INFLUENCE OF HEAVY METAL CONTAMINATION ON SOIL ENZYME ACTIVITIES

Samuel Alina Dora*, Blidar Cristian Felix*, Domuța Cornel, Șandor Maria**, Vușcan Adrian**, Borza Ioana**, Brejea Radu****

* University of Oradea, Department of Plant Biology, 1 Universității St., Oradea, Romania
**University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., Oradea, Romania,
e-mail: samuelalina@rdslink.ro

Abstract

Hazardous heavy metal pollution of soils is one of the most important environmental problems throughout the world. In fact, heavy metals have a significant toxicity for humans, animals, plant and microorganisms. Our results indicate that enzymatic activities are a great utility for understanding the negative impacts of heavy metals in soils.

Actual and potential dehydrogenase and catalase activities were determined in the 0-10, 10-20, 20-30 and 30-40 cm layers of a soil polluted with heavy metals. Comparing the soil enzyme activities in different seasons, it was found that seasonal differences were registered with higher soil enzymatic activities in the spring and in lower activities in the winter. The enzymatic indicators of soil quality calculated from values of enzymatic activities determined in the six places showed the order: NE/S > SE/E > NE/M > SE/M > NE/B > SE/B. This order means that by determination of dehydrogenase and catalase activities, which are considered as indicators of the global and respiratory activity of soil, valuable information can be obtained regarding fertility status of soils.

Key words: catalase, dehydrogenase, heavy metal, soil quality

INTRODUCTION

Heavy metal pollution of soil is an increasingly urgent problem all over the industrialized world (Garan G. et al, 2007; Kelly J.J. et al, 2003). Heavy metals, unlike organic contaminants, are generally immutable, not degradable and persistent in soil (Viti C. et al, 2006; Baker L.R. et al, 2011). Several studies have demonstrated the adverse effects of heavy metal contamination on plant growth (White C. et al, 2005) as well as on the size, structure (Peng J.F. et al, 2009) and functional diversity of soil microbial populations (Renella G. et al, 2003; Renella G. et al, 2005).

Microorganisms have co-existed with metals since the beginning of life. This is reflected by the presence of a wide range of heavy metals in the active sites of many enzymes (Marzadori C. et al, 1996; Lee I.S. et al, 2002), where the chemical properties of specific metal cations have been recruited for catalyzing key metabolic reactions and for maintaining protein structures. These metals are considered as essential micronutrients because they are required in minute amounts for normal cell metabolism (Kumpiene J. et al, 2008; Wang Y. et al, 2007). However, other metals seem to serve no biologically relevant function. All metals, when present at high concentration can damage cell membranes, block and inactivate enzymes (Costa-Martinez V. and Tabatabai M.A., 2000).

In recent years, pollution of soils by heavy metals has increased steadily as a result of increased application of organic amendments (Castaldi P. et al, 2005; Castaldi P. et al, 2009; Tejada M. et al, 2011), uncontrolled dumping, etc. Considering the toxicity of heavy metals to organisms, their presence will affect the microbiology of the soil ecosystem (Hiroki M., 1992). For this reason in the last decade, great effort has been put into the study of amendments capable of heavy metal adsorption, immobilization (Fawzy E.M. et al, 2008) or precipitation in order to reduce their bio-available fractions in the soil solution and, therefore, their negative effect on soil biological properties (Dobler R. et al, 2000; Garcia-Gil J.C. et al, 2000; Masciandaro G. et al, 2004).

The current literature indicates that soil enzymatic activities react faster than physical variables and/or after any chemical change in the soil and, therefore may be useful as early indicators of the various biological changes that may occur in soil (Pérez de Mora A. et al, 2005; Wei-yu S. et al, 2009).

Although there is much information about the adverse effects of heavy metals on soil enzymatic activities, there is not much information on how these activities evolve when the soil is contaminated with various metals at different rates (Minojosa M.B. et al, 2004; Mulligan C.N. et al, 2001). Therefore, the objective of this work is to study the effect of heavy metal contamination on some enzymatic activities involved in intracellular metabolism which will inform us about the impact of these heavy metals on soil biology.

MATERIAL AND METHOD

This experiment is part of a larger research initiative covering the use of micro biota in the overall regeneration of tailing ponds. Research studies reveal that remedial and restoration of vegetation in areas polluted with heavy metals, areas to which tailing ponds, could be enabled by a clever selection of tolerant species of plants. Within this framework we monitor the role that microorganisms 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 2008 to October 2009 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 (Schinner F. et al, 1996). 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. Dehydrogenase activities are expressed in mg of triphenyl-formazan (TPF) produced from 2, 3, 5 - triphenyltetrazolium chloride (TTC) by 10 g of soil in 24 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. The reaction mixtures consisted of 3.0 g soil, 2 ml H_2O_2 3% and 10 ml phosphate buffer. It suffered incubation at 37º C for 1hour. 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 according to the methods described in (Sachs L., 2002).

RESULTS AND DISSCUSIONS

The results of the enzymological analyses are presented in Fig. 1 – 6.

When the results obtained in the four soil layers were considered together, the actual and potential dehydrogenase and catalase activities were the highest in spring. These findings are valid for both slopes at all levels.

In accordance with (Renella G et al, 2005; Marian M. et al, 2008b), our results indicate that enzymatic activities are of great utility for understanding the negative impacts of heavy metals in soils. Enzymatic activities permit the immediate detection of changes in the quality of soils resulting from their contamination by heavy metals, since these activities are linked closely by the cycles of nutrients.

Currently there are various interpretations that try to explain the negative interactions between heavy metals and the soil biochemical properties, the great majority of them indicating that these interactions do not depend directly on the soil pH. It was found (Renella G. et al, 2003) that negative effects on the biological properties of soil contaminated by heavy metals can possibly be a consequence of a decrease in the time that substrates are available to the microorganisms, a lower synthesis and/or liberation of the extracellular enzymes. On the other hand, it is known that the different metallic ions differ in their capacity to act as inhibitors of diverse soil enzymes.

The results obtained in this work suggest that dehydrogenase and catalase can serve as a good indicator of heavy metal contamination. Since these enzymes have an intracellular origin, it is found fundamentally in the viable microorganisms resistant to heavy metal contamination. Thus, its activity is related to the presence of live microorganisms and their oxidative capacity (Teyada M. et al, 2011).

Since soil contamination by heavy metals implies a serious environmental problem, in recent years different techniques have been developed for their immobilization or elimination. Usually, techniques such as phytoremediation or immobilization with diverse organic substances (Marian M. et al. 2008a; Marian M. et al, 2009) are employed. However, the immobilization of these metals by organic substances, due to their great adsorption power, is of special interest, since this adsorption will depend on the chemical characteristics of the organic matter. In all cases, this immobilization of the metals implies a decline in their concentration in the soil solution and, as a consequence, in their mobility.

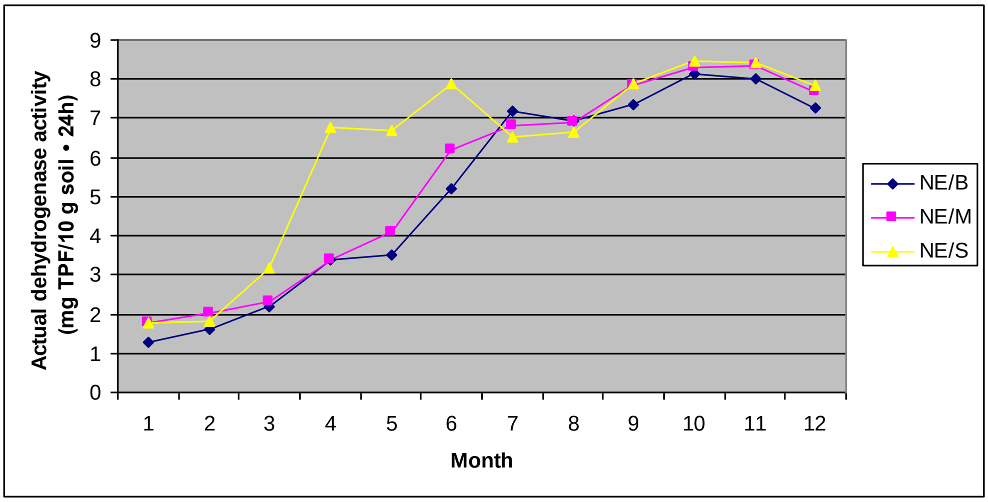

Fig. 1. Evolution of the actual dehydrogenase activity during the twelve months on the NE slope

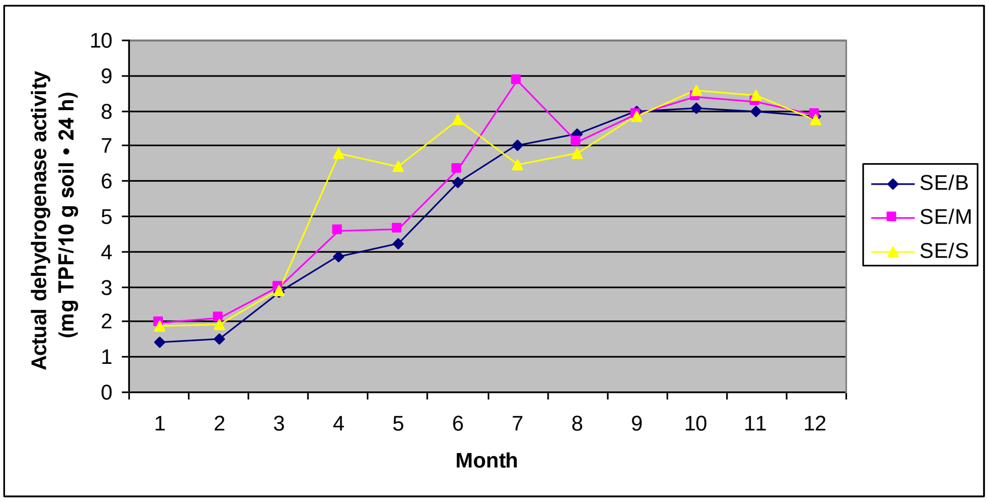

Fig. 2. Evolution of the actual dehydrogenase activity during the twelve months on the SE slope

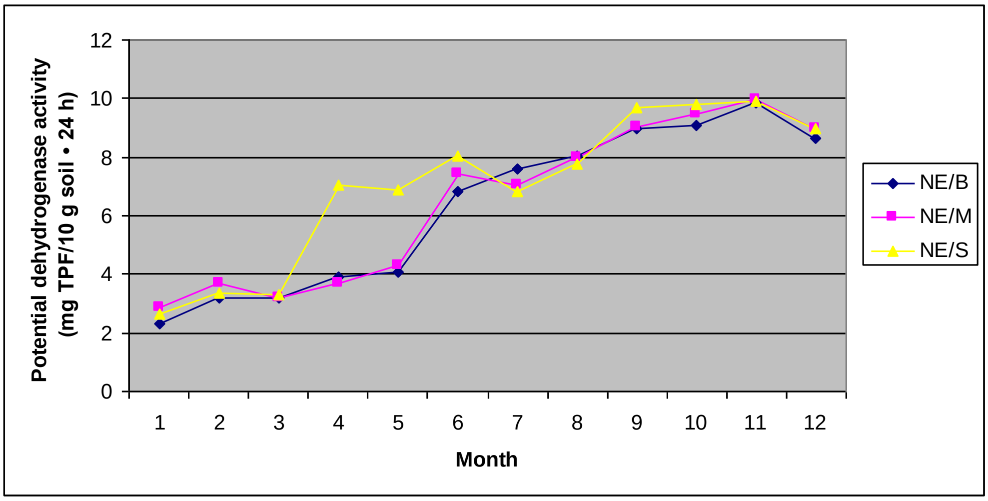

Fig. 3. Evolution of the potential dehydrogenase activity during the twelve months on the NE slope

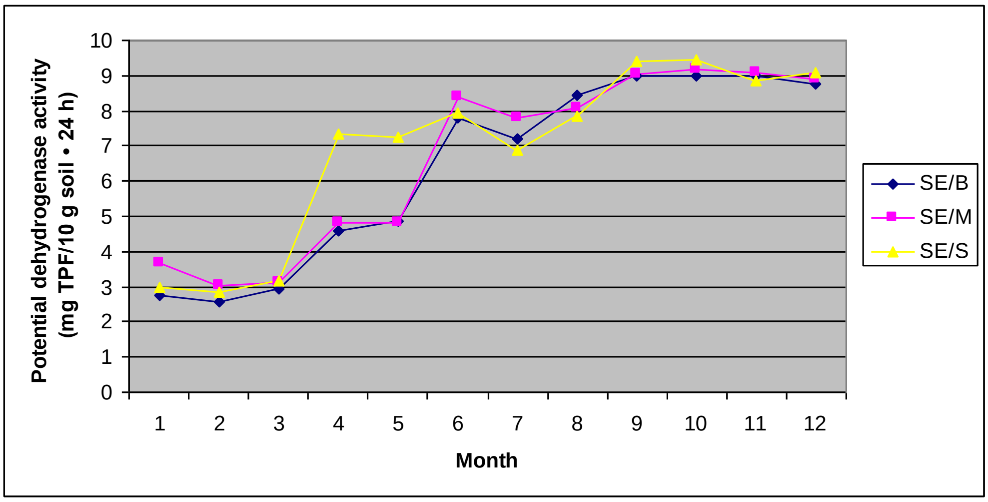

Fig. 4. Evolution of the potential dehydrogenase activity during the twelve months on the SE slope

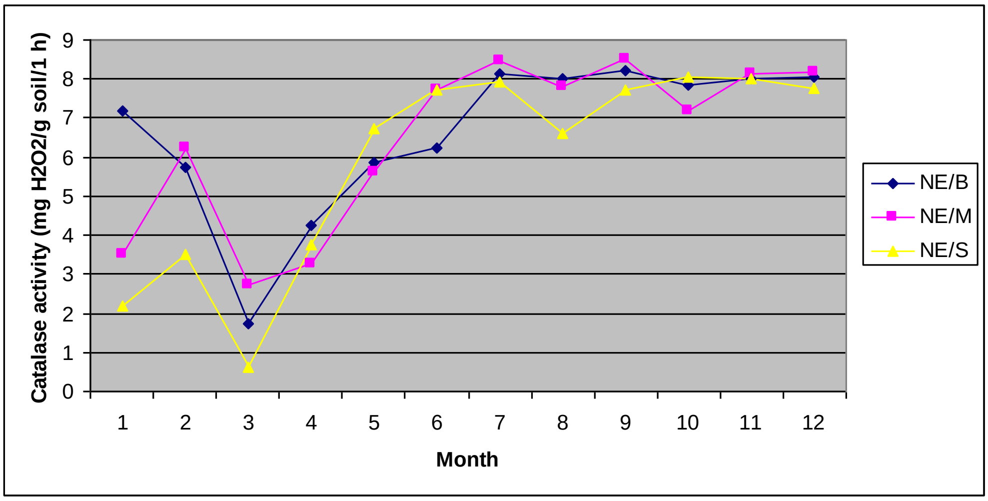

Fig. 5. Evolution of the catalase activity during the twelve months on the NE slope

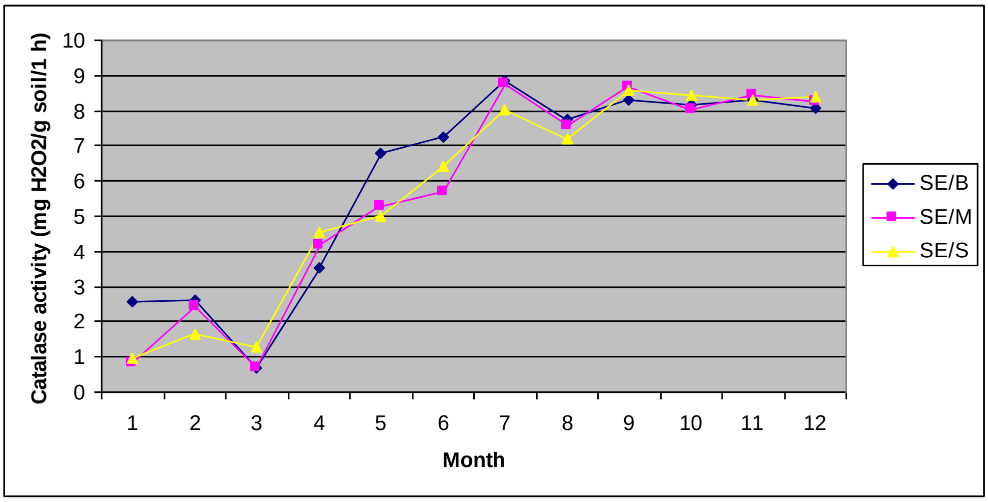

Fig. 6. Evolution of the catalase activity during the twelve months on the SE slope

Enzymatic indicators of soil quality

Significant ($p < 0.05$ to $p < 0.01$) and unsignificant ($p > 0.05$ to $p > 0.10$) differences were registered in the soil enzymatic activities depending on the kind of enzymatic activity and on the sample place. Based on these differences the following decreasing orders of the enzymatic activities could be established in the soil of the six places:

- actual dehydrogenase activity:
  NE/S > SE/S > SE/M > SE/B > NE/M > NE/B ;
- potential dehydrogenase activity:
  NE/S > SE/S > SE/M > NE/M > SE/B > NE/B ;
- catalase activity :
  NE/B > NE/M > SE/B > NE/S > SE/M > SE/S.

It is evident from these orders that each of the six places presented either a maximum or a minimum value of the three soil enzymatic activities. Consequently, these orders do not make it possible to establish such an enzymatic hierarchy of the places which takes into account each activity for each place. For establishing such a hierarchy, we have applied the method suggested in (Samuel A.D. et al, 2008). 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 place. The higher the enzymatic indicator of soil quality, the higher the position of place is in the hierarchy.

Table 1

Enzymatic indicators of soil quality

Position	Place	Enzymatic indicator of soil quality
1	NE/S	289.08
2	SE/S	284.98
3	NE/M	278.69
4	SE/M	277.87
5	NE/B	273.94
6	SE/B	272.85

Table 1 shows that the first four positions are occupied by those places in which actual dehydrogenase activity, potential dehydrogenase activity and catalase activity, respectively, were the highest. The places NE/B and SE/B occupying the last positions can be considered as the least enzyme-active soil.

CONCLUSIONS

Dehydrogenase and catalase activities are widely used to evaluate the metabolic activity of soil microbial communities and some evidences indicated that these activities are more sensitive indicators of the effects of heavy metal on soil microbial properties than other soil parameters.

Seasonal differences were registered, the most intense activities being noticed in spring.

The enzymatic indicators of soil quality calculated from the values of actual and potential dehydrogenase and catalase activities determined in the six places showed the order: NE/S > SE/S > NE/M >SE/M >NE/B >SE/B.

Acknowledgements

We have conducted our research work and subsequent analyses within the framework of the 52144/01.10.2008 PNCDI II Project.

REFERENCES

1. Baker L.R., White P.M., Pierzynski G.M., 2011, Changes in microbial properties after manure, lime and bentonite application to a heavy metal – contaminated mine waste. Appl. Soil Ecol., no. 48, pp. 1-10.
2. Castaldi P., Santana I., Melis P., 2005, Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. Chemosphere, no. 60, pp. 365-371.
3. Castaldi P., Melis P., Silvetti M., Deiana P., Garan G, 2009, Influence of pea and wheat growth on Pb, Cd and Zn mobility and soil biological status in a polluted amended soil. Geoderma, no. 151, pp. 241-248.

4. Costa-Martinez V., Tabatabai M.A., 2000, Enzyme activities in a limed agricultural soil. Biol. Fertil. Soils, no. 31, pp. 85-91.
5. Dobler R., Saner M., Bachofen R., 2000, Population changes of soil microbial communities induced by hydrocarbon and heavy metal contamination. Bioremediat. J., no. 4, pp. 41-56.
6. Fawzy E. M., 2008, Soil remediation using in situ immobilisation techniques Chem. Ecol., no. 24, pp. 147-156.
7. Garan G., Castaldi P., Santona L., Deiana P., Melis P., 2007, Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil. Geoderma, no. 142, pp. 47-57.
8. Garcia-Gil J.C., Plaza C., Soler-Rovira P., Polo A., 2000, Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biol. Biochem., no. 32, pp. 1907-1913.
9. Hiroki M., 1992, Effects of heavy metal contamination on soil microbial population. Soil Sci. Plant. Nutr., no. 38, pp. 141-147.
10. Kelly J.J., Häggblom M., Tate R.L., 2003, Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter as indicated by analysis of microbial community phospholipid fatty acid profiles. Biol. Fertil. Soils, no. 38, pp. 65-71.
11. Kumpiene J., Lagerkvist A., Maurice C., 2008, Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – a review. Waste Manage, no. 28, pp. 215 – 225.
12. Lee I.S., Kim O.M., Chang Y.Y., Bac B., Korean H.H., Back K.H., 2002, Heavy metal concentrations and enzyme activities in soil from a contaminated Koran shooting range. J. Biosci. Bioeng., no. 94, pp. 406-411.
13. Marian M., Varga C., Mihaly-Cozmuța L., Mihaly-Cozmuța A., Mihalescu L., Roșca-Mare, O., Blidar C.F., 2008a, Evaluation of the phytoremediation potential of the *Quercus petraea* in tailing ponds. Stud. Univ. Vasile Goldiș, Life Sci. Seri., no. 18, pp. 41-51.
14. Marian M., Varga C., Mihaly-Cozmuța L., Mihaly-Cozmuța A., Mihalescu L., Roșca-Mare, O., Blidar C.F., 2008b, Variance analysis on different tree species depending on soil type-uncontaminated and heavy metals contaminated ones. Analele Univ. Oradea, Fasc. Biol., no. 15, pp. 41-46.
15. Marian M., Varga C., Mihaly-Cozmuța L., Peter A., Mihaly-Cozmuța A., Boltea D., 2009, Evaluation of the phytoremediation potential of the *Populus tremula* in tailing ponds. Buletin USAMV Agriculture, no. 66(2), pp. 124-131.
16. Masciandaro G., Ceccanti S., Benedicto S., Lee H.C., Cook F., 2004, Enzyme activity and C and N pools in soil following application of mulches. Can. J. Soil Sci., no. 84, pp. 19-30.
17. Marzadori C., Ciavatta C., Montecchio D., Gessa C., 1996, Effects of lead pollution on different soil enzyme activities. Biol. Fertil. Soils, no. 22, pp. 53-58.
18. Minojosa M.B., Garcia-Ruiz R., Viňegla B., Carreiro J.A., 2004, Microbiological rates and enzyme activities as indicators of functionality in soils affected by the Aznalcóllar toxic spill. Soil Biol. Biochem., no. 36, pp. 1637-1644.
19. Mulligan C.N., Yong R.N., Gibbs B.F., 2001, Remediation technologies for metal contaminated soils and groundwater: an evaluation. Engin. Geo., no. 60, pp. 193-207.
20. Peng J.F., Song Y.H., Yuan P., 2009, The remediation of heavy metals contaminated sediment. J. Hazard. Mater., no, 161, pp. 633-640.
21. Pérez de Mora A., Ortega-Calvo J.J., Cabrera F., Madejón E., 2005, Changes in enzyme activities and microbial biomass after “in situ” remediation of heavy metal contaminated soil. Appl. Soil Ecol., no. 28(2), pp. 125-137.

22. Renella G., Ortigoza A.I.R., Laudi I., Nannipieri P., 2003, Additive effects of copper and zinc on cadmium toxicity on phosphatase activities and ATP content of soil as estimated by the ecological dose (ED_{50}). Soil Biol. Biochem., no. 35(9), pp. 1203-1210.
23. Renella G., Mench M., Gelsomino A., Laudi I., Nannipieri P., 2005, Functional activity and microbial community structure in soils amended with bimetallic sludges. Soil Biol. Biochem., no. 37(8), pp. 1498-1506.
24. Sachs L., 2002, Angewandte Statistik Anwerdung Statisticher Methoden. Springer, Berlin, pp. 189-195.
25. Samuel A.D., Domuţa C., Ciobanu C., Şandor M., 2008, Field management effects on soil enzyme activities. Rom. Agric. Res., no. 25, pp. 61-68.
26. Schiner F., Őhlinger R., Kandeler E., Margesin R., 1996, Methods in Soil Biology. Springer, Berlin, pp. 235-243.
27. Tejada M., Parrado J., Hernandez T., Garcia C., 2011, The biochemical response to different Cr and Cd concentrations in soils amended with organic wastes. J. Hazard. Mater., no. 185, pp. 204-211.
28. Viti C., Mini A., Ranalli G., Lustrato G., Giovannetti L., 2006, Response of microbial communities to different doses of chromate in soil microcosms. Appl. Soil Ecol., no. 34, pp. 125-139.
29. Wang Y., Shi J., Wang H., Lin Q., Chen X., Chen Y., 2007, The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity and community composition near a copper smelter. Ecotox. Environ. Safety, no. 67, pp. 75-81.
30. Wey-yu S., Hong-So S., Hua L., Ming-an S., Sheng D., 2009, Progress in the remediation of hazardous heavy metal polluted soils by natural zeolite. J. Hazard. Mater., no. 170, pp. 1-6.
31. White C., Tardif J.C., Adkins A., Staniforth R., 2005, Functional diversity of microbial communities in the mixed boreal plain forest of central Canada. Soil Biol. Biochem., no. 37, pp. 1359-1372.