Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 1142–1147
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Response of soil catalase activity to chromium contamination

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Received 20 December 2007; revised 04 May 2008; accepted 06 June 2008

Abstract

The impact of chromium(III) and (VI) forms on soil catalase activity was presented. The Orthic Podzol, Haplic Phaeozem and Mollic Gleysol from different depths were used in the experiment. The soil samples were amended with solution of Cr(III) using $CrCl_3$, and with Cr(VI) using $K_2Cr_2O_7$ in the concentration range from 0 to 20 mg/kg, whereas the samples without the addition of chromium served as control. Catalase activity was assayed by one of the commonly used spectrophotometric methods. As it was demonstrated in the experiment, both Cr(III) and Cr(VI) have an ability to reduce soil catalase activity. A chromium dosage of 20 mg/kg caused the inhibition of catalase activity and the corresponding contamination levels ranged from 75% to 92% for Cr(III) and 68% to 76% for Cr(VI), with relation to the control. Catalase activity reached maximum in the soil material from surface layers (0–25 cm), typically characterized by the highest content of organic matter creating favorable conditions for microorganisms.

Key words: chromium; catalase; enzymatic activity; inhibition

DOI: 10.1016/S1001-0742(08)62394-3

Introduction

Many enzymes, produced by microorganisms in soil, can be involved in C, P and N cycles in soil (Tabatabai, 1994; Shiyin *et al*., 2004). Natural and anthropogenic factors may affect directly and indirectly the activities of enzymes in soil, which was reviewed by Gianfreda and Bollag (1996). Among anthropogenic factors, heavy metals and pesticides are of primary importance because they can be either directly introduced to soil or transferred in other ways (Dick, 1997; Shiyin *et al*., 2004). Metals are the natural constituents of soil materials (Kakkar and Jaffery, 2005). The use of metals by humans was, and still is, accompanied by increasing inputs of metals into soils with different types of waste (Welp, 1999).

Chromium is one of the heavy metals that can be hazardous (Cervantes *et al*., 2001; Hu *et al*., 2003; Stępniewska *et al*., 2004). This element is highly toxic non-essential metal to microorganisms and plants (Cervantes *et al*., 2001; Kakkar and Jaffery, 2005). The migration of chromium from a pollution source both into soils and into surface- or ground-waters, as well as its toxicity, mainly depend on its speciation (Bartlett and James, 1988; Bagchi and Stohs, 2002; Parks *et al*., 2004; Stçpniewska *et al*., 2004). The commonly existing forms of chromium are the trivalent and the hexavalent. The oxidized Cr(VI) form is highly soluble in water, whereas the reduced Cr(III) form is almost insoluble in water and immobile in the soil environment (Bartlett and James, 1988; Cervantes *et al*., 2001; Desjardin *et al*., 2002). There are conflicting results with regard to the form of chromium which is taken up and accumulated by plants (Cervantes *et al*., 2001; Mangabeira, 2004). However, many reports highlighted the negative impact of heavy metals on enzyme activities in soils (Kandeler *et al*., 1996; Stuczynski *et al*., 2003; Tokunaga *et al*., 2003; Chaperon and Sauve, 2007; Liu *et al*., 2007).

Soil enzyme activities are considered to be sensitive to pollution and have been proposed as indicators for soil degradation (Trasar-Cepeda *et al*., 2000). Catalase (hydrogen peroxide oxidoreductase, EC 1.11.1.6) is an intracellular enzyme found in all aerobic bacteria and most facultative anaerobes, but absent in obligate anaerobes (Trasar-Cepeda *et al*., 2000; Anderson, 2002; Shiyin *et al*., 2004). It is well known that the products of oxygen reduction, such as hydrogen peroxide, superoxide radical, and hydroxyl radical, can be highly toxic for cells and might damage cellular macromolecules.

Catalase can split hydrogen peroxide into molecular oxygen and water and thus prevent cells from damage by reactive oxygen species (Yao *et al*., 2006). This enzyme can be found in all aerobic microorganisms, plant and animal cells (Alef and Nannipieri, 1995). Although it was one of the first isolated and purified enzymes, its physiological function and regulation are still poorly understood. Catalase activity may be related to the metabolic activity of aerobic organisms and has been used as an indicator of soil fertility (Gianfreda and Bollag, 1996; Shiyin *et al*.,

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2004; Trasar-Cepeda *et al*., 2007). Catalase activity is very stable in soil and shows a significant correlation with the content of organic carbon decreasing with soil depth (Alef and Nannipieri, 1995).

This study was designed to investigate the effect of chromium contamination on the soil enzymes environment with reference to its oxidation state. Specifically, the effect induced by Cr(III) and Cr(VI) on catalase activity was tested in Orthic Podzol, Haplic Phaeozem and Mollic Gleysol material under laboratory conditions. It might be worth mentioning that this report is the first one studying the effect of Cr pollution on catalase activity in soils, although there might have been similar studies with relation to enzyme activities other than catalase or metals other than Cr.

1 Materials and methods

1.1 Soil samples

The experiment was performed under laboratory conditions using the Orthic Podzol, Haplic Phaeozem, and Mollic Gleysol soil taken from the bank of soils maintained by the Institute of Agrophysics of the Polish Academy of Sciences in Lublin, Poland (Gliński *et al*., 1991). The main characteristics of the soils are presented in Table 1. The soil materials were analysed by both Flame Atomic Absorption Spectroscopy (FAAS) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS) for chromium content (AAS spectrophotometer, Z-8200, Hitachi, Japan). The presence of chromium in the control samples was below the detection limit (ca. 0.1 mg/kg), whereas the remaining soil samples were treated with Cr(III) using $CrCl_3$ aqueous solution and with Cr(VI) as $K_2Cr_2O_7$ solution. Four chromium dosages of 2, 5, 10, and 20 mg/kg were used to amend the soils. The incubation of the samples took 24 h at 30°C.

1.2 Assaying of soil catalase activity

Catalase activity was measured by back-titrating residual H_2O_2 with $KMnO_4$ (Johnson and Temple, 1964; Roberge, 1978). Two grams of soil samples were added to 40 mL distilled water with 5 mL of 0.3% hydrogen peroxide solution. The mixture was shaken for 20 min and then 5 mL of 1.5 mol/L H_2SO_4 were added. Afterwards the solution was filtered and titrated using 0.02 mol/L $KMnO_4$. The reacted amount of 0.02 mol/L $KMnO_4$, calculated per gram of dry soil (Minczewski and Marczenko, 1973), was used to express the activity of catalase.

All determinations of enzymatic activities were performed in triplicates, and all values reported are their averages.

1.3 Data analysis

The significance of soil amendment with Cr(III) or Cr(VI) or their combination on soil catalase activity was evaluated using ANOVA (Statgraphics 3.0) with chromium treatment level and chromium form as the factors at the confidence level of 95%.

2 Results

2.1 Statistical analysis

The results of laboratory experiments revealed a strong relationship between soil catalase activity and Cr presence in the range of 2–20 mg/kg. Statistical processing of the data was conducted, with the determination of the mean values of catalase activity and standard deviations (Table 2). In each case, the value of P was lower than 0.0001, showing statistically significant differences between average activities of catalases tested and chromium dosage at the 95% confidence interval.

2.2 Influence of Cr(III) or Cr(VI) on soil catalase activity

Response of catalase activity to Cr(III) or Cr(VI) contamination in Orthic Podzol is shown in Fig. 1. The highest recorded catalase activity was at the value of 0.35 mL of (0.02 mol/L $KMnO_4$)/g in the control sample at surface layer, and amounted for 0.2 mL of (0.02 mol/L $KMnO_4$)/g in the subsoil. Higher values of enzymatic activity of controls were associated with biotic and abiotic natural processes, which took place in soil. Conversely, the introduction of Cr to the soil samples must have clearly disturbed the metabolic homeostasis of microorganisms, which led to the reduction in the enzymatic activity. Cr(III) and Cr(VI) supplementation was the primary factor to inhibite enzymatic activities. The highest Cr(III) and Cr(VI) addition (20 mg/kg) limited enzymatic activity by 83% and 68%, respectively. Reduction of catalase activity as a consequence of Cr presence was stronger in subsoil (50–60 cm), than in the upper layer (0–25 cm).

The impact of Cr forms on catalase activity in Haplic Phaeozem is presented in Fig. 2. Samples used as controls (without Cr addition) were characterized by approximately 30% lower values of catalase activity (0.24 mL of (0.02 mol/L $KMnO_4$)/g in surface layer) in comparison with Orthic Podzol. In this case a stronger inhibition of catalase activity by the Cr(III) than by the Cr(VI) is well illustrated.

Table 1 Selected characteristics of soils

Soil type	Depth (cm)	Granulometric composition (%)			Organic matter (%)	pH in H_2O
		1–0.02 mm	0.02–0.002 mm	< 0.002 mm		
Orthic Podzol	0–25	75	22	3	1.19	5.86
	50–60	77	10	13	0.33	5.91
Haplic Phaeozem	0–25	56	36	6	1.21	7.15
	50–60	50	27	24	0.22	7.17
Mollic Gleysol	0–25	85	14	1	2.19	7.42
	50–60	97	1	2	0.4	7.8

Table 2 Effect of particular factors on soil catalase activity

Chromium dose (mg/kg)	Orthic Podzol		Haplic Phaeozem		Mollic Gleysol	
	Mean	SD*	Mean	SD	Mean	SD
Cr(III) layer (0–25 cm)						
0	0.3466 a	0.0251	0.2566 a	0.0208	0.2300 a	0.0100
2	0.1766 b	0.0208	0.1300 b	0.0200	0.1700 b	0.0264
5	0.1133 c	0.0057	0.0766 c	0.0152	0.1166 c	0.0208
10	0.0833 cd	0.0057	0.0500 cd	0.0100	0.0600 d	0.0100
20	0.0566 d	0.0152	0.0300 d	0.0100	0.0466 d	0.0115
Cr(III) layer (50–60 cm)						
0	0.2166 a	0.0251	0.1933 a	0.0115	0.1800 a	0.0200
2	0.1200 b	0.0264	0.0500 b	0.0100	0.1233 b	0.0251
5	0.0860 c	0.0115	0.0366 bc	0.0115	0.0833 c	0.0208
10	0.0466 d	0.1154	0.0233 cd	0.0057	0.0566 cd	0.0152
20	0.0400 d	0.0100	0.0140 d	0.0026	0.0366 d	0.0115
Cr(VI) layer (0–25 cm)						
0	0.3633 a	0.0152	0.2566 a	0.0208	0.2300 a	0.0100
2	0.2200 b	0.0200	0.1476 b	0.0035	0.2033 a	0.0208
5	0.1500 c	0.0200	0.0380 c	0.0450	0.1266 b	0.0208
10	0.1066 cd	0.0152	0.0700 c	0.0062	0.0966 b	0.0208
20	0.1266 d	0.0208	0.0596 c	0.0020	0.0566 c	0.0115
Cr(VI) layer (50–60 cm)						
0	0.1463 a	0.1107	0.1933 a	0.0115	0.1800 a	0.0200
2	0.1350 a	0.0132	0.1233 b	0.0115	0.1533 a	0.1527
5	0.1033 a	0.0115	0.0900 c	0.0100	0.1200 b	0.0200
10	0.0966 a	0.0208	0.0586 d	0.0040	0.0833 c	0.0057
20	0.0700 a	0.0200	0.0733 cd	0.0115	0.0400 d	0.0100

Mean values followed by the same letter are not significantly different at 5% confidence interval. * 95% half interval of confidence.

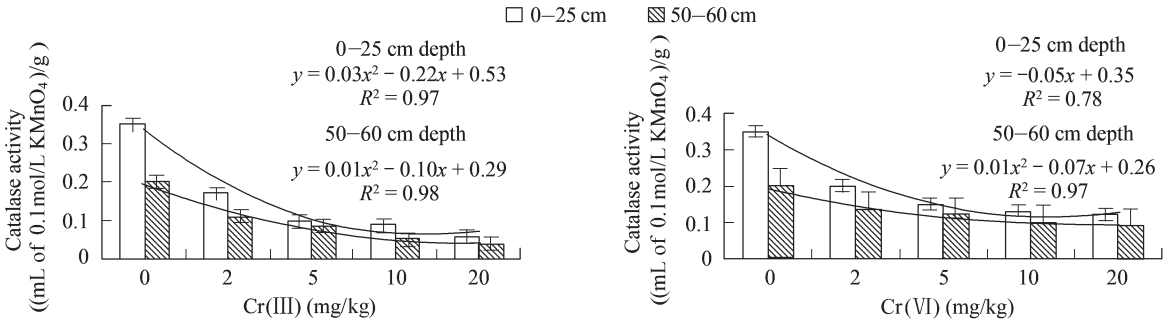

Fig. 1 Effect of Cr(III) and Cr(VI) addition on catalase activity in Orthic Podzol.

Catalase activity with response to the same dosages of both chromium forms (20 mg/kg each) was inhibited by 92% in the case of Cr(III), and by 76% in the presence of Cr(VI) with reference to the control.

The effect of Cr(III) and Cr(VI) supplement on catalase activity in Mollic Gleysol is presented in Fig. 3. This kind of soil, characterized by the highest content of organic matter, reached 2.19% and 0.4% in the surface and subsoil, respectively (Table 1).

Catalase activity in the control samples achieved the same values as in Haplic Phaeozem 0.24 mL (0.02 mol/L $KMnO_4$)/g in the surface layer and 0.19 mL (0.02 mol/L $KMnO_4$)/g in the subsoil). In the samples enriched with 2, 5, 10 mg/kg of Cr(III) or Cr(VI), catalyse activities were inhibited by 37.5%, 58.4%, and 66.7%, respectively. Maximal dosage of Cr(III) resulted in the decrease in catalase activity by approximately 75%. Slightly higher values of the enzymatic activity in the samples enriched with Cr(VI) were noted.

Cr(III) caused a stronger inhibition of soil catalase activity than Cr(VI) regarded as more dangerous for living organisms. Moreover, the values of soil catalase activity exhibited a declining trend along with the increasing depth in the soil profile. The highest values of enzymatic activity arrived at their maximum in surface layers (0–25 cm) and were reduced by 20%–40% in subsoil (50–60 cm). This phenomenon might be linked to the humus content, which is smaller in the deeper layers, than in the surface layers, where the optimal conditions for microbial life occur.

Ecological dose (ED_{50}) is the toxicant concentration that inhibits a microbe-mediated ecological process by 50% (Babich *et al*., 1983; Speir *et al*., 1995). ED_{50} was calculated when a 50% drop in the enzyme activity was observed, amounting for 2 mg/kg for Cr(III), and ranged between 5–10 mg/kg for Cr(VI).

3 Discussion

Many studies related to the toxicity of heavy metals or pesticides on enzyme activities in soil are available in the literature (Speir *et al*., 1995; Kandeler *et al*., 1996; Stuczynski *et al*., 2003; Chaperon and Sauve, 2007). This is partly because enzymes are highly sensitive to metals and the methodology for their determination is rapid,

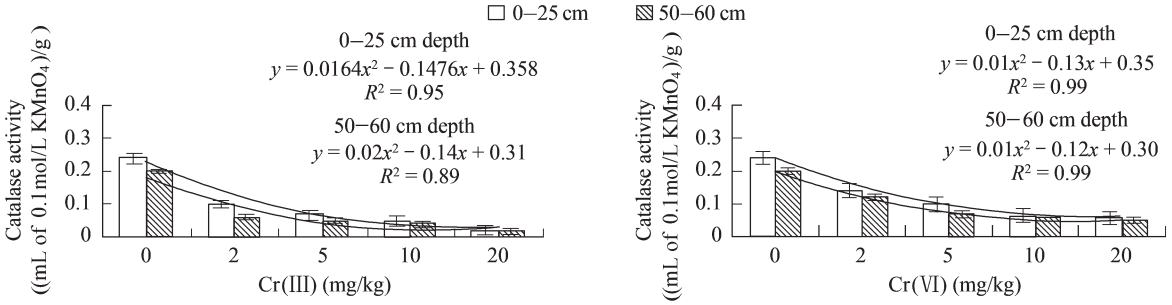

Fig. 2 Effect of Cr (III) and Cr (VI) addition on catalase activity in Haplic Phaeozem.

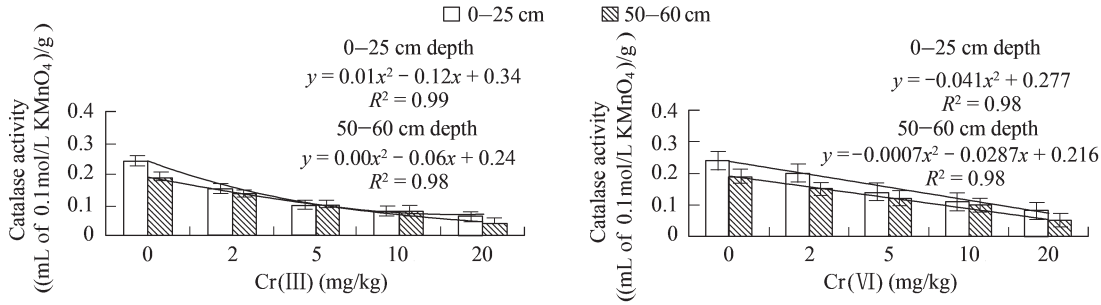

Fig. 3 Effect of Cr (III) and Cr (VI) addition on catalase activity in Mollic Gleysol.

simple and inexpensive (Chaperon and Sauve, 2007). Enzyme activities in soil are related to active soil biomass, and have been suggested as sensitive indicators of soil quality (Guwy *et al*., 1999).

Soil enzymes might behave differently when exposed to a pollutant (Qian *et al*., 2007). Liu *et al*. (2007) reported a negative correlation between soil enzymatic activities and Pb and Cd contents in wastewater-irrigated soil, where soil catalase activity decreasing alongside with increasing dosages of Pb and Cd added to investigated soils. Therefore, catalase can be considered as the biochemical index which reflects the degree of soil Pb and Cd pollution. Moreover, they stated that the influence of Cd on soil enzymatic activities was greater than that of Pb. The effect of Zn, Pb and Cu on soil catalase activity was examined also by Turski *et al*. (1993) and Alloway (1995). They found that the catalase activity increases to a limit point, above which it starts to drop. These limitation values are dependent on the soil type and the soil position in the profile. In upper layers the critical values were estimated as follows: 7.5 (Zn), 0.75 (Pb), 0.125 (Cu) mg per 1000 g of soil, whereas in subsoil amounted 10, 0.5, 0.1 mg per 1000 g soil for Zn, Pb and Cu, respectively. The activity of catalase was strongly limited by the metal content after exceeding the values mentioned above, and it exhibited a drop with increase in depth. These observations are consistent with our studies, where soil catalase activity showed a declining trend with depth in soil profile. Similar results were also confirmed by Alef and Nannipieri (1995). Negative correlation between heavy metals and soil catalase, urease and other enzyme activities were demonstrated by Kizilkaya *et al*. (2004). The inhibitory effect of Cr(VI) and (III) on urease activity was shown by Samborska *et al*. (2004). They found that the inhibiting effect of Cr(III) was much stronger than that of Cr(VI), which is agreeable with results presented at this work with regards to catalase activity, which is much more reduced by Cr(III) than Cr(VI) in comparison with urease activity. Increase in the Cr(III) concentration quickly reduced the enzyme activity. The ED_{50} for urease was 0.2 mg Cr(III)/kg, whereas at 0.4 mg Cr(III)/kg the total inhibition of urease activity was stated (Samborska *et al*., 2004). Probably, the stronger influence of Cr(III) might be connected with the ability of Cr(III) to modify the structure of enzymes through reactions with carbonyl and sulfhydryl groups of enzymes causing modifications of their activities (Samborska *et al*., 2004). Such modifications of the enzyme activities caused by the displacement of magnesium by Cr(III) has been reported by Cervantes *et al*. (Cervantes *et al*., 2001).

Catalase is active over a wide pH range, and its activity does not drop until the pH is below 3.5 (Guwy *et al*., 1999). In our experiment, pH in all the tested samples ranged from 5.86 to 7.8.

Pascual *et al*. (1998) observed that a low dose of organic materials added to soil did not improve soil catalase activity with respect to the non-amended soil, while the high dose of these materials (with the exception for the sewage sludge) involved the significant increase in catalase activity. This suggests that the presence of a minimum amount of fresh organic matter is needed to activate soil catalase activity, which was also confirmed by Kizilkaya *et al*. (2004).

As to other reports, the phenomena of soil enzymes stimulated and inhibited were coexistent, and the fluctuation extent was different among the variety of enzyme types (Qian *et al*., 2007). It has been reported that the mostly investigated enzyme-complexes are those attained by the adsorption interaction of enzyme molecules with pure clays, humus materials or humus-like compounds (Rao *et al*., 2000; Qian *et al*., 2007). Potentially toxic substances such as heavy metals and pesticides are introduced to soil deliberately or inadvertently. Their addition to soil

influences the biological activity of soil as well as many (bio)chemical and physical processes (Shiyin *et al*., 2004). Our experiments on the influence of Cr(III) and Cr(VI) on soil catalase activity revealed that the presence of these substances in soil has an inhibitory effect on enzymatic activities, specifically catalase activity. Stępniewska *et al*. (2004) determined the sorption capacity of several soils for trivalent chromium. They estimated that the values of adsorbed Cr(III) ranged from 9.72×10^{-4} mol/100 g for Eutric Cambisol to 9.95×10^{-4} mol/100 g for Haplic Phaeozem. Among the soils studied, samples with the highest content of organic matter exhibited the greatest ability to sorb Cr(III), and the addition of 52 mg/kg of Cr(III) to soil-water suspensions resulted in almost complete sorption of it (Stępniewska *et al*., 2004). This finding can explain the different fate of pollutants in the environment, as well as heavy metal impact on a soil enzyme activity.

Pesticides or heavy metals may also attach to active sites of enzymes or determinate the substrate accessibility to the active site and cause chemical or conformational changes of enzymatic structure (Nannipieri and Gianfreda, 1998). Qian *et al*. (2007) found that validamycin (an antibiotic with fungicide action) at the rate of 240 mL/kg caused a significant decrease (14%) in soil catalase activity. No obvious influence on catalase activity after application of acetamprid (chloronicotine pesticide) from 0.5 to 50 mg/kg after 14 days was observed in the studies of Yao *et al*. (2006). Shiyin *et al*. (2004) found that soil catalase activities at different dose of pesticides and their hydrolysates will recover from stimulative effect, and reach the level of the enzyme activity of a blank soil sample after about 35 days.

4 Conclusions

Our results indicate that the highest soil catalase activity arrives at maximal values in surface layers, where of the abundant organic matter is responsible for the favorable conditions preferred by living microorganisms. Catalase activities in subsoil (50–60 cm) were lower by 43%, 16.7%, and 21% than at surface layers in Orthic Podzol, Haplic Phaeozem and Mollic Gleysol, respectively. The present findings also suggest that both Cr(III) and Cr(VI) forms have the ability to reduce soil catalase activity. However, Cr(III) caused a stronger inhibition of enzymatic activity than Cr(VI). The catalase activity inhibition seems to be correlated with higher dose of both Cr(III) and Cr(VI) supplements in case of Mollic Gleysol, Orthic Podzol and Haplic Phaeozem. ED_{50} for Cr(III) was estimated at 2 mg/kg, and ranged from 5 to 10 mg/kg for Cr(VI).

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