

Effect of Heavy Metals on Seed Germination of *Trigonella foenum-graceum* L.

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ABSTRACT- Contamination of soil by heavy metals is an ecological problem on a global level, this contamination affects agricultural crops in the area concerned. In the present study, Copper, Zinc and Chromium being heavy metals have been assessed for their injurious effects on seed germination and seedling growth of *Trigonella foenum-graceum* L. solutions of the heavy metals were prepared in concentrations ranging from 1, 3, 5, 10, 50, 100, 200, 300, 500 ppm for irrigating the seeds of the crop to be germinated in Petri-dishes for seven days. The young seedlings were studied for their response based on seed vigour index, length of radicle, length of plumule and fresh weight against seeds germinated using distilled water as control. It was observed that toxic effect of heavy metals on fenugreek growth was as follows: Cr>Cu>Zn on the basis of a decrease in germination percentage and overall poor health of the seedling.

Key-words- Chromium, Copper, Zinc, Germination, *Trigonella foenum-graceum* L., Toxicity

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INTRODUCTION

Heavy metals are not pollutants but a component of the earth's crust, it's the excessive rise of anthropogenic activities over the past few decades has led to the contamination of soil and water presenting us with a problem of heavy metal pollution. Heavy are metallic elements with an atomic weight more than five and they are highly toxic even at low concentration. Plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage and disturbance of cellular ionic homeostasis (1).

Some plant species have capacity to grow in the metal contaminated soil and accumulate elevated amount of heavy metals (hyper-accumulation) as an eco-physiological adaptation in metalliferous (2). Sources of Cu contamination include mining and smelting, urban, industrial and agricultural wastes, and the use of agrochemicals. Copper is present in many forms in soils, with free Cu²⁺ activity considered to be the best indicator of bioavailability (3). The effect of Cu toxicity is largely on root growth and morphology.

Chromium is an environmental pollutant that ranks seventh in abundance within the earth crust (4). Chromium is used on a large scale in many industries, including metallurgical, electroplating, production of paints and pigments, tanning, wood preservation, chromium chemicals production, pulp and paper production (5).

Zinc is an essential nutrient with a vital role as a cofactor of enzymes, synthesis of chlorophyll and carotenoids, regulation of cytoplasmic concentration of nutrients, albeit in high doses it causes abiotic stress and affects plant growth negatively. Considerable work on plants and heavy metals has been reviewed by several authors (6-7). Germination and early seedling growths have been regarded as critical phases, which are greatly influenced by stressful conditions (8). Growth changes are the first most obvious reactions of plants under stress. Heavy metals uptake and accumulation in plants have been shown to result in negative effects on plant growth (9). Several plants such as *Amaranthus viridis* L., *Oryza sativa* L., *Vigna radiata* L., *Abelmoschus esculentus*, *Brassica juncea* L. (10) have been studied for their phytotoxic responses to chromium as well as for their phyto-remediation potential.

The present study was conducted to investigate and compare the detrimental effects of varying concentrations of chromium, copper and zinc on *Trigonella foenum-graceum* L. to understand the response of this plant to metal stress and illustrate its tolerance potential for the same.

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MATERIALS AND METHODS

The research was conducted over a period of 10 months at the department of Botany, Rizvi College of arts, science and commerce, Mumbai, India. Seeds of *Trigonella foenum-graecum* L. were obtained from Namdeo Umaji Agritech (India) Pvt. Ltd. Different concentration solutions of Chromium, Copper and Zinc was prepared using anhydrous potassium dichromate (K₂Cr₂O₇), Copper sulphate pentahydrate (CuSO₄.5H₂O) and Zinc sulphate monohydrate (ZnSO₄) of analytical grade obtained from Loba Chemie.

1, 3, 5, 10, 50,100,200,300,500 ppm solutions were prepared for each metal. Ten surface sterilized seeds uniform in colour, weight and size were placed on a Petri dish (9 cm diameter) on double-layered Whatman filter paper No. 1. The seeds were sterilized using Bavistin solution for 5 minutes followed by a through rinse, using distilled water. The filter paper was moistened with varying concentrations of heavy metal solutions, 5mL on the first day followed 2 ml on alternate days for 7 days. Triplicates of each treatment in completely randomized designed were studied along with a separate control series using distilled water.

Germination indices i.e. total germination (also known as final germination percentage) (GT) and Seedling vigour index (SVI) (11), length of the radical, length of the plumule and fresh weight were recorded at the end of seven days.

Total Germination: the final Germination percentage 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 (%). The total germination (GT) was calculated using the following formula:

GT = No. of seeds germinated/Total seeds x 100

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

RESULTS AND DISCUSSION

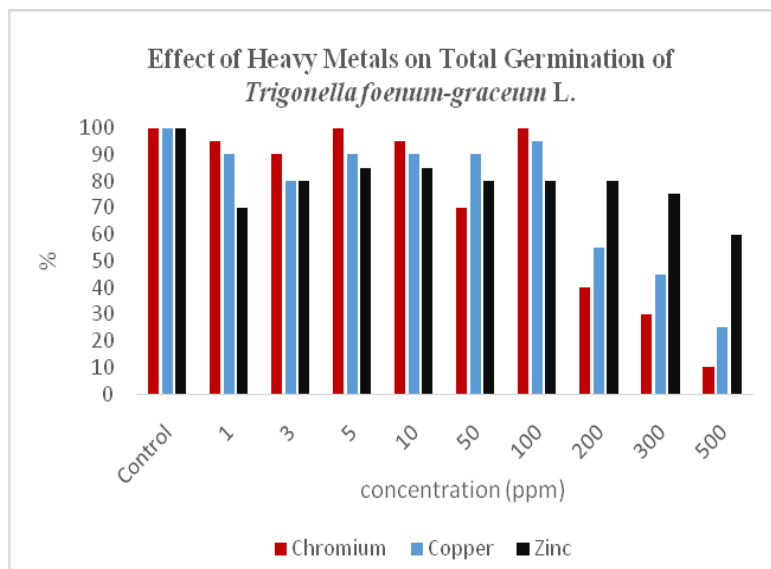
Effect of Heavy Metals on Total Germination of *Trigonella foenum-graecum* L.

A general trend observed in the present study reveals that higher concentration of Chromium, Copper and Zinc solutions adversely affected the germination of selected angiospermic plants (Table1). Chromium interferes with several metabolic processes, causing toxicity to plants as exhibited by reduced seed germination or early seedling growth (12). Metabolic alteration has also been reported in plants as the direct effect on enzymes and metabolites or by its ability to generate ROS (13). Even tough Cu and Zn are considered to essential micro nutrients, there is a clear decrease in total germination when subjected to higher concentrations. The effects of copper on germination of fenugreek remained fairly unaffected by concentrations

below 100ppm with a noticeable drop in the germination percentage thereafter. 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 (14). Protease activity increases simultaneously with the chromium treatment which could also contribute to the reduction in germination of chromium treated seeds (14).

Table 1: Effect of Heavy Metals on Total Germination of *Trigonella foenum-graceum* L.

ppm	Total germination (%)		
	Cr ⁶⁺	Cu ²⁺	Zn ²⁺
Control	100	100	100
1	95	90	70
3	90	80	80
5	100	90	85
10	95	90	85
50	70	90	80
100	100	95	80
200	40	55	80
300	30	45	75
500	10	25	60



Effect of Heavy Metals on radicle emergence of *Trigonella foenum-graecum* L.

Treatments with varying concentration of Cr⁶⁺ showed a significant effect on the emergence of radical (Table 2). A progressive decrease was observed with an increase in concentrations, the average length of radicle was fairly unaffected at 1, 3 and 5ppm although the detrimental effects of chromium were observed from 50-500 mg.kg⁻¹ with slight to no emergence of radicle respectively. Death of all the seedlings was observed at 300 and 500 ppm. The roots subjected to higher concentrations of Cr⁶⁺ were observed to be brownish in colour in comparison with control. Decrease in root growth in presence of Cr⁶⁺ can be

explained by inhibition of root cell division and/or elongation, which might have occurred as a result of tissue collapse and consequent incapacity of the roots to absorb water and nutrients from the medium (15) combined with extension of cell cycle (16). A reduction in the root surface and root hair could induce further stress viz. decrease in absorption of water, establishment of seedling. In comparison, copper and zinc were found to be less harmful, as the seeds germinated at higher concentrations of 300 and 500 ppm with an extremely reduced root growth. This reduction in root growth of seeds treated with copper could be attributed to the inhibition of amylase and other hydrolytic enzymes. Root growth is particularly sensitive to the Cu and Zn toxicity (17). (18) has studied the effect of copper (Cu) and zinc (Zn) toxicity on growth of mung bean (*Phaseolus aures* Roxb. cv VC- 3762) in a solution culture.

Table 2: Effect of Heavy Metals on Length of radicle of *Trigonella foenum-graceum* L.

Length of radicle (cm)			
ppm	Cr ⁶⁺	Cu ²⁺	Zn ²⁺
Control	3.24±1.25	3.24±1.25	3.24±1.25
1	2.85±1.54**	3.09±1.59	2.41±0.55
3	3.64±1.31^	2.05±0.89**	2.56±0.53
5	2.16±0.92**	2.88±1.37	3.37±1.01
10	2.19±0.92**	2.48±0.75**	2.78±0.49
50	1.28±0.65**	1.59±0.80*	2.15±0.42
100	0.89±0.38**	0.86±0.26*	1.60±0.48
200	0.5±0.16**	0.46±0.49*	1.83±0.46

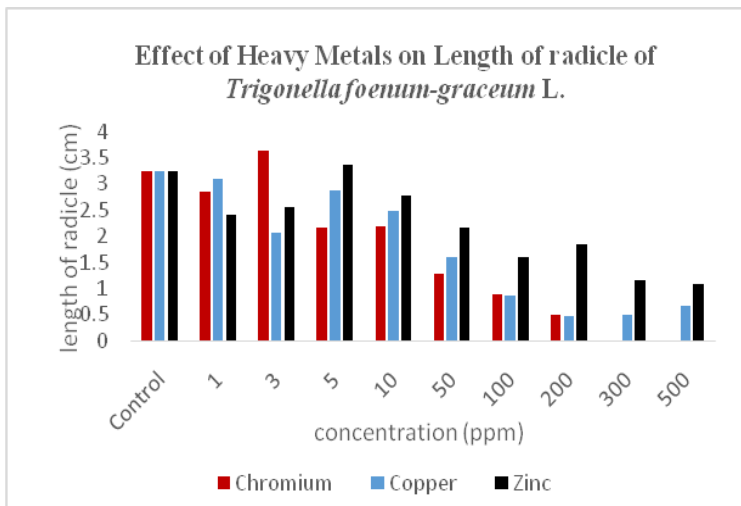
Sat p<.01 **NS p<.05 ^ NS at p<.01 Cr*S at p<.001 **S at p<.05 Zn*S at p<.001 **S at p<.05 Cu

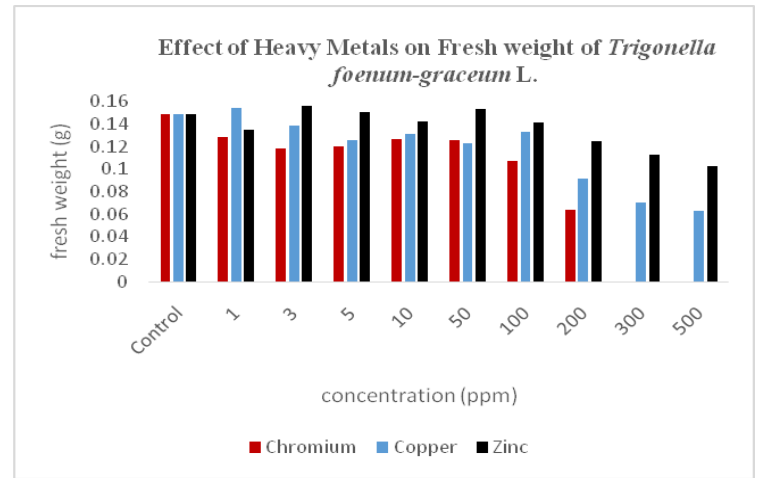
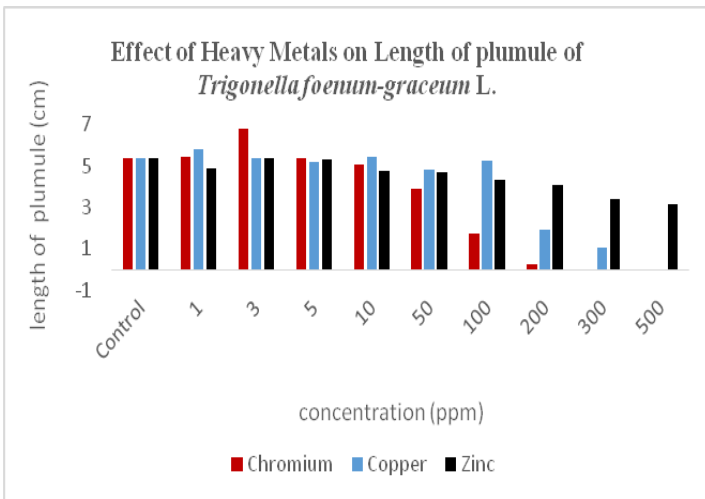
Effect of Heavy Metals on length on plumule of *Trigonella foenum-graceum* L.

All metal treatments affected the development and growth of plumule, the shoot lengths of fenugreek were significantly reduced compared to control treatment (Table 3). In the case of chromium, the shortest shoots (0.24 cm) were observed at 200ppm concentration, however further increase in concentrations caused a total failure of seed germination. Copper and zinc developed shoots with the least average length of (1.93 cm) and (4.07 cm) respectively at 200ppm. Reduction in shoot and root lengths could be due to excess accumulation of salts in the cell wall, which modifies the metabolic activities negatively and limits the cell wall elasticity (19). Root length was more affected than shoot length by both copper and zinc concentrations. The inhibition of root elongation caused by heavy metals may be due to metal interference with cell division, including inducement of chromosomal aberrations and abnormal mitosis (20). These results agreed with findings on the Cluster Bean (21); wheat (22); the Psyllium (23); and on Mung bean (24). Heavy metal accumulation was markedly higher in roots as compared to shoots, while both showed a gradual decrease in growth, the effect was more pronounced in roots (25).

Table 3: Effect of Heavy Metals on Length of plumule of *Trigonella foenum-graceum* L.

Length of plumule (cm)			
ppm	Cr ⁶⁺	Cu ²⁺	Zn ²⁺
Control	5.38±1.60	5.38±1.60	5.38±1.60
1	5.46±1.16*	5.8±1.15	4.9±0.86
3	6.84±2.03*	5.46±1.45	5.37±0.84
5	5.37±1.34**	5.22±2.21	5.31±1.01
10	5.09±2.08**	5.44±0.89	4.77±0.47
50	3.915±1.71*	4.85±0.9	4.71±0.73
100	1.76±0.86*	5.24±1.0	4.33±1.15
200	0.24±0.08*	1.93±0.09*	4.07±0.70





Effect of Heavy Metals on fresh weight of *Trigonella foenum-graceum* L.

The influence of heavy metals on fenugreek also shows a gradual decrease in the fresh seedling weight with an increase in concentration (Table 4). The results of germination experiments for chromium report a decrease in the root growth and shoot growth, subsequently decrease the fresh weight of the plant, 1 ppm Cu shows an increase in fresh seedling weight (0.1547g) whereas 3 ppm of Zn concentration also shows an increase in the fresh weight followed by a decrease in weight thereafter, hence these concentrations can be considered as optimal threshold levels beneficial for plant growth. Excess copper induced an increase in heavy metals have been reported to impair the growth of new roots and seedling establishment (26) by subjecting the plant to stress induced by inhibition of root cell division, root elongation or the extension of cell cycle in the roots due to absorption of chromium, higher concentrations of copper increase the production of hydrogen peroxide (H₂O₂) and lipid per-oxidation, thereby causing oxidative stress.

Table 4: Effect of Heavy Metals on Fresh weight of *Trigonella foenum-graceum* L.

Fresh weight (g)			
ppm	Cr ⁶⁺	Cu ²⁺	Zn ²⁺
Control	0.1488±0.03	0.1488±0.03	0.1488±0.03
1	0.1283±0.03**	0.1547±0.018	0.1350±0.016
3	0.1188±0.04^	0.1385±0.041**	0.1559±0.046
5	0.1206±0.04^	0.1258±0.027**	0.1503±0.024
10	0.1267±0.04**	0.1317±0.026	0.1421±0.014
50	0.1255±0.03^	0.1227±0.025**	0.1539±0.022
100	0.1069±0.02*	0.1330±0.018*	0.1415±0.028
200	0.0641±0.002*	0.0919±0.018*	0.1249±0.025

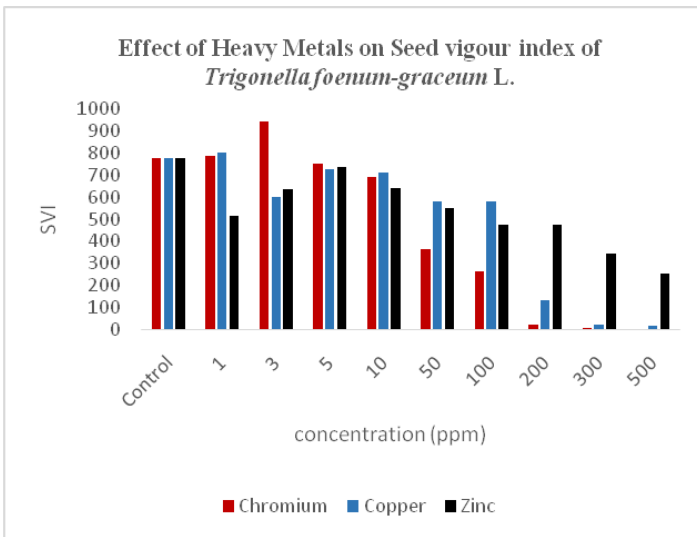
Effect of Heavy Metals on SVI of *Trigonella foenum-graceum* L.

The negative influence of heavy metals on SVI can be attributed to the decline in germination percentage, the length of radicle and plumule and fresh weight. A drastic reduction in the average SVI was observed with chromium treatments (Table 5) from the value of 789.925 recorded at 1 ppm followed by a spike at 3 ppm (943.2) to 2.5 at 500 ppm.

Increasing concentrations of zinc and copper showed decreased seed vigour compared to the control samples. Increasing zinc concentrations from control to 500ppm decreased SVI 775.8 to 253.5. Similarly, for copper a drastic drop was observed 800.55 to 16.5 when the concentrations were increased from 1 ppm to 500ppm. However, for all three metals 10ppm can be considered as a threshold concentration. Similar results were reported by (27) and (28); stating that increasing metal concentrations decreased tolerance and seedling vigour indexes in wheat and rapeseed and by (29) that there was a reduction in vigour index in four genotypes of soybean at 5-200 mg·L⁻¹ concentration of Chromium, with respect to control application stating that Increasing concentration of heavy metal significantly reduce the strength of germination as compare to the lowest concentration of heavy metal which have the least harmful influence on the germination (30).

Table 5: Effect of Heavy Metals on Seed vigour index of *Trigonella foenum-graceum* L.

Seed Vigour Index (SVI)			
ppm	Cr ⁶⁺	Cu ²⁺	Zn ²⁺
Control	775.8	775.8	775.8
1	789.925	800.55	516.95
3	943.2	601.2	634.8
5	753.5	729	738.65
10	692.075	713.25	641.75
50	363.65	579.6	548.8
100	265.5	579.975	475.2
200	23.2	132	472.8
300	7.5	22.275	344.25
500	2.5	16.5	253.5



CONCLUSIONS

The result of this study showed that increasing concentrations of heavy metals (Cr^{6+} , Cu^{2+} and Zn^{2+}) inhibited seed germination and limited seedling growth. Of the selected heavy metals, the seedlings were more sensitive to chromium stress than copper and zinc. The overall inhibitory effect on seedling growth based on the seed vigour index which was more pronounced in chromium treatment. This information can be a useful factor in finding the tolerance and uptake limit of *Trigonella foenum-graceum* Lat different concentrations.

The toxicity of copper and zinc increased considerably at ≥ 50 ppm concentrations. Moreover, copper has more toxic effect than zinc at high concentrations on seedling growth. These findings can be used practically for selection and for application with caution of pesticides with high concentrations of copper and zinc. Results of the study are useful indicators of tolerance to some extent for plantation and safe limits for consumption of *Trigonella foenum-graceum* L. contaminated areas. However, extended research is necessary in metal contaminated soil and water, to determine and overcome the detrimental effect of different metals in the environment on plants.

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