J. Environ. Science & Engg. Vol. 56, No. 3, p. 309-314, July 2014

Effect of Increasing Concentrations of Chromium on Soil Enzymatic Activities and Soil Respiration

MASOOMA QUAZI AND N. C. AERY+

The effects of various concentrations of chromium on the activity of dehydrogenase, catalase and soil respiration were studied after 14, 28, 56 and 112 days of incubation period. Chromium was applied as $K_2Cr_2O_7$ in the amounts of 0, 25, 50, 100, 200, 400, 800 ìg g^{-1} of soil. Soil contamination with Cr decreased the activity of dehydrogenase, catalase and soil respiration. About 36.92% and 44.88% inhibition in the activity of dehydrogenase was caused by 50 and 100 ìg g^{-1} Cr addition respectively, after 14 days of incubation. Catalase activity significantly ($P < 0.001$) decreased with applied doses of chromium after 14 days of incubation. A significant inhibition in soil respiration was observed after 56 days of incubation with applied doses of chromium. These results suggest that soil respiration, dehydrogenase and catalase enzyme activities are suitable parameters for the toxicity test.

Key words : *Chromium, dehydrogenase, catalase, soil respiration*

Introduction

Chromium is the 10th abundant element in the earth's mantle and persists in the environment as either Cr (III) or Cr (VI). Cr (VI) is toxic to both plants and animals being a strong oxidizing agent, corrosive, and potential carcinogen [1-2]. Chromium has a harmful effect on soil microorganisms by depressing their biological activity[3] as well as modifying the environment in which they live[4]. The activities of soil enzymes support biochemical processes which are essential for the maintenance of soil fertility. They are important in catalyzing several important reactions necessary for the life processes of micro-organisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling[5]. Soil enzyme assays have been considered as one of the cheapest and easiest techniques and used as sensors for measuring the degree of soil pollution. Soil enzyme inhibition depends on the nature and concentration of heavy metals[6].

Soil dehydrogenase activity reflects the working of a group of intracellular enzymes that are present in living soil microbes. These enzymes take part in many metabolic reactions involved in oxidative energy transfer[7]. Dehydrogenase activities in soils provide correlative information on the biological activity and microbial populations in the soil. Catalase is an intracellular enzyme found in all aerobic bacteria and most facultative anaerobes, but absent in obligate anaerobes[8]. Catalase can split hydrogen peroxide into molecular oxygen and water and thus prevent cells from damage by reactive oxygen species[9]. Catalase activity may be related to the metabolic activity of aerobic organisms and has been used as an indicator of soil fertility[10].

Soil respiration, measured as CO_2 evolution or O_2 consumption, is one of the easiest, general and frequently used parameters for measuring the decomposition of organic compounds in soil[11]. It is commonly applied to characterize the microbiological status of soil[12], and is furthermore discussed as potential, integrative bioindicator of soil health or soil quality[13]. It has been recognized that high concentrations of heavy metals can have deleterious effects on organic matter decomposition and physiological processes of soil microbes[14] and plants[15, 16, 17].

Keeping this in view, a pot trial was undertaken to determine the effects of various concentrations of Cr on soil dehydrogenase and catalase enzyme activity, and soil respiration so that effect of this metal could be minimized and soil fertility could be maintained.

Materials and methods

The experimental soil was sandy-loam type with alkaline (8.7) pH. The main characteristics of experimental soil are presented in **Table 1**. Cr was applied as potassium dichromate at doses of 25, 50, 100, 200, 400, and 800µg g^{-1} Cr of soil. Different concentrations of chromium as potassium dichromate were prepared separately by taking corresponding amounts (calculated on the basis of molecular weight) of the chemical per kilogram of soil sample. Pots without the added Cr constituted the control.

Experiment was set during the month of May. Three replicates were prepared of each concentrations and its moisture content was brought up to 60% water holding capacity by pouring distilled water and maintained throughout the incubation period. Activity of soil enzymes was measured

Laboratory of Geobotany & Biogeochemistry, Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur- 313 001, India

+ Corresponding author : e-mail : ncaery@yahoo.com

Table 1 : Physico-chemical characteristics of experimental soil

Parameters	Value
Soil pH	8.7
Soil moisture (%)	15.34
Soil texture	Sandy-Loam
WHC (%)	51.73
Bulk density (g/cc)	0.98
Specific gravity	4.09
Porosity (%)	76.25
Organic carbon (%)	0.84

after 14, 28, 56 and 112 days of incubation period. The soil dehydrogenase enzyme activity was determined by Lenhard's method modified by Casida *et al.*[18]. The substrate of dehydrogenase was 3% aqueous TTC (2, 3, 5, Triphenyl Tetrazolium Chloride) solution. Soil incubation was carried out for 24 hours at 30°C and the enzyme activity was measured at a wavelength of 485 nm with the help of a Shimadzu UV-1700 spectrophotometer (Schimadzu, Columbia, Maryland). The activity was expressed as μg TPF g^{-1} of soil 24 h^{-1}. Soil catalase enzyme activity was determined by the method given by Roberge[19] with slight modification (0.1 N $KMnO_4$ was used instead of 0.02 M $KMnO_4$).

Soil respiration was determined by measuring CO_2 evolution from soil by method of Pramer and Schmidt[20] with slight modification (0.1 N NaOH and 0.1 N HCl were used instead of 0.5 N NaOH and 0.5 N HCl).

Statistical analysis

The raw data were analyzed using Origin 6.1 software for the computation of correlation coefficient (r), and regression equation (Y).

Results

Chromium showed an inhibitory effect on soil dehydrogenase activity. Soil dehydrogenase activity significantly decreased with applied concentrations of chromium for incubation period of 14 and 112 days (**Table 2**). Even the lowest Cr concentration (25 μg g^{-1}) decreased the activity of dehydrogenase by nearly 29% after 14 days of incubation. At 100 μg g^{-1} concentration of Cr, maximum reduction (44.88% and 43.25%, respectively) in enzymatic activity was observed after 14 and 56 days of incubation. After 112 days of incubation period, the lowest and the highest enzyme inhibition were observed in 25μg g^{-1} and 100μg g^{-1} levels, which were 61.79 and 83.70% respectively, compared to control. The overall enzyme activity decreased with increasing incubation periods (**Fig. 1**).

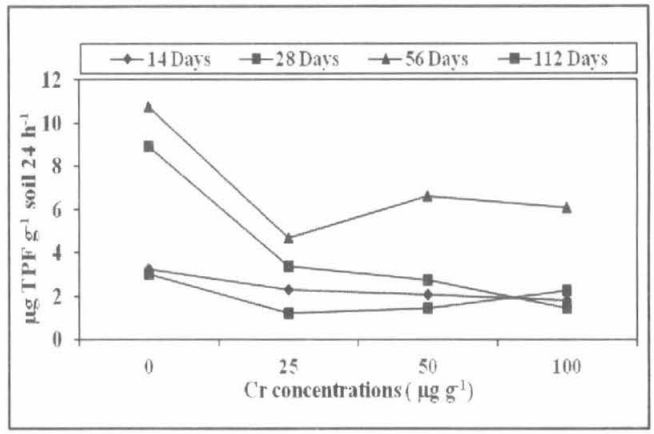

Fig. 1: Effect of Chromium as potassium dichromate on soil dehydrogenase activity in experimental soil after 14, 28, 56 and 112 days of incubation period

A very highly significant ($P < 0.001$) inhibition in catalase activity was observed with applied doses of chromium after 14 days of incubation (**Table 3**). At lowest applied dose of chromium (25 μg g^{-1}) catalase activity was inhibited by 6.41%, 6.90% and 18.04% after 14, 28 and 56 days of incubation periods, respectively. Maximum reduction in catalase activity was observed for highest applied dose of chromium (800μg g^{-1}), which was 51.48%, 53.96% and 48.05% after 14, 28 and 56 days of incubation periods, respectively. Applied doses of chromium significantly and negatively correlated with catalase activity for 112 days of incubation. For applied doses of chromium, inhibition in the catalase activity was higher in the first two weeks but the inhibition decreased with incubation time (**Fig. 2**).

In the present study, soil respiration decreased in Cr contaminated soils as compared to uncontaminated soils. The CO_2 evolution ranged from 2.07 to 4.5 mg CO_2-C/100g soil. Increasing levels of Cr decreased the amount of CO_2 evolution. The CO_2 evolution decreased for all incubation periods ranging

Table 2 : Relationship between soil applied Cr concentrations (X) and soil dehydrogenase activity (Y)

Parameters (Days)	Regression equation	Correlation coefficient (r)	Significance
14	Y=2.9316-0.01315x	-0.8772	Significant ($0.01 < P < 0.05$)
28	Y=2.1364-0.00341x	-0.1812	Not Significant
56	Y=8.43-0.03183x	-0.5222	Not Significant
112	Y=6.96-0.0648x	-0.8422	Significant ($0.01 < P < 0.05$)

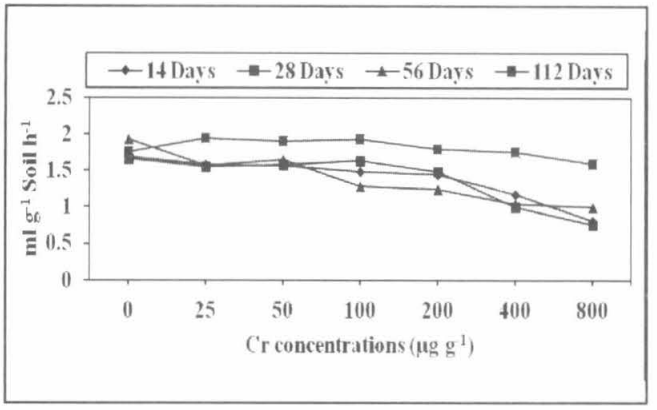

Fig. 2 : Effect of Chromium as potassium dichromate on soil catalase activity in experimental soil after 14, 28, 56 and 112 days of incubation period

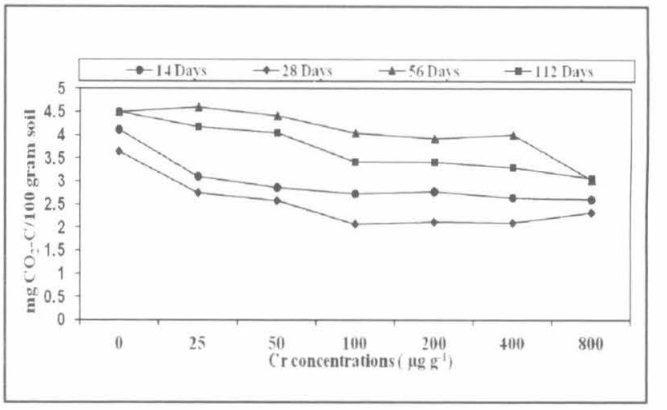

Fig. 3: Effect of Chromium as potassium dichromate on soil respiration activity in experimental soil after 14, 28, 56 and 112 days of incubation period

from 0 to 800 µg Cr g^{-1}. CO_2 evolution decreased by 36.40%, 36.26%, 32.44% and 32%, respectively after 14, 28, 56, 112 days of incubation period at Cr level of 800 µg g^{-1} soil (**Fig. 3**). Soil respiration showed a highly significant inhibition with applied doses of chromium after 56 days of incubation periods (**Table 4**).

Discussion

Heavy metals are regarded as inhibitors of microbiological activity of soil. They cause quantitative and qualitative changes in the composition of microflora and in enzymatic activity[21]. They can inhibit enzymatic activities by interacting with the enzyme substrate complexes, denaturing the enzyme protein and interacting with its active sites[22].

Dehydrogenase enzyme activity reflects the total range of activity of soil microflora and may be used as a good indicator of microbiological activity[23-24]. Frankenberger *et al.*[25] reported that slightly higher quantities of heavy metals might stimulate the activity of soil enzymes, whereas in larger doses they will have an inhibitory effect on soil enzymatic activity. Dehydrogenase activity can be inhibited up to 90% by high soil contamination with heavy metals[26]. Motuzas *et al.*[27] found a significant decrease in the activities of dehydrogenases (95-98%), urease (65-97%), saccharase (55-77%) and soil respiration intensity (38-65%) in polluted soils as compared to unpolluted soil. In the present investigation, chromium also inhibited the activity of soil dehydrogenase (**Fig.1**). As dehydrogenase is an intracellular enzyme[6] its activity may be contributed to the inhibition of microbial growth and reduction in microbial number. Chundawat and Aery[28] investigated the effects of various concentrations of Cd on soil dehydrogenase enzyme activity and soil respiration. The maximum soil dehydrogenase activity was observed at 100 ppm concentration of cadmium after 15 days of incubation, beyond this level a gradual reduction was observed. Higher doses (1600 ppm) of cadmium significantly inhibited soil respiration after 30 days of incubation period[28]. Wyszkowska[29] observed that the effect of Cr (VI) on the activity of dehydrogenase, urease, acid phosphatase and alkaline phosphatase depends

Table 3 : Relationship between soil applied Cr concentrations (X) and soil catalase activity (Y)

Parameters (Days)	Regression equation	Correlation coefficient (r)	Significance
14	Y=1.6251-0.00103x	-0.9928	Very highly significant ($P < 0.001$)
28	Y=1.6460-0.00117x	-0.9626	Highly significant ($0.001 < P < 0.01$)
56	Y=1.5996-9.3749x	-0.8002	Possibly significant ($0.05 < P < 0.10$)
112	Y=1.8899-3.5213E-4x	-0.8184	Significant ($0.01 < P < 0.05$)

Table 4 : Relationship between soil applied Cr concentrations (X) and soil respiration (Y)

Parameters (Days)	Regression equation	Correlation coefficient (r)	Significance
14	Y=3.2010-9.82456E-4x	-0.5365	Not significant
28	Y=2.7034-8.59649E-4x	-0.4422	Not significant
56	Y=4.4655-0.00172x	-0.9393	Highly significant ($0.001 < P < 0.01$)
112	Y=4.0328-0.00147x	-0.7915	Possibly significant ($0.05 < P < 0.10$)

on the rate of soil contamination with chromium and its inoculation with *Bradyrhizobium* bacteria. A negative correlation was observed between the degree of soil contamination with chromium and the activity of dehydrogenase, urease, acid phosphatase and alkaline phosphatase. Even the lowest rate of chromium (10 mg Cr kg^{-1} of soil) was enough to depress the activity of dehydrogenases by nearly 20% in non-inoculated and 7% in inoculated soil. The highest concentration of chromium (150 mg Cr kg^{-1} of soil) in both non-inoculated and inoculated soil caused a nearly complete inhibition of the activity of dehydrogenases.

Aerobic bacteria use catalase for protection against by-products formed during oxygen-dependent metabolism. There are few reports regarding the effect of Cr addition on soil catalase activity, but there are no reports indicating the effect of Cr doses ranging from 0 to 800 μg g^{-1} and for varying incubation periods (14, 28, 56 and 112 days). Stepniewska *et al.*[30] observed that the presence of Cr (VI) in soil has an inhibitory effect on catalase activity. In the sample enriched with 2, 5 and 10 mg kg^{-1} of Cr (VI), activity of catalase was inhibited by 37.6%, 58.4% and 66.7%, respectively. Inhibition in the activity of catalase was 76%, over the control, at 20 mg kg^{-1} Cr (VI) dose[30]. Liu *et al.*[31] reported a negative correlation between soil catalase activity and increasing dosage of heavy metals in wastewater irrigated soil. Negative correlations between heavy metals and soil catalase, urease and other enzyme activities were also demonstrated by Kizilkaya *et al.*[32]. In the present study, a negative correlation was observed between soil catalase activity and increasing concentrations of chromium. A highly significant inhibition in soil catalase activity was observed with applied doses of chromium after 28 days of incubation period (**Table 3**). Inhibition in catalase activity may be due to both lower synthesis and/or release of extracellular enzymes by microbes or to the inhibition of extracellular enzymes[33]. Belyaeva *et al.*[22] elucidated contrast results and found that catalase activity was not markedly inhibited by heavy metals. The highest inhibitory effect of chromium on catalase activity may be due to the sudden exposure of the microbes to heavy metal in the first two weeks. Later on the microbes may have adapted to the polluted environment, and the enzyme activity tended to recover[33].

Bing *et al.*[34] observed the influence of various Cr concentrations (0.05, 0.25, 0.50, 1.00 and 2.00 g kg^{-1}) on the activity of soil catalase, polyphenol oxidase, dehydrogenase and alkaline phosphatase in the incubation experiment with a period of 35 days. All the tested Cr concentrations significantly inhibited dehydrogenase activity by over 70% after 30 days. The activity of alkaline phosphatase was slightly inhibited while no obvious effect was observed on the activity of catalase in soil. Wyszkowska *et al.*[35] observed that chromium applied at doses of 0, 40, 80, 120 mg kg^{-1} of soil, adversely affected the activity of soil dehydrogenase, urease and acid and alkaline phosphatase enzymes.

Verma *et al.*[11] observed that the CO_2 evolution in Cr contaminated soils with sugarcane trash ranged from 6.8 to 74.6 μg g^{-1} soils. The rate of soil respiration decreased with increasing heavy metal concentration. Maximum soil respiration was recorded at 7 days of incubation period and decreased with increasing incubation period. In clay soils, the addition of Cr at 10 μg g^{-1} or more decreased CO_2 evolution, over the control. After 120 days of incubation period CO_2 evolution decreased by 46.6, 48.7 and 71.4% in sandy, loam and clay soils, respectively, at Cr levels of 1000μg g^{-1} soil. In the present investigation, increasing concentrations of chromium showed a highly significant negative correlation with soil respiration, after 56 days of incubation (**Table 4**). A decreased rate of soil respiration related to increased incubation period may be attributed to the concentrations of heavy metals, which would bring down the soil microbial activity[36]. D' Ascoli *et al.*[37] showed a significant reduction from 15% to 41% in soil potential respiration with higher chromium contamination (500-5000 mg Cr kg^{-1}). Hattori[38] indicated that CO_2 production was significantly depressed in heavy metal contaminated soil. Doelman and Hannstra[39] have also observed that long term heavy metal contamination in soil has harmful effects on soil microbial activity, especially microbial respiration.

Conclusion

Results indicate that high concentrations of chromium had a detrimental effect on soil health. The soil enzyme activities significantly altered with the exposure of chromium as evidenced by the reduction in dehydrogenase and catalase activities. The decreasing rate of soil respiration reflects the fact that all the applied doses of chromium are toxic to soil microbial population, hence the measurement of CO_2 evolution appears to be a sensitive indicator for Cr pollution.

Acknowledgement

The authors are thankful to University Grants Commission, New Delhi for providing financial assistance in the form of meritorious fellowship to Masooma Quazi.

References

1. Banerjee M, Mishra S and Chatterjee J, Scavenging of nickel and chromium toxicity in *Aulosira fertilissima* by immobilization: Effect on nitrogen assimilating enzymes, *Electron J Biotechnol*, **7(3)**, 302-309 (2004).

2. Megharaj M, Avudainayagam S and Naidu R, Toxicity of hexavalent chromium and its reduction by bacteria isolated

from soil contaminated with tannery waste, *Curr Microbiol*, **47(1)**, 51-54 (2003).

3. Barabasz W, Chmiel M J, Galus A and Paœmionka I, Ekotoksykologia chromu, *Chemia i In¿ynieria Ekologiczna*, **5(8-9)**, 665 (1998).

4. Babich H, Stotzky G and Schiffenbauer M, Comparative toxicity of trivalent and hexavalent chromium to fungi, *B Environ Contam Toxicol*, **28**, 452-459 (1982).

5. Dick R P, Sander J A and Eash N S, Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley Peru, *Agric Ecosyst Environ*, **50**, 123-131(1994).

6. Shun-hong H, Bing P, Zhi-hui bY, Li-yuan C and Li-cheng, Z, Chromium accumulation, microorganism population and enzyme activities in soils around chromium-containing slag heap of steel alloy factory, *Trans Nonferrous Met Soc China*, **19**, 241-248 (2009).

7. Smith E L, Hill R L, Lehman I R, Lefkowitz R J, Handler P and White A, *Principles of Biochemistry: General Aspects* (McGrow-Hill, Singapore) 1983.

8. Trasar-Cepada C, Leiros M C, Seoane S and Gil-sotres F, Limitations of soil enzymes as indicators of soil pollution, *Soil Biol Biochem*, **32**, 1867-1875 (2000).

9. Yao X H, Min H, Lu Z H and Yuan H P, Influence of acetamprid on soil enzymatic activities and respiration, *Eur J Soil Biol*, **42**, 120-126 (2006).

10. Shiyin L, Lixiao N, Panying P, Cheng S and Liansheng W, Effects of pesticides and their hydrolysates on catalase activity in soil, *B Environ Contam and Tox*, **72**, 600-606 (2004).

11. Verma R K, Yadav D V, Singh C P, Suman A and Gaur A, Effect of heavy metals on soil respiration during decomposition of sugarcane (*Saccharum officinarum* L.) trash. in different soils, *Plant Soil Environ*, **56(2)**, 76-81 (2010).

12. Stenberg B, Pell M and Torsrensson L, Integrated evaluation of variation in biological, chemical and physical soil properties, *Ambio*, **27**, 9-15 (1998).

13. Gregorich E A, Carter M R, Angers D A, Monreal C M and Ellert B H, Towards a minimum data set to assess soil organic matter quality in agricultural soils, *Can J Soil Sci*, **74**, 367-385 (1994).

14. Chaney W R, Kelley J M and Strickland R C, Influence of cadmium and zinc on carbon dioxide evolution from litter and soil from a black oak forest, *J Environ Qual*, **7**, 115-119 (1978).

15. Aery N C and Rana D K, Interactive effects of Zn, Pb and Cd in Barley, *Journal of Environ Science & Engg*, **49(1)**, 71-76 (2007).

16. Mukhopadhyay N and Aery N C, Effect of Cr (III) and Cr (VI) on the growth and physiology of *Triticum aestivum* plants during early seedling growth, *Biologia, Bratislava*, **55(4)**, 403-408 (2000).

17. Aery N C and Jagetiya B L, Relative toxicity of Cadmium, Lead and Zinc on barley, *Commun Soil Sci Plant Anal*, **28(11 & 12)**, 949-960 (1997).

18. Casida L E, Klein D A and Santoro R, Soil dehydrogenase activity, *Soil Sci*, **98**, 371-378 (1964).

19. Roberge M R, Methodology of soil enzyme measurement and extraction, in *Soil Enzymes*, edited by R G Burns (Academic Press, New York) 1978, 341-373.

20. Pramer D and Schmidt E L, in *Experimental Soil Microbiology* (Burgess, Minneapolis, Minnesota) 1964, 6-13.

21. Pacha J, Wp³yw trójwartoœciowego chromu na aktywnoœæ wybranych enzymów w glebie, *Acta Biol Siles*, **3(20)**, 95 (1986).

22. Belyaeva O N, Haynes R J and Birukova O A, Barley yield and soil microbial and enzyme activities as affected by contamination of two soils with lead, zinc or copper, *Biol Fert Soils*, **41(2)**, 85-94 (2005).

23. Nannipieri P, Grego S and Ceccanti B, Ecological significance of the biological activity in soil, *Soil Biol Biochem*, **6**, 293-355 (1990).

24. Skujins J, Extracellular Enzymes in Soil, *Crit Rev Microbiol*, **4**, 383-421 (1976).

25. Frankenberger W T Jr, Johanson J B and Nelson C O, Urease activity in sewage sludge-amended soils, *Soil Biol Biochem*, **15**, 543-551 (1983).

26. Tresar-Cepeda C, Leiros C, Gil-Sotres F and Seoane S, Toward a biochemical quality index for soil: An expression relating several biological and bio-chemical properties, *Biol Fert Soils*, **26**, 100-106 (1998).

27. Motuzas A, Vaisvalavicius R, Zakarauskite D, Grigaliuniene K and Batkus V, Experimental studies on soil enzymes changes under the critical heavy metals accumulation in luvisols and albeluvisols, *Latvian Journal of Agronomy*, **8**, 65-70 (2005).

28. Chundawat M and Aery N C, Influence of various concentrations of cadmium on soil dehydrogenase enzyme

activity and soil respiration, *J Pure Appl Microbiol*, **2(1)**, 235-238 (2008).

29. Wyszkowska J, Soil contamination by chromium and its enzymatic activity and yielding, *Polish J Environ Stud*, **11(1)**, 79-84 (2002).

30. Stepniewska Z, Wolinska A and Ziomek J, Response of soil catalase activity to chromium contamination, *J Environ Sci*, **21(8)**, 1142-1147 (2009).

31. Liu S, Yang Z, Wang X, Gao R and Liu X, Effects of Cd and Pb pollution on soil enzymatic activities and soil microbiota, *Front Agric China*, **1**, 85-89 (2007).

32. Kizilkaya R, Askin T, Bayrakli B and Saglam M, Microbiological characteristics of soils contaminated with heavy metals, *Eur J Soil Biol*, **40**, 95-102 (2004).

33. Khan S, Cao Q, Hesham A E, Xia Y and He J, Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb, *J Environl Sci*, 834-840 (2007).

34. Bing P, Shun-hong H, Zhi-hui Y, Li-yuan C, You-ze X and Chang-qing S, Inhibitory effect of Cr(VI) on activities of soil enzymes, *J Cent South Univ Technol*, **16**, 0594-0598 (2009).

35. Wyszkowska J, Kucharski J, Jastrzebska E and Hlasko A The biological properties of soil as influenced b chromium contamination, *Polish J Environ Stud*, **10(1** 37-42 (2001).

36. Heal O W, Latter P M and Howson J, A study of the rat of decomposition of organic matter, in: *Productic Ecology of British Moors and Mountane Grasslan* edited by O W Heal & D F Perkins (Springer-Verlag, Berli 1978, 136-159.

37. D' Ascoli R, Pascale R A D, Nappa V, Castaldi S a Rutigliano F A, *Effect of increasing concentrations of (III) on soil microbial community*, paper presented XIV Congresso della Societa Italiana di Ecologia, Sien 2-4 october, 2004.

38. Hattori H, Influence of heavy metals on soil microbi activities, *Soil Sci Plant Nutr*, **38**, 93-100 (1992).

39. Doelman P and Hannstra L, Short-term and long- ter effects of Cd, Cr, Cu, Ni, Pb and Zn on microbial respirati in relation to abiotic soil factors, *Plant Soil*, **79**, 317-3 (1984).