

## ECOLOGICAL AND TECHNOLOGICAL IMPLICATIONS OF THE BIODIVERSITY SPECIFIC TO LACUSTRINE ECOSYSTEMS FROM THE OLTENIA SECTOR OF THE DANUBE LOWLAND

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**Abstract.** The construction of a 1,158 km long dam on the Romanian course of the Danube led to the disappearance of more than 400,000 hectares of lakes, ponds, marshes. The Danube was not dammed between the kms 817 and 665, and is characterized by structural and functional diversity of lacustrine ecosystems. This sector, covering 56,425 ha, is a sample of the former floodplain, which preserves biocoenotic structures specific to wetlands. Eutrophication is one of the fundamental factors affecting the structure and functionality of lacustrine ecosystems. The benthos facies consists of a thick layer of organic silt and vegetal detritus, explaining the clogging tendency of the lakes. The main groups of benthic invertebrates are Oligochaeta, Chironomidae, Plecoptera, Bivalves. Gastropods represent the dominant group of the benthic production with 37 species. Thus, the development of technological processes based on the activity of indigenous microorganisms demonstrated their involvement in the biogeochemical cycles, by: chemical-bacterial solubilisation of industrial wastes and precipitation of metal ions from industrial effluents; concentration and removal of heavy metals from industrial waste waters. Acidophilic microorganisms present in soil and water, through the mineralization of organic substances, ensure the circulation of organic matter, releasing mineral elements used by plants for their nutrition. The role of acidophilic microorganisms in reduction of soluble iron sulfide and other metal sulfides to insoluble ions is well established. The presence of other species in the microbial culture has proven useful in increasing the oxidation and reduction rate of heavy metals from extreme environments. The study of the way in which acidophilic microorganisms interact in extreme environmental conditions has a strong influence over the technologies used for the remediation of environments polluted with heavy metallic ions. The knowledge of the degree of spread use of acidophilic microorganisms is a benchmark in establishing the influence of extreme environmental conditions on the processes of bioaccumulation and solubilization of heavy metal ions by their action on metabolic processes in the microbial cell.

**Keywords:** eutrophication, lacustrine ecosystems, acidophilic microorganisms, enzymes, extreme environments.

**Rezumat. Implicațiile ecologice și tehnologice ale biodiversității specifice ecosistemelor lacustre din sectorul oltean al Luncii Dunării.** Construcția unui dig în lungime de 1158 Km pe cursul românesc al Dunării a determinat dispariția a peste 400.000 ha de lacuri, bălți și mlaștini. În zona inundabilă a Dunării, între km 817-665, Dunărea nu a fost îndiguită și se caracterizează prin diversitatea structurală și funcțională a ecosistemelor lacustre. Acest sector, în suprafață de 56.425 ha este un eșantion al zonei inundabile ce a dispărut și care conservă structurile biocenotice specifice zonelor umede. Eutrofizarea constituie unul dintre factorii fundamentali care afectează structura și funcționalitatea ecosistemelor lacustre. Faciesul bental este alcătuit dintr-un strat gros de măt organic și detritus vegetal, ceea ce explică tendința de colmatare a lacurilor. Principalele grupe de nevertebrate bentonice sunt: oligochetele, chironomidele, plecopterele, bivalvele. Gasteropodele constituie grupul dominant al producției bentale, fiind identificate 37 specii. În acest context, dezvoltarea proceselor tehnologice bazate pe activitatea microorganismelor autohtone au demonstrat implicarea lor în ciclurile biogeochimice globale prin: solubilizarea chimico-bacteriană a deșeurilor industriale, precum și precipitarea ionilor metalici din efluentii industriali; concentrarea și îndepărtarea metalelor grele din ape reziduale industriale. Microorganismele acidofile prezente în sol și ape, prin acțiunea de mineralizare a substanțelor organice, asigură circulația materiei organice în natură, eliberând elementele minerale și redându-le plantelor pentru nutriția lor. Rolul microorganismelor acidofile în reducerea sulfurilor de fier solubile și a altor sulfuri metalice la ioni insolubili este bine stabilit. Prezența celorlalte specii în cultura microbiană s-a dovedit utilă în creșterea vitezei de oxidare și reducere a metalelor grele din medii extreme. Studiul modului în care microorganismele acidofile interacționează între ele în condiții extreme de mediu are o influență puternică asupra tehnologiilor utilizate pentru remedierea mediului cu ioni de metale grele. Cunoașterea gradului de răspândire a microorganismelor acidofile constituie un punct de reper în stabilirea influenței condițiilor extreme de mediu asupra proceselor de bioacumulare și biosolubilizare a ionilor de metale grele prin acțiunea lor asupra proceselor metabolice de la nivelul celulei microbiene.

**Cuvinte cheie:** eutrofizare, ecosisteme lacustre, microorganismele acidofile, enzime, medii extreme.

### INTRODUCTION

Along the Romanian Danube sector, a 1,158 km long dam was constructed, that separated the Danube from its area prone to floods. The construction of the dam determined the disappearance of lakes, marshes and bogs over a surface of 400,000 ha. It was one of the most aggressive human activities that changed the ecology of a vast territory (ANTIPA, 1910). Between the kms 817 and 665, the Danube was not dammed and is characterized by the structural and functional diversity of the lacustrine ecosystems located in southern Romania.

This sector, covering 56,425 ha, is a sample of the floodplain that disappeared; thus, it still preserves the biocoenotic structures specific to wetlands affected by industrial activities (CIOBOIU & CISMAȘIU, 2016). The main ecosystem components in the area between the localities Cetate and Dăbuleni in the sector of the Danube Floodplain are: lakes (Fântâna Banului, Hunia, Maglavit, Golenți, Desa-Ciuperceni, Bistreț, Cârna, Călugăreni), ponds (Arcear, Manginița, Țarova, Balta Lată), brooks (Milu's brook), a series of swamps and channels (Fig. 1). These ecosystems are subject to anthropogenic eutrophication process and industrial pollution (CIOBOIU et al., 2020).

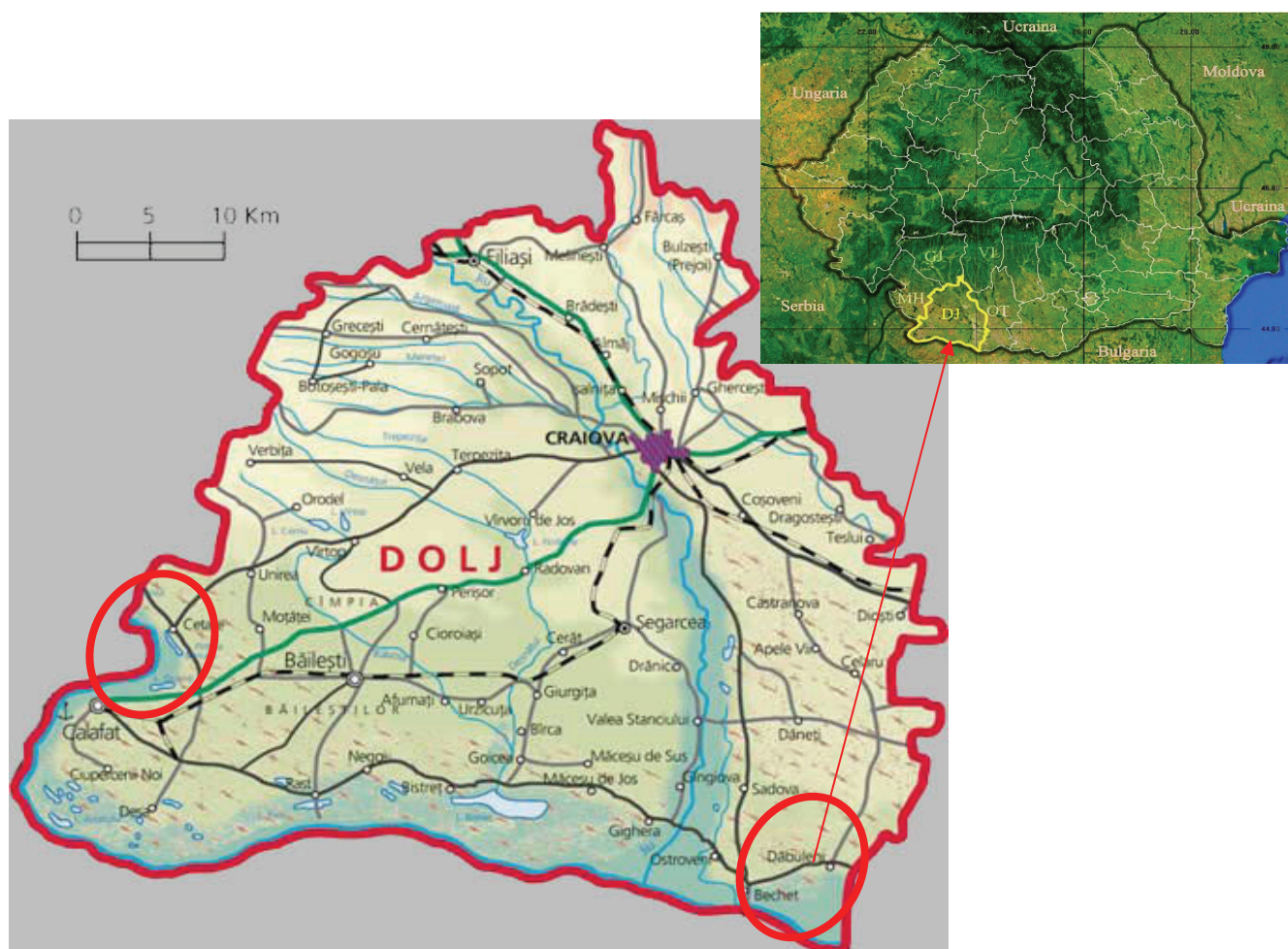


Figure 1. Location of the lacustrine ecosystems in the floodplain of the Danube from the southwestern of Romania (from Google Earth, accessed: March 11, 2020 in CIOBOIU et al., 2020).

The acute pollution of the river produced ecological changes in the structure and functionality of the lacustrine ecosystems in this sector. As a result of eutrophication, water chemistry is characterized by high levels of nutrients ( $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ) which explains the mass development of aquatic and paludous macrophytes, as well as of phytoplankton. This process induced by the increasing quantities of inorganic and organic nutrients transported by the river waters determined the modification of natural evolutionary tendency of all biocenotic structures. Due to the enhanced eutrophication, the lakes are in an advanced process of the biological clogging. The clogging of the lakes leads to the appearance of these marshlands covered by paludous vegetation, reduction of depth and surface, algal blooms that harm fish and human health (BREZEANU et al., 2011; PANDEY & FULEKAR, 2012).

## MATERIALS AND METHODS

With a view to assessing the hydrobiological characteristics, an extensive research program was carried out. Based on the existing data in the specialized literature and on our own research, a study was drawn up, which allowed the monitoring of the lacustrine ecosystems biodiversity from the studied sector (BREZEANU et al., 2011; CIOBOIU & BREZEANU, 2014; CISMAȘIU & CIOBOIU, 2016; CIOBOIU et al., 2020; 2022).

Samples were taken in order to render the physical-chemical characteristics of water and the planktonic and benthic structures. An important component of the investigated lacustrine ecosystems is represented by indigenous microorganisms. The performed studies showed that microorganisms from extreme environments are highly resistant to the metallic ions present in them, having a greater capacity to reduce and oxidize the organic and inorganic compounds from the industrial habitats. This type of the extracellular metabolic adaptation could be used in the bioremediation of contaminated industrial sites from Romania (JOHNSON & HALLBERG, 2008; CISMAȘIU et al., 2015; CISMAȘIU & CIOBOIU, 2021).

## RESULTS AND DISCUSSIONS

**Water chemistry** is characteristic to eutrophic ecosystems. The pH values vary between 6.5 and 8.5 (slightly alkaline). The large amount of nitrates and nitrites is induced by the nutrient ( $1.5-7 \text{ mg/l NO}_3^-$ ;  $0.007-0.161 \text{ mg/l PO}_4^{3-}$ ) input as mineral and organic fertilizers are intensively used in the neighbouring agricultural fields. Among the cations, we mainly remark calcium ( $\text{Ca}^{2+}$ ) that originates in the sedimentary rocks found at the bottom of the lakes and in the treatments applied to the agricultural plots from the area (CIOBOIU & PLENICEANU, 2005).

**The structure of planktonic and benthic biocoenoses.** The effect of the eutrophication process is manifested through the excessive development of phytoplankton and paludous and aquatic macrophytes. Bacillariophyceae (56.21 %) and Chlorophyceae (28.92 %) are dominant groups within phytoplankton, while Cyanophyta intensively develop in summer (Table 1). 121 species were identified, the most common being: *Diatoma elongatum*, *D. vulgare*, *Synedra acus*, *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*, having an average numerical density of over 80,000 specimens/l (BREZEANU et al., 2011; MOLDOVEANU & FLORESCU, 2013; CIOBOIU et al., 2020).

Table 1. The number of species belonging to the taxonomic groups of phytoplankton.

No.	Taxonomic groups	Number of species	%
1	Cyanophyta	10	8.26
2	Euglenophyceae	5	4.13
3	Heterokontae	3	2.48
4	Bacillariophyceae	68	56.21
5	Chlorophyceae	35	28.92

Paludous and aquatic macrophytes occupy an important place in the bioeconomy of ecosystems, with the dominant species being *Phragmites communis*, *Typha angustifolia*, *Nuphar luteum*, *Nymphaea alba*, *Rorippa amphibia*, *Polygonum amphibium*, *Iris pseudacorus*, *Equisetum arvense*, *Euphorbia palustris*, *Scirpus lacustris*, *Carex riparia*, *Ranunculus aquatilis*, *Salvinia natans*, *Stratiotes aloides*, *Myriophyllum spicatum*, *Hydrocharis morsus-ranae* (Table 2). An overall evaluation of the biomass production showed that 85,200 Kg/ha/year wet biomass can be obtained. It is a proof of the trophic capacity of the ecosystems (DIHORU & ARDELEAN, 2009; CISMAȘIU et al., 2016; CIOBOIU & CISMAȘIU, 2018; GAVRILESCU et al., 2018; ZANFIR et al., 2019; CIOBOIU et al., 2022).

Table 2. Paludous and aquatic macrophyte species.

SPECIES	
PALUDOUS	AQUATIC
<i>Phragmites communis</i> Trin.	<i>Lemna minor</i> L.
<i>Typha angustifolia</i> L.	<i>Nymphaea alba</i> L.
<i>Typha latifolia</i> L.	<i>Nuphar luteum</i> L.
<i>Scirpus lacustris</i> L.	<i>Polygonum amphibium</i> L.
<i>Heleocharis palustris</i> L.	<i>Potamogeton natans</i> L.
<i>Juncus effusus</i> L.	<i>Potamogeton crispus</i> L.
<i>Mentha aquatica</i> L.	<i>Potamogeton perfoliatus</i> L.
<i>Mentha longifolia</i> L.	<i>Potamogeton pectinatus</i> L.
<i>Iris pseudacorus</i> L.	<i>Salvinia natans</i> L.
<i>Carex riparia</i> L.	<i>Stratiotes aloides</i> L.
<i>Carex hirta</i> L.	<i>Schoenoplectus mucronatus</i> L.
<i>Ranunculus aquatilis</i> L.	<i>Myriophyllum spicatum</i> L.
<i>Ranunculus repens</i> L.	<i>Ceratophyllum submersum</i> L.
<i>Polygonum hydropiper</i> L.	<i>Hydrocharis morsus-ranae</i> L.
<i>Pastinaca sativa</i> L.	<i>Glyceria maxima</i> L.
<i>Vicia peregrina</i> L.	<i>Rorippa amphibia</i> (L.) Besser
<i>Equisetum arvense</i> L.	
<i>Euphorbia palustris</i> L.	

Besides phytoplankton, zooplankton is an important part of the organic production. Its development is correlated with that of phytoplankton, the latter being the main food source of zooplankters. The dominant groups are rotifers, copepods and cladocerans (BREZEANU et al., 2011; CIOBOIU et al., 2019). In the case of rotifers, whose numerical density exceeds 86 thousand specimens/l, the dominant species are: *Brachionus angularis*, *B. calyciflorus*, *Keratella cochlearis*, *Polyarthra vulgaris*, *P. major*, *Filinia longiseta*, *Asplanchna priodonta*, *Synchaeta pectinata*. Copepods that have a significant contribution to the production of zooplankton (over 20,000 specimens/l in the Maglavit lake) are often represented by the following species – *Acanthocyclops vernalis*, *Cyclops vicinus*, *Eudiaptomus gracilis*, *Mesocyclops leukarti*. Cladocerans are a group with a large share in the zooplankton of eutrophic lakes. The dominant and common species are: *Diaphanosoma brachiurum*, *Moina micrura*, *Bosmina longirostris*, *Leptodora kindi*, *Sida cristalina*, *Daphnia cucullata*, *Chydorus sphaericus*, *Leydigia acanthoscelidis*.

The benthos floodplain structure is determined by the structure of benthic facies. Macrophytes that grow excessively in most lakes and ponds are the main cause of the accumulation of large amounts of detritus so that most of the bottom surface is made up of a muddy and detritic facies.

The main benthic groups are Oligochaeta (dominant species *Limnodrilus* sp., *Pelosclex ferox*, *Stylaria lacustris*, *Branchiura* sp.), Chironomida (*Chironomus plumosus*, *Tendipes semireductus*, *Polypedilum* sp., *Cricotopus* sp., *Procladius* sp.), Plecoptera (*Amphinemura standfussi*, *Capnia bifrons*, *Leuctra fusca*, *L. nigra*, *Nemoura cambica*, *N. cinerea*, *Perla marginata*, *Protonemura intricata*), bivalves (*Unio pictorum*, *Anodonta piscinalis*, *Sphaerium lacustre*, *Dreissena polymorpha*), Hirudinea (*Piscicola geometra*, *Hirudo medicinalis*), Ephemeroptera (*Palingenia* sp.), odonates (*Gomphus pulchellus*), beetles (*Hydrophilus* sp.), Trichoptera (*Hydropsyche ornatula*, *Setodes* sp.), Gammarida (*Pontogammarus crasus*, *Dichrogammarus vilosus*), Corophiidae (*Corophium* sp.).

A major role in the biological production from the lacustrine ecosystems in the area is represented by the populations of gastropods, preponderantly the benthic organisms that respond actively to the heterogeneity of the microhabitats they inhabit. A number of 37 species was identified (Table 3), among which *Viviparus acerosus*, *Radix balthica*, *Physella (Costatella) acuta*, *Lymnaea stagnalis*, *Planorbarius corneus* are characteristic to the eutrophic lacustrine ecosystems from the area prone to floods (CIOBOIU, 2015).

Table 3. The taxonomic diversity of gastropods.

CLASS GASTROPODA Cuvier 1798	
SUBCLASS PROSOBRANCHIA Milne Edwards 1848	
ORDER CYCLONERITIDA Rafinesque 1815	
Family Neritidae Rafinesque 1815	<i>Theodoxus danubialis</i> C. Pfeiffer 1828 <i>Theodoxus fluviatilis</i> Linnaeus 1758 <i>Theodoxus transversalis</i> (C. Pfeiffer 1828)
ORDER MESOGASTROPODA (Thiele 1925)	
Family Viviparidae Gray 1847	<i>Viviparus acerosus</i> Bourguignat 1870 <i>Viviparus viviparus</i> Linnaeus 1758
Family Valvatidae Thomson 1840	<i>Valvata cristata</i> (O. F. Muller 1774) <i>Valvata (Cincina) piscinalis</i> O. F. Muller 1774 <i>Borysthenia naticina</i> (Menke 1845)
Family Lithoglyphidae Troschel 1857	<i>Lithoglyphus naticoides</i> C. Pfeiffer 1828
Family Bithyniidae Gray 1849	<i>Bithynia tentaculata</i> Linnaeus 1758 <i>Bithynia (Codiella) leachii</i> (Sheppard 1848)
Family Thiaridae Troschel 1857	<i>Esperiana esperi</i> (Ferussac 1829) <i>Esperiana (Microcolpia) daudebardii</i> (Prevost 1821) <i>Esperiana (Microcolpia) daudebardii acicularis</i> Ferussac 1823
SUBCLASS PULMONATA Cuvier 1817	
ORDER BASOMMATOPHORA A. Schmidt 1855	
Family Physidae Fitzinger 1833	<i>Physa fontinalis</i> (Linnaeus 1758) <i>Physella (Costatella) acuta</i> (Draparnaud 1805) <i>Aplexa hypnorum</i> (Linnaeus, 1758)
Family Lymnaeidae Rafinesque 1815	<i>Lymnaea stagnalis</i> (Linnaeus 1758) <i>Stagnicola palustris</i> (O. F. Muller 1774) <i>Stagnicola corvus</i> Gmelin 1788 <i>Radix auricularia</i> (Linnaeus 1758) <i>Radix ampla</i> (Draparnaud 1805) <i>Radix balthica</i> (Linnaeus 1758) <i>Galba truncatula</i> (O. F. Muller 1774)
Family Ancylidae Rafinesque 1815	<i>Ancylus fluviatilis</i> O. F. Muller 1774
Family Acroloxidae Thiele 1931	<i>Acroloxus lacustris</i> (Linnaeus 1758)
Family Planorbidae Rafinesque 1815	<i>Planorbis planorbis</i> (Linnaeus 1758) <i>Anisus (Anisus) septemgyratus</i> (Rossmassler 1835) <i>Anisus (Anisus) spirorbis</i> (Linnaeus 1758) <i>Anisus (A.) vortex</i> (Linnaeus 1758) <i>Bathyomphalus contortus</i> (Linnaeus 1758) <i>Gyraulus albus</i> (O. F. Muller 1774) <i>Armiger crista</i> (Linnaeus 1758) <i>Segmentina nitida</i> (O. F. Muller 1774) <i>Hippeutes complanatus</i> (Linnaeus 1758) <i>Planorbarius corneus</i> (Linnaeus 1758)
ORDER STYLOMMATOPHORA A. Schmidt 1855	
Family Succineidae Beck, 1837	<i>Oxiloma elegans</i> (Risso 1826)

The distribution according to the benthic facies shows us that the largest number of species populates the muddy-detritus (24 species) bottom, near the shore, in shallow areas. These areas present the best possible feeding conditions. Gastropods find abundant food on the coarse detritus, on the leaves fallen in the water but still unaffected by putrefaction on which a rich periphyton develops and on the silt that is rich in organic substances. The lowest diversity of species was determined in the areas where the substrate is predominantly sandy (10 species).

As for the presence of bivalent heavy metals in sediments and shell of fresh water snails, it was found that levels of Mn<sup>2+</sup>, Fe<sup>2+</sup> and Cu<sup>2+</sup> can accumulate which are higher than the environment admissible values (0.001 – 0.01

mg/l), in accordance with accepted limits of international standards. The results obtained in the lakes from the Cetate-Calafat sector showed up the ability of snail species such as *Lymnaea stagnalis*, *Stagnicola palustris*, *Planorbarius corneus* to accumulate the metal ions of the type  $Mn^{2+}$ ,  $Fe^{2+}$  and  $Cu^{2+}$  depending on the concentrations of such ions from the soil. Also, the performed experiments showed increased tolerance of different species, such as *Radix balthica* and *Viviparus acerosus*, to the presence of bivalent metal ions originating from industrial waste processing activities (CISMAȘIU et al., 2016; GAVRILESCU et al., 2018).

The development of technological processes based on the activity of indigenous microorganisms demonstrated their involvement in the biogeochemical cycles, by: chemical-bacterial solubilisation of industrial wastes and precipitation of metal ions from industrial effluents; concentration and removal of heavy metals from industrial waste waters. Acidophilic microorganisms present in soil and water, through the mineralization of organic substances, ensure circulation of organic matter, releasing mineral elements used by plants for their nutrition. The role of acidophilic microorganisms in the reduction of soluble iron sulfide and other metal sulfides to insoluble ions is well established. The presence of the other species in the microbial culture has proven useful in increasing the oxidation and reduction rate of heavy metals from extreme environments. The study of how acidophilic microorganisms interacting between them in extreme environmental conditions has a strong influence over technologies used for the remediation of environments polluted with heavy metallic ions. The knowledge of the degree of spread use acidophilic microorganisms is a benchmark in establishing the influence of extreme environmental conditions on the processes of bioaccumulation and solubilization of heavy metal ions by their action on metabolic processes in the microbial cell.

Also, the reduction of  $SO_2$  from coal can have many applications in fields such as: environmental protection in former mining perimeters and coal processing plants, as well in adjacent areas through the bioremediation of environments contaminated with sulphates and metal ions, in agriculture, food industry, chemical and pharmaceutical industries (WANG & CHEN, 2009; TEODOSIU-BELEUȚĂ et al., 2020). The obtained results showed the increasing efficiency of the process of degradation of organic substances under the action of heterotrophic bacteria belonging to the *Acidiphilium* genus in the presence of salts existing in industrial contaminated sites in Romania (Fig. 2).

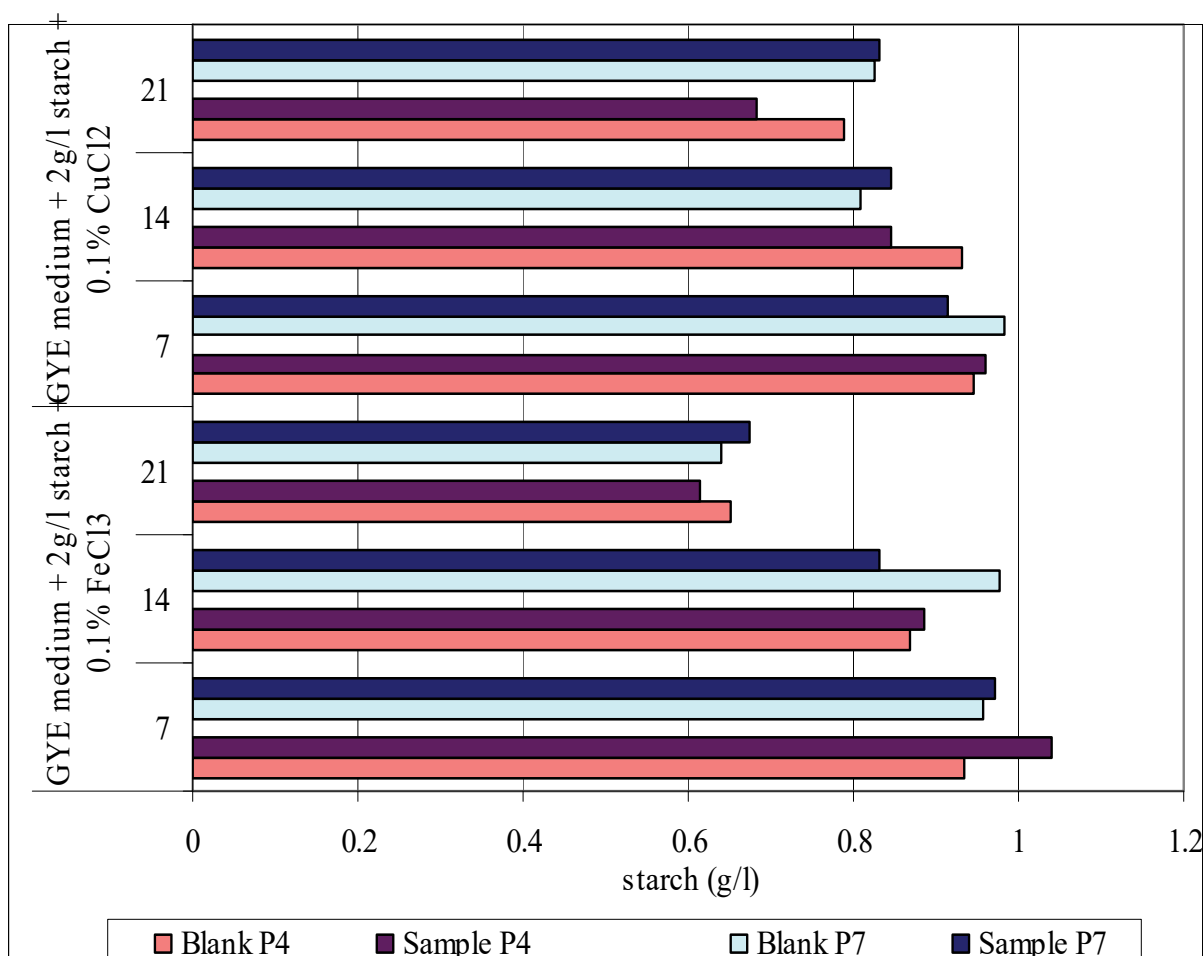


Figure 2. The starch degradation of the extracellular enzymatic activity of *Acidiphilium* populations in a GYE environment with 0.1%  $FeCl_3$ , respectively 0.1%  $CuCl_2$ , and 2g/l starch.

## CONCLUSIONS

Taking into account the present state of the Danube within the territory of Romania, as induced by diking, and the disappearance of the largest part of its natural floodplain, the studied ecosystems represent an *area* that still preserves the specific biocoenotic structures.

The presented data showed the biodiversity evolution of the lacustrine ecosystems subjected to the process of eutrophication, as well as the increased tolerance of snail species to the presence in the environment of bivalent metal ions originating from the industrial activities of solid waste processing. The experiments suggest that the efficiency of the bioaccumulation process is strongly influenced by the type of metal ions, as well as the concentration in the water and sediment. Our studies illustrate the toxic effects of metal ions on living organisms and especially biotechnology processes effectiveness in the reduction of the concentrations of heavy metals in the wastewater by classical and modern systems.

Understanding the mechanisms by which the acidophilic bacteria of the genus *Acidiphilium* are involved in the fixation of heavy metals is very important in terms of the development of microbial processes of concentration and removal of these metals from aqueous solutions. Thus, the knowledge of the chemical reactions that take place during the fixation of metal ions leads to the achievement of an efficient control of the parameters of the bioremediation process of the industrially polluted areas from the Oltenia sector of the Danube Lowlands.

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