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Response to Cadmium stress in *Vigna radiata* L., *Trigonella foenum-graceum* L., *Oryza sativa* L. and *Pennisetum glaucum* L.

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Abstract: Industrialization has resulted in the heavy metal contamination of agricultural soil. In the recent past, extensive studies on heavy metals are being carried out throughout the world, to understand the disturbances caused in the ecosystems. In the present study, Cadmium was assessed for its injurious effect on seed germination and seedling growth of *Vignaradiata*L., *Trigonella foenum-graceum* L., *Oryzasativa*L. and *Pennisetumglaucum*L. The seeds growing in petri dishes were exposed to cadmium in increasing concentrations of 1,3,5,10,50,100,200,300,500 ppm. Each treatment was replicated in a randomised design and observed over a period of 7 days. The seedlings were studied for their response based on germination rate, seed, vigour index, length of radicle, length of plumule and fresh weight against seeds germinated using distilled water as control. The intensity of inhibitory effect on all other parameters was directly proportional to the concentration of cadmium solution employed and inhibition was prominent from 50 ppm onwards. Based on the overall health of the seedling, the observed toxic effect of Cadmium was pronounced in *Trigonella foenum-graceum* L. >*Oryza sativa* L. >*Pennisetum glaucum* L. >*Vigna radiate* L.

Key words: cadmium; seed germination; toxicity; crop.

Introduction

Heavy metals are primarily components of the earth's crust, not pollutants the significant rise in 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 metals are metallic elements with a relatively high density that are required in very small amounts for the maintenance of human and animal health. Due to their high atomic density of 4g/cm^3 heavy metals are toxic even at low concentrations, causing damage to the organism by accumulating and displacing vital nutrients in the tissues, thereby disrupting organ function. Some heavy metals are required for plant growth; however, at higher levels it becomes toxic to plants. Cadmium is a non-essential element that negatively affects plant growth and development. It is released into the environment by power station, heating systems metal working industry, urban traffic, mining activities. It is widely used in electro plating pigments, plastic stabilisers, Ni-Cd batteries. (Sanita di Toppi, L and Gabbrielli, R., 1999.) Cadmiumcould also enter the soil or water from spills or leaks at hazardous waste sites if large amounts of dissolved cadmium were present at the site where it was potentially available to rooted plants. (Munzuroglu O., Geckil H. 2002. Ozdener Y., Kutbay H.G. 2009. Prodanovic O. *et al.,* 2012. Salvator *et al.,* 2008.). It is recognised as an extremely significant pollutant due to its high toxicity and large solubility in water. (Pinto A.P *et al.,* 2004). Greger (1999) reported that the uptake of Cd, both by roots and stems increased with increasing metal concentration in the external medium but the uptake was not linear in correlation with the concentration increase. Similarly, Ishikawa *et al.,* (2006) evaluated the ability of *Brassica juncea* L., which has already been recognized as a plant suitable for metal phytoremediation, and of several other cultivated plant species (maize, rice, and sugar beet), to extract cadmium from soils with moderately low levels of Cd contamination. Results by Moosavi *et al.,* (2012) in canola (*Barassica napus*), wheat (*Triticum aestivum*) and safflower (*Carthamus tinctorious*) showed that the percentage of seed germination, root and shoot length decreased as concentrations of solution increased with No germination was observed at 1000 ppm of cadmium level. There was no seedling growth at 350 and 500 ppm of cadmium and lead concentration. The present study was conducted to investigate and compare the detrimental effects of varying concentrations of cadmium (Cd^{2+}) on seed germination, root and shoot growth of*Vigna radiate* L., *Trigonella foenum-graceum* L., *Oryza sativa* L. and *Pennisetum glaucum* L. The aim of our study was to understand the response and sensitivity of these plants to metal stress and illustrate its tolerance potential for the same by determining the degree of inhibition of seedling growth.

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Material and Methods

Seeds, Planting material and stock solution: Seeds of *Vigna radiata* L., *Trigonella foenum-graceum* L., *Oryza sativa*

L. and *Pennisetum glaucum* L. were studied for the effects of Cd^{2+} on seed germination were from Pyramid Seeds by Namdeo Umaji Agritech (India) Pvt. Ltd. For the germination studies: Ten surface sterilized seeds uniform in colour, weight and size were placed on a Petri dish (9 cm diameter) on double-layered filter paper. The seeds were sterilized using Bavistin @ 200mg in 100ml distilled water for 5 minutes followed by a through rinse again, 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. 1, 3, 5, 10, 50,100,200,300,500 ppm of Cd was prepared from Cadmium sulphate for the treatment of seeds of various crops.

Germination indices i.e. Total germination (GT) and Seedling vigour index (SVI) (Abdul-Baki A, Anderson J.D. 1973), were selected and recorded for this study. Seed germination was observed after 24 hours, for a constant percentage of germination; other growth attributes viz. length of the root and shoot (cm) and fresh weight (g) of the seedlings was recorded on a digital balance after a period of 7 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: Seed vigour helps understand the potential for emergence and development of seedlings in field conditions. Compared to GT, SVI being more sensitive is an 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.

Results and Discussion

Effect of cadmium toxicity on seed germination

The present study showed a general trend where higher concentrations of cadmium solutions adversely affected the germination of selected angiospermic plants (table 1). The total germination of the selected showed significant difference compared to control. Amongst the four species the germination percentage of *Trigonella foenum-graceum* L. were lower than the other three. In case of *Vigna radiata* L. germination was not significantly affected by cadmium treatment showing better tolerance. The effects on germination of *Oryza sativa* L. and *Pennisetum glaucum* L. are fairly comparable for concentrations below 50ppm with a noticeable drop in the germination percentage thereafter. The observed reduction in seed germination can be attributed to alterations of selective permeability properties of cell membrane (Breckle S.W. and Kahle H., 1992) as well as the accelerated breakdown of stored food materials in seed (Heidari M., Sarani S, 2011)

Effect on SVI

The decrease in the total germination percentage and early seedling growth has contributed to the negative influence on seed vigour of all four species. (Table 2) A decline in the average SVI was observed from the highest recorded value of 1951.5 in *Vigna radiate* L. to 102.4 in *Trigonella foenum-graceum* L. stating the overall health of the selected crops was significantly different among cadmium treatment and control. Studies have shown that seed coats are effective barriers to metals and can prevent contamination of embryos until the seed coat is torn apart by the germinating embryonic root (Munzuroglu O., Geckil H. 2002). Increasing concentration of cadmium decreased seed vigour compared to the control samples, for *Pennisetum glaucum* L. and *Oryza sativa* L. drop was observed 1362.5 to 519.75 and 1034.5 to 386.6 respectively when the concentrations were increased from control to 10ppm.

Effect on seedling growth

Table 3, 4 and 5 show the affected development of seedlings of all plant species upon treatment with varying concentration of cadmium. Cd in cells gets associated with cell walls and middle lamella and increases the cross-linking between the cell wall components, resulting in the inhibition of the cell expansion growth (Poschenrieder C. *et al.,* 1989). Based on the observations, detrimental effects of cadmium were from 50 to 500ppm with slight to no emergence of radicle respectively, therefore no data was recorded at higher concentrations as all plants were dead at germination stage. The seedlings showed browning of roots at higher concentrations of Cd^{2+} with the effect being most pronounced at 50ppm. Cadmium treatment at 10ppm showed a significant decrease in radicle emergence in comparison with control, with a pronounced effect on *Trigonella foenum-graceum* L. (1.28 cm) than the other crops. Root length of all the species were inhibited but *Vigna radiata*L. (5.02 cm) performed better under Cd stress compared to others. Shoot length follow similar trend as root length against Cd stress. It was also observed that further increase in concentration of Cd from 1 to 10ppm remarkably reduced shoot length with maximum inhibition at 50ppm. Shoot length in Cd stress decreased in order; *Trigonella foenum-graceum* L. (1.28 cm) >*Oryza sativa* L. (3.17 cm) >*Pennisetum glaucum* L. (4.09 cm) >*Vigna radiate* L. (6.21cm). The reduction in shoot length could be attributed to the adverse effect of heavy metal on the reduction in meristematic cells present in this region and some enzyme contained in cotyledon and endosperm and thereby, reduction the cell elongation and cell expansion (Houshmandfar A, Moraghebi F. 2011). The results of germination experiments report a decrease in the root growth and shoot growth, subsequently decreasing the fresh weight of the plant. Amongst the monocotyledonous plants least seedling weight was reported in *Oryza sativa* L. at 0.0303g. Cd toxicity leads to protein degradation through amino acid metabolism resulting in decreased plant growth (Dinakar N, *et al.,* 2008.).

Table 1: Effect of Cadmium on Total Germination

Cadmium	Total germination (%)			
Ppm	***Vigna radiata* L.**	***Trigonella foenum-graceum* L.**	***Pennisetum glaucum* L.**	***Oryza sativa* L.**
Control	100	100	100	100
1	100	90	85	85
3	90	70	85	90
5	100	60	75	65
10	100	80	90	75

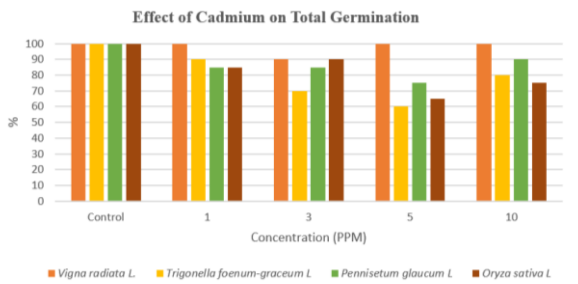


Table 2: Effect of Cadmium on Seed vigour index

Cadmium	Seed Vigour Index (SVI)			
Ppm	***Vigna radiata* L.**	***Trigonella foenum-graceum* L.**	***Pennisetum glaucum* L.**	***Oryza sativa* L.**
Control	2358	1059	1362.5	1034.5
1	1951.5	467.55	887.4	595.42
3	1263.15	336.7	1013.6	601.2
5	1335	194.7	520.12	337.02
10	1124	102.4	519.75	386.6

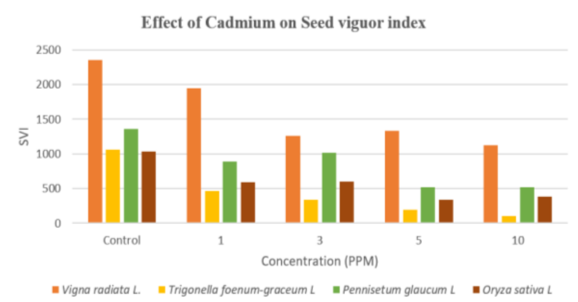


Table 3: Effect of Cadmium on Length of Radicle

Cadmium	Length of Radicle (cm)			
Ppm	***Vigna radiata* L.**	***Trigonella foenum-graceum* L.**	***Pennisetum glaucum* L.**	***Oryza sativa* L.**
Control	11.24±1.88	3.9±0.90	7.63±3.10	5.75±0.97
1	9.32±0.88*	2.24±0.58*	4.69±0.56*	2.89±0.65*
3	6.05±1.08*	1.5±0.50*	5.72±1.34*	2.66±0.61*
5	6.29±0.88*	1.28±0.25*	2.94±0.90*	1.9±0.61*
10	5.02±0.79*	1.28±0.38*	1.68±0.47*	1.98±0.61*

*significant at $p < .01$

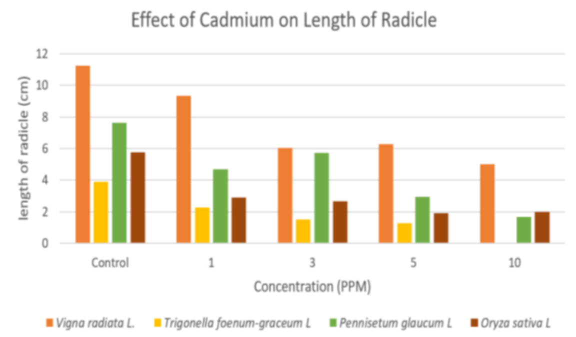


Table 4: Effect of Cadmium on Length of Plumule

Cadmium	Length of Plumule (cm)			
Ppm	***Vigna radiata* L.**	***Trigonella foenum-graceum* L.**	***Pennisetum glaucum* L.**	***Oryza sativa* L.**
Control	12.04±1.36	6.69±0.72	5.95±1.10	4.77±0.40
1	10.19±1.23*	2.95±0.31*	5.74±0.61**	4.11±0.58*
3	7.98±1.27*	3.3±0.37*	6.2±1.09**	4.02±0.61*
5	7.06±1.23*	1.96±0.55*	3.99±1.02*	3.28±0.45*
10	6.21±0.86*	0.0±0.0*	4.09±0.83*	3.17±0.30*

* significant at $p < .01$** not significant at $p < .05$

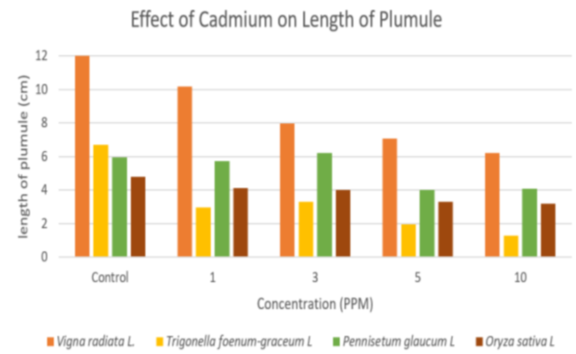
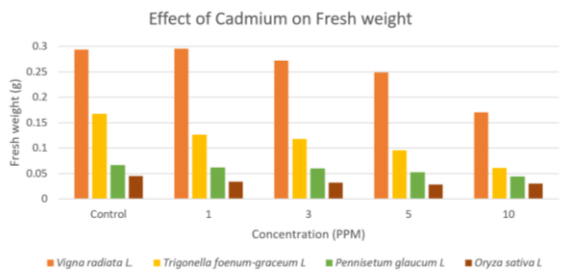


Table 5: Effect of Cadmium on Fresh weight

Cadmium	Fresh weight (g)			
Ppm	***Vigna radiata* L.**	***Trigonella foenum-graceum* L.**	***Pennisetum glaucum* L.**	***Oryza sativa* L.**
Control	0.2937±0.057	0.1673±0.030	0.0663±0.015	0.0451±0.004
1	0.2959±0.056**	0.1262±0.023*	0.0617±0.016#	0.0338±0.004*
3	0.2725±0.051**	0.1182±0.016*	0.0602±0.010#	0.0323±0.005*
5	0.2488±0.066#	0.0958±0.007*	0.052±0.010*	0.0286±0.004*
10	0.1703±0.053*	0.0606±0.010*	0.044±0.008*	0.0303±0.005*

* significant at $p < .01$ ** not significant at $p < .05$ #significant at $p < .05$



Conclusion

The results Obtained from this study revealed that cadmium had a toxic effect on germination percentage and seedling growth of *Vigna radiata* L., *Trigonella foenum-graceum* L., *Oryza sativa* L. and *Pennisetum glaucum* L. Heavy metal cadmium at all tested concentrations produced a negative effect ongrowth parameters as compared with control. The seedlings treated with Cd^{2+}wereseriously inhibited where the root growth was affected more than shoot growth. Plantdefence strategies exist to cope with heavy metal toxicity via reduced uptake into the cell, sequestration into vacuoles by the formation of complexes but mechanisms by which germinating seeds combat heavy metal stress remains largely unknown. (DalCorso G, Farinati S,2010) In l contaminated areas, further research is needed to determine the levels of heavy metals in the environment and its effect on plants, results of these findings can be useful indicators of metal tolerance, developing strategies to overcome stress by understanding the biochemistry of heavy metal toxicity leading to better agricultural yield, thereby lowering the risk to man by contaminated produce.

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