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Effect of chromium on germination in some crops of India.

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Abstract

Industrial development and scientific advancement has contaminated the environment, as a result one of the major pollutants in the environment are heavy metals and their introduction in air, water and agricultural soil. In recent years, chromium has become a major environmental pollutant as it widely used in making alloys, electroplating of stainless steel, production of pigments. Many studies have been carried out on heavy metals to understand their detrimental effects in the ecosystem. In the present study, Chromium was selected to find out its harmful effects on seed germination and seedling growth of *Vigna radiata* L, *Trigonella foenum-graceum* L, *Oryza sativa* L, *Sorghum vulgare* L and *Pennisetum glaucum* L. Seeds of the selected plants were grown in petri dishes and treated with varying concentrations of Chromium solution. Each treatment was replicated in a randomised design and observed for 7 days. The developing seedlings were studied for their total rate of germination, seed vigour index, length of radicle, length of plumule and fresh weight compared to a set of seeds germinated using distilled water as control. It was observed that the harmful effects of chromium on all the parameters were directly proportional to the concentration of solution employed, with the inhibition of growth being pronounced from 50 ppm onwards. Based on the response of the plants the toxic effect of chromium was seen in the following order, *Trigonella foenum-graceum* L>*Oryza sativa* L>*Pennisetum glaucum* L>*Sorghum vulgare* L> *Vigna radiata* L.

Keywords: Chromium, Seed germination, Toxicity, Crop.

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Introduction

Heavy metals are substances naturally occurring in the Earth's crust; it is the significant rise in human activities that has caused soil contamination by heavy metals. Heavy metals are elements with a high density of 4 g/cm^3; some of these are required in trace amounts and vital for maintaining human health. Heavy metals disrupt the functioning of an organ by accumulating in the tissues thus displacing vital nutrients. Some heavy metals are essential for growth; however at higher levels it becomes toxic to living organisms. Chromium is one such nutrient required for sugar and fat metabolism in humans, whereas the role of chromium in plant growth and its uptake pathway are not yet fully understood. Plant uptake of Cr (III) is a passive process, that is, no energy expenditure is required by the plant [1,2]. The uptake of Cr (VI) is thought to be an active mechanism performed by carriers for the uptake of essential elements such as sulphate [3,4]. Other studies have reported that plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage and disturbance of cellular ionic homeostasis, in fact disrupting the physiology and morphology of plants [5].

An experiment was designed and conducted for studying the effects of chromium on five species of plants; the present study is a comparative report of the unfavourable effects of varying concentrations of chromium on seed germination, root and shoot growth and fresh weight of *Vigna radiata* L, *Trigonella foenum-graceum* L, *Oryza sativa* L, *Sorghum vulgare* L and *Pennisetum glaucum* L. The aim of our study was to understand and compare the response and sensitivity of these plants to heavy metal stress to illustrate their tolerance potential.

Materials and Methods

For understanding the effects of chromium on germination plants of Vigna radiata L, *Trigonella foenum-graceum* L, *Oryza sativa* L, *Sorghum vulgare* L and *Pennisetum glaucum* L. were grown from seeds. The seeds of all the plants were bought from American hybrid Seed Company. The plant species were identified from the Botanical Survey of India.

Ten seeds of uniform colour and size were placed on a double-layered filter paper in each of the petri dishes having a diameter of 9 centimeters. Preceding the placement in petri dishes, the seeds were sterilized using bavistin solution prepared by dissolving 200 mg of bavistin powder in 100 ml distilled water. The seeds were kept in this solution for 5 minutes followed by a through rinse using distilled water.

The filter paper was moistened with varying concentrations (1, 3, 5, 10, 50, 100, 200, 300, 500 ppm) of heavy metal solutions, over a period of 7 days. For the treatment 5 mL of solution was added on the first day followed by 2 ml on alternate days or depending on the level of moisture every day. Triplicates of each treatment were studied in completely randomized design along with a separate set of control using distilled water.

A stock solution of chromium (1000 ppm) was prepared using Potassium dichromate ($K_2Cr_2O_7$) of analytical grade obtained from Loba Chemie. Accurately weighed 2.828 g of 99.9% analytical grade $K_2Cr_2O_7$ in 1000 mL of distilled water to obtain 1000 ppm of Cr stock solution. In order to obtain desired concentrations of 1, 3, 5, 10, 50, 100, 200, 300, 500 ppm for the treatment of seeds of selected crops, the stock solution was further diluted.

Germination was observed after 24 hours and for further 7 days to record a constant percentage of germination. Total germination (GT) and Seedling vigour index (SVI) [6], length of the radicle (cm), length of the plumule (cm) and fresh weight (g) were parameters selected and recorded for this study. Total germination (GT) which is a measure of the time for a population of seeds to germinate in order to estimate its viability and is expressed as a percentage and it was calculated using the formula GT=no. of seeds germinated/total seeds x 100. Length of the radicle (cm) and length of the plumule (cm) of the seedlings were noted after a period of 7 days using a standard centimetre scale. Fresh weight (g) of each individual seedling was recorded using a digital balance.

Seed vigour index (SVI) vigour helps understand the potential for emergence and development of seedlings in field conditions. SVI is considered to be a sensitive and important component of germination studies as it provides a better understanding of seed damage and deterioration and response to stressors.

Seedling vigour index was calculated by following formula:

SVI=Germination% × Seedling length (cm)

Seedling length=RL+SL where RL is root length (cm), SL is shoot length.

Statistical analysis of the data was done using SPSS software ver. 11.0. The data was processed using excel 2013 for paired t-test to establish the level of significance at 0.01 and 0.05.

Results and Discussion

Effect of chromium on total germination

As per the data in Table 1 higher concentrations of chromium severely affected the germination of selected plants, as compared to control. Amongst the five species, the germination percentage of *Trigonella foenum-graceum* L was recorded to be the lowest whereas, *Vigna radiata* L showed higher tolerance to chromium treatment in comparison to others. The effects on germination of *Pennisetum glaucum* L and *Sorghum vulgare* L are fairly comparable across all the concentrations, with a significant drop in the germination percentage from 200 ppm onwards. *Oryza sativa* L fared poorly with increasing concentrations of chromium, with no germination recorded at 300 and 500 ppm. The reduced germination of seeds under Cr stress would be due to the depressive effect of Cr on the subsequent transport of sugars to the embryo axis. Protease activity increases simultaneously with the chromium treatment which could also contribute to the reduction in germination of chromium treated seeds [7].

Table 1. *Effect of chromium on total germination (%).*

Concentration in ppm	*Vigna radiata L*	*Trigonella foenum-graceum L*	*Pennisetum glaucum L*	*Sorghum vulgare L*	*Oryza sativa L*
Control	100	100	100	100	100
1	100	95	65	100	95
3	100	90	65	95	100
5	100	100	95	90	100
10	100	95	95	85	95
50	100	70	95	70	95
100	100	100	90	90	55
200	55	40	50	55	10
300	70	30	50	60	0
500	40	10	40	45	0

Effect of chromium on length of radicle

Based on the values recorded in Table 2, detrimental effects were observed from 50 to 500 ppm. A slight emergence of radicle was observed above 50 ppm for plant studied except *Vigna radiata* L, with a recorded length of (1.99 ± 0.98) cm at 300 ppm. Heavy metal treatment from 50 ppm onwards drastically decreased the radicle emergence in all the plants. *Trigonella foenum-graceum* L and *Oryza sativa* L showed no radicle emergence above 200 ppm. Similar values were recorded in *Pennisetum glaucum* L. (0.41 ± 0.26) cm and *Sorghum vulgare* L (0.42 ± 0.36) cm at 50 ppm. In plants, roots are the first organs to come into contact with toxic elements and they usually accumulate more metals than shoots [8-10] thus inhibiting the growth and roots. Roots accumulate higher amount of chromium than the shoot. It could be due to immobilization of chromium in the vacuoles of the root cells, thus rendering it less toxic, which may be a natural toxicity response of the plant [11]. Based on the work of several researchers, major morphological effects of heavy metals on roots can be decrease in root elongation, root hair collapse, and decrease in the number of roots [12-14].

Table 2. *Effect of chromium on length of radicle (cm).*

Concentration in ppm	*Vigna radiata L.*	*Trigonella foenum-graceum L.*	*Pennisetum glaucum L.*	*Sorghum vulgare L.*	*Oryza sativa L.*
Control	11.54 ± 1.88	3.9 ± 0.90	7.63 ± 3.10	8.21 ± 1.96	5.75 ± 0.97
1	10.75 ± 1.89$^{\#}$	2.85 ± 1.54*	6.13 ± 4.44^{ns}	7.92 ± 1.63^{ns}	4.85 ± 1.25°
3	11.43 ± 2.88^{ns}	3.64 ± 1.31^{ns}	9.89 ± 2.75°	3.44 ± 1.77*	2.58 ± 0.67*
5	11.18 ± 2.07 ^{ns}	2.16 ± 0.92*	7.12 ± 3.84^{ns}	4.82 ± 1.75*	1.70 ± 0.69*
10	9.86 ± 1.40*	2.19 ±.092*	3.30 ± 1.66*	3.04 ± 0.66*	1.01 ± 0.42*
50	6.59 ± 2.54*	1.28 ± 0.65*	0.41 ± 0.26*	0.42 ± 0.36*	0.34 ± 0.18*
100	5.44 ± 1.89*	0.89 ± 0.38*	0.32 ± 0.32*	0.16 ± 0.05*	0.17 ± 0.11*
200	2.07 ± 1.30*	0.5 ± 0.16*	0.17 ± 0.18*	0.14 ± 0.15*	0.2 ± 0.00*
300	1.99 ± 0.98*	00 ± 00	0.14 ± 0.60*	0.11 ± 0.03*	00 ± 00
500	0.08 ± 0.70*	00 ± 00	0.13 ± 0.05*	0.1 ± 0.5*	00 ± 00

$^{*}p<.01$ $^{\#}p<.10$^{ns} -not significant $^{\circ}p<.05$

Effect of chromium on length of plumule

Shoot length gradually decreased with the increase in chromium concentrations (Table 3). The highest length was observed in control *Vigna radiata* L seedlings (12.04 ± 1.36) cm. More pronounced effect on seedling growth was observed above 50 ppm chromium concentrations.

Shoot length due to heavy metal stress decreased in order; *Trigonella foenum-graceum* L>*Oryza sativa* L>*Pennisetum glaucum* L>*Sorghum vulgare* L>*Vigna radiata* L. Decrease in shoot length is obvious since destruction of root cells by Cr may cause decrease in nutrient and water mobility from root to shoot [15].

Table 3. *Effect of chromium on length of plumule (cm).*
$^{*}p<.01$ #p<.10 ns-not significant $^{\circ}p<.05$

Concentration in ppm	***Vigna radiata*** **L**	***Trigonella foenum-graceum*** **L**	***Pennisetum glaucum*** **L**	***Sorghum vulgare*** **L**	***Oryza sativa*** **L**
Control	12.04 ± 1.36	6.69 ± 0.72	5.95 ± 1.10	6.74 ± 2.51	4.77 ± 0.40
1	5.15 ± 1.52*	5.46 ± 1.16*	3.98 ± 1.74*	7.78 ± 1.00°	4.6 ± 0.54^{ns}
3	5.65 ± 1.55*	6.84 ± 2.03^{ns}	5.13 ± 1.29°	4.95 ± 1.75*	4.02 ± 0.59*
5	4.67 ± 1.80*	5.37 ± 1.34*	4.58 ± 1.29*	6.18 ± 1.66^{ns}	3.87 ± 0.74*
10	3.65 ± 1.18*	5.09 ± 2.08*	4.91 ± 1.19*	5.265 ± 0.72*	3.96 ± 0.96*
50	3.24 ± 1.56*	3.915 ± 1.71*	2.96 ± 1.67*	3.25 ± 1.29*	2.13 ± 0.85*
100	2.36 ± 1.17*	1.76 ± 0.86*	2.4 ± 1.07*	2.175 ± 1.01*	0.79 ± 0.41*
200	1.00 ± 0.79*	00 ± 00	1.27 ± 0.72*	1.94 ± 0.72*	0.32 ± 0.08*
300	0.81 ± 0.86*	00 ± 00	0.94 ± 0.40*	1.07 ± 0.59*	00 ± 00
500	1.01 ± 0.04*	00 ± 00	0.49 ± 0.46*	0.565 ± 0.52*	00 ± 00

Effect of chromium on fresh weight

With a reported decrease in the root growth and shoot growth, subsequently the fresh weight of the plant is bound to decrease. Amongst the monocotyledonous plants minimum seedling weight was recorded in *Pennisetum glaucum* L at (0.0199 ± 0.00) g and *Oryza sativa* L at (0.0212 ± 0.001) g, whereas in dicotyledonous plants *Trigonella foenum-graceum* L showed a low of (0.0641 ± 0.002) g (Table 4). This decrease in biomass productivity might be attributed to a disruption in nitrogen metabolism of seedlings under chromium stress [16].

Table 4. *Effect of chromium on fresh weight (g).*
*p<.01 #p<.10 ns-not significant $^{\circ}$p<.05

Concentration in ppm	***Vigna radiata* L**	***Trigonella foenum-graceum* L**	***Pennisetum glaucum* L**	***Sorghum vulgare* L**	***Oryza sativa* L**
Control	0.2937 ± 0.05	0.1673 ± 0.03	0.0663 ± 0.01	0.1214 ± 0.01	0.0451 ± 0.004
1	0.2379 ± 0.06*	0.1283 ± 0.03*	0.0642 ± 0.02^{ns}	0.1266 ± 0.03^{ns}	0.0401 ± 0.00*6
3	0.2195 ± 0.05*	0.1188 ± 0.04*	0.0462 ± 0.01*	0.0941 ± 0.03*	0.0321* ± 0.006*
5	0.2302 ± 0.06*	0.1206 ± 0.04*	0.0629 ± 0.03^{ns}	0.0849 ± 0.02*	0.0386 ± 0.005*
10	0.2091 ± 0.04*	0.1267 ± 0.04*	0.0444 ± 0.01*	0.0791 ± 0.01*	0.0328 ± 0.005*
50	0.2295 ± 0.06*	0.1255 ± 0.03*	0.0321 ± 0.01*	0.0767 ± 0.02*	0.0293 ± 0.003*
100	0.2261 ± 0.04*	0.1069 ± 0.02*	0.0296 ± 0.01*	0.0666 ± 0.03*	0.0249 ± 0.002*
200	0.1599 ± 0.03*	0.0641 ± 0.002*	0.0199 ± 0.00*	0.0447 ± 0.00*	0.0212 ± 0.001*
300	0.1406 ± 0.04*	00 ± 00	0.0178 ± 0.00*	0.0465 ± 0.00*	00 ± 00
500	0.1260 ± 0.04*	00 ± 00	0.0141 ± 0.00*	0.0372 ± 0.01*	00 ± 00

Effect of chromium on seed vigour index

The decrease in the total germination percentage, length of radicle and plumule has a negative influence on seed vigour of all species. A decline in the average SVI was observed from the highest recorded value of 1587 in *Vigna radiata* L at 1 ppm to 72.60 at 500 ppm (Table 5). In *Trigonella foenum-graceum* L the overall health was significantly affected based on the drop in seed vigour index from 789.92 to 2.50. Increasing concentration of chromium decreased seed vigour compared to the control samples, for *Pennisetum glaucum* L, *Sorghum vulgare* L. ranging from 1570 to 29.92. As no germination was recorded in *Oryza sativa* L. at 300 and 500 ppm the SVI calculated is zero. The findings of this study were in agreement with other researchers who reported increasing metal concentrations decreased rapeseed seedling vigour indexes [17-20].

Table 5. *Effect of Chromium on Seed Vigour Index (SVI).*

Ppm	***Vigna radiata* L**	***Trigonella foenum-graceum* L**	***Pennisetum glaucum* L**	***Sorghum vulgare* L**	***Oryza sativa* L**
Control	2358	1059	1362.5	1495.5	1034.5
1	1587	789.92	657.47	1570	898.2
3	1699.5	943.2	974.35	797.05	660.5
5	1586	753.5	1111.97	990.45	557.5
10	1352	692.07	780.9	705.92	472.1
50	984	363.65	321	256.9	235.6
100	781	265.5	244.8	210.15	52.8
200	168.8	23.2	72	114.67	5.2
300	196.7	7.5	54.25	70.8	0
500	72.6	2.5	25.2	29.92	0

Conclusion

The results of this study showed that chromium at all concentrations had a negative effect on all the growth parameters in comparison to control. It can be concluded that the level of chromium at and above 200 ppm proved to be lethal to all the crops selected. Based on the comparison of the toxic effect on germination percentage and overall seedling growth, *Vigna radiata* L, has coped best to heavy metal stress, while *Trigonella foenum-graceum* L, and *Oryza sativa* L seedlings were seriously inhibited. The accumulation of chromium in plants depends on its oxidation state, studies have reported, the chromium accumulation was higher when contaminated with Cr (VI), as compared Cr (III). It has also been reported that the absorption mechanisms of Cr(III) and Cr(VI) are different suggesting that use of metabolic inhibitors decreases the uptake of Cr(VI) but that was not the same for Cr(III).

Since the plants selected in this study were common crops, our results can be considered as indicator of tolerance potential for chromium, in order to devise strategies to overcome stress by understanding the biochemistry of heavy metal toxicity, for their potential to be planted in contaminated sites and lower the risk to man of ingesting a contaminated produce as soil contaminated by chromium is a major concern for sustainable agriculture.

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