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Soil dehydrogenase activity in the presence of chromium (III) and (VI)

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A b s t r a c t. The paper presents the influence of chromium forms (III) and (VI) on the soil dehydrogenase activity. Enzyme activities can be considered effective indicators of soil quality changes resulting from environmental stress or management practices. It was found that chromium compounds have detrimental effects on soil dehydrogenase activity. After the addition of chromium, a rapid and significant decrease in enzymatic activities was observed.

K e y w o r d s: dehydrogenase activity, chromium, microorganisms, soil

INTRODUCTION

Metals are natural constituents of soil materials. The use of metals by humans was, and still is, accompanied by increasing inputs of metals into soils through different type of wastes (Welp, 1999). Major sources of chromium include the metal finishing industry, petroleum refining, leather tanning, iron and steel industries, production of inorganic chemicals, textile manufacturing and pulp production. Because metals persist in soils and their leaching is a very slow process, they tend to accumulate in soils (Irha *et al*., 2003).

Chromium is one of the heavy metals and has oxidation states ranging from chromium (II) to chromium (VI). Chromium compounds are stable in the trivalent state and occur in nature in this state in ores such as ferrochromite, whilst chromium (VI) is usually produced from anthropogenic sources (Cervantes *et al*., 2001). Hexavalent chromium compounds in the form of chromate CrO_4^{2-} and $Cr_2O_7^{2-}$ have been used in a wide variety of commercial processes (Turick *et al*., 1996). Upon the reduction of chromium (VI) to trivalent chromium (III), the toxic effects are significantly decreased in humans, animals and plants because of a decrease in the solubility and bioavailability of chromium (III) (Turick *et al*., 1996). The reduction of the highly toxic and mobile Cr (VI) to the less toxic and less mobile Cr (III) is likely to be useful for the remediation of contaminated waters and soils. This problem has stimulated interest in microorganisms as alternatives to conventional methods, due to their eco-friendly nature. Cr (III) is transformed to Cr (VI) mainly inside root cells, but also in the aerial part of the plant (Cervantes *et al*., 2001). Roots accumulate 10-100 times more Cr than shoots and other tissues (Cervantes *et al*., 2001). As a consequence, inhibition of growth, photosynthesis and respiration processes in plants and microorganisms are observed. Reduction of soluble Cr (VI) to insoluble Cr (III) occurs only within the surface layer of aggregates with higher available organic carbon and higher microbial respiration (Tokunaga *et al*., 2003). Thus, spatially resolved chemical and microbiological measurements are necessary within anaerobic soil aggregates to characterize and predict the fate of chromium contamination (Tokunaga *et al*., 2003). Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances, and show a quick response to the induced changes (Quilchano and Maranon, 2002). Therefore, enzyme activities can be considered effective indicators of soil quality changes resulting from environmental stress or management practices (Quilchano and Maranon, 2002). Studies of enzyme activities provide information on the biochemical reactions occurring in soil (Masciandro *et al*., 2000).

Dehydrogenase is considered an indicator of overall microbial activity because it occurs intracellularly in all

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T a b l e 1. The characteristics of the soils investigated

Soil type	Depth (cm)	Granulometric composition (%)				$C_{org.}$ (%)	pH_{H2O}
		1-0.1	0.1-0.01	0.01-0.001	<0.002		
Haplic Luvisol	10-20	70	14	9	7	0.53	6.05
Eutric Cambisol	0-25	88	6	4	2	0.29	4.72
Mollic Gleysol	20-30	41	27	25	7	0.84	6.52

living microbial cells, and it is linked with microbial oxidoreduction processes (Madejon *et al*., 2001; Quilchano and Maranon, 2002). However, the relationship between an individual biochemical property and the total microbial activity is not always obvious, especially in the case of complex systems like soils, where both microorganisms and processes involved in the degradation of organic compounds are highly diverse (Quilchano and Maranon, 2002).

The study was designed to indicate, under laboratory conditions, which is the effect of the two most common forms of chromium [Cr (III), Cr (VI)] on dehydrogenase activity in three different type of soils.

MATERIALS

The characteristics of the soils investigated are reported in Table 1. The study was performed on soils from the bank of Polish soils, gathered in the Institute of Agrophysics of the Polish Academy of Sciences, organized in 1980-1983.

Determination of soil dehydrogenase activity in soils is based on the use of soluble tetrazolium salts [2,3,5- triphenylotetrazolium chloride (TTC)], as an artificial electron acceptor, which are reduced to red–coloured formazans, extracted, and then determined colorimetrically. Lendhard (1956) was the first to introduce the reduction of 2,3,5-TTC to triphenyloformazane as a standard procedure to measure soil dehydrogenase activity (Welp, 1999). Since then, the TTC method has been widely used to investigate the effects of organic and inorganic toxicants on the soil microflora (Welp, 1999).

The design of the experiment consisted of a complete block with three sample replicates. The air dry soils were amended with Cr (III) as $CrCl_3$ in the concentration range from 0 to 20 mg kg^{-1}, and with Cr (VI) as $K_2Cr_2O_7$ in the range from 0 to 100 μg kg^{-1}. Non-amended soil samples were used as a control. Soils were incubated for 20 h at 20°C in darkness. The triphenyloformazane (TPF) formed by the reduction of TTC was extracted by adding 20 ml of ethanol, then the soil mixture was vigorously shaken by hand for 1 min and finally filtered through filter paper. The TPF concentration was measured spectrophotometrically by means of HITACHI UV-VIS U-2001 at 485 nm, and the results were expressed as nmol TPF g^{-1} soil min^{-1}.

Dehydrogenase activity was calculated as follows:

$$A = \frac{c_f V 10^6}{M_f \; m \, t} W, \qquad (1)$$

where: A – soil dehydrogenase activity, c_f – concentration of 1,3,5-triphenyloformazan (mg ml^{-1}), V – volume of added solutions: 25 ml ethanol + 1 ml TTC + 4 ml distilled water, M_f – molar mass of triphenyloformazan = 301 g mol^{-1}, m – soil mass = 6 g, t – time = 20 h, W – dampness coefficient.

RESULTS AND DISCUSSION

The study was performed on three soils, different in type and properties, which were amended with Cr (III) and Cr (VI) compounds. Dehydrogenase activity was determined by the Johnson and Casida method, using $CaCO_3$ and TTC (2,3,5-triphenylotetrazolium chloride).

Figure 1a shows the variations of dehydrogenase activity with different chromium (III) and (VI) concentrations in the Haplic Luvisol, Fig. 1b presents the same relationship in the Eutric Cambisol, whilst Fig. 1c in the Mollic Gleysol. The results obtained in this study confirm the fact that chromium compounds have detrimental effects on soil dehydrogenase activity. After the addition of chromium, rapid and significant decrease in enzymatic activities was observed in each of the investigated soils. Non-amended soils samples were used as a control, and their enzymatic activity were estimated as 100%. Dehydrogenase activity in the controls was similar for the investigated soils. However, for the Eutric Cambisol control sample, the value of dehydrogenase activity was a little higher (0.0415 nm g^{-1} min^{-1}) than in the Haplic Luvisol soil, where the same value came to 0.0104 nm g^{-1} min^{-1}. The highest value, on the level of 0.2314 nm g^{-1} min^{-1}, was observed in the Mollic Gleysol. The Haplic Luvisol (Fig. 1a) seemed to be more sensitive to chromium contamination than the other two soils which were taken into account in the experiments. Dehydrogenase activity in that type of soil was reduced to 11-13% in the samples enriched in Cr (III) forms (Fig. 1a). The more dangerous form of Cr (VI) was less harmful for dehydrogenases in the Haplic Luvisol, because enzymatic activity equaled 89% with a 1μg kg^{-1} addition and decreased to the value of 19% with the highest supplement of Cr (VI) (Fig. 1a). One possible explanation for this fact is that the

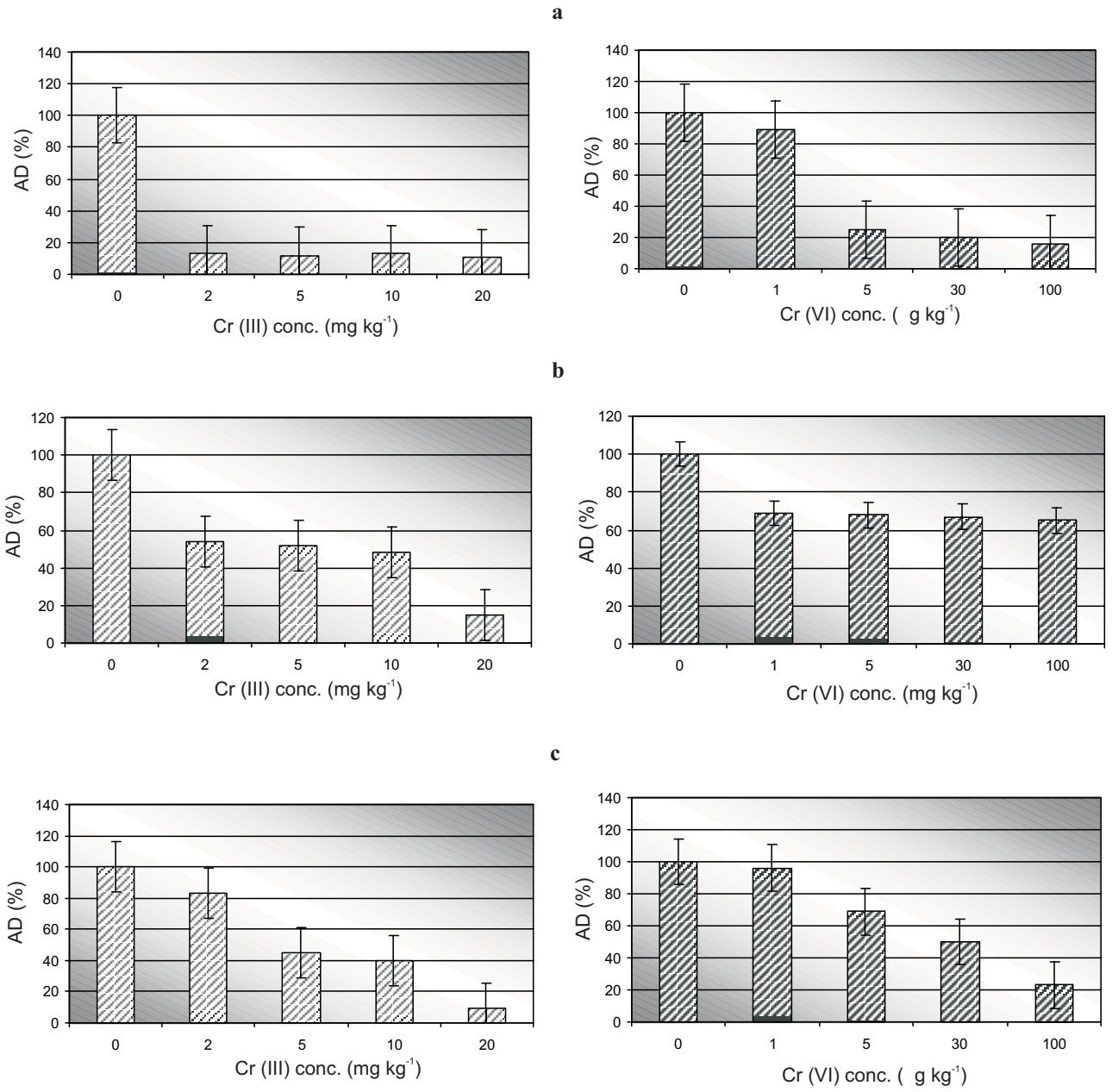

Fig. 1. The variations of dehydrogenase activity (AD) at different chromium (III) and (VI) concentrations in the: Haplic Luvisol (a), Eutric Cambisol (b), and Mollic Gleysol (c).

more dangerous form of Cr (VI) was reduced to the less toxic form of Cr (III) by microorganisms living in the soil.

The initial decrease in the dehydrogense activity in the Eutric Cambisol (Fig. 1b) as well as in the Mollic Gleysol (Fig. 1c) was more noticeable for the samples which were amended with the Cr (III) supplement. The 2 mg kg^{-1} Cr (III) concentration caused the reduction of dehydrogenase activity to 53% in relation to the control. The significant decline to 48% of activity at the range of 10 mg kg^{-1} was estimated. As a result of adding 20 mg kg-1 Cr (III) concentration, a gradual drop of enzymatic activity to 15% was observed (Fig. 1b). Cr (VI) compounds seemed to inhibit dehydrogenase activity at the permanent level of 69-65% in spite of the fact that the concentration of chromium increased (Fig. 1b).

Changes in dehydrogenase activity in the Mollic Gleysol soil are shown in Fig. 1c. Enzyme activity was gradually slowed down after Cr (III) as well as Cr (VI) supplement. 5 mg kg^{-1} Cr (III) concentration caused a 50% drop of dehydrogenase activity, whereas with 20 mg kg^{-1} Cr

concentration the activity of microorganisms came down to 9%. A similar situation was observed in the presence of an addition of Cr (VI). 30 μg kg^{-1}Cr (VI) concentration reduced enzymatic activity to the level of 49%, whilst the highest dose (100 μg kg^{-1}) was the reason of dropping the activity to the standard of 23%.

The values for dehydrogenase activity obtained with the different chromium dosage combinations are shown in Table 2. In the Eutric Cambisol soil, dehydrogenase activity reached a maximum of 0.0056 nm g^{-1} min^{-1} with a 2 mg kg^{-1} trivalent chromium supplement, while after adding the hexavalent chromium form at the rate of 1 μg kg^{1} the enzymatic activity increased to the value of 0.00716 nm g^{-1} min^{-1}. The lowest appreciation in the activity of oxidoreductase enzymes was found after the highest addition of both Cr (III) and Cr (VI) in the three investigated soils. The point of maximum dehydrogenase activity in the Haplic Luvisol was reached at 2 and 10 mg $kg^{-1}$of Cr (III) addition and came to 0.005377 nm g^{-1} min^{-1}, whereas the peak of 0.03705 nm $g^{-1}min^{-1}$ occurred as a result of the supplement of hexavalent chromium at the rate of 1 μg kg^{-1}. The highest value for dehydrogenases in the Mollic Gleysol containing the 2 mg kg^{-1} Cr (III) concentration was estimated on the level of 0.1929 nm g^{-1} min^{-1}, and in the case of the 1 μg kg^{-1} Cr (VI) concentration – on the level of 0.2233 nm g^{-1} min^{-1}. The lowest values of enzymatic activities were the effect of maximum Cr (III) and (VI) doses.

The increase of enzymatic activity observed at low chromium doses can be explained by a shortage of this nutrient element in the investigated soil samples. The results obtained in this study confirm the fact that, generally, chromium supplements cause a drop of enzymatic activity in the soil environment.

CONCLUSIONS

1. The application of trivalent as well as hexavalent chromium compounds had a noticeable negative effect on soil dehydrogenase activity.

2. Dehydrogenase activity in the Eutric Cambisol which contained a smaller level of organic matter was less sensitive to chromium contamination.

3. A significant and gradual drop of enzymatic activity in proportion to increasing chromium concentration in the Eutric Cambisol soil was found.

4. The Haplic Luvisol, in which dehydrogenase activity was reduced to 11-13% after trivalent chromium addition, was more sensitive to chromium supplement than the Eutric Cambisol and Mollic Gleysol.

5. The most dangerous form of hexavalent chromium seemed to be less harmful for the dehydrogenases activity in the Haplic Luvisol.

6. A significant positive linear relationship between the dehydrogenase activity and increasing Cr (III) and (VI) concentration in the Mollic Gleysol was found.

7. The differences in microorganisms activity can be due to differences in the composition of microorganisms living in the investigated soil samples.

8. The lowest values of dehydrogenase activities were the effect of increasing Cr (III) and (VI) doses.

T a b l e 2. Dehydrogenase activity in soils amended in different Cr forms

Soil type	Conc. Cr (III) (mg kg^{-1})	Dehydrogenase activity (nm g^{-1} min^{-1})	Conc. Cr (VI) (mg kg^{-1})	Dehydrogenase activity (nm g^{-1} min^{-1})
Haplic Luvisol	0	0.04151	0	0.04151
	2	0.00537	1	0.03705
	5	0.00507	5	0.01045
	10	0.00537	30	0.00836
	20	0.00448	100	0.01224
Eutric Cambisol	0	0.01040	0	0.01040
	2	0.00560	1	0.00716
	5	0.00540	5	0.00712
	10	0.00500	30	0.00700
	20	0.00160	100	0.00677
Mollic Gleysol	0	0.24140	0	0.23140
	2	0.19290	1	0.22330
	5	0.10600	5	0.16120
	10	0.09820	30	0.11640
	20	0.02240	100	0.05370

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