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CHROMIUM ACCUMULATION AND ITS EFFECTS ON OTHER MINERAL ELEMENTS IN *AMARANTHUS VIRIDIS* L.

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Chromium accumulation and its effects on other mineral elements in *Amaranthus viridis* L. were investigated using inductively coupled plasma atomic emission spectrometry (ICP-AES) and resin adsorption. The aim was to understand why *A. viridis* can grow well in soils heavily contaminated by Cr, what the forms of Cr in soils and residues are, and what effects Cr has on the distribution of Mn, Fe, Cu and Zn in the plant. The results indicated that *A. viridis* is not a hyperaccumulator, although it can grow well in soil containing a high concentration of Cr. The Cr concentration in plant tissues from a contaminated site was about 11 times higher than in those from an uncontaminated site. At both the contaminated and uncontaminated sites, Cr was accumulated primarily in its shoots, and in roots in much lower concentrations. The levels of Cr in *A. viridis* tissues were as follows: leaf > root > stem. Cr occurred predominantly as Cr(III). There was very little Cr(VI) in the polluted soil where *A. viridis* grew close to a waste heap discarded by a chromium(VI) production factory. The possible mechanisms for Cr accumulation of *A. viridis* and the effects of Cr on uptake and accumulation of the other mineral elements in *A. viridis* are briefly discussed.

Key words: *Amaranthus viridis* L., chromium, copper, iron, manganese, zinc, hyperaccumulation.

INTRODUCTION

Chromium (Cr) is the 21st most abundant element in the earth's crust (Krauskopf, 1979). It occurs in nature in bound forms that constitute 0.1–0.3 mg/kg of the earth's crust. Cr has several oxidation states ranging from Cr(-II) to Cr(+VI). Cr exists predominantly in the +III and +VI oxidation states. The most stable oxidation state of Cr is +III, and under most prevailing environmental conditions Cr(VI) is rapidly reduced to Cr(III). The intermediate states of +IV and +V are metastable and rarely encountered (Zayed and Terry, 2003).

In recent years, contamination of the environment by chromium has become a major concern. Chromium is used on a large scale in many different industries, including metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production, and pulp and paper production (Zayed and Terry, 2003). These industries have be-come especially large contributors of Cr pollution, which can ultimately have significant adverse biological and ecological effects. Very high levels of Cr(VI) contamination (14,600 mg/kg in ground water and 25,900 mg/kg in soil) were reported at the United Chrome Products site in Corvallis, Oregon (Krishnamurthy and Wilkens, 1994).

Chromium enters the food chain through consumption of plant material. A high concentration of Cr has been found to be harmful to vegetation. As the chromium concentration in plants increases, it adversely affects several biological parameters. Ultimately there is loss of vegetation, and land sometimes becomes barren (Dube et al., 2003).

Symptoms of Cr phytotoxicity include inhibition of seed germination or of early seedling development, reduction of root growth, leaf chlorosis and depressed biomass (Sharma et al., 1995). There are many studies on Cr toxicity in crop plants. Chromium significantly affects the metabolism of plants such as barley (*Hordeum vulgare*) (Ali et al., 2004), citrullus (Dube et al., 2003), cauliflower (Chatterjee and Chatterjee, 2000), vegetable crops (Zayed et al., 1998), wheat (*Triticum aestivum* cv. HD2204) (Sharma et al., 1995) and maize (*Zea mays*) (Sharma and Pant, 1994). The subcellular localization of Cr as found by electron energy loss spectroscopy (EELS) and electron spectroscopic

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imaging (ESI) suggested that Cr is accumulated mainly in the cell wall and vacuoles (Liu and Kottke, 2003).

Tianjin is a big industrial city in P.R. China. There are some disused plants there. The Cr(VI) residues and the soils in this investigation were collected at one chemical plant that had produced potassium dichromate ($K_2Cr_2O_7$); the waste heap is ~40 years old and occupies ~100 m^2. The soils next to the Cr(VI) residue heap contain 1941.82 mg/kg Cr, 417.69 mg/kg Mn, 19,254.79 mg/kg Fe, 34.00 mg/kg Cu and 819.50 mg/kg Zn, and are covered by vegetation. Most significant is the abundant occurrence of *Plantago asiatica, Phragmites australis* (Cav.) Trin., *Kochio scoparia* (L.) Schrad., *Scirpus planiculmis* F. Schmidt, *Amaranthus viridis* and other specific vegetation (Wang et al., 2002). No plants have grown on the waste heap itself, however, because of the very high Cr concentration (25,635.79 mg/kg DW).

To examine why *Amaranthus viridis* can grow well in such an environment, what forms of Cr occur in the soils and waste, and what the effects of Cr are on the distribution of Mn, Fe, Cu and Zn in plants, we made a study employing inductively coupled plasma atomic emission spectrometry (ICP-AES), resin adsorption and standard addition techniques.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF PLANT AND SOIL SAMPLES

Amaranthus viridis was harvested and the soils below its roots were collected from an uncontaminated site and a contaminated site. The samples from the contaminated site were collected ~15 m from a waste Cr(VI) heap of a disused chemical plant in Tianjin, P.R. China. The chemical plant produced potassium dichromate 40 years ago. The samples from the uncontaminated site were collected from the grounds of Tianjin Normal University. Roots, stems and leaves of *A. viridis* and the soils from both sites were dried for 3 days at 45°C, for 1 day at 80°C, and again for 12 h at 105°C in an oven, and used for element analysis. Before element analysis the soil samples were sieved (80 mesh sieve).

ANALYSIS OF TOTAL Cr AND SEVERAL MINERALS IN PLANTS AND SOILS

All the plants and soil samples were prepared using the wet-digestion method (Piper, 1942). Concentrations of Cr, Mn, Fe, Cu and Zn were analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES; LEEMAN LABS INC., New Hampshire, USA) as described by Duan (2003).

TABLE 1. Percentage of Cr(III) and Cr(VI) in total extractive Cr of contaminated soils and residues [%]

	Total extractive Cr (μg /g DW)	Cr(III) (μg /g DW)	Cr(VI) (μg /g DW)
Soils	1545.41 ±34.32 [100%]	1302.05 ±71.49 [84.25%]	243.37 ±18.60 [15.75%]
Residues	25,635.79 ±77.76]100%]	10,800.50 ±56.76 [42.13%]	14,835.29 ±57.49 [57.87%]

PREPARATION OF SOIL AND WASTE RESIDUE SAMPLES

Residues from the Cr(VI) waste heap and soil samples from the contaminated site where *Amaranthus viridis* grows were collected. The contaminated site's soils and residues underwent wetting-drying cycles at room temperature to prevent reduction and oxidization between Cr(VI) and Cr(III).

ANALYSIS OF Cr FORMS IN SOILS AND RESIDUES

To extract the total available Cr from soils or residues, we used the modified extraction method of Risser and Baker (1990) employing 10% HCl (v/v), and a modification of the resin adsorption method (Yu et al., 2004) to analyze the forms of Cr [Cr(VI) or Cr(III)] in soils and residues in solution. In determining total Cr in extracts and Cr(III) in absorbed solutions, the standard addition technique (Liu et al., 1996) was used for verification, followed by ICP-AES determinations and analysis. Standard stock solutions (0.1 M) were prepared by dissolving high-purity potassium dichromate ($K_2Cr_2O_7$) and chromium nitrate [$Cr(NO_3)_3$]. Working standards were prepared by serial dilution with deionized water, and acidified to 1% (v/v) HNO_3.

The resins (D301-T) were immersed in pure alcohol and then 1 M HCl and 1 M NaOH for 12 h each. The resins were thoroughly rinsed with deionized water before each immersion. Lastly they were rinsed with deionized water again, and then air-dried at room temperature before adsorption, as modified by Yu et al. (2004).

In brief, 10 g of soils and residues (passed through an 80 mesh sieve) were each placed in a 1000 ml flask, and 250 ml 10% HCl (v/v) was added to each flask. The flasks were then shaken at 150 rpm at 25°C for 24 h. At the end of that period, the supernatant solution was filtered and transferred to clean beakers. Nine 5 ml aliquots of soil extracts were transferred to nine 50 ml flasks. Three of them were diluted to 50 ml with deionized water. The others were diluted to 50 ml after the addition of

TABLE 2. Concentrations of several metal elements in *Amaranthus viridis* L. and soil from contaminated and uncontaminated sites [μg /g DW±SD]

Type	Element	Soil	Total amount	Root (%)	Stem (%)	Leaf (%)
Uncontaminated site	Cr	51.06±2.07a	5.34	1.71±0.93bc (32)	1.35±0.19b (25)	2.28±1.05c (43)
	Mn	369.16±4.62a	52.77	13.35±0.24b (25)	11.97±0.37c (23)	27.45±0.54d (52)
	Fe	17,219.12±227.13a	458.39	103.45±1.75b (23)	106.15±2.02b (23)	248.79±2.71c (54)
	Cu	29.85±0.95a		0.73±0.06b	0.00c	3.49±0.17d
	Zn	123.67±0.97a	125.13	39.19±1.00b (31)	34.97±1.24c (28)	50.97±4.03d (41)
Contaminated site	Cr	1941.82±22.49a	58.68	21.17±2.93b (36)	13.30±2.07b (23)	24.21±1.04b (41)
	Mn	417.69±11.70a	117.24	20.50±0.37b (18)	17.89±0.30c (15)	78.85±0.92d (67)
	Fe	19,254.79±81.33a	371.44	67.78±0.59b (18)	93.20±3.10c (25)	210.46±0.21d (57)
	Cu	34.00±0.50a		0.92±0.11b	0.00c	4.18±0.53d
	Zn	819.50±1.88a	200.37	85.46±1.28b (43)	46.91±0.30c (23)	68.00±1.47d (34)

20 ml 10 ppm standard Cr(VI) to three flasks and Cr(III) to the other three. Aliquots (5 ml) of residue extracts were transferred to nine 100 ml flasks. Three of those were diluted to 100 ml directly; three others first had 10 ml 100 ppm standard Cr(VI) added, and the other three 10 ml 100 ppm standard Cr(III). Then 10 ml from each flask (18 flasks) was transferred to clean flasks containing the prepared resins. All 36 flasks were then shaken at 150 rpm at 25°C. The concentrations of Cr in all flasks were analyzed by ICP-AES.

Each treatment was done in triplicate for statistical validity. The data were expressed as means ± standard deviation and analyzed by ANOVA using SigmaPlot 8.0 (Jandel Scientific Corporation). The test of equality of averages using the t-test was also applied. Differences were considered statistically significant at $p < 0.05$.

RESULTS

SPECIATION OF Cr

In the analytical results shown in Table 1, the total available Cr concentration in soils was found to be 1545.41 μg/g DW, and for Cr(III) it was 1302.05 μg/g DW. Total available Cr in residues was 25,635.79 μg/g DW, and Cr(III) was 10,800.50 μg/g DW. The concentrations of Cr(VI) in soils and residues were 15.75% and 57.87% of total available Cr, respectively.

CONTENT OF Cr AND OTHER METALS IN PLANT TISSUES AND SOILS FROM CONTAMINATED AND UNCONTAMINATED SITES

At both the contaminated and uncontaminated sites, *Amaranthus viridis* accumulated chromium primarily in shoots, and concentrated it in roots (Tab. 2). Cr levels in *A. viridis* were as follows: leaf > root > stem. As seen from Table 1, the Cr concentration in contaminated soil was 38 times higher than in uncontaminated soil. Accordingly, *A. viridis* growing on contaminated soil accumulated ~11 times more Cr than those growing at the uncontaminated site.

In *Amaranthus viridis* from the uncontaminated site, manganese concentrations in leaves were highest, followed by roots and stems (Tab. 2). The Mn levels in seedlings from the contaminated site showed a similar tendency, but the level of Mn in leaf as a percentage of total Mn in the plant was much higher (Tab. 2). Iron was located mainly in leaves, then in stems and roots in the seedlings from both the contaminated and uncontaminated sites (Tab. 2). Copper was not detected in stem; it accumulated mainly in leaves and slightly in roots from both the contaminated and uncontaminated sites. Zinc content in leaf and stem from the contaminated site was higher than from the uncontaminated site, and much higher in roots from the contaminated site than from the uncontaminated site (Tab. 2). Zinc levels in *A. viridis* from the uncontaminated

site followed the order leaf > root > stem, but from the contaminated site the order was root > leaf > stem (Tab. 2).

The levels of Mn, Fe, Cu and Zn in soils at the contaminated site were higher than at the uncontaminated site (Tab. 1). Accordingly, in the plants the concentrations of Mn, Cu and Zn were higher at the contaminated site than at the uncontaminated site. Iron concentrations in roots, stems and leaves were low in plants from the contaminated site, and somewhat higher in those from the uncontaminated site (Tab. 2).

DISCUSSION

The bioavailability and toxicity of Cr in the soil depends on its speciation. Chromium exists mainly in the +3 and +6 oxidation states (Zayed and Terry, 2003). The intermediate +4 and +5 states are metastable and rarely encountered. Chromium(III) is largely present in soil as relatively unavailable, insoluble oxides of Cr and Cr-Fe. The prevailing view is that the Cr(VI) form is more toxic and more mobile than the Cr(III) form. There have been conflicting reports on the uptake of Cr(III) and Cr(VI) by plant roots (Skeffington et al., 1976). However, both forms, Cr (III) and Cr (VI), are now thought to be taken up by plants. The two ions do not share a common uptake mechanism: the uptake of Cr(III) is largely a passive process, while that of Cr(VI) is considered active, and the uptake of Cr(VI) is mediated by sulfate carrier but with lower affinity (Skeffington et al., 1976).

In general, plants have a low capacity to absorb and translocate Cr (Barceló and Poschenrieder, 1997). Different vegetable crops vary in their ability to accumulate Cr in tissues (Zayed et al., 1998). In plants grown on soils after long-term application of sewage sludge containing Cr, its level in leaves seldom exceeded a few μg/g DW. Even in plants adapted to Cr-rich serpentine soils, the mean Cr content does not exceed 45 μg/g DW (Juste and Mench, 1992). However, a limited number of serpentinophytes apparently have a relatively high capacity to absorb Cr. In this investigation, *Amaranthus viridis* grown in soil with a high Cr level (1941.82 μg/g DW) accumulated only 35.72 μg Cr/g DW in shoots. The definition of hyperaccumulation has been discussed by many authors (e.g., Baker and Brooks, 1989; Baker et al., 2000, Baker and Whiting, 2002). Most have recognized standard criteria based on metal concentrations in above-ground tissues per dry biomass of plant material sampled from the natural habitat (Pollard et al., 2002). According to the results from the present investigation, *A. viridis* cannot be considered a hyperaccumulator. It did accumulate Cr, mainly in stems and leaves, but could not absorb and accumulate large amounts of Cr.

The level of Mn in leaves of *A. viridis* from the contaminated site was significantly higher ($p < 0.05$) than from the uncontaminated site, although the Mn concentration in the contaminated soil was only slightly higher than from the uncontaminated soil. This may suggest that compensatory mechanisms are engaged, promoting Mn uptake and accumulation in leaves in order to reduce Cr toxicity. Manganese ions activate several enzymes in plant cells. The most important role of Mn in green plants is in the process of splitting H_2O and releasing O_2 (Hagemeyer, 1999). Bowler et al. (1992) indicated that Mn is involved in some metalloproteins.

Iron is required for the functioning of a range of enzymes, especially those involved in oxidation and reduction processes, for synthesis of the porphyrin ring (chlorophyll and heme biosynthesis), reduction of nitrite and sulphate, N_2-fixation (as part of leghemoglobin) (Rengel, 1999). The visible toxic symptoms of Cr(VI) are superficially somewhat similar to those of Fe deficiency (Sharma et al., 1995); this is related in part to the ability of Cr to displace other metals (particularly Fe) from physiologically important centers, producing Fe deficiency (Hewitt, 1983; Pandey and Sharma, 2003). In fact, Cr has been successfully substituted into several Fe-metalloproteins such as the Fe^{3+}-carrying proteins transferrin and cytochrome P450 (Cupo and Wetterhahn, 1985). These toxic effects of Cr are further supported by our finding of restricted Fe uptake and accumulation in *A. viridis* associated with the presence of Cr at the contaminated site.

Zinc is an essential mineral element for plant nutrition and normal growth. The distribution of Zn in different organs of *A. viridis* growing at the contaminated site differed from that in plants from the uncontaminated site. The level of Zn in soil at the contaminated site was 6.63 times higher than at the uncontaminated site, and 1.60 times higher in seedlings from the contaminated site than from the uncontaminated site, indicating that Cr promotes the uptake of Zn. These results do not agree with Barceló et al.'s (1985) findings on experimental administration of Cr(VI) in bean plant growing in nutrient solution, in which inhibition of Cu, Zn and Fe translocation was observed. In our study, *A. viridis* had been exposed to Cr for a long period, and the increased level of Zn may have been the result of compensatory mechanisms to bring down Cr toxicity and increase Cr tolerance. The work of Broadley et al. (2001) indicates that phylogeny influences the trait of metal accumulation in angiosperms, and implies that traits have evolved that affect the shoot content of more than one metal. The evolution of traits influencing the shoot content of Cr, Cd and Pb has also affected the shoot content of Cu and Zn.

Chromium is involved in numerous physiological functions as a component of several plant enzymes, with minimum requirements generally in the range of 1–5 μg/g in plant tissue depending on the species (Marschner, 1995). Various interactions can occur when plants are exposed to unfavorable concentrations of Cr (Dube et al., 2003). In this investigation, however, the content of Cu in roots, stems and leaves differed little between *A. viridis* from the uncontaminated site and plants exposed to Cr for a long period at the contaminated site. Perhaps the negative effect of Cr on Cu in *A. viridis* from the contaminated site is compensated by other mechanisms or is not serious enough to be detectable.

There are reports that the presence of high levels of Cr diminish the uptake of Fe, Zn and Mn in maize (Sharma and Pant, 1994), greatly reduce the uptake of Fe, Ca, Mn, Cu, Mg and Zn in sugar beet (Terry, 1981), interfere with uptake of Ca, K, Mg, Pb, B and Cu in soybean (Turner and Rust, 1971), and decrease levels of Fe and Zn and increase Mn in bush bean (Barceló et al., 1985). Cunningham et al. (1975) found that the presence of high levels of chromium reduced Cu, Mn and Zn intake in corn.

A number of soil processes and factors may affect the form and biomobilization potential of Cr. Chromium is present in soils mostly as insoluble $Cr(OH)_3$ or as Cr(III) adsorbed to soil components, which prevents Cr leaching into groundwater or its uptake by plants (Bartlett and Kimble, 1976). Cr(VI) is readily transformed to Cr(III) as a result of reduction by Fe(II) in solution and at mineral surfaces, by sulfur compounds, or by soil organic matter under most soil conditions (Fendorf, 1995). Chromium(III) has been found to be readily absorbed by macromolecular clay compounds; also, humic acids contain donor groups forming stable Cr(III) complexes, especially when they produce chelate rings, and adsorption of Cr(III) to humic acids renders it insoluble, immobile and unreactive (James, 1996). Although Cr(III) can oxidize to Cr(VI), especially in the presence of manganese oxides, oxidation usually occurs only in moist conditions, and not appreciably in dry soils. Cr(III) can thus be expected to be the predominant form. Cr(III) is largely present in soil as relatively unavailable, insoluble oxides of Cr (Kotaś and Stasicka, 2000). This can explain why *A. viridis* can grow well and absorb and accumulate a small amount of Cr in such a contaminated environment. The lower proportion of Cr(VI) to total available Cr in the soils analyzed in the present investigation by standard addition and resin adsorption methods, accords with the findings of Kotaś and Stasicka (2000). Generally, only a very small fraction of total Cr content in soils is determined to be extractable Cr available to the plant.

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