Ovidius University Annals of Chemistry Volume 20, Number 1, pp. 127-131, 2009

Analysis of hexavalent chromium uptake by plants in polluted soils

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Abstract Concentration levels of hexavalent chromium in contaminated soil and in *Zea mays* plant parts were determined and Cr(VI) bioaccumulation and bioconcentration capacity of this plant were discussed. *Zea mays* seeds were sown in 40 mg Cr(VI)/kg dw polluted soil. After harvesting it was observed that hexavalent chromium concentrations in plant organs decreased in the following order: roots > stems > leaves. This means that *Zea mays* roots have the greatest tendency to concentrate Cr(VI), the concentration in these plant parts being 11.7 times greater than in the surrounding soil. The translocation factor (TF), bioaccumulation factor (BAF) and the bioconcentration ratio (BCR) were determined and they confirmed that hexavalent chromium was slowly translocated within the plant from the roots to stems, and very slowly further translocated to leaves. The results of this study indicate that *Zea Mays* is not a good hexavalent chromium phytoextractor from soils with 40 mg Cr(VI)/kg dw content.

Keywords: hexavalent chromium, toxic metals, polluted soils, translocation, bioaccumulation, bioconcentration.

1. Introduction

In recent decades there has been increasing concern with heavy metal contamination, because of their toxicity to microorganisms, plants, and animals, but also because metals, unlike most organic contaminants, are non-biodegradable and can accumulate in living tissues [1]. A variety of anthropogenic sources, such as mining processing, electroplating, wood preservation, iron and steel production, pigment manufacture, smelters, power station industry, production and application of metal-containing pesticides, can lead to soils acquiring heavy metal contents substantially in excess of natural levels. Some of these metals, at relatively low concentrations, may stimulate the biological life [2,3], while increased concentrations in the environment can be detrimental to a variety of living species [4-7]. The toxic potential of heavy metals in soil depends on soil composition, particularly on amount and type of clay minerals, organic matter and iron and manganese oxides [8]. All these properties influence metal mobility and availability, and therefore, influence their release and their interaction with other components of the ecosystem, such as plants. Chromium is an important metal that is usually encountered in the environment at oxidation states of (+III) and (+VI) [9]. Each of these oxidation states has very different biological and toxicological properties [10]. Hexavalent chromium has a high solubility, being a well-established carcinogen based upon animal, human, and in vitro assessment data [11-13]. On the contrary, the reduced form of chromium, Cr(III), is much less toxic and usually precipitates as hydroxides [13,14].

Plant species have different responses to heavy metal pollution of soils. Although it may exist a relationship between heavy metal accumulation and plants tolerance, many plant species grow on contaminated soils and yet do not accumulate metals [15]. Plants that possess the ability to tolerate, uptake and accumulate high levels of metals in their biomass are termed as hyperaccumulators [16]. However, even if not harmful for those plants, toxic metals can be hazardous for human or animal health due to metal concentration throughout the food chain [17]. Whyle hexavalent chromium can easily cross

ISSN-1223-7221

the cell membranes, using a mechanism involving carriers of essential anions such sulfate and phosphate [18], trivalent chromium does not utilize any specific membrane transport mechanism [19]. The objective of the study was to determine the concentration levels of hexavalent chromium in *Zea mays* plants growing in a Cr(VI) contaminated soil and to analyze the Cr(VI) bioaccumulation and bioconcentration capacity of this plant.

2. Experimental

2.1. Soil preparation and characterization

Soil applied in this research was collected from the arable horizon (0-30 cm depth) of a public garden located in Timisoara, Romania. The soil was homogenized, air-dried under room temperature, passed through 2.5 mm mesh and analyzed for: 1) cation exchange capacity and anion exchange capacity, by ammonium chloride method, 2) organic carbon by Walkley-Black method, 3) carbonates, by gravimetric method with HCl, 4) pH, at a ratio soil:water = 1:2.5, 5) Cr(VI) total content, by aqua regia digestion method. One 0.5 L plastic pot was filled with 300 g of soil which was then rehydrated with 100 mL 120 mg Cr(VI)/L, in order to obtain a final soil concentration of 40 mg/kg dw. Potassium dichromate solution was used as a source of Cr(VI). This concentration was selected because it's within the range of relevant concentrations for Cr(VI) polluted soils, according to the Romanian legal standards (maximum allowed Cr(VI) concentration for protected (residential) soils = 10 mg/kg dw) [20]. After the addition of Cr(VI) solution the soil was allowed to equilibrate for a period of 15 days and, afterwards, 10 seeds of *Zea mays* were sown on the pot surface.

2.2. Extraction of Cr(VI) from plant samples

Plants were allowed to grow for 40 days, after which they were removed from soil. Immediately following harvesting, the rhizosphere soil adhering to the roots was gently shaken and plants were rinsed in deionized water to remove any other adhering particles. The clean plant samples were then separated in different parts (roots, stems and leaves) and dried in an oven at 80^o C for two days till constant weight was reached. Samples of dried roots, stems and leaves were ashed in a muffle furnace at 600°C for 6 h, in order to pre-concentrate the Cr(VI) prior to its analysis [21]. The ash was then dissolved and made up to volume with a mixture of 2 M HCl and 1 M HNO_3, filtered and analyzed for Cr(VI).

2.3. Extraction of bioavailable Cr(VI) and total Cr(VI) from soil samples

Bioavailability is considered as the fraction of the total contaminant in the interstitial water and soil particles that is available to the receptor organism. In this study, the bioavailable Cr(VI) fraction in soil was considered to be the exchangeable Cr(VI) fraction, which is the fraction that is not tightly bound to soil. To extract this fraction a modified procedure proposed by James and Bartlett [22] was followed. Five grams of air-dried soil were transferred to 50 mL 0,005 M KH_2PO_4 and 0,05 M K_2HPO_4, mixed in a rotary shaker at 200 rpm, and allowed to equilibrate for a period of 24 hours. Thereafter, the soil suspension was centrifuged at 2500 rpm for 15 minutes and the supernatant was collected, made up to volume with the same KH_2PO_4 + K_2HPO_4 mixture and sent for Cr(VI) analysis. For the extraction of total Cr(VI), 2 g air-dried soil were transferred to 40 ml aqua regia (HCl : HNO_3 = 3:1). The vials were loosely capped and left to stand for 16 h. The next day the mixture was digested 2 h at 85^o C under reflux conditions. The extract was then cooled, filtered, made up to volume with HNO_3 and sent for Cr(VI) analysis [23,24].

2.4. Determination of Cr(VI) concentration in plant and soil extracts

Cr(VI) concentration in aqueous extracts was measured by the 1,5-diphenylcarbazide colorimetric method, based on the purple complex formed by Cr(VI) in the presence of 1,5-diphenylcarbazide. The color was fully developed after 15 min and the absorbance was measured at 540 nm in a 1 cm long glass cell using a Jasco V 530 spectrophotometer [25].

3. Results and Discussions

The results of the soil characterization are presented in Table 1. From this table it can be seen that the original soil applied in this study did not contain hexavalent chromium prior to spiking with

this pollutant. It also can be seen that the soil has a much lower anion exchange capacity compared with the cation exchange capacity. This could influence the bioavailability of Cr(VI) in soil, knowing that this oxidation state of chromium exists, as a function of pH, as bichromate or chromate anions.

Table 1. Original soil characterization

Cation exchange capacity (mval/100 g soil)	45
Anion exchange capacity (mval/100 g soil)	10
Carbonates (g/kg)	25
pH_{water}	7.7
Total organic matters (%)	7.2
Total organic carbon (%)	4.5
Cr(VI) (mg/kg dw)	-

Table 2. Bioavailable and total Cr(VI) concentration in prepared soil

Bioavailable Cr(VI) concentration (mg/kg dw)	2.9
Total aqua regia Cr(VI) concentration (mg/kg dw)	38.1

Table 3. Cr(VI) concentration in plant tissues

Plant tissue	Cr(VI) concentration (mg/kg dw)
Root	33.9
Stem	29.1
Leaf	6.1
Shoot	12.8
Total	15.1

The bioavailable and total Cr(VI) concentrations in the Cr(VI) spiked soil are presented in Table 2. It can be noticed that the bioavailable (exchangeable) Cr(VI) represent only a small percent (7.5%) from the total Cr(VI) concentration. The soil pH has a strong influence on the mobility of anions and their uptake by plants. In general, the retention of anionic metals in soil increases with the decrease of soil pH [26].

A good correlation was observed between the total Cr(VI) added to soil and the total Cr(VI) concentration determined by the aqua regia digestion method, the difference between the two concentrations being within 5% of the total concentration. Cr(VI) concentrations in plant tissues are presented in Table 3. Within the plant, the concentrations largely differ between different parts of the plant.

The highest Cr(VI) concentration was found in roots and the lowest in leaves. The *Zea mays* translocation factor (TF), the ratio of element concentration in shoot tissue to element concentration in root tissue, which estimates the translocation efficiency of a plant, was determined according to Eq. (1) [27]:

$$TF = mg\ Cr(VI)/kg\ dw\ shoot\ /\ mg\ Cr(VI)/kg\ dw\ root \quad (1)$$

For hyperaccumulator plants TF is typically greater than 1 [27]. Therefore, it is obvious that Cr(VI) was slowly translocated within the plant from the roots to stems, and very slowly further translocated to leaves. These results are in accord with other studies which also reported highest chromium accumulation in roots [28].

According to Shanker et al. [29] the high Cr(VI) concentration in roots is due to Cr(VI) immobilization in the vacuoles of the root cells. In biomagnification in *Zea mays* plants, the bioaccumulation factor (BAF) and the bioconcentration ratio (BCR) were determined.

Bioaccumulation is the process by which a chemical is taken up by an organism, either from direct exposure to a contaminated medium, or by consumption of contaminated food [23].

Bioconcentration refers to the absorption or uptake of a chemical from the media to concentrations in the organism tissues that are greater than in surrounding environment [30].

Biomagnification is a special case of bioaccumulation whereby the concentration of a chemical increases from one level in the food chain to another. The soil to plant BAF describes bioaccumulation as the ratio of the concentration of a chemical inside an organism to the concentration in the surrounding environment. For plants, BAF can be determined according to Eq. (2) [23]:

$$BAF = mg\ Cr(VI)/kg\ dw\ plant\ /\ mg\ Cr(VI)/kg\ dw\ soil \quad (2)$$

The above-ground-plant/soil bioconcentration ratio (BCR_{abg-s}), which represents the ratio of the

concentration of chemical in above-ground vegetation (shoot) to the concentration in contaminated soil, was calculated as [30]:

$$BCR_{abg-s} = mg\ Cr(VI)/kg\ dw\ plant / mg\ Cr(VI)/kg\ dw\ soil \quad (3)$$

The below-ground-plant/soil bioconcentration ratio (BCR_{root-s}), which represents the ratio of the concentration of chemical in below-ground vegetation to the concentration in soil solution, can be calculated as [30]:

$$BCR_{root-s} = mg\ Cr(VI)/kg\ dw\ plant / mg\ Cr(VI)/L\ soil\ solution \quad (4)$$

For this study Cr(VI) concentration in soil solution was approximated by the bioavailable Cr(VI) concentration in soil. The values of BAF, BCR_{abg-s} and BCR_{root-s} are presented in Table 4. From this table it can be seen that *Zea mays* roots have the greatest tendency to concentrate Cr(VI), the concentration in roots being 11.7 times greater than in the surrounding soil solution. However, despite the fact that *Zea mays* is capable to concentrate Cr(VI), according to US EPA only persistent chemicals (half-life greater than 30 days) having a BCR greater than 1000 tend to bioaccumulate [31].

Table 4. Calculated values of TF, BAF, BCR_{abg-s} and BCR_{root-s}.

TF	0.37
BAF	0.39
BCR_{abg-s}	0.33
BCR_{root-s}	11.69

4. Conclusions

In this study, concentration levels of hexavalent chromium in polluted soil and in *Zea mays* plants growing in this soil were determined. Experimental results revealed that hexavalent chromium concentrations largely differ between different parts of the plant. The highest Cr(VI) content was detected in roots of plants. Progressive Cr(VI) decrease in plant tissue was observed, with lowest Cr(VI) concentration in leaves. This can be due to the fact that Cr(VI) was slowly translocated within the plant from the roots to stems, and very slowly further translocated to leaves. A significant correlation was observed between the total Cr(VI) added to soil and the total Cr(VI) concentration determined by the aqua regia digestion method. Our results suggest that *Zea Mays* is not a good hexavalent chromium phytoextractor from soils with 40 mg Cr(VI)/kg dw content. However, since no visible phytotoxic symptoms were observed at this concentration, we will investigate with separate studies the capacity of this plant to extract and accumulate hexavalent chromium from soils contaminated with higher amounts of this metal.

5. Acknowledgments

This work was supported by Romanian Ministry of Education and Youth, through PN II Exploratory Research Project: "Innovative technologies for the removal of hexavalent chromium from wastewaters by reuse of scrap iron", CNCSIS code 1031/2008.

6. References

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