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MYCORRHIZAL FUNGI INCREASE CHROMIUM UPTAKE BY SUNFLOWER PLANTS: INFLUENCE ON TISSUE MINERAL CONCENTRATION, GROWTH, AND GAS EXCHANGE

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ABSTRACT

As a potential phytoremediation system for phytoextraction of chromium (Cr), we evaluated the influence of the arbuscular mycorrhizal fungus *Glomus intraradices* on leaf tissue elemental composition, growth and gas exchange of sunflower (*Helianthus annuus* L.). Sunflower seedlings were either inoculated with mycorrhizal fungi (*AM*) or non-inoculated (*Non-AM*) and then exposed to two Cr species: {12 mmol of trivalent cation (Cr^{+3}) [Cr(III)] or 0.1 mmol of divalent dichromate anion (Cr_2O_7^-) [Cr(VI)]}. Both Cr species depressed plant growth, decreased stomatal conductance (g_s) and net photosynthesis (A).

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However, Cr(VI) was more toxic than Cr(III) as indicated by early reduction in g_s , and greater reduction in plant growth and arbuscule formation. Mycorrhizal fungi helped to partially alleviate Cr toxicity as indicated by greater growth in plants treated with either Cr species, and increased gas exchange of Cr(III) plants. With Cr(III), Cr concentrations were the same in tissue of *AM* and *Non-AM* plants, while *Non-AM* Cr(VI) treatments had higher Cr concentration than *AM* plants; however with both Cr species, the larger-sized *AM* plants had greater total Cr accumulation on a whole-plant basis. Chromium reduced leaf elemental nitrogen (N), phosphorus (P), and potassium (K), and increased aluminum (Al), iron (Fe), and zinc (Zn) uptake. Chromium-treated *AM* plants had higher K and decreased levels of Fe and Al than *Non-AM* plants. Except for the depression of arbuscular formation in Cr(VI) treated plants, high colonization occurred with either Cr species. While higher P may have contributed to mycorrhizal benefits with Cr(III) plants, it does not account for any *AM* benefits observed with Cr(VI) plants.

INTRODUCTION

Phytoremediation utilizes biological organisms for phytoextraction or removal of plant biomass containing concentrated levels of heavy metals taken up from polluted soils. Phytoremediation is an alternative to conventional chemical and physical methods of treating contaminated soils.^[1]

Essential heavy metals include zinc (Zn), copper (Cu), manganese (Mn), cobalt (Co), but no biological functions in plants are currently known for cadmium (Cd), lead (Pb), mercury (Hg), or chromium (Cr). Chromium is a heavy metal with risk to human health. Its presence in agricultural soils can be attributed to the use of organic wastes as fertilizer and the use of wastewater for irrigation.^[2] Sites for wood-treatment/preservation, steel processing, aluminum alloys, electroplating, and microbial growth inhibition such as cooling towers of power plants, may be contaminated with Cr. At a Department of Energy (DOE) nuclear weapons dismantling plant (Pantex) near Amarillo, Texas, there is Cr contamination of soils. Work is underway to contain and remove the contamination, thus preventing Cr from entering the perched aquifer below. Conventional means for cleaning up contaminated sites are chemical and physical removal (excavation/disposition) techniques that are quite costly. Very little data was available on Cr uptake by agronomic crops until recently.^[3]

Heavy metals are not degraded biologically in soil. In soils they occur as free metal ions, exchangeable metal ions, soluble metal complexes (sequestered

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to ligands), organically bound metals, precipitated or insoluble compounds such as oxides, carbonates and hydroxides, or they may form part of the silicate structure (indigenous soil content).^[4] Mycorrhizal fungi have greatest impact on elements with narrow diffusion zones around plant roots, including heavy metals and phosphorus (P).^[5,6] Mycorrhiza enhance the uptake of Cu,^[7] Zn,^[5,8,9] nickel (Ni),^[10] Cd,^[11,12] Pb,^[13] and other metals.^[9]

Mycorrhiza increase nutrient and water uptake, alleviate cultural and environmental stresses, and enhance disease resistance and plant health.^[14–19] An important arbuscular mycorrhizal genus is *Glomus*, which colonize a variety of host species,^[6] including sunflower.^[20]

Mycorrhizal fungi are a direct link between soil and roots, and consequently of great importance in phytoremediation—potentially enhancing heavy metal availability and plant tolerance. Little is known of the ability of mycorrhizal fungi to enhance plant tolerance or phytoaccumulation of Cr. Furthermore information is lacking on the influence of mycorrhiza on the uptake of other essential plant macro- and micronutrients in soils contaminated with Cr. It is known that Cr predominantly exists in two forms in soil: as a trivalent cation $\{(Cr^{3+}) [Cr(III)]\}$ and divalent dichromate anion $\{(Cr_2O_7^{2-}) [Cr(VI)]\}$.^[21,22,23] Chromium (III) readily precipitates in soil, whereas greater environmental pollution problems occur with the more mobile and toxic Cr(VI).^[24,25]

Hence, this research was conducted to determine the effects of *Glomus intraradices* (Schenck and Smith) in the phytoremediation of Cr using sunflower seedlings (*Helianthus annuus* L.). Sunflower is a high biomass plant with high metal accumulating ability, but low Cr tolerance compared to other agronomic crops.^[3] Objectives of this research were to determine if *G. intraradices* could enhance Cr uptake and increase plant tolerance as determined by plant development and gas exchange of container-grown sunflower seedlings. The ability of *G. intraradices* to modify Cr effects on tissue mineral concentration was also determined.

MATERIALS AND METHODS**Cultural Conditions**

This study was conducted under glasshouse conditions. Sunflower seedlings (*Helianthus annuus* L., hybrid 571 m Triumph Seeds, lot M24159) were planted into 1.7 L pots. The container medium was composed of 1 part coarse sand : 1 part sandy loam soil (v/v) with a textural analysis of 85% sand, 10% clay, and 5% silt, low organic matter (1.01%), and low nutrient levels ($1 \mu\text{g N g}^{-1}$, $3 \mu\text{g P g}^{-1}$, $27 \mu\text{g K g}^{-1}$, $547 \mu\text{g Ca g}^{-1}$, $42 \mu\text{g Mg g}^{-1}$, $3 \mu\text{g Zn g}^{-1}$,



3.5 $\mu\text{g Mn g}^{-1}$, 0.07 $\mu\text{g Cu g}^{-1}$, 3.5 $\mu\text{g Na g}^{-1}$, and 5 $\mu\text{g S g}^{-1}$). The container medium was previously steam pasteurized on two consecutive days for 5 hours per day at 80°C. Pots were irrigated as needed and fertilized twice weekly after shoots emerged using 200 mL per pot of modified Long Ashton nutrient solution [LANS (26)] to supply P at 11 $\mu\text{g P mL}^{-1}$.

The experiment was initiated on October 24, 1997 and terminated on December 12, 1997. Environmental plant culture conditions included: minimum and maximum temperatures of 26.2 and 32.7°C, respectively. Maximum photosynthetic photon flux (PPF) was 653 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Relative humidity ranged from mean low 60.1% to 98.3%.

Half the plants were not colonized (*Non-AM*) and the remainder inoculated with the mycorrhizal fungus *G. intraradices* Schenck and Smith (*AM*), which was applied at 2000 spores per plant. The inoculum was applied as a band at the bottom third of the container, then covered with soil and the roots of the sunflower seedlings were allowed to grow into the band and colonize. The inoculum was a commercial product supplied by Premier-Tech, Rivière-du-Loup, Quebec, Canada. We have used this isolate to enhance growth of other plant species.^[27]

Chromium Application

Seedlings were two weeks old at transplanting and initiating the experiment. Two weeks after transplanting, Chromium was applied weekly over a five-week period. At each Cr application approximately 200 ml soil drenches of 12 mmol of CrCl_3 $\{(\text{Cr}^{+3}) [\text{Cr(III)}]\}$ and 0.1 mmol of $\text{Na}_2\text{Cr}_2\text{O}_7$ $\{(\text{Cr}_2\text{O}_7^-) [\text{Cr(VI)}]\}$ were applied to the containerized plants. The 120-fold difference between Cr(III) and Cr(VI) was based on a preliminary study screening morphological and physiological responses. The Cr(III) is less toxic than Cr(VI), hence lower levels were used with the later.

Assessment of Plant Growth, Leaf Elemental Analysis, and Gas Exchange

Final growth measurements of ten plants per treatment ($n = 10$) were taken 50 days after transplanting, and included plant height, leaf area, leaf, flower, shoot, root and total plant dry mass (DM). The mycorrhizal inoculation effect (MIE) was calculated by the formula $\text{MIE (\%)} = (\text{total plant DM of AM} - \text{total DM of Non-AM}) (\text{total plant DM of Non-AM})^{-1} \times 100$.^[28] Leaves were analyzed for macro- and microelements with an inductively coupled atomic emission spectrophotometer (3510 ICP, J.R. Peters, Fogelsville, PA). From 10 plants per

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treatment, newly matured leaves were pooled (plants #1–#3, #4–#6, and plants #7–#9) into three composite samples for tissue analysis ($n = 3$).

Measurements were taken with a LiCor 6200 Portable Photosynthesis System (LiCor, Inc., Lincoln, NE). Data were recorded between 9:00 and 11:00 at 19 and 50 days after planting (5 and 36 days, respectively, after initiation of Cr treatments) to assess gas exchange on four plants per treatment from two newly matured leaves per plant ($n = 8$). Measurements included net photosynthesis (A) and stomatal conductance (g_s). Measurements were made with ambient CO_2 concentration at about $360 \mu\text{L L}^{-1}$ under $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PPF supplied from a 1000 W high pressure sodium vapor lamp filtered through 5 cm of water enclosed in a Plexiglas box.^[29]

Chromium Analysis

Measurements of Cr content ($\mu\text{g g}^{-1}$) were made on individual plants ($n = 3$). Plants were harvested and roots washed with running tap water. Roots, shoots, and leaves were then separated and oven dried for three days at 80°C . Samples were then ground into fine powder using a Tecator high-speed mill (Foss Tecator AB, Hoganas, Sweden).

The conditions used for digestion of dried sunflower tissues were modified from Cary and Olsen.^[30] Five milliliter of concentrated HNO_3 was added to approximately 0.25 g of dried plant sample in a 50 mL digestion tube and allowed to stand overnight at room temperature. The digestion tubes were placed in a Tecator heating block and heated to 150°C for one hour. Tubes were removed from the heating block and allowed to cool at least 15 min, and then 2 mL of 30% H_2O_2 was added to each tube. The tube contents were then mixed by swirling and then heated for 2 more hours at 150°C . After cooling, the solution was diluted to 50 mL total volume and the upper clear portion was used for Cr measurement. During dilution, NH_4Cl was added at 2% (w/v) to each sample and standard to control interference caused by Fe during spectrophotometric analysis. Similarly, CaCl_2 was added at 0.5% to overcome P interference of Cr measurement.

Digested samples were analyzed for Cr under the following manufacturer's recommended standard conditions on a Perkin Elmer 3100 EDS Atomic Absorption/Emission Spectrophotometer, using a fuel rich air-acetylene flame, 10 cm burner head, 357.9 nm wavelength, 0.7 nm slit and 20 mA lamp current. Some digests were diluted in order to fall into the linear calibration range of 0 to 5 mg L^{-1} . The mean of three replicate readings was recorded for each sample and was used for all Cr content calculations.



Assessment of Mycorrhiza Development

For *AM* analysis of roots, 1 cm root segments from 10 plants per treatment were sampled at harvest and pooled to assess colonization percentage after clearing and staining of the root samples.^[31] Ten 1 cm stained root pieces were placed on each slide and three microscopic observations per 1 cm root piece at 40X were made at the top, the middle and the bottom of each root piece. There were 10 slides per treatment ($n = 300$ observations per treatment). The presence of arbuscules, vesicles and hyphae was recorded and statistically analyzed.

Statistical Design

The experiment was a 2×3 factorial in a completely randomized design with two mycorrhizal levels (*AM*, *Non-AM*) and three levels of chromium: Cr(III), Cr(VI), and a control. There was one sunflower seedling per container with each container as a single replicate. All data were analyzed using Analysis of Variance (ANOVA).^[32]

RESULTS

Both Cr species reduced plant growth parameters (plant height, leaf area, leaf, shoot, root, and total plant DM) (Table 1). The most toxic treatment to plants was 0.1 mmol Cr(VI) which caused the greatest growth reduction. In general, Cr-treated *AM* plants had greater leaf, shoot, root, and total plant DM than *Non-AM* plants (Table 1). Plant height and leaf area were greater in Cr(VI) treated *AM* than *Non-AM* plants. High (+56%) and very high (+75%) mycorrhizal dependency occurred with Cr(III) and Cr(VI), respectively, as determined by MIE.

Within five days after the first Cr application (19 days after the experiment initiation), there was a 60% reduction in stomatal conductance (g_s) of Cr(VI) treated plants (Table 2). However net photosynthesis (A) was not initially affected (Table 2). After the initial Cr(III) treatment, g_s was higher in *AM* than *Non-AM* plants, however gas exchange was not negatively affected by cumulative Cr(III) treatments until later. By day 50 of the experiment (36 days after the initial Cr treatment), both A and g_s of Cr(III) and Cr(VI)-treated plants were drastically reduced (Table 2). At the experiment termination *AM*-inoculated Cr(III) plants had higher gas exchange than *Non-AM* plants, while *AM* did not significantly affect the gas exchange of Cr(VI) plants.



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Table 1. Effect of Chromium (Cr) and Mycorrhiza (AM) on Growth and Development of Sunflower (*H. annuus* L.) Plants

Chromium	Concentration (mmol)	AM	Plant Height (cm)	Leaf Area (cm ²)	Leaf Dry Mass (g)	Shoot Dry Mass (g)	Root Dry Mass (g)	Total Plant Dry Mass (g)	Mycorrhizal Inoculation Effect (%)
Control	0	Yes	54.6 ± 0.9 ^a	501 ± 18	1.3 ± 0.1	1.7 ± 0.1	0.8 ± 0.2	3.8 ± 0.2	36
		No	59.4 ± 1.2	332 ± 13	0.9 ± 0	1.4 ± 0.1	0.5 ± 0	2.8 ± 0.2	
Cr(III)	12	Yes	35.4 ± 2.4	121 ± 25	0.4 ± 0	0.7 ± 0.1	0.3 ± 0.2	1.4 ± 0.3	56
		No	33.4 ± 1.7	92 ± 9	0.3 ± 0	0.5 ± 0	0.1 ± 0	0.9 ± 0.1	
Cr(VI)	0.1	Yes	23.5 ± 1.1	59 ± 5	0.2 ± 0	0.3 ± 0	0.2 ± 0	0.7 ± 0.1	75
		No	21.2 ± 0.6	47 ± 3	0.1 ± 0	0.2 ± 0	0.1 ± 0	0.4 ± 0	
Significance									
Cr			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	—
AM			NS	NS	NS	NS	0.0139	0.0052	—
Cr × AM			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	—

^aMeans with standard error and ANOVA; *n* = 10.^bMIE is the mycorrhizal inoculation effect (total plant DM of AM – total DM of Non-AM) (total plant DM of Non-AM)⁻¹ × 100.

**Table 2.** Effect of Chromium (Cr) and Mycorrhiza (AM) on Net Photosynthesis (*A*) and Stomatal Conductance (*g_s*) of Sunflower (*H. annuus* L.) 5 and 36 Days After Initiation of Cr Treatment

Chromium	Concentration (mmol)	AM	Day 5		Day 36	
			<i>A</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	<i>g_s</i> ($\text{mmol m}^{-2} \text{s}^{-1}$)	<i>A</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	<i>g_s</i> ($\text{mmol m}^{-2} \text{s}^{-1}$)
Control	0	Yes	21.4 ^a	1.0 ± 0.1	29.7 ± 1.7	0.7 ± 0
		No	20.0	0.9 ± 0.1	33.4 ± 2.1	0.8 ± 0.1
Cr(III)	12	Yes	22.5	1.2 ± 0.1	16.1 ± 1.5	0.2 ± 0
		No	21.9	0.9 ± 0.1	10.2 ± 1.5	0.1 ± 0
Cr(VI)	0.1	Yes	22.8	0.4 ± 0	13.6 ± 1.2	0.1 ± 0
		No	22.2	0.3 ± 0	12.2 ± 1.4	0.1 ± 0
Significance						
Cr			NS	0.0001	0.0001	0.0001
AM			NS	0.0107	NS	NS
Cr × AM			NS	NS	0.0124	0.0357

^aMeans with standard error and ANOVA; *n* = 8.

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Both Cr species reduced leaf tissue N, P, and K, and increased Al, Fe, and Zn (Table 3). Non-Cr treated *AM* plants had greater K and lower Fe than *Non-AM* plants, while Ca, magnesium (Mg), boron (B), Cu, Mn, molybdenum (Mo), and sodium (Na) were not affected (data not presented). Tissue P was the same among non-Cr treated *AM* and *Non-AM* plants. Tissue P was only higher in *AM* plants treated with Cr(III).

Root, shoot and leaf elemental concentration ($\mu\text{g g}^{-1}$) of Cr were greater with Cr(III) than Cr(VI) plants (Table 4). Both Cr species had highest concentrations in the root and lowest in leaves. *AM* plants had similar and lower Cr tissue concentration with Cr(III) and Cr(VI) treatments, respectively (Table 4). However on a whole plant basis, with their larger biomass, *AM* plants had greater total plant Cr accumulation (total μg) than *Non-AM* plants (Table 5).

No colonization occurred with non-inoculated plants, so the data are not presented. The Cr levels used had no effect on hyphae, vesicles or total colonization. The only *AM* depression occurred with Cr(VI) plants which had a reduction in root arbuscule formation (Table 6). Nonetheless, high colonization levels of 72% and 83% were achieved, respectively, in Cr(III) and Cr(VI) treated plants.

DISCUSSION

This is one of the first reports on the influence of *AM* on Cr accumulation and tissue concentration of other essential plant macro- and micronutrients of sunflower plants grown in soil contaminated with Cr. This study shows the potential importance of mycorrhizal sunflower plants in the phytoremediation of Cr. While sunflower has high biomass production and will hyperaccumulate Cr, it also has a low Cr tolerance compared to other agronomic species.^[3] To enhance the phytoextraction of Cr, we used a low P fertility level ($11 \mu\text{g P mL}^{-1}$) to allow greater Cr uptake. Phosphate (H_2PO_4^-) will inhibit CrO_4^{2-} adsorption in equilibrated soil suspensions.^[33-36] Thus, P and Cr are competitive species for surface sites. The utilization of *AM* to colonize a Cr-hyperaccumulating host plants has obvious advantages. Compared to *Non-AM*, *AM* plants can increase uptake of less soluble metals, help alleviate nutritional stress, including P-stress, and enhance resistance to disease, drought, and other abiotic stresses encountered in Cr phytoremediation sites.

Both Cr species depressed plant growth, decreased stomatal conductance (g_s) and net photosynthesis (A); however, Cr(VI) was more toxic. Within five days after the first Cr application, there was a rapid drop in g_s of Cr(VI) treated plants. Mycorrhizal fungi helped to partially alleviate Cr toxicity as indicated by greater plant growth, and increased g_s and A of Cr(III) plants. While non-Cr treated *AM*

**Table 3.** Effect of Chromium (Cr) and Mycorrhiza (AM) on Leaf Mineral Concentration of Sunflower (*H. annuus* L.)

Chromium	Concentration (mmol)	AM	Nitrogen (g kg ⁻¹)	Phosphorus (g kg ⁻¹)	Potassium (g kg ⁻¹)	Aluminum (μg g ⁻¹)	Iron (μg g ⁻¹)	Zinc (μg g ⁻¹)
Control	0	Yes	55 ± 2 ^{a, b}	3.0 ± 0.2	62 ± 1	27 ± 2	79 ± 2	146 ± 8
		No	53 ± 1	2.6 ± 0.2	55 ± 1	32 ± 3	88 ± 2	144 ± 7
Cr(III)	12	Yes	40 ± 1	1.5 ± 0.1	55 ± 2	65 ± 5	95 ± 4	261 ± 18
		No	38 ± 1	0.9 ± 0	49 ± 1	147 ± 56	120 ± 20	315 ± 17
Cr(VI)	0.1	Yes	47 ± 2	1.6 ± 0.1	50 ± 2	83 ± 4	93 ± 4	323 ± 17
		No	48 ± 2	1.8 ± 0.1	45 ± 2	163 ± 12	126 ± 5	289 ± 16
Significance								
Cr			0.0001	0.0001	0.0001	0.0037	0.0670	0.0001
AM			NS	0.0170	0.0001	0.0118	0.0302	NS
Cr × AM			NS	0.0085	NS	NS	NS	0.0065

^aMeans with standard error and ANOVA.^bLeaves from were pooled from three plants as one replication, with three replications per treatment $n = 3$.



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Table 4. Effect of Chromium (Cr) and Mycorrhiza (*AM*) on Cr Concentration in Plant Tissues of Sunflower (*H. annuus* L.)

Chromium	Concentration (mmol)	<i>AM</i>	Concentration of Chromium		
			Root ($\mu\text{g Cr g}^{-1}$ DM)	Shoots ($\mu\text{g Cr g}^{-1}$ DM)	Leaves ($\mu\text{g Cr g}^{-1}$ DM)
Control	0	Yes	48 \pm 13 ^a	0 \pm 0	0 \pm 0
		No	20 \pm 5	0 \pm 0	0 \pm 0
Cr(III)	12	Yes	4,051 \pm 471	2,336 \pm 687	135 \pm 10
		No	5,392 \pm 1,397	2,404 \pm 564	132 \pm 20
Cr(VI)	0.1	Yes	447 \pm 87	75 \pm 4	50 \pm 2
		No	818 \pm 52	98 \pm 16	67 \pm 9
Significance					
Cr			0.0001	0.0001	0.0001
<i>AM</i>			NS	NS	NS
Cr \times <i>AM</i>			0.0001	0.0004	0.0001

^aMeans with standard error and ANOVA; $n = 3$ with 3 to 4 plants pooled per replication.

sunflower plants had greater growth than *Non-AM* plants, the MIE was low. However, with Cr(III) and Cr(IV) treatments the MIE greatly increased, i.e., the plants became, respectively, highly and very highly mycorrhizal dependent.^[28] The 1.6-fold and 2.1-fold increase of MIE in Cr-treated plants suggests that *AM* were enhancing more than just plant growth.

High levels of Cr reduced leaf tissue P levels. This is explained in part because P and Cr are competitive species, as mentioned previously.^[23,33,34,36] Tissue P was the same among non-Cr treated *AM* and *Non-AM* plants. However, though P concentration was similar ($0.30 \pm 0.02\%$ vs. $0.26 \pm 0.02\%$) between *AM* and *Non-AM* plants it does not necessarily mean that P was not involved in growth enhancement. Sometime leaves are very conservative in their P concentration. When more P becomes available, plants produce greater leaf area and thus maintain the same tissue concentration levels.^[37] Furthermore, there was greater total plant acquisition of P since the root and total plant biomass were greater with *AM* than *Non-AM* plants. The critical range for leaf tissue P in sunflower is 2.2 to 2.5 g P kg⁻¹, whereas concentrations from 1.5 to 2.0 g P kg⁻¹ are considered marginal.^[38] Hence while non-Cr treated sunflower plants were P adequate (2.6 to 3.0 g P kg⁻¹), Cr reduced tissue P to marginal levels (1.8 to 0.9 g P kg⁻¹). While *AM* increased tissue P levels in Cr(III) plants (which in part may explain *AM* enhancement of growth and gas exchange), tissue P levels were

**Table 5.** Effect of Chromium (Cr) and Mycorrhiza (AM) on Total Cr Uptake in Plant Tissues of Sunflower (*H. annuus* L.)

Chromium	Concentration (mmol)	AM	Accumulation of Chromium			
			Root ($\mu\text{g Chromium}$)	Shoots ($\mu\text{g Chromium}$)	Leaves ($\mu\text{g Chromium}$)	Total Plant ($\mu\text{g Chromium}$)
Control	0	Yes	38 ± 11^a	0 ± 0	0 ± 0	38 ± 11
		No	10 ± 3	0 ± 0	0 ± 0	10 ± 3
Cr(III)	12	Yes	$1,323 \pm 322$	$1,519 \pm 293$	5 ± 5	$2,897 \pm 382$
		No	556 ± 93	$1,356 \pm 288$	38 ± 5	$1,950 \pm 325$
Cr(VI)	0.1	Yes	99 ± 8	22 ± 2	10 ± 1	131 ± 8
		No	70 ± 3	20 ± 4	7 ± 2	97 ± 12
Significance						
Cr			0.0003	0.0001	0.0001	0.0001
AM			NS	NS	NS	0.0001
Cr \times AM			0.0001	0.0001	0.0001	0.0001

^aMeans with standard error and ANOVA; $n = 3$ with 3 to 4 plants pooled per replication.



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Table 6. Effect of Chromium (Cr) on % Arbuscules, Hyphae, and Vesicles in Root Cortical Cells of Mycorrhizal Sunflower (*H. annuus* L.)

Chromium	Concentration (mM)	Arbuscules ^a (%)	Hyphae (%)	Vesicles (%)	Total Colonization (%)
Control	0	26.0 ± 4.7	68.7	34.7	68.7
Cr(III)	12	21.7 ± 6.2	72.0	41.3	72.0
Cr(VI)	0.1	7.5 ± 1.4	82.3	30.3	82.7
Significance ($P \leq$)		0.041	NS	NS	NS

^aData represents a mean of 300 observations ($n = 300$) with standard error and ANOVA.

still depressed (deficient), which is reflected in lower gas exchange and plant growth compared to non-Cr treated plants.

Both Cr species reduced leaf tissue N and K, and increased Al, Fe, and Zn. Critical N levels in sunflower are around 48 g N kg^{-1} .^[38] While Cr depressed N levels, only Cr(III) plants were deficient. The lower N levels in Cr(III) relative to Cr(VI) plants may be due to the dilution effect, because Cr(III) plants had a larger biomass than Cr(VI).^[39] AM did not enhance tissue N. While Cr reduced K levels, all treatments were well above the critical level of K (26 g K kg^{-1}) for sunflower. The levels of Zn in all treatments were within levels are considered adequate (40 to $100 \text{ } \mu\text{g g}^{-1}$).^[38] AM plants had lower Fe than *Non-AM* sunflower plants. There are reports that Fe- and P-deficient plants can better transport Cr from roots to shoots.^[24,40] Iron- and P-deficiency induce accumulation of organic acids (i.e. citric acid),^[41] which may play an important role in Cr translocation. However, while Cr(III) *Non-AM* sunflower plants had marginal P concentrations, there was no difference in tissue Cr concentration and total Cr accumulation (roots, leaves, total plant) was less than in AM plants. Mycorrhiza can increase P uptake by sunflower.^[20] In a non-mycorrhizal study of Cr in tomato plants, there was a nonsignificant trend of decreased leaf P at higher Cr levels.^[42]

This is one of the first reports of Cr reducing plant g_s and A . Chromium is thought to alter plant membrane systems, but the physiological and metabolic responses are not well understood. The primary toxic effect seems to be membrane damage because of the high oxidative potential of Cr(VI). It has been suggested that Cr is retained in vacuoles and cell walls of roots and that Cr reaching leaves may be principally from Cr(III) retained in the cell wall, but mechanisms of Cr hyperaccumulation are not well known.^[43] Chromium is reported to have a direct effect on roots and an indirect effect on leaves,^[43] Cr(VI) acts principally on plant roots causing intense growth inhibition. Chromium



damage to root systems could account for lower g_s and A . Stomatal conductance had greater sensitivity to Cr-stress (7- to 8-fold reduction) than A (a 2- to 3-fold reduction) with both Cr species at the termination of the experiment. Chromium toxicity has been reported to cause leaf chlorosis in upper leaves, which we also observed in sunflower. This can lead to reduced gas exchange.

Photosynthesis in sunflower can also be diminished by both sub- and supra-optimal P nutrition.^[44] In this study, the non-Cr treated *AM* and *Non-AM* sunflower plants had similar P, g_s and A . The A observed with non-Cr stressed plants was in the same range reported for sunflower under optimal conditions.^[45] However, sunflower plants treated with both Cr species had lower tissue P and subsequently lower g_s and A by the end of the experiment. While *AM* plants treated with Cr(III) had greater leaf tissue P and subsequently higher gas exchange than *Non-AM* treatment, *AM* were ineffective in enhancing gas exchange in Cr(VI) treatments—which is explained in part by comparably deficient P levels between *Non-AM* and *AM* plants.

Only Cr(VI) treated *AM* plants had reduced arbuscule formation. None of the Cr treatments caused significant depression of vesicle and hyphae formation, and total colonization remained high. The reduction of arbuscule formation with the more toxic Cr(VI) treatment is significant in that arbuscules in the root cortical cells are important sites for ion exchange between mycorrhizal hyphae and the host plant. Decreased arbuscule formation is also correlated with less active *AM* symbiosis.^[37]

Chromium concentration was greatest in the roots, intermediate in shoots and lowest in leaves. Chromium is predominately accumulated in plant roots,^[24] and in *Thlaspi sp.* was reported to be immobilized in the roots.^[46] In a non-mycorrhizal study of tomato plants, the transport of Cr(III) from the roots to the aerial parts of the plant was very low, with high Cr concentration in roots, and little in the aerial portions.^[42] No transport from the roots to tomato fruits was detected. However, in sunflower significant amounts of Cr accumulated in shoots and leaves. Greater phytoextraction would occur if roots were harvested in field sites, i.e., utilizing potato digging equipment or other modified harvesters.

Tissue elemental levels of Cr were greater with Cr(III) than Cr(VI) plants; in part this may be because of the 120-fold greater level of Cr(III) applied. Despite the lower tissue level, the more soluble Cr(VI) caused greater stress and reduced plant growth than Cr(III). The 60% reduction in g_s in Cr(VI) plants within the first five days of treatment could also have contributed to lower final Cr uptake. The low solubility of Cr(III) plus strong retention on soil surfaces limits its bioavailability and mobility in soil and water.^[23] Greater phytoextraction of Cr(VI) than Cr(III) was reported in a non-mycorrhizal study of *Brassica juncea* (L.) Czern and *Thlaspi caerulescens*.^[1] Chromium is toxic for agronomic plants at about 0.5 to 5.0 $\mu\text{g mL}^{-1}$ in nutrient solution and 5 to 100 $\mu\text{g g}^{-1}$ in soil.^[47]

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AM sunflower plants had greater Cr-accumulating ability than *Non-AM* plants. While Cr concentration was not greater with *AM* plants (Table 4), on a whole plant basis the larger biomass *AM* plants had greater Cr accumulation than *Non-AM* plants (Table 5). The Cr(VI) is a strong oxidant that is easily reduced to Cr(III) and other intermediate states.^[25] For plants to hyperaccumulate Cr(III) from soil, plants have to be efficient in solubilizing Cr in soil, efficient in the absorption of soluble Cr, translocation and compartmentalization of absorbed Cr within the plant to minimize toxicity. Hence, the advantage of utilizing mycorrhizal systems.

Since *AM* can occur in sites contaminated with Zn, Pb, and Cd there may be metal tolerance by these fungi.^[48,49] Extraradical hyphae of mycorrhiza can transport Cd from soil to plants, but the transfer from fungus to plant is restricted due to fungal immobilization.^[12] Mycorrhizal contribution to metal tolerance of host plants is poorly documented.^[4] While metal tolerant mycorrhizal plants can grow in heavily polluted soil, the extent *AM* contribute to plant metal tolerance via accumulation of heavy metals in roots and limited translocation to shoots is uncertain.^[49] Within the mycorrhizal root, the mechanisms of tolerance or of alleviation of metal stress by *AM* have yet to be separated from plant tolerance. There are also differences in efficiency among *AM* species on plant metal uptake, such as with P, Zn, and Mn.

In conclusion, this study shows the benefit of the mycorrhizal sunflower system in phytoremediation of Cr. *AM* enhanced plant accumulation and tolerance to Cr. Most of the metal-accumulating wild plants are relatively small in size and have slow growth rates—thus their potential for phytoextraction is limited.^[1] The optimum plant-mycorrhizal system for phytoextraction of Cr should not only be able to tolerate and accumulate high levels of Cr in harvestable parts but also have a rapid growth rate and the potential to produce high biomass in the field. The high biomass, rapid-growing sunflower-*Glomus* association offers excellent opportunities for phytoextraction. While we used a commercially available mycorrhizal isolate, there are options for isolating and selecting mycorrhiza from Cr-contaminated sites.

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