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THE QUALITATIVE AND QUANTITATIVE MICROBIOTA STRUCTURE IDENTIFIED IN THE BOZANTA MARE TAILING POND

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Abstract. Tailing pond, produced from extraction and processing of heavy metals ores, not only damage native vegetation, thus leading to large areas of derelict land, but are also sources of metal contaminants in local water, air and land. Microorganisms can be important biosorbents for heavy metal remediation of contaminated soils and wastewaters. The presence of some beneficial fungi stimulated plant growth and protected the plant from metal toxicity. To establish the appropriate bioremediation method, we identified some fungi and bacteria species localized in different points and at different depths.
Keywords: tailing ponds, CFU (colony forming units), microflora, bioremediation, metal toxicity

INTRODUCTION

The tailings pond used for storage of sterile from mining activities may have negative influences on environment reflected in water quality, air, vegetation and general aspect of a region if appropriate measures are not being taken (*Beinsan and al.2009).*

Tailing pond, produced from extraction and processing of heavy metals ores, not only damage native vegetation, thus leading to large areas of derelict land, but are also sources of metal contaminants in local water, air and land. Mines produce large amounts of waste because the ore is only a small fraction of the total volume of the mined material.

Long-term application of such wastewater may result in the accumulation of heavy metals in soil and exert a selection pressure on soil microbiota and could pose a public health risk. (Zafar et al. 2007). Therefore it is important to explore microbes from such ecological niches for use in metal biosorption since conventional technologies such as ion exchange, chemical precipitation, reverse osmosis or evaporative recovery are often ineficient and very expensive especially for metals at low concentration (Spriniti et al.,1995; Zhou, 1999). In recent years, the biosorption process has been studied extensively using microbial biomass as a biosorbent for heavy metal removal (Zafar et al. 2007) .

The soil microbial community is responsible for most nutrient transformations in soil, regenerating minerals that limit plant productivity. Fungi and bacteria are the two groups that dominate the microbial decomposer community, and, crudely defined, they share the function of decomposing organic matter in soil, indicating that there is a strong potential for interaction. **(Rousk and al. 2009***).* When tailing ponds soil microflora is an important element to develop according to substrate composition, but equally can change some parameters of substrate such as chemical composition and pH. This is because bacteria that develop chemosintetizante oxidative mineral substrate.

The presence of an efficient and adequate microflora in soil increases the chances of superior plants to survive, supporting efficiently in time the creation of a well formed vegetal layer. It is proved that the microflora in soil, but especially the mycorrhized fungi stimulate the density of roots in herbaceous and ligneous plants, thus making them more efficient in stabilizing and consolidating the ground layer (Ryszka, et al.2007).

We consider that the young plants on tailing ponds are susceptible to find in the edaphic ground layer species of fungi with which to create new types of mycorrhizae.

Mining tailings sites in arid and semi-arid environments remain barren of vegetation following deposition due to a combination of factors including metal toxicity, acidic pH, poor soil structure, low nutrient levels, and stressed microbial communities (Mendez and Maier 2008).

The mining activity and procession in Maramures zone, during the last centuries, generated vast areas covered with sterile waste dumps and decantation pond, some of them being included in the urban and rural perimeter. All of these represents, at the same time serios environmental problems and challenges for their reconstruction and reinstalation in the natural landscape. This study focuses on the identifying and inventory of the microorganisms species, bacteria and fungi from the sterile substratum.

The presence of the tailing deposits conduct not only the water pollution but also the air pollution, having as immediate effect the transformation, the degradation or the total destruction of the structures of the biocenoses from the adjacent zones, affecting both the terrestrial and aquatic flora and fauna (Jelea et al. 2007).

MATERIALS AND METHODS

1. Site description. For the interests of our case study we have selected the tailing pond in Bozanta Mare, built in 1977 and covering 1,050,000 m^2. It has 30 m in depth and an embankment of 18-20°. The *Remin Tailing pond* is the result of sedimentation of water filled with sterile from the flotation of ore. This pond, now in preservation, is partly covered with vegetation either inherited from the previous attempts to ameliorate the area or from the spontaneous settlement of some species, due to a primary succession. Winds easily transport the fine particles in the dam. There were a few attempts to consolidate the pond by planting trees, of which we mention the bur (*Cirsium lanceolatum*) and acacia (*Robinia pseudaccacia*). For the above mentioned *Preserved Tailing Pond*, Table 1 includes the physical characteristics of the underlying soil.

Table 1. Characteristics of soil

Nr.	Parameter	Value	Nr.	Parameter	Value
1	Texture	Sandy clay loam	8	Particle composition, %	
				> 0.2 mm	3
2	Type	Alluvial		0.2 ÷ 0.2 mm	4
3	Organic matter, g· kg^{-1}	0.57		0.1 ÷ 0.05 mm	18
4	Organic carbon, g· kg^{-1}	0.38		0.05 ÷ 0.02 mm	10
5	Water holding capacity, mm/cm depth of soil	38.7		0.02 ÷ 0.01 mm	15
6	Cationic exchange capacity (CEC), cmol· kg^{-1}	12.6		0.01 ÷ 0.015 mm	11
				< 0.005 mm	39
7	Mineralogic composition:		9	Parameters of sandy clay loam	
	Quartzite (sand)	40-45		Natural humidity, %	27-37
	Clay	20-25		Plasticity, %	56-61
	Feldspar	10-15		Porosity, %	60-17
	Sulphides	7 – 8		Cohesivity factor, Kpa	22.46
	Sericite, Carbonates,	23 – 7		Specific weigh, KN· m^{-3}	24.13

(Marian et al 2009).

This study is part of wider research. I watched the total number of microorganisms (bacteria and fungi) encountered in flotation tailings to determine the degree of contamination on different depths.

We have collected samples from the tailing pond. Soil samples from the 0 –10, 10 –20, 20 –30 and 30 – 40 cm depths were collected in 28 February 2010 of the slope NE at three levels (base -B, middle -M and high -S) in the tailing pond in Bozanta Mare (Maramures County).

It is known that soil bacteria are mostly attached to soil particles, so is necessary their separation and their suspension in a solution. For this purpose will make a dispersion of soil in water or other dispersant. Glassware and equipment has been sterilized for one hour at 180^{0}C and cultural environment, Thornton, and saline was sterilized by autoclaving for 15 minutes at 121^0 C. Average sample of tailings from different depths was weighed 1 g and added in 10 ml saline suspension mechanically agitating for 1 hour. From this suspension 1 ml is transferred into a test tube which contains 9 ml of sterile saline, thoroughly homogenized and then transfer 1ml from tube 1 to tube 2, mix and proceed further along the same a series of five tubes. After execution of decimal dilutions was performed to determine the number of microorganisms by cultivation method on cards. Inoculation of plates was carried out in depth by integrating of inoculum in mass plate, over which liquid culture medium was added and cooled to 45^{0}C. After seeding Petri dishes were placed in an incubator at a temperature of 28^{0}C, the result reading is done after 3-5 days.

RESULTS AND DISCUSSIONS

The results of the microbiologycal analyses are presented in Table 2-4. To the presence of a more important number of fungi than bacteria due to unfavorable substrate conditions. This evidence may be due to fungi and natural phenomena (wind, rain, erosion) that favors movement of mold spores. Findings are valid for slope NE at all levels.

Table 2. Influence of the deeph on the CFU extracted from steril NEs

Microorganisms	Depth sampler from NEs (cm)			
	No. of microorganisms x 10^3/g flotation tailings			
	0-10	10-20	20-30	30-40
Bacteria	1500	5000	0	0
Fungi	8950	6250	6850	900

Table 3. *Influence of the deeph on the CFU extracted from steril NEm*

Microorganisms	Depth sampler from NEm (cm)			
	No. of microorganisms x 10^3/g flotation tailings			
	0-10	10-20	20-30	30-40
Bacteria	2500	2000	0	0
Fungi	93500	27500	15000	2250

Table 4. Influence of the deeph on the CFU extracted from steril NEb

Microorganisms	Depth sampler from NEb (cm)			
	No. of microorganisms x 10^3/g flotation tailings			
	0-10	10-20	20-30	30-40
Bacteria	0	0	0	0
Fungi	38000	54500	19500	97500

In the figure 1- 4 is presents variation of CFU (Fungi) in different points tailing pond at 0-10; 10-20; 20-30; 30-40 cm depth.

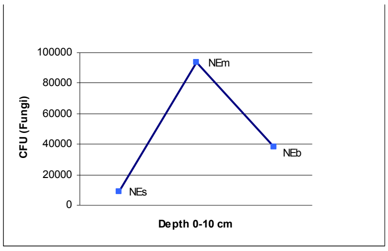

Figure 1. Variation of CFU (Fungi) at 0-10 depth

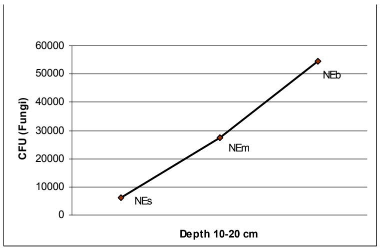

Figure 2. Variation of CFU (Fungi) at 10-20 depth

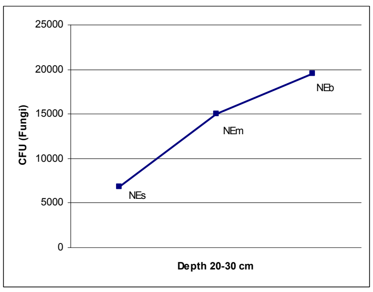

Figure 3. Variation of CFU (Fungi) at 20-30 depth

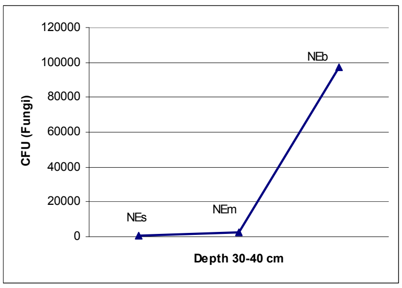

Figure 4. Variation of CFU (Fungi) at 30-40 depth

Fungi occur over a wide pH range (pH 1.0–11.0) and have been detected in acid habitats like volcanic springs, acid mine drainage or acid industrial wastewaters. The presence of some beneficial fungi stimulated plant growth and

Fungi occur over a wide pH range (pH 1.0–11.0) and have been detected in acid habitats like volcanic springs, acid mine drainage or acid industrial wastewaters. The presence of some beneficial fungi stimulated plant growth and protected the plant from metal toxicity.

In this regard, interactions among metals, fungi and plants have attracted attention because of the biotechnological potential of fungi for metal removal directly from polluted soils or the possible transfer of accumulated metals to higher plants, and the toxicity of heavy metals toward fungi metabolism and growth. It was interesting to observed that CFU (colonies forming units) is high enough/g flotation tailings.

The amount of microbiota, quantified by CFU, in the middle and upper point decrease with depth, results confirmed by the literature, excepting the bottom of the tailing pond where the CFU increase with depth. This behavior could be explained due to the substratum which is a mixture of soil and mining sterile in comparison with middle and upper which consist only in sterile. The presence of the soil stimulates the microbiota growth.

From developed colonies (Figure 5) by microscopic examination could be seen predominantly iron- and sulfur-oxidizing bacili, and the most common mold was *Penicillium* sp. (Figure 6 and 7). *Penicillium* sp. are known to produce spores able to tolerate dry conditions (Corry, 1987).

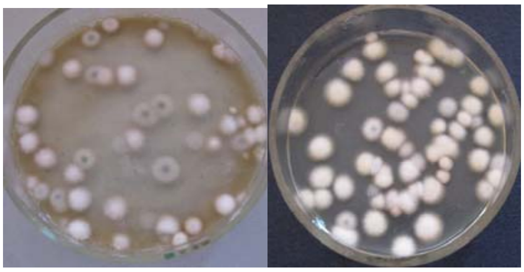

Figure 5. Macroscopic views of the different species of fungal colonies existent in the tailing pond sterile

Figure 6. Iron- and sulfur-oxidizing bacteria

Figure 7. *Penicillium sp.*

The microbial population in the tailing pond steril is restricted against garden soil, for example where was significantly influenced by aplication of different types of organic manure, bioinoculants and inorganic fertilizers. Here maximum bacterial population can be 64,16 CFU×10^6/g and fungal 37,66 CFU×10^3/g (Shashidhar et al. 2009). In the tailing ponds the microorganismes have adapted to low organic carbon content, almost undetectable phosphate and nitrogen content, high metal content, moisture stress, low heterotrophic counts, and high iron and sulfur oxidizer counts (Stevenson and Cole, 1999). Autotrophic iron- and sulfur- oxidizers were ability to create an acidic environment in the tailings and impede revegetation (Schippers et al., 2000).

Southam and Beveridge (1992, 1993) and Schippers et al. (2000) have shown that unamended bulk tailings contained high numbers (up to 10^{6} MPN g^{-1} dry tailings) of iron- and sulfur-oxidizing bacteria while heterotrophic bacteria ranged from as low as 10^{1} to 10^{5} CFU g^{-1}. (Monica O. Mendez et al. 2007).

CONCLUSIONS

This study demonstrate the presents of microorganism in the tailing pond steril can be used to indicate the potential for and success of a mine tailings revegetation. The plants on tailing ponds can to find in the edaphic ground layer species of fungi with which to create new types of symbiosis.

The tailings ponds under preservation still remain threats for environment, due their physically and chemically instability. One of the most important problem must be solved concerns avoiding of soil mobility. In this end, biological rehabilitation of pond should be considers in all ecosystem's elements: trees layer, grassy layer and microbiota of soil. Simbiotic relationships settled between all these contribute not only to stop the mobility of soil but also to improving the soil structure.

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