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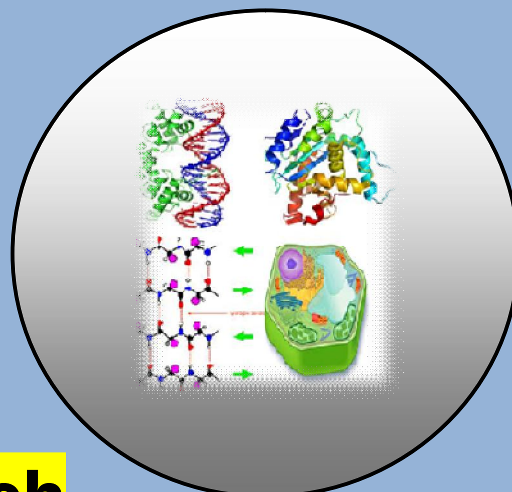

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RESEARCH PAPER

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Comparative Effects of Cadmium and Lead on the Cowpea (*Vigna sinensis* L.) Plants

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ABSTRACT

Contamination of agricultural environment by heavy metals now becomes ever growing worldwide concern because of the transportation of heavy metals in food chain. Specific attention is given to Cadmium (Cd) and Lead (Pb) as both are widely used in different industries, thus contaminating agricultural land. In toxic metal research, till date more attention was given to common cereal or oil yielding plants, therefore limited data was available for other important plants. In the present study effects of these two metals were observed in important food and animal feed crop of tropical and subtropical area viz. Cowpea (Vigna sinensis L.). Cowpea is an important economic crop in many developing regions as it has high protein content, adaptability to different types of soil and intercropping systems, resistance to drought, and ability to improve soil fertility and prevent erosion. Studies on comparative effects of Cd and Pb on Vigna sinensis shows that although both metals induce toxic effects but Pb induced more drastic changes in metabolic parameters viz. chlorophyll, protein and sugar contents. Antioxidative enzymes such as catalase and peroxidase showed varied pattern due to Cd and Pb toxicity.
Keywords: Lead (Pb), Cadmium (Cd), Cowpea, Vigna sinensis, Anti oxidative enzymes and Heavy metals.

INTRODUCTION

Heavy metals are given special attention throughout the world due to their toxic effects on man, animals and vegetation. Several cases of diseases, malfunctions and malformations due to metal toxicity have been reported throughout the world. There is extensive literature available on accumulation of heavy metals and their effects in ecosystem and their components. Uptake of toxic heavy metals by plants grown in polluted land has been one of the principal focuses of Environmental Science Research since the outbreak of itai-itai disease (Adriano, 2001).

The contamination of agricultural environment by heavy metals now become ever growing worldwide concern because of the transportation of heavy metals in food chain. Transferring of potentially toxic metals from soil to plants has been well documented (Nagajyoti et. al., 2010; Schumacher et. al., 1997).

Adriano (1986) reported that naturally cadmium (Cd) and Lead (Pb) occur in all plants but have not been shown to be essential for plant metabolism and probably taken up passively by roots and usually confined to roots (Cunningham et.al., 1996). According to Andres Schutzendubel & Andrea-Polle (2002), based on their chemical and physical properties, three different molecular mechanisms of heavy metal toxicity can be distinguished. First is production of reactive oxygen species by auto-oxidation and Fenton reaction; this reaction is typical for transition metals such as iron or copper. Second is blocking of essential functional groups in bio-molecules, this reaction has mainly been reported for non-redox reactive heavy metals such as cadmium and mercury and third one is displacement of essential metal ions from bio-molecules. It is interesting fact that even when the concentration of heavy metal is same in different kind of soils, damages to crop plant differ depending on the kind of soil. In a specific soil, no damage may occur even at the concentration of 100 ppm while in other soil, same concentration may cause severe damage to plants. Zenk (1996) reported that generally all heavy metals in excess supply cause metabolic disorders in plants. These disorders include imbalance in chlorophyll, protein and sugar content and disturbed activities of certain anti-oxidative enzymes viz. Catalase and peroxidise. The toxic effects of most metals can be due to their ability to disrupt the function of essential biological molecules such as protein, enzyme and DNA. In some cases this involves displacing chemically related metal ions that are required for important biological function such as cell growth, division and repair.

Studies on the contamination of Cd and Pb in soil and its effect on plants, have so far been restricted to highly industrialized temperate regions and the availability of quantitative data on the fate of these pollutants in soils of sub-tropical and tropical regions are limited, except some common cereal plants viz. Rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Shukla et. al., 2003; Kaneta et. Al., 1983). In comparison with advanced countries of the world, India is still lagging behind in search of scientific solutions of toxicity problems. In this context, the present study was designed to find out the toxic effects of Cd and Pb in Cowpea plant (*Vigna sinensis* L.). Cowpea is a food and animal feed crop grown in the semi-arid tropics covering Africa, Asia, United States and Central and South America. It originated and was domesticated in Southern Africa and was later moved to East and West Africa and Asia. The grains contain 25% protein, and several vitamins and minerals. The plant tolerates drought, performs well in a wide variety of soils, and being a legume, replenishes low fertility soils when the roots are left to decay. It is grown mainly by small-scale farmers in developing regions where it is often cultivated with other crops as it tolerates shade. It also grows and covers the ground quickly, preventing erosion. Cowpea's high protein content, its adaptability to different types of soil and intercropping systems, its resistance to drought, and its ability to improve soil fertility and prevent erosion makes it an important economic crop in many developing regions. The sale of the stems and leaves as animal feed during the dry season also provides a vital income for farmer (IITA, 2014).

MATERIAL AND METHODS

Experiment was carried out in sand culture conditions. Plants of Cow pea (*Vigna sinensis*) were grown in controlled glass house condition in pots having purified sand in replicates. Controlled plants were provided only basal nutrient solution whereas test plants were treated with different doses of cadmium chloride and lead acetate along with basal nutrient solution. Plants were observed daily for some abnormal changes in the form of chlorosis, necrosis, browning of leaf tissue and other typical toxic symptoms resulted due to cadmium and lead exposure.

The composition of basal nutrient solution was the same as given by Hewitt, 1966. Macro and micronutrient solutions were prepared by using A.R. (Analytical Reagents) grade chemicals. Graded levels of cadmium chloride and lead acetate were super imposed on basal nutrient solution and were supplied in the doses of 0.25, 0.5 and 1.0 mM metal salt. The basal nutrient solutions (control) along with respective treatment of cadmium chloride and lead acetate were supplied regularly for proper growth of plants.

As far as morphological parameters were concerned, numbers of plant branches were counted in each pot and their mean values were taken. After completing the experiment period i.e. 60 days plants were harvested for taking fresh and dry matter yield. For evaluating the fresh weight of plants, they were taken out from plastic containers, washed with running water followed by distilled water. Fresh weight of plants was taken after soaking them with the help of blotting sheet. Then they were cut into pieces and kept in oven at 70 °C for 3 days after which the dry weight of each plant was measured with the help of electronic balance. For metabolic parameters, fresh leaf was ground with sand in ice chilled pestle and mortar kept in ice bath. 1 g of leaf tissue was extracted in 10 ml of glass distilled water. The homogenate was filtered through two folds of muslin cloth with the help of Buchner funnel and stored at freezing temperature in refrigerator. Leaf extract was used for estimation of various metabolic parameters.

Chlorophyll concentration was measured by the method of Ptering, 1940, Protein and total sugars were estimated by the methods of Lowry et al. 1950 and Dubias et al. 1956 respectively. The activities of antioxidative enzymes were measured in the fresh leaf extracts. Catalase was assayed by the method of Bisht, 1972, a modified method of Euler and Josephson 1927, while that of peroxidase was measured by the method of Luck (1963).

RESULTS AND DISCUSSION

Growth parameters and visible symptoms

In general Cadmium (Cd) and Lead (Pb) produce typical phytotoxic effects. They induce chlorosis of younger leaves followed by older leaves and as a consequence inhibited plant growth. But in particular Cd treatment stimulates growth of Cowpea plants at 0.25 mM level although a reduction was noted at higher doses. It is interesting to note that at lowest dose i.e. 0.25 mM Cd, increased shoot length and excessive branching was observed as compared to control plants but dry weight of treated plants was reduced (Table 1). Excessive chlorosis was noticed as a consequence of long term continuous treatment of $CdCl_2$ to Cow-pea plants. Some red and brown spots were found to be developed in the inter and intra-veinal regions of the leaves.

Necrosis of leaves was also observed at higher doses. After long period of exposure, rapid basal leaf shedding was observed. In contrast to Cd, Pb treatment causes a reduction in growth at lowest dose of 0.25 mM but at higher doses i.e. 0.5 mM and 1.0 mM, growth was found to be stimulated in terms of shoot length and excessive branching. Probably the cause of elongation is reversal of dwarfism due to excessive synthesis of gibberellins caused by Pb-stress conditions. As in the case of Cd, Pb also causes reduced dry weight at all treatment doses as well as chlorosis, necrosis and development of small brown patches in the inter and intra-veinal regions of leaves (Table 1).

It has been observed by various workers in previous literature that the effect of heavy metals differs from plant to plant but in general almost all heavy metals produce typical phytotoxic effects (Bisht *et.al.* 1976, Zenk 1996, Tandon and Gupta 2002). They induce chlorosis of younger leaves followed by older leaves and as a consequence inhibited plant growth. The results of present study are in conformity with earlier reports. The plants express chlorotic symptoms due to interference in Fe-metabolism. Disturbance in Fe metabolism attributes to Fe deficiency resulting in chlorotic responses of plants. It has been observed during present investigation that toxic effects on plant growth is directly proportional to increasing doses of heavy metals, but in some instances, Cd and Pb promoted the visible growth of plant i.e. shoot length and excessive branching but fresh weight and dry weight of plant reduced. Growth suppression was also reported by several workers (Dixit *et al.* 2001, Liao *et al.* 2003, Singh *et al.* 2006). The main factor responsible for reduced growth of plants is associated with abnormal transport of essential nutrients including Zn, K, Fe, Mg etc. Cd and Pb are generally not considered as essential elements for the growth of plants but they appear to stimulate plant growth in some plants in small amounts (Jiang and Liu, 2010) as observed in case of Cowpea (*Vigna sinensis* L.). Probably the induction in growth at lower doses might be related with the reversal of dwarfism due to excessive synthesis of gibberellins (growth hormone) resulted due to heavy metal stress conditions. In an earlier study, Pb was reported to enhance root and shoot length in Gram at 2.0 mM and 0.5 mM doses respectively (Tandon and Gupta, 2002).

Effect on chlorophyll

Chlorophyll concentration was found to be reduced at all the given doses of Cd and Pb i.e. 0.25, 0.50 and 1.0 mM as compared to control plants in a dose dependent manner (Fig. 1). Lead has more drastic effect on chlorophyll concentration as compared to cadmium. Inhibition in the biosynthesis of chlorophyll in leaves under Cd and Pb stresses as found in this study was already reported by various workers (Stobart et al. 1985; Shukla et al. 2003; Liu et al. 2008; Piotrowska et al. 2009; Singh et al. 2010; Cenkci et al. 2010). Adverse effects of heavy metals on Chlorophyll and Fe concentration in plants were also reported by Behera and Mishra (1983), Gallego *et al.* (1996) and Baryla *et al.* (2001). Chlorophyll inhibition is believed to result from the many indirect effects of lead and Cadmium like distorted chloroplast ultrastructure, decreased ferredoxin NADP+ reductase and delta-aminolevulinic acid dehydratase (ALAD) activity, chlorophyll synthesis inhibition (Gupta et al. 2009; Cenkci et al. 2010), impaired uptake of essential elements such as Mn and Fe (Chatterjee et al. 2004; Gopal and Rizvi 2008) and substitution of divalent cations by lead (Gupta et al. 2009; Cenkci et al. 2010).

However, these different effects vary by plant species. Generally, chlorophyll b is more sensitive than chlorophyll a (Xiong et al. 2006). The mechanism of chlorophyll breakdown into phytol, magnesium and the primary cleavage product of the porphyrin ring occur in four consecutive steps. This reaction is catalyzed by chlorophyllase, Mg-dechelatase, pheophorbide oxygenase, and red chlorophyll catabolite reductase.

Effect on Protein

Protein concentration was found to be decreased at lowest Cd treatment i.e. 0.25 mM but an enhancement was noted at higher doses (Fig 2). In case of Pb treatment Protein concentration was adversely affected at all the doses but maximum reduction was found at 0.5 mM Pb concentration (Fig.2). Although effect of cadmium and lead on the total concentration of protein is unclear, but high concentrations of these metals may decrease the protein pool (Chatterjee et al. 2004; Mishra et al. 2006; Garcia et al. 2006; Piotrowska et al. 2009). This quantitative decrease in total protein content is the result of several effects like acute oxidative stress of reactive oxygen species (ROS) (Piotrowska et al. 2009; Gupta et al. 2009), modification in gene expression (Kovalchuk et al. 2005), increased ribonuclease activity (Gopal and Rizvi 2008), protein utilization by plants for the purposes of metal detoxification (Gupta et al. 2009), and diminution of free amino acid content (Gupta et al. 2009) that is correlated with a disturbance in nitrogen metabolism (Chatterjee et al. 2004).

However, certain amino acids, like proline, increase under metal stress (Qureshi et al. 2007). Such proteins play a major role in the tolerance of the plant. In contrast, low concentrations of these metals may increase total protein content (Mishra et al. 2006). This protein accumulation may defend the plant against metal stress (Gupta et al. 2010), particularly for proteins involved in cell redox maintenance.

Effect on Sugar content

Sugar concentration was adversely affected at 0.25 mM and 0.50 mM Cd level but an enhancement in sugar content was observed at 1.0 mM Cd (Fig. 3). In contrast to Cd treatment, enhanced total sugar concentration was noted at 0.25 and 0.50 mM Pb treatment, but significant increase was found only at 0.25 mM Pb treatment. Reduction in sugar content was observed at 1.0 mM Pb treatment (Fig. 3). Singh et al. (2006) have already reported reduced sugar concentration at excess supply of Cd and Cr. This is in conformity with the findings of this study. Heavy metals such as Cd and Pb are responsible for developing water stress conditions to plants and as a result, relative water content (RWC) of plants gets decreased which negatively affect the process of photosynthesis causing reduced sugar content in plants. Lang et al. 1995 reported reduced CO_2 assimilation due to Cd stress condition which is responsible for decreased sugar content in leaves. Significant increase in the total sugars content with increasing concentration of heavy metals have been also reported by some previous workers (Bisht et al. 1976; Agarwala et al. 1977). This might be due to heavy metal stress restrict proper translocation of sugar from leaf to root which results in excess storage of sugar in leaf tissue.

Effect on Antioxidative enzymes

Activity of two anti-oxidative enzymes viz. catalase (CAT) and peroxidise (POD) were measured for the Cd and Pb treated plants. The activity of catalase enzyme was found to be significantly reduced at all the doses of Cd treatment but in case of other Fe-enzyme, various pattern of results have been expressed.

Peroxidise activity get enhanced at a dose of 0.25 mM Cd but an inhibition was recorded at 0.5 mM and 1.0 mM doses (Fig. 4). The presence of Pb in growth medium drastically changed the activity of Fe-enzymes in Cowpea plants. Catalase activity was found to be adversely affected at all the doses of Pb but in case of peroxidise enzyme, an enhancement in activity was observed at 0.25 mM and 1.0 mM Pb concentration but activity of peroxidase enzyme was reduced at exposure of 0.50 mM Pb (Fig. 5).

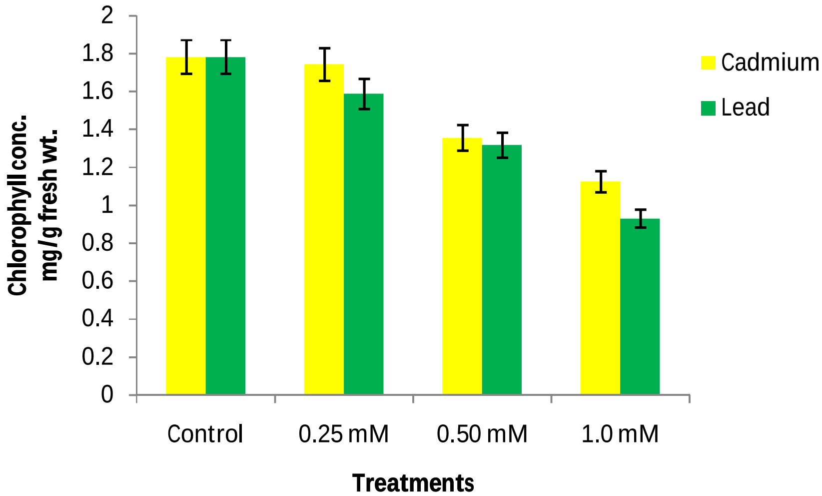

Fig. 1. Effect of different treatments of Cd and Pb on the chlorophyll concentration of *Vigna sinensis* L. Plants

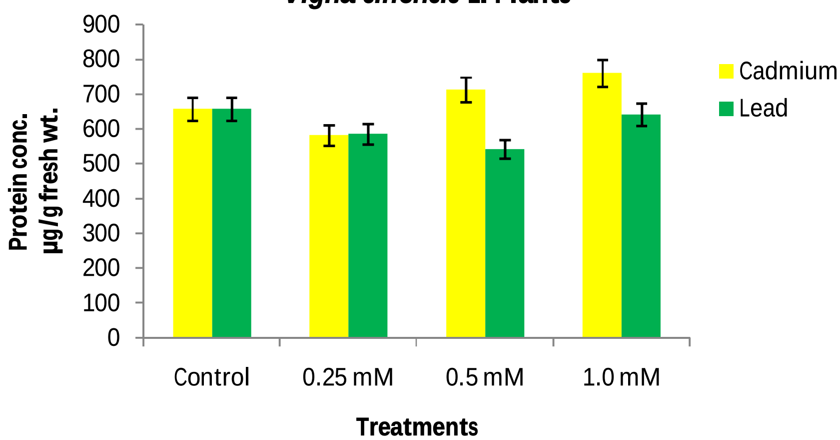

Fig 2. Effect of different treatments of Cd and Pb on the Protein concentration of *Vigna sinensis* L. Plants

The activities of two iron enzymes viz. catalase and peroxidase were differentially affected by different doses of cadmium and lead. The activity of both antioxidative enzymes viz., POD and CAT showed variable trends during the present investigation. In some cases, the activity as found to be enhanced while in other cases it was found to be suppressed.

Heavy metal pollution induces plant to produce more peroxidase and enhances POD activity. Higher POD activity reflects more serious damages happened on plant organs. Liao et al. (2003) observed increased POD activity in *Vicia faba* plant in heavy metal stress condition.

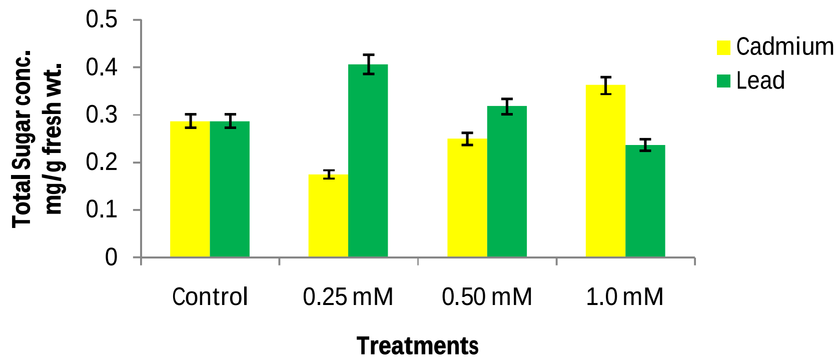

Fig 3. Effect of different treatments of Cd and Pb on total Sugar concentration of *Vigna sinensis* L. Plants

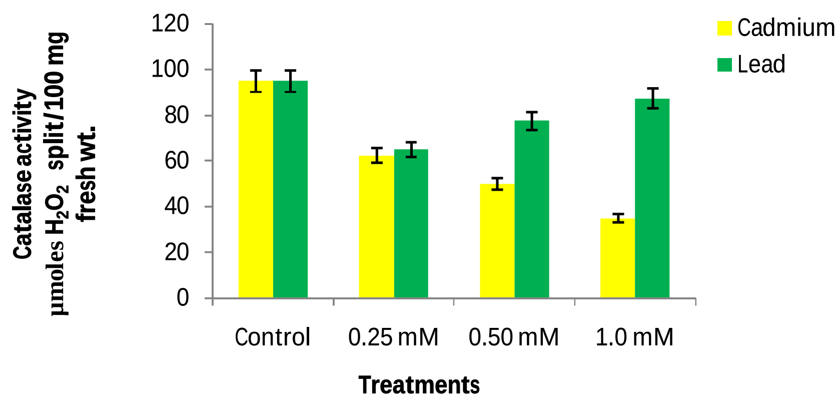

Fig 4. Effect of different treatments of Cd and Pb on the Catalase activity of *Vigna sinensis* L. Plants

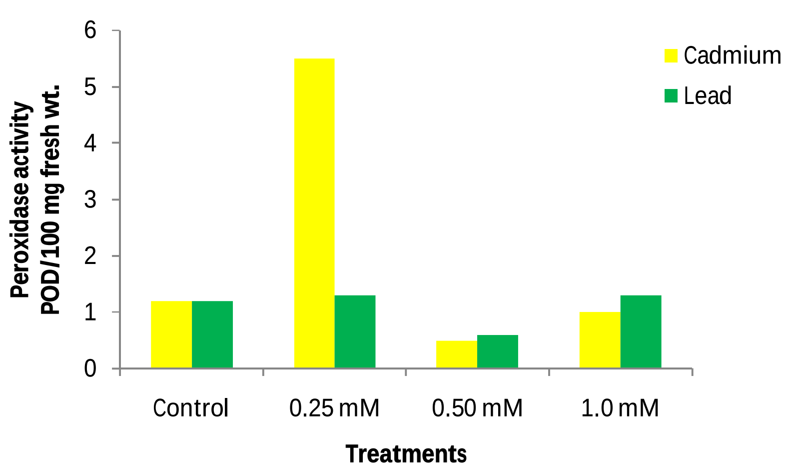

Fig 5. Effect of different treatments of Cd and Pb on the Peroxidase activity of *Vigna sinensis* L. Plants

Table 1. Effect of different doses of Cadmium (Cd) and Lead (Pb) on the Shoot length and biomass of *Vigna sinensis* L.

S.No.	Treatments	Shoot length (cm)	Fresh Weight (gm.)	Dry Weight (gm)
1.	Control	45.85±2.60	66.0±1.50	9.0±080
2.	0.25 mM Cd	49.65±1.80	33.0±2.60	3.7±0.40
3.	0.50 mM Cd	40.30±1.55	28.0±2.20	3.4±0.30
4.	1.0 mM Cd	36.95±2.30	25.0±1.70	3.1±0.20
5.	0.25 mM Pb	35.85±2.1	30.0±2.20	3.6±0.20
6.	0.50 mM Pb	53.95±2.10	39.0±2.50	4.8±0.50
7.	1.0 mM Pb	54.70±2.35	42.0±3.20	5.4±0.30
8.	CD Value at 5% P	1.380	3.335	1.628

Values represent the mean±SD of two replicates
CD represents Critical difference between two observations

Enhanced activity of these antioxidative enzymes might be due to the generation of metal ion induced H_2O_2 and ROS (Reactive oxygen species). An elevated activity of antioxidative enzymes indicates the excessive heavy metal stress conditions in plants and the changes in the activity of enzymes can be correlated with the plant species and heavy metal type. Catalase activity might be suppressed due to the reduced supply of iron for the synthesis of Catalase enzyme. This might be due to the fact that during heavy metal stress conditions, the transport of essential elements gets blocked which might have resulted into reduced catalase activity. A decrease in catalase activity was also reported by Somashekaraiah et al. 1992.

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