THE EFFECTS OF ECOLOGICAL FACTORS ON THE STRUCTURE OF THE COMMUNITIES OF ORGANISMS PRESENT IN THE INDUSTRIAL POLLUTED AREAS FROM OLTENIA REGION

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Abstract. The authors' research on industrial contaminated biotopes in Oltenia have revealed the significant presence of certain physiological groups of organisms (plants, invertebrates) adapted to different living environments, which gained perfect tolerance to the ecological characteristics of the substrates. The results of the performed experiments showed that between the growth and multiplication of bacteria in acidic media there is a close correlation highlighted by their maximum tolerance at different metal ion concentrations under certain pH and temperature conditions. Knowing the diversity of microbiota in industrially contaminated areas is a point of view in determining the influence of physico-chemical conditions on the evolution of planktonic and benthic organisms in Romania industrial areas. The analyzed data emphasized significant differences in the distribution of microbiota in areas contaminated by industrial waste in close correlation with the adaptation of the microorganisms to the presence of salts in the culture medium existing in their sampling sites.

Keywords: industrial waste, biodiversity, Turceni thermal power plant, Jiu Valley.

Rezumat. Efectele factorilor ecologici asupra structurii comunităților de organisme existente în zonele poluate industrial din regiunea Oltenia. Cercetările autorilor asupra biotopurilor contaminate industrial din Oltenia au evidențiat prezența cu pregnanță a anumitor grupe fiziologice de organisme (plante, nevertebrate) adaptate la diferite medii de viață, care au dobândit o perfectă toleranță la caracteristicile ecologice ale substratelor. Rezultatele experimentelor efectuate au arătat că între creșterea și multiplicarea bacteriilor în mediile acide există o strânsă corelație evidențiată prin toleranța maximă la diferite concentrații de ioni metalici a acestora în anumite condiții de pH și temperatură. Cunoașterea diversității microbiotei din zone contaminate industrial reprezintă un punct de vedere în stabilirea influenței condițiilor fizico-chimice asupra dinamicii evoluției organismelor planctonice și bentonice din areale industriale ale României. Datele analizate au evidențiat diferențe semnificative privind distribuția microbiotei din areale poluate cu deșeuri industriale în strânsă corelație cu adaptarea microorganismelor la prezența în mediul de cultură a sărurilor existente în situsurile de prelevare a acestora.

Cuvinte cheie: deșeuri industriale, biodiversitate, termocentrala Turceni, Valea Jiului.

INTRODUCTION

Discovering ecological relationships between acidophilic bacteria and the rest of the microbial world shows a special interest because they have had a strong impact on the field of microbiology in at least three directions. They have a specific physiology for extreme environments, are biotechnologically important in the remediation of industrially contaminated areas in Romania and are involved in several ecological processes related to mineralization and deposition of ores. In this context, due to their great metabolic capacity, carried out over millions of years, acidophilic microorganisms have an important role in the history of the planet, causing the accumulation of huge amounts of mineral substances, such as $CaCO_3$, sulfur deposits and iron compounds, through which they contributed to the change of the Earth's appearance. In these processes, apart from the detoxification function, acidophilic bacteria, due to their intense metabolic activity and high S / V ratio, can act as important vectors for the introduction of heavy metals into trophic networks.

In the last decade, research on bio-sulphurization of coal occurred on a large scale, based on the activity of acidophilic chemolithotrophic bacteria of the genus Acidithiobacillus in the conversion of the valences of solid metals into their water-soluble forms. Bacteria (*Acidithiobacillus* sp.), yeasts (*Rhodotorula*, *Trichosporon)*, flakes (*Eutrepia*), amoebae and protozoa (*Amoeba protes*, *Trachelomonas* sp.) are part of the microbial biocoenosis found in acid mine waters. Bacteria of the genus *Acidithiobacillus* contribute to acidification of ecosystems leaching successively (CAVAZZA & BRUSCHI, 1995; KLEIN, 1998).

The formation of extracellular polymeric substances plays an important role in the attachment of *Acidithiobacillus ferrooxidans* on mineral surfaces, as well as on sulfur, pyrite or covel. In this context, ore bioprocessing is expected to grow on a large scale, as well as ore types that may be so processed. Different strains, species or even genes of acidophilic bacteria as members of microbial consortia may become the most important oxidation agents of industrial waste.

The reductive dissolution of ferric iron sulphide ores containing iron-oxidizing under the action of acidophilic bacteria can be used to remove impurities from the iron oxides of mining waste. Other possible applications include the use of acidophilic microorganism iron-reducing bacteria, for example *Acidiphilium* sp., also for the controlled bioremediation of acidic waste water (***. Order 161/2006). The chronic pollution with inorganic substances in the industrial contaminated areas of Oltenia is evidenced by the presence of organisms adapted to a certain concentration of sulphides of the heavy iron, copper, zinc and nickel ions, which reflect the numerical densities specific to their ecological conditions.

In this context, the plant species, invertebrates and micro-organisms present in these polluted areas are biological indicators for metal ion, calcium and sodium chloride pollution, as well as for sulfur compounds (***. Order 756/1997). In this respect, the species *Viviparus acerosus*, *Radix balthica*, *Lymnaea stagnalis* are bioindicators of the industrial contaminated media in Oltenia because they signal the occurrence of negative changes in the ecological structures and functions of lacustrine systems. ((MURARIU, 2002; CIOBOIU & CISMAȘIU, 2016).

MATERIALS AND METHODS

Studies have shown that chemical and bacterial solubilization of industrial waste does not correspond to a single mechanism, but different mechanisms depending on the nature of the metal, the nature of microorganisms and physico-chemical conditions under which the respective bacterial culture acts. The solutions of ferric salts, oxygen and sulfuric acid represent the chemical oxidizing factors of their heavy metals, oxides and sulphides. The action of ferric sulphite solutions on sulphurous minerals results in the dissolution of the sulphide molecule followed by the oxidation of the sulfuric ion, the removal of the metal ion in the solution and the reduction of iron (DONATO et al., 1993; GAZSO et al., 1995; MONROY – FERNANDEZ, 1995).

The involvement of acidophilic bacteria in controlled oxidation processes of the metallic ion from industrial wastes was highlighted by selective methods for the determination of Fe^{2+}, Fe^{3+} and SO_4^{2-} from the culture liquid, as well as from the hard coal samples subjected to the microbiological removal method of the inorganic sulfur from industrial wastes from Turceni thermal power plant, located at the confluence between the Jilţ and the Jiu Rivers (CISMAȘIU et al., 2000; CISMAȘIU, 2009).

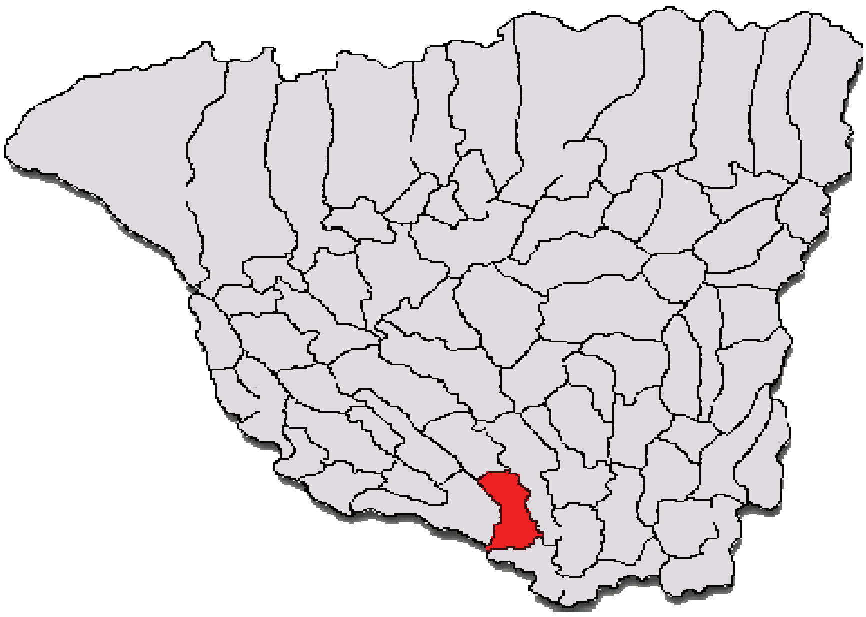

Figure 1. Location of Turceni Thermal Power Plant on the territory of Gorj County (Google Earth, accessed: March 5, 2017).

The biological methods are based on the ecological particularities of the species of microorganisms, plants and invertebrates present in the continental aquatic ecosystems (flowing waters and lakes) and the structure of organism populations depending on ecological factors of the environment contaminated with organic and inorganic substances (DUNCAN et al., 1995; CISMAȘIU, 2005; VIDYALAKSHMI et al., 2009; CIOBOIU & CISMAȘIU, 2016).

RESULTS AND DISCUSSION

The results show that the system of saprobes demonstrates the water quality status in relation to the organic substance content in the water and sediment mass. The physico-chemical factors also influence the presence or absence of certain species of planktonic and benthic organisms, as well as their numerical density. The method used in this system and its application in the assessment of the ecological status of industrial systems is the only indicator that reflects the structure of community organisms existing in waters contaminated with organic and inorganic compounds (***. Order 161/2006).

In this context, the biological indicators for iron inorganic compounds are ferruginous bacteria (ferrobacteria) and some protozoa (*Trachelomonas hispida*, *Antophisa vegetans*). In the case of pollution with hydrogen sulphide and sulfur compounds, it was noticed he presence of bacteria species involved in the biogeochemical circuit of sulfur (colorless, red and green sulfobacteria), as well as of ciliate species (*Plagiophyla nasuta*, *Lagynus elegans*, *Saprodinium dentatum*, *Pelodinium reniforme*). In the presence of calcium salts, there were noticed the algae species of *Vaucheria deboryana*, *Chaetophora elegans* and the species of macrophages *Potamogeton* sp., *Ranunculus* sp., *Ceratophyllum* sp., *Elodea canadensis*, as well as some Lepidoptera larvae (*Pericama decipens*, *P. trifasciata*). In case of sodium chloride, there have been identified halophilic organisms, including diatoms such as *Navicula longirostris*, *Nitzschia apicullata*, *Navicula salinarum*, *N. minuscula*, *Diatoma elongatum* and rotifers *Colurella dicentra*, *Redolia fenica*, *Lecane ychtioura*, *Brachionus plicatilis* (BREZEANU et al., 2011; CIOBOIU, 2014).

Studies carried out in industrially contaminated areas from Oltenia showed the presence of groups of organisms represented by species of microorganisms, invertebrates and plants, which, due to the adaptation to the physico-chemical properties of the areas polluted with organic and inorganic substances, have become biological indicators of the concerned areas under Order no. 756/1997 on the approval of the Regulation on the assessment of environmental pollution. In this context, the industrial waste subjected to microbiological processes for the removal of inorganic sulphur came from the Jiu Valley coal mining basins and the Oltenia energy complex (Fig. 2).

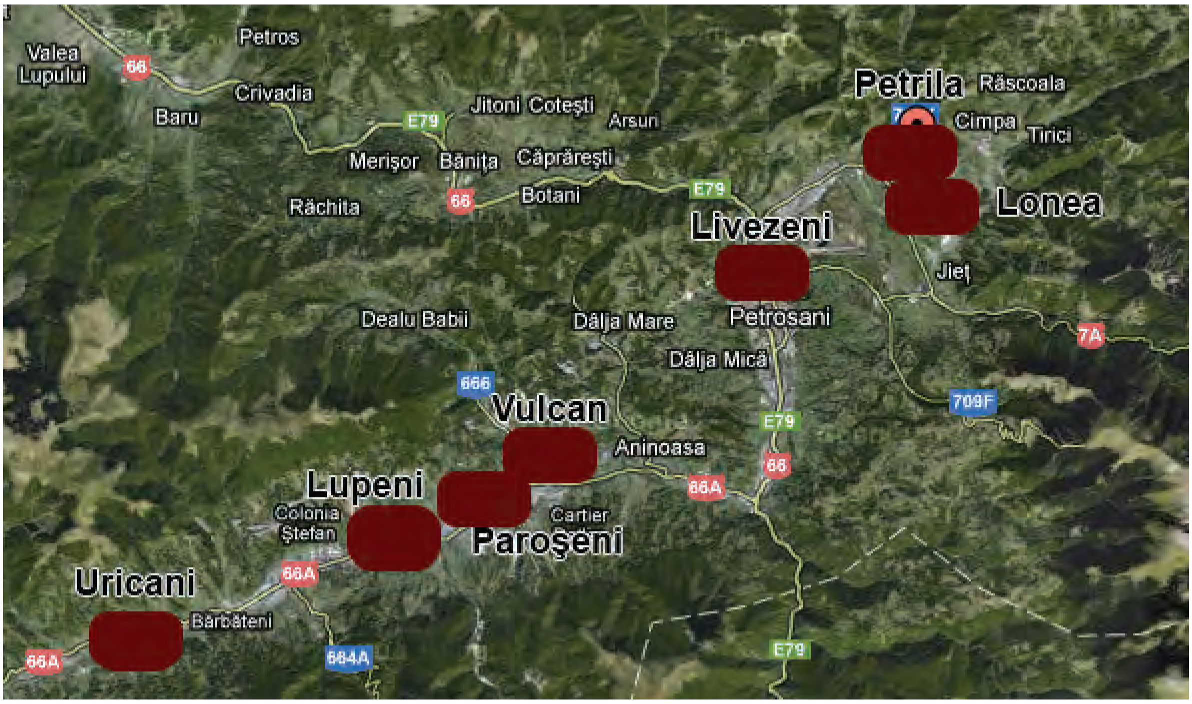

Figure 2. Map of the lignite processing basins in the Jiu Valley (Google Earth, accessed: March 9, 2017).

Our analyses revealed the existence of areas with specific characteristics in the influence of ecological factors on the structure of biodiversity in the saprobe systems affected by industrial contamination from Rovinari and Turceni thermal power plants (Fig. 3).

Polysaprobic area - a region with the highest organic load in the form of unsaturated proteins, hydrogen sulphide and carbon dioxide, lacking almost totally or totally dissolved oxygen, which determines the anaerobic character of the biochemical and microbiological processes of the degradation of substances. The biological indicators for this area are mainly bacteria (*Beggiatoa alba*, *Thiothrix nivea*, *T. violacaea*, *Chromatium okenii*, *Pelagloea chlorina*, *Chlorobacterium aggregatum*, *Sphaerotilus natans*), a small number of cyanobacterial species (*Anabaena constricta*, *Oscillatoria chlorina*, *O. putrida*, *O. lauterbornii*), ciliates (*Metopus contortus*, *Caenomorpha medusula*, *Saprodinium dentatum*), turbid worms (*Tubifex tubifex*) and some Chironomidae (*Chironomus thumi*).

Mesosaprobic area - middle contamination area, subdivided into the sub-zones: **α mesosaprobic**, where the reduction processes predominate and **ß mesosaprobic**, where the oxidation processes predominate. Organic substances in the form of amino acids and poorly oxidized nitrogen compounds are characteristic to this area. Oxygen is present, but also there are small amounts of H_2S and CO_2. The number of saprophytic germs, however, is lower compared to the polysaprobic area, but is quite large: about 100,000 / cm^3 (Fig. 3).

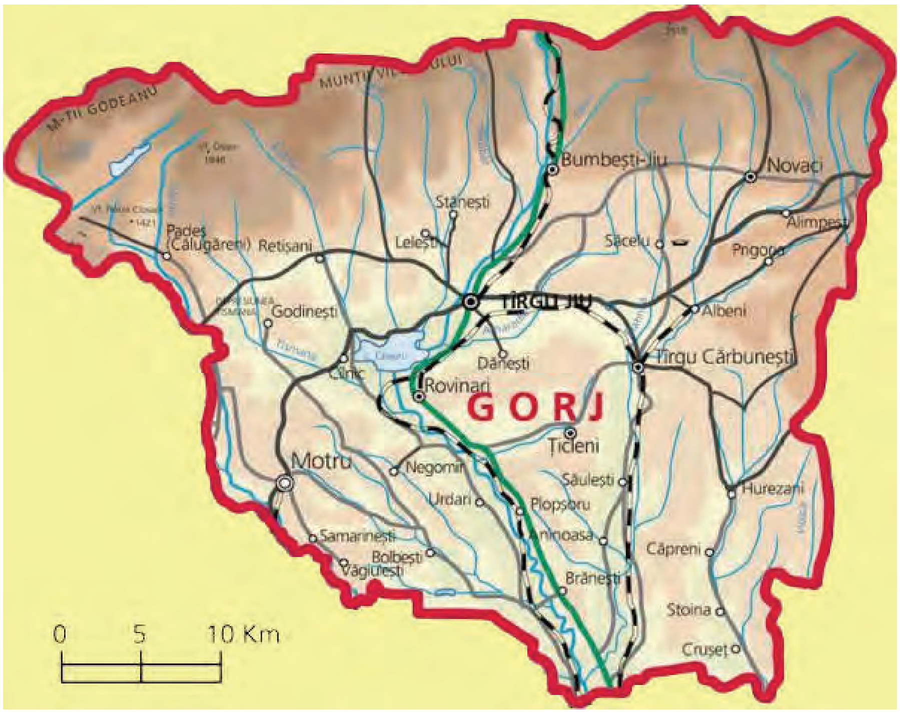

Figure 3. The map of Gorj County with the delimitation of Rovinari and Turceni thermal power plants (Google Earth, accessed: March 5, 2017).

In the α mesosaprobic subarea, with highly polluted water, the amount of organic matter in degradation is slightly lower (CBO5 is between 5 and 10 mg/l). Here, it can occur the *algal bloom* phenomenon. The diversity of organisms is greater, as they are adapted to strong pH oscillations and solvated oxygen concentrations. There are present cyanobacteria *Oscillatoria*, *Phormidium*, diatoms such as *Nitzschia*, *Cyclotella*, *Stephanodiscus*, *Leptomitus*, fungus such as *Fusarium aquaeductum*. Among invertebrates, there can be met hirudines such as *Herpobdella octoculata*, clams such as *Sphaerium corneum*. There are filamentous algae such as *Spyrogyra*, *Cosmarium* and phanerogams such as *Ceratophyllum*.

In the β mesosaprobic subarea, the self-purification process is advanced, the mineralization of organic substances almost complete, and the amount of oxygen does not fall below 50% saturation. The number of bacteria is below 100,000 / cm^3 and the number of plants and animals increases. The most characteristic biological indicators are cyanobacteria such as *Mycrocystis*, *Oscillatoria nostoc*, *Melosira diatoms, Diatoma*, *Fragilaria*, *Pinularia*; there appear flagellates such as *Synura uvella*, *Uroglena volvox*, ciliates such as *Paramecium*, *Didinium*, *Vorticella*, rotifers such as *Brachionus urceus*, *Monostyla lunaris*, Oligochaeta such as *Stylaria lacustris*, *Dendrocellum lacteum*, gastropods such as *Ancylus fluviatilis*, *Lymnaea stagnalis*, *Radix auricularia*, *R. balthica*, *Planorbarius corneus*, bivalves such as *Pisidium cinereum*, *Anodonta cygnaea*, larvae of dipterum insect such as *Cloëon dipterum*, *Hydropsyche lepida*. The aquatic macrophytes *Elodea*, *Lemna*, *Myriophillum*, *Ceratophyllum* are present, and the planktonic algae multiply, often causing algal bloom.

The oligosaprobic area corresponds to the ecosystems with clean water, the organic substances being completely oxidized. The water is clear, the dissolved oxygen within the saturation limits. In this area, the number of bacteria decreases below 100 / cm^3. These ecosystems are populated with a large number of species, but a low number of individuals. Here, there are identified hundreds of algae species, among which characteristic are diatoms such as *Cyclotella*, *Pinularia*, *Synedra*, *Surirella spiralis*, *Nitzschia linearis*, Chlorophyceae such as *Ulotrix*, *Vaucheria debaryana*, *Acacia leucosis*, Rhodophyceae such as *Lemania annulata*, *Batrachospermum vagum*. There are present species of water moss such as *Fontinalis antipyretic* and *Cinclidotus aquaticus*, numerous species of flagellates, ciliates, rotifers, bivalves, gamarids and larvae of Ephemeroptera such as *Rhithrogena*, *Ecdyonurus*, *Oligoneuria*, Plecoptera such as *Perla*, *Taenyopterix* and Trichoptera such as *Setodes*, *Agapetus*, *Leptocerus*. On the back of the stones, it can be found *Planaria gonocephala* (MĂLĂCEA, 1969; BREZEANU et al., 2011; CIOBOIU, 2014).

In Romania, the extraction and processing of the crude oil determined and still determine serious chronic and accidental pollution of the environment. Therefore, as a result of the progress of the petrochemical industry, large areas from Romania are exposed to the impact of petroleum hydrocarbons. Different bacterial strains with the ability to tolerate and degrade toxic hydrocarbons were previously reported. Furthermore, these bacteria were able to produce various secondary metabolites, such as surfactants and pigments (STANCU 2014; 2015; 2016).

The use of acidophilic microorganisms isolated from industrially contaminated sites in Romania involved in the reduction of metal ion content in mining waters and sediments as well as industrial wastes is argued by numerous international publications. At the same time, it is considered that the realization of bioremediation techniques in polluted areas with inorganic substances based on the activities of the microorganisms, invertebrates and plants are economically feasible. (DONATO et al., 1993; CISMAŞIU et al., 2002; TOMUŞ & CISMAŞIU, 2014).

CONCLUSIONS

Increasing the efficiency of bacteriological and chemical solubilization techniques of industrial wastes is interconnected with the direct interaction of microorganisms and solid surfaces of industrial waste subjected to the action of the microbiological method of leaching in accordance with Order 756/1997. Also, the obtained results revealed the direct correlation between the ecological characteristics of the saprobe system and the physico-chemical factors that influence the distribution, as well as the activity, the species of planktonic and benthic organisms from industrially contaminated fields of Oltenia in accordance with Order 161/2006.

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REFERENCES

BREZEANU GH., CIOBOIU OLIVIA, ARDELEAN A. 2011. *Ecologie acvatică*. Vasile Goldiş University Press. Arad. 406 pp.

CAVAZZA C. & BRUSCHI M. 1995. Iron oxidation by *Thiobacillus ferrooxidans* characterization of two electron transfer proteins. *Biochemistry, Genetics and Molecular Ecology of Bioleaching Microorganisms.* Proceedings of the International Biohydrometallurgy Symposium IBS-95 edited by T. Vargas, C. A. Jerez, J. V. Wiertz and H. Toledo. University of Chile. **2**: 97-107.

CIOBOIU OLIVIA. 2014. *Structurile și funcțiile unui sistem bazinal de câmpie. Structura și producția populațiilor de gastropode*. Edit. Antheo. Craiova. 194 pp.

CIOBOIU OLIVIA & CISMAŞIU CARMEN MĂDĂLINA. 2016. The impact of anthropogenic factors on the biocenotic reconstruction of industrial ecosystems from Oltenia Plain. *Oltenia. Studii şi comunicări. Ştiinţele Naturii*. Muzeul Olteniei Craiova. **32**(1): 149-159.

CISMAȘIU CARMEN MĂDĂLINA. 2005. The biosolubilization metals of the pyrite concentrates with cultures of *Thiobacillus ferrooxidans* resistant to high concentrates of metallic ions. *Proceedings of the Institute of Biology*. Romanian Academy. Bucharest. **7**: 147-158.

CISMAŞIU CARMEN MĂDĂLINA. 2009. The acidophilic microorganisms diversity present in lignite and pit coal from Paroseni, Halânga, Turceni mines. *The Annals of Oradea University, Biology Fascicle*. Universitaria Press. Oradea. **16**(2): 60-65.

CISMAŞIU CARMEN MĂDĂLINA., VOICU ANCA, LĂZĂROAIE MIHAELA, LAZĂR I. 2000. The influence of temperature on the metabolism of the acidophylic chemolythotrophic iron oxidizing bacteria, interested for bioremediation of polluted environment with metallic ions. *Conferința Națională de Biotehnologie și ingineria mediului*. Facultatea Ingineria Mediului si Biotehnologie. Universitatea Valahia Târgovişte. **1**: 155-159.

CISMAŞIU CARMEN MĂDĂLINA, POPEA FLORINA, TONIUC MARIA. 2002. The tolerance of the acidophilic chemolithotrophic ironoxidizing bacteria at the different concentrations of Zn^{2+} and Cu^{2+}. *Proceedings of the Institute of Biology*, Romanian Academy. **4**: 225-234.

DONATO DE PH., MUSTIN C., BENOIT R., ERRE R. 1993. Surface oxidized species, a key factor in the study of bioleaching processes. *Part I: An approach to spatial distribution of iron and sulphur species present on pyrite surface*. Biohydrometallurgical Technologies edited by A. E. Torma, J. E. Wey and V. L. Lakshmanan. The Minerals, Metals & Materials Society. Paris. **1**: 163-174.

DUNCAN J. R., BREDY D., STOLL A., WILHELMI B. 1995. Heavy metal removal from industrial effluents by immobilized yeast and algal biomass. *Biochemistry, Genetics and Molecular Ecology of Bioleaching Microorganisms*. Proceedings of the International Biohydrometallurgy Symposium IBS-95 edited by T. Vargas, C. A. Jerez, J. V. Wiertz and H. Toledo. University of Chile. **2**: 237-246.

GAZSO L., SZENDROI A., HIDVEGI E. J. 1995. Bacterial desulphurization of Hungarian coal. *Biosorption, Bioaccumulation and Treatment of Coal, Oil and Effluents*. Proceedings of the International Biohydrometallurgy Symposium IBS-95 edited by T.Vargas, C. A. Jerez, J. V. Wiertz and H. Toledo. University of Chile. **2**: 359-366.

KLEIN J. 1998. Technological and economic aspects of coal biodesulfurisation. *Biodegradation*. Springer. Berlin. **9**: 293-300.

MĂLĂCEA I. 1969. *Biologia apelor impurificate*. Edit. Academiei R. S. R. București. 245 pp.

MONROY - FERNANDEZ M. G., MUSTIN C., DONATO DE PH., BERTHELIN J., MARION P. 1995. Bacterial behavior and evolution of surface oxidized phases during arsenopyrite oxidation by *Thiobacillus ferrooxidans*. *Biohydrometallurgical Processing*. Edited by T. Vargas, C. A. Jerez, J. V. Wiertz and H. Toledo. University of Chile. **1**: 57-66.

MURARIU D. 2002. Muzeele de Ştiinţele Naturii şi biosfera / Natural Science Museums and Biosphere. *Oltenia. Studii şi comunicări. Ştiinţele Naturii.* Muzeul Olteniei Craiova. **18**: 5-8.

TOMUŞ N. & CISMAŞIU CARMEN MĂDĂLINA. 2014. Clean technology for coals desulphuration to reduce SO_2 emission from their burning in thermal power plants. *Buletin CENTIREM*. Edit. Universitas Petroșani. **8**: 60–64.

STANCU MIHAELA MARILENA. 2014. Physiological cellular responses and adaptations of *Rhodococcus erythropolis* IB_{BPo1} to toxic organic solvents. *Journal of Environmental Sciences*. Springer. Berlin. **26**(10): 2065-2075.

STANCU MIHAELA MARILENA. 2015. Solvent tolerance mechanisms in *Shewanella putrefaciens* IBB_{Po6}. *Water, Air, and Soil Pollution*. Springer Verlag. Stuttgart. **226**(39): 1-16.

STANCU MIHAELA MARILENA. 2016. Response mechanisms in *Serratia marcescens* IBB_{Po15} during organic solvents exposure. *Current Microbiology*. Springer. Berlin. **73**(6): 755-765.

VIDYALAKSHMI R., PARANTHAMAN R., BHAKYARAJ R. 2009. Sulphur Oxidizing Bacteria and Pulse Nutrition – A Review. *World Journal of Agricultural Sciences*. Elsevier. New Delhi. **5**(3): 270-278.

***. Ord. 756/1997. Ordinul nr. 756 pentru aprobarea Reglementării privind evaluarea poluării mediului. Anexa 1. Reglementări privind poluarea solului. Valori de referință pentru compuși anorganici în sol. Emisă de Ministerul Apelor, Pădurilor și Protecției Mediului și publicată în Monitorul Oficial. 15 pp. (Accessed February, 2017).

***. Ord. 161/2006. Ordinul nr. 161 din 7 februarie 2016 pentru aprobarea Normativului privind clasificarea calităţii apelor de suprafaţă în vederea stabilirii stării ecologice a corpurilor de apă publicat în MONITORUL OFICIAL nr. 511 din 13 iunie 2006 din Legea apelor nr. 107/1996 cu modificările şi completările ulterioare. 161 pp. (Accessed February, 2017).

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