THE RESEARCH ON THE METABOLIC CHARACTERISTICS OF GASTROPODS AND THE ACIDOPHILIC BACTERIA TO IDENTIFY POTENTIAL TECHNOLOGICAL IMPLICATIONS

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Abstract. The study is a component part of the collaboration convention numbers 1797/20.05.2015, respectively 1402/21.05.2015 between the Institute of Biology Bucharest, the Department of Microbiology and the Museum of Oltenia Craiova, Department of Natural Sciences, on the topic: The biodiversity of the microbiota from areas with industrial contamination of Oltenia and potential biotechnological applications in order to reduce it. The researches focused on the following objectives: the structural and functional particularities of the lakeside salvation in the Preajba Valley river basin and the floodplain area of the Danube (Km 811-661); industrial pollution and biological clogging as a result of water evaporation due to climate change, as well as the accumulation of metal ions in *Viviparus acerosus* and *Radix balthica* species; desulphurization of coal from Oltenia using acidophilic bacteria of the *Acidithiobacillus* and *Acidiphilium*. The obtained results revealed the increased tolerance of the gastropods species to the presence of metal ions from the industrial activities of solid waste processing in the environment, *Viviparus acerosus* and *Radix balthica* species being bioindicators of the industrial contaminated media in Oltenia Plain because it signals early the occurrence of some negative changes within the lake ecosystems.

Keywords: Preajba Valley, Danube Delta, populations of gastropods, acidophilic bacteria, Oltenia Plain.

Rezumat. Cercetări privind caracteristicile metabolice ale gasteropodelor şi bacteriilor acidofile cu implicaţii în identificarea potenţialului tehnologic. Studiul este o parte componentă a conventiei de colaborare numerele 1797/20.05.2015 respectiv 1402/21.05.2015 dintre Institutul de Biologie Bucureşti, Departamentul de Microbiologie şi Muzeul Olteniei Craiova, Secţia de Ştiinţele Naturii, având ca temă: Biodiversitatea microbiotei din areale cu contaminare industrială ale Olteniei şi potenţiale aplicaţii biotehnologice în scopul diminuării acestuia. Cercetările au vizat următoarele obiective: particularităţile structurale şi funcţionale ale salbei de lacuri din bazinul hidrografic Valea Preajba şi zona inundabilă a Dunării (Km 811-661); poluarea industrială şi colmatarea biologică, ca urmare a evaporării apei în urma schimbărilor climatice, precum şi acumularea de ioni metalici la specii de gasteropode *Viviparus acerosus*, *Radix balthica*; desulfurizarea cărbunilor din Oltenia folosind bacterii acidofile din genurile *Acidithiobacillus* şi *Acidiphilium*. Rezultatele obţinute au evidenţiat toleranţa crescută a speciilor de gasteropode la prezenţa în mediul înconjurător a ionilor metalici proveniţi din activităţile industriale de prelucrare a deşeurilor solide, speciile *Viviparus acerosus* şi *Radix balthica* fiind bioindicatori ai mediilor contaminate industrial din Câmpia Olteniei deoarece semnalizează timpuriu apariţia unor modificări negative în cadrul ecosistemelor lacustre.

Cuvinte cheie: Valea Preajba, Lunca Dunării, populaţii de gasteropode, bacterii acidofle, Câmpia Olteniei.

INTRODUCTION

Research conducted as part of a comprehensive national program of knowledge of the representative ecosystems of Romania, makes an important contribution to defining the role and place of a system of lowlands basin - the Preajba Valley within Oltenia Plain from Dolj County and the lake ecosystems in the floodplain of the Danube.

The assessment of the biodiversity of the organism communities present in natural and anthropogenic ecosystems is of particular importance in the global context of the disappearance of many species of organisms as a result of human activity. Due to human-induced changes in natural habitats, the identification and protection of natural species is now an international priority (BREZEANU et al., 2011; CIOBOIU, 2014a; b).

This study presents more research on aquatic, lentic and loamy ecosystems in the floodplain areas of the Danube in the Oltenian sector and the Preajba Valley basin system. This research demonstrates the importance of the Danubian-Carpathian space in preserving specific biodiversity.

In the Oltenian sector, according to the results, the algae register the greatest diversity, bacillus and chlorophyll being dominant. Marsh and aquatic macrophytes have an important place in the bioeconomy of the ecosystems in the studied areas. The studies have revealed the existence of 24 fauna invertebrate groups, protozoa, rotifers, copepods, cladocerans, Oligochaeta, gastropods, bivalves, amphipods, dragonfly, Chironomidae being dominant. In these ecosystem assemblies, gastropod species play an important role among consumers, as they represent an accumulation factor and mass transfer towards higher order energy consumers, such as fish.

THE STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF THE LAKES FROM PREAJBA VALLEY UNDER THE INFLUENCE OF CLIMATE CHANGES

The research approach has started from the concept that a basin system presents a wide variety of geographic, physicochemical, and ecological relationships in particular. In these context, taking into account the Preajba Valley basin system, the study was of a priority character because it presents for the first time the structural-functional characteristics of

such a lacustrine complex. The system, enclosed in the Jiu river basin, comprises a network of terrestrial and aquatic ecosystems with an area of 30 Km^2. In this space, there is grouped a variety of ecosystems: small hills covered by pastures and meadows, farmland and a complex river system: springs, streams, rivers, marshes, small reservoirs.

The construction of dams and the formation of lakes have a double effect on a river from the ecologic viewpoint: a positive one, by enhancing the diversity of ecosystems and biodiversity types and another by the imbalance of biocoenotic structures and the deregulation of the physiology and behavior of some populations (especially fish reproduction migration or survival of fish). The same situation is encountered in the course of the Preajba Valley river: on the one hand, the enrichment of the natural environment with new elements of the landscape and the appearance of the lake ecosystem with specific population structures and, on the other, the degradation of the rheophilic ecosystem downstream the dam, as a result of the reduction of the water flow below the minimum required for the preservation and operation of the rheophilic biocoenotic structures.

Referring to the hydrographical and hydrological characteristics of the area, it was revealed that the hydrographic network is the result of the influences of the relief, the lithological structure, the climate and the soil. It should be noted that the Preajba Valley complex, consisting of the main course - the 9.6 km long Preajba river and 6.8 km of its tributary, the Bătrâna Valley, has an area of 20.2 Km^2. The main source of river supply is the 50 springs located at the morphological contact between Romanați Plain and the high terrace of the Jiu River. The 14 lakes formed through the barrage of the Preajba River and its tributary, the Bătrâna Valley, In relation to the precipitation dynamics and the use of lake water for fishery purposes, in some periods, upstream lakes (I - IV) as a result of the evaporation process have a pronounced decrease in water surface. They get the character of marshlands. The surface of the lakes is mainly determined by the width of the valley and the major bed (the former meadow), as well as by the length of the dam and its height (CIOBOIU, 2002; 2011; 2014a).

The peculiarity of this hydrographic basin consists in the fact that it runs in the east-west direction, contrary to the directions of the other hydrographic basins in Oltenia Plain. The importance of this pool derives from the fact that, being 6 km south of Craiova, this area offers a recreational area with multiple functions (watering, sport fishing, etc.). The area is not affected by strong sources of pollution; however, due to the penetration of mineral nutrients from the neighboring agricultural fields where fertilizers are used and the spillage of a small amount of domestic waste water, the lakes have a high degree of eutrophication (CIOBOIU & PLENICEANU, 2005).

The water chemistry is characteristic of the eutrophic ecosystems. In terms of surface water quality conditions, the small lakes of the Preajba river fall in the second category (bicarbonate - sulphato - calcic - magnesium) and can be used for fish farming, as well as for tourist and leisure purposes (CIOBOIU & CHICIUDEAN, 2003; CIOBOIU & BREZEANU, 2009).

THE BIOLOGICAL CLOGGING FOLLOWING WATER EVAPORATION FROM CLIMATE CHANGE AND ACCUMULATION OF METALLIC IONS IN GASTROPODS SPECIES

An important component of these industrially contaminated ecosystems is represented by microorganisms. The microbiological analysis of the affected ecosystems was focused on establishing the presence of the main physiological groups of chemioautotrophic and heterotrophic microorganisms. Chemioautotrophic microorganisms are primary producers able to use chemical energy to produce complex organic substances from inorganic substances (nitrifying bacteria, colorless sulfurous bacteria). The heterotrophic microorganisms are involved in the detritic chain by decomposing the organic residues of the primary producers (CIOBOIU & CISMAŞIU, 2016a; b; c).

In addition to industrial pollution, the lagoon ecosystems on the Preajba Valley river are subject to the anthropogenic eutrophication process. In the increasing eutrophication, lakes are in an advanced process of biological clogging due to water evaporation induced by climate change. Eutrophication effect is manifested by excessive growth of phytoplankton (78 species) and marsh and aquatic macrophytes (34 species). Bacillaryophiceae and chlorophyceae are the dominant phytoplankton groups and, in summer, cyanobacteria also have an intensive development.

The analyses performed illustrate the ability of *Viviparus acerosus* and *Radix balthica* species to accumulate metal ions Fe^{2+}, Mn^{2+} and Zn^{2+} in direct correlation with the concentration of the respective ions in the soil. Also, our studies have shown the increased tolerance of the species of snails (for example species of gills snails such as *Viviparus acerosus*) in the presence of metal ions in the environment coming from the solid waste of industrial processing (CIOBOIU, 2002; CISMAŞIU et al., 2015b; CIOBOIU & CISMAŞIU, 2016b; CIOBOIU et al., 2017). These snail species are bioindicators of industrially contaminated environments in Oltenia Plain because they signal early the appearance of negative changes in lacustrine ecosystems. Although Pb^{2+} and Cd^{2+} are not considered essential for life, they are concentrated in some aquatic organisms by leaching from the aquatic environment. Significant differences were found between the heavy metallic concentrations of water and the meat mass of the aquatic organisms present in these waters. The concentrations of Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+} and Fe^{2+} from the Preajba lakes were found below the detection limit (0.001 – 0.01 mg/l), according to orders 756/1997 and 161/2006 (***. Ord. 756/1997; ***. Ord. 161/2006).

In this context, it has been hypothesized that such models HM transfer and accumulation in biota can be used as a fingerprint for detection and characterization of biochemical risk of environmental pollution in Oltenia Plain. Consequently, river damming and the formation of reservoirs, induce profound hydrological, geomorphological,

hydrochemical modifications of the ecosystem and in accordance with these modifications, modifications of the structures and functions of the plant and animal populations (BREZEANU & GÂŞTESCU, 1996; BREZEANU et al., 2011). Such changes are also evident in the Preajba Valley river basin, especially within its limits, the unsettled segment of the river (the one in the upper sector) being considered as a witness.

Among the microorganisms capable of removing metallic ions there are also the heterotrophic bacteria of the *Acidiphilium* sp. acidophilic bacteria that have the capability to form a polysaccharide gelatin capsule on the surface of the cells. The mechanism of attracting metallic ions by these bacteria is biosorption, retaining relatively large amounts of dissolved metals through electrical charge attraction.

The use of the heterotrophic bacteria isolated from industrial sites contaminated with metal ions to reduce the heavy metal content is substantiated by numerous publications and it is considered that the development of a technology cost of this group of microorganisms is very good for the bioremediation processes (BOONSTRA et al., 1999; BOSECKER, 1999; CISMAŞIU, 2010; CISMAŞIU et al., 2015b; 2016; 2017; CIOBOIU & CISMAŞIU, 2018a; b).

THE COAL DESULPHURIZATION PROCESSES FROM OLTENIA UNDER THE ACTION OF HETEROTROPHIC AND CHEMOLITHOTROPHIC ACIDOPHILIC BACTERIA

It is now accepted that starting with 3.5 million years ago, that is from the appearance of life on earth, some bacteria release or extract metal elements from the rock that go into solution, a phenomenon called bioleaching or biosolubilization or bio-extraction, and other groups of bacteria fix and accumulate metals, a phenomenon called biosorption, bioaccumulation or biofixation (LAZĂR & DUMITRU, 1998; BRANDL, 2001; LAZĂR, 2001).

The indigenous microorganisms are opposed to the imbalance caused by temporary environmental changes (e.g. industrial wastewater spill into soil or natural waters). To reduce the imbalance in the normal processes involved homeostasis made by consumers. The adverse effects of heavy metals on the aquatic ecosystem are manifested: (1) quantitative: reduction of water transparency and clogging of filters at capture, (2) chemical: change of O_2 level, CO_2 level, pH values and extreme conditions, (3) the modification of the biocoenosis structure (CIOBOIU & CISMAŞIU, 2016).

The acidophilic chemolithotrophic bacteria of the *Acidithiobacillus* genus, such as s sulfur- and iron-oxidizing bacteria, have a versatile metabolism because they release organic compounds in the culture medium that can accumulate in inhibitory amounts for bacterial growth. In this regard, heterotrophic bacteria of the genus *Acidiphilium* can remove this inhibition by the metabolism of organic materials. This phenomenon is the reason why mixed cultures of acidophilic iron-oxidant and heterotrophic bacteria have a higher leaching capacity compared to pure cultures. The activity of *Acidithiobacillus ferrooxidans* in the oxidation of the ferrous iron may be controlled by several parameters: H_2SO_4 production, decrease of pH, CO_2 consumption, the biomass growth or directly by measuring the passage of Fe^{2+} to Fe^{3+}. A field widely approached internationally with immediate practical applications is the use of microorganisms in microbiological recovery of metals. This method can be applied to ores and poor concentrates, as well as mining waste that accumulates over time and cannot be processed by classical hydrometallurgical methods (TOMUŞ & CISMAŞIU, 2014; GAVRILESCU et al., 2017).

The changing iron valence can be measured by changing the redox potential, which is a good indicator for determining microorganisms and allow the study of their physiology. Bacterial cultures of *Acidithiobacillus ferrooxidans* (9 populations), selected on the basis of their resistance to very high concentrations of ferrous iron (18 g/l Fe^{2+}), Cu^{2+} and Zn^{2+} (5000 ppm), were used in the desulfurization experiments (CISMAŞIU, 2010). The biooxidation processes of inorganic sulfur from lignite (Halânga mine) and coal (thermal power mind) for different values of the ratio of the solid / liquid (5 g/100 ml - 10 g/100 ml) in the presence of *Acidithiobacillus ferrooxidans* bacterial cultures under continuous stirring (KARAVAIKO, 1988; KLEIN, 1998; CISMAŞIU, 2015a).

The mutualism process has a special ecological significance because the metabolic activity and limits of physiological tolerance of the population of acidophilic bacteria involved in mutua relations are very different from those of the individual population. In the case of acidic environments the association is so close that the populations of associated microorganisms behave as a unitary population. In this context, the natural communities of microorganisms of a wide variety of common species, often as dense populations, are relatively stable and difficult to disrupt.

In the solubilization process of metal ions, an important role is played by the acidophilic heterotrophic bacteria and yeast, which is based on the elimination of organic acids in the environment, as well as metabolic products which together form soluble compounds in the water. An example of mutualism in industrial ecosystems is the iron cycle between sulfur and iron-oxidizing chemolithotrophs (using iron as an electron donor) and heterotrophic iron-reducing bacteria (using iron as an electron acceptor) in situations where oxygen concentrations dissolved varies spatially or temporarily (MURARIU, 2002; CISMAŞIU, 2012; CISMAŞIU et al., 2015a; b).

The best results of coal desulphurization have been obtained by the combined use of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*. The presence of other species in desulphurization cultures has proven useful in increasing the level and yield of coal desulphurization. Also, some of these species (including heterotrophic bacteria) can metabolize simple fragments of organic sulfur by removing a low percentage of organic sulfur left in the coal matrix (MONROY-FERNANDEZ et al., 1995; VIDYALAKSHMI et al., 2009).

DISCUSSION

Different studies illustrate the toxic effects of metals on living organisms and particularly effective technologies for reducing concentrations of metals in the waste water, the treatment of classical and modern systems thereof. The reduction of SO_2 in coal can have many applications in the fields of environmental protection in the former mining areas and facilities for the processing of coal, and in the zones adjacent to the bioremediation of media contaminated by sulfates and metal ions, in agriculture, food industry, in the chemical and pharmaceutical industry (BOSECKER, 1999; TOMUŞ & CISMAŞIU, 2014).

With regard to the physical-geographical, hydrological and biocoenotic factors, it can be concluded that the Preajba Valley basin system is a true *natural laboratory*, which demonstrates how anthropogenic factors cause the change of natural ecosystems and contribute to the building of other structural and functional relationships within the complex of ecosystems (FIRA & NĂSTĂSESCU, 1977; VICOL, 2011; CIOBOIU, 2014a; b).

The composition and distribution of the biocoenotic and population structures of the Valea Preajba Basin system operate in accordance with the particularities of ecosystem organization. In this regard, species of diatoms, ciliates, testos, ostracodes, gamarides, chironomides, ephemeropters, heteropters were identified in the rhitron area (including springs and small streams). In the potamon area, the structure of biocenoses is made up of representatives of all aquatic ecosystem groups (pelagial and benthic communities) in the potamon area (the hill and river sector of the Valea Preajba river basin). Thus, species of diatoms, ciliates, Testacea, ostracods, Gammaridae, Chironomidae, Ephemeroptera, Heteroptera were identified in the rhitron area (including springs and small streams). In the potamon area (the hilly sector of the Preajba Valley river basin), the structure of biocoenoses is made up of representatives of all aquatic ecosystem groups (pelagic and benthic communities). In the phytoplankton, there were identified over 70 species of algae, while in the zooplankton 65 species of ciliates, rotifers, cladocerans, copepods. Zoobenthos is composed of 13 major groups of invertebrates, some with a large number of individuals (Chironomidae 4,600 ind./m^2, ostracods 1,750 ind./m^2, Ephemeroptera 213 ind./m^2, Plecoptera 226 ind./m^2). Gastropods constitute the dominant group in the benthic structure (18 species have been identified, of which 5 are new species for the Oltenia fauna) and have an important role in the functioning of the habitats they inhabit. In the lakes of the basin, the ichthyofauna consists of a number of 14 species in which the cyprinids are dominant (CIOBOIU & NICOLESCU, 1999; CIOBOIU 2014a, b; GOGA, 2016; GAVRILESCU et al., 2017).

The research objectives are the results obtained under the collaborative convention between the Institute of Biology Bucharest of the Romanian Academy, the Department of Microbiology and the Museum of Oltenia Craiova on the topic: The biodiversity of the microbiota from areas with industrial contamination of Oltenia and potential biotechnological applications in order to reduce it. Also, some results were achieved in the project RO1567-IBB05 of the Institute of Biology Bucharest, Romanian Academy.

CONCLUSIONS

In anthropogenic environments, native microorganisms exist in the form of mixed populations, interacting with each other, both positively and negatively. Their presence is evidenced by the products of metabolism rather than by the accumulation of biomass. The accumulation of products with inhibitory activity and the phenomena of antagonism contributes together with the quantitative changes of nutrients to the emergence of new communities of microorganisms that underlie the succession of populations in an ecosystem. In this whole ecosystem, gastropod populations have an important role among consumers, representing a factor of accumulation and transfer of mass and energy towards higher order consumers - fish.

The populations of *Viviparus acerosus* and *Radix balthica* are one of the reference factors for the accumulation of Cu^{2+} and Cd^{2+} heavy metals. Bioaccumulation is strongly dependent on the amount of metal present in the living environment of sediments. The freshwater snails can accumulate higher levels of Cu^{2+} and Cd^{2+} concentrations than the average and they are generally recognized as "macroconcentrator" HMS for these species.

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