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Research article

THE EFFECT OF HEAVY METALS ON ENZYME ACTIVITIES IN SPONGE IRON INDUSTRY POLLUTED SOIL

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ABSTRACT: Present study assessed the effect of heavy metals on soil enzyme activities like amylase, invertase, dehydrogenase and urease of open shrub lands present at 1 km, 1.5 km, 2 km and 2.5 km distance away from Bhushan sponge iron industry, in Rengali block of Sambalpur district, Odisha. The heavy metals studied were Cd, Ni, and Cr, whose concentrations in the above sites were much more than the safety limits prescribed by WHO/FAO (2007). Highest amount of heavy metals were recorded in the site nearest to the plant i.e.1km away from the plant. Soil enzymes secreted by microorganisms, animals and plant roots below the soil play an important role in the biochemical functioning of soil like organic matter formation and degradation, nutrient cycling and energy transformation by catalyzing numerous chemical, physical and biological reactions. The site containing lowest heavy metals (5.6mg/Kg, 413.0mg/Kg, and 1006.3mg/kg of Cd, Ni, and Cr respectively) showed highest enzyme activities with respect to other experimental sites. A negative correlation was observed between heavy metal concentrations and soil enzyme activities. Further, the study indicated higher soil enzyme activities in the post-monsoon period in comparison to pre-monsoon period. When compared to the enzyme activities in the soil of open shrub lands present at the bottom of Dehari hill in Jyoti Vihar of Burla town, 32 km away from the present study site (control site), the enzyme activity of the present study was very low. The percentage inhibition of different enzyme activities in experimental sites with respect to control site were 91% and 80% for amylase, 93% and 91% for invertase, 97% and 96% for cellulase, 90% and 85% for dehydrogenase , 90% and 79.5% for urease activity in premonsoon and postmonsoon period respectively. The results indicate that the heavy metals in such high concentrations inhibit the activity of enzyme secreting flora and fauna in soil, as excessive amounts of heavy metals disrupt the homeostasis of soil by interfering with the control mechanisms at genetic level. Thus, the decline in soil enzyme activity reflects the soil degradation potential of the heavy metals released from sponge iron industry. Therefore, it is highly essential to take immediate remediation measure in order to protect the health of soil flora and fauna in these areas.
Key words: Soil enzymes activity, heavy metals, safety limit, correlation

INTRODUCTION

The contamination of soils by heavy metals is a significant problem, which leads to negative influence on soil characteristics and limitation of productive and environmental functions [9]. The advancement in industrial and technological progress negatively affects the environment by polluting and degrading the soil. One such industry is sponge iron where sponge iron is made from reduction of iron and is used as a raw material in making steel. Most of sponge iron plants in India are coal- based and are concentrated in the central eastern belt of the country including Odisha because of the easy availability of iron and coal in this belt [12]. Ash is a hazardous waste emitted from sponge iron industry and has tremendous impact on soil properties. The coal fly ash emitted from the furnace of coal based sponge iron industry contains heavy metals like arsenic, cadmium, cobalt, copper, chromium, nickel, lead, zinc and mercury [17]. Thus, deposition of fly ash in the environment surrounding sponge iron plant leads to accumulation of heavy metals in the soil.

The ecological problem of soil degradation due to sponge iron industry is of extreme importance in present global scenario. Sponge Iron plants release hazardous pollutants like cadmium, nickel, hexavalent chromium (http://www.minesandcommunities. org/article.php?a=5099) which ultimately reach soil and cause harm to soil biota. Metals entering the soil accumulate in it and remain as long lasting pollutant. Once the metal enters the soil, there is uptake by the plants, some amount is immobilised in the soil matrix and rest moves with water to ground water sources [10, 7]. The study of heavy metals in soil is of great concern not only for their toxicity to soil biota but also their immobilisation with different organic and inorganic colloids. In the immobilised form they can persist for long time before being again available to living organisms including plants [20]. Soil is a slow moving medium and the heavy metals migrate in it slower than they do it in water and air; because of which these toxicants increase gradually in the soil [28].

The measurement of soil enzyme activities can give information about specific processes in soil and some time acts as an indicator of soil fertility [2, 19]. Most of the soil enzymes (intracellular and extra cellular) are proteins of microbial origin, and heavy metals affect the number, diversity and microbial activity of soil microorganisms [26]. The high level of heavy metal concentration in soil slows down the speed of growth and reproduction of microorganisms present in it. This results in slower growth of microorganisms with lower diversity and also subsequent resistant to heavy metals with decreased biological activities. Thus, higher concentration of heavy metals in soil negatively affects the activity of soil enzymes, and therefore, the knowledge of various enzyme activities can provide information about the degradation of soil due to heavy metal contamination [27].

There is a cluster of eight large and medium scale sponge iron industries in Sambalpur district [13]. The SPCB had entrusted NEERI to conduct the study in a 45 km radius area with Rengali in Sambalpur district as the epicentre. Earlier, Indian Institute of Technology (IIT), Kharagpur, had conducted a similar survey, based on which some industries, in the first phase, were given closure notice [11]. But till now no such steps have been taken. People residing near the sponge iron industries are continuously complaining about the low productivity of rice crop and fruit bearing trees in these areas [14]. Several literatures are available regarding role of vegetation in reclamation of waste dumps [16]. The soil of crop fields and open forest surrounding Bhushan sponge iron industry (a large scale industry in Rengali block of Sambalpur district) was found to be highly polluted with heavy metals like Cd, Cr, and Ni [24]. Currently no information is available regarding the activity of soil enzymes as affected by heavy metal pollution due to sponge iron industry. In the present study we have investigated soil enzyme activities on the top soil (0-10cm depth) at different open shrub lands near Bhusan sponge iron industry, during premonsoon and postmonsoon period of 2011.

MATERIAL AND METHODS

Study sites

The study sites are open shrub lands surrounding the Bhushan sponge iron industry. These sites are located between latitude 21°44' to 21°46' N and longitude 84°01' to 84°03' E at Rengali Block of Sambalpur district of Odisha (Fig 1). The study was carried out in premonsoon (April) and postmonsoon (October) period of 2011.The mean annual rainfall was 1460.8mm and most of its fell during monsoon season which lasted from June to September. The relative humidity varied from 21 to 87%. The open shrub lands present at the bottom of Dehari hill of JyotiVihar Campus in Burla town of Sambalpur district present 32 km away from experimental sites was considered as control site. Some of the common herb, shrub species found in both experimental and control sites were *Aristida setacea* Retz.**,** *Cynodon dactylon* (L.)Pers, *Eragrostis ciliaris* (L.), *Evolvulus alsinoides* (L.). These grass and non grass species have slow growth with small biomass, and therefore not suited for phytoextraction [31].

As the predominant wind directions in April and October were North- East, East, and South-East, the sampling sites were chosen in these directions which were all open shrub lands. For present study four different locations were chosen and designated as site 1, site II, site III, site IV which corresponds to sites located at 1 km, 1.5 km, 2 km and 2.5 km distances from the stack of the sponge iron plant. The soil samples from 0-10 cm depth were packed in plastic zipper bag, brought into the laboratory and stored at 4 ºC before analysis. The enzyme analyses were made within four weeks after sampling because storage beyond four weeks can cause decline in enzyme activities [1].

Enzyme activities

The amylase, invertase, and cellulase activities were measured by dinitro salicylic acid method [26] and were expressed as µg glucose g^{-1} dry soil hr^{-1}. The estimation of dehydrogenase activity was done by tryphenyl formazan method [3] and was expressed in µg TPF g^{-1} dry soil hr^{-1}. Method used for the assay of urease activity involved the estimation of ammonium nitrogen released on incubation of soil with buffered urea solution [25]. The amount of NH_4^+-N released by the action of urease was measured at 625 nm and is expressed as µg NH_4^+-N g^{-1} dry soil hr^{-1}.

Soil heavy metal study was done during premonsoon period. The heavy metal content in the soil samples were analysed by AAS (Perkin Elemer-3110) after digesting the samples with conc. Hydrochloric acid, Perchloric acid and Nitric acid.

Data Analysis

Variation pattern examined graphically after the study of different enzyme activities were subjected to two way ANOVA with replicates to find out the significance of the enzyme activities with respect to different sites and different seasons. Pearson's Correlation coefficient was calculated to find out the correlation between heavy metal concentrations and rate of enzyme activities.

RESULTS

Heavy metals

The heavy metal contents (Cd, Ni, and Cr) of the present study sites (open shrub lands) have been presented in Table 1. The Cd, Ni, Cr content of the experimental sites at different distances varied from 5.6 to 6.01, 413.0 to 429.75, and 1006.3 to 1186.5 mg/Kg dry soil respectively. These values were much higher than the safe limits prescribed for Indian soil [29]. However no data was available for safe limit of Cr in Indian soil. Therefore, for Cr, safe limit for European soil was considered for the comparison. The Cd, Ni, Cr content of control site were 0.29, 25.67, 56.78 mg/Kg respectively. Highest concentration of all the heavy metals were recorded at 1km away from the stack of the sponge iron plant and the concentrations decreased with the increase in distance from the plant.

Enzyme activity

Figure 2 reveals the amylase, invertase, cellulase, dehydrogenase and urease activity in experimental sites and control site. All the enzyme activities were significantly low in experimental sites when compared to control site in both the seasons. Further, the enzyme activities were significantly high (at $p < 0.001$) during postmonsoon period when compared to premonsoon period and increased with increasing distance from the plant. By taking the average values of enzyme activities of experimental sites it has been recorded that the percentage inhibition of different enzyme activities in experimental sites with respect to control site were 91% and 80% for amylase, 93% and 91% for invertase, 97% and 96% for cellulase, 90% and 85% for dehydrogenase, 90% and 79.5% for urease activity in premonsoon and postmonsoon period, respectively. All the enzyme activities with increasing distance from the sponge iron plant showed significant negative correlation with heavy metals in the respective top soils away from the industry (Table 2) which indicates that with the increase in distance from the stack of the industry, the heavy metal contents decreased and the enzyme activities increased.

Table 1 Heavy metal concentration in control and experimental sites near Bhushan steel plant

Distance	Heavy metal content (mg/kg)		
	Cd	Ni	Cr
1.0km	6.01±0.38	429.75±19.3	1186.5±133.0
1.5km	5.97±1.6	420.5±3.5	1166.5±35.35
2.0km	5.7±0.4	418.5±5.0	1119.75±48.8
2.5km	5.6±0.68	413.0±24.8	1006.3±203.7
Control, 32 km	0.29±0.08	25.67±4.89	56.78±5.6
Safe limits for Indian soil	3-6	75-150	150*

*indicates the safe limit for European soil (EU 2002) [7].

Table 2 Correlation between Enzyme activities and heavy metal concentrations

Heavy metal concentration (mg/kg)	Enzyme activities				
	Amylase	Invertase	Cellulase	Dehydrogenase	Urease
Cd	− 0.97*	− 0.88*	-0.98*	− 0.974*	− 0.99*
Ni	− 0.89*	− 0.94*	-0.87*	− 0.99*	− 0.90*
Cr	− 0.91*	− 0.97*	-0.94*	− 0.93*	− 0.97*

*represents significance at p<0.001,

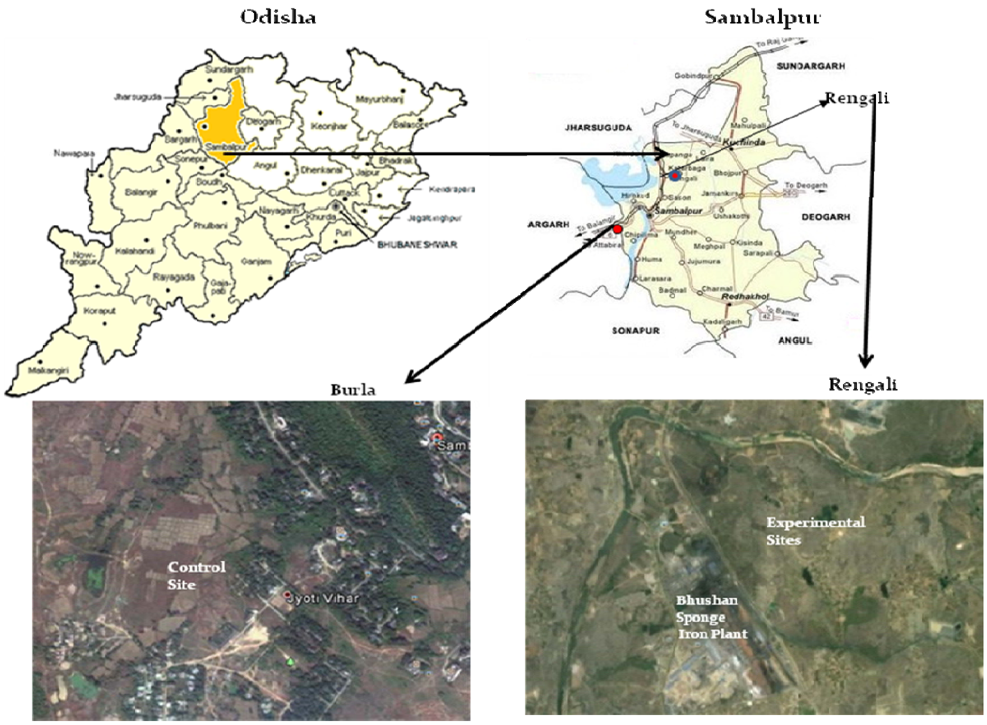

Figure 1 Experimental site at Rengali block and Control site at Jyoti Vihar, Burla of Sambalpur District of Odisha (Source www.google.earth)

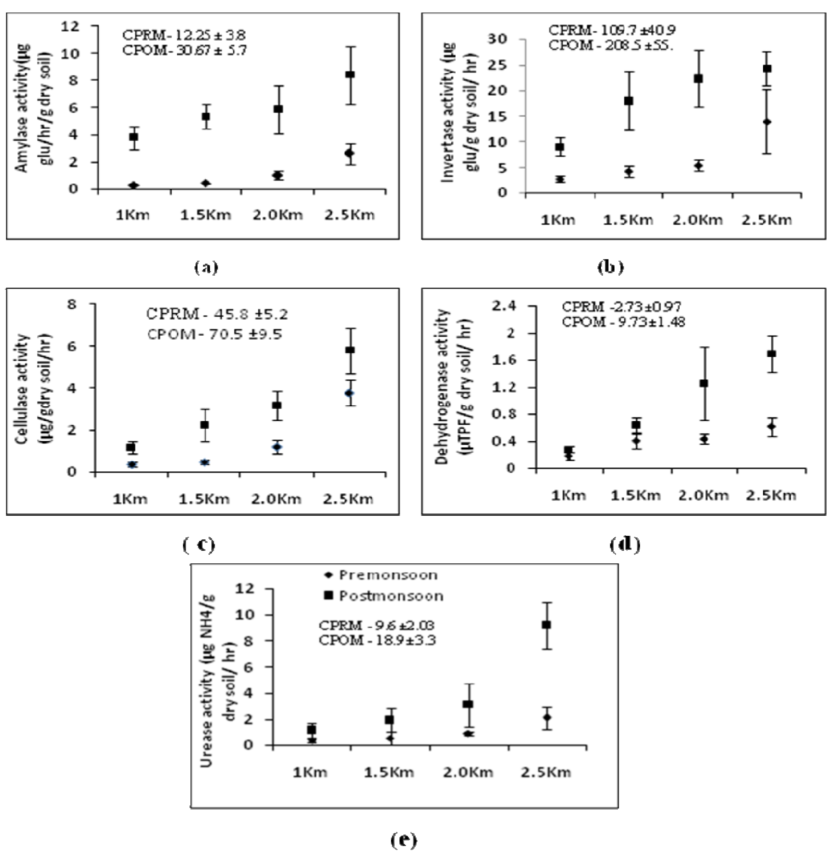

Figure 2 Different soil enzyme activities during premonsoon and postmonsoon period: (a) Amylase activity, (b)Invertase activity, (c) Cellulase activity (d) Dehydrogenase activity, (e) Urease activity, CPRM and CPOM values represent the premonsoon and postmonsoon values for different enzyme activities of control soil, respectively

DISCUSSION

In the present study, site I was located adjacent to an unpaved road built by Bhushan limited for the transport of waste materials from the plant to the dumping site. So, large amount of dust along with ash are deposited on the soil of site I. Significantly high concentration of heavy metals like Cd, Ni, and Cr in site I and gradual decrease of the concentration of these heavy metals with the increasing distance from the stack of the plant suggested that site I was heavily polluted with heavy metals in comparison to other sites. Pollution of soil environment with heavy metals, its effect on the soil microorganisms [15] and further on their enzymatic activity depends, apart from other things on the soil pH, the content of organic and mineral colloids and on metal type and its chemical properties [21] which are responsible for the variations observed in the different enzyme activities in various sites. Soil enzyme activities have potential to provide a unique integrative and reliable biological assessment of soil because of their relationship to soil biology, ease of measurement and rapid response to change in soil management [6].They are influenced by soil temperature, moisture content, vegetation cover, period of sampling [23]. Dehydrogenases are enzymes that indicate rather closely the soil biological activity. Their activity can be inhibited up to 90% by high soil contamination with heavy metals [27]. Urease activity is also sensitive to soil contamination with heavy metals [5]. 80% to more than 90% inhibition of enzyme activity was recorded in experimental sites with respect to control sites in the present study, which supports the above findings. The heavy metals also inhibit enzymatic reaction by bonding themselves to substrate, creating complexes with substrate, blocking reactive functional groups of enzymes or reacting with an enzyme-substrate complex [18]. These mechanisms were possibly responsible for such significant inhibition of soil enzyme activities in the experimental sites in the present study. Strong negative correlation of Cd, Cr, and Ni with all the studied enzyme activities suggests that the microbes secreting these enzymes are very much sensitive to the heavy metal concentration in soil and their activities are strongly inhibited by all the three heavy metals (Cd, Ni, Cr). Nowak et al. [21] reported that the enzyme activity in the soil decreases with increase of heavy metal ions concentration in the soil. The present findings indicating significant increase in enzyme activities with increase in distance from the plant site is in agreement with the above findings. Sahoo et al. [24] reported Low OC, TN, available Na and significantly high heavy metals like Cr, Ni, Cd in soils near the Bhushan Sponge Iron Industry. Thus low nutrient content and high heavy metal concentration (6.01mg/Kg, 429.75mg/Kg, and 1186.5mg/kg of Cd, Ni, and Cr respectively) in the vicinity of sponge iron plant might be responsible for lowest enzyme activities at 1 km distance from the stack of the plant.

Climatic condition controls the soil enzyme activity. In the present study, the enzyme activities were found to be significantly more in post-monsoon periods than the pre-monsoon periods in all the experimental sites. This is supposed to be due to increase in soil moisture content from 2% to 30%, optimal temperature (35^{0}C), high organic matter content in post monsoon which favoured the maximum microbial activities that lead to the higher enzyme activities. Rain fall resulting in mobilization and leaching of heavy metals down the profile along with the above factors seems to be responsible for higher enzyme activity in the top soil during postmonsoon in comparison to premonsoon period.

CONCLUSION

In Rengali block of Sambalpur district of Odisha, the heavy metal content in the soil nearer to sponge iron industry is well above the safety limit prescribed by WHO/FAO (2007). The enzyme activities are vigorous in control site, but inhibited in the experimental sites. Such inhibition could be attributed to heavy metal contamination of the soil by the fall out of pollutants from the sponge iron industry as the level of enzymatic activity gradually increases, at every 500 m between 1km to 2.5km distance from the stack, with the decrease in heavy metal concentration. Giller et al. [30], suggested that there is a need for experimentation beyond simple laboratory studies to provide a better understanding of the long-term effects of heavy metal toxicity to microorganisms that are responsible for enzyme secretion in field soils, and how such effects are regulated, which can then be used to guide future policies for the environmental protection of soils. Therefore, for any industrial site, field investigation in relation to metal toxicity and enzyme activity in soil is inevitable for the management of the environment.

REFERENCES

[1] Benjamin, L. T., Tania, E. R., 2010. Stability of hydrolytic enzyme activity and microbial phosphorus during storage of tropical rain forest soils, Soil Biology & Biochemistry, 42 (3), pp 459-465.
[2] Burns, R. G. 1978. Enzyme activity in soil: some theoretical and practical considerations. In: R.G.Bums (ed) Soil enzymes. Academic, London: pp 295–340.
[3] Casida, L. E. J. R., Klein, D. A. Sntoro, D. 1964. Soil Dehydrogenase activity. Soil Science. 98, pp 371-378.
[4] Castaldi S., Rutigliano F.A., Virzo De Santo A. 2004, Suitability of soil microbial parameters as indicators of heavy metal pollution. Water, Air and Soil Pollution, 158, pp 21–35.

[5] Chung, Rang, Z., Cong, TU., Huai Man, C. 1999. Effect of combined heavy metal pollution on nitrogen mineralization potential, urease and phosphatase activities in a Typic Udic Ferrisol. Pedosphere, 9, (3) pp 251-258.

[6] Dick, R. P. 1997. Soil enzyme activities as integrative indicators of soil health. In: C. E. Pankhurst, B.M. Doube, V. V. S. R. Gupta (Eds.). Biological Indicators of Soil Health, CAB International, Wellingford, pp 121–156.

[7] Dudka, S., Diotrowska, M., Chlopecka, A. 1994. Effect of elevated concentrations of Cd and Zn in soil on Spring wheat yield and the metal contents of the plants. Water Air Soil Pollution, 76, pp 333-341.

[8] European Union. 2002. Heavy Metals in Waste, European Commission, DG ENV. E_3, Final Report, February 2002.

[9] Friedlova, M. 2010. The influence of heavy metals on soil biological and chemical properties, Soil and Water Research,5 (1), pp 21-27.

[10] Hapke, H. J. 1991. Effects of metals on domestic animals in: Metals and their compounds in the environment, E. Merian, (ed), VCH publisher, Inc., New York, USA, pp 1438.

[11] http://articles.timesofindia.indiatimes.com/2012-10-21/bhubaneswar/346272821 sponge - iron- neeri-industries.html

[12] http://ejatlas.org/conflict/sponge-iron-plants-in-odisha-india

[13] http://orissa.oriyaonline.com/sambalpur_industries.html.

[14] http://www.globalissues.org/news/2010/02/05/4435.

[15] Kucharski, J. and Wyszkowska, J. 2004. Interrelationship between number of microorganisms or spring barley yield and degree of soil contamination with copper. Plant, Soil and Environment, 50, pp 243-249.

[16] Kullu, B., Behera, N. 2011. Vegetation succession on different age series sponge iron solid waste dumps with respect to top soil application. Research Journal of Environmental and Earth Science,3 (1), pp 38-45.

[17] Llorent, J. F., Fernandez-Turiel, J. L., Querol, X. 2001. The fate of trace elements in a large coal-fired power plant. Environmental Geology, 40 (5), pp 405-416.

[18] Mikanova, O. 2006. Effects of heavy metals on some soil biological parameters. Journal of Geochemical Exploration, 88, pp 220-223.

[19] Mishra P.C., Mohanty, R. K., Dash, M. C. 1979. Enzyme activities in subtropical surface soils under pasture. Indian Journal of Agriculture and Chemistry.1, pp 25-27.

[20] Nannipieri P., Badalucco L., Landi L., Pietramellara G. 1997, Measurement in assessing the risk of chemicals to the soil ecosystem. In: J.T. Zelikoff (ed.): Ecotoxicology: Responses, Biomarkers and Risk Assessment. OECD Workshop. SOS Publ. Fair Haven, New York, pp 507–534.

[21] Nowak, J., Niedzwiecki, E. and Dziel, M. 1999. Heavy metals influence on soil enzyme activity changes (in Polish) Roczniki Gleboznawcze, L (1/2), pp 61-68.

[22] Ross, D. J. 1976. Invertase and Amylase activities in ryegrass and white clover plants and their relationships with activities in soils under pastures, Soil Biol Biochem, 8, pp 351-356.

[23] Ross, D. J., Roberts, H. S. 1970. Enzyme activnies and oxygen uptakes of soils under pasture in temperature and rainfall sequences. Journal of Soil Science. 21, pp 368-381.

[24] Sahoo, S., Pattanayak, S. K., Sethi, J. R., Haripal, K., Panda, A. R. 2013. Heavy metal content and physicochemical characteristics of soils in open forest and crop fields near Bhushan sponge iron plant in Sambalpur district of Odisha, India, International Journal of Scientific Research. 2 (8). Pp 206-2010.

[25] Schinner F., Ohlinger, R., Kandeier, E. Margensin, R. (Eds) 1995. Methode in Soil Biology. Springer-Verlag, Berlin Heidelberg, pp 93-97.

[26] Simon T. 1999. The effect of increasing rates of nickel and arsenic on the growth of radish and soil microflora. Rostlinna vyroba, 45, pp 421–430.

[27] Tresar-Cepeda C., Leiros C., Gil-Sotres F. and Seoane, S. 1998. Toward a biochemical quality index for soil: An expression relating several biological and biochemical properties. Biology and Fertility of Soils, 26, pp 100-106.

[28] Vladislav G., Neli M. S., Alecaunder, A. N. 2003. Physicochemical aspects of contamination of soil and hydrosphere with heavy metals, Chemistry for sustainable Development,11, pp 787-801.

[29] WHO/FAO 2007. Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission 13th Session. Report of the Thirty Eight Session of the Codex Committee on Food Hygiene. Houston, United States of America, ALINORM 07/30/13.

[30] Giller, K E., Witter, E., Mcgrath, S. P. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: (Soil Biol. Biochem. Vol. 30, No. 10/11, pp. 1389-1414.

[31] Brooks RR. 1998. Plants that hyperaccumulate heavy metals.384 pp. Wallingford: CAB International. £55 (hardback). Greenland DJ. 1997, pp 384