RESEARCH REGARDING THE ENZYMOLOGICAL ACTIVITIES OF THE TECHNOGENIC SOIL FROM MARAMURES COUNTY

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Abstract. *This paper summarizes our research work regarding the dynamics of vegetation growth of miscellaneous species of trees planted and monitored in the particular environment of the tailing pond in Bozanta Mare (Maramures County). The structure of soil bearing high content of heavy metals and cyanides considerably impacts the ecologic conditions of tailing ponds. Aspects related to soil characteristics (such as structure, size of particles, porosity, texture, chemical composition) are included. In the framework of our experiment we have planted seedlings belonging to four species of trees: Quercus petraea, Populus tremula, Betula verrucosa, Salix caprea. Our aim was to study the evolution of enzyme activities. Our contribution, based on the outcomes of our research, consists in the formulation of functional correlations spotted between cormophites and enzyme activities, between the species of trees and their environmental underlying conditions, with the overarching goal to optimize the activities undertaken in order to alleviate the tailing ponds inherent to mining activities.*

Key words: catalase, dehydrogenase, techonogenic soils, recultivation

1. Introduction

Tailing ponds entail highly – complex environment issues (of a chemical, biological, technological and social nature) stemming from the high content of harmful components but particularly because of the impending dangers that such components inflict on environment and health. Only the joint work of experts in various fields, proposing innovative technologies and services, could enable for a solution to be reached, as the classic approaches considered up to now proved to be far from enough.

Research studies reveal that remedial and restoration of vegetation in areas polluted with heavy metals, areas to which tailing ponds belong, could be enabled by a clever selection of tolerant species of plants as well as by selecting tolerant mycorrhizic fungi**.**

Technogenic soils are soils that form during the technical and biological recultivation of overburdens, tailings and other spoils and wastes resulting from mining and other industrial activities. At the same time, all these wastes constitute

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a dangerous source of environmental pollution [4, 12].
The evolution of technogenic soils is the process of transforming all these wastes into agricultural or forest soils or into soils used for other purposes (parks etc). Simultaneously, this process is accompanied by reduction or elimination of the polluting effects of wastes on the environment [3, 4].
The practical importance of this process is growing because the development of mining and other industries leads to increasing amounts of wastes and, therefore, the recultivation of wastelands becomes more and more a major economic necessity [13, 18].
The evolution of technogenic soils, which reflects the efficiency of recultivation, is studied using many physical, chemical and biological methods [19, 20]. Enzymological methods have also been applied and it has been found that the level of enzymatic activity is a good indicator of the degree of evolution of technogenic soils [6, 7].
The present paper deals with the enzymatic potential in the profiles (0–40 cm) of soil contaminated and uncontaminated with heavy metals.

2. Materials and methods

This experiment is part of a larger research initiative covering the use of micro biota in the overall regeneration of tailing ponds. Within this framework we monitor the role microorganisms (as well as fungi) could play in terms of supporting superior species to grow and to improve their rate of development under the poor environmental circumstances in tailing ponds.
Soil samples from the 0–10, 10–20, 20–30 and 30–40 cm depths were collected monthly from November 2010 to April 2011 of two slopes (NE and SE), at three levels (base - B, middle - M and high - S) in the tailing pond in Bozanta Mare (Maramures County).
The soil samples were allowed to air-dry, then ground and passed through a 2 mm sieve and, finally, used for enzymological analyses.
Enzymological analyses
Two enzymatic activities (actual and potential dehydrogenase) were determined according to the methods described in [2, 15, 17]. The reaction mixtures consisted of 3.0 g soil, 0.5 ml TTC (2,3,5- triphenyltetrazolium chloride) and 1.5 ml distilled water or 1.5 ml glucose solution, respectively, for potential dehydrogenase. All reaction mixtures were incubated at 37º C for 24 hours. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm. Dehydrogenase activities were expressed in mg of triphenylformazan (TPF) produced (from 2,3,5-triphenyltetrazolium chloride, TTC) by 10 g of soil in 24 hours.
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hours.
Catalase activity has been determined using the permanganometric method. The same technique was used for the determination of nonenzymatic catalytic activity, but the soil samples have been thermically inactivated by autoclaving [16]. The reaction mixtures consisted of 3.0 g soil, 2 ml H_2O_2 3% and 10 ml phosphate buffer. It suffered incubation at 37° C for 1 hour. Catalase and nonenzymatic catalytic activities are expressed as mg of H_2O_2 decomposed by 1g of soil in 1 hour.
The activity values were submitted to statistical evaluation by the two *t* test [14] and the correlations between the enzymatic activities were determined according to the methods described in [16].

3. Results and discussions

The results of the enzymological analyses are presented in Fig. 1-6.
It was found that each enzyme activities decreased with sampling depth.
When the results obtained in the four soil layers were considered together, the catalase, actual and potential dehydrogenase activities were the highest in April. These findings are valid for both slopes at all levels.

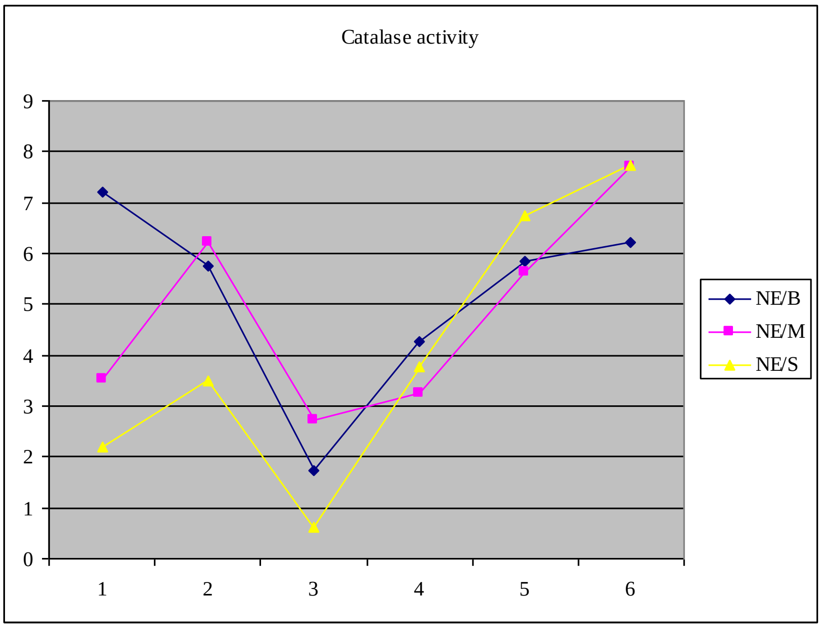

Fig. 1. Evolution of the catalase activity during the six months on the NE slope

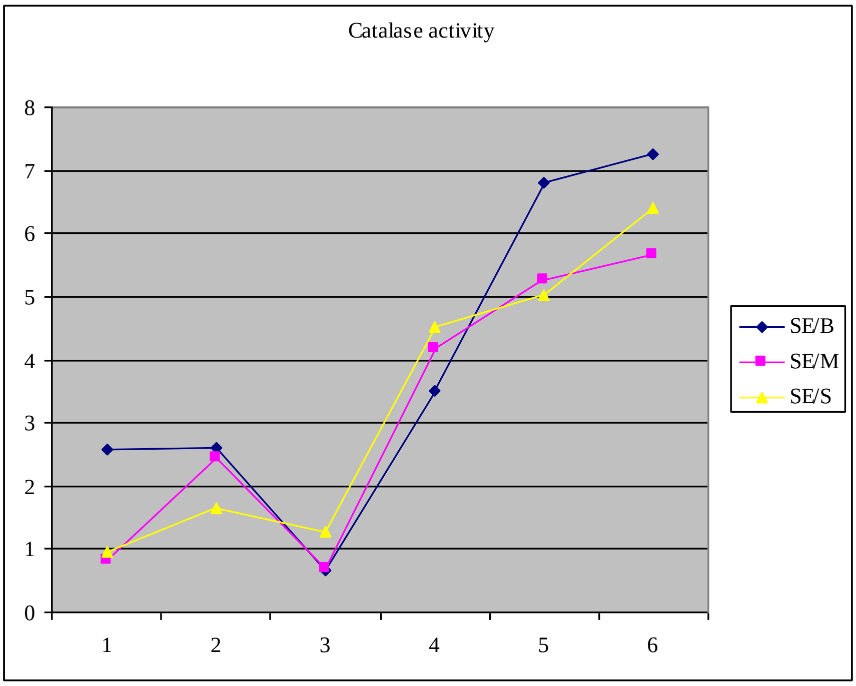

Fig. 2. Evolution of the catalase activity during the six months on the SE slope

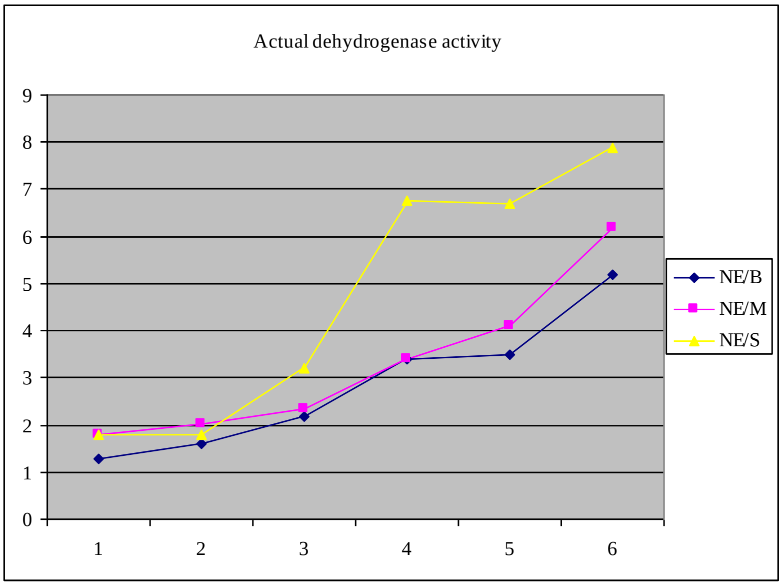

Fig. 3. Evolution of the actual dehydrogenase activity during the six months on the NE slope

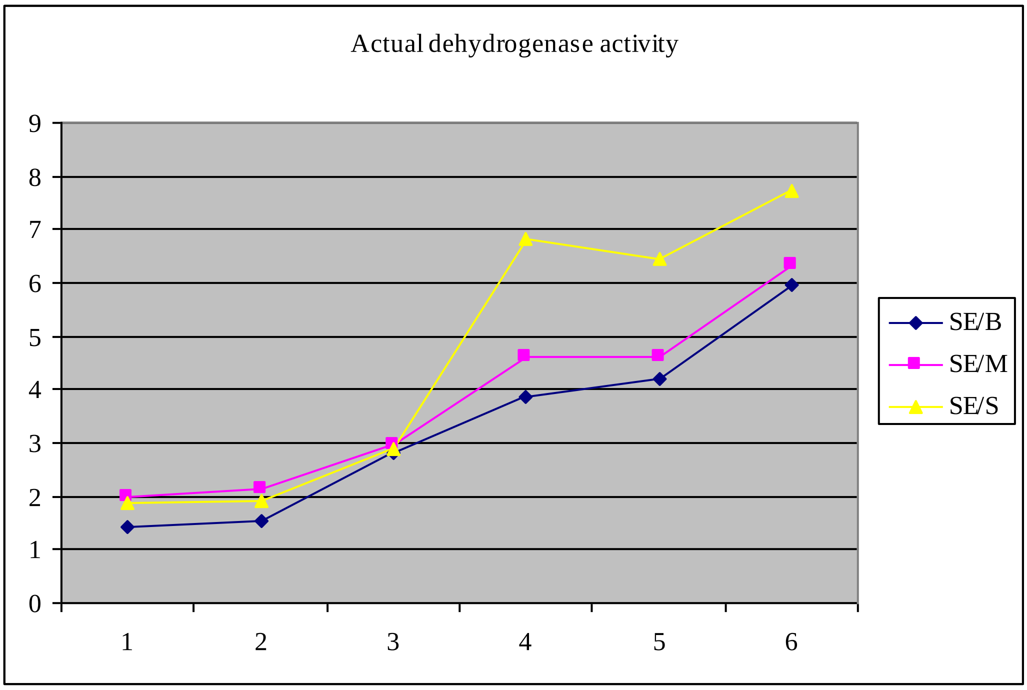

Fig. 4. Evolution of the actual dehydrogenase activity during the six months on the SE slope

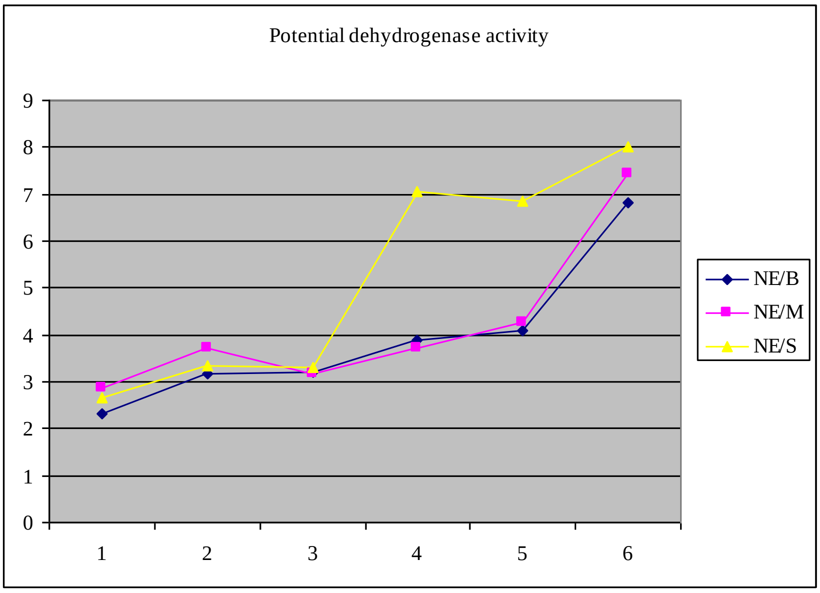

Fig. 5. Evolution of the potential dehydrogenase activity during the six months on the NE slope

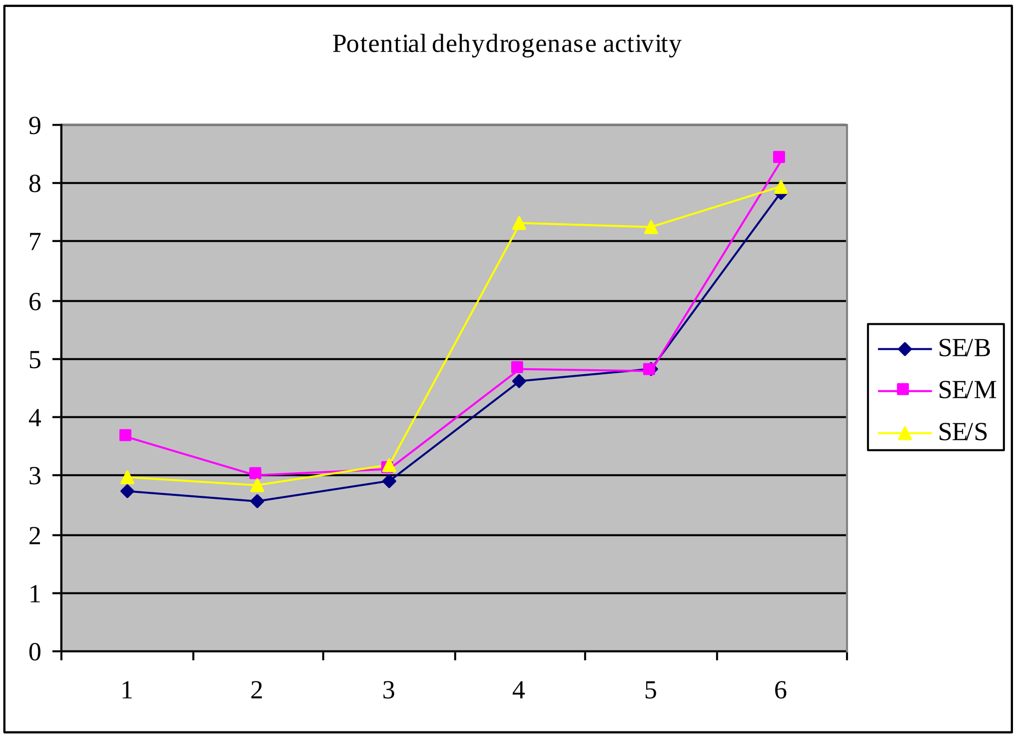

Fig. 6. Evolution of the potential dehydrogenase activity during the six months on the SE slope

Enzymatic indicators of soil quality

Significant ($p < 0.05$ to $p < 0.001$) and unsignificant ($p > 0.05$ to $p > 0.10$) differences were registered in the soil enzymatic activities depending on the sample area and the period of sampling. Based on these differences the following decreasing orders of the enzymatic activities could be established in the soil of the six plots:

November: NE/B > NE/M >SE/B > SE/M > SE/S > NE/S;
December: NE/B > NE/M > NE/S > SE/M > SE/S > SE/B;
January: NE/M > NE/B > SE/S > NE/S > SE/M > SE/B;
February: SE/S > NE/S > SE/M > NE/S > NE/M > NE/B;
March: NE/S > SE/S > SE/M > NE/M > NE/B > NE/S;
April: NE/M > SE/S > SE/B > NE/S > NE/B > SE/M.

For establishing such a hierarchy, we have applied the method suggested in [17]. Briefly, by taking the maximum mean value of each activity as 100% we have calculated the relative (percentage) activities. The sum of the relative activities is the enzymatic indicator which is considered as an index of the biological quality of the soil in a given plot. The higher the enzymatic indicator of soil quality, the higher position of plot is in the hierarchy. Table 1 shows that the first positions are occupied by those plots in which enzymatic activities were the highest.

Table 1. Enzymatic indicators of soil quality

Position	Plot	Enzymatic indicator of soil quality
1	NE/M	1904.82
2	NE/B	1838.43
3	SE/S	1804.61
4	NE/S	1778.81
5	SE/B	1597.17
6	SE/M	1364.21

The observation is in agreement with other studies. For example [9,10] observed that the ecologic rehabilitation of tailing ponds is a complex process, depending on many variables whose sharper definition would require interdisciplinary correlations as regards the physical–chemical particularities of the soil, orographycs factors, and especially micro soil factors that impact the changes of micro climatic parameters, systematic studies, physiologic and ecologic studies on the various groups of organisms.

Conclusions

(1)The results are in good agreement with the literature data [1, 5, 17] and constitute novelties for the enzymological characterisation of a soil from bauxite mine spoils.
(2)The literature reviewed [4, 8, 11] shows that application of enzymological methods makes it possible to indicate the degree of evolution of technogenic soils, the transformation of overburdens and other spoils and wastes into agricultural and forest soils, the efficiency of the recultivation measures applied.
(3)In comparison with microbiological parameters, the enzymes are more synthetic indicators of the evolution of technogenic soils because they reflect a) due to their accumulation in form of humic complexes, the past of technogenic soils, and b) due to their catalytic activity, which plays a key role in nutrient cycles, the present biological status of these soils.

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