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Effect of heavy metals on seed germination and plant growth on Grass pea plant (Lathyrus sativus)

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Abstract: Heavy metals are important contaminants in the soil, metal pollution is continuously increasing and the root cause is the anthropogenic behavior which interferes with the environmental activities. Metal ions are gaining increased attention due to their importance in mineral nutrition and toxicity studies. Plants have been used for leaching of minerals and also in phytoremediation of toxic metal ions from polluted effluents. Especially short living plants like leguminacea family plants absorb more metals from soil and routinely used in phytoremediation. In this current study Lathyrus sativus germinating seeds were grown on hoglands minimal medium having various concentrations of cadmium and copper were separately grown up to 10 days. The shoot and root were used for estimation of heavy metals by AAS. Interestingly Lathyrus sativus shoots and roots absorb metals exponentially, it reveals that plant having the capacity of resistance to heavy metal.

Key words: Lathyrus sativus, Phytoremediation, Cadmium sulphate, Metal toxicity.

I. Introduction:

Metal contamination is always increasing chiefly by human activities which interferes with the environment and makes conditions toxic for living organism. Some plant species have capacity to grow in the metal contaminated soil and accumulate high amount of heavy metals (hyper-accumulation) as an eco-physiological adaptation in metaliferous soil. *Phaseolus vulgaris* has been reported a good accumulator of lead and cadmium. The mechanisms involved in heavy metal tolerance may range from exclusion, inclusion and accumulation of heavy metals depending on the plant species (1-3). Distinct concentrations of metals induce different biochemical responses in plants. In sensitive plants, high concentrations of these metals inhibit enzymes involved in photosynthetic reaction (4-5). Brassica juncea (Indian mustard), a high biomass producing plant can accumulate lead (Pb), chromium (Cr VI), cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), boron (B) and selenium (Se) (6-7). Even trace elements have been shown to have toxic effect on different plant traits such as leaf, stem, root flower etc. (8).

Excessive amount of toxic element usually caused reduction in plant growth (9). The wide spread use of cadmium in a large number of products and industrial process has resulted in severe environmental contamination. Our knowledge is still insufficient to explain the mechanism of cadmium toxicity and more especially the interaction of cadmium with important biochemical processes in plant growth. An attempt has been made to evaluate the effect of increasing levels of cadmium toxicity on germination and seedling vigor of Lathyrus. *sativus.* Reports on gradual decrease in plant growth of *Dalbergia sissoo with* increasing of cadmium level (10). Studies on higher concentration of cadmium and chromium (100-250 ppm) affected germination and early growth performance of *Allium cepa* (11). The reason of reduced seedling length in metal treatments could be the reduction in meristematic cells present in this region and some enzymes contained in the cotyledon and endosperms. So when activities of hydrolytic enzyme are affected, the food does not reach to the radicle and plumule affecting the seedling length.

Copper is widely prevalent in our environment and was considered as a vital element for all living organisms including plants (12-13). Copper occurs in the environment as hydrated ionic species, forming complex compounds with inorganic and organic legends. Subsequent relating to diet studies has demonstrated that copper and other metals are essential for optimal growth of plants and animals (14). Living organisms require certain metals for their growth and metabolism and so, they evolved an appropriate uptake mechanism for metals.

The effects of toxic substances on plants are dependent on the amount of toxic substance taken up from the given environment. The toxicity of some of the metals may be large enough that plant growth is retarded before large quantities of the element can be Trans located (15). Lead and cadmium treatment showed significant effect on seedling dry weight, which was evident in the poor growth of roots and aerial parts (16). Cadmium treatment at 25 ppm concentration showed reduction in root growth, stunned growth. For the determination of trace element quantities in natural products, the material has to be digested first. Commonly used methods for digestion involve heating samples with mixtures of HNO_3, $HClO_4$ and H_2SO_4 are used (17). However, HCl, HF, and H_2O_2 are also used, depending on the material and on the element under consideration. During the process of sample digestion by these methods, some elements were lost through volatilization, even when a digestion bomb was used. A newer technique uses microwave radiation for sample digestion in high-pressure digestion vessels using HNO_3, HCl, H_2SO_4 and H_2O_2. Several laboratories use microwave radiation for sample dissolution process because it provides rapid sample dissolution. However, here too losses are unavoidable.

A new sample treatment was proposed for wear metals in lubricating oil assisted by a microwave oven as can be seen; each material has to be digested according to its composition. Steel, for example, cannot be digested with HCl for its sulfur content since sulfur will be lost as H_2S. A sample for selenite determination has to be digested first with HNO_3 and $HClO_4$ but then HCl has to be used in order to reduce selenate to selenite. Thus, the digestion procedure will depend on the elements under consideration, on the method used for the determination, and on the volatility of the element. The dependence of losses on digestion time has been studied for some trace elements in cauliflower. With 30 h of digestion all the selenium was lost. Similar losses were observed for Ti(IV), Cr(III) and Zn with longer (6-9 h) digestion times for the electrochemical determination of As and Se in tuna fish,13a wet digestion procedure of about 18 h using HNO_3 and $H_2\ SO_4$ has been applied. After the addition of HCl and heating for 30 min more, the recovery was 84% for selenium. Microwave digestion is excellent for routine analysis of known trace elements in well-known samples. However, there are many cases in which open wet digestion has to be used. With microwave ovens only 0.1-0.5 g sample quantities can be digested; thus the final volume and the concentrations will be small. When an unknown sample has to be analyzed for its trace element contents, wet digestion will be preferred since large amounts of sample such as 5-10 g can be digested at a time. Using larger quantities enables samples that reflect the average composition of the bulk of the material.

As can be seen from the literature, in all digestion procedures, either microwave oven or open wet digestion, there are cases of low recoveries. Thus, if the behavior of ions during digestion is known, precautions can be taken. The aim of this work was to investigate the effect of digestion time and type of acids on the recovery of elements. Wet open digestion was applied to 21 elements, using various acids and digestion times; care was taken to minimize losses. Differential pulse polarography (DPP) was used throughout the study for the trace element determinations. Heavy metals such as Pb, Cd, Cu and Hg (18), are micro-pollutants and of special interest as they have both health and environmental significance due to their persistence, high toxicity and bio-accumulation characteristics. Harmful effects of Cu in water: Water turns blue-green in color as the corroded Cu inside of the pipes and mix together with the water as a precipitate. Cu in a very high quantity is toxic and may cause vomiting, diarrhea and loss of strength. In a long term, the toxicity can cause liver damage, kidney failure and ultimately death while the short term effect is gastrointestinal distress.

II. Materials and Methods:

In the present investigation, effect of cadmium and copper was evaluated on Lathyrus sativus plants with respect to their growth and biochemical parameters. In this all chemicals were pure and analytical grade.

2.1. Seed collection:

The *Lathyrus sativus (LS)* seeds were collected from farmer Sangareddy, Medak district, Telangana, India. Seeds with uniform size were chosen for this experiment. The seeds were sterilized with 3% v/v formaldehyde solution for 5 minutes to avoid fungal contamination and washed thoroughly thrice with distilled water. The seeds were immersed in 3% v/v formaldehyde/deionized water for 5 minutes to avoid fungal contamination. After that, the seeds were washed with deionized water and placed in plastic glass of one-pint capacity. The present study was taken up with Cadmium sulfate ($3CdSO_4 . 8H_2O$) and Copper sulfate ($CuSO_4 . 5H_2O$) with concentrations of 0, 5, 10, 20, 40, and 80 ppm.

Seeds were placed in modified Hoagland's Plant Growth Media with 1% Agar agar and were treated separately with 2 mL solution of 5, 10, 20, 40, and 80 ppm cadmium sulfate and copper sulfate. Control treatments were supplied with distilled water. Each treatment was replicated three times for statistical purposes. The seeds were set under a photoperiod of 12 hrs and 25/18 °C day/night temperature for each treatment, the pH was adjusted to 5.3.

2.2. Preparation of metal solution:

Different concentrations of cadmium sulfate ($3CdSO_4 . 8H_2O$) and copper sulfate ($CuSO_4 . 5H_2O$) with concentrations of 0, 5, 10, 20, 40, and 80 ppm were prepared and used for this experiment.

2.3. Pot culture experiments:

Seeds were placed in Modified Hoagland's Plant Growth Media with 1% agar and were treated separately with 2 mL solution of 5, 10, 20, 40, and 80 ppm cadmium sulfate and copper sulfate. Control treatments were supplied with distilled water. Each treatment was replicated three times for statistical purposes. The seeds were set under a photoperiod of 12 hrs and 25/18 °C day/night temperature for each treatment, the pH was adjusted to 7.3.

2.4. Atomic Absorption Spectroscopy:

Modified method of Kaiser et. al acid digestion method (19), one ml of perchloric acid and 5 ml of HCl were added to conical flask containing 0.1 g of plant material and kept on san bath at 100°c for digestion in fume hood. After that 1: 1 ratio of (2.5 ml each) of acid mixture of nitric acid and hydrochloric acid was added and digested until the acids were driven off. Finally 5 ml of hydrochloric acid was added and kept on sand bath for dry. The digest was cooled, diluted with milli pore water, filtered through Whattman No.42 filter paper and made up to 10 ml. The solution was directly, aspirated to an Atomic Absorption spectrophotometer (Perkin Elmer-2280), with air/acetylene flame for estimating copper and cadmium.

2.6. Phytotoxicity:

The plant samples collected after 2 weeks and various growth indices such as root and seedling length were measured. % Phytotoxicity for shoot and root of 2 weeks old seedlings were calculated by the following formula (20).

$$\% \text{ of Phytotoxicity} = \frac{\text{Shoot or root length of control- Shoot or root length of treatment}}{\text{Shoot or root length of control}}$$

Phytoaccumulation of cadmium and copper content of the Lathyrus sativus plants were estimated using Atomic absorption spectrophotometer.

III. Results and discussion:

3.1. Effect of Cadmium and Copper on shoot and root length of *Lathyrus sativus* plant and its phytotoxicity (%):

The root and shoot length of *Lathyrus sativus* plants after 2 weeks of growth under cadmium and copper stress are represented in Table.1 and 2. The root and shoot length of *LS* plants were different with different levels of cadmium and copper in the media. The compartmentalization and accumulation of heavy metal occurs in the vacuoles of root cells thus limiting the heavy metal transportation to shoots. So heavy metals are stored predominantly in roots, consequently root cell metabolism disorder and depressed the root growth [20]. Similar reduction in growth under various metal treatments was reported for aluminum on green gram [21], cadmium on Pisum sativus [22], lead on wheat [23] and nickel on black gram [24].

The % phytotoxicity of shoot and root of *Lathyrus sativus* plants under cadmium and copper treatment showed an increasing trend with increasing cadmium and copper concentration in case of all cultivars. The highest % phytotoxicity value of shoot was found at 80 ppm concentration of cadmium and copper. In case of % phytotoxicity of root similar observation was found but highest % phytotoxicity was found at 80 ppm cadmium and copper concentration in all the plants (Table.1 and 2).

Table: 1 Effect of different concentrations of copper on shoot and root length, Phytotoxicity % of shoot and root:

copper treatment (ppm)	**Shoot length (mm)**	**Root length (mm)**	**Phytotoxicity % of shoot**	**Phytotoxicity % of root**
Control	350 ± 0.3	14 ± 0.6	--	--
5	160 ± 0.2	10 ± 0.3	54.28	28.57
10	80 ± 0.4	8 ± 0.1	77.14	42.85
20	75 ± 0.1	5 ± 0.4	78.57	64.28
40	55 ± 0.5	2 ± 0.2	84.28	85.71
80	46 ± 0.6	0 ± 0.0	86.85	100

Table: 2 Effect of different concentrations of Cadmium on shoot and root length, Phytotoxicity % of shoot and root:

cadmium treatment (ppm)	**Shoot length (mm)**	**Root length (mm)**	**Phytotoxicity % of shoot**	**Phytotoxicity % of root**
Control	350 ± 0.3	14 ± 0.6	--	--
5	250 ± 0.4	12 ± 0.1	28.57	14.28
10	190 ± 0.1	10 ± 0.2	45.71	28.57
20	160 ± 0.3	5 ± 0.5	54.28	64.28
40	70 ± 0.2	3 ± 0.6	80	78.57
80	0 ± 0.0	0 ± 0.0	100	100

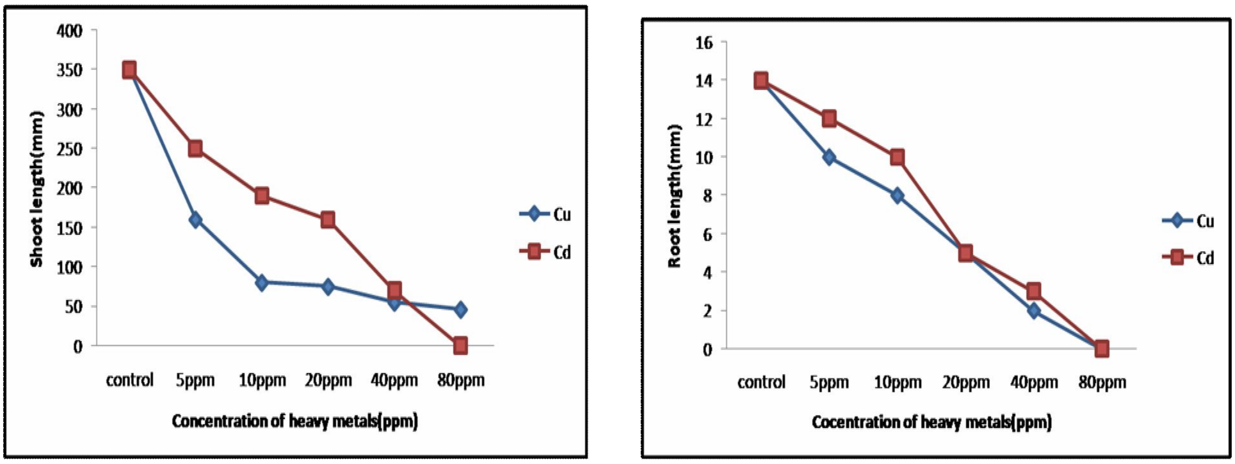

Fig: A) Graphical representation % of phytotoxicity of Lathyrus sativus shoots. B) Graphical representation % of phytotoxicity of Lathyrus sativus shoots

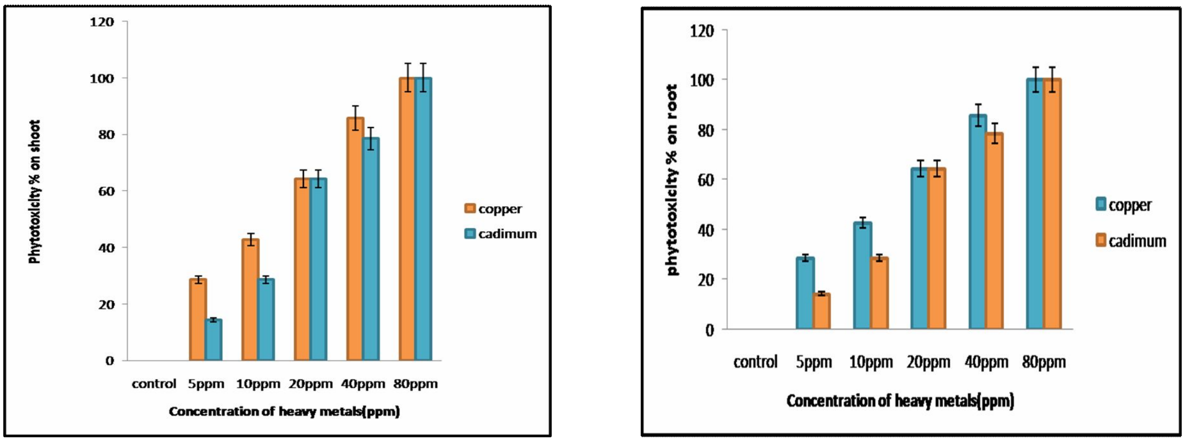

Fig: 2 C) Concentrations of copper and cadmium in shoots of *Lathyrus sativus* plant. D) Concentrations of copper and cadmium in roots *Lathyrus sativus* plant

3.2. Uptake and accumulation of cadmium on Lathyrus sativus root:

Cadmium content of root in *Lathyrus sativus* plants is recorded in Table 3. Maximum cadmium content of *Lathyrus sativus* root was 1.28 ppm observed at 80 ppm cadmium level in the media. The minimum cadmium content of Lathyrus sativus root was 0.02 ppm, observed in 10 ppm cadmium level in the media.

3.3. Uptake and accumulation of cadmium on Lathyrus sativus shoot:

Cadmium content of stem in *Lathyrus sativus* plants is recorded in Table 3. Maximum cadmium content of *LS* shoot was 21.3 ppm observed at 80 ppm cadmium level in the media. The minimum cadmium content of *LS* shoot was 0.001ppm observed in 5 ppm cadmium level in the media.

3.4. Uptake and accumulation of copper on Lathyrus sativus root:

Copper content of root in *Lathyrus sativus* plants is recorded in Table 4. Maximum copper content of *LS* root was 2.25 ppm observed at 80 ppm copper level in the media. The minimum copper content of *LS* root was 0.87 ppm observed in 10 ppm copper level in the media.

3.5. Uptake and accumulation of copper on Lathyrus sativus shoot:

Copper content of root in *Lathyrus sativus* plants is recorded in Table 4. Maximum copper content of *LS* root was 35.9 ppm observed at 80 ppm copper level in the media. The minimum copper content of *LS* root was 0.5 ppm observed in 5 ppm copper level in the media.

Table: 4 Concentration of cadmium entered in to shoots and roots:

Treatment cadmium sulfate (ppm)	Uptake of cadmium by shoot (ppm)	Uptake of cadmium by root (ppm)
Control	--	--
5	0.001	0
10	0.51	0.02
20	1.4	0.12
40	5.08	0.85
80	21.3	1.28

Table: 4 Concentration of copper entered in to shoots and roots

Treatment copper sulfate (ppm)	Uptake of copper by shoot (ppm)	Uptake of copper by root (ppm)
Control	-	-
5	0.5	0

10	1.4	0.87
20	5.8	1.2
40	21.3	2.1
80	35.9	2.25

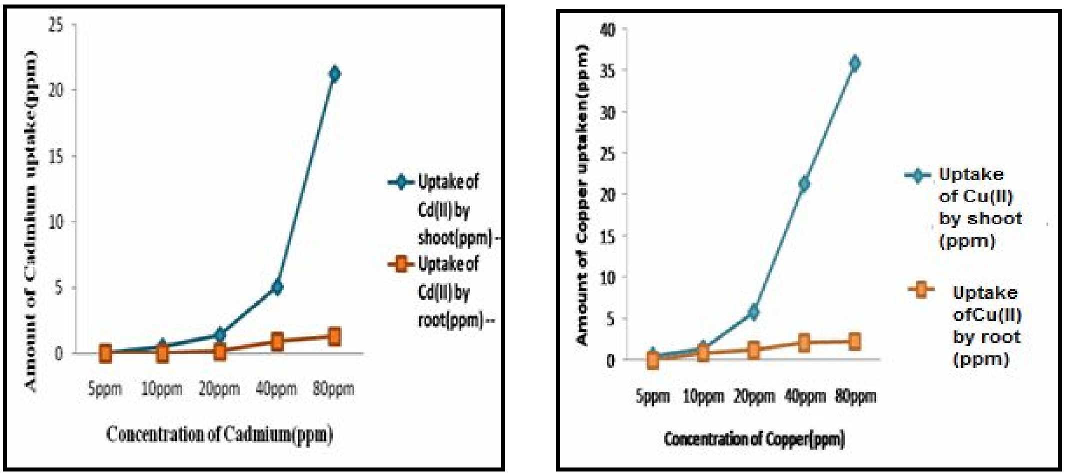

Fig: 3. A) Uptake and accumulation of copper and cadmium on *Lathyrus sativus* shoot B) Uptake and accumulation of copper and cadmium on *Lathyrus sativus* root.

Conclusion:

Phytoremediation is one of the best come within reach of for removing the heavy metals from contaminated soil. Metal pollution leads to biomagnifications through food chain and causes the many disorders and sometimes causes death to animals and human beings. Removal of heavy metals by cheapest plants like leguminacea is one of the finest, eco friendly and cost effective methods. In this present study *Lathyrus sativus* plant absorbs more than 60 ppm of heavy metals like copper and cadmium. As this is cheapest plant easily grown and removed, could be the best method in phytoremediation like other Fabaceae plants.

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