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Soil Contamination by Chromium and Its Enzymatic Activity and Yielding

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Abstract

The effect of chromium (VI) on the activity of dehydrogenases, urease, acid and alkaline phosphatases and the yield of lupine was assayed in a pot experiment. Chromium was applied as $K_2Cr_2O_7$ in the amounts of 0, 10, 20, 30, 40, 50, 100 and 150 in mg · kg^{-1} of soil. The tests were conducted on brown leached soil from slightly dusty clay sand of pH in 1 M KCl 5.80. Soil was inoculated with nitragine containing *Bradyrhizobium* bacteria.

Hexavalent chromium was found to inhibit the activity of dehydrogenases, urease, acid and alkaline phosphatases and to depress the yield of above-ground parts of lupine, yield of roots, number and weight of root nodules. The negative effect of chromium occurred in both inoculated and non-inoculated soil. The potential biochemical soil fertility index derived from the activity of the soil enzymes and organic carbon content was negatively correlated with the rate of chromium (VI) contamination of soil. On the other hand, the index was positively correlated with the yield of lupine.

Keywords: chromium, soil enzymes, potential biochemical index of soil fertility, lupine yielding

Introduction

Excessive accumulation of heavy metals in soils is a side effect of the development of civilisation. Contamination of the environment with heavy metals has become one of the major ecological issues in the past few years. Chromium is one of those heavy metals the concentration of which is steadily increasing due to industrial growth, especially the development of metal, chemical and tanning industries [1]. Other sources of chromium permeating the environment are air and water erosion of rocks, power plants on liquid fuels, brown and hard coal, and industrial and municipal waste [2]. Although there is no risk of chromium contamination on a global scale, local permeation of the metal to soil, water or the atmosphere might result in excessive amounts of this pollutant in biogeochemical circulation [3, 4]. Chromium in soil and water is usually present as trivalent or hexavalent ions. The occurrence of chromium as tri- or hexavalentions depends mainly on soil pH, granulometric composition, redox potential and content of humus [5]. Trivalent chromium is weakly soluble in highly acid and alkaline soils, whereas hexavalent Cr dissolves well in acid and alkaline soils. Cr (VI) in soil is reduced to Cr (III), which is not well available for plants. Chromium (VI) has a harmful effect on soil microorganisms by depressing their biological activity [6]. Like other heavy metals, chromium may influence the enzymatic activity of soils by affecting soil microorganisms as well as by modifying the environment in which they live, and which is rich in many enzymes [7, 8].

The purpose of this study was to assess the effect of hexavalent chromium compounds on biological properties of soil and on the yield of lupine. An attempt was also made to diminish possible toxic effects produced by chromium on the biological life in soil by inoculating soil with symbiotic bacteria.

Methods

The tests were performed in a greenhouse in plastic pots (in five replications), which were filled with 2.5 kg leached brown soil formed of slightly dusty clay sand of pH in 1 KCl equal 5.80. Constant fertilisation was applied, with the following amounts of macro- and microelements as pure element in mg · kg^{-1} of soil: P – 100 [K_2HPO_4]; K – 150 [K_2HPO_4 + KCl], Mg – 50 [$MgSO_4 \cdot 7H_2O$], Zn – 5 [$ZnCl_2$], Cu – 5 [$CuSO_4 \cdot 5H_2O$], Mn – 5 [$MnCl_2 \cdot 4H_2O$], Mo – 5 [$Na_2MoO_4 \cdot 2H_2O$], B – 0.33 [H_3BO_3]. Against such fertilisation background, the effect of hexavalent chromium applied as $K_2Cr_2O_7$ on enzymatic activity of soil and the growth and development of cv. Markiz lupine was assayed. Prior to being put in pots, soil was mixed with mineral fertilisers and, in the relevant objects, with chromium in the following quantities: 0, 10, 20, 30, 40, 50, 100 and 150 in mg · kg^{-1} of soil. The tests were conducted in two series, with and without soil amendment with symbiotic bacteria *Bradyrhizobium* sp. (lupini). Shortly before sowing, one dose of nitragine was mixed in 1 dm^3 H_2O to obtain a suspension, which was applied to lupine seeds (1.0 cm^3 per seed).

During the whole vegetative period of plants (60 days) moisture was maintained on a constant level of 60% of water capillary capacity. Lupine was harvested at the phase of flowering to determine the yield of above-ground parts, yield of roots, number and weight of root nodules. Soil samples were taken at the same time to determine the activity of dehydrogenases (Deh) by Lenhard's method modified by Casidy *et al*. [9], urease (Ure) by Gorin and Chine Chang's method [10], acid phosphatase (Pac) alkaline phosphatase (Pal) according to Tabatabai and Bremner's method [11]. The content of organic carbon in soil was also determined using the method of Tiurin [12]. Because potassium dichromate did not modify the content of organic carbon in soil, the latter results are not reported in this paper, other than the average carbon content in soil contaminated with chromium (VI) in the series without nitragine (0.37%) and in the inoculated objects (0.44%). In addition, based on the enzymatic activity of soil and carbon content, a potential soil fertility index (Mw) was computed from the formula:

$$M_w = \left(\frac{Ure}{10} + Deh + Pal + Pac\right) \cdot \%C.$$

The results were elaborated statistically using Duncan's test. The following were calculated: Pearson's simple correlation coefficients between the activity of soil enzymes and lupine yield, regression equations, determination coefficients and correlation coefficients between the rate of chromium and yield of lupine. All statistical calculations were carried out with the help of the software package Statistica [13].

Discussion of Results

Enzymatic activity of soil is a reliable measure of current biological status. Naturally occurring amounts of heavy metals do not disturb the biochemical balance of soil. Slightly higher quantities of heavy metals might stimulate the activity of soil enzymes, whereas in larger

Table 1. Effect of chromium (VI) on enzymatic activity and and biochemical index of soil fertility (Mw) in the dry matter of soil from lupine.

Dose Cr [mg · kg^{-1} of soil]	Dehydrogenases [cm^3 H_2 · kg^{-1}]		Urease [mg N-NH_4 · kg^{-1} · h^{-1}]		Phosphatase [mmol PNP · kg^{-1}]			
					acid		alkaline	
	–R	+R	–R	+R	–R	+R	–R	+R
0	1.53	1.16	5.87	5.05	1.25	1.15	1.21	0.84
10	1.23	1.08	7.62	6.39	0.95	1.10	0.98	0.68
20	0.71	1.01	7.04	5.51	0.95	0.97	0.95	0.61
30	0.70	0.93	6.71	5.40	0.90	0.81	0.58	0.62
40	0.64	0.63	6.35	5.34	0.88	0.80	0.57	0.54
50	0.60	0.60	5.48	5.23	0.79	0.68	0.57	0.54
100	0.55	0.41	5.24	4.87	0.64	0.63	0.56	0.54
150	0.14	0.10	3.88	2.95	0.55	0.50	0.38	0.36
$\bar{x}$	0.76	0.74	6.02	5.09	0.86	0.83	0.72	0.59
LSD*	a – 0.15; b – n.s.; a x b – 0.21		a – 0.57; b – 0.28; a x b – n.s.		a – 0.06; b – 0.03; a x b – 0.09		a – 0.10; b – 0.05; a x b – 0.15	

–R – objects withouth nitragina inoculum, +R – objects with nitragina inoculum,

*a – dose of chromium, b – nitragina inoculum, axb – interaction, n.s. – not significant difference; r – correlation coefficients.

Table 2. Correlation coefficients between soil enzymatic activity and biochemical index of soil fertility contaminated with chromium (VI).

Variable	Without nitragina	With nitragina	$\bar{x}$
Deh	-0.82*	-0.96**	-0.92**
Ure	-0.86*	-0.86*	-0.87*
Pac	-0.79*	-0.88*	-0.83*
Pal	-0.90*	-0.90*	-0.92**
Mw	-0.92	-0.96	-0.96

Deh – dehydrogenases; Ure – urease; Pac – phosphatase acid; Pal – phosphatase alkaline; Mw – biochemical index of soil fertility.
Correlation coefficients significant difference for * $p < 0.05$; ** $p < 0.01$.

dosages they will have an inhibitory effect [14, 15]. Heavy metals, however, are regarded as inhibitors of enzymatic and microbiological activity of soil. This is because if added to soil (whether on purpose or by accident) they cause quantitative and qualitative changes in the composition of microflora and in enzymatic activity [16]. The range of impact heavy metals have on microorganisms, soil enzymes and crops depends on the physicochemical properties of metals and soil. The results obtained by the authors showed that hexavalent chromium modified the activity of all the analysed soil enzymes (Tab. 1). The effect of Cr (VI) on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase depended on the rate of soil contamination with chromium and its inoculation with *Bradyrhizobium* bacteria. Chromium had an inhibitory influence of dehydrogenases, urease and acid and alkaline phosphatases. The inhibitory effect of chromium was correlated with the amendment of soil with nitragine. It was stronger in nitragine inoculated soil and weaker in the soil without symbiotic bacteria. In both series of experiments a negative correlation was observed between the degree of soil contamination with chromium and the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase (Tab. 2). For either series of tests the correlation coefficient between the dose of Cr (VI) and activity of the enzymes ranged from -0.83 for alkaline phosphatase to -0.92 for dehydrogenases and acid phosphatase. Of all the soil enzymes, dehydrogenases were the least tolerant to the contamination of soil with hexavalent chromium. 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. The activity of acid and alkaline phosphatases decreased at higher rates of soil contamination with Cr (VI). Less unambiguous data were attained on the activity of urease. Chromium applied at 10 to 40 mg Cr · kg^{-1} of soil stimulated, while higher rates of the pollutant inhibited the activity of urease. The fact that hexavalent chromium inhibits the activity of all the analysed enzymes finds further confirmation in some earlier research [17, 18, 19], in which the negative effect of chromium was found to be related mainly to the type of soil use and degree of its contamination.

The results of the analyses reported hereby prove that activity of individual enzymes is quite a sufficient indicator of soil fertility. However, a more accurate understanding of the biochemical changes occurring in soil can be attained using the potential biochemical soil fertility index (Mw), which comprises the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase as well as content of organic carbon [20]. The potential soil fertility index thus computed decreased at higher rates of hexavalent chromium contamination (Fig. 1) and was significantly negatively correlated with chromium. The correlation coefficient between the rate of chromium (VI) and the potential biochemical index of soil fertility in the objects without nitragine was – 0.92, and in the soil amended with the inoculant – 0.96. It is worth pointing to high correlations between the activity of particular soil enzymes and between the potential soil fertility index versus the yield of above-ground parts, yield of roots and weight and number of nodules on 1 root of lupine (Tab. 3).

The experiments revealed that lupine, as much as soil enzymes, is not tolerant to hexavalent chromium. Toxicity of chromium was correlated with the degree of soil contamination (Tab. 4). Negative correlation was observed between the rate of chromium applied and the yield of above-ground parts (r = -0.93), roots (r = -0.97), weight of nodules (r = -0.68) and number of nodules on 1 root (r = -0.88) in both inoculated and non-inoculated soil. Such negative influence of chromium on plants was confirmed by the regression equations and determination coefficients between the rate of chromium and yield of lupine (Fig. 2). Symptoms of toxicity involving disturbed water balance (wilting), chlorosis of new leaves and damage of the growing point and roots were observed on the objects contaminated with the lowest rate of chromium (10 mg Cr · kg^{-1} of soil), only to become more evident as the concentration of the metal in soil increased. In the objects polluted with 100 mg Cr · kg^{-1} of soil, plants became necrotic at the stage of seedlings, and in the soil treated with 150 mg Cr · kg^{-1} of soil, the emergence of plants was inhibited. Inoculation with symbiotic bacteria

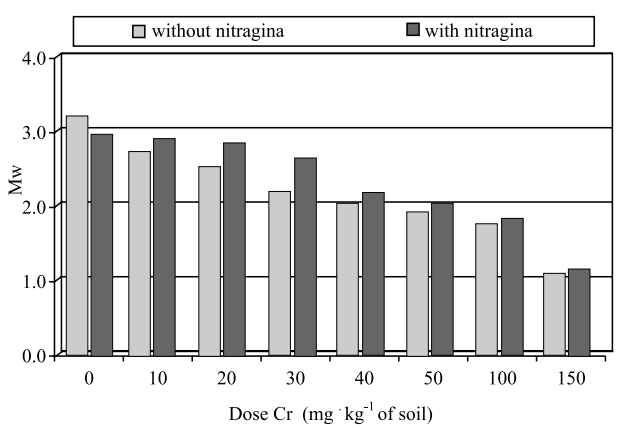

Fig. 1. Biochemical index of soil fertility in soil contaminated of chromium (VI).

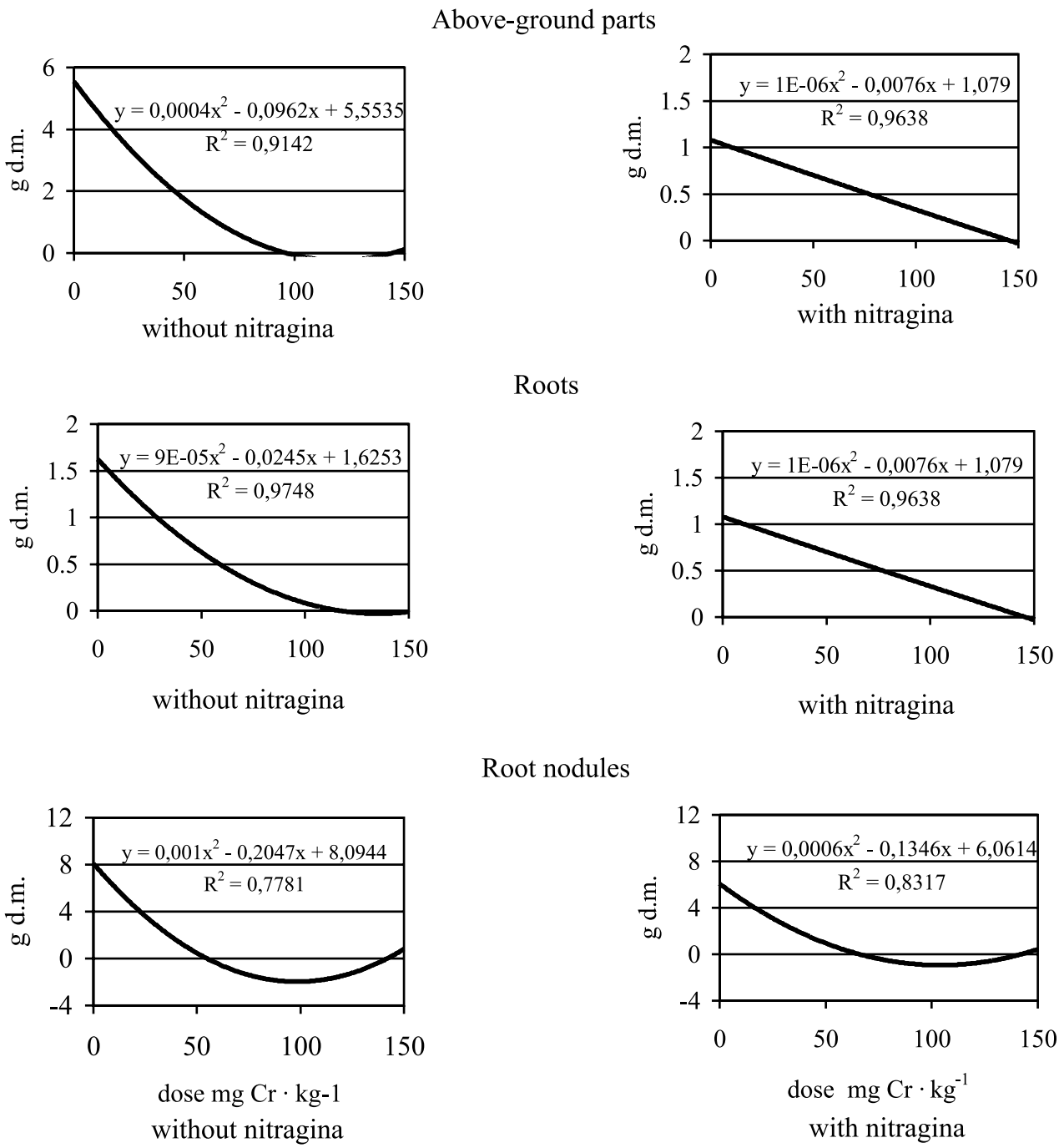

Fig. 2. Regression equations for lupine yield (g d.m. per pot).

Table 3. Correlation coefficients between soil enzymatic activity and biochemical index of soil fertility and yield of lupine.

Variable	Above-ground parts		Roots		Weight of nodules		Number of nodules	
	–R	+R	–R	+R	–R	+R	–R	+R
Deh	0.94**	0.92*	0.90*	0.91*	0.93**	0.80*	0.75*	0.83*
Ure	0.57	0.76*	0.74	0.82*	0.32	0.38	0.78*	0.72
Pac	0.97**	0.87*	0.93**	0.86*	0.84*	0.88*	0.60	0.67
Pal	0.89*	0.86*	0.88*	0.83*	0.87*	0.91*	0.71	0.59
Mw	0.94**	0.91*	0.94**	0.90*	0.84*	0.75*	0.75*	0.83*

* explanations as in Table 2

Table 4. Influence of chromium (VI) on yield of above-ground parts and roots of lupine and on the weight and number of nodules on 1 root.

Dose Cr [mg · kg^{-1} of soil]	Above-ground parts [g d.m. pot^{-1}]		Roots [g d.m. pot^{-1}]		Nodules [mg d.m. roots^{1}]		Number of nodules	
	–R	+R	–R	+R	–R	+R	–R	+R
0	6.52	4.17	1.63	1.08	10.62	7.95	6.63	6.00
10	4.25	3.26	1.47	1.02	5.98	3.72	14.75	6.75
20	2.84	3.09	1.10	0.84	1.20	2.70	5.31	8.94
30	2.56	2.98	0.82	0.82	1.14	1.80	4.08	9.63
40	2.49	2.86	0.82	0.83	1.15	1.49	3.00	5.58
50	2.38	2.81	0.78	0.82	0.57	1.54	3.00	5.33
100	0.11	0.72	0.03	0.23	0.02	0.04	0.13	0.56
150	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$\bar{x}$	2.64	2.49	0.83	0.71	2.58	2.41	4.61	5.35
r	-0.87*	-0.98**	-0.94**	-0.98**	-0.63	-0.74	-0.71	-0.85*
LSD*	a – 0.51; b – n.s.; a x b – 0.73		a – 0.19; b – 0.09; a x b – 0.26		a – 1.00; b – n.s.; a x b – 2.00		a – 2.79; b – n.s.; a x b – 3.21	

* explanations as in Table 1.

not only failed to improve the yields, but also stimulated toxic influence of chromium applied at the lowest rate (10 mg Cr · kg^{-1} of soil) on the growth and development of lupine plants.

Both the data reported in this paper and the relevant references [2, 18, 21, 22] clearly show if present in excess chromium has a negative influence on the growth and development of plants. Chromates (chromium VI) seem to produce a much stronger effect, which is probably due to the fact that they permeate through cytoplasmatic membranes more efficiently than trivalent chromium ions. Therefore, hexavalent chromium caused a depression in the photosynthetic productivity and prolongs water stress (Barcelo quoted in [2]).

Conclusions

1. Hexavalent chromium has a negative effect on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. The negative effect occurred irrespective of nitragine inoculation.

2. The response of lupine to soil contamination with chromium depended on the rate of chromium. Inoculant containing symbiotic bacteria of the type Bradyrhizobium turned out to be ineffective in remediation of soil contaminated with chromium.

3. Potential biochemical index of soil fertility derived from the activity of dehydrogenases, urease, acid and alkaline phosphatases as well as the content of organic carbon was negatively correlated with the degree of soil contamination with chromium (VI) and positively with yield of lupine.

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