (*Dendrocopos syriacus*)
- Whitw Wagtail (*Motacilla alba*)
- Robin (*Erithacus rubecula*)
- Nightingale (*Luscinia megarynchos*)
- Black Redstart (*Phoenicurus ochruros*)
- Redstart (*Phoenicurus phoenicurus*)

- Blackbird (*Turdus merula*),
- Song Thrush (*Turdus philomelos*)
- Fieldfare (*Turdus pilaris*)
- Redwing (*Turdus iliacus*)
- Blackcap (*Sylvia atricapilla*)
- Lesser Whitethroat (*Sylvia curruca*)
- Garden Warbler (*Sylvia borin*)
- Chiffchaff (*Phylloscopus collybita*)
- Marsh Tit (*Parus palustris*)
- Blue Tit (*Parus caeruleus*)
- Great Tit (*Parus major*)
- Nuthatch (*Sitta europaea*)
- Golden Oriole (*Oriolus oriolus*)
- Jay (*Garrulus glandarius*)
- Starling (*Sturnus vulgaris*)
- House Sparrow (*Passer domesticus*)
- Tree Sparrow (*Passer montanus*)
- Caffinch (*Fringilla coelebs*)
- Greenfinch (*Carduelis chloris*)
- Goldfinch (*Carduelis carduelis*)
- Serin (*Serinus serinus*)
- Saskin (*Carduelis spinus*)
- Hawfinch (*Coccothraustes coccothraustes*)
- Common Cuckoo (*Cuculus canorus*)
- Long-eared Owl (*Asio otus*)
- Green Woodpecker (*Picus viridis*)
- Grey-headed Woodpecker (*Picus canus*)
- Barn Swallow (*Hirundo rustica*)
- House Martin (*Delichon urbica*)
- Wren (*Troglodytes troglodytes*)
- Spotted Flycatcher (*Muscicapa striata*)
- Collared Flycatcher (*Muscicapa collaris*)
- Goldcrest (*Regulus regulus*)
- Long-tailed Tit (*Aegithalos caudatus*)
- Hort-toed Tree Creeper (*Certhia brachydactyla*)
- Red-backed Shrike (*Lanius collurio*)
- Rook (*Corvus frugilegus*)
- Hooded Crow (*Corvus corone cornix*)
- Magpie (*Pica pica*)
- Jackdaw (*Corvus monedula*)
- Mallard (*Anas platyrhynchos*)
- Peregrin Falcon (*Falco peregrinus*)
- Kestrel (*Falco tinnunculus*)
- Black-headed Gull (*Larus ridibundus*)

Conclusions

The disturbances caused by the urbanization of certain areas, more or less expanded, against natural habitats, have different effects on the different bird species:

- this phenomenon can have negative effects on some bird species, which are less adaptable, which can affect biodiversity;
- on the other hand, for birds with a greater adaptation capacity, urbanization determines the appearance of an environment where they can develop and live in good conditions;
- a smaller number of birds have adapted and live in this environment, compared to the same territory when there was a natural habitat, but the number of these populations can be bigger;
- urbanization can have another effect which is considered positive – because of a milder microclimate, it determines some migratory species to remain in these places over winter.

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REVIEW CONCERNING THE NATIVE AND ALIEN FISH SPECIES IN THERMAL LAKE PEŢEA (BĂILE 1 MAI, BIHOR, ROMANIA)

Claudiu GAVRILOAIE*, Corina BERKESY**

Abstract: In this paper we discuss about the ichthyofauna from the protected area Peţea lake (Bihor county, Romania), reviewing the data from available literature and also bringing some original data. We will insist upon the alien fish species both introduced by human or entered in a natural way through the hydrographical network and their relationships with the native fish species, and, especially with thermal rudd, *Scardinius racovitzai.* During 1861-2010 in Peţea lake were observed 27 fish species and varieties, belonging to 23 genera and 5 families. In present, in the lake there are only 16 fish species, from which 6 are native (including the thermal rudd), and 10 are alien species. From the native ones, the most important species is, obviously, *S. racovitzai.* Due to relative neighbourhood of this protected area with human settlements, all the fish species are permanently exposed to anthropic impact, but of major concern is, of course, the thermal rudd, *S. racovitzai.*
Key words: *Peţea lake, ichthyofauna, alien species, native species, endemic, protection.*

Introduction

In north-west of Romania, near the city of Oradea, there is a small subtropical oasis: Băile 1 Mai, where it lies, at an altitude of 140 m, the lake and the rivulet Peţea. The rivulet flows into Crişul Repede river, after it crosses Sânmartin and Oradea localities. The rivulet springs from the place named „Ochiul Ţiganilor" and collects the water of several thermal springs. On the biggest one has formed the lake Peţea, with a surface of 4100 sm, a maximum depth of 3,5 m and a constantly temperature around 30-31 °C (Paucă and Vasiliu, 1933) (fig. 1).

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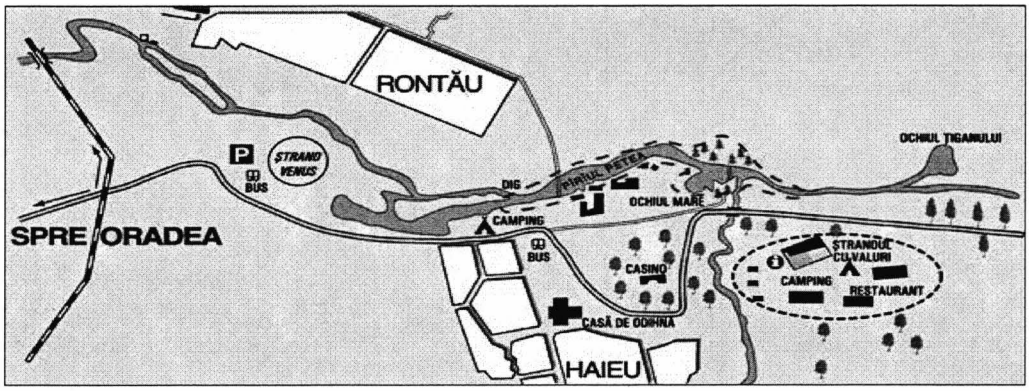

Fig. 1. The lake and the rivulet Peţea (www.aventurilapescuit.ro)

In the warm water of the lake live three endemic species: the thermal waterlily *Nymphaea lotus* var. *thermalis*, (Marossy, 1974; Olteanu, 1977), the snail *Melanopsis parreyssi*, a survivor of the last ice age (Bănărescu, 1969 and 1981), and, finally, a fish species, the thermal rudd *Scardinius racovitzai* (Bănărescu, 1964 and 1981; Bănărescu et al., 1960) (fig. 2). All these three species have a special scientific importance and, due to their presence, the lake Peţea has now a natural reserve statute (Marossy, 1974).

Because the lake is very close to human settlements, there are major difficulties to assure the protection for the three endemic species mentioned above. One of a major problems consists in that some aquarium owners from Oradea have introduced in the lake several aquarium plants and fish species and, also, an american turtle species which originates in Florida, USA - *Trachemys scripta elegans* (Gavriloaie and Rusu, 2010). These introductions have become, unfortunatelly, a constant phenomenon.

The living beings are placed on the Earth in relation with environmental conditions, and their spreading depends on climatic, hydrographic and orographic barriers, which limit or even make impossible the movement of living beings from a region to another. Thus, as a result of the geographic isolation, the evolution patterns from an area to another are different. From ancient times, the humans interfered with these evolution patterns through the carrying some species on the whole planet.

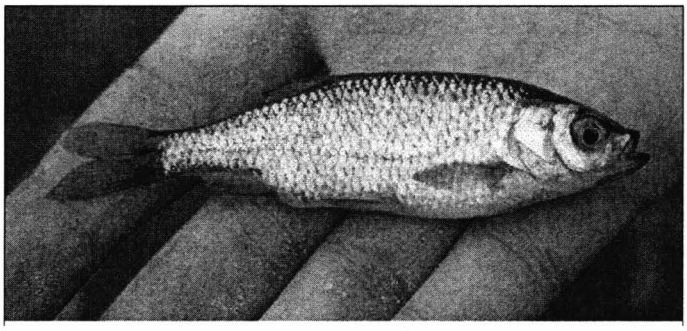

Fig. 2. *S. racovitzai* - thermal rudd

Let us see what alien species in opposition with the native ones does it mean? In the scientific papers the following definitions are prevalent used (Copp et al., 2005):

• **native, indigenous** – this refers to a taxon (species, sub-species, race or variety)

that occurs naturally in a geographical area, with dispersal occurring independent of human intervention, whether direct or indirect, intentional or unintentional.

• **non-native, non-indigenous, alien, exotic** – this refers to a species, sub-species, race or variety (including gametes, propagules or part of an organism that might survive and subsequently reproduce) that does not occur naturally in a geographical area, i.e. it did not previously occur there or its dispersal into the area was mediated or facilitated directly or indirectly by humans, whether deliberately or unintentionally.

The aquatic ecosystems are much more vulnerable to alien species than the terrestrial ecosystems (Lodge et al., 1998).

Large scale introductions of fish species into areas outside their native range is a comparatively recent phenomenon. Some transfers of fish may be considered ancient, in that they date from the middle ages in Europe, but the majority of such movements date from the end the 19th century. So, since 1850 the pattern of introductions has shown a steady increase, until 1960. After this year the rate of new introductions has apparently declined (Welcomme, 1988). The reasons fro such introductions were varoius: food sources, ornamental, the filling of empty ecological niches, biological control, accident etc. (Gavriloaie, 2007b).

In this paper we discuss about the ichthyofauna from lake Peţea; we make a survey of the scientific papers regarding the fish species from lake Peţea and we also bring some new information related to the topic, collected in 2007 and 2008 from the area. We dwell on alien fish species and their relations with the native ones, and especiallly, with the endemic thermal rudd - *S. racovitzai*. All fish species which were observed only in the rivulet Peţea by the various authors can reach the lake Peţea as well, so we did not make a separation between these species and those which were observed in the lake.

Results and discussions

In table 1 we have noted all fish species which were observed in lake Peţea since 1861 until 2010, in chronological order.

Table 1. Fish species from lake and rivulet Peţea

Nr.	Species	Family	Citation in scientific papers (in chronological order)	The origin in Peţea ecosystem
1	*Cyprinus carpio*	Cyprinidae	Antal, 1861; Paucă and Vasiliu, 1933; Bănărescu, 1964; Telcean and Cupşa, 1999; Mag et al., 2005; Gavriloaie et al., 2009	native species
1[1]	*Cyprinus carpio* var. koi		Mag et al., 2005; Petrescu-Mag and Petrescu-Mag, 2007; Iacob and Petrescu-Mag, 2008	ornamental variety, introduced by human
2	*Carassius carassius*		Bănărescu, 1964	native species
3	*Carassius auratus*		Petrescu-Mag and Petrescu-Mag, 2007; Iacob and Petrescu-Mag, 2008; Gavriloaie et al., 2009	asian ornamental species, introduced by human
4	*Carassius gibelio*		Telcean and Cupşa, 1999; Mag et al., 2005; Gavriloaie et al., 2009	alien species of asian origin, entered in natural way through hydrographical network
5	*Barbus petenyi*		Paucă and Vasiliu, 1933	native species
6	*Rhodeus amarus*		Paucă and Vasiliu, 1933	native species
7	*Gobio obtusirostris*		Paucă and Vasiliu, 1933; Bănărescu et al., 1960; Telcean and Cupşa, 1999; Mag et al., 2005	native species
8	*Pseudorasbora parva*		Telcean and Cupşa, 1999; Mag et al., 2005	alien species of asian origin, entered in natural way through hydrographical network
9	*Rutilus carpathorossicus*		Bănărescu, 1964	native species
10	*Leuciscus leuciscus*		Bănărescu et al., 1960	native species
11	*Squalius cephalus*		Antal, 1861; Paucă and Vasiliu, 1933; Bănărescu et al., 1960; Mag et al., 2005	native species
12	*Scardinius racovitzai*		Müller, 1958; Bănărescu et al., 1960; Bănărescu, 1964, 1981; Mag et al., 2005; Gavriloaie et al., 2009	endemic native species
13	*Alburnus alburnus*		Bănărescu et al., 1960	native species

14	*Aspius aspius*		Antal, 1861	native species
15	*Vimba carinata*		Bănărescu et al., 1960	native species
16	*Chondrostoma nasus*		Bănărescu et al., 1960	native species
17	*Misgurnus fossilis*	Cobitidae	Bănărescu, 1964	native species
18	*Cobitis danubialis*		Paucă and Vasiliu, 1933; Bănărescu, 1960, 1964	native species
19	*Sabanejewia balcanica*		Bănărescu, 1960, 1981	native species
20	*Ictalurus nebulosus*	Ictaluridae	Bănărescu et al., 1960	alien species of north-american origin, entered in natural way through hydrographical network
21	*Poecilia reticulata*	Poeciliidae	Falka and Gavriloaie, 2005; Mag et al., 2005; Gavriloaie, 2007a, 2007b; Petrescu-Mag and Petrescu-Mag, 2007; Iacob and Petrescu-Mag, 2008; Gavriloaie et al., 2009	south-american ornamental species, introduced by human between 1981 and 1989
22	*Poecilia sphenops*		Mag et al., 2005; Petrescu-Mag and Petrescu-Mag, 2007; Iacob and Petrescu-Mag, 2008; Petrescu-Mag et al., 2008; Gavriloaie et al., 2009	central and south-american ornamental species, introduced by human
23	*Xiphophorus helleri*		Mag et al., 2005; Petrescu-Mag and Petrescu-Mag, 2007; Iacob and Petrescu-Mag, 2008; Gavriloaie et al., 2009	central and north-american ornamental species, introduced by human
24	*Macropodus opercularis*	Belontiidae	Mag et al., 2005; Petrescu-Mag and Petrescu-Mag, 2007; Iacob and Petrescu-Mag, 2008; Lozinsky, 2009	asian ornamental species, introduced by human
25	*Trichogaster trichopterus*		Petrescu-Mag and Petrescu-Mag, 2007; Iacob and Petrescu-Mag, 2008; Lozinsky, 2009	asian ornamental species, introduced by human
26	*Betta splendens*		Lozinsky, 2009	asian ornamental species, introduced by human

As we may observe in table 1, in the rivulet and lake Peţea were signaled since 1861 until 2010, 27 fish species and varieties belonging to 23 genera and 5 families. Among them, one species is endemic (*S. racovitzai*), 15 are native species (*C. carpio, C. carassius, B. petenyi, R. amarus, G. obtusirostris, R. carpathorossicus, L. leuciscus, S. cephalus, A. alburnus, A. aspius, V. carinata, C. nasus, M. fossilis, C.*

danubialis, S. balcanica), 3 are alien species entered in lake through the hydrographic network (*C. gibelio, P. parva, I. nebulosus*), and 8 are ornamental or/and aquarium species introduced by humans (*C. carpio* var. koi, *C. auratus, P. reticulata, P. sphenops, X. helleri, M. opercularis, T. trichopterus, B. splendens*).

Among the native species, *C. carassius* and *M. fossilis* had dramatically declined in the last decades in Romanian waters (Bănărescu, 1994) and it seems they are no longer present in lake Peţea. Then, *A. aspius* is quoted only once by Antal (1861) and we may presume this species in no longer present in lake Peţea. The native species observed by Bănărescu et al., (1960) at the confluence of rivulet Peţea with Crişul Repede river, or *R. carpathorossicus* quoted by Bănărescu (1964) are not quoted later by Bănărescu or others authors, so we presume they enter only occasionally in rivulet Peţea, but they do not reach the lake because its warm water. It is surprising the presence in the lake of the reofilic species *S. balcanica* (Bănărescu et al., 1960; Bănărescu, 1981). So, it seems in lake Peţea are present nowadays only four native species: *C. carpio, G. obtusirostris, S. cephalus, S. racovitzai,* to which we probably may add the *C. cobitis danubialis* and *S. balcanica,* so in the most optimistic way, a total of six native species.

Regarding the alien species entered in natural way through the hydrographic network, it seems that *I. nebulosus* disappeared from the lake (Petrescu-Mag et al., 2008); instead, *P. parva* and *C. gibelio* are constant species in the lake. It is known that these two species, once entered in a new habitat, they practically can not be removed (Gavriloaie and Falka, 2006; Gavriloaie and Chiş, 2006; Gavriloaie, 2008).

All the ornamental and aquarium species (*C. carpio* var. koi, *C. auratus,* the three poeciliides and the three belontiides) were introduced in lake Peţea by humans. Without the *C. carpio* var. koi, *C. auratus*, the other 6 species are now naturalized in the lake, but the species *M. opercularis, T. trichopterus* and *B. splendens* hardly survive in winter. Unfortunately, in the lake are constantly introduced all these ornamental species, especially in spring season.

So, in lake Peţea there are now 16 fish species and varieties, from which 6 are native (*G. obtusirostris, S. cephalus, S. racovitzai, C. cobitis danubialis, S. balcanica,*) and 10 are alien species (. *P. parva, C. gibelio, C. auratus, C. carpio* var koi, *P. reticulata, P. sphenops, X. helleri, M. opercularis, T. trichopterus, B. splendens*).

From the native species, the most important is, undoubtedly, the endemic thermal rudd - *S. racovitzai.* Bănărescu (1994) considers it little vulnerable, according to IUCN classification. Though, there are several factors which may lead to the diminishing of this species' individuals number. None of the alien fish species can consume directly the adults of thermal rudd, instead *C. gibelio* and *X. helleri* are potential consumers of rudd's fries and eggs. The north-american turtle *Trachemys scripta elegans* it is a potential consumer of adult thermal rudds. In the same connection, some of the alien fish species may consume the same food items as the *S. racovitzai,* but this topic needs further research. Another threat consists in that many

anglers fish regularly in the lake and we had observed some thermal rudd in the captures.

Conclusions

The lake and rivulet Peţea comprise a remarcable flora and fauna, with numerous species protected at European level. Among fishes, between 1861 and 2010 were quoted 27 species from 23 genera and 5 families, the most important species being the endemic thermal rudd - *S. racovitzai.* Nowadays, in the lake there are only 6 native fish species and 10 alien fish species, most of them being introduced by humans. Upon the lake Peţea there is a constantly anthropic impact (angling, picnic, cattle watering), but the most important threat regards the alien plant, fish and turtle species. The alien fish species have the greatest impact upon the native ones, and because of these aliens the native species may decline in number.

It is necessary to protect better the Peţea lake against the human impact.

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