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EFFECTS OF TRIVALENT CHROMIUM (Cr-III) ON *VIGNA MUNGO* L. SEEDLINGS

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Tannery effluents consist of toxic chemicals including chromium, which is a heavy metal. Presence of heavy metals in growth media causes damage to plants. In the present study experiments were conducted to understand the effects of chromium (III) on *Vigna mungo* L. seedlings in pot culture experiments. Seed germination was largely affected by high concentrations of chromium (500 mg Cr/kg soil & above). Median Lethal Dose (MLD) in terms of LD 50 was found to be at 1500 mg Cr/kg soil, wherein 50% seeds germinated. Maximum retardation of growth was 29% in both shoots and roots. Analysis of chromium revealed that all parts of the seedlings showed the presence of chromium. But, most of the chromium was retained by roots (80%). Furthermore, the chromium was present in trivalent state in all the parts. EDTA in the growth media aggravated the toxic effects induced by chromium.

Key Words : Chromium, *Vigna mungo*, Seedling.

One of the important industrial waste problems facing the country relates to pollution. Environmental pollution due to tannery waste water is a matter of concern. Tannery effluents consist of toxic chemicals including chromium heavy metal. Heavy metals occur naturally and are added to environment by man's activities. They have attracted much attention because they are non-degradable, perisist in nature for extended period of time and become toxic to animals and plants even at low concentrations (Goyer & Mehlman, 1977). Many crop plants tolerate trace amounts of both heavy metals and non-heavy metals present in soil and surface water. Presence of excessive amounts of heavy metals like copper, mercury, zinc in growth media causes damage to plants, the degree and extent depending on the concentration of the metal and other environmental factors. Heavy metals like copper, mercury, zinc in higher amounts are toxic to plants. besides causing retarded growth, chromium is known to interfere with biochemical processes of plants during germination (Swamy *et al.*, 1984, 1992 a and b, Nag *et al.*, 1980 1981). In this paper, an attempt has been made to understand the effects of chromium (III) on *Vigna mungo* L. during germination in pot culture experiments.

MATERIALS AND METHODS

Healthy seeds of *Vigna mungo* L c.v. - T.9 of uniform size were selected and surface sterlised with 0.1% mercuric chloride for 5 minutes and washed thouroughly with distilled water to remove traces of mercuric chloride. Later, the seeds were soaked for 6 hours in distilled water and sown 1/2" below the soil level for germination in pots filled with river sand + red soil + manure (1.2 kg dry weight) in the ratio of 1:1:2. Each pot contained 25 seeds and three replicates were maintained for each treatment. 200 ml of the treatment solution (0, 100, 200, 300, 400, 500, 1000, 1500 ppm) was evenly dispersed on the surface of the soil for 6 days. Water was added to make the soil approximately to field capacity. Total of 1200 ml treatment solution was received by each pot. Seedlings were harvested after 6 days by which time the germination was complete. Percentage germination, growth of roots and shoots, their fresh and dry weights were found out. The pH of the soil before and after the experiments was measured by ISI method.

To study the effects of EDTA, one lower concentration of chromium solution (non-toxic) and one higher concentration (toxic) were selected. The required amount of Na_2 EDTA was mixed with the basic chromium sulphate solution. For e.g., 1.074 gms of Na_2EDTA is required to complex the chromium present in 1 liter of 100 ppm chrome liquor.

Analysis of chromium :

(a) Plant material : The dried seedlings were wet digested with a mixture of nitric acid, sulphuric acid and perchloric acid (10:1:4) and chromium was

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Table 1. Effects of chromium (III) on growth.

Treatment (Cr-III) (ppm)	Percent germination	Shoot Fresh weight (mg)+	Shoot Dry weight (mg)+	Shoot Percent dry matter	Shoot Growth (cms)++	Shoot Retardation (percentage)
Control	100	246.5	20.8	8.4	9.5 + 0.20	-
100	100	238.0	20.6	8.5	9.3 + 0.23	-
200	100	218.0	18.6	8.5	9.5 + 0.20	-
300	100	190.0	16.3	8.6	9.4 + 0.21	-
400	100	181.0	16.0	8.4	9.3 + 0.24	-
500	96	172.8	15.2	8.7	8.9 + 0.20	6.6 @
1000	78	162.2	14.5	8.9	8.3 + 0.23	12.9@
1500	52	148.0	14.1	9.5	6.7 + 0.33	29.0@

Treatment (Cr-III) (ppm)	Percent germination	Root Fresh weight (mg)+	Root Dry weight (mg)+	Root Percent dry matter	Root Growth (cms)++	Root Retardation (percentage)
Control		162.9	8.5	5.2	21.1 + 2.9	-
100		159.0	8.2	5.2	21.4 + 3.7	
200		145.1	8.0	5.5	20.3 + 3.4	4.7 @
300		140.0	8.1	5.9	18.3 + 3.8	12.2@
400		132.2	7.4	5.6	18.5 + 2.9	12.3@
500		121.4	6.5	5.3	17.8 + 2.8	12.6@
1000		102.2	5.7	5.6	17.0 + 1.9	19.4@
1500		83.5	5.6	5.6	15.0 + 1.6	29.0@

\+ Average of 3 replicates
++ Numbe rof Samples - 10
@ Significantly different at p = 0.05

estimated by spectrophotometric method using diphenyl carbazide reagent method (Jackson, 1965; Snell and Snell, 1959).

(b) Soil: The soil chromium was extracted by using N/10 HCl and analysed as per the above method.

RESULTS AND DISCUSSION

Metals play vital role in the growth and development of plants. They may act as co-factors of some enzymes or help in the formation of intermediate metabolites. However, excess of metals especially heavy metals like chromium when present in the growth media are toxic to plants.

Pots received a total of 0-1500 mg/Cr/kg soil over the growth period (Table - 1). Seed germination was largely affected by high concentrations of chromium (1000 mg Cr/kg soil and above) (Table - 1). MLD in terms of LD50 was found to be at 1500 mg/kg soil, wherein 50% of the seeds germinated. The germination was altogether inhibited at 2000 mg Cr/per kg soil.

Application of chromium up to 500 mg Cr/kg soil did not exhibit significant effect on the growth of shoots and roots. The maximum retardation of

Table 2. Uptake and distribution of chromium (III)

Treatment Cr (III) in ppm	Shoot per plant gm	Shoot ppm	Shoot percent distribution	Root per plant gm	Root ppm	Root percent distribution
Control	nil	nil	nil	nil	nil	nil
100	0.8	4.35	22.2	2.8	32.40	77.8
200	1.8	10.87	24.3	5.6	61.07	75.7
300	1.9	11.49	21.8	6.8	73.60	78.2
400	2.0	12.58	22.7	6.8	75.14	77.3
500	2.0	14.68	21.7	7.2	91.63	78.3
1000	2.5	15.73	22.9	8.4	125.00	77.1
1500	2.9	19.25	23.8	10.4	226.00	76.2

Average of 3 samples

growth was 29% both in root and shoot as noticed in the highest dose studied.

It is interesting to note that though germination and inhibitionof growth was not marked at lower concentrations (up to 200 mg Cr/kg soil) there was a tendency to show decreased yield in terms of fresh and dry weight of seedling root and shoot. Nevertheless, percentage of dry matter increased in chromium treated seedlings (Table 1).

50% inhibition of root fresh weight was at 1500 mg Cr/kg soil. Such reduction was not observed in shoots (Table 1).

Dry weight never decreased to 50% in both root and shoot even in the highest dose (1500 mg Cr/kg soil) (Table 1).

It appears that chromium toxicity induces dehydration of the tissues even under natural conditions (i.e. in soils). Such an observation has been made in seedlings grown in laboratory conditions in mungbean seedlings, when treated with trivalent chromium and with hexavalent chromium on various plants (Mukherji & Roy, 1978, Swamy *et al,* 1984).

Morphology : Except for the stunted growth of roots and shoots no other obnormalities were observed. However, in extreme cases of toxicity the coiling of the seedlings, as observed in laboratory conditions, were seen (Swamy *et al,* 1984).

Uptake and distribution of chromium

All parts of the seedlings showed the presence of chromium (root, shoot including primary leaves). Most of the chromium was retained by roots and could accumulate chromium up to 226 ppm (80%),

Table 3. Effects of Cr-EDTA on seedlings.

Treatment (Chromium (III) in ppm)	Per-cent germi-nation	Fresh weight (mg/ plant part)	Dry weight (mg/ plant part)	Growth (length in cm)
(a) **SHOOT**				
1000 Cr	76	152.21	13.80	8.5 ± 0.31
1000 Cr + Na_2 EDTA (50% complex)	68	143.30	13.06	8.0 ± 0.26δ
1000 Cr + Na_2 EDTA (100% complex)	- No germination -			
Control I	100	243.80	20.35	9.48 ± 0.30
Control II	98	249.80	20.10	9.38 ± 0.26
Control III	96	238.40	20.00	9.36 ± 0.35
(b) **ROOT**				
1000 Cr	-	106.23	6.27	17.23 ± 2.10
1000 Cr + Na_2 EDTA (50% complex)	-	83.10	5.46	13.80 ± 2.60δ
1000 Cr + Na_2 EDTA (100% complex)	- No germination -			
Control I	-	156.10	8.34	20.33 ± 3.20
Control II	-	150.20	8.29	20.00 ± 2.20
Control III	-	148.25	8.31	19.90 ± 2.30

Control I : Water
Control II : EDTA (contained same amount of EDTA used for 50% complex)
Control III : EDTA (contained same amount of EDTA used for 100% complex)
δ : Significantly different at p = 0.05 when compared to respective chromium treatment (1000.Cr).

while shoots accounted for 20 ppm as observed in the highest dose (1500 mg Cr/kg soil) (Table 2). Furthermore, the chromium was present in trivalent form in all the parts of the seedlings. Most of the chromium, like other heavy metals, is generally retained within the roots in higher plants; and as is well known, roots serve as barrier to translocation of many trace elements as well as those that are not essential. Such increased accumulation of chromium in roots has also been observed earlier by various authors (Swamy *et al.*, 1984, Mukherji and Roy, 178; Wallance *et al.*, 1976; Turner & Rust, 1971; Sykes *et al.*, 1981).

Accumulation of chromium in roots is an established fact when grown either in nutrient media or in soil. However, in the present study the translocation of chromium to shoots appeared to be moderately high.

Analysis of soil before and after the treatments indicated that there was a gradual decrease in pH of the soil which was significant in higher dosages of chromium.

Table 4. Uptake of chromium by Cr-EDTA treated seedlings.

Treatment (chromium (III) in ppm)	Root		Shoot	
	ppm Cr	Total concen-tration (µg)	ppm Cr	Total concen-tration (µg)
100 Cr	29.90	2.5	7.32	0.70
100 Cr _ Na_2 EDTA (50% complex)	28.00	2.6	14.45	1.43
100 Cr + Na_2 EDTA (100% complex)	30.30	2.6	19.60	1.94
1000 Cr	133.90	9.0	19.80	2.40
1000 Cr + Na_2 EDTA (50% complex)	130.40	8.2	34.60	4.60

Effect of EDTA :

The effects were studied at two different levels (a) lower concentration (100 ppm), wherein chromium was not toxic (b) higher concentration (1000 ppm), wherein chromium was already toxic (Table 3 & 4).

In lower concentrations tested 100 ppm Cr) increased availability of EDTA in the form of Cr-EDTA complex (50% and 100% complex) did not induce any toxic effects, except for increased translocation of chromium to shoots. Hence, the data on uptake of chromium only are furnished (Table 4). In higher concentration tested (1000 ppm) wherein the chromium was already toxic, EDTA aggravated the toxic effects (Table 3 & 4). This is evident from the following observations.

(i) Percent germination was reduced in seedlings treated with EDTA - Cr complex (65% in 50% EDTA complex and total inhibition of germination in 100% cr-EDTA complex) compared to treatments containing only chromium (76% germination).

(ii) Fresh and dry weight, length of root and shoot decreased in seedlings treated with Cr-EDTA complex.

(iii) Increased absorption and translocation of chromium in seedlings treated with EDTA complex.

Chromium as such is not mobile in soil and readily adsorbed on to clays, oxides etc., and most forms of chromium are readily immobilised and thus become unavailable for uptake by plants. But,

Cr-EDTA complex is not only soluble but available for absorption and it is highly mobile in the plant body compared to trivalent chromium. Therefore, the increased availability of EDTA in soils ensures increased formation of Cr-EDTA complex, which in turn is readily taken up by roots and translocated to shoots. Consequently, toxicity is enhanced in the seedlings resulting in death in higher concentrations (Wallace *et al.*, 1976).

In general, the seedlings could tolerate higher amounts of chromium applied to soil and the toxic effects in higher concentrations were inhibition of root and shoot and were dose dependent. Furthermore, the complexing of chromium with EDTA aggravated the toxic effects induced by chromium. The author is thankful to the Director, C.L.R.I., Madras for the facilities.

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