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Toxicity assessment of soil amended with tannery sludge, trivalent chromium and hexavalent chromium, using wheat, oat and sorghum plants

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ABSTRACT

This work assessed the effect of soil amended with tannery sludge (0, 500, 1000, 2000, 4000 and 8000 mg Cr kg^{-1} soil), Cr^{3+} as $CrCl_3 \cdot 6H_2O$ (0, 100, 250, 500, 1000 and 2000 mg Cr kg^{-1} soil), and Cr^{6+} as $K_2Cr_2O_7$ (0, 25, 50, 100, 200 and 500 mg Cr kg^{-1} soil) on wheat, oat and sorghum plants.

Seed germination, seedling growth (root and shoot) and Cr accumulation in dry tissue were measured. Toxicological parameters; medium effective concentration, no observed adverse effect concentration and low observed adverse effect concentration were determined. Root growth was the most sensitive assessment of Cr toxicity ($P < 0.05$). There was a significant correlation ($P < 0.0001$) between Cr accumulation in dry tissue and toxic effects on seedling growth. The three Cr sources had different accumulation and mobility patterns; tannery sludge was less toxic for all three plant species, followed by $CrCl_3 \cdot 6H_2O$ and $K_2Cr_2O_7$.

1. Introduction

Anthropogenic chromium (Cr) sources contribute greatly to current Cr pollution in the environment. Cr production has significantly increased since the 1950s and in 2000 was estimated as 105.4 million tons [1].

Cr used in several industrial processes has attained wide public and regulatory attention because of toxicity to environmental ecosystems in certain oxidation states. Cr oxidation states vary between −2 and +6, but only the +3 and +6 states are stable under commonly observed environmental conditions [2,3]. Hexavalent chromium (Cr^{6+}) is a known human carcinogen and is mobile, whereas trivalent chromium (Cr^{3+}) is comparatively less toxic and relatively immobile [3,4].

The leather industry contributes 40% of total industrial Cr-pollution [5,6]. The worldwide Cr-contamination of soils has arisen predominantly from the common practice of land-based disposal of tannery wastes under the assumption that the dominant species in the waste is the thermodynamically stable Cr^{3+}. However, significant levels of toxic Cr^{6+} could be detected in surface water and ground water in India, China and Australia [3]. In polluted soils in India total Cr levels as high as 30,000 mg kg^{-1} were reached [7].

The US Environmental Protection Agency (US-EPA), the US Food and Drug Administration and the Organization for Economic Cooperation and Development require phytotoxicity testing of new chemical products [8]. Recently, there has been increasing demand for assessing the ecological risks of soil contamination, using phytotoxicity tests as important tools for risk assessment and environmental monitoring of Cr-polluted soils [9].

Phytotoxicity tests generally use toxicological endpoints such as seedling growth, biomass production, and percent of seed germination [10]. There is increasing interest in using other measurement parameters based on physiological and biochemical biomarkers, such as photosynthesis, chlorophyll fluorescence and enzymatic activities in plant tissues [10–12].

The US-EPA [13] recommends several plants as biomarkers for toxicity assessment in terrestrial and aquatic ecosystems. These plants include tomato, cucumber, lettuce, soybean, cabbage, oat, perennial ryegrass, onion, carrot and maize. Other species of economic or ecological importance to the region of impact may also be appropriate for testing.

In León Valley, Guanajuato, México, wastewater is an important resource for farmers because it is a reliable source in water-scarce conditions, and represents a large part of their water supply, especially during the dry season [14]. Wastewater (including tannery effluents) and tannery sludge provide nutrients for crops, avoiding the need to purchase commercial fertilizers. For such reasons, the risk associated with the consumption of Cr-contaminated crops by cattle and humans, emphasizes the importance of studies using

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plants of agronomic interest. Crops' nutritional requirements and culture conditions are better known than those for plant that are Cr-hyperaccumulators. The aim of this work was to determine the effect of tannery sludge and the main environmental Cr species (Cr^{3+} and Cr^{6+}) on plant species of agronomic interest, such as wheat, oat and sorghum.

2. Materials and methods

2.1. Test soil, tannery sludge, chemicals and test seeds

For toxicity assays a Cr-free garden soil was used. Soil was air-dried at room temperature and sieved through a 2-mm mesh. The results of soil analysis were: pH 6.7, cation exchange capacity 32.5 cmol kg^{-1}, total N 0.51% (w/w), total C 7.04% (w/w), sand 84%, silt 12% and clay 4%. Tannery sludge was collected from a leather industry in León Guanajuato, México; sludge analysis showed pH 8.65, total N 0.76% (w/w), total C 7.62% (w/w) and Cr 65 016 mg kg^{-1} dry weight. $CrCl_3{\cdot}6H_2O$ (Sigma–Aldrich CAS No. 10060-12-5) and $K_2Cr_2O_7$ (J.T. Baker CAS No. 7778-50-9) were used as Cr^{3+} and Cr^{6+} sources, respectively.

Oat (*Avena sativa*) and forage sorghum-sudan grass (*Sorghum bicolor* × *Sorghum sudanense*) seeds were purchased in an agricultural store. Wheat (*Triticum aestivum*) seeds were kindly provided by Centro Internacional del Mejoramiento del Maíz y Trigo (México). Seeds were selected for uniformity and damaged ones discarded. Germination tests using control soil (no Cr added) had nearly 90% seed germination.

2.2. Experimental setup

Seeds were disinfected in 0.1% $HgCl_2$ for 2 min to avoid fungal contamination; later they were washed three times with deionized water prior to use [15]. Tests were conducted in 24-well cell culture plates [16]. One seed was placed inside each well and covered with 1.5 g of soil amended with tannery sludge or Cr source. Soil was adjusted to 40% moisture content [8]. At each concentration, 96 seeds were sown and a control with no Cr added was established. Tests were conducted for 7 days in a growth chamber (CAMCRE-11520-SEV) at 28 ± 2 °C and 60% relative humidity. Seeds were incubated in darkness until 65% of the control seed germinated and roots were at least 20 mm long [13]. Then 16 h/8 h light/darkness photoperiod was applied, using 30 W cold-light lamps (1600 Lux). Deionized water was added as necessary to maintain soil surface moisture.

There were three different treatments: adding tannery sludge, $CrCl_3{\cdot}6H_2O$ (starting from a 20,000-mg L^{-1} Cr solution) for Cr^{3+} and $K_2Cr_2O_7$ (starting from a 10,000-mg L^{-1} Cr solution) for Cr^{6+}. Several concentrations were assayed until the highest concentration tested resulted in >50% reduction in seedling growth. In the definitive test, seeds were exposed to six concentrations to determine the medium effective concentration (EC_{50}). The concentrations on a dry-soil basis were: 0, 100, 250, 500, 1000 and 2000 mg kg^{-1} soil for Cr^{3+} (an additional 4000 mg kg^{-1} soil Cr^{3+} concentration was used for oats); and 0, 25, 50, 100, 200 and 500 mg kg^{-1} soil for Cr^{6+}. Soil was amended with 0, 7.70, 15.40, 30.80, 61.60 and 123.20 g tannery sludge kg^{-1}. Representative samples were successively digested with three volumes of HNO_3 and analyzed with the 7000A EPA-Method [17] to obtain concentrations of 0, 500, 1000, 2000, 4000 and 8000 mg Cr kg^{-1} soil. There were four replications for each concentration.

Seed germination, root and shoot length, and Cr accumulation in dry tissue were measured. The bioaccumulation factors (BAFs) soil–root and root–shoot were defined as the Cr concentration in roots divided by Cr concentration in soil, and Cr concentration in shoots divided by Cr concentration in roots, respectively [8].

2.3. Cr determination

For total Cr determination, seedlings were thoroughly washed with deionized water to remove adhered soil. Once separated into roots and shoots, seedlings were oven-dried at 70 °C for 48 h [8]. The dried, milled and weighed vegetable material was digested with HCl:HNO_3 2:1 (v/v) in a digestor (DigiPrep). A blank without sample was used, having only the addition of the acid mixture. The digested samples were analyzed by atomic absorption spectrometry (GBC-Avanta) using the 7000A EPA-Method [17].

To ensure precision and accuracy of metal analyses, every 20 samples analyzed, a known-concentration standard was included.

2.4. Statistical analysis

An analysis of variance (ANOVA) was used to determine significant differences among treatments. Dunnett's multiple comparison method was used to calculate the minimum significant differences between control and treatment means [8]. Through Dunnett's test the toxicological parameters No Observed Adverse Effect Concentration (NOAEC) and Low Observed Adverse Effect Concentration (LOAEC) were calculated for seed germination and seedling growth. EC_{50} (Medium Effective Concentration) was determined by expressing root and shoot growth as percentages of controls by the Spearman–Karber Method [18] and the Spearman–Karber Method Software [19]. To establish the relationships between phytotoxicity and dry-tissue Cr accumulation a Pearson's correlation analysis was carried out. Statistical analyses were performed with the SAS System Software ver. 6.12 [20] at $P < 0.05$.

3. Results and discussion

3.1. Seed germination

Germination of wheat, oat and sorghum seeds exposed to tannery sludge Cr are presented as percentage of control means (Fig. 1a). In tannery sludge treatments wheat germination was affected at ≥4000 mg Cr kg^{-1}, the minimum concentration of any adverse effect (LOAEC) using Dunnett's multiple comparison ($P < 0.05$). For oat and sorghum, LOAEC was 2000 mg Cr kg^{-1} soil. Oat seed germination was severely diminished (84% respect to control) at 4000 mg Cr kg^{-1} soil, and at 8000 mg Cr kg^{-1} both oat and sorghum seed germination was suppressed.

Sorghum and wheat germination was markedly affected at 500–1000 mg Cr^{3+} kg^{-1} soil, respectively (Fig. 1b). Cr^{3+} had no significant effect on oat germination below 4000 mg kg^{-1} soil. Cr^{6+} significantly affected wheat and sorghum germination at the maximum concentration of 500 mg kg^{-1} soil (Fig. 1c). Oat germination did not differ significantly between Cr^{6+} treatments and the control (no Cr added).

Results indicate that Cr toxicity is dependent on the plant and the Cr source. Thus, seed germination may not be a sensitive test for evaluating toxicity, because at most of the Cr levels there were no toxic effects, at least for tannery sludge Cr and Cr^{6+} treatments. Previously, seed germination was found to be resistant to cadmium toxicity at most levels used [8], suggesting that germination is insufficiently sensitive to assess toxicity.

There are several literature reports demonstrating that low Cr concentrations do not significantly affect seed germination. Cr^{6+} added as 0.5 mmol L^{-1} $K_2Cr_2O_7$ did not affect proportion of pea seed germination [21]. However, 40 mg kg^{-1} Cr^{6+} as $K_2Cr_2O_7$ reduced alfalfa seed germination by 50% [22]. Seed germination

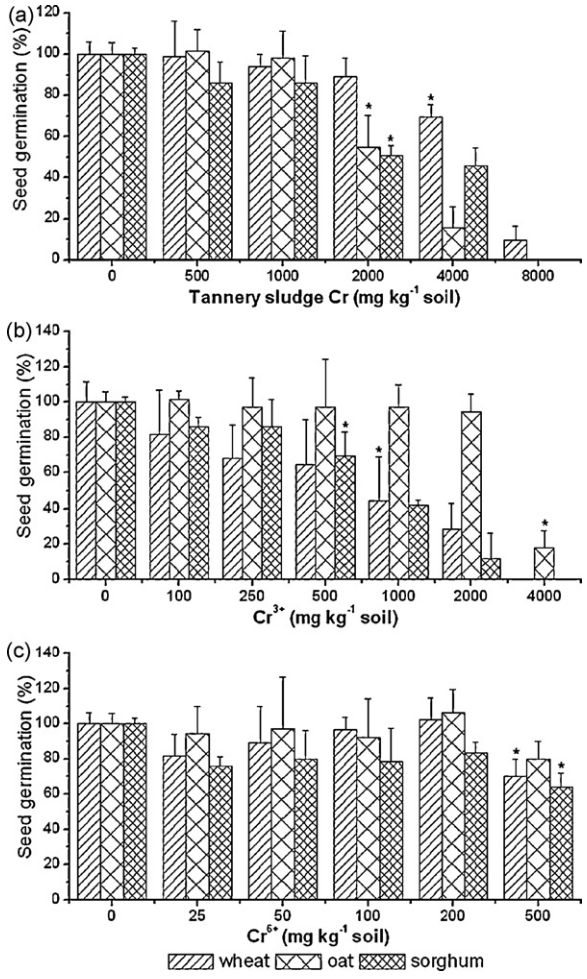

Fig. 1. Germination of wheat, oat and sorghum seeds exposed to (a) tannery sludge, (b) Cr^{3+}, and (c) Cr^{6+} for 7 days. Values are percentages of control mean (no Cr added). Bars represent standard deviations of four replicates. Asterisks represent LOAEC.

and growth of beans were substantially affected at high levels of Cr^{6+} in soil (500 mg kg^{-1}) [23].

The reduced seed germination under Cr stress could be a depressive effect of Cr on the activity of amylases and subsequent transport of sugars to the embryo axes [24]. On the other hand, protease activity increases with the Cr treatment, which could also contribute to reducing germination of Cr-treated seeds [24].

3.2. Root and shoot growth

In tannery sludge treatments the root and shoot lengths of wheat, oat and sorghum decreased as Cr concentrations increased (Fig. 2a and b). The root lengths of three plant species were reduced by 50% compared to controls (no Cr added) at nearly 4000 mg Cr kg^{-1} soil, corresponding to EC_{50} (Table 1). In wheat plants, root and shoot growth were similarly sensitive (i.e. there were equivalent values of NOAEC, LOAEC and EC_{50}). A toxic effect of tannery sludge Cr on root growth could have directly affected shoot growth considering there was no Cr translocation from roots to shoots (Table 2). Oat and sorghum shoot growth was more sensitive to Cr than root growth. Oat shoot growth was the most affected, starting from LOAEC of 500 mg Cr kg^{-1} soil.

As for tannery sludge treatments, root and shoot lengths of wheat and sorghum seedlings (Fig. 2c and d) decreased as Cr^{3+} concentrations increased. Nevertheless, in oat plants the toxic effects were barely notorious at most high Cr levels, so that including an additional 4000 mg Cr^{3+} kg^{-1} soil concentration was necessary to establish the EC_{50}. Thus, oat seedling growth was the most resistant to Cr^{3+} toxicity compared to wheat and sorghum (Table 1).

Even though Cr^{3+} is reported as the less mobile Cr specie, plants are impaired in a concentration and a time-dependent manner, as well as by the Cr^{3+} application medium. $CrCl_3$ caused drastic inhibition of root growth of celery plants at concentrations of 0.01–1 mmol L^{-1}, and root length progressively decreased as Cr^{3+} concentrations increased [25].

Seedling growth in Cr^{6+} treatments (Fig. 2e and f) showed different behavior to tannery sludge and Cr^{3+}. For the three plants, root and shoot length did not proportionally decrease as Cr^{6+} concentrations increased. For wheat root growth the LOAEC was 25 mg Cr^{6+} kg^{-1} soil (Table 1), however, at 50 mg Cr^{6+} kg^{-1} soil there was no significant differences to controls. In fact, at this concentration wheat shoot length was ca. 23% greater than controls and at 100 mg Cr^{6+} kg^{-1} soil there was a 20% stimulatory effect on shoot growth. Peralta et al. [22] also found stimulating effects with applied $K_2Cr_2O_7$; the roots of alfalfa plants exposed to 5 mg kg^{-1} of Cr^{6+} grew more than control lengths by 166%. Roots of oat and sorghum plants were affected by >100 mg Cr^{6+} kg^{-1} soil. There was a deleterious effect at the highest Cr^{6+} concentration (500 mg kg^{-1} soil) on shoot growth of wheat and oats.

Contrary to treatments with other Cr sources, roots in Cr^{6+} were much more sensitive than shoots, with a difference in EC_{50} of more than twice in wheat and sorghum. This could be due to Cr first accumulating in roots, so that root growth inhibition is the first effect of the heavy metal's presence in plants [10]. Wong and Bradshaw [26] reported that for several metals, shoots continued to grow after root growth had ceased. In the present study leaves of wheat exposed to Cr^{6+} had chlorotic lesions appearing from 50 mg kg^{-1}. There was no chlorosis in oat and sorghum leaves at any Cr^{6+} concentration. Sharma et al. [27] observed chlorosis in wheat leaves at low Cr^{6+} concentrations.

According to EC_{50} values (Table 1), Cr exerted the following toxicity order to the three plant species: Cr^{6+} > Cr^{3+} > tannery sludge, as it was necessary higher concentrations of Cr-tannery sludge than Cr^{3+} and Cr^{6+}, to diminish more than 50% the root and shoot length of the plants.

The predominant Cr species in tannery sludge is Cr^{3+} [3], for which reason similar behavior could be expected as for Cr^{3+} in the $CrCl_3 \cdot 6H_2O$ treatments. However, Cr in tannery sludge is predominantly adsorbed in its organic fraction and also in the organic fraction of soils amended with the sludge [3]. Likewise, Gupta and Sinha [28] found that most Cr was present in the carbonate and residual fractions of soil amended with tannery sludge, which can diminish Cr availability for plants. Differences in toxicity levels (NOAEC, LOAEC and EC_{50}) of $K_2Cr_2O_7$ (Cr^{6+}), $CrCl_3 \cdot 6H_2O$ (Cr^{3+}) and tannery sludge can be explained by Cr mobility and bioavailability in soils.

3.3. Cr accumulation and transport

In treatments with different Cr sources and for all three species (Tables 2–4), the Cr accumulation was mainly in roots. Generally, Cr accumulates more in roots than shoots [3]. Singh et al. [29] applied tannery sludge to tomato plants and found that Cr mainly accumulated in roots. For wheat and sorghum seedlings with the three Cr treatments the increases in Cr accumulation in dry root tissue was dependent on Cr concentrations in soil.

Mishra et al. [30] found greater accumulation in rice roots than in plant aerial parts for both Cr^{3+} and Cr^{6+}; accumulation in plant tissue was also dependent on Cr concentration in soil. In tannery

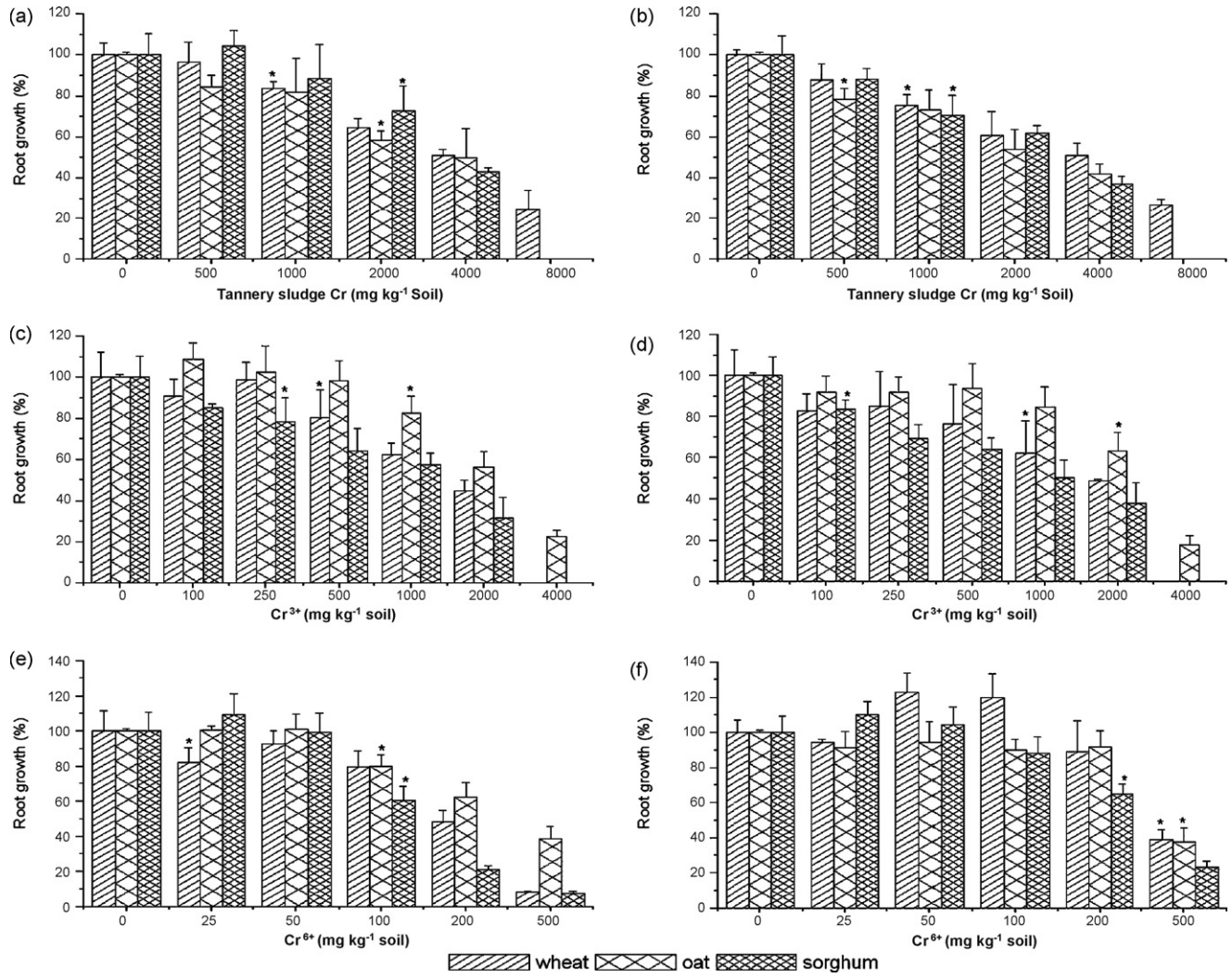

Fig. 2. Growth of wheat, oat and sorghum seedlings exposed to (a, b) tannery sludge, (c, d) Cr^{3+}, and (e, f) Cr^{6+} for 7 days. Values are percentages of control mean (no Cr added). Bars represent standard deviations of four replicates. Asterisks represent LOAEC.

sludge treatments the least Cr accumulation was in oat. At root level more Cr was accumulated in wheat than in sorghum. In spite of this, Cr translocation from root to shoot only occurred in sorghum seedlings starting from 1000 mg Cr kg^{-1} soil.

For Cr^{3+} and Cr^{6+} treatments, there was important Cr translocation from root to aerial parts of the three species. As shown in Tables 1–3, the highest soil–root BAFs were for Cr^{6+} treatments, followed by Cr^{3+} and then tannery sludge Cr. This indicates that

Table 1
Toxicity of three different Cr sources in wheat, oat and sorghum seedlings

Cr source	NOAEC (mg kg^{-1}) DW		LOAEC (mg kg^{-1}) DW		EC_{50} (mg kg^{-1}) DW	
	Root	Shoot	Root	Shoot	Root	Shoot
Wheat						
Tannery sludge	500	500	1000	1000	3595.81	3643.23
Cr^{3+} ($CrCl_3{\cdot}6H_2O$)	250	500	500	1000	1631.14	1811.41
Cr^{6+} ($K_2Cr_2O_7$)	<25	200	25	500	186.86	408.72
Oat						
Tannery sludge	1000	<500	2000	500	4000.00	2546.80
Cr^{3+} ($CrCl_3{\cdot}6H_2O$)	500	1000	1000	2000	2216.84	2334.91
Cr^{6+} ($K_2Cr_2O_7$)	50	200	100	500	316.23	405.52
Sorghum						
Tannery sludge	1000	500	2000	1000	3402.67	2786.84
Cr^{3+} ($CrCl_3{\cdot}6H_2O$)	100	<100	250	100	1089.01	1025.06
Cr^{6+} ($K_2Cr_2O_7$)	50	100	100	200	126.12	274.37

NOAEC and LOAEC were calculated by Dunnett's multiple comparison method. Spearman–Karber Method (Hamilton, 1977) and Spearman–Karber Method Software [13] at $P < 0.05$ were used to determine EC_{50}. DW, dry weight.

Table 2
Cr accumulation and transport in wheat seedlings

Cr source	Cr soil concentration (mg kg^{-1})	Total Cr root (μg g^{-1}) DW	Total Cr shoot (μg g^{-1}) DW	BAF (soil–root)	BAF (root–shoot)
Tannery sludge	0	Nd	Nd		
	500	6.96 ± 3.32	Nd	0.014	
	1000	17.26 ± 2.75	Nd	0.017	
	2000	28.15 ± 3.41	Nd	0.014	
	4000	37.58 ± 2.79	Nd	0.009	
	8000	47.61 ± 0	Nd	0.006	
Cr^{3+}	0	Nd	Nd	0.014	0.733
	100	1.41 ± 0.81	1.03 ± 2.06	0.007	0.989
	250	1.86 ± 0.02	1.84 ± 0.03	0.005	1.231
	500	2.60 ± 1.30	3.20 ± 1.95	0.010	0.650
	1000	10.09 ± 1.57	6.56 ± 0.56	0.012	0.532
	2000	24.64 ± 3.26	13.10 ± 0.28		
Cr^{6+}	0	Nd	Nd		
	25	1.69 ± 0.88	0.92 ± 0.39	0.068	0.546
	50	5.25 ± 1.94	1.51 ± 0.94	0.105	0.288
	100	4.93 ± 1.32	1.87 ± 1.09	0.049	0.380
	200	10.93 ± 2.39	8.22 ± 1.99	0.055	0.751
	500	26.24 ± 4.95	20.65 ± 0.36	0.052	0.787

Cr accumulation values represent the means of four replications ± S.D. DW, dry weight. Nd, not detected.

Table 3
Cr accumulation and transport in oat seedlings

Cr source	Cr soil concentration (mg kg^{-1})	Total Cr root (μg g^{-1}) DW	Total Cr shoot (μg g^{-1}) DW	BAF (soil–root)	BAF (root–shoot)
Tannery sludge	0	Nd	Nd		
	500	2.33 ± 0.59	Nd	0.005	
	1000	1.50 ± 0.99	Nd	0.002	
	2000	8.71 ± 1.04	Nd	0.004	
	4000	4.73 ± 0.66	Nd	0.001	
Cr^{3+}	0	Nd	Nd		
	100	11.04 ± 2.13	3.18 ± 0.45	0.110	0.288
	250	9.57 ± 1.96	7.62 ± 2.83	0.038	0.797
	500	14.90 ± 1.94	8.46 ± 4.52	0.030	0.567
	1000	13.01 ± 1.40	12.99 ± 4.73	0.013	1.000
	2000	13.00 ± 4.03	10.45 ± 2.36	0.007	0.804
	4000	45.00 ± 7.34	13.23 ± 0.68	0.011	0.294
Cr^{6+}	0	Nd	Nd		
	25	1.43 ± 0.15	2.53 ± 1.32	0.057	1.769
	50	3.17 ± 1.89	2.31 ± 0.01	0.063	0.728
	100	9.20 ± 0.80	3.07 ± 1.16	0.092	0.333
	200	17.97 ± 1.72	2.08 ± 0.74	0.090	0.116
	500	52.50 ± 4.00	15.18 ± 5.47	0.105	0.289

Cr accumulation values represent the means of four replications ± S.D. DW, dry weight. Nd, not detected.

Table 4
Cr accumulation and transport in sorghum seedlings

Cr source	Cr soil concentration (mg kg^{-1})	Total Cr root (μg g^{-1}) DW	Total Cr shoot (μg g^{-1}) DW	BAF (soil–root)	BAF (root–shoot)
Tannery sludge	0	Nd	Nd		
	500	4.94 ± 1.27	Nd	0.010	
	1000	18.81 ± 3.06	0.19 ± 0.04	0.019	0.010
	2000	22.19 ± 0.00	3.70 ± 1.23	0.011	0.167
	4000	26.50 ± 5.09	7.77 ± 2.42	0.007	0.293
Cr^{3+}	0	Nd	Nd		
	100	3.04 ± 0.52	0.97 ± 0.00	0.030	0.320
	250	11.12 ± 2.73	4.24 ± 1.04	0.044	0.381
	500	15.81 ± 5.42	5.26 ± 0.83	0.032	0.332
	1000	25.00 ± 0.00	6.11 ± 2.67	0.025	0.244
	2000	21.10 ± 1.98	15.75 ± 1.39	0.011	0.747
Cr^{6+}	0	Nd	Nd		
	25	20.01 ± 3.15	1.23 ± 0.18	0.800	0.061
	50	20.19 ± 3.47	3.14 ± 0.00	0.404	0.156
	100	42.81 ± 2.98	6.95 ± 2.85	0.428	0.162
	200	57.99 ± 1.09	11.28 ± 2.83	0.290	0.194
	500	50.74 ± 1.51	6.72 ± 1.64	0.101	0.132

Cr accumulation values represent the means of four replications ± S.D. DW, dry weight. Nd, not detected.

greater Cr mobility and therefore availability in soils, increases Cr accumulation in plants. However, especially in oat and sorghum seedlings, once Cr^{3+} (less mobile than Cr^{6+}) accumulated in roots, there was remarkable Cr transport to plant aerial parts, as indicated by root–shoot BAFs.

Differences in toxicity, mobility and Cr accumulation in plants, depend on the contamination source and the chemical species (Cr^{3+} or Cr^{6+}). Chromate and dichromate are negatively charged anions, therefore, they cannot easily be adsorbed onto soil organic matter [31], conferring them great mobility in addition to their high solubility [3]. Cr^{6+} is actively transported in plants by metabolic processes [6]. Kleiman and Cogliatti [32] suggest that chromate penetrates root cells using the same system of transport as sulfates. Cr^{3+} is probably transported in a passive way and its mobility is very limited. However, it can easily enter the plant system if it is organically complexed in the rhizosphere [6]. The interaction with organic acids greatly contributes to the incorporation of Cr^{3+} in plants [33].

In experiments with the three Cr sources (tannery sludge, $CrCl_3 \cdot 6H_2O$ and $K_2Cr_2O_7$) and for the three plant species, there was a highly significant negative correlation ($R = -0.70$ to -0.92, $P < 0.0001$) between Cr accumulation in dry tissue and toxic effects on root growth. That means that accumulation increase corresponded with reduced root growth. There was no correlation between Cr accumulation and seedling dry weight for any treatment (data not shown). Root and shoot biomass of barley decreased as a function of Cr concentration in plant dry tissues [10]. Han et al. [1] found a direct relationship between dry biomass and Cr concentration in leaves of *Brassica juncea*, suggesting this relationship as an indicator of Cr toxicity. However, *B. juncea* is a hyperaccumulator plant and could have different behaviors to plants of agronomic interest.

4. Conclusions

In the present study, root growth was the most sensitive assessment for toxicity of Cr-polluted soil, compared to shoot growth and seed germination. Cr^{6+} was more mobile in soil than Cr^{3+} and tannery sludge Cr, and caused greater toxicity effects on wheat, oat and sorghum seedlings at lower Cr concentrations. Tannery sludge was the least toxic for the three plant species tested, resulting in higher EC_{50s} than for Cr^{3+} and Cr^{6+}.

Cr accumulated mostly in roots and not shoots. Under the assayed conditions, only in sorghum plants there was Cr translocation from root to shoot, even though this was very limited when applying tannery sludge. Importantly, the three plant species showed Cr translocation from roots to shoots in the Cr^{6+} and Cr^{3+} treatments. There was a highly significant negative correlation between Cr accumulation in dry tissue and toxic effects at root growth level.

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