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DEHYDROGENASE ACTIVITY IN THE SOILS CONTAMINATED BY CR(III) AND CR(VI) COMPOUNDS

Our aim was to investigate the changes in the soil dehydrogenase activity as a result of contaminating soil environment by Cr(III) and Cr(VI) compounds. The experiment was performed on the samples (soil dump and bottom sediment) collected from the tannery waste lagoon in Lubartów (eastern part of Poland). The Eutric Cambisol and Haplic Luvisol soils were taken from the bank of Polish mineral soils gathered in the Institute of Agrophysics of the Polish Academy of Sciences in Lublin. They were enriched with Cr(III) under laboratory conditions; Cr(VI) forms were analyzed as well. The soils tested were chosen because of their domination in the area of waste lagoon subjected to reclamation. It was revealed that soil dehydrogenase activity rose in the concentration range from 2 to 5 mg of Cr(III) dm^{-3} only in the sample of bottom sediment. A similar tendency was found for dehydrogenase in soil dump, bottom sediments and Eutric Cambisol soil samples with Cr(VI) concentration ranging from 1 to 5 $\mu g\ dm^{-3}$. Chromium salts in a higher concentration inhibite enzymatic activity in the soils tested.

1. INTRODUCTION

Chromium accumulation in the soils has an adverse effect on the growth of living organisms [4]. The behaviour of chromium in natural environment depends on its oxidation level. The Cr(III) compounds are stable in a trivalent state, and at neutral pH they can occur as the relatively inactive Cr_2O_3 or $Cr(OH)_3$ sediments [2]. Cr(VI) compounds are considered to be toxic and carcinogenic for all living organisms. They are strong oxidants highly mobile both in water and in soil; moreover, they are readily soluble, especially in anion forms [3].

Chromium compounds can have a detrimental effect on soil microorganisms and their enzymatic activity, which is responsible for the fertility of soil [5].

Soil enzymes are mostly produced by microorganisms (bacteria, algae, fungi), plant roots, micro- and mesofauna [3]. It is widely known that the activity of dehydrogenases,

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which take part in a substrate oxidation in respiration processes, is an effective parameter for evaluation of soil fertility. Dehydrogenase is an enzyme found in the cytoplasm or structures made of plasma membranes. Dehydrogenase activity is also considered to be a very sensitive indicator of contamination of soil by heavy metals.

The aim of this study was to examine the effect produced by two most common forms of chromium (Cr(III), Cr(VI)) on the activity of soil dehydrogenase.

2. MATERIALS

2.1. TANNERY WASTE LAGOON

The samples for test were taken in the area nearby to the town of Lubartów in the eastern part of Poland. Tannery waste lagoon occupying up 6 ha is located in a direct neighbourhood of the Wieprz river and arable land. The soil samples were collected from soil dump and bottom sediments of the Wieprz river.

2.2. SOIL MATERIALS

The main characterictics of the soil materials taken from the bank of Polish mineral soils being gathered in the Institute of Agrophysics of the Polish Academy of Sciences in Lublin are presented in table 1.

Table 1

Selected properties of the soils investigated

	Granulometric composition [%]						
Type of soil	Depth [cm]	1–0.1	0.1–0.01	0.01–0.001	<0.002	Organic matter [%]	pH in H_2O
Eutric Cambisol-hydrogenic silt	15–20	5	42	41	12	0.92	5.01
Haplic Luvisol-loamy sand	5–15	5	62	24	9	0.80	6.78

3. METHODS

3.1. CHROMIUM CONCENTRATION MEASUREMENT – GFAAS TECHNIQUE

Environmental soil samples (soil dump, bottom sediments) were preserved by means of H_2SO_4 (0.5 cm^3 per 0.5 dm^3 of water), before measurement. Then, they were

filtered and analyzed with Z-8200 Hitachi spectrophotometer using Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) method.

3.2. DETERMINATION OF SOIL DEHYDROGENASE ACTIVITY

Dehydrogenase activity was determined by the Johnson and Casida method [1] based on the use of TTC (2,3,5-triphenylotetrazolium chloride). A 6 g soil sample was introduced into 50 cm^3 glass flasks and then 1 cm^3 of 3% aqueous solution of TTC, 120 mg of $CaCO_3$ and 4 cm^3 of distilled water were poured into the flasks. The soils were incubated for 20 hours at 30 °C in a thermostatic chamber. Then the samples were extracted with 25 cm^3 of ethanol and filtered. Absorbance was measured with HITACHI UV-VIS U-2001 spectrophotometer at 485 nm. All determinations of enzymatic activities were performed in triplicates for the soils enriched with Cr(III) in the form of $CrCl_3$ in the concentration range from 0 to 20 mg kg^{-1} as well as enriched with Cr(VI) as $K_2Cr_2O_7$ in the range from 0 to 100 μg kg^{-1}.

4. RESULTS AND DISCUSSION

The results obtained on the basis of the laboratory experiment are presented in table 2. Statistical processing of the data (correlation analysis and ANOVA) was conducted, which allowed determining mean values of dehydrogenase activity and standard deviations. In each case, the value of *P* was lower than 0.0001 which testified to statistically significant differences between average activities of dehydrogenases tested and the chromium dose at the 95% confidence interval.

In the control conditions, environmental samples (soil dump, bottom sediments) without chromium supplement were characterized by similar activities of dehydrogenases. Their enzymatic activities were found to be on the level of 0.1133 nmol g^{-1} min^{-1} in the soil dump and 0.1330 nmol g^{-1} min^{-1} in the bottom sediments. Cr(III) compounds were responsible for stronger inhibition of enzymatic activity in the soil dump sample compared to Cr(VI) salts. The doses of 2 and 5 mg of Cr(III) kg^{-1} of dry weight of soil caused the 98% and 96% decrease in dehydrogenase activity, respectively. A 35% reduction of this activity compared to the control sample was observed at the sample of 10 mg of Cr(III) kg^{-1} of dry weight of soil. The highest dose of 20 mg of Cr(III) kg^{-1} of dry weight of soil caused inhibition of dehydrogenase activity by 89.5% in comparison to the control value. An increase in the enzymatic activity of dehydrogenase in the soil dump sample in the presence of Cr(VI) ions whose concentrations reached 1, 5, 30 μg kg^{-1} of dry weight of soil was higher by 131, 105 and 27%, respectively. A considerable inhibition of dehydrogenase activity at 100 μg dose of Cr(VI) per 1 kg of dry weight of soil was found. In these conditions it was as low as 0.0186 nmol g^{-1} min^{-1}, which was 16.4% of enzymatic activity in the control conditions.

Table 2

The effect of particular factors on soil dehydrogenase activity; mean value, SD (95% half interval of confidence)

Dehydrogenase activity (nmol g^{-1} min^{-1})						
Type of soil	Cr(III) dose (mg kg^{-1} d.w.)	Mean	Standard deviation	Cr(VI) dose (μg kg^{-1} d.w.)	Mean	Standard deviation
Soil dump	0	0.1133	0.1520 (a)	0	0.1133	0.1527 (a)
	2	0.0013	0.0006 (a)	1	0.2626	0.0020 (b)
	5	0.0045	0.0005 (a)	5	0.2323	0.0015 (c)
	10	0.0283	0.0021 (b)	30	0.1446	0.0015 (d)
	20	0.0120	0.0020 (c)	100	0.0186	0.0015 (e)
Bottom sediment	0	0.1330	0.0020 (a)	0	0.1330	0.0020 (a)
	2	0.1630	0.0080 (b)	1	0.1446	0.0015 (b)
	5	0.1503	0.0021 (c)	5	0.1453	0.0040 (c)
	10	0.1043	0.0015 (d)	30	0.1186	0.0025 (d)
	20	0.0017	0.0001 (e)	100	0.1053	0.0021 (d)
Eutric Cambisol-hydrogenic silt	0	0.0073	0.0002 (a)	0	0.0073	0.0002 (a)
	2	0.0048	0.0003 (b)	1	0.0116	0.0002 (a)
	5	0.0054	0.0001 (b)	5	0.0120	0.0002 (a)
	10	0.0049	0.0002 (c)	30	0.0076	0.0004 (b)
	20	0.0018	0.0003 (d)	100	0.0069	0.0001(b)
Haplic Luvisol-loamy sand	0	0.0216	0.0011 (a)	0	0.0216	0.0011 (a)
	2	0.1540	0.0040 (a)	1	0.0170	0.0003 (b)
	5	0.1556	0.0045 (b)	5	0.0072	0.0003 (c)
	10	0.0343	0.0015 (c)	30	0.0244	0.0003 (d)
	20	0.0246	0.0020 (c)	100	0.0011	0.0001 (e)

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

In the samples of bottom sediment taken from the Wieprz river, an increase in the dehydrogenase activity by 22 and 13% was found at the 2 and 5 mg doses of Cr(III) per 1 kg of dry weight of soil, respectively. Cr(VI) forms in the concentrations of 1 and 5 μg kg^{-1} increased an enzymatic activity by 8 and 9%, respectively, compared to the control sample as well.

An initial increase in enzymatic activity of dehydrogenases at low chromium doses can be explained by a shortage of this nutrient in a barren soil [2]. However, higher doses of chromium compounds have an adverse effect on oxydoreductases, which manifested itself as an inhibition of their activity. Based on the laboratory analysis of the soil samples taken from the bank of Polish mineral soil it can be confirmed that generally chromium compounds have detrimental effect on the activity of soil enzymes, which is compatible with MADEJON [3] and OBBARD [5] observations.

5. CONCLUSIONS

The experiments carried out revealed that both Cr(III) and Cr(VI) compounds changed the activity of soil dehydrogenases. Generally, chromium inhibited soil enzymatic activity, which was also confirmed by other authors [3]–[5]. Only in the case of barren soils, poor in mineral components, an advantageous influence of low element concentrations (2–5 mg of Cr(III)kg^{-1}, 1–30 μg of Cr(VI) kg^{-1}) was detected. Such enzymatic activity in this case can be explained by shortage of this metal as a nutrient element in the soil samples investigated.

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AKTYWNOŚĆ DEHYDROGENAZOWA GLEB ZANIECZYSZCZONYCH ZWIĄZKAMI Cr(III) I Cr(VI)

Zbadano zmiany aktywności dehydrogenaz glebowych zachodzące pod wpływem zanieczyszczeń środowiska glebowego związkami Cr(III) i Cr(VI). Próby pobrano z hałdy glebowej oraz osadu dennego rzeki Wieprz na terenie składowiska odpadów garbarskich koło Lubartowa. Analizowano również gleby bielicowe i brunatne pobrane z Banku Gleb PAN, które wzbogacono chromem w warunkach laboratoryjnych. Wyboru gleb dokonano, kierując się ich dominacją na terenie rekultywowanego składowiska odpadów. Stwierdzono, że aktywność dehydrogenazowa rosła dla Cr(III) w zakresie stężenia 2–5 mg dm^{-3} tylko w próbie osadu dennego oraz dla Cr(VI) w zakresie 1–5 μg dm^{-3} w próbach hałdy, osadu dennego oraz gleby brunatnej. Wyższe dawki wprowadzanego pierwiastka powodowały inhibicję aktywności enzymatycznej badanych gleb.