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Original Research Article

Eco-toxicological studies of a pulse crop (*Vigna radaita*, L.) with lead stress.

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Abstract: In the present study, the ecotoxicological effects of Lead nitrate is evaluated taking a locally cultivated pulse crop *Vigna mungo* L. The effective concentration of the toxicant was determined taking in to consideration the emergence of shoot. LC_{50} was found out to be about 380 mg/l. The germination data showed that there is direct impact of concentration of lead nitrate on the germination of seeds. Root & shoot growth of seedlings was worst effected with exposure to lead nitrate. Roots are more affected then the Shoot Morphologically they look different from normal roots by their colour and shape. Effect of different concentration of lead nitrate was visible in different pigment concentration of leaves. With increase in the concentration of the toxicant expose to the seeds there were decline in chlorophyll-a, chlorophyll-b total chlorophyll, carotenoid and phaeophytin content of leaves. This was a clear indication of fall in the growth rate of the plant, as a fall in the pigment content had direct impact on photosynthesis. There was increase in the amino acid contents of roots and shoots with increase in the concentration of the toxicant. The impact of lead nitrate to soluble sugar in seedlings showed a decrease within increase on the concentration of lead nitrate. It was found out that there was increase in the DNA content of root and shoot tissues percentage change of DNA content shoot tissue was marginally high to that of the control it was bit higher in the root tissues. The percentage change in the RNA content of both the tissues showed an increasing trend. The change in the protein content of root and shoot tissue showed an increasing trend was seen in both the tissue expose to the toxicant.
Keywords: Chlorophyll, lead nitrate, mung bean, seedlings, toxicity

INTRODUCTION:

Lead is one of the most common heavy metal contaminant in the environment [1] and has gained considerable importance as a potent environmental pollutant in almost all. Excessive lead accumulation in plant tissue impairs various morphological, physiological, and biochemical functions in plants, either directly or indirectly, and induces a range of deleterious effects. It caused phytotoxicity by changing cell membrane permeability, by reacting with active groups of different enzymes involved in plant metabolism and by reacting with the phosphate groups of ADP or ATP, and by replacing essential ions. In addition, lead strongly inhibits seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, and water and protein content. The negative effects that lead has on plant vegetative growth mainly result from the following factors: distortion of chloroplast ultra-structure, obstructed electron transport, inhibition of Calvin cycle enzymes, and impaired uptake of essential elements. Under lead stress, plants possess several defense strategies to cope with lead toxicity. Such strategies include reduced uptake into the cell; sequestration of lead into vacuoles by the formation of complexes; binding of lead by phytochelatins, glutathione, and amino acids; and synthesis of osmolytes. The major processes affected are seed germination, seedling growth, photosynthesis, plant water status, mineral nutrition, and enzymatic activities [2] and the enzymes involved in those and lead interferes with several physiological and biochemical processes; photosynthesis being one of the most affected.

The present work is designed to study the effects of the heavy metal Pb on the growth and photosynthetic efficiency, physiological and biochemical aspects of a widely cultivated pulse *Vigna mungo* L. (OUM 11-5 Kamdev) cultivated in the Berhampur locality. This work is a part of the present effort to review stress by different types of metal on different plant species in this laboratory.

MATERIALS AND METHODS:

Test Organism:

The test organism for the present study is a commonly cultivated pulse *Vigna mungo* L. The variety

used in the present work is (OUM 11-5 Kamdev). Pure seeds were procured from the Central Pulse Research Station, OUAT, Ratanapur, and Berhampur. It is an annual herb, usually cultivated after rice is harvested, in the month of Nov-December and harvested in the month of March-April. One of the purposes of choosing the test organism is to consider the impact of roadside pollution of Lead.

Test Chemical:

Lead Nitrate Pb $(NO_3)_2$, was used in the work was of a guaranteed reagent from Thomas & Baker, India. First stock solution of 1000mg/L was prepared by taking 1gm of test chemical in 1litre of distilled water. Subsequent dilutions were made using distilled water and solutions of 100,150, 200, 250, 300, 350, 400, 500, 600, 700, 800, 900 mg/l were made. Fresh test solutions were prepared each time experiments were performed.

Morphological Studies:

Growth of the seedlings was measured by taking the root and shoot on the 7^{th} day of inoculation.

Determination of LC $_{50}$:

LC $_{50}$ (Lethal Concentration 50%) was calculated by the considering shoot emergence of Mung bean seeds in test solutions of 100,150, 200, 250, 300, 350, 400, 500, 600, 700, 800, 900 and 1000mg/I concentrations. Observations were presented in Table 1. LC $_{50}$ values were calculated from data using HPSS regression analysis. $Pb(NO_3)_2$ of Concentrations 100mg/l, 300mg/l, 500mg/l, 700mg/l and 900mg/l were taken for exposing the seeds and distilled water as control and observations of different parameters and recorded in the study.

Biochemical Studies:

The total chlorophyll (chl), chlorophyll-a (chl-a), chlorophyll-b (chl-b) and caretonoid content was measured by the method given by Arnon [3]. The Phaeophytin content was measured by recording the absorbance of the extract at 645, 663nm and the values were calculated by using the formula given by Vernon [4]. The amino acid content was determined by the method of Moore and Stein [5]. Estimation of protein was carried out following the method of Lowry *et al.;* [6]. The DNA and RNA content was estimated by following the method of Schneider [7]. The sugar content was measured by method [8].

Statistical Analysis

Statistical analysis for determination of Pearson's correlation was carried out using SPSS 17.0, while the tabulation and computation of the data was made using MS Excel.

RESULTS:

A dose response curve was prepared to find out the lethal concentrations of the test chemical (Fig.-1). LC_{50} value was found out to be 460 mg/l.

Seedling Growth:

Data clearly indicated a sharp decline in the root and shoot length of the seedlings. Ratio of Root & Shoot lengths and their percent changes were given in Fig. No.1 and 2. Decreasing trend was seen in the root and shoot length corresponding to increase in concentration of the test chemical.

There were visible morphological changes seen in the roots which appeared different from that of the control. Roots appeared to be swollen in higher concentrations of test chemical and developments of lateral roots were less in comparison to the control.

Pigment Contents:

Pigments viz. Chlorophyll-a, Chlorophyll-b, total Chlorophyll Phaeophytin and Carotenoids were presented in Fig. No.3

There is significant decline in the chlorophyll-a , chlorophyll-b and Total Chl.pigment with the increase in the concentration of lead nitrate. The percent change in the amount of chlorophyll-a of the exposed seedlings of 100mg/l showed a fall by 52.67% of control. The highest fall was recorded at the 900mg/l exposure, a fall by 83.64% of the control in the pigment. Effect of the toxicant on the contents in comparison to that of the control and their percent change against control.

Effects on the carotenoid and Phaeophytin contents of the exposed seedlings showed clear trends of decline in the carotenoid content with increase in the concentration of test chemical.

Effects on the Biomolecules:

Effect of lead nitrate on the amino acid contents on the root and shoot tissues are presented in Table No. -1. There is an increase in the amino acid contents in shoot and root of the seedlings with increase in the concentration of toxicant except in the concentration of 700mg/l of $Pb(NO_3)_2$ .Mung seedlings growing in lead nitrate treatment showed soluble sugar contents of root and shoot tissues decreasing in comparison to the control. DNA content of root tissues marginally increase by 2.67, 27.33, 26.67 and 28 % to the control value exposed lead nitrate. However there was decline in the DNA content by 4% in the highest concentration of 900mg/l of exposed mung bean seedlings. In the control shoot DNA content was found out to be 0.014mg/g which marginally increased with increase in the concentration of the test chemical. Highest amount of DNA (0.155mg/g) was found in shoot tissue exposed to 900mg/l of lead nitrate. There was increase in the RNA content with increase in the concentration of lead nitrate however a slight decline is observed in 500mg/l and 700mg/l. A decrease in the RNA content by 35.13% was seen with exposure to 100mg/l but there was an increase in the subsequent higher

concentrations. Protein contents of root tissue and percentage change of the exposed tissue to that of the shows decline in the Protein content.

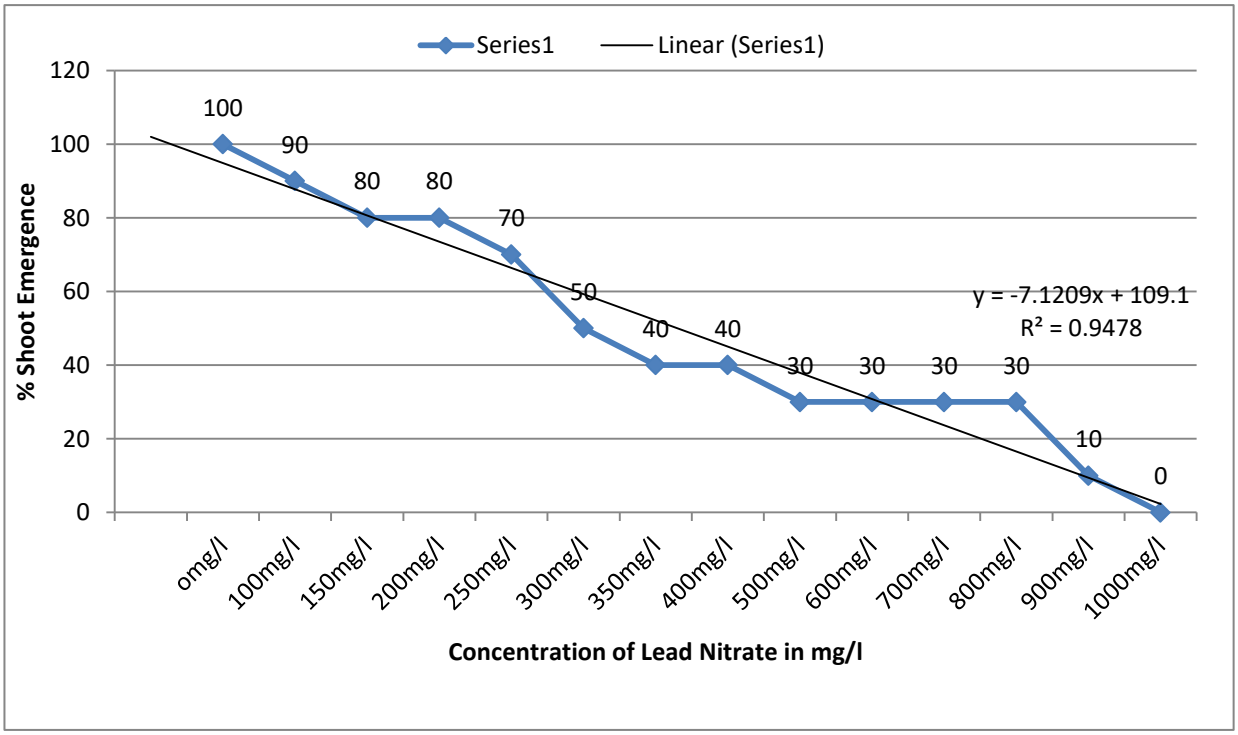

Fig 1: Dose response curve of percentage change of shoot emergence of *Vigna mungo* L. seeds treated with different concentrations of lead nitrate on 7th day Shoot emergence.

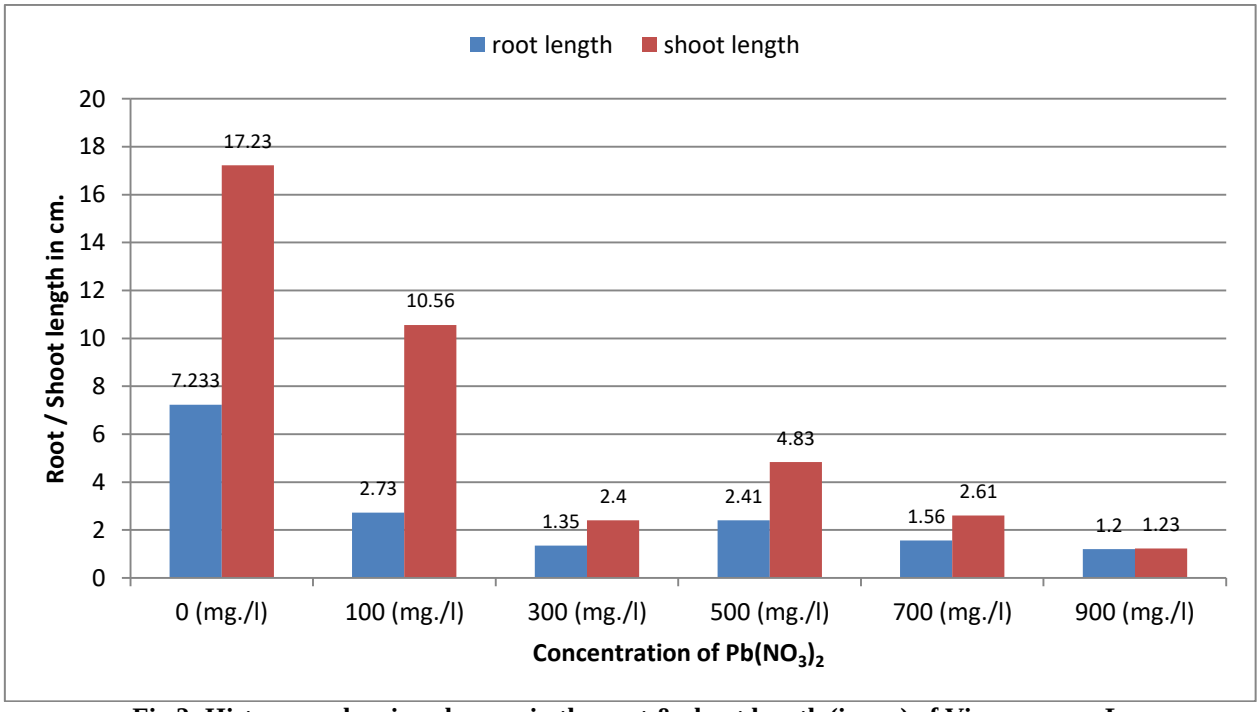

Fig 2: Histogram showing changes in the root & shoot length (in cm) of Vigna *mungo* L. Seedlings of control and lead nitrate treated seeds.

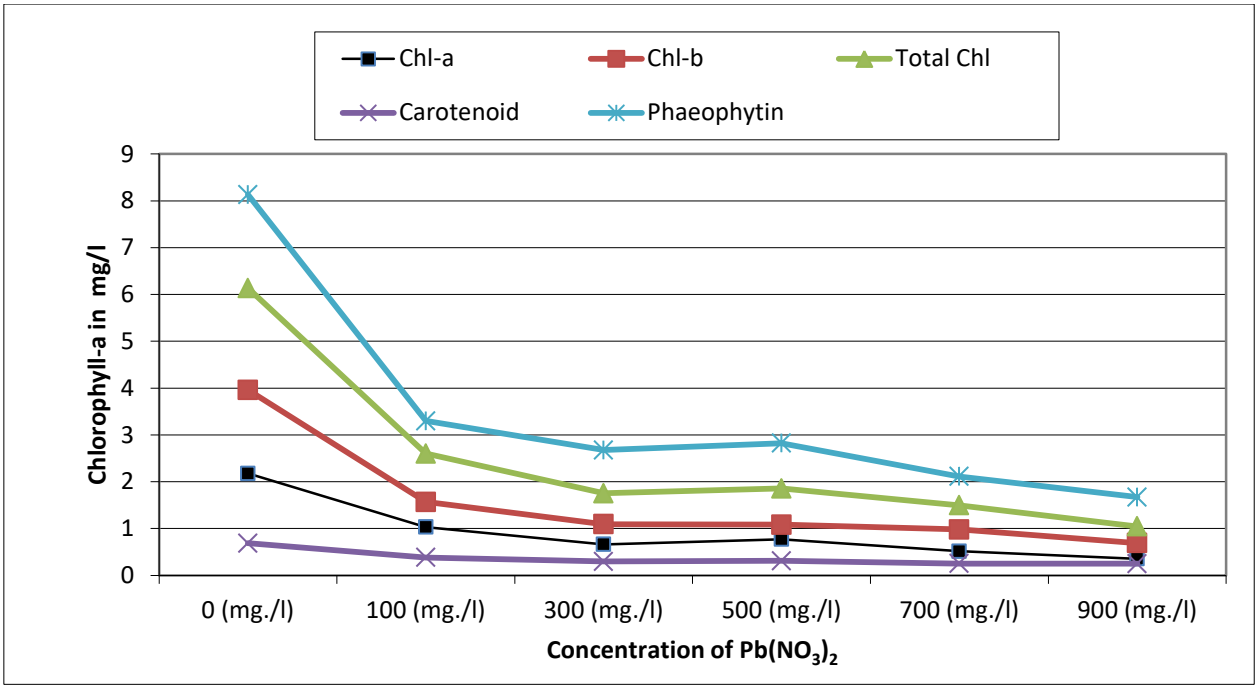

Fig 3: Effect of lead nitrate on change in the Chl-a, Chl-b, Total Chl., Carotenoid and Phaeophytin contents (in mg/g) in leaves of *Vigna mungo* L. seedlings exposed to different concentrations of lead nitrate.

DISCUSSION:

Generally, Pb toxicity is associated largely with roots of the plant as compared to other aerial parts of the plant. This can be attributed to higher accumulation of Pb in the cell walls of the root in contrast to other parts of the plant [9-11]. Such growth retardation might have been contributed by disturbances in various physiological and biochemical processes [12, 9].

Hussain *et al.*, [12] reported a continuous decrease in germination (10−100 %) of *Zea mays* with the increasing Pb concentration compared to the control. Pb has been found to decrease the seed germination in *Arachis hypogeae* [13, 14]. Pb contamination in plant environment affects germination of seeds and exerts deleterious effects on the growth and metabolism of plants [13, 15-17]. The permeability of lead through the seed coat and its impact on seed germination was studied by Wierzbicka & Obidzińska [18]. Results of the present investigation are in accordance to the findings of the above works. In the present study, chlorophyll-a, chlorophyll-b and total chlorophyll content in the exposed leaves declined with the increase in concentration of the lead nitrate. The percent change in the total chlorophyll was from 2.65 – 1.004% is in confirmation to the data presented by Zengin and Munzuroglu [19] & Singh *et al.;* [20].

Photosynthetic pigments (chlorophyll a and chlorophyll b significantly. Chlorophyll contents are reduced by high concentration of Pb, because Pb prevents the incorporation of Fe (iron) in phyto porphyrin ring of chlorophyll molecule, so cause reduction in chlorophyll contents [21]. Reductions in the level of photosynthetic pigments, including Chl-a, Chl-b and carotenoids, after exposure to heavy metals, including Pb, has been observed in many plant species [22-24]. The reduction of Chlorophyll b was more than overall Chlorophyll content. This can be associated with the alteration in pigment composition of photosynthetic approach that possesses lower level of light harvesting chlorophyll proteins the LHCPS [25, 26]. Photosynthesis in higher plants is more sensitive to heavy metal treatments, affecting biosynthesis of cholorphyll and accessory pigments [26-29]. It can be assumed that lead may inhibit chlorophyll biosynthesis by impairing the uptake of essential photosynthetic pigment elements, such as magnesium, potassium, calcium and in [23].

There might be increase or decrease in macro-molecules of plant tissues exposed to toxicants [30]. In the present study there was increase in soluble sugar, DNA, RNA and protein contents and decrease in amino acid contents. With exposure to lead DNA double strand breaks, induced by reactive oxygen species can lead to chromosome fragments. Earlier studies have shown that Pb increases the free radical level in cells [31]. Which has several deleterious effects on the crucial macromolecule of cell such as DNA. Decline in DNA and RNA content of *Phaseolus vulgaris* to lead exposure was reported by Jana & Choudhuri [32], Hamid *et al.*, [33] who also found a decrease in DNA and RNA content with heavy metal stress Elements such as Cu, Ni, Cd and Pb have been reported to decrease RNA synthesis and to activate ribonuclease

(RNase) activity, leading to further decrease in RNA content [34].

Pb alters the content of macromolecules (proteins and carbohydrates) and activities of related hydrolyzing enzymes. Singh *et al.;* [20] reported Pb-induced increase in protein and carbohydrates content in *B. campestris* roots. Suzuki *et al.;* [35] suggested that these metal-induced proteins play a significant role either in detoxification and/or in the maintenance of heavy metal homeostasis. Kaur *et al.;* [36] correlated the enhanced the contents of water soluble proteins and carbohydrates with the reduced activities of proteases and amylases in wheat radicle in a dose-dependent manner after 24 hr of Pb exposure. Induction in protein content is possible due to induction of stress proteins [37] under lower metal exposure.). Kratovalieva and Cvetanowska, [38] reported an increase in total sugars in tomato under the influence of Pb. Increased carbohydrates content indicated either failure of the plant to hydrolyze carbohydrates or *de-novo* synthesis of enhanced carbohydrates under Pb-stress. Many studies showed that the protein content of plants was decreased by Pb accumulation [22, 39- 41].

Table 1: The effect of lead on biochemical parameters of shoots and roots in *Vigna mungo* L Seedlings of 7- days old

Concentration of Lead Nitrate	Amino acid, mg/g		Sugar, mg/g		DNA ,mg/g		RNA, mg/g		Protein,mg/g	
	SHOOT	ROOT	SHOOT	ROOT	SHOOT	ROOT	SHOOT	ROOT	SHOOT	ROOT
0 (mg./l)	4.850	4.500	2.707	3.797	0.140	0.150	1.950	2.335	6.110	4.205
100 (mg./l)	5.440	6.640	1.720	2.710	0.143	0.154	1.265	3.674	5.840	6.960
300 (mg./l)	7.330	8.815	2.006	2.852	0.144	0.191	4.150	3.892	7.320	7.460
500 (mg./l)	6.440	10.945	2.111	2.964	0.147	0.190	4.059	3.782	8.260	4.930
700 (mg./l)	7.280	10.635	1.890	2.794	0.143	0.192	4.638	3.624	7.340	5.920
900 (mg./l)	9.390	11.350	2.416	3.339	0.155	0.144	5.210	4.380	1.620	5.550

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