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EFFECT OF DIFFERENT HEAVY METAL STRESS ON SEED GERMINATION PARAMETERS IN *VIGNA UNGUICULATA* (L.) WALP.

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ABSTRACT

Vigna unguiculata L. (cowpea) is legume vegetable which is good source for proteins. The cultivation is affected by various biotic as well as abiotic factors. Heavy metals cause both positive and adverse effects on the growth of the plant. In the present study, effects of various concentrations (10, 50, 100, 200, 500 and 1000 ppm) of $CdSO_4$, $CuSO_4$, $PbNO_3$, $NiSO_4$ and $ZnSO_4$ were observed on seed germination parameters in *Vigna unguiculata* L. results revealed that cadmium and lead affect negatively the germination and seedling growth while copper, zinc and nickel increased germination and growth at their lower concentrations and inhibited at higher concentrations.

Keywords: *Vigna unguiculala,* heavy metals, seed germination, seedling growth

Introduction

Bioaccumulation of heavy metals within biological systems has been linked to their status as a class of non-biodegradable elements. Pollution from heavy metals is a serious environmental issue on a global scale (**Sha and Reddy, 2017**). Scientists say that high levels of Pb in soils typically have a negative impact on crop yields (**Patra *et al*., 2004**). Lead (Pb) can induce withering, stunted growth in plants by interfering with a variety of vital activities, including photosynthesis, water absorption, and mitosis, even at extremely low concentrations. Some researchers have found that even trace amounts of metals including cadmium, mercury, lead, tin, arsenic, and chromium can be fatal to living organisms (**Salt *et al*., 1995**).

Industrial wastes are a major source of heavy metals, which have caused several ecological problems (**Gu *et al.*, 2014**). Children exposed to multiple heavy metals, for instance, showed signs of growth retardation (**Zeng *et al.*, 2008**). Rocks, soil, and water all contain these metals in trace amounts. However, due to industrialization's anthropogenic activities, the environment becomes contaminated, which has consequences for both the food chain and human health (**Jamal *et al.,* 2006**). Plants play a crucial role in ecosystems by facilitating the movement of metals from their nonliving abiotic surroundings into their living biotic ones (**Chojnacka *et al.,* 2005**).

About 7 million hectares are dedicated to growing cowpea (*Vigna unguiculata* L. Walp.), a grain legume, vegetable, and fodder crop that thrives in warm to hot climates across Africa, Asia, and the Americas. The widespread promotion of cowpea for its high protein content has provided many people with a means of subsistence through its production. Therefore, research into how growing cowpea might affect soils contaminated with heavy metals as a result of human activities is warranted. The importance of addressing environmental issues is paramount.

In the present study, we tried to find out the effects of different heavy metals on seed germination and its parameters on *Vigna unguiculata* L.

Materials and methods

Collection of Seeds: Certified seeds of *Vigna unguiculata* (L.) Walp., were collected from the Durgapura Agriculture Research Station, Jaipur.Seeds were kept in glass-corked containers.

Figure 1: Seeds of *Vigna unguiculata* L.

Surface Sterilization and Germination: The seeds were initially chosen for comparable requirements (seed size and colour), became germ-free by using 0.1% $HgCl_2$ for 2 min, rinsed 3 times by using deionised water, and then submerged for 2 hrs. in solutions with various concentrations of $CdSO_4$, $CuSO_4$, $PbNO_3$, $NiSO_4$ and $ZnSO_4$ (10, 50, 100, 200, 500 and 1000 ppm). As a control, seeds were submerged in distilled water for 2 hrs. Seeds were picked out and left to grow on Petri dishes lined with filter paper dipped in individually mentioned above metallic solutions after all treatments. For each concentration of each heavy metal, triplicates with ten seeds each, were maintained. The metallic solution was employed to moisten the filter paper. The tests were done for 10 days in the laboratory conditions of temperature (25±2°C) and disperse light.

Morphometric Parameters: The number of germinations was assessed on the tenth day of the experiment, along with observations of the seedling growth variables like shoot length, root length, fresh weight, were measured for each experiment.

RESULTS AND DISCUSSION

Different heavy metals (Cd, Pb, Zn, Cu and Ni) affected the seed germination, shoot-root length, and fresh weight of seedlings at different level. Results are shown in figure 2. The detailed summary of the results has been described below-

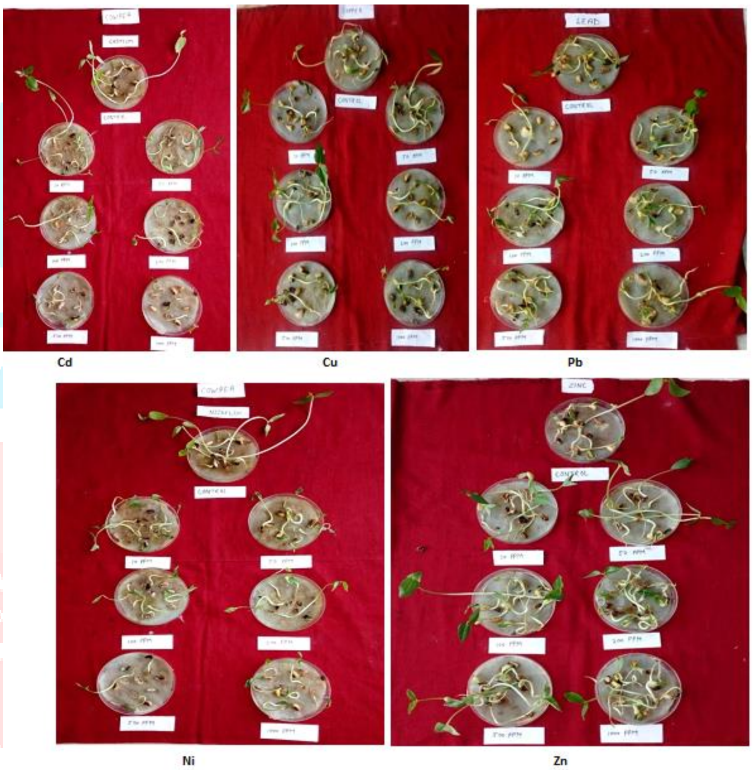


Figure 2: Photographs showing effects of heavy metal stress on seed germination parameters of *Vigna unguiculata* L.

Seed Germination

Effects of the selected heavy metals on seed germination percentage of *Vigna unguiculata* are shown in Table 1 and graphically represented in Fig 3.

Cd and Pb negatively affect seed germination at all concentrations. Seed germination (%) was decreased from 73.3 % to 30 % with increased concentrations, 10 to 1000 ppm of Cd which weresignificantly lower, compared to untreated seeds (80%). The Pb treatment showed a maximum reduced seed germination percentage at 1000 ppm. Pb caused decreased seed germination percentage, from 73.3 % to 23.3 % at the concentration of 10 ppm to 1000 ppm.These values were found significant in comparison to the control when analyzed statistically.

Treatment of seeds with Ni, Zn, and Cu showed enhanced seed germination at lower concentrations (10 ppm to 100 ppm for Ni, and 10 ppm to 200 ppm for Zn and Cu), at higher concentrations, lowered seed germination per cent was observed. The maximum seed germination with Ni treatment was found to be 86.6 % at 100 ppm while the minimum (33.3%) at 1000 ppm. For both Zn and Cu, the maximum seed germination (86.6%) was observed at 200 ppm and minimum at 1000 ppm (33.3%) for Zn and 43.3% for Cu. For Ni, Zn and Cu, seed germination did not increase significantly at lower concentrations but decreased significantly at higher concentrations. The level of toxicity for seed germination at 1000 ppm concentration in *Vigna unguiculata* was in the order of Pb>Cd>Ni>Zn>Cu.

Shoot Length

Effects of heavy metals on shoot length in *Vigna unguiculata* are shown in Table 2, fig 4. Results revealed that Cd and Pb showed a negative impact on shoot length on the plant while Ni, Cu, and Zn showed a positive effect at lower concentrations and a negative effect at higher concentrations. With Cu and Zn treatment, the length of the shoot was increased significantly compared to the control (4.56%) ranging from 4.66 and 4.83 cm at 10 ppm to 200 ppm (4.83 cm and 5.8 cm) after that reduced significantly at 1000 ppm respectively. Shoot length was reduced maximum from 4.61cm to 1.4 cm, 4.43 cm to 1.2 cm for Cd and 4.1 cm to 0.7 cm for Pb at 10 to 1000ppm. Cu showed the minimum reduction of shoot length at 1000 ppm (4 cm) as compared to other heavy metals. At higher concentrations of Pb and Cd, shoot length decreased significantly compared to control while no significant difference was observed in shoot length among different concentrations. The level of toxicity was maximum for shoot length in *Vigna unguiculata* in order of Pb>Cd>Ni>Zn>Cu

Root Length

Effects of the selected heavy metals on seed germination percentageof *Vigna unguiculata* are shown in Table 3 and graphically represented in Fig 5. A slight difference was observed in root length at 10 ppm (3.61 cm for Cd, 3.92 cm for Cu, 3.32 cm for Pb, 3.12 cm for Ni and 3.90 cm for Zn) in comparison to control (3.79 cm). The maximum root length was observed in the treatment of Zn and Cu at 200 ppm around 5.17and 5.31 cm, respectively. Pb affected the root length negatively, as the concentration increase the length of the root was reduced significantly. The impact of lead treatment was more toxic at 1000 ppm (0.38 cm) in comparison to other heavy metals.

The level of toxicity was maximum for root length in *Vigna unguiculata* in order of Pb>Cd>Ni>Cu>Zn.

Fresh Weight

Results showing effects of heavy metals on fresh weight in *Vigna unguiculata* are shown in Table 4 and graphically represented in Fig 6. Results showed that in control plants, 0.329 g fresh weight was obtained which was decreased with treatment of Cd and Pb at their all concentration. With treatment of Ni, Cu, and Zn, fresh weight was increased at their lower concentrations and decreased at higher concentrations. Cd and Pb showed a slight decrease in weight at 10 ppm (0.281 and 0.294g respectively) in comparison to the control (0.329 g) but Cu, Ni and Zn showed a significant increase in weight at 10 ppm (0.339, 0.357 and 0.359 g respectively). At the highest concentration, the maximum reduction in weight was observed in Cd (0.074 g) followed by Pb (0.078 g),Ni (0.153 g), Zn (0.271 g) and Cu (0.292 g). The reduction in fresh weight was influenced by different heavy metals in descending order Cd> Pb>Ni>Zn>Cu.

Table 1: Showing the Effect of Heavy Metals on Seed Germination (%) on *Vigna unguiculata.* (Values are means of three replicates each)

Heavy Metals Concentration (ppm)	**Seed Germination (%) at Different Heavy Metals**				
	Cd	**Cu**	**Pb**	**Ni**	**Zn**
Control	80	80	80	80	80
10	73.3	80	73.3	83.3	80
50	63.3	83.3	70	83.3	83.3
100	60	86.6	63.3	86.6	83.3
200	53.3	86.6	56.6	63.3	86.6
500	43.3	60	43.3	40	40
1000	30	43.3	23.3	33.3	33.3
F value	Between treatment				1.1364^{NS}
	Between concentrations				17.421*

Table 2: Showing the Effect of Heavy Metals on Shoot Length (cm) in *Vigna unguiculata.* (Values are means of three replicates each)

Heavy Metals Concentration (ppm)	Shoot Length (cm) at Different Heavy Metals				
	Cd	Cu	Pb	Ni	Zn
Control	4.56	4.56	4.56	4.56	4.56
10	4.43	4.66	4.1	4.61	4.83
50	3.8	4.80	3.8	5.7	4.86
100	3.6	4.80	3.2	5.8	5.2
200	2.4	4.83	3.0	4.8	5.8
500	1.7	4.4	2.1	2.2	4.9
1000	1.2	4	0.7	1.4	2.8
F value	Between treatment				3.5327^{NS}
	Between concentration				3.5327^{NS}

Table 3: Showing the Effect of Heavy Metals on Root Length (cm) in *Vigna unguiculata.* (values are means of three replicates each)

Heavy Metals Concentration (ppm)	Root Length (cm) at Different Heavy Metals				
	Cd	Cu	Pb	Ni	Zn
Control	3.79	3.79	3.79	3.79	3.79
10	3.61	3.92	3.32	3.12	3.90
50	3.50	4.21	3.02	3.46	4.35
100	3.38	4.57	2.38	4.18	4.83
200	2.43	5.31	2.14	3.82	5.17
500	2.15	4.44	0.76	3.46	4.68
1000	1.20	3.12	0.38	2.58	3.23
F value	Between treatment				8.3716*
	Between concentration				1.7063^{NS}

Table 4: Showing the Effect of Heavy Metals on Fresh Weight (g) in *Vigna unguiculata.* (values are means of three replicates each)

Heavy Metals Concentration (ppm)	Fresh Weight (g) at Different Heavy Metals				
	Cd	Cu	Pb	Ni	Zn
Control	0.329	0.329	0.329	0.329	0.329
10	0.281	0.339	0.294	0.357	0.359
50	0.274	0.383	0.213	0.378	0.416
100	0.253	0.411	0.189	0.401	0.448
200	0.227	0.492	0.129	0.349	0.494
500	0.142	0.348	0.122	0.242	0.342
1000	0.074	0.292	0.078	0.153	0.271
F value	Between treatment				7.2174*
	Between concentration				6.431*

DISCUSSION

Those heavy metals in the soil solution or dissolved by root exudates that plants can absorb are the soluble components (**Blaylock and Huang, 2000**). Heavy metals are required for plant growth and maintenance, yet plants can't handle too much of any one metal, and plants' ability to amass critical metals also allows them to accumulate other nonessential elements (**Djingova and Kuleff, 2000**). Some of the direct toxic effects caused by high metal concentration are the inhibition of cytoplasmic enzymes and the damage to cell structures due to oxidative stress, both of which are detrimental to the plant as metals cannot be broken down when concentrations within the plant exceed optimal levels (**Assche and Clijsters, 1990; Jadia and Fulekar, 1999**). The indirect toxicity comes from the displacement of necessary nutrients at plants' cation exchange sites (**Taiz and Zeiger, 2002**). Heavy metals had a knock-on effect on plant growth because of their toxic effects on soil microbes.

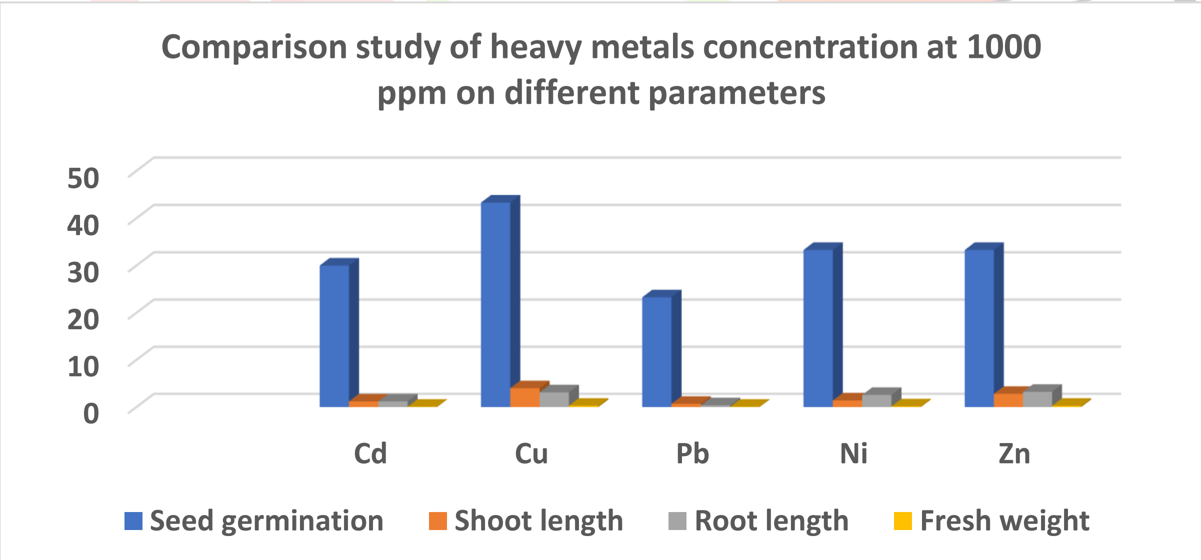
The effect of cadmium on seed germination was explained by **Huybrechts *et al.*, (2019)**. Cadmium (Cd), which inhibits the metabolic reactivation, adversely affects hydrolyzing enzyme levels, starch mobilization, and seed imbibition. Additionally, it may have an impact on the levels of phytohormones like ethylene (ET), gibberellic acid (GA), auxin (AUX), abscisic acid (ABA), and auxin as well as redox signaling via calcium (Ca), mitogenactivated protein kinases (MAPKs), and transcription factors (TFs). They both are essential to the germination of seeds.

According to **Sarcari *et al.*, 2002**, Lead toxicity prevents seeds from germinating and stunts the growth of young plants. Lead reduces root and shoot dry mass, root and shoot length, tolerance index, and germination percentage in both species. Concentrations of Pb accumulate close to the endodermis after first moving radially throughout the root cortex. When it comes to Pb, the endodermis provides a partial barrier between the root and the shoot. This may help explain why it has been found that Pb accumulates more in roots than in stems and leaves (**Rafia *et al.*, 2006**). In the present study, the maximum toxicity of heavy metals on different parameters was observed at 1000 ppm concentration (Fig 1).

Germination, root, leaf, and stem growth, photosynthesis, biomass, and pigment content are all known to be suppressed by the high copper level (**Mallick et al., 2010**). Numerous researchers have studied zinc's impact on plant germination, growth, and development (**Grejtovsky, 2006; Gunes, 2000; Gyana and Premananda, 2003**). Zinc was found to slow down the germination process and prevent the growth of leaves, stems, and roots across a wide range of plant species

According to **Shweti *et al.*, (2018**), In trace amounts, nickel (Ni) is an indispensable plant nutrient for germination and development. When the concentration is high, it can be harmful to plants and soil. Tolerance, however, is species-specific in terms of its effectiveness.

Fig 1 Comparison study of heavy metals concentration at 1000 ppm on different parameters



CONCLUSION

The present investigation found that various heavy metals have variable effects on germination rates and other seed characteristics. Smaller amounts of lead and cadmium were shown to be poisonous, while lower concentrations of copper, zinc, and nickel enhanced germination of seeds and seedling growth but were harmful at larger concentrations.

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