EFFECT OF METAL TOXICITY ON PLANT GROWTH AND METABOLISM OF *DATURA STRAMOINUM* L. PLANT

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ABSTRACT: *Contamination of soil by heavy metals is of widespread occurrence as a result of human, agricultural and industrial activities. Among heavy metals, lead and cadmium are harmful pollutants that accumulates in soils and sediments. Although lead is not an essential element for plants, it gets easily absorbed and accumulated in different plant parts. Uptake of lead in plants is regulated by PH, particle size and cation exchange capacity of soils as well as by root exudation and other physio chemical parameter. Excess of lead causes a number of toxicity symptoms in plants e.g. stunted growth, chlorosis and blackening of root system. Pb inhibits photosynthesis, imbalance of mineral nutrition and water balance, charges hormonal status and affects membranes structure and permeability. This paper addresses various morphological, physiological and biochemical effects of lead and cadmium toxicity on growth of Datura plant.*

INTRODUCTION

Phytoremediation is the use of green plants to clean-up contamination from soils, sediments, water and hazardous waste sites. The idea of using metal-accumulating plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the past300 years on wastewater discharges (Chaney *et al*., 1997). A general, visual reference concerning plant-based mechanisms used to remediate the environment. Phytoremediation is a cost-effective, environmentally-friendly, aesthetically-pleasing environmental pollutant removal approach and is most suitable for developing countries (Ghosh and Singh, 2005). The technologies based on the phytoremediation technique can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or air (Raskin*et al*., 1994; Salt *et al*., 1998). Plants with exceptional metal-accumulating capacity are known as hyper accumulator plants. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body.

The success of green technology in phytoremediation, in general, is dependent upon several factors. First, plants must produce sufficient biomass while accumulating high concentrations of metal. In some cases, an increased biomass will lower the total concentration of the metal in the plant tissue, but allows for a larger amount of metal to be accumulated overall. Second, the metal-accumulating plants need to be responsive to agricultural practices that allow repeated planting and harvesting of the metal- rich tissues. Thus, it is preferable to have the metal accumulated in the shoots as opposed to the roots, for metal in the shoot can be cut from the plant and removed. The availability of metals in the soil for plant uptake is another limitation for successful phytoremediation.

Plants as phytoremediators

The principal application of phytoremediation is for lightly contaminated soils and waters where the material to be treated is at a shallow or medium depth and the area to be treated is large. This will make agronomic techniques economical and applicable for both planting and harvesting. In addition, the site owner must be prepared to accept a longer remediation period. Plants that are able to decontaminate soils does one or more of the following: 1) plant uptake of contaminant from soil particles or soil liquid into their roots; 2) bind the contaminant into their root tissue, physically or chemically; and 3) transport the contaminant from their roots into growing shoots and prevent or inhibit the contaminant from leaching out of the soil.

Heavy metal as harmful pollutant

Heavy metals are among the contaminants in the environment. Beside the natural activities, almost all human activities also have potential contribution to produce heavy metals as side effects. Migration of these contaminants into non contaminated areas as dust or leachates through the soil and spreading of heavy metals containing sewage sludge area few examples of events contributing towards contamination of the ecosystems (A Gaur *et al.*,2004).

Source and effect of heavy metal on environment

Heavy metals are conventionally defined as elements with metallic properties and an atomic number >20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil (M. M. Lasat.2000). Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni, and Co, while others have unknown biological function, such as Cd, Pb, and Hg (A gaur *et al.*, 2004). Metal pollution has harmful effect on biological systems and does not undergo biodegradation. Toxic heavy metals such as Pb, Co, Cd can be differentiated from other pollutants, since they cannot be biodegraded but can be accumulated in living organisms, thus causing various diseases and disorders even in relatively lower concentrations (E. Pehlivan*et al.*, 2009). Heavy metals, with soil residence times of thousands of years, pose numerous health dangers to higher organisms. They are also known to have effect on plant growth, ground cover and have a negative impact on soil microflora (S Roy *et al.*, 2005). It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be transformed into nontoxic compounds (A gaur *et al.*, 2004).

Effect of Cadmium on Plant

The heavy metal, Cd is commonly released into the arable soil from industrial processes and farming practices and has been ranked No. 7 among the top 20 toxins (yang *et al.,* 2004). Even at low concentrations, Cd is toxic for most of the plants at concentrations greater than 5–10 μg Cd g^{-1} leaf dry weight, except Cd-hyper accumulators which can tolerate Cd concentrations of 100 μg Cd g^{-1} leaf dry weight (Reeves RD *et al.,* 2000 &Verbruggen N *et al.,*2009). In spite of its high phytotoxicity, Cd is easily taken up by plant roots and transported to above-ground tissues (DalCorso G *et al.*, 2010 & Liu F *et al.*, 2010).

Being highly mobile in phloem (Benavides MP *et al.*, 2005), Cd can be accumulated in all plant parts which causes stunted growth, chlorosis, leaf epinasty, alters the chloroplast ultrastructure, inhibits photosynthesis, inactivates enzymes in CO_2 fixation, induces lipid peroxidation, inhibits pollen germination and tube growth, and also disturbs the nitrogen (N) and sulfur (S) metabolism and antioxidant machinery (Mishra *et al.*, 2005). Cd can also inhibit the activity of several groups of enzymes such as those of the Calvin cycle, carbohydrate metabolism and phosphorus metabolism. The effect of Cd on nitrate and sulfur assimilation has been studied in several plants showing an inhibition of the nitrate uptake rate and the activity of the enzymes involved in the N assimilation pathway (Hernandez E *et al.*, 1998 & Hasan SA *et al.*, 2008).

Effect of Lead on Plant

Availability and Uptake of Lead by Roots

The rhizosphere is where interactions take place between roots and soils constituents (Lynch and Whipps, 1990).When a root absorbs water or nutrients from soil, ions and molecules move toward this organ both by mass flow with soil water and by diffusion (Robinson, 1991). Pb may be present in different fractions in the soils. It was previously thought that Pb had low solubility and availability for plant uptake because it forms precipitates with phosphates, sulfates, and chemicals in the rhizosphere (Blaylock and Huang, 2000). These geo-chemical forms of Pb in soils affect its solubility, which directly influences its mobility. However, roots produce and excrete protons, exudates and several metabolites, which can modify the soil pH and thus interfere with the dissolution processes and formation of soluble metal–organic complexes (Leyval and Berthelin, 1991).

Lead uptake is greatly affected by rhizospheric processes. (Lin *et al.,* 2004) explained the ability of Oryza sativa L. to absorb high levels of Pb from soil by a decrease in soil pH due to root exudates, solubilization of Pb by rhizosphere microorganisms and complexation of Pb with organic matter at the soil–root interface. These authors also found larger amounts of extractable Pb in the rhizosphere than in bulk soil, pointing to the involvement of root activities in changes in Pb availability (Lin *et al.*, 2004). Uptake of and tolerance to Pb depends on root system conditions.

PHYTOTOXICITY

To evaluate meaningful physiological and biochemical effects of toxicity, one must know the metals which are phytotoxic in nature and interactions with other metals (Cunnigham J.D*et al.*, 1975)before starting a phytotoxicity experiment one should be fully aware of the movement of the metal including its absorption

and translocation in the plant system. Availability of metal in the soil depends on soil adsorption strength as well as plant effectors such as root exudates for metal chelation or reduction. Metal phytotoxicity can result only if metals can move from the soil to root systems (Foy C.D*et al*., 1978). Phytotoxicity levels of zinc in different crop plants were reported by many workers (Chardonnens A.N*et al*., 1999,Staker E.V*et al*., 1941). The most significant phytotoxicity symptoms were stunting of growth, chlorosis and reduction in biomass yield. The phytotoxicity caused by a wide variety of metals has been well documented; however, models designed to quantify the relationship between exposure to metal ions and progressive yield losses are lacking (Taylor G.J*et al*., 1971).
The present experiment was undertaken to investigate a change in the level of growth, and biochemical aspects like, Total Protein, Amino Acid, Total Sugar and Reducing Sugar, Starch, Amino Acid and Chlorophyll Content in *Datura Stramonium* L. selected plant wastreated with Cd and Pb in order to contribute to an understanding of *D. stramonium*L.adaptation to environmental stress.This crop may further be useful in soil reclamation through the process of phytoremediation.

MATERIALS AND METHODS

Selected plant variety

Scientific name	:*Datura stramonium*L.
Synonyms	:Thron apple, Jimson weed
Common names	:Dhanturo, Devil's weed
Family	:Solanacae

Field experiment

Study area and experimental procedure

The study area was Botanical garden, Department of Botany, Gujarat University, Ahmedabad. Seeds of *Datura stramonium* L. Was purchased from local nursery of Ahmedabad. The soil was collected from the botanical garden and analyzed for soil basic properties and specially to detect the available Cadmium and lead content.
Treatment of heavy metals of different salts of lead and cadmium in Specific concentration was given to selected plant and after interval of 20 days data was taken to study the effect of heavy metal, growth parameters and physiology of the plants.

- The experiments consist of 3 treatments with 8 replicates for each treatment.
- 8 pots watered with distill water for control
- 8 pots watered with distill water containing lead acetate at a concentration of 50 mg/l
- 8 pots watered with distill water containing cadmium acetate at a concentration of 50 mg/l
- The treatment of lead acetate and cadmium acetate given to plant at interval of 10 days respectively for 3 months and vegetative growth and biochemical test were done respectively after every 20 days.

Measurement of physiological parameter

Germination – Seeds of *Datura Stramonium* L.were sown in the soil substrates. Seeds were placed under the surface of the soil substrate in pots, eight replicates for each pot, and twenty four pots for each treatment. The pots were watered with tap water daily till seed germination. Only daylight was used for illuminating.
Plant length – The length of plants was measured every week regularly. At the end of the experiment all plants were excavated from all the sets of treatments, partitioned into shoots and roots, carefully washed with distilled water.
Morphological parameters to be investigated include plant height, number of leaves, leaf area, fresh and dry weight of plant parts, RGR, LWR, and NAR. The parameters will be determined after treatment given to plants. Physico – chemical analysis of water and soil samples was done.
The chlorophyll content – chlorophyll content was measured at every interval of 20 days. Fresh leaves was taken from treated and controlled plant, washed with distilled water and small cuttings were homogenized in 80% acetone. After completing the procedure determination of the absorbance of the sample was carried out at wavelengths of 663 and 646 nm.

Biochemical estimation of *Datura stramonium* L.

Analysis of Biochemical test like reducing sugar, total sugar, amino acid, protein and starch test was done. This parameters were studied to investigate the content present in treated and controlled plant.

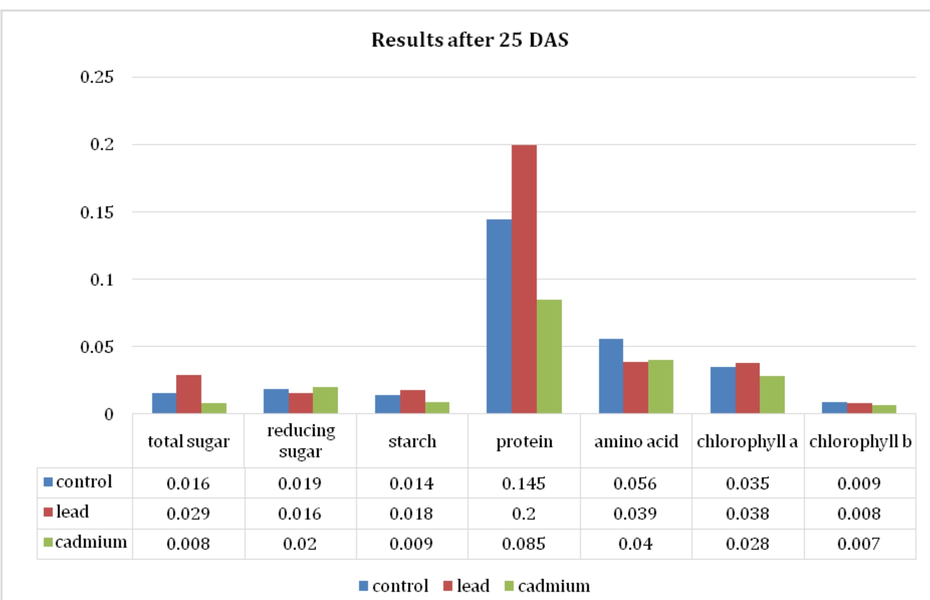
RESULT AND DISCUSSION

1.1 Table Showing Growth Parameter at 25 DAS at 50 ppm Concentration

Growth parameter fresh weight (mg)	control	lead	cadmium
Leaf weight	22.3	52.5	54.9
Shoot weight	23.2	44.9	63
Root weight	4.7	6.5	5.8
Root length	1.5	3.9	4.5
Shoot length	6.3	8.2	5.06
No. of leaves	2	4	4

1.2 Table Showing Biochemical Parameters at 25 DAS at 50 ppm Concentration

Biochemical estimation	control	lead	cd
Chlorophyll a	0.035	0.038	0.028
Chlorophyll b	0.009	0.008	0.007
Total sugar	0.016±0.003	0.029±0.002	0.008±0.001
Reducing Sugar	0.019±0.007	0.016±0.002	0.020±0.008
Starch	0.014±0.006	0.018±0.002	0.009±0.001
Protein	0.145±0.001	0.200±0.002	0.085±0.001
Amino acid	0.056±0.014	0.039±0.007	0.040±0.028



1.3 Graph Showing Biochemical Results After 25 DAS

After 25 DAS leaf and shoot weight of cadmium treated plant was higher compare to other treated plant. Root weight was higher in lead treated plant. However increasing in number of leaves was similar in lead and cadmium treated plant compare to control plant.

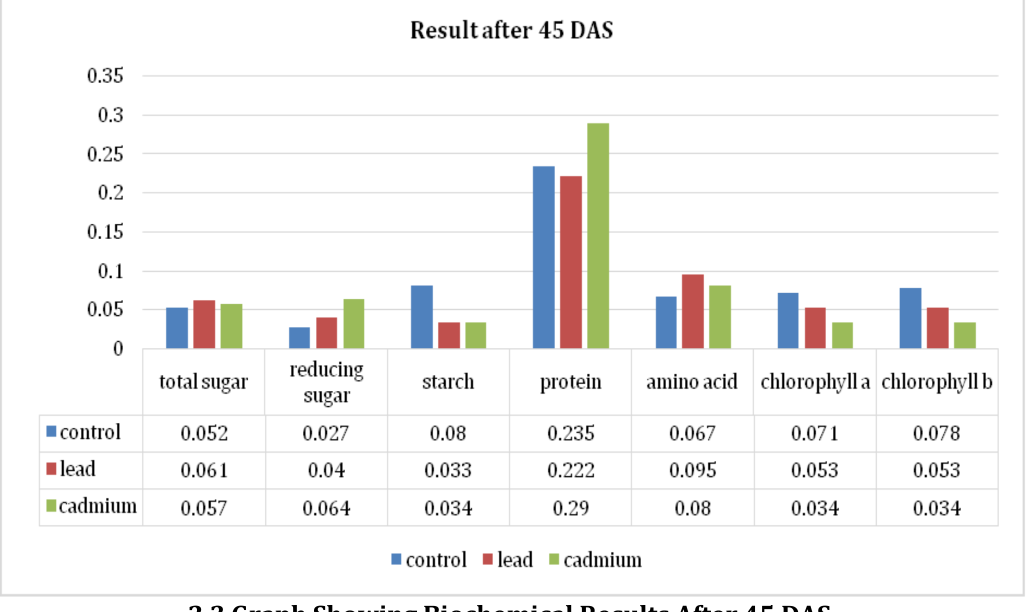
From biochemical analysis we estimated that, presence of chlorophyll a was higher lead treated plant and chlorophyll b was in control plant. Presence of total sugar and reducing sugar was higher in lead and control plant. Presence of starch was higher in lead treated plant. Control plant had higher value of protein and amino acid.

2.1 Table Showing Growth Parameter at 45 DAS at 50 ppm Concentration

Growth parameter fresh weight (mg)	control	lead	cadmium
Leaf weight	199	424.3	246.5
Shoot weight	139	228.8	172.5
Root weight	22.2	39.9	24.8
Root length	4.8	5.7	4.4
Shoot length	11.5	13.6	11.7
No.of leaves	7	8	7

2.2 Table Showing Biochemical Parameters at 45 DAS at 50 ppm Concentration

Biochemical estimation	control	lead	cadmium
Chlorophyll a	0.071	0.053	0.034
Chlorophyll b	0.078	0.078	0.016
Total sugar	0.052±0.002	0.061±0.004	0.057±0.001
Reducing Sugar	0.027±0.002	0.040±0.001	0.064±0.002
Starch	0.080±0.002	0.033±0.005	0.034±0.006
Protein	0.235±0.002	0.222±0.003	0.290±0.003
Amino acid	0.067±0.005	0.095±0.008	0.080±0.003



2.3 Graph Showing Biochemical Results After 45 DAS

After 45 DAS leaf, root, shoot weight and number of leaves was higher in lead treated pants.
From biochemical analysis we estimated that value of total sugar and amino acid was higher in lead treated plant. Reducing sugar and protein value was higher in cadmium treated plant. Control plant had higher value of starch.

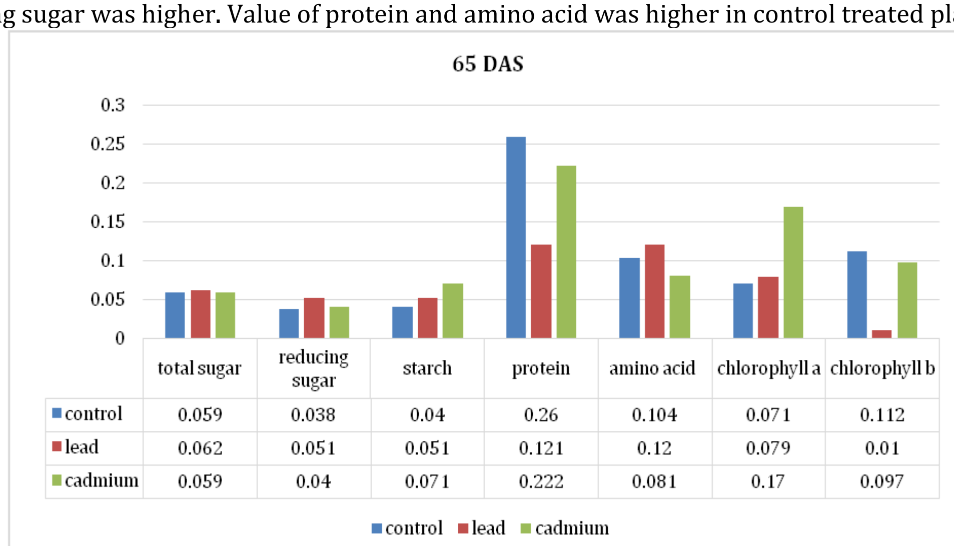
3.1 Table Showing Growth Parameter at 65 DAS at 50 ppm Concentration

Growth parameter fresh weight (mg)	control	lead	cadmium
Leaf weight	1041.5	948	498.9
Shoot weight	1248.4	1426.9	1060.7
Root weight	142.5	100.4	128
Root length	11.7	6.6	5.1
Shoot length	23	18	17
No.of leaves	11	9	8

3.2 Table Showing Biochemical Parameters at 65 DAS at 50 ppm Concentration

Biochemical estimation	control	lead	Cadmium
Chlorophyll a	0.071	0.079	0.170
Chlorophyll b	0.112	0.010	0.097
Total sugar	0.059±0.003	0.062±0.003	0.059±0.001
Reducing Sugar	0.038±0.0008	0.051±0.001	0.040±0.0005
Starch	0.047±0.012	0.051±0.001	0.071±0.003
Protein	0.260±0.004	0.121±0.003	0.222±0.002
Amino acid	0.104±0.0003	0.120±0.015	0.081±0.015

After 65 DAS leaf weight, root weight, shoot length, root length and number of leaves was higher in control treated plant. From biochemical analysis we estimated that value of chlorophyll a and starch was higher in cadmium treated plant. Value of chlorophyll b was higher in control plant. In lead treated plant total sugar and reducing sugar was higher. Value of protein and amino acid was higher in control treated plant.



3.3 Graph Showing Biochemical Results After 65 DAS

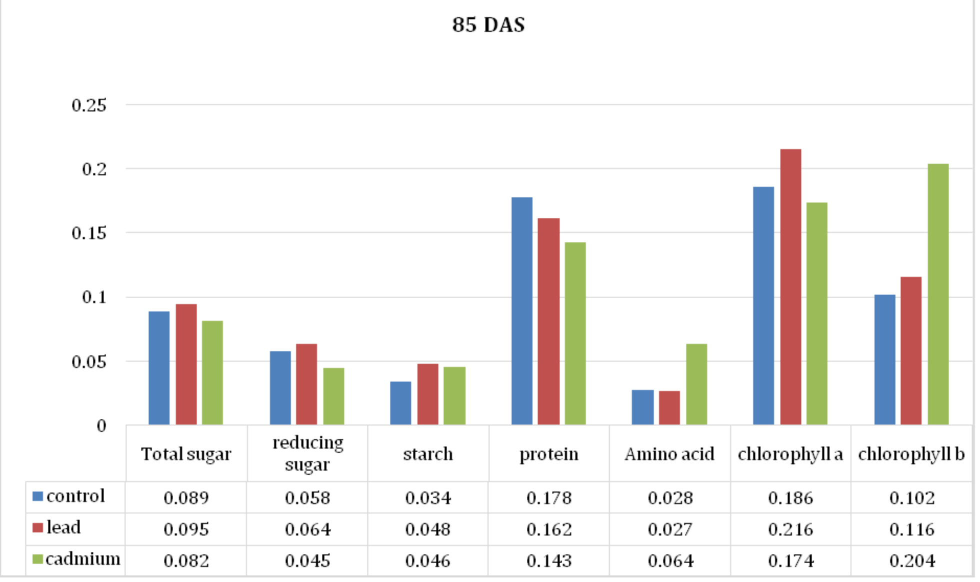
4.1 Table Showing Growth Parameter at 85 DAS at 50 ppm Concentration

Growth parameter fresh weight (mg)	control	lead	cadmium
Leaf weight	4341.8	7952	5546.9
Shoot weight	7305.4	12099	8284
Root weight	2065.9	2460.4	1969.5
Root length	27.4	10.2	8.7
Shoot length	10.3	27.4	28.3
No. of leaves	10	16	13

4.2 Table Showing Biochemical Parameters at 85 DAS at 50 ppm Concentration

Biochemical estimation	control	lead	cadmium
Chlorophyll a	0.186	0.216	0.174
Chlorophyll b	0.102	0.116	0.204
Total sugar	0.089±0.001	0.095±0.002	0.082±0.0001
Reducing Sugar	0.058±0.002	0.064±0.003	0.045±0.0001
Starch	0.034±0.004	0.048±0.003	0.046±0.003
Protein	0.178±0.005	0.162±0.004	0.143±0.0001
Amino acid	0.028±0.0001	0.027±0.0001	0.064±0.0003

After 85 DAS leaf, shoot, root weight and number of leaves was higher in lead treated plant. From biochemical analysis we estimated that chlorophyll a was higher in lead treated plant. Chlorophyll b and amino acid was higher in cadmium treated plant. Value of total sugar, reducing sugar and starch was higher in lead treated plant. Control has higher value of protein.



4.3 Graph Showing Biochemical Results After 85 DAS

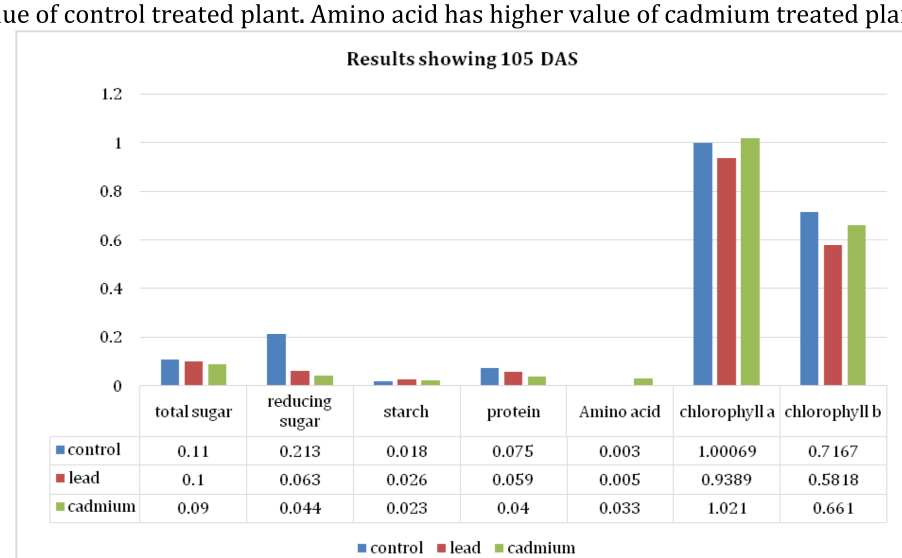
5.1 Table Showing Growth Parameter at 105 DAS at 50 ppm Concentration

Growth parameter fresh weight (mg)	control	lead	cadmium
Leaf weight	25000.9	28299.8	25787.9
Shoot weight	5689.3	43428.4	57789.4
Root weight	8442.6	11937.8	12814.4
Root length	15.6	13.5	15.6
Shoot length	55	51.3	48.7
No. of leaves	32	28	26

5.2 Table Showing Biochemical Parameters at 105 DAS at 25 ppm Concentration

Biochemical estimation	control	lead	cadmium
Chlorophyll a	1.00069	0.9389	1.021
Chlorophyll b	0.7167	0.5818	0.6610
Total sugar	0.110±0.0002	0.100±0.0002	0.090±0.0003
Reducing Sugar	0.213±0.002	0.063±0.002	0.044±0.002
Starch	0.018±0.001	0.026±0.001	0.023±0.0001
Protein	0.075±0.002	0.059±0.0003	0.040±0.0002
Amino acid	0.003±0.0001	0.005±0.0001	0.033±0.0001

After 105 DAS leaf weight was higher in lead treated plant. Shoot weight and root weight was higher in cadmium treated plant. However root length was similar in control and cadmium treated plant. Shoot length and number of leaves was higher in control treated plant. From biochemical analysis we estimated that, value of chlorophyll a was similar in control and cadmium treated plants. Value of total sugar and reducing sugar was similar in control and lead treated plant. Value of starch was higher in lead treated plant. Control has higher value of control treated plant. Amino acid has higher value of cadmium treated plant.



5.3 Graph Showing Biochemical Results After 105 DAS

The reduction in plant growth caused by heavy metal toxic effects on plants can bedetermined as reduced growth rate or decreased biomass production (BEGONIA *et al*., 1998). Negative effects of metals exposure on reduction in growth have been often observed by several authors (ARDUINI *et al*., 2004; GUO *et al*., 2007; VERNAY *et al*., 2008; CAO *et al*., 2009; OZTURK *et al*., 2010). Some of them (ARDUINI *et al*., 2004; CAO *et al*., 2009; OZTURK *et al*., 2010) determined that low metal content has stimulative effect on biomass production. For example in the case of *L. usitatissimum,*presence of low Cu, Cd and Zn concentrations stimulated the biomass production

But the same metal concentrations decreased the biomass production in *C. chinensis*. It shows that plants of *C. chinensis*are more sensitive to the metal toxicity than plants of *L. usitatissimum*.

A common response of plants to metal stress is a decrease of photosyntheticpigments (chlorophylls and carotenoids) in leaves of plants (MONTEIRO *et al*., 2009). The reduction at the levels of total chlorophyll, chlorophylls a, b and carotenoids on exposure to heavy metals has been observed in many species treated with different metals (MACFARLANE and BURCHETT, 2001; EKMEKÇI *et al*., 2008; GHNAYA *etal*., 2009; EKMEKÇI *et al*., 2008; MONFERRÁN *et al*., 2009). However, some authors observed no changes or slight increase of photosynthetic pigments in leaves at low metal content (ZHOU and QIU, 2005; GUPTA and SINHA, 2009). In our study, total chlorophyll, chlorophyll *a* and chlorophyll *b* contents were not different from the control for all plants of *L. usitatissimum*under investigation. On the other hand, the chlorophyll content in the leaves of *C. chinensis*was slightly decreased by the metal mixture in comparison with controls

CONCLUSION

Phytoremediation is a potential remediation technology for cleaning-up polluted soils and water. Research related to this relatively new and promise technology needs to be widened by better understanding heavy metal-induced stress in plants. Symptoms of stress in plants depend on the particular metal (yet metal combination can act differently), plant species, but also on preliminary adaptation and other factors. The evaluation of heavy metal toxicity in plants using the biochemical tests is rather complex process. The influence of metals on physiological parameters is not uniform and changes of these parameters are difficult to be generalized. The combination of measurements of various physiological parameters is necessary to evaluate the metal stress in plants. It is desirable to prepare a standard protocol for clear stress evaluation for the researchers engaged in the field of phytoremediation without background in plant physiology.

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