STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF MICROORGANISMS INVOLVED IN PROCESSES OF METAL IONS CONTROLLED BIOREDUCTION IN ORDER TO RECONSTRUCT BIOCENOTIC STRUCTURES

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Abstract. The extreme environments represent a particular relevance to the study of ecological and evolutionary relations between microorganisms. The positive interactions in polluted environments with inorganic substances are particularly significant in case of the degradation of recalcitrant substances or metabolites; they are products of the solubilisation of the various compounds of the habitat, by making them available for other organisms. The implications of acidophilic heterotrophic microorganisms in the development of metal removal processes prevent the accumulation of heavy metal ions in the trophic chains and persistence in nature. The effects of pollution translate into changes in the flora and fauna. The negative impact of heavy metals may be increased by some environmental conditions. It was found that at a certain concentration of heavy metallic ions, temperature increase reduced by half of the fish survival times. This study was focused on the isolation of acidophilic bacteria as pure cultures and the reduction of heavy metallic ions from environments contaminated with organic compounds using autotrophic and heterotrophic microorganisms isolated from mining waster waters and tailings preloaded from representative areas in Romania.

Keywords: mining effluents, anions, organic substances, amylases.

Rezumat. Caracteristicile structurale şi funcţionale ale microorganismelor implicate în procese de bioreducere controlată a ionilor metalici în vederea reconstrucţiei structurilor biocenotice. Mediile extreme prezintă o importanţă deosebită pentru studiul relaţiilor ecologice şi evolutive dintre microorganisme. Interacţiunile pozitive în medii poluate cu substanţe anorganice sunt deosebit de importante în cazul degradării substanţelor recalcitrante sau a metaboliţilor; sunt produsele solubilizării diferiţilor compuşi ai habitatului, făcându-le disponibile pentru alte organisme. Implicaţiile microorganismelor heterotrofe acidofile în dezvoltarea proceselor de îndepărtare a metalelor previne acumularea ionilor metalici în lanţurile trofice şi persistenţa în natură. Efectele poluării determină schimbări în floră şi faună. Impactul negativ al metalelor grele poate fi intensificat prin anumite condiţii de mediu. S-a constatat faptul că, la o anumită concentraţie de ioni metalici creşterea temperaturii reduce la jumătate timpul de supravieţuire a peştilor. Acest studiu a fost axat pe izolarea bacteriilor acidofile în cultura pură şi reducerea ionilor de metale grele din medii contaminate cu substanţe organice utilizând microorganisme autotrofe şi heterotrofe izolate din ape reziduale industriale şi steril minier din zone reprezentative din România.

Cuvinte cheie: efluenţi minieri, anioni, substanţe organice, amilaze.

INTRODUCTION

In Romania, the wastewater coming from copper ore operating and processing activities is collected in a settling pond or lake, through a series of effluents that start both in the surface exploitation area of the copper sulphide ores and from the flotation where these ores are processed. In this pond, as a result of a natural process of bioleaching due to the sulfur- and iron oxidizing bacteria, there occurs a drastic decrease in pH and an increase in the concentration of heavy metals (CISMAȘIU, 2009).

The activity of optimization has been materialized through a series of laboratory experiments which mainly rely on microbial cells affinity for certain surfaces. The best known microbial restraint methods are: a) inclusion in inert materials (exp. agar); b) adsorption on an inert solid material; c) immobilization by affinity links between biological molecules; d) cell immobilization via covalent or coordinative bonds with materials other than the inert ones. Among the materials used as immobilization supports we can mention: the agar, the molecular sieves, the activated carbon, the volcanic tuffs, the ion exchange resins, the zeolite, the silica gel. In case of using ion-exchange resins we can get a bacterial cell separation out of a mixture (consortium) based on affinities different from some anion or cationic ion exchangers. The anionic ion exchange resins at neutral pH adsorb the Gram- bacteria and desorbs the Gram + bacteria, while the cationic ion exchanger adsorb the Gram + bacteria and desorbs the Gram + bacteria at low pH. Also, the most used neutral supports for immobilizing the whole bacterial cells are: the activated carbon, the molecular sieves, the volcanic tuffs, the ceramics and the agar (BAKER & BANFIELD, 2003; CISMAŞIU, 2001; CISMAȘIU et al., 2010).

The bacterial biofilm is defined as a microbial population included in a matrix adherent to the surfaces or interfaces of some solid supports in liquid medium. The physiological cooperation is the main advantage of this structure. Cellular joining and exuberant production of polysaccharide matrix create specific microclimate conditions for each of the biofilm bacteria. The biofilm is a community that works in a coordinated way, so it is more efficient than the mixed populations of floating planktonic microorganisms. The submerged biofilters method is used in order to speed up the water depollution. By this method, the water mass is passed through filters made of different materials in granular form. Bacterial biofilms are formed on the surface of these granules (DJUKIC & MANDIC, 2006; CISMAŞIU, 2009).

The behaviour of primary producers (macrophytes, phytoplankton and periphyton) initiates the concentration of biogenic substances, turning them into mass plant material source of energy for consumers. In these processes, much of the accumulated stock of material and energy in all trophic levels and the links are in revert to the chain circuit by primary producers. In this context, they have the main role of bacterial populations, which converts organic mass degradation resulting from dead plants and animals and elemental substances in the circuit resumes. In these circumstances a large part of the organic mass have accumulate in the benthal form of the degraded organic material – detritus. In this ecosystem, gastropod populations have an important role among consumers, which constitute a factor of accumulation and transfer of mass and energy by the consumers of higher order – fish (CIOBOIU, 2014).

MATERIAL AND METHODS

1. The microbial system of the field target

Acidophilic heterotrophic microorganisms, due to their ability to adsorb and concentrate heavy metallic ions from industrial waste waters into the cells, could represent a performance source of bacteria in biotechnological processes in order to eliminate the metallic ions (CISMAȘIU, 2001; 2009).

The researches on the biocenotic structures of the vegetable and animal populations in the chain of lakes of the Valley Preajba have revealed essential peculiarities of the organization and functioning of these ecosystems (CIOBOIU, 2002; BREZEANU et al., 2011).

Water samples were collected (250 ml) in sterile bottles to determine the chemistry using the DR 2000 spectrophotometer analysis and sediment samples from the reservoir habitat Preajba Valley (Oltenia Plain) were analysed for determining the levels of chromium, nickel, zinc, lead, cadmium, copper, manganese, and iron using flame atomic absorption spectrophotometer Avanta GBC, 5378 SN.

Samples are taken in containers of clean inert material (glass, plastic and paper); after sampling, shredded organic material in the mortar dries to about 70 ^{0}C. There were also determined, by the same method, the concentrations of heavy metals in *Viviparus acerosus* shells, the dominant species in the lakes, especially downstream (Fig. 1).

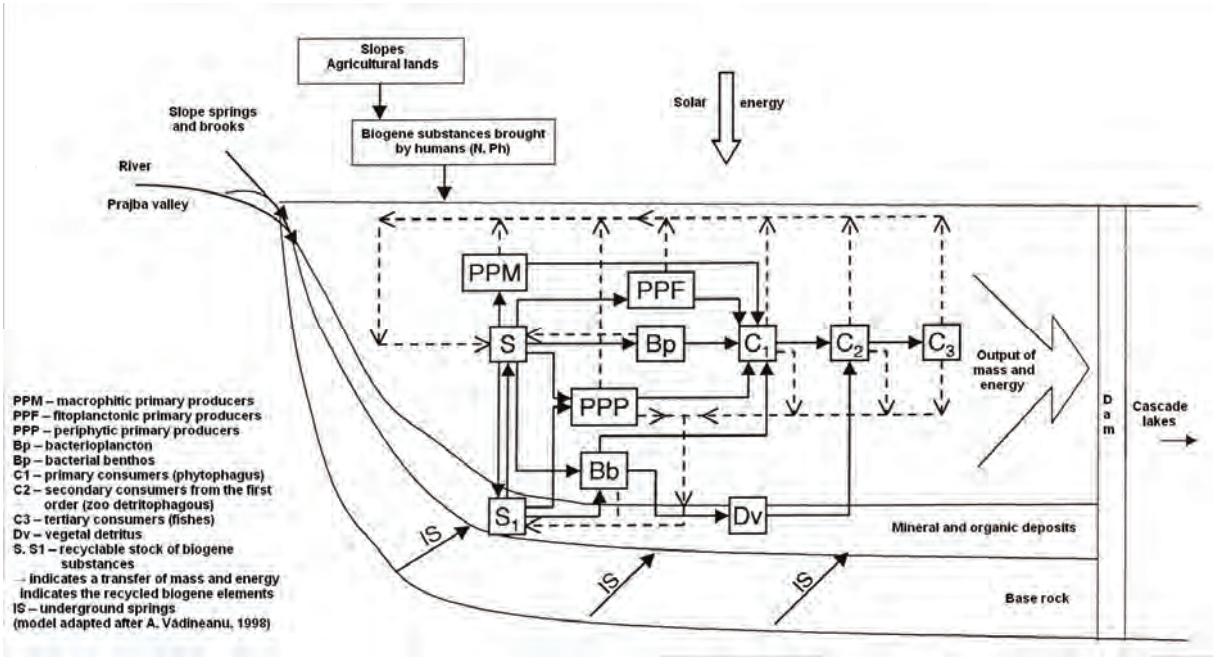

Figure 1. Scheme of the homomorphous model of the functional structures specific to the small reservoirs from Preajba Valley (Oltenia Plain) (after BREZEANU et al., 2011).

2. Selection of the field test

In laboratory conditions, the tolerance study of the bacterial cultures to different concentration of Cu^{2+}, $Zn^{2+}$and Ni^{2+} permitted the selection of strains and populations, which show an increased resistance to these ions towards improving the biosorption processes. Several studies have shown significant variations regarding the assimilative capacity of heavy metallic ions between different genera, species and even among strains of the same species (CISMAȘIU, 2010).

Mineralization made in order to shift the metals in solution is achieved in Ethos microwave type D, power 1000W, equipped with Teflon tubes, programmable and occurs as follows:

- weigh approximately 0.5g, 1g dry organic material, graded and placed in Teflon tube;

- 3 ml of nitric acid 65%, 2 ml HCl and 1 ml hydrogen peroxide 37% in the tube and then left standing a few minutes;

- mineralized samples filtered through quantitative filter paper, each fresh acid extract is collected in one 25 ml; volumetric flask, add 5 ml of each sample added 5ppm standard gauge, and adjust the volume with distilled water;

- aspirate the standard solutions in ascending order of concentration and blank (zero) to construct the calibration curve, the following wavelengths type atomic absorption spectrometer Avanta (GBC) equipped with flame burner for air/acetylene lamps determined corresponding hollow cathode metals (Table 1).

Table 1. Synoptic data of wavelength for some heavy metals determined.

Metallic ions determinates	**λ (nm)**
Pb^{2+}	217
Cd^{2+}	228.8
Cu^{2+}	324.7
Ni^{2+}	232

RESULTS AND DISCUSSION

In natural and human environments the acidophilic microorganisms exist in the form of mixed populations, interacting with each other, both positively and negatively. Their presence is highlighted more by metabolites (the most obvious are the reddish deposits rich in ferric iron) and not by the accumulation of biomass; in others it is the reverse. The latter is seen in the formation of gelatin macro structures - "acid filaments", widespread in all sites in the world (especially in underground areas).

Biosorption or biofixation is a process that unfolds rapidly and the differentiations occurring in the ability of microorganisms to fix different metal ions depend on the specificity of the microorganisms to different metal ions, on the cell wall composition and structure, as well as on the ion exchange reaction that occurs in the cell wall. For extracellular accumulation of metal ions from the industrial effluents, especially for those coming from the extraction and processing of minerals, the microbial biotechnologies such as biosorption or bioaccumulation processes are based on microbial systems, live or killed (dried), free or immobilized on various adsorbents.

Acidophilic bacteria were tested for their efficiency in order to improve reduction processes of metal ions in different physic-chemical environment characteristics such as pH, temperature, organic and inorganic substratum on their development and extracellular enzymatic activities. Our results indicate the maximum hydrolysis activities of organic substances from bacterial cultures belonging to the *Acidiphilium* genus which was determined in selective medium with 0.3% organic compounds and pH 3.0 in the first 7 days of incubation at 28^0C; these features are correlated with physic-chemical terms of mining sites (Figs. 1-4).

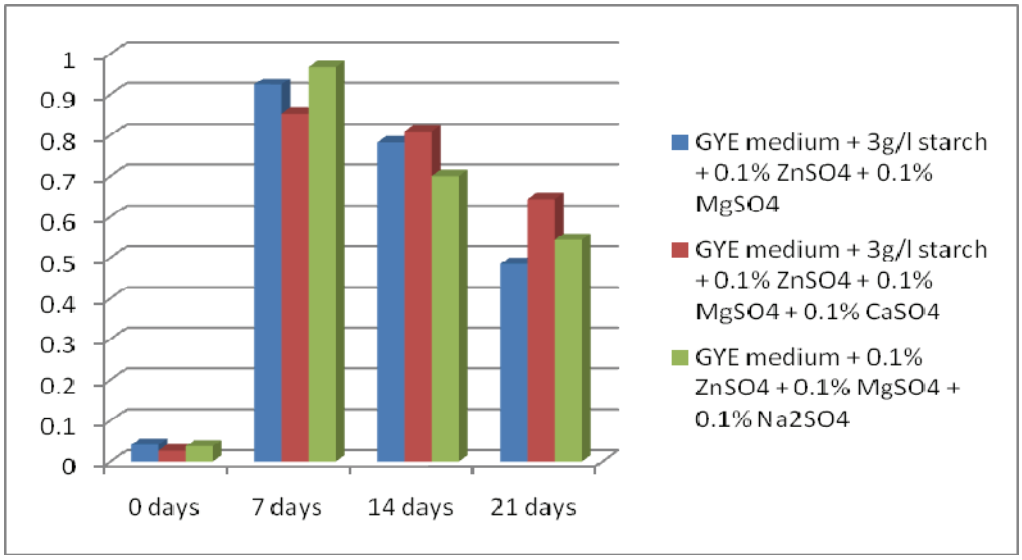

Figure 1. The development of the *Acidiphilium* population in GYE medium with 0.1% $ZnSO_4$ in the presence of environmental cations (Ca^{2+}, Mg^{2+} and Na^{+}) at 3g/l starch.

In spatially organized ecosystems, the degree of cooperating between cooperative microorganisms depends on the concentration and diffusion gradients of dissolved substances, such as nutrients and metabolites. Based on the results of the physic-chemical characterization of samples taken from the mining effluents, it was considered that this study is necessary. It is about the optimization of the biosorption process of the metal ions contained in mining effluent samples, using industrial water samples from the tailings pond, which has a much higher degree of pollution in sulphates and heavy metal ions such as iron, copper, zinc, nickel (Figs. 1-2).

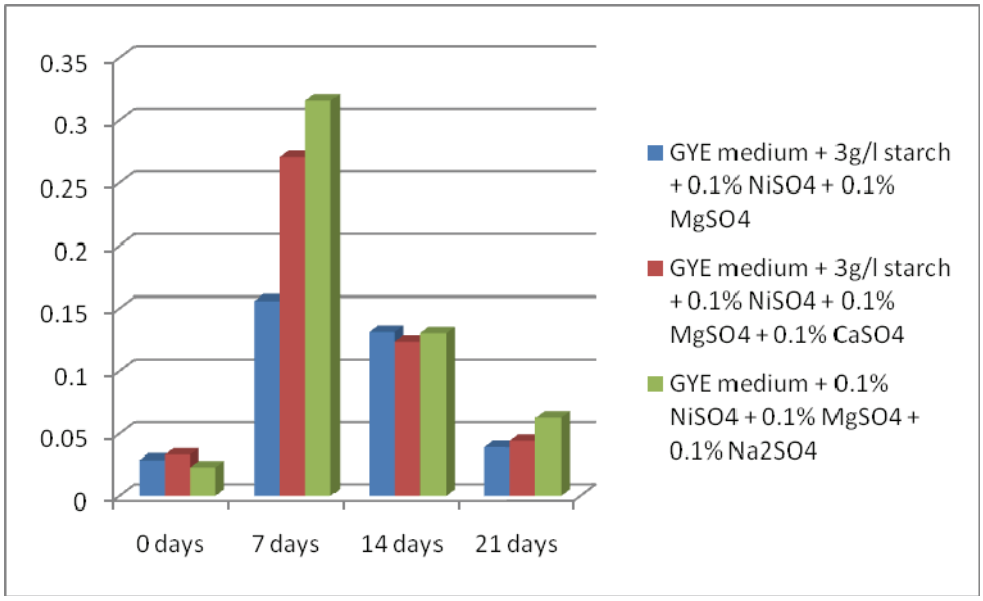

Figure 2. The development of the *Acidiphilium* population in GYE medium with 0.1% $NiSO_4$ in the presence of environmental cations (Ca^{2+}, Mg^{2+} and Na^{+}) at 3g/l starch.

The main microorganisms involved in the bioaccumulation processes of heavy metallic ions from industrial waste waters are acidophilic heterotrophic bacteria, which form a heterogeneous group of bacteria belonging to the *Acidiphilium* genus. From this point of view, these bacteria isolated from polluted environment are able to take up heavy metallic ions from the studied area through some mechanisms such as: the formation of organic acids, the reactions of reduction, the excretion of organic complexion agents (Figs. 3-4).

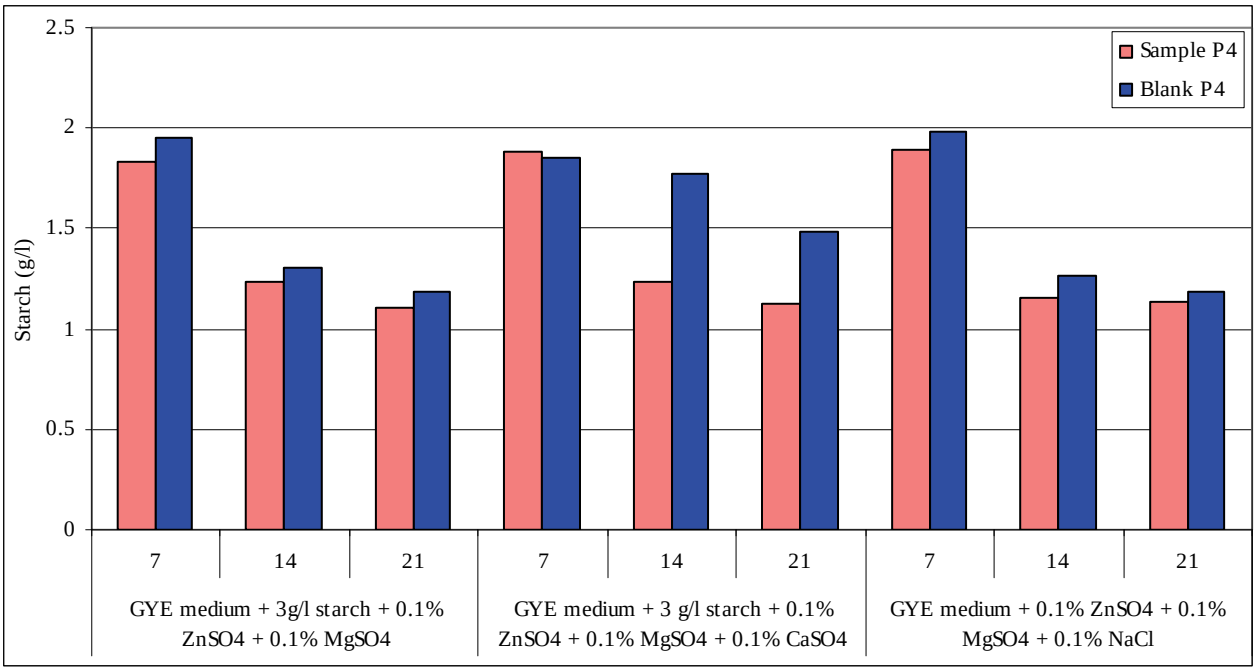

Figure 3. The starch degradation activity of the *Acidiphilium* population in GYE medium with 0.1% $ZnSO_4$ in the presence of environmental cations (Ca^{2+}, Mg^{2+} and Na^{+}).

Studies have shown that water and sediment samples taken from areas situated downstream the tailings pond contain a richer and more diverse microflora compared to the mine effluent that is collected in that tailings pond. The results of the microbiological analysis correlate with the physic-chemical characteristics of the water and sediment samples from the mining effluents, meaning that the mining effluent collected in the tailings pond is much more polluted with metal ions, with a more acidic pH value, compared to the one taken from a creek located at a distance of 3-5 km downstream (Figs. 1-4).

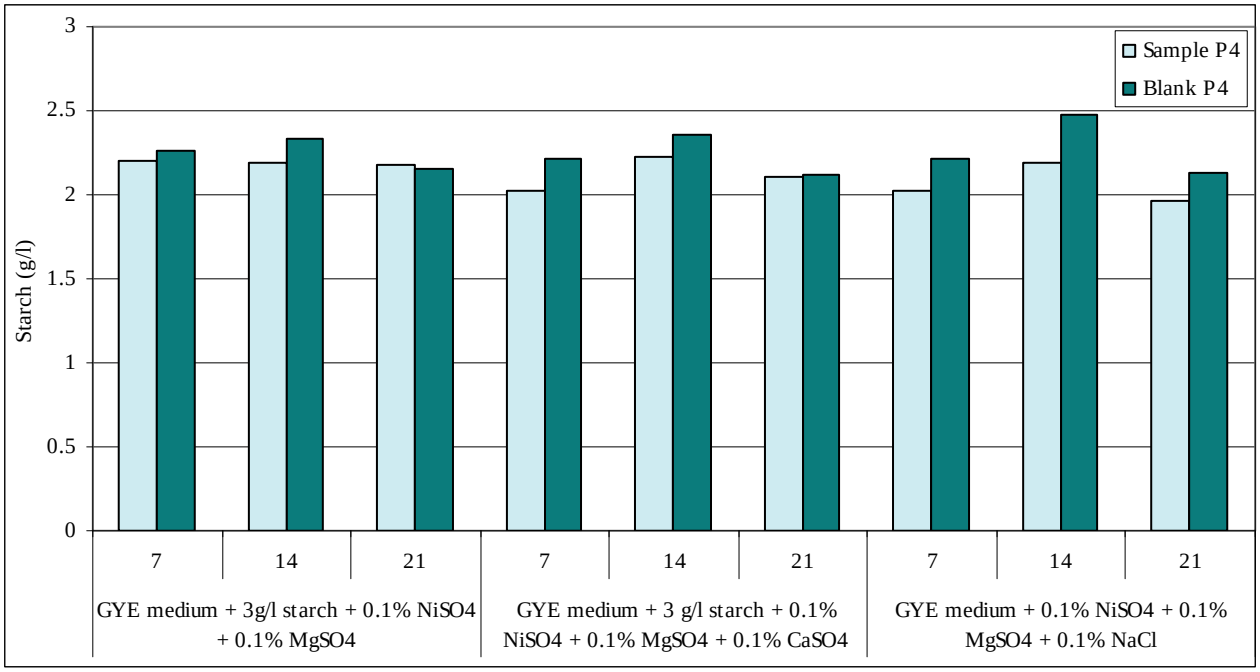

Figure 4. The starch degradation activity of the *Acidiphilium* population in GYE medium with 0.1% $NiSO_4$ in the presence of environmental cations (Ca^{2+}, Mg^{2+} and Na^{+}).

It was felt that the essential elements that determine the development of the planktonic and benthic communities are hydrologic factor such as the Preajba River and adjacent springs on the one hand and on the other trophic factor determined by nutrient intake. These two elements, hydrological factors and other nutrients in terms of stimulating factors (solar energy, temperature, etc.) trigger the mechanisms that lead to the establishment of trophic structures and relationships, transfer of material and energy in the ecosystem. Pb^{2+} and Cd^{2+} are not considered essential for life, but they are concentrated in some aquatic organisms in the aquatic environment.

We found significant differences between the concentrations of heavy metals in water and aquatic organisms present in those waters (Fig. 5). The concentrations of Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Fe^{3+} in the reservoirs of the Preajba Valley found below the limit of detection. Also, pH values range from 7.29 to 8.64 (slightly alkaline range) in accordance with bicarbonates content (414-695 mg/l). Among cations, there stands primarily calcium (Ca^{2+}) the origin of which is considered in the sedimentary rocks of the basin lakes and the amendments applied within farmlands. Ions of calcium, magnesium with carbonates, bicarbonates and sulfates present in the waters of the lakes, are due to higher hardness of the total water (over 30 degrees German).

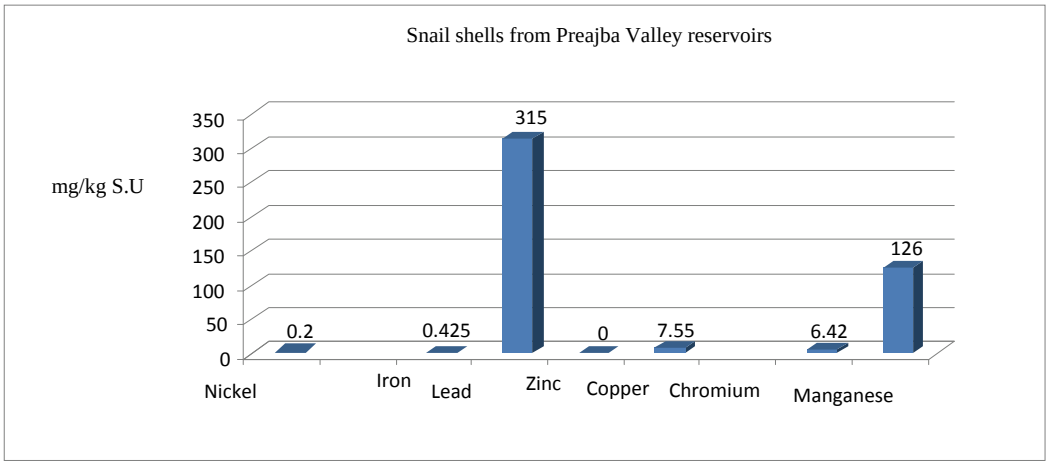

Figure 5. Concentrations of heavy metallic ions from snail shells of *Viviparus acerosus*.

Water chemistry varies in relation both with natural springs and streams and anthropogenic nutrient loads carried by rainwater coming from the neighbouring agricultural areas (CIOBOIU & PLENICEANU, 2005). The chemical composition of eutrophic water ecosystems is specified; the concentrations of the nutrient medium (NO_3^- and PO_4^{3-}) is 18.5 mg / l or 7.9 mg / l (CIOBOIU & CHICIUDEAN, 2003). In terms of the quality requirements for surface

waters, the small reservoirs from the Preajba river falls into the category II (bicarbonate - sulphate - calcium - magnesium) which means if can be used for fish farming (apart from Salmonids) and tourist and recreational purposes (CIOBOIU & BREZEANU, 2009; CISMAŞIU et al., 2010).

Positive interactions are cooperative relationships that increase the growth speed of the associated organisms. They prevail in low-density populations and when the growth speed is below the optimum. Colony forming provides not only aggregation of individual organisms, but also a more efficient use of available resources. Overall, the accumulation of products with inhibitory activity and the antagonism phenomena contribute (together with the quality and quantity changes of nutrients) to the appearance of changes in the microorganism community structure, underlying the succession of populations in an ecosystem (BAKER & BANFIELD, 2003; DJUKIC & MANDIC, 2006; CISMAŞIU, 2012).

It can be concluded that bioaccumulation is heavily dependent on the quantity of metal present in the living environment of sediment. We hypothesized that such patterns of accumulation and transfer HM in biota can be used as a fingerprint for the detection and characterization of biochemical risk of environmental pollution in a specific area (DALLINGER et al., 2001; NIANZHI, 2003; NICA et al., 2012).

CONCLUSIONS

The cooperating interactions have a great ecological importance, especially in extreme situations. The formation of colonies of microorganisms is probably an adaptation based on cooperative interactions in population. The production of extracellular enzymes by some members of the colonies makes the substrates available to all the population members.

Productions of organic and inorganic acids alter the natural environment, which becomes inaccessible to susceptible microorganisms. One example is the productions of organic acids by the acidophilic heterotrophic bacteria belong to the *Acidiphilium* genus, which lowers the pH value of mine draining water at 2.5-4.5. These waters prevent the growth of both acid-sensitive microorganisms in the water courses in which they are discharged and the microorganisms themselves in that habitat. In the metallic ions biosorption process there can occur both mediated metabolic phenomena and no metabolic phenomena, depending on the chemical and physiological reactions involved in reduction processes. These reactions depend on the physiological conditions of the cells, on the chemical state of the metal ions in the impact with the microbial cells, and all of these are strongly influenced by the chemical conditions of the environment and the presence of other metals.

The natural communities of microorganisms consisting of a great variety of species that live in common, frequently as dense populations, are relatively stable and difficult to disrupt. The indigenous microorganisms oppose the imbalance produced by ecological temporary changes (exp. discharge of wastewater into the soil or natural water). In reducing this imbalance to the normal values there occur homeostasis processes taken by predators. Our study illustrates the efficiency of degradation of inorganic substances under the enzymatic activity of acidophilic microorganisms in the presence of the above mentioned experimental conditions for biocenotic structures reconstruction of representative miming areas in Oltenia Region. Because freshwater snails can accumulate higher concentrations of Cu^{2+} and Cd^{2+} than the average, they are generally recognized as "macroconcentrator" HMS for these species. By ranking the 18 species identified in lakes according to the biomass, it was found that the percentage is higher in the populations of *Viviparus acerosus*, 147 g/m^2 to 649 g/m^2, this species representing a reference factor in the accumulation of heavy metals.

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