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THE UPTAKE AND TRANSLOCATION OF TRI- AND HEXA-VALENT CHROMIUM AND EFFECTS ON THE GROWTH OF OAT IN FLOWING NUTRIENT SOLUTION AND IN SOIL

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Summary

ri- and hexa-valent chromium (Cr) were both absorbed by oat and were equally toxic when ıpplied at 2 to 200 μM in nutrient solution. The proportion of Cr translocated to the shoots as similar, regardless of the form supplied to roots.

Adding 750 μg of hexa-valent Cr per gram of either acid or alkaline soil resulted in large, toxic ›ncentrations of Cr in soil solution. The same amount of trivalent Cr gave smaller Cr ›ncentrations in the soil solution, with more Cr present from acid than from alkaline soils. ievertheless, increased uptake of Cr and reduced plant yields occurred on both soil types with ıded trivalent Cr.

These findings are discussed in relation to the chemistry of Cr in soils and the potential hytotoxicity of the common types of Cr-contaminated substrates.

Introduction

'he accepted view that trivalent Cr [Cr(III)] is less toxic to plants than the exa-valent form [Cr(VI)] (Peterson and Girling, 1981) derives from experiments ı which nutrient solution culture techniques were used to compare the uptake and ›xicities of nominally equal amounts of the two forms of the element. However, ıe solubility of Cr(III) [but not Cr(VI)] in nutrient solutions depends on both H and the concentration of other salts present. Much smaller amounts of Cr(III) :main soluble with increased pH and nutrient solution concentration (Fig. 1), but ıis has not been considered by previous workers. For instance, all previous ›mparisons of the toxicities of Cr(III) and (VI) have been made either at a H > 5·5 or at unspecified values (Myttenaere and Mousny, 1974; Verfaillie, 1974; ettersson, 1976; Cary, Allaway and Olson, 1977; Wallace, Alexander and 'haudhry, 1977; Chetelat, 1978; Hara and Sonoda, 1979; Lee, Sturgis and ·andin, 1981). Some workers used solutions without Ca, the presence of which :abilizes cell membranes, or without other nutrient ions which are normally resent in the root environment (Breeze, 1973; Skeffington, Shewry and Peterson, 976). These omissions may have influenced the observed rates of Cr uptake into ıe plant.

Previous views on the relative toxicities of Cr(III) and Cr(VI) are therefore ıbject to doubt and, in an attempt to resolve this uncertainty, plants were grown ı a flowing nutrient culture system which enabled the pH of the solution to be ontrolled and therefore equal concentrations of dissolved Cr(III) and Cr(VI) to e maintained. Plants of oat were grown at pH 4·5 in a flowing one-tenth-strength utrient solution, thus minimizing the precipitation of Cr(III) whilst supplying

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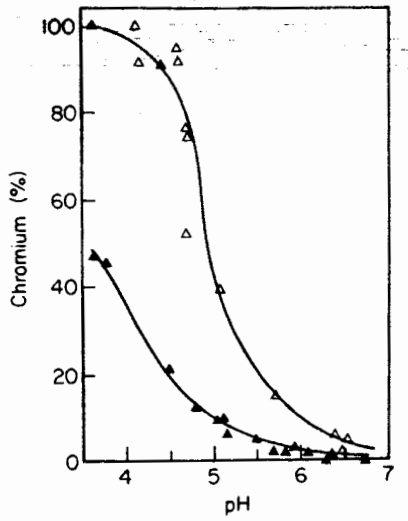

Fig. 1. Percentage of Cr added to nutrient solution remaining soluble after 24 h. Initial concentration 200 μM Cr(III); ▲—▲, full strength solution; △—△, one-tenth dilution. Solution used was that of Rorison (see Table 30c, Hewitt, 1966).

adequate nutrients. The aim was to measure the uptake and translocation of Cr of two valencies at equal concentrations and to determine their relative toxicities. Oat was also grown on soil at two pH values to which either tri- or hexa-valent Cr had been added to examine the uptake of Cr(III) and also to determine the extent of reduction of Cr(VI) in soil at low and high pH.

Materials and Methods

Solution culture

'Seed' of oat (*Avena sativa* L. cv. Manod) was germinated in trays on filter paper dampened with de-ionized water. Seven days after germination, six seedlings were transplanted into each of 35 polyethylene culture vessels of one litre capacity containing flowing nutrient solution (composition given in Table 1).

Fresh nutrient solution was pumped from 20 l reservoirs into the bottom of each vessel at 50 ml h^{-1}. Solution leaving the vessels by an overflow at the top was discarded. The treatments, Cr(III) and Cr(VI), each at 2, 20 and 200 μM plus Cr-free controls, were replicated five times and the vessels were fully randomized in a growth room (20 °C day, 16 °C night, 16 h daylength, from warm white fluorescent tubes plus tungsten bulbs giving a mean photon flux density of 500 $\mu E\ m^{-2}\ s^{-1}$).

The concentration and valency of Cr remaining in solution at the end of the experiment was determined on centrifuged samples of solution; Cr(VI) was determined by the absorptiometric method of Bloomfield and Pruden (1980) and total Cr by atomic absorption spectrophotometry.

Plants, harvested weekly for 4 weeks, starting after 2 weeks' growth, were washed in de-ionized water, and the roots and shoots were separated and dried at 90 °C for 48 h before weighing. The plants were digested in nitric/perchloric acids prior to determining Cr by atomic absorption spectrophotometry.

Plant growth on Cr-treated soil

Soils with long-term equilibrium pH values of 3·9 and 7·6 [measured in 1:1

Table 1. *Composition of nutrient solutions (after Rorison, see Table 30c of Hewitt, 1966)*

Element	Concentration in solution (μM)	Source
Ca	200	$Ca(NO_3)_2 . 4H_2O$
N	400	
P	100	$NH_4H_2PO_4$
N	100	
Mg	100	$MgSO_4 . 7H_2O$
S	100	
Fe	5	Fe–EDTA
Mn	0·9	$MnSO_4 . 4H_2O$
B	5	H_3BO_3
Mo	0·015	$(NH_4)_6Mo_7O_{24} . 4H_2O$
Zn	0·15	$ZnSO_4 . 7H_2O$
Cu	0·15	$CuSO_4 . 5H_2O$
Variables, according to treatment		
K	0, 180, 198, 200	KCl
Cr(III)	200, 20, 2, 0	$KCr(SO_4)_2$
Cr(VI)	200, 20, 2, 0	$K_2Cr_2O_7$
H_2SO_4	Used to adjust pH to 4·5	

(w/v) mixture with water] were collected from plots on Hoosfield, Rothamsted, and were sieved to 2 mm. Solutions of either $KCr(SO_4)_2$ or $K_2Cr_2O_7$ were added in amounts equivalent to 750 μg Cr per gram of dry soil. Acidification of the soil by $KCr(SO_4)_2$ was prevented by adding 0·24 g and 0·14 g of $Ca(OH)_2$ to the acid and alkaline soils, respectively.

Subsamples of 350 g soil were put into 9 cm plastic pots with fine nylon gauze placed over the drainage holes. Basal nutrients were pipetted on to the soil as a solution containing $MgSO_4$, NH_4NO_3 and KH_2PO_4 (25 mg Mg, 33 mg S, 28 mg N, 78 mg K and 62 mg P per pot). The soil was then thoroughly mixed and wetted to 50 % water-holding capacity (25 % water by weight). Five replicate pots comprising controls (no Cr), Cr(III) and Cr(VI) treatments at each pH were incubated for 10 days in a randomized block in a green house. After this equilibration, two 7-day-old pregerminated oat seedlings were planted per pot and grown for 35 days during April–May. An extra 56 mg N as NH_4NO_3 solution was added to each pot after 15 and 25 days growth.

After harvesting, the plants were washed and dried and the roots and shoots weighed separately. The shoots were digested for analysis described above.

To determine the Cr-contents of the soil solutions at harvest, approximately 30 % of the total soil water was displaced from the soil by dripping de-ionized water on to the soil surface at 1 ml min^{-1} (using a Watson–Marlowe peristaltic pump) (see Adams, 1974). The first 5 ml of solution were discarded and the remainder was centrifuged at 35 000 g for 15 min and decanted into polypropylene bottles. The concentrations of Cr(VI) and total Cr in these soil solutions were determined as described above. Iron hydroxides precipitated when the solutions from the acid soil were treated with the KOH/EDTA reagent (Bloomfield and Pruden, 1980) so that the Cr(VI) contents of these solutions could not be determined.

Results

Yields

In nutrient solutions in which equal concentrations of both valencies of Cr were maintained (Table 2), both forms of Cr were toxic – growth almost ceased at 200 μg and was slowed greatly at 20 μm. The growth of roots was inhibited at concentrations > 2 μM Cr(III) and > 20 μM Cr(VI) [Fig. 2(a)], and, at each concentration of Cr, Cr(III) was more inhibitory than Cr(VI). The growth of

Table 2. *Measured valency and concentration of Cr remaining in solution at the end of the solution culture experiment*

Treatment	Total Cr (μM)	Cr(VI) (μM)
2 μM Cr(III)	2·0	—
20 μM Cr(III)	19·2	—
200 μM Cr(III)	192·2	—
2 μM Cr(VI)	2·0	1·9
20 μM Cr(VI)	19·4	18·5
200 μM Cr(VI)	188·3	181·7

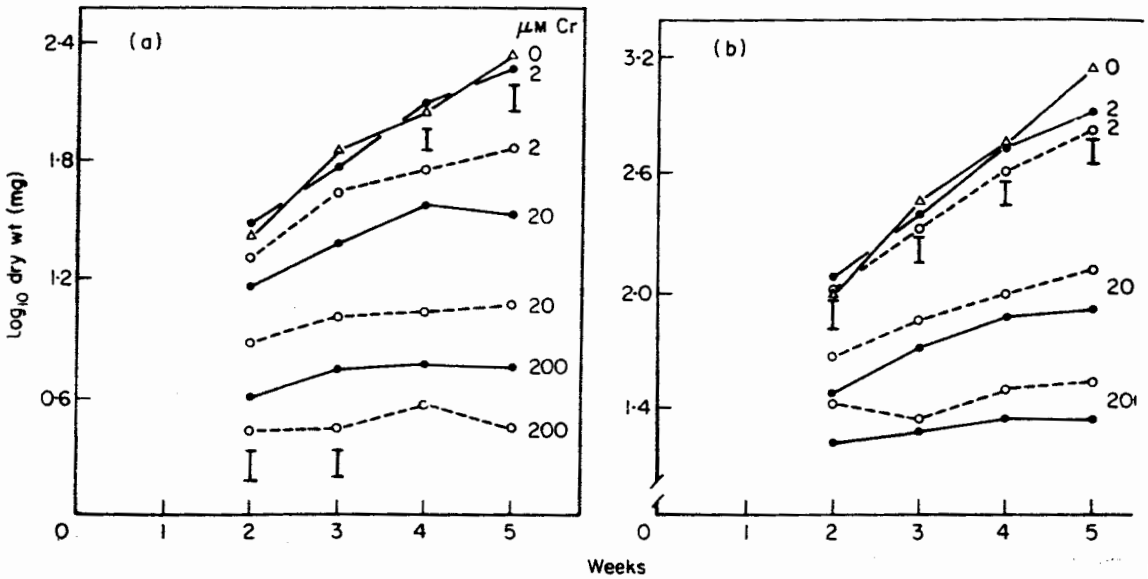

Fig. 2. Yields of (a) roots and (b) shoots of oat grown in nutrient solutions containing Cr(III), ○---○ or Cr(VI), ●—●. Points are means of five replicates, vertical bars = least significant difference $P < 0{\cdot}05$.

shoots was decreased by 20 μM or more of both valencies of Cr; Cr(VI) decreas yields of shoots more than Cr(III) [Fig. 2(b)]. The differential effects of Cr(I and Cr(VI) on growth of roots and shoots were reflected in changes in t root:shoot ratios (Table 3).

All plants died when grown on Cr(VI)-treated soil, probably because of the lar concentraion of dissolved Cr (Table 4). Within the limits of the experimen techniques used, with the Cr(VI)-treated soil at pH 7·6, all Cr in the soil soluti

Table 3. *Ratio of root weight to shoot weight of oat grown for 5 weeks in nutrient solutions containing Cr(III) or Cr(VI)*

Cr treatments (μM)	Valency (III)	Valency (VI)
0	0·158	0·158
2	0·074	0·235
20	0·086	0·395
200	0·112	0·215

Values are means of five replicates.
L.S.D. ($P < 0·05$) 0·035.

Table 4. *Results of experiment with oat grown on soil amended with 750 μg Cr(VI) or Cr(III) per gram of soil at two pH values*

	Soil amended with: Cr VI		Cr III		Control (no Cr)	
pH ...	3·9	7·6	3·9	7·6	3·9	7·6
Soil solution						
Total Cr concentration (mM)	1·02*	6·79	0·07	0·05	—	—
[Cr(VI)] (mM)	—	6·92	—	0	—	—
Yield of shoot (mg)	0	0	35	156	181	776
Yield of root (mg)	0	0	5	44	24	341
Cr concentration in shoot ($\mu g\ g^{-1}$)	—	—	144	15	0	0

* All figures in the table are means of five replicates after incubation of the soil for 10 days, then plant growth for 35 days.

was Cr(VI). However, with the acid soil, the total Cr content of the soil solution was much lower. Additions of Cr(III) resulted in lower concentrations of Cr in the soil solutions of both soils. At both pH values the plants were stunted compared with the controls and development of lateral roots was inhibited.

Uptake of chromium

Concentration of Cr in roots increased with increased concentration of Cr in nutrient solutions but its valency had no significant effect [Fig. 3(a)]. Concentration of Cr in the shoots also increased with increasing concentration of Cr in the nutrient solution but very large values were obtained only at 200 μM Cr [Fig. 3(b)]. Cr(VI) tended to give larger Cr concentrations in the shoots than Cr(III).

The transport index for Cr (proportion of whole plant Cr content which was transported to the shoot) remained fairly constant within each treatment during the experimental period and was more dependent on the concentration at which Cr was supplied than on its valency (Fig. 4).

Plants grown on soil amended with Cr(III) absorbed and translocated Cr to the shoots, with the greatest uptake at low pH (Table 4).

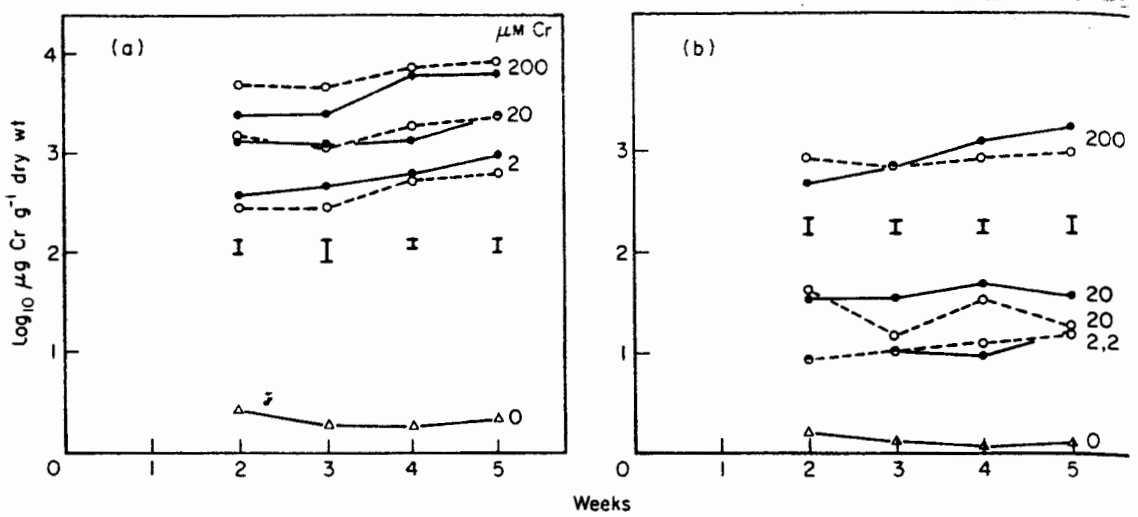

Fig. 3. Concentration of Cr in (a) roots and (b) shoots of oat grown in nutrient solutions containing Cr(III), ○---○ or Cr(VI), ●—●. Points are means of five replicates, vertical bars = least signicant difference $P < 0{\cdot}05$.

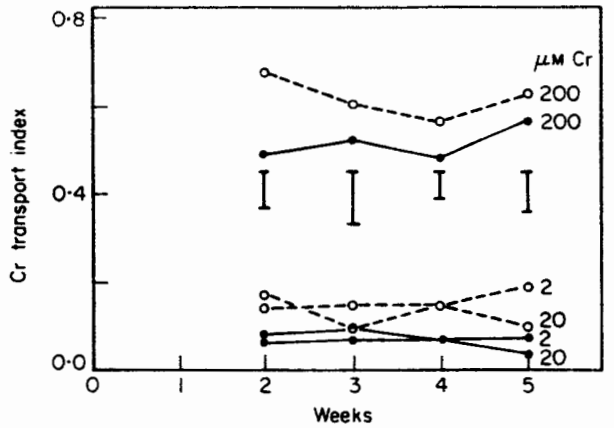

Fig. 4. Cr transport index (shoot Cr content/whole plant Cr content) of oat grown in nutrient solutions containing Cr(III), ○---○ or Cr(VI), ●—●. Points are means of five replicates vertical bars = least significant difference $P < 0{\cdot}05$.

Discussion

Solution culture

In solution culture, the oat plants absorbed both forms of Cr and showe equally severe symptoms of toxicity at a range of concentrations of both forms Root and shoot weights of Cr-treated plants were much smaller than those o control plants even at the lowest concentration of Cr(III) or (VI) used.

Figure 5 shows that, despite the different chemical properties of the two ions there were no great differences in their specific absorption rates. Studies usin inhibitor have indicated that uptake of Cr(VI) is 'active' whereas that of Cr(III is 'passive' (Skeffington *et al.*, 1976). The two forms therefore probably do no share a common uptake mechanism, and also interconversion of the two forms prio to uptake is unlikely. None the less, in the present experiment at equa concentrations the uptake of the two forms was almost equal.

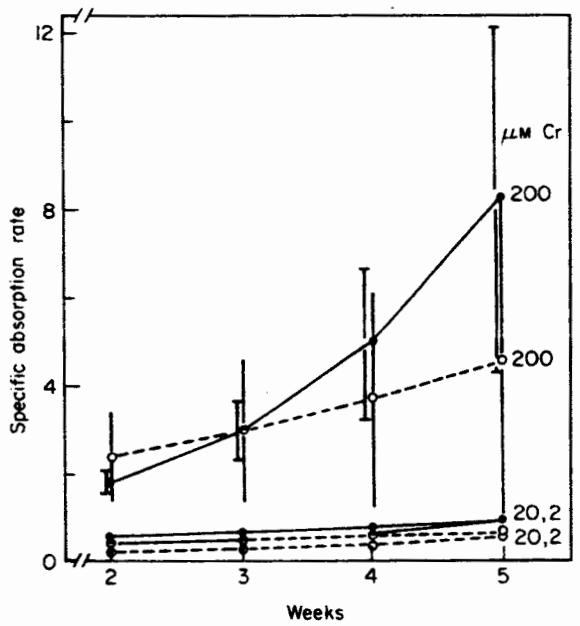

Fig. 5. Instantaneous specific absorption rate (calculated uing the method of Hunt and Parsons, 1974) of chromium in mg Cr mg root D.M.$^{-1}$ by oat grown in nutrient solutions containing Cr(III), O—O or Cr(VI), ●—●. Points are means of five replicates, vertical bars are confidence intervals ($P < 0{\cdot}05$).

The table of variance ratios from the analyses of variance (Table 5) summarizes the effects of the two different ions and their concentrations on plant growth and Cr uptake. Only in the analysis of root:shoot ratios was valency the stronger factor (i.e. largest variance ratio) and the next largest variance ratio for the effect of valency was for root dry wt. These large effects were due to the effect that Cr(III) had in reducing root growth. The concentration of Cr in the medium had large effects on all the plant characteristics except root:shoot ratios, emphasizing that the amount present, not the form of Cr, was the factor which had the greatest influence, particularly on measurements involving the shoots. The lack of major differences with Cr(III) or (VI) makes it likely that one form is causing the observed effects inside the plant. An hypothesis which would explain the above effects would be that interconversion of the two forms takes place within the roots of the plants and, though this process may not be complete (Skeffington *et al.*, 1976), one form may then predominate, particularly in transport to the shoot.

Plant growth and uptake of Cr on Cr-treated soil

Cr(III). The experiment using solution culture showed that dissolved Cr(III) is as toxic as Cr(VI) and it was therefore of interest to investigate whether Cr(III) toxicity could be demonstrated in soil.

The concentration of Cr in soil solutions from Cr(III)-treated soils exceeded the concentration which gave 50% inhibition of root growth in solution culture by approximately 30-fold. Although a large proportion of the metal in the soil solution may have been in unavailable complexed forms, the yields of roots and shoots were reduced by more than 80% at both pH values. Because of the precipitation of Cr(III) hydroxide at pH 7·6, more Cr was present in the soil solution at pH 3·9 than at pH 7·6, leading to greater concentrations of Cr in the shoots of plants grown at pH 3·9 than at pH 7·6, the concentrations of Cr in shoots

Table 5. *Variance ratios from analyses of variance after 5 weeks in solution culture with probabilities*

Measurements	Effects, with degrees of freedom in brackets**** Valency (V) (1)	Concentrations (C) (2)	V × C (3)
Log root dry wt	103·87**	529·66**	1·56 NS
Log shoot dry wt	8·50*	566·50**	7·77**
Root:shoot ratio	501·74**	40·65**	51·51**
Root Cr concentration	0·40 NS	257·65**	7·72*
Shoot Cr concentration	17·38**	648·60**	2·60 NS
Total shoot Cr / Total whole plant Cr	9·60*	167·29**	0·46 NS

* $P = 0{\cdot}01$.
** $P = 0{\cdot}001$.
*** Residual degrees of freedom = 28.

exceed previously published critical Cr-concentration limits of 3 to 10 μg Cr g^{-} dry matter (Mortvedt and Giordano, 1975; Dijkshoorn, van Broekhoven anc Lampe, 1979).

Serpentine soils and some sewage sludges contain large amounts of Cr but thes may not necessarily be associated with large amounts of 'available' Cr (Peterson 1976). As the Cr(III)-containing minerals of the serpentine soils are highl refractory and the soil pH is frequently > 7, the likelihood of large concentration of Cr equilibrating with the soil solution is reduced, and unequivocal cases of C toxicity in plants growing on these soils have not been reported. However, plant growing on these soils may be tolerant of Cr or even accumulators (Peterson, 1976)

Sewage sludge can contain as much as 8800 μg g^{-1} Cr (Berrow and Webber 1972) and is a potential source of hazardous quantities of Cr when it is dispose of on agricultural land. Chromium in sewage sludges exhibits the chemistry anc immobility of trivalent Cr compounds, which are presumably precipitated durin the sludge-formation process (Grove and Ellis, 1980; Williams *et al.*, 1980). Som workers have reported little effect of Cr in sewage on plant growth (Mortvedt anc Giordano, 1975; Zwarich and Mills, 1979), but Dowdy and Larson (1975) founc increased Cr uptake from sludge-amended acidic and calcareous soils; the uptak was greater by a factor of four from the acid soil than from the calcareous soil Therefore, uptake and toxicity of Cr(III) from sludge will depend on the pH o the sludge/soil mixture and the total Cr loading.

Cr(VI). As expected, Cr(VI)-treated soil was extremely toxic (in this cas lethal) to the test plants at both pH values, though at low pH less Cr was detecte in the soil solutions than at high pH. Reduction of Cr(VI) to Cr(III) and sorptio of Cr(VI) in soil both increase with decreasing pH (Bloomfield and Pruden, 1980) This accounts for the lower concentration of Cr in the solution from the acid soil the acid soil.

Chromate wastes represent potential sources of Cr-toxicity, with water-solubl concentrations of 10 to 6000 μg Cr g^{-1} dry soil [95 % as Cr(VI)] reported (Breeze 1973). Reduction of Cr(VI) to Cr(III) by chemical means has been suggested a

method of ameliorating this type of waste but the substrate must not be allowed o become too acid, as chromic(III) hydroxide would then dissolve and chromium vould accumulate in and reduce the yields of plants growing on the waste.

Conclusions

Both forms of Cr are toxic when they are available to the root, thus toxicity problems can occur when (a) soil is contaminated with Cr(VI) and the pH is high – under these conditions very little Cr(VI) is adsorbed or reduced in soil and therefore present in the soil solution – and (b) when soil is contaminated with Cr(III) or Cr(VI) and the pH is low – in this case Cr(VI) will be reduced to Cr(III) which will equilibrate with the soil solution. The amount available to plants will depend on the precise conditions, of which pH and the magnitude of the contamination are most important. Problems of Cr toxicity have been reported under conditions of high Cr(VI) availability or at low pH where sufficient soluble Cr(III) is present. At higher pH values, as with aluminium, precipitation of the Cr(III) hydroxide makes the metal ion unavailable to plants.

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