HEAVY METAL INFLUENCE ON THE EVOLUTION OF THE PLANKTONIC AND BENTHIC DIVERSITY OF ORGANISMS PRESENT IN CONTAMINATED INDUSTRIAL ECOSYSTEMS OF OLTENIA PLAIN

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Abstract. Ecology of extreme environments is an area of interdisciplinary research aimed at increasing the efficiency of processes to reduce the concentration of heavy metals from contaminated industrial areas. Authors' research on the influence of heavy metals on the dynamics of planktonic and benthic organisms from the industrially contaminated aquatic ecosystems in Oltenia Plain revealed a numerical abundance of the species of microorganisms, invertebrates and plants linked to the presence and distribution of metal ions. By analyzing the biocenotic structures of the lakes in Cetate – Calafat sector, it was found to have a high degree of trophicity reflected by the quantitative and qualitative composition of communities of planktonic and benthic organisms. With regard to the presence of bivalent heavy metals in sediments and shells of freshwater snails, it resulted that they can accumulate higher level of Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} than the admissible environmental values (0.001-0.01 mg/l) according to Order no. 161/2006. The performed analyzes illustrate the capacity of the pulmonary snails species such as *Radix balthica* and *Lymnaea stagnalis* to accumulate metal ions type Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} in direct correlation with the concentration of the respective ions in the soil.

Keywords: invertebrates, microorganisms, metal extraction, technological processes.

Rezumat. Influența metalelor grele asupra evoluției diversității de organisme planctonice și bentonice prezente în ecosistemele contaminate industrial din Câmpia Olteniei. Ecologia mediilor extreme este un domeniu de cercetare interdisciplinar având ca scop creşterea eficienței proceselor tehnologice de reducere a concentrației de metale grele din zone contaminate industrial. Cercetările autorilor privind influenţa metalelor grele asupra dinamicii organismelor planctonice şi bentonice din ecosisteme acvatice contaminate industrial din Câmpia Olteniei au evidenţiat o abundenţă numerică a speciilor de microorganisme, nevertebrate şi plante în strânsă concordanţă cu prezenţa şi distribuţia ionilor metalici. Analizând structurile biocenotice ale lacurilor din sectorul Cetate - Calafat s-a constatat că au un grad ridicat de troficitate reflectat prin componenţa cantitativă şi calitativă a comunităților de organisme planctonice şi bentonice. În ceea ce priveşte prezenţa metalelor grele bivalente în sedimente și cochilia melcilor de apă dulce a rezultat că pot acumula niveluri mai ridicate de Mn^{2+}, Fe^{2+}, Cu^{2+} și Zn^{2+} decât valorile admisibile de mediu (0,001 – 0,01 mg/l), în conformitate cu Ordinul nr. 161/2006. Analizele realizate ilustrează capacitatea speciilor de melci pulmonaţi *Radix balthica* şi *Lymnaea stagnalis* de a acumula ioni metalici de tip Mn^{2+}, Fe^{2+}, Cu^{2+} şi Zn^{2+} în corelație directă cu concentraţia ionilor respectivi din sol.

Cuvinte cheie: nevertebrate, microorganisme, extracția metalică, procese technologice.

INTRODUCTION

Research conducted within a comprehensive knowledge national program of some reprezentative ecosystems for Romania territory, makes an important contribution to the establishment of the hydrobiological particularities of some lakes in Oltenia Plain subject to industrial contamination. In this respect, between Cetate and Calafat localities, on the site of former meanders or abandoned branches of the Danube, there appeared several lakes: Banului Fountain, Hunia, Maglavit, Golenți (Fig. 1). The area between the Drincea Valley to the West and the Jiu Valley to the East is part of the RIFPA0074 Maglavit avifaunistic protection site, with an area of 3,562.6 ha. The lakes are natural, developed in the lower sections of the alluvial plain, with a large surface and shallow waters, being used for irrigation and pisciculture. The dimensions of the lakes are: Banului Fountain with an area of 310 ha and depth of up to 2.6 m; Maglavit – 48 ha and the deep of 2.4 m; Golenți – 165 ha and depths up to 3.2 m. Their connection with the Danube is still preserved, the water supply being made directly from the river (CIOBOIU, 2014; 2015).

The main factors of industrial contamination in Romania consist in the discharge of untreated waste water and the lack of infrastructure organized on methods of reducing the concentration of metal ions in the areas polluted with heavy metals. In this context, a field widely approached at international level with immediate practical applications is the use of acidophilic microorganisms in microbiological recovery of metals. Bacteria in the sulfur cycle can be used to remove heavy metals. The formation of iron sulphide is an important reaction from biogeochemical point of view because low temperatures leads to the formation of pyrite. In addition, the presence of iron and pyrite sulphide can affect the solubility of other dissolved metals (Cu^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+}) by absorption and co-precipitation reactions. H_2S produced by sulphate-reducing bacteria reacts with free or adsorbent metal ions, which precipitates in the form of insoluble metal sulfides. The process can be used for the concentration of heavy metals and recover their side, but also for the treatment of industrial effluents for the purpose of bioremediation (FAUR & GEORGESCU, 2009; TOMUŞ et al., 2015).

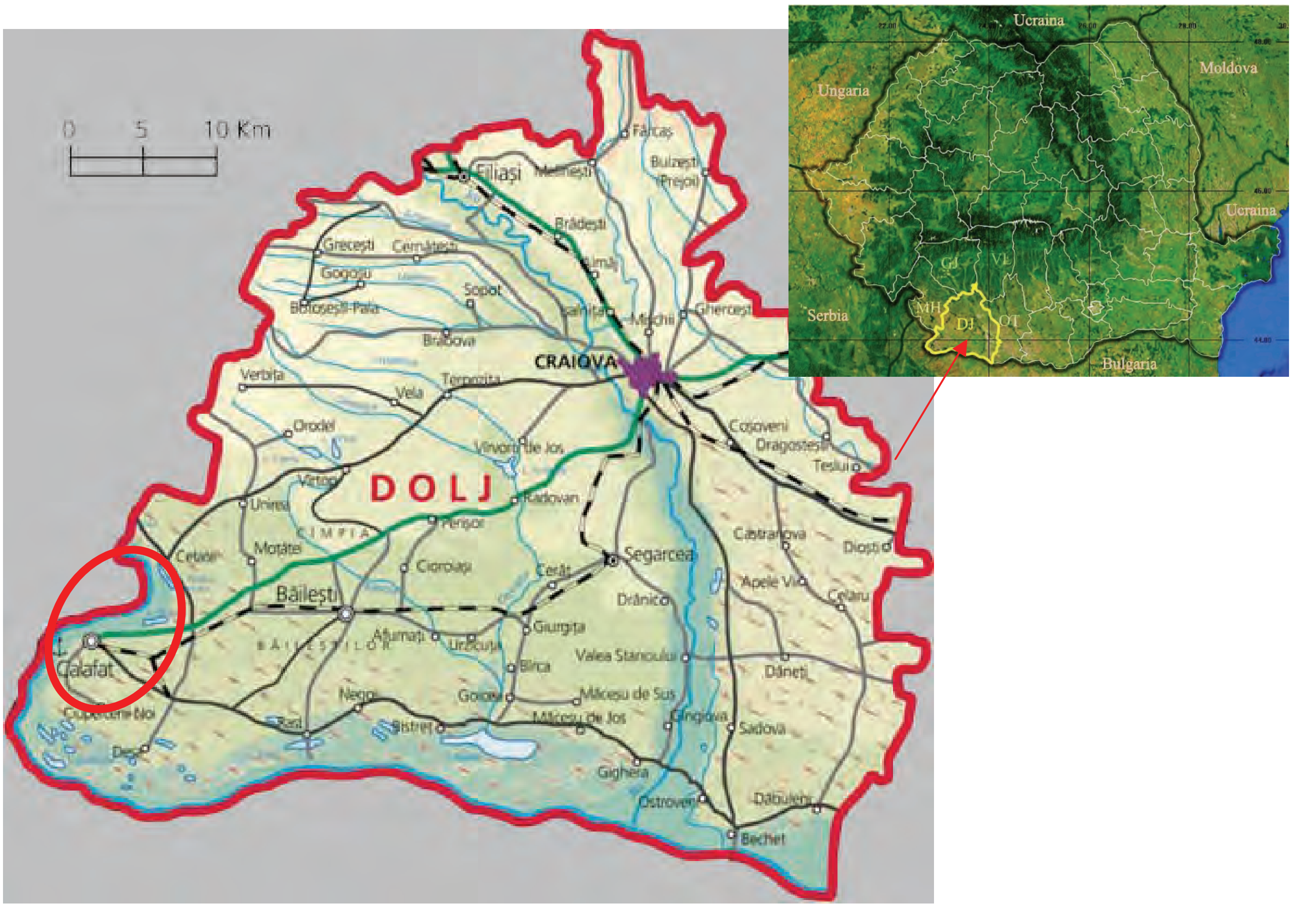

Figure 1. The location of the lakes in Cetate - Calafat sector from Oltenia Plain (from Google Earth, accessed: March 11, 2017).

MATERIAL AND METHODS

Mineralization carried out in order to translate the metal solution is performed in Ethos mineralizer, microwave type D, 1000W power, equipped with Teflon tubes, programmable and it occurs in several stages. About 1 g of dried and ground organic material is weighted and inserted into the Teflon tube and then added 65% nitric acid 3 ml, 2 ml and 1 ml of HCl 37% hydrogen peroxide; afterwards, it is left a few minutes to settle. The mineralized samples are filtered through quantitative filter paper and standard solutions are aspirated in ascending order of concentration and the blank (zero) to construct the calibration curve at the following wavelengths of the Avanta atomic absorption spectrometer (GBC) equipped with an air / acetylene flame burner and cavity cathode lamps corresponding to the determined metals (Pb^{2+} - 217 nm; Cd^{2+} - 228.8 nm; Cu^{2+} - 324.7 nm; Ni^{2+} - 232 nm) (CIOBOIU & CISMAȘIU, 2016).

The activity of acidophilic microorganisms in technological processes of remediation of industrial contaminated areas in Romania is conditioned by a series of physical and chemical factors because it competes for the nutrient substrates containing both organic and inorganic electron donors (LAZĂR et al., 2004; JOHNSON & HALLBERG, 2008; FURTUNĂ et al., 2009).

RESULTS AND DISCUSSION

Water chemistry is characteristic to eutrophic ecosystems (Table 1). The pH values are from 6.5 to 8.5 (slightly alkaline). The high amount of nitrates and nitrates is due to nutrient intake as a result of the administration of mineral and organic fertilizers on neighboring agricultural land. Among cations, calcium (Ca^{2+}) is primarily remarked; its origin is considered to be the sedimentary rocks composing the lakes basins, as well as the amendments applied to agricultural land in the area (CIOBOIU, 2014).

Table 1. Physical-chemical composition of the water of the lakes in the sector Cetate-Calafat (average values).

No.	Analysed indicators	Measured values	Admitted values Ord. 161/2006 - Quality Class II	Analysis method	Used equipments
1.	Concentration of hydrogen ions (pH), unit. pH	7.2	6.5 – 8.5	STAS 6325-75	pH-meter WTW 330i, series 08090178
2.	Electric conductivity µS/cm, max.	1100	-	STAS 7722-84	Cond WTW 340i, series 08082507
3.	Total hardness, German degrees, max.	27.50	-	STAS 3026-76	-

4.	Fixed residue, mg/dm^3, min./max.	550	750	STAS 3638-76	Analytic balance type KERN 770 Series 17308244
5.	Ammonia (NH_4), mg/dm^3, max.	0.102	1.0	STAS 6328-85	Spectrophotometer DR 2000, series no. 930700025411
6.	Calcium (Ca^{2+}), mg/dm^3, max.	55	100	STAS 3662-62	-
7.	Magnesium (Mg), mg/dm^3, max.	87	50	STAS 6674-77	-
8.	Nitrites (NO_2), mg/dm^3, max.	<0.01	0.1	Method 571	Spectrophotometer Lovibond PC spectro Series 100510
9.	Nitrates (NO_3), mg/dm^3, max.	108	13	Method 355	Spectrophotometer DR 2000, series no. 930700025411
10.	Chlorides (Cl), mg/dm^3, max.	64	50	STAS 3049-86	-
11.	Oxidizable organic substance CCOCr (O_2) mg O_2/dm^3, max	4.3	25	STAS 3002-85	-

Analyzing the biocoenotic structures of Cetate-Calafat lacustrine ecosystems, it was noticed that they have a high degree of trophicity reflected by the quantitative and qualitative composition of the planktonic and benthic communities, a specific character of the ecosystems in Oltenia Plain. In the composition of phytoplankton there were identified the following species - *Diatoma elongatum*, *Synedra acus*, *S. ulna*, *Amphora ovalis*, *Ceratoneis arcus*, *Gyrosigma acuminatum*, *Scenedesmus quadricauda*, *Pediastrum duplex*, *P. boryanum* (CIOBOIU & NICOLESCU, 1999; DINU & BREZEANU, 2014).

Together with phytoplankton primary producers, macrophytes represent an important part of the biological production, the dominant species being *Phragmites communis*, *Typha angustifolia*, *Scirpus lacustris*, *Mentha aquatica*, *Carex riparia*, *Lemna minor*, *Nuphar luteum*, *Potamogeton crispus*, *P. natans*, *Myriophyllum spicatum* (DIHORU & ARDELEAN, 2009).

Zooplankton consists of the following groups: Ciliata, Rotifera, Cladocera, Copepoda. The dominant and frequent species are: *Brachionus angularis, Filinia longiseta, Synchaeta pectinata*, *Acathocyclops vernalis, Cyclops vicinus, Mesocyclops leukarti*, *Diaphanosoma brachiurum, Bosmina longirostris, Sida cristalina, Daphnia cucullata, Chydorus sphaericus, Leydigia acanthoscelidis*. The main benthic groups are Oligochaeta (*Peloscolex ferox, Stylaria lacustris*), Chironomidae (*Chironomus plumosus*, *Tendipes semireductus*), bivalves (*Unio pictorum*, *Anodonta piscinalis*, *Sphaerium lake, Dreissena polymorpha*), caddisflies (*Hydropsyche ornatula, Setodes* sp.), Gammaridae (*Gammarus roeselli*, *Pontogammarus crasus, Dicherogammarus vilosus*) (CIOBOIU, 2015).

An important role in organic production of lake ecosystems in the region is played by the populations of gastropods, mainly benthic organisms that respond actively to the heterogeneity of the microhabitats they inhabit. A number of 25 species have been identified, among which *Viviparus acerosus*, *Radix balthica*, *Physella* (*Costatella*) *acuta*, *Lymnaea stagnalis*, *Planorbarius coneus* are characteristic of the eutrophic lake ecosystems in Oltenia Plain.

Regarding the presence of bivalent heavy metals in sediments and the freshwater snail shell, it has been found that they can accumulate higher levels of Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} than the admissible environmental values (0.001-0.01 mg/ l), with Order no. 161/2006 (Table 2). The analyzes performed in the lacustrine ecosystems in the studied sector illustrate the ability of *Radix balthica* and *Lymnaea stagnalis* species to accumulate Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} metal ions in direct correlation with the concentration of the respective ions in the soil (Tables 3; 4).

The aforementioned analyzes are consistent with the literature data that have shown that abiotic ecological factors represented by temperature, acidity, organic substrate concentrations and dissolved heavy metals have a great influence on the extracellular metabolic activity of acidophilic bacteria as they develop under specific environmental conditions (GRAHAM, 1996; GIANFREDA & RAO**,** 2004; TOMUŞ et al., 2015).

Table 2. The concentrations of heavy metals in the water of lakes compared with the maximum allowable studied Order no. 161/2006**.

Metals	Unit of measurement	Lakes of the Cetate-Calafat sector	CMA cf. Ord. 161/2006	Analysis method	Aperture
Cr	mg/ l	<0.003	0.1	Specific method of working in the GBS-Avanta atomic absorption spectrometer user manual	Flame Atomic Absorption Spectrometer Avanta GBC, SN 5378
Ni	mg/ l	0.016	0.1		
Zn	mg/ l	<0.005	0.05		
Pb	mg/ l	<0.01	0.01		
Cd	mg/ l	0.001	0.005		
Cu	mg/ l	<0.01	0.01		
Mn	mg/ l	<0.001	0.01		
Fe	mg/ l	<0.005	0.1		

**Order no. 161 of February 7, 2016 for the approval of the Normative regarding the classification of surface water quality in order to establish the ecological state of water bodies published in the Official Gazette no. 511 of June 13, 2006 of the Water Law no.107/1996 with subsequent amendments and completions.

Table 3. Concentrations of metals from the soil and shells of the pulmonate snail *Radix balthica*.

No.	**Metals** (mg/kg/su)	**Hunia (sol)**	**Snail (shells)**
1	Fe	0,148	0,124
2	Mn	0,0075	0,014
3	Ni	0,0045	< SLD
4	Cr	< SLD	< SLD
5	Cu	< SLD	< SLD
6	Zn	0.0005	0.006
7	Cd	0.0015	< SLD

Note: SLD – below the limit of detection

Table 4. Concentrations of metals from soil and shells of pulmonic snails *Lymnaea stagnalis*.

No.	**Metals** (mg/Kg/SU)	**Maglavit (soil)**	**Snails (shells)**	**The method of analysis**	**The used apparatus**
1	Iron	0.27	180	The working method specified in the user manual of the spectrometer by the atomic absorption – Avanta GBS	The spectrometer of the atomic absorption with flame Avanta GBC, SN A 5378
2	Manganese	0.008	187		
3	Nickel	< SLD	0.475		
4	Chromium	< SLD	4.07		
5	Copper	< SLD	8.1		
6	Zinc	0.006	0.115		
7	Cadmium	0.0015	0.1		
8	Lead	< SLD	0		

Note: SLD – below the limit of detection

Deepening the knowledge of the diversity and action of organisms on different organic and inorganic substrates in industrially contaminated areas has allowed the development of technological processes with low pollutant effects (LAWRENCE et al., 1996).

The obtained data revealed that in industrial ecosystems, gastropod populations have a particular role among consumers, as they are an important factor in the process of accumulation of metal ions in waters and sediments. These species are bioindicators of industrially contaminated environments in Oltenia Plain because it signals early the appearance of negative changes in industrial contaminated ecosystems. Also, studies have shown the increased tolerance of the bacteria of the species present in the environment acidophilic the divalent metal ions derived from industrial solid waste processing (Figs. 1-3).

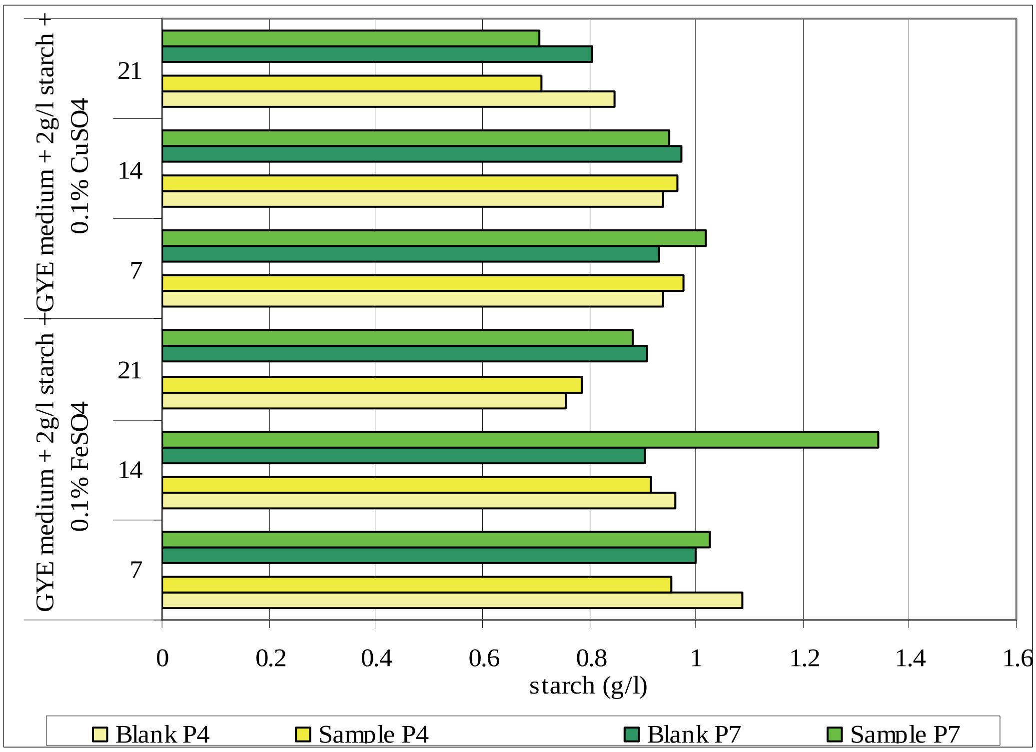

Figure 1. The starch degradation of the extracellular enzymatic activity of *Acidiphilium* populations in GYE medium with 0.1% $FeSO_4$, respectively 0.1% $CuSO_4$, and 2g/l starch.

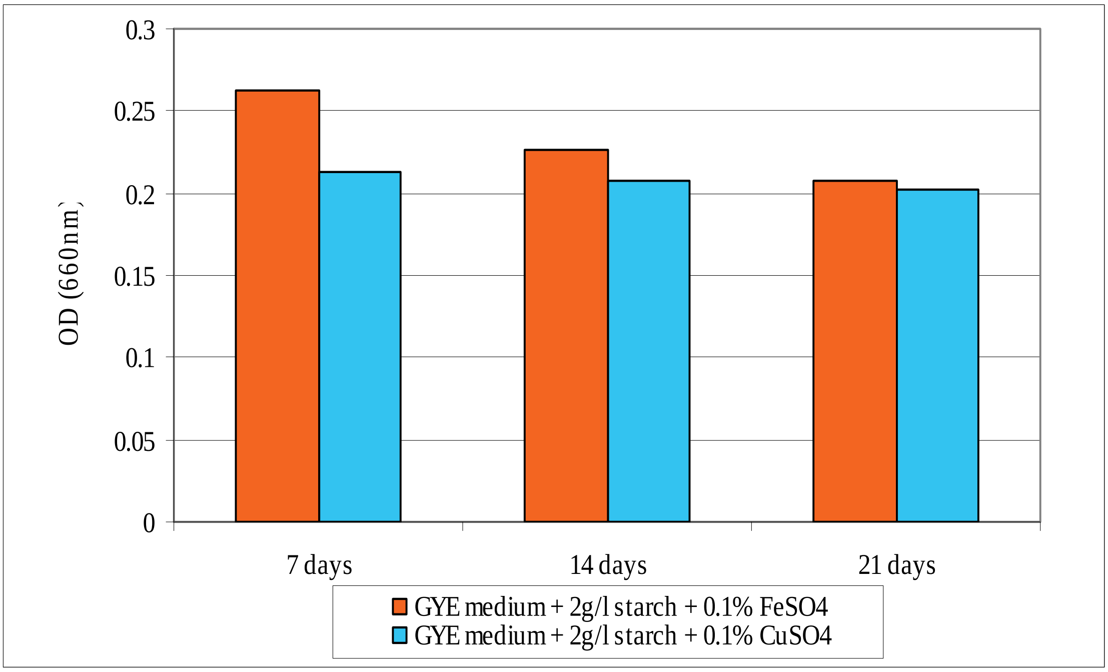

Figure 2. The bacterial density of the P_4 populations in GYE medium with 0.1% $FeSO_4$, respectively 0.1% $CuSO_4$, and 2g/l starch.

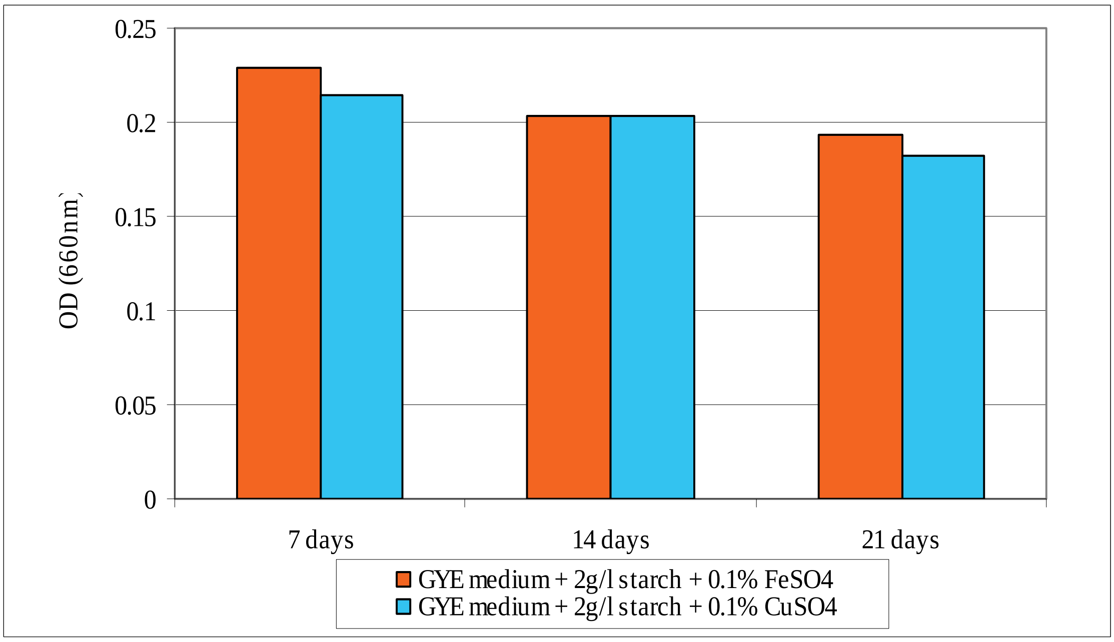

Figure 3. The bacterial density of the P_7 populations in GYE medium with 0.1% $FeSO_4$, respectively 0.1% $CuSO_4$, and 2g/l starch.

CONCLUSIONS

Cetate-Calafat lake ecosystems have a high degree of trophicity reflected by the quantitative and qualitative composition of the planktonic and benthic communities. Our studies illustrate the toxic effects of metal ions on living organisms and, in particular, the efficiency of biotechnological processes in reducing heavy metal concentrations in wastewater through their classical and modern systems of treatment.

The use of tolerant microorganisms at increased concentrations of metal ions in their biosolubilization and biosorbtion processes is efficient in technological applications of bioremediation in the industrially contaminated areas from Oltenia Plain. Also, studies have shown the increased tolerance of these snail species to the presence in the environment of bivalent metallic ions from industrial solid waste processing activities.

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