Research Article

EFFECT OF REACTIVE MATERIALS ON THE CONTENT OF SELECTED ELEMENTS IN INDIAN MUSTARD GROWN IN CR(VI)-CONTAMINATED SOILS

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ABSTRACT

Reactive materials represent a promising agent for environmental co-remediation. The research was aimed to determine the influence of hexavalent chromium in doses of 0, 25, 50, and 150 mg Cr(VI)·kg^{-1} of soil as well as zero valent-iron, and lignite additives on the content of macroelements in the Indian mustard (*Brassica juncea* L.). The average accumulation of the analysed elements in Indian mustard grown in Cr(VI) contaminated soil were found to follow the decreasing order Mg>Na>P>Ca>K. Soil contamination at 150 mg Cr(VI)·kg^{-1} of soil led to the highest increase in magnesium, calcium, sodium, and potassium content in Indian mustard. The application of zero-valent iron had a positive influence on the average Na and K content of the tested plant. The application of lignite had a positive influence on the average magnesium, sodium and calcium content in the above-ground parts of the studied plant. In the non-amended treatments (without reactive materials), the increasing rates of chromium (VI) had an explicitly positive effect on the content of phosphorous and sodium in Indian mustard.

Keywords: chromium, Indian mustard, macroelements, soil.

INTRODUCTION

Chromium (Cr) is a trace and lithophilic element; it ranks 20th in terms of frequency of occurrence, comprising 0.037% of the Earth's crust. It also ranks fourth in regards to availability among 29 elements regarded as important due to their biological role and toxicity. The total amount of chromium in the natural environment is complemented by an additional, approximately 50% higher that from natural sources, amount of chromium created as an effect of anthropogenic activity [Ščančarab and Milačič, 2014].

Trivalent chromium is adsorbed on cell membranes, and is quickly immobilized by proteins, while hexavalent chromium is able to enter the cell where it undergoes transformation into a trivalent ion, which in reaction with inner-cell substances can lead to genetic changes [Jabłońska-Czapla, 2015]. As a result of this phenomenon, chromium is bound in the root cells and poorly transported from the root system to the above-ground parts of plants. Therefore, Cr(VI) ions are more biologically toxic, and absorbed faster by plants than Cr(III) ions [Naseem et al., 2015].

Chromium compounds belong to a group of chemicals whose toxicity to man is classified depending on their oxidation state and solubility, with hexavalent chromium compounds held responsible for strong carcinogenic and toxic effects [Salnikow et al., 2008]. For humans, the toxic dose of soluble chromates Cr^{3+} or Cr^{6+} absorbed through the alimentary tract is around 5 g, and the main symptoms of poisoning are nausea, diarrhoea and haemorrhagic diathesis, which consequently lead to the development of hepa-

tonecrosis and necrosis of uriniferous tubules, as well as some damage to the haematopoietic system [Rowbotham et al., 2008]. As a result, they are classified by the International Agency for Research on Cancer as B-2 group compounds [IARC, 1990], that is substances which are carcinogenic and mutagenic to humans. Hexavalent chromium is toxic mainly because of its strong oxidizing properties and due to the formation of free radicals when Cr(VI) in cells is reduced to Cr(III). Studies on mutagenicity prove that chromium (VI) is genetically active [Wrobel et al., 2015], and its toxicity is closely connected with its oxidizing properties. The final mutagenic form of this metal is thought to be chromium (III), derived from reduction of chromium (VI) within the cellular nucleus and formation of complexes of chromium (III) [Banks et al., 2006].

The objectives of this study were to determine the effects of Cr(VI) on the content of trace elements in the Indian mustard (*Brassica juncea* L.). Moreover, an attempt was also made to determine the ability of reactive materials i.e., zero-valent iron (ZVI), lignite and mixture of zero-valent iron and lignite (ZVI/Lignite), on the content of some elements in the analysed plant.

MATERIAL AND METHODS

The experiment was assessed under the conditions of a pot experiment in an acclimatized greenhouse, with two factors and fourfold replication. The first factor was the addition of increased doses of Cr(VI) to soil (0, 25, 50, and 150 mg·kg^{-1}), introduced in a form of chemically pure aqueous solutions of $K_2Cr_2O_7$ (Sigma-Aldrich). The second factor consisted of the addition of two reactive materials, i.e. ZVI, lignite, and the mixture of ZVI/lignite (3.0% w/w). Soils without nickel and amendments (0.0%) were designated as the control. Non-polluted soil for the pot experiment were collected at a depth of 0-20cm from farmland in the vicinity of Olsztyn, Poland, (53°35'45''N, 19°51'06'E). The chemical properties of the soil are shown in Table 1. The soil was air-dried, passed through a 1-cm sieve and packed into 20-cm-diameter and 26-cm-height experimental pots (10 kg soil per pot); it was then used for physical and chemical analysis, as well as N, P, K, Mg, Ca, Na concentration analysis.

The polyethylene pots were maintained under natural day/night conditions; during the day

(14h), the air temperature was 26±3°C and approximately ten degrees lower (16±2°C) at night (10h), with a relative humidity of 75±5%. The plants were watered every other day with distilled water to 60% of the maximum water holding capacity of the soil. The plants were harvested after 100 days, and soil and sorbents were collected.

The seeds of *B. juncea* (L.) cv. Małopolska, were obtained from an authorized Seed Production Centre in Olsztyn, Poland (OLZNAS-CN Sp. z o.o.), and were planted at the quantity of *n*=5 per pot. The soil was fertilized with a macro- and micronutrient fertilizer mixture (g kg^{-1}) containing N-26%, K_2O-26%, B-0.013%, Cu-0.025%, Fe-0.05%, and Mn-0.025%. The above-part of Indian mustard was harvested in the flowering phase and plant material samples were collected for laboratory tests.

In the laboratory, plant samples were thoroughly rinsed, first with tap water and then with deionized water to remove dust and soil particles.

Table 1. Physical and chemical parameters of the experimental soil

Soil chemical parameters		
1	pH	4.80
2	Hydrolytic acidity (mmol·kg^{-1})	33.75
3	Sum of exchangeable bases (mmol·kg^{-1})	62.20
4	Cation exchange capacity (mmol·kg^{-1})	95.95
5	Base saturation (%)	64.80
Organic matter		
1	Organic carbon (g·kg^{-1})	7.13
2	Total N (g·kg^{-1})	1.04
3	Carbon:Nitrogen	6.85
4	N-NH$_4^+$ (mg·kg^{-1})	21.18
5	N-NO$_3^-$ (mg·kg^{-1})	9.88
Soil texture (%)		
1	2.0–0.05 mm	86.6
2	0.05–0.002 mm	11.2
3	0.002 mm	2.2
Trace metal (mg kg^{-1})		
1	Nickel	4.05
2	Copper	8.49
3	Chromium	10.95
4	Zinc	24.21
5	Lead	16.33
6	Manganese	210.9
Available forms (mg kg^{-1})		
1	Phosphorous	46.6
2	Potassium	8.20
3	Magnesium	33.9

After oven drying (60 °C, 48 h) the plants were weighed (DW) and powdered using an analytical mill (A11 IKA, Germany) preceding the chemical analyses. The samples were kept at an ambient temperature until analysis. All reagents were of analytical reagent grade unless otherwise stated. Ultra-pure (UP) water (Millipore System, USA) of 0.055 $\mu S \cdot cm^{-1}$ resistivity was used for preparing the solutions and dilutions.

Total nitrogen content was tested for by means of Kjedahl's method after mineralization in concentrated sulfuric (VI) acid using hydrogen peroxide as a catalyst [Bremner, 1965]. Phosphorus (P) was assessed by colorimetric analysis, using the vanadium-molybdenum method (Cavell, 1955); sodium (Na), calcium (Ca), potassium (K) - atomic emission spectrometry, AES method [Szyszko, 1982], magnesium (Mg) – atomic absorption spectrometry, AAS method [Szyszko, 1982].

Statistical analysis was performed using the software Statistica [StatSoft, 2010]. Differences of means between treatments were tested by ANOVA and comparisons of means using LSD test, at $p=0.05$. The means and standard deviations (±SD) of five replications are reported.

RESULTS AND DISCUSSION

The major cations in plant cells are potassium (K), calcium (Ca), and magnesium (Mg) [Maathuis, 2009]. When soil pH becomes acid, the phytoavailability of these elements usually increases due to the replacement of cations on soil binding sites by H^+ ions [Pinto and Ferreira, 2015]. According to the literature [Collin et al. 2014; Kabata-Pendias, 2011] trace elements have a strong and varied influence on the content of macroelements in plants, which was confirmed by our studies. The analysis of results showed that the content of phosphorous, sodium, potassium, calcium, and magnesium in the Indian mustard (*Brassica juncea* L.) was influenced by the level of chromium (VI), as well as the addition of zero-valent iron or lignite (Figure 1).

Phosphorus (P) contained in the plant tissues occurs mainly in a form of inorganic compounds (ortho- and pyrophosphates), and in organic forms, especially in phospholipids, sugar-phosphates and nucleoproteins [Konieczynski and Wesolowski, 2007]. Indian mustard from this experiment had phosphorus concentrations range from 1.61 to 2.70 $g \cdot kg^{-1}$ (Figure 1). Applying Cr(VI) to soil contributed to increased levels of phosphorus in plants as compared to the control series - without amendments. Crops in the study group without reactive materials and exposed to Cr(VI) doses of 50 $mg \cdot kg^{-1}$ soil were found to have the highest phosphorus content in their aboveground parts. The addition of mixture zero-valent iron and lignite (ZVI/Lignite) caused a 23% decrease in the average content of the above mentioned element in Indian mustard as compared to the control series. The results confirm researches of Kalembasa and Wysokiński [2002] where addition of brown coals' ash caused decrease of
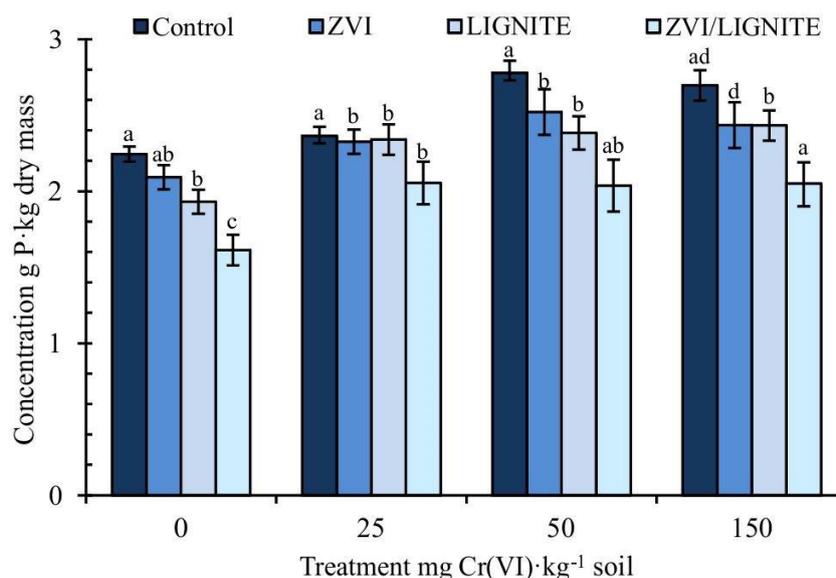
Figure 1. Effect of Cr(VI) contamination on phosphorus (P) content in Indian mustard (g·kg⁻¹dry mass); mean ± SD, n=3; Columns marked with different letter indicate significant differences between members of the same set (Tukey test, P < 0.05)

contents of phosphorus in plants. In our recent study [Wyszkowski and Radziemska 2013] the average phosphorous content in oat (*Avena sativa* L.) straw was the highest in objects with Cr(III) (+34%) and Cr(VI) (+27%) upon the application of organic matter. Moreover, application of mineral reactive materials, i.e. zeolite and halloysite to heavy metals contaminated soil led to positive influence on the average phosphorus content of *Zea mays* L. [Radziemska et al., 2013].

The application of chromium (VI) to soil on the whole led to increased potassium (K) contents in plants, as compared to the control series - without reactive materials (Figure 2). Leszczyńska and Kwiatkowska-Malina [2011] showed that the potassium contents in roots of rye, significantly decreased, influenced by addition of lignite in comparison with control object. The accumulation of potassium in the grain of the oat was negatively correlated with the increasing contamination of soil with hexavalent chromium reported by Wyszkowski and Radziemska [2013]. Moreover, according to Kozera et al. [2006], heavy metals in the soil may decrease potassium content in potato tubers. In the presented studies, among the substances added to the soil to alleviate Cr(VI) contamination, zero-valent iron (ZVI) turned out to be the best, leading to an 8% increase in the average content of the described element in the test plant as compared to the control series. An analogical situation was observed in the case of natural lignite additive, although their influence was smaller. In soils contaminated at the level of

50 Cr(VI)·kg^{-1} with the addition of zero-valent iron (ZVI), the content of the analyzed element in Indian mustard plants was approximately 5% higher than in plants grown in soil free of contamination and additives.

Calcium (Ca) is usually present at high concentrations in the cytosol of root cells [Pittman, 2011]. Atriola-Fortuny and Fuller [1982] reported that the influence of chromium (VI) on plants is indirectly restricted by the presence of organic substances and calcium in the subsoil. Calcium content in the tested plant was significantly influenced by the dose of chromium (VI) as well as the reactive materials added to the soil (Figure 3). The content of calcium in the described plant in the control series - without reactive additives, was positively correlated with increasing doses of hexavalent chromium. In this series, plants grown in soil contaminated by Cr(VI) at a level of 150 mg·kg^{-1} was characterized by the highest calcium content. The addition of zero-valent iron (ZVI) (+11%) and mixture zero-valent iron and lignite (ZVI/Lignite) (12%) led to the highest decrease in the average content of the analysed element in relation to pots without neutralizing additives. In soils contaminated at the level of 150 mg Cr(VI)·kg^{-1} with the addition of lignite, the content of the analysed element in Indian mustard plants was approximately 25% higher than in plants grown in soil free of contamination and additives. Leszczyńska and Kwiatkowska-Malina [2011] demonstrated that the addition of lignite to soil causes a higher content of calcium in all parts of rye (*Secale cereale)*.
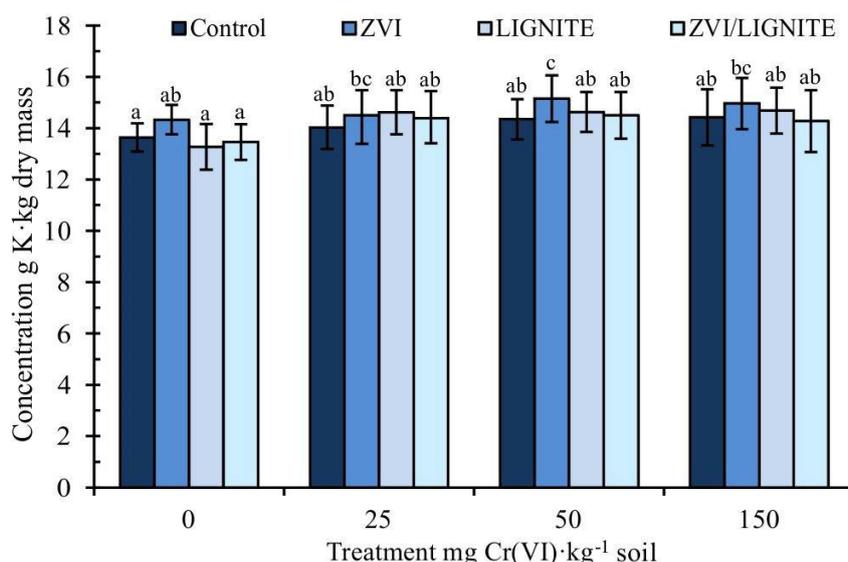
Figure 2. Effect of Cr(VI) contamination on potassium (K) content in Indian mustard (g·kg^{-1}dry mass); mean ± SD, n=3; Columns marked with different letter indicate significant differences between members of the same set (Tukey test, P < 0.05)
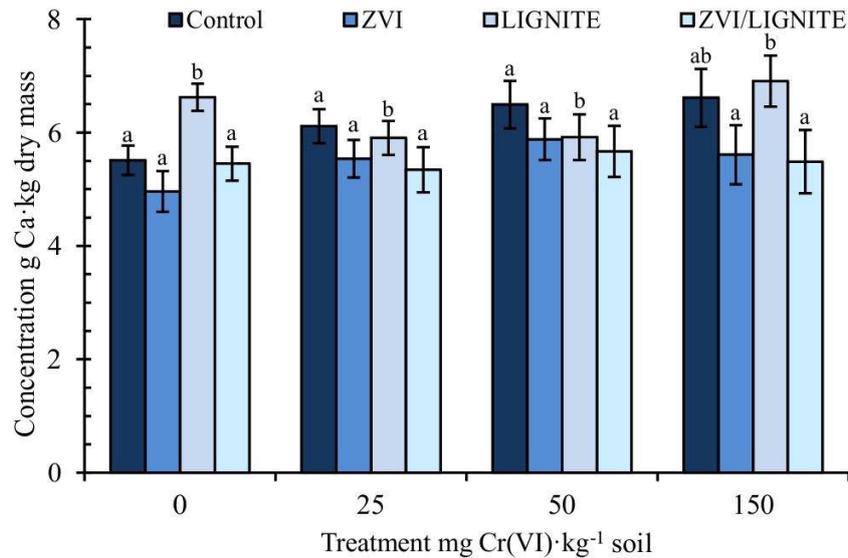
Figure 3. Effect of Cr(VI) contamination on calcium (Ca) content in Indian mustard (g·kg^{-1}dry mass); mean ± SD, n=3; Columns marked with different letter indicate significant differences between members of the same set (Tukey test, P < 0.05)
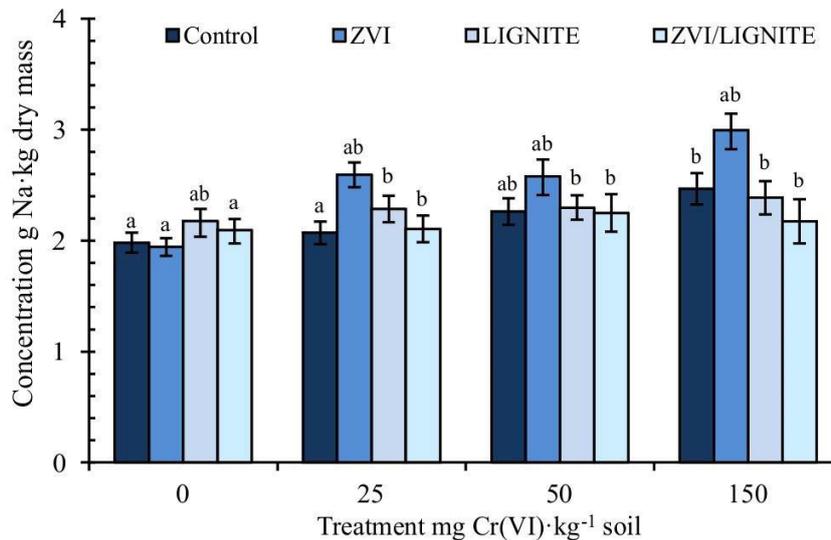
Figure 4. Effect of Cr(VI) contamination on sodium (Na) content in Indian mustard (g·kg^{-1}dry mass); mean ± SD, n=3; Columns marked with different letter indicate significant differences between members of the same set (Tukey test, P < 0.05)

The chromium hexavalent dose as well as the addition of ZVI, Lignite and mixture of ZVI/Lignite shaped sodium (Na) content in Indian mustard (Figure 4). Plant from this experiment had sodium concentrations range from 1.94 to 3.00 g·kg^{-1}. Higher doses of chromium (VI) in the control series resulted in a significant increase of sodium content in the aerial parts of the described plant, with the highest dose of such contamination (150 mg·kg^{-1} of soil) increasing sodium content by 20%. The influence of reactive materials used in the experiment was reflected in the sodium content of the Indian mustard. Mixture of zero-valent iron and lignite (ZVI/Lignite) nega-

tively influenced the average content of this element. The calcium content in the analysed plant was particularly and strongly affected by the addition of zero-valent iron (+ 13%) and, to a lesser extent, by lignite (+ 4%). In soils contaminated at the level of 150 mg Cr(VI)·kg^{-1} with the addition of mixture of zero-valent iron and lignite (ZVI/Lignite), the content of the analysed element in Indian mustard plants was approximately 11% lower than in plants grown in the soil free of additives. The data presented by Leszczyńska and Kwiatkowska-Malina [2011] showed that organic matter from different sources did not have an effect on the content of sodium in some parts of rye
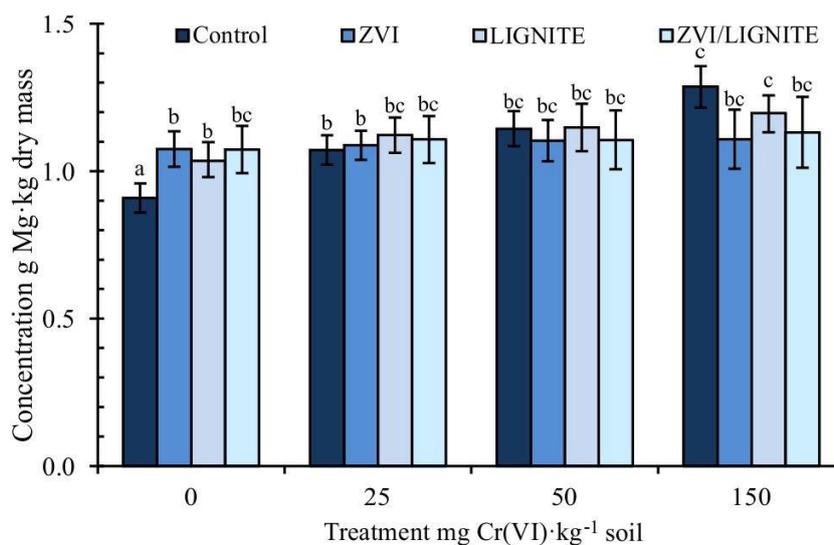
Figure 5. Effect of Cr(VI) contamination on magnesium (Mg) content in Indian mustard (g·kg⁻¹dry mass);
mean ± SD, n=3; Columns marked with different letter indicate significant differences between
members of the same set (Tukey test, P < 0.05)

in variants of soil contaminated with, or without the addition of heavy metals.

Magnesium (Mg) has a number of key functions in plants. The contamination of soil with hexavalent chromium, as well as the application of reactive materials had a significant effect on the content of magnesium in the Indian mustard (Figure 5). The uptake of magnesium is strongly influenced by the availability of other cations, i.e. calcium, potassium, and NH_4^+ [Römheld and Kirkby, 2007]. Indian mustard from the present experiment was found to have magnesium concentrations ranging from 0.91 to 1.20 g·kg⁻¹. Crops in the study group without reactive materials and exposed to a Cr(VI) dose of 150 mg·kg⁻¹ soil were found to have the highest magnesium content in their above-ground parts. The application of mixture zero-valent iron and lignite (ZVI/Lignite) had a positive influence on the average magnesium content in the above-ground parts of Indian mustard. When compared to the control series, lignite was shown to have the most beneficial effect on magnesium content in the analysed plant.

CONCLUSIONS

The reactive materials applied in the experiment, i.e. zero-valent iron (ZVI), lignite, and mixture zero-valent iron and lignite (ZVI/Lignite) significantly shaped the content of phosphorus (P), calcium (Ca), sodium (Na), magnesium (Mg), and potassium (K) in the Indian mustard. The average accumulation of macronutrients

in tested plant grown in Cr(VI)-contaminated soil was found to follow the decreasing order of Mg>Na>P>Ca>K.

In the control series (without the addition of reactive materials), the differences in phosphorous, calcium, sodium, magnesium and potassium content were positively correlated with the increasing doses of Cr(VI)-contamination. Soil contamination at 150 mg Cr(VI)·kg⁻¹ of soil led to the highest increase in magnesium, calcium, sodium, and potassium content in Indian mustard. The application of zero-valent iron (ZVI) had a positive influence on the average sodium and potassium content of this plant species. Moreover, the application of lignite had a positive influence on the average magnesium, sodium and calcium content in the above-ground parts of the studied plant. In the non-amended treatments (no reactive materials), the increasing rates of chromium (VI) had an explicitly positive effect on the content of phosphorous and sodium in Indian mustard.

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