INVESTIGATIONS REGARDING STRUCTURAL AND BIOCHEMICAL PARTICULARITIES OF THE MICROORGANISMS AND INVERTEBRATES ADAPTED TO HARSH ENVIRONMENTS

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Abstract. Research conducted in lake ecosystems representative of the Romanian territory of the Oltenia Plain has highlighted the fact that invertebrate species make up populations with high numerical densities and biomass. The phytophilic fauna is dominated by the larvae of chironomids, coleoptera, ephemeroptera, heteroptera, gastropods. In the areas near the banks, where the facies is composed of plant detritus and mud, there are a large number of chironomids, ephemeroptera, plecoptera, oligochaetes and gastropods. A smaller diversity of benthic invertebrates has been observed in deep areas where the facies is sandy-muddy. Numerous protozoa, gastropods, tendiped larvae, trichoptera, odonata, chironomids and gamarids have been identified in areas abundant in macrophytes and plant detritus. Under current conditions, an important role in the biological production of lake ecosystems in the lower Jiu sector is played by populations of gastropods, predominantly benthic organisms that actively respond to the heterogeneity of the microhabitats they populate. Following research conducted on 16 species of gastropods identified in lakes, depending on biomass, it was found that the largest share belongs to the populations of *Viviparus acerosus*, *Lymnaea stagnalis*, *Stagnicola palustris*, *Radix balthica* and *Planorbarius corneus*, between 147 g / m^2 and 649 g / m^2. Also, the performed studies showed the increased tolerance of the lung snail species *Radix balthica*, *Lymnaea stagnalis*, *Planorbarius corneus* to the presence in the environment of bivalent metal ions coming from the industrial activities of solid waste processing. These species are indicators of contaminated environments in the lower industrial sector of the Jiu due to early signals of the emergence of negative changes in lake ecosystems.

Keywords: chemolitoautotrophic and chemoorganotrophic bacteria, invertebrates, metallic ions, biotechnology.

Rezumat. Investigații privind particularitățile structurale şi biochimice ale microorganismelor şi nevertebratelor adaptate la medii ostile. Cercetările efectuate în ecosisteme lacustre reprezentative pentru teritoriul României din Câmpia Olteniei au scos în evidență faptul că speciile de nevertebrate alcătuiesc populaţii cu densități numerice și de biomasă crescute. Fauna fitofilă este dominată de larvele de chironomide, coleoptere, efemeroptere, heteroptere, gasteropode. În zonele din apropierea malurilor, unde faciesul este alcătuit din detritus vegetal şi mâl sunt prezente un număr mare de chironomide, efemeroptere, plecoptere, oligochete si gasteropode. O diversitate mai restrânsă de nevertebrate bentonice a fost observată în zonele adânci unde faciesul este nisipos-mâlos. În zonele abundente în macrofite şi detritus vegetal au fost identificate numeroase protozoare, gasteropode, larve de tendipedide, trichoptere, odonate, chironomide şi gamaride. În condițiile actuale, un rol important în producția biologică a ecosistemelor lacustre din sectorul inferior al Jiului o au populațiile de gasteropode, organisme preponderent bentonice care răspund activ la heterogenitatea microhabitatelor pe care le populează. În urma cercetărilor efectuate la 16 specii de gasteropode identificate în lacuri, în funcţie de biomasă s-a constatat că ponderea cea mai mare revine populațiilor de *Viviparus acerosus, Lymnaea stagnalis*, *Stagnicola palustris*, *Radix balthica* şi *Planorbarius corneus*, între 147 g/m^2 si 649 g/m^2. De asemenea, studiile efectuate au evidenţiat toleranţa crescută a speciilor de melci pulmonaţi *Radix balthica*, *Lymnaea stagnalis*, *Planorbarius corneus* la prezenţa în mediu a ionilor metalici bivalenţi proveniţi din activităţile industriale de prelucrare a deşeurilor solide. Aceste specii reprezintă bioindicatori ai mediilor contaminate industrial din sectorul inferior al Jiului deoarece semnalizează timpuriu apariția unor modificări negative în cadrul ecosistemelor lacustre.

Cuvinte cheie: bacterii chemolitotrofe si heterotrofe, nevertebrate, ioni metalici, biotehnologie.

INTRODUCTION

The researches carried out within an extensive national program of knowledge of some representative ecosystems for the Romanian territory bring an important contribution to the establishment of the structural-functional particularities of a protected lake complex from the Jiu river basin, subject to anthropogenic impact (FIRĂ & NĂSTĂSESCU, 1977; BREZEANU et al., 2011; CIOBOIU, 2014; GOGA & MITITELU-IONUŞ, 2018; CIOBOIU et al., 2019; MARINESCU & MITITELU-IONUŞ, 2019; MITITELU-IONUŞ et al., 2021; MURARIU & MAICAN, 2021; RĂDUCA et al., 2021). The protected area of national interest the Lacustrine Complex Adunaţii of Geormane (code: 2393) is included in the Continental biogeographical region, in the forest-steppe area of the Oltenia Plain, the land unit of Lunca Jiu-Jieț. It covers the territory of the localities Bratovoeşti and Teasc on an area of 102 ha and is characterized by the presence of lake ecosystems, respectively Victoria and Marica lakes, surrounded by swampy areas, the surface can be extended to 111.25 ha, due to the evacuation channel that ensures the connectivity between these two lakes (Fig. 1).

The economic implications of ecological effects are identified as long as they can be determined within acceptable limits. A variety of ecological processes are affected and altered by air pollution. Such processes include community succession and retrogression, nutrient biogeochemical cycling, photosynthetic activity, primary and secondary productivity, species diversity and community stability (FIRĂ & NĂSTĂSESCU, 1977; CUŞA, 1996; MEHEDINȚU et al., 1996; POPEA et al., 2004). The study of research on acidophilic, chemolithotrophic and chemoorganotrophic bacteria is very current due to the importance of these bacteria in biotechnology and in the decontamination of the environment. Through their activity, acidophilic, chemolithotrophic and chemoorganotrophic bacteria cause changes in the pH of the culture medium, as well as the redox potential. Also, various substances of an organic nature can develop during the metabolic activity, which have oxidizing and reducing properties. In order to reduce the contamination, it is necessary to slow down or stop the bacterial leaching of the heavy metals from the sulfur ores under the indirect action on the growth process of the reduction of ferrous iron from the culture environment. Acidophilic microorganisms, optionally thermophilic, catalyze important leaching reactions of some metals from poor ores, coal and acid mine waters (KARAVAIKO, 1988; JOHNSON, 1999; MEHEDINȚU et al., 1996; POPEA et al., 2004; CISMAŞIU & CIOBOIU, 2021).

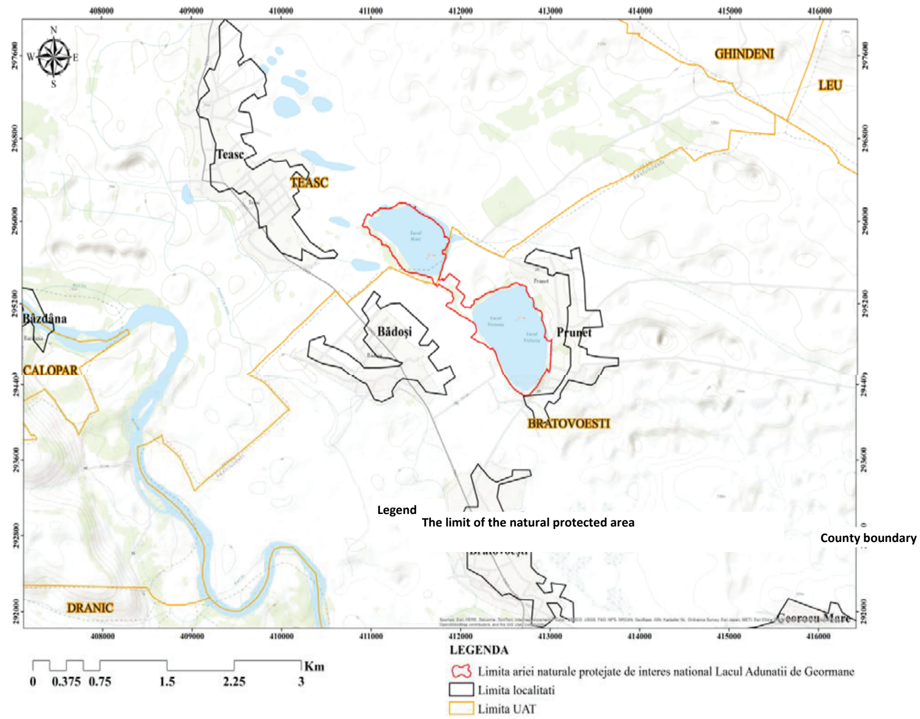

Figure 1. Sketch of Lacustrine Complex Adunații of Geormane in the lower sector of the Jiu (Google Earth, accessed: February 18, 2021).

MATERIAL AND METHODS

In order to evaluate the ecological characteristics, an extensive research program was carried out consisting of field observations, seasonally, in the period 2016-2018. Samples were collected for the physico-chemical analyses of water and sediment, as well as qualitative samples for the determination of planktonic and benthic communities. The following physico-chemical parameters were determined: pH with Hanna pH meter, electrical conductivity with Hach conductivity meter, dry fixed residue 105^0C, dissolved oxygen (O_2), organic matter quantity determined by CCO-Mn, nitrates, nitrites, ammonium, sulphates, total phosphorus (total P), sulphates with spectrophotometer DR2010 and chlorophyll (GAVRILESCU et al., 2017). Also, by means of spectrophotometer with atomic absorption type DR 2000 and with Avanta GBC flame, SN A 5378 concentrations of metal ions were highlighted in the shells and meat mass of the species such as *Viviparus acerosus, Lymnaea stagnalis, Stagnicola palustris, Radix balthica* and *Planorbarius corneus,* dominant in the lacustrine ecosystems, which were subjected to elementary analyses by the mineralization process.

Methods of microbial mobilization are as follows: (a) inclusion in organic and inorganic materials, (b) sorption on a solid, inert material, (c) mobilization by affinity bonds between organic and inorganic molecules, (d) mobilization cells by covalent bonds. Through their activity, acidophilic microorganisms cause qualitative and quantitative changes in the nutrients in the environment in accordance with the pH values of the environment, as well as the potential for oxidation and reduction. Also, during the metabolic activity, these microorganisms can develop useful substances with inhibitory activity and antagonism (KARAVAIKO, 1988; JOHNSON; 1999; POPEA et al., 2004; CISMAŞIU, 2012; GAVRILESCU & POPESCU; 2012; CÎRSTEA et al., 2015; CISMAŞIU & CIOBOIU, 2021).

RESULTS AND DISCUSSIONS

Water chemistry. The analysis of the main physical and chemical indicators reveals the formation conditions of the chemical composition of the water and the mineralization stage of these lacustrine ecosystems. The chemical composition of the water is characteristic to eutrophic ecosystems. According to the ionic balance and the anion and cation content, the water of the lakes belongs to the bicarbonate-sulphato-calcic-magnesium category, characteristic to the mixed mineralization stage. From the point of view of quality conditions for surface waters, the lakes fall into category II and can be used for fish farming (BUCURESCU et al., 2007; CIOBOIU & CISMAȘIU, 2016).

The physical and chemical analyses carried out in water and sediment samples, over the years 2016-2017, revealed that the pH ranges from 7.5 to 8.4 pH units, fixed residue 195 mg/dm^3, chlorides 21 g/l, and hardness varies between 10.66-12.7 degrees Ge. Using the values of the *chlorophyll a* concentration as a quality indicator, the eutrophication degree of water was estimated according to Order 161/2006, the lakes being eutrophic (8-25 µg/l in Lake Victoria, > 25 µg/l in Marica).

The concentrations of Ni^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Fe^{2+} in the waters of the lakes located in the lower Jiu sector were found to be below the detection limit admitted by international standards (0.001 – 0.01 mg / l) (Table 1).

Table 1. Concentrations of heavy metals in the water of the studied lakes, compared to the maximum admissible values of Ord. 161/2006.

No.	The analyzed indicator (mg/Kg/SU)	Victoria	Marica	Limits a admissible laccording to Ord. 161/2006	The analysis method	The used apparatus
1	Fe^{2+}	< 0.005	0.012	0.1	The working method specified in the user manual of the spectrometer by atomic absorption –Avanta GBS	The spectrometer of the atomic absorption with flame Avanta GBC, SN A 5378
2	Mn^{2+}	< 0.001	0.004	0.01		
3	Ni^{2+}	0.016	0.012	0.1		
4	Cr^{2+}	< 0.003	< 0.003	0.1		
5	Cu^{2+}	< 0.01	< 0.01	0.01		
6	Zn^{2+}	< 0.005	< 0.005	0.05		
7	Cd^{2+}	< 0.001	0.001	0.005		
8	Pb^{2+}	< 0.01	< 0.01	0.01		

The structure of biocenoses from lakes. By analysing the biocenotic structures of the lacustrine ecosystems it was found that they have a high degree of trophicity reflected by the quantitative and qualitative composition of the planktonic and benthic communities, specific to the ecosystems in the Oltenia Plain. The results highlight that a special place in the structure of biocenoses belongs to the periphyton, in whose composition the largest number of species belong to bacillariophyceae and chlorophyceae. The most common species are*: Diatoma elongatum, D. vulgare, Synedra accus, S. ulna, Amphora ovalis, Ceratoneis arcus, Gyrosigma acuminatum, Scenedesmus quadricauda, S. acuminatus, Pediastrum duplex, P. boryanum, P. simplex, Cymatopleura solea, Navicula cineta, Cymbella affinis, C. lanceolata, Microcystis aeruginosa, Euglena viridis*, with an average numerical density of over 80 thousand specimens / l.

They are abundant , especially on the shores of Lake Victoria, plated with concrete plates and on palustris macrophytes, periphyton, where bacterial populations and numerous micro-invertebrates (especially protozoa) live alongside algae and represent the preferred food for gastropods.

The paludous and aquatic macrophytes occupy an important place in the bioeconomics of the ecosystems, with the dominant species being *Phragmites communis, Typha angustifolia, Nuphar luteum, Nymphaea alba, Rorippa amphibia, Poygonium amphibium, Iris pseudacorus, Equisetum arvense, Equishorpus palustris, Euphorpia palustris, Ranchus palustris, Salvinia natans, Stratiotes alloides, Myriophylum spicatum, Hydrocharis morsus-ranae*. An overall assessment of biomass production has shown that 85200 Kg / ha / year of wet biomass can be obtained. It is a proof of the trophic capacity of the ecosystems (Fig. 2) (CIOBOIU, 2011).

In zooplankton, the dominant species are rotifers and cladocers. Species of invertebrates from the coastal areas with abundant macrophytes make up populations with increased number densities and biomass. Phytophile fauna is dominated by larvae of chironomides, coleoptera, ephemeroptera, heteroptera, gastropods. In the areas near the banks, where the facies consist of vegetal debris and mud, a large number of chironomides, ephemeropters, plecoptera, oligochaeta and gastropods are present. A narrower diversity of benthic invertebrates has been observed in deep areas where the facies are sandy-muddy. In these areas abundant in macrophytes and plant detritus, numerous protozoa, gastropods, tendipedidae larvae, trichoptera, odonates, chironomides and gamarides have been identified (Table 2).

Under the current circumstances, an important role in the biological production of the lacustrine ecosystems in the region is played by the population of gastropods, mainly benthic organisms that respond actively to the heterogeneity of the microhabitats in which they inhabit (Table 3). Following the research carried out on the 16 species of gastropods found in these lakes, depending on the biomass, it was found that the largest share belongs to the *Viviparus acerosus, Lymnaea stagnalis*, *Stagnicola palustris*, *Radix balthica* and *Planorbarius corneus* populations, between 147 g/m^2 and 649 g/m^2.

Figure 2. Surfaces of lakes covered with macrophytes (original).

Table 2. Structure of the zoobenthos (mean values).

Taxonomic group	Marica			Victoria		
	No. Individuals / m^2	Abundance %	Frequency %	No. Individuals / m^2	Abundance %	Frequency %
Chironomidae	3963	45,4	100	750	20,4	100
Gammarida	2506	25,6	33	1740	19,25	90
Ostracoda	267	2,8	66	420	12,5	66
Heteroptera	213	2,2	100	125	4,2	100
Gastropoda	386	4,1	66	270	5,6	66
Bivalvia	53	0,5	33	34	0,25	31
Cladocera	175	5,4	23	127	5,4	33
Copepoda	156	4,5	80	290	8,5	33
Ephemeroptera	160	1,7	66	247	7,8	66
Plecoptera	226,5	6,45	16,5	403	11,9	33
Isopoda	26	0,3	33	50	1,6	66
Oligochaeta	26,5	0,95	16,5	53	1,7	33
Hirudinea	13	0,1	33	27	0,9	33
TOTAL	9317	100,00		3027	100,00	

Table 3. The species of Gastropods from the Lacustrine Complex.

CLASS GASTROPODA Cuvier, 1798	
SUBCLASS PROSOBRANCHIA Milne Edward, 1848	
ORDER MESOGASTROPODA Thiele, 1925	
Family Viviparidae Gray, 1847	*Viviparus acerosus* Bourguignat, 1870 *Viviparus viviparus* Linnaeus, 1758
Family Valvatidae Thomson, 1840	*Valvata (Cincina) piscinalis* O. F. Muller, 1774
SUBCLASS PULMONATA Cuvier, 1817	
ORDER BASOMMATOPHORA A. Schmidt, 1855	
Family Physidae Fitzinger, 1833	*Physa fontinalis* (Linnaeus, 1758) *Physella (Costatella) acuta* (Draparnaud, 1805)
Family Lymnaeidae Rafinesque, 1815	*Lymnaea stagnalis* (Linnaeus, 1758) *Stagnicola palustris* (O. F. Muller, 1774) *Stagnicola corvus* Gmelin, 1788 *Radix auricularia* (Linnaeus, 1758) *Radix ampla* (Draparnaud, 1805) *Radix balthica* (Linnaeus, 1758) *Galba truncatula* (O. F. Muller, 1774)
Family Planorbidae Rafinesque, 1815	*Planorbis planorbis* (Linnaeus, 1758) *Anisus (Anisus) spirorbis* (Linnaeus, 1758) *Anisus (Disculifer) vortex* (Linnaeus, 1758) *Planorbarius corneus* (Linnaeus, 1758)

With regard to the presence of heavy metals in sediment, shell and meat mass of snails from fresh water, it has been found that they can accumulate higher levels of Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} than the allowable values of the environment (0.001 - 0.01 mg / l), according to Order 756/1997, Order 161/2006 and the Law number 278/2013.

The analyses carried out in the Victoria lake and the Marica pond from the lacustrine complex illustrate the ability of lung snails species such as *Viviparus acerosus* and *Stagnicola palustris* to accumulate metal ions of Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} in direct correlation with the concentration of the respective ions in the soil.

Also, the performed studies have been shown that the increased tolerance of these snail species (for example the species of lung snails such as *Radix balthica*, *Lymnaea stagnalis*, *Planorbarius corneus*) in the presence of bivalent metal ions coming from the industrial activities of solid waste processing (CISMAŞIU, 2009; CÎRSTEA et al., 2015; CISMAŞIU et al., 2018; CIOBOIU & CISMAŞIU, 2018; CIOBOIU et al., 2019; MURARIU & MAICAN, 2021).

These species represent bioindicators of the industrially contaminated environments in the lower sector of Jiu River because they signal the early occurrence of negative changes within the lacustrine ecosystems (Tables 4; 5).

Table 4. Concentrations of metals from the soil and shells of the branchiate snail *Viviparus acerosus*.

No.	The analysed indicator (mg/Kg/SU)	Victoria (soil)	Snails (shells)	Method of analysis	The used equipment
1	Iron	4590	305	Acid mineralization Working method specified in the user manual atomic absorption spectrometer GBS-Avanta	Avanta GBC atomic absorption spectrometer SN A 5378
2	Manganese	192	123		
3	Nickel	5.2	0.422		
4	Chromium	9.05	6.32		
5	Copper	16.5	12.2		
6	Zinc	20.1	7.05		
7	Cadmium	0.015	0.1		
8	Lead	< SLD	0		

Note: SLD – below detection limit

Table 5. Concentrations of metals from the soil, shells and table meat of the pulmonate snail *Stagnicola palustris*.

No.	The analysed indicator (mg/Kg/SU)	Marica (soil)	Snails (shells)	Snails (table meat)	Method of analysis	The used equipment
1	Iron	3272	290	137.5	Acid mineralization Working method specified in the user manual atomic absorption spectrometer GBS-Avanta	Avanta GBC atomic absorption spectrometer SN A 5378
2	Manganese	237	197	147		
3	Nickel	8.2	1.725	0.675		
4	Chromium	8.95	6.07	4.22		
5	Copper	12.5	10.1	9.32		
6	Zinc	20.17	6.125	7.35		
7	Cadmium	0.19	0.17	0.30		
8	Lead	0.0015	0.0013	< SLD		

Note: SLD – below detection limit

According to the above considerations, selected *Acidithiobacillus (Thiobacillus) ferrooxidans* chemolithotrophic bacteria with a high tolerance to high concentrations of iron, copper and zinc may be used in the solubilization of sulphur ores (pyrite, arsenopyrite) from gold concentrates, especially those refractory to classic cyanide processes Also, acidophilic heterotrophic bacteria of the *Acidiphilium* genus and yeasts, with increased resistance to copper, zinc, nickel and chromium will be able to be used with increased efficiency for the depollution of residual liquid effluents and sediments with high concentrations of heavy metals.

The results regarding the effect of physical and chemical factors on the growth and oxidation / reduction capacity of some compounds specific to acidophilic microorganisms led to the establishment of optimal growth and activity parameters, as well as the identification of populations of interest for further experiments. Also, the obtained results demonstrated the higher efficiency of removing metal ions when using acidophilic microorganisms, heterotrophic bacteria of the *Acidiphilium* genus and yeasts.

These experiments performed in laboratory conditions allowed the collection of acidophilic microorganisms, chemolitoautotrophic and chemoorganotrophic, with important properties in terms of involvement in technological processes of recovery / removal of metal ions from harsh environments (RUSU & ROJANSCHI, 1980; WICHLACZ & UNZ, 1981; HAWKSWORTH, 1992; ZARNEA, 1994; RAWLINGS, 1999; CISMAȘIU et al., 2015).

CONCLUSIONS

The evaluation of the structural-functional features of some representative ecosystems for the territory of Romania made an important contribution when defining the place and the role of the lacustrine complex of Adunatii de Geormane, respectively the Victoria-Marica lakes in the lower sector of the Jiu River.

The physical, chemical, hydrological and biodiversity factors award the lakes a special ecological character for the Oltenia Plain. The lacustrine ecosystems have a high degree of trophicity reflected by the quantitative and qualitative composition of planktonic and benthic communities, among which the gastropods represent one of the most important components as numerical density and biomass.

16 species of gastropods have been identified in all the eutrophic ecosystems from the perimeters of of the lacustrine complex, depending on the biomass. The biggest share belongs to the populations of *Viviparus acerosus, Lymnaea stagnalis*, *Stagnicola palustris*, *Radix balthica* și *Planorbarius corneus*, between 147 g/m^2 and 649 g/m^2. These species have the ability to accumulate metal ions of the type Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} and represent indicators of environments contaminated with heavy metals because they signal early anthropogenic changes in the lacustrine ecosystems of south-western Romania.

In industrial environments, acidophilic microorganisms exist as populations that interact with each other both positively and negatively. The presence of these populations is evidenced both by metabolic products and by the accumulation of biomass. In this sense, there is a direct correlation between the extracellular accumulation of metal ions by acidophilic microorganisms in natural and artificial environments, as well as the amount of biomass in those environments. The synthesis of extracellular enzymes by populations has a special ecological importance in extreme environmental conditions. Overall, the accumulation of products with antagonistic activity contributes together with the quantitative changes of the inorganic substances to the appearance of some changes in the structure of the associates of acidophilic microorganisms.

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