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Lead induced changes in DNA and protein content in *Vigna radiata* L.

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Abstract
Pollution of heavy metals in environment is a growing problem worldwide and currently it has reached at an alarming rate. This heavy metal pollution affects the major threat to Agricultural sector. Lead (Pb) is extensively used in electronic and other industries as an important heavy metal. In the present study phytotoxic effect of heavy metal lead determine on *Vigna radiata* L. seedling. The different concentrations of lead (25ppm; 50ppm; 75ppm and 100ppm) were used. After the 7 days seedling were harvested and measured the fresh and dry weights, water content, length, chlorophyll content, protein, and changes in DNA content. Further, protein of control and treated seedlings was characterized by SDS-PAGE. Treatment with Pb showed that decrease in dry weight, water content, length and chlorophyll content was observed while DNA and protein concentration increased with Pb concentrations.

Keywords: DNA, Lead, mungbean, protein

Introduction
Heavy metals are major environmental pollutants; their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons because these elements can enter in food chains and the biological cycle (Dugar and Bafna, 2013; Asati *et al*., 2016) [9, 3]. Heavy metals are one of the major sources of soil pollution. Heavy metal pollution of the soil is caused by various metals, especially Cu, Ni, Cd, Zn, Cr and Pb (Karaca *et al*., 2010) [15]. Lead (Pb) is one of the most distributed abundant toxic elements in the soil. Plants absorb Pb from solution in the soil through their roots and translocation in to whole plant, the maximum concentration of $Pb2^+$ is accumulated within roots in an insoluble form (Wierzbicka *et al*., 2007) [31]. Lead can affect the physiological process of plant such as seed germination, plant growth, water status; photosynthesis and nitrate assimilation (Sharma and Dubey, 2005) [28]. Metal-contaminated soils not only reduce crop production, but also affect life indirectly via edible parts of crop plants. In the present study, *Vigna radiata* was selected as a legume plant; commonly known as Mungbean. It is the third most important pulse crop in India, in an area of 3.44 million hectares with annual production of 1.54 million tons and average productivity of 461 kg ha^{-1} (Kalaria, 2017; Verma *et al*., 2017) [14, 30]. Like many plants, mungbean at seed germination and seedling stages are sensitive to environmental factor. The presence of heavy metals in the soil is also an important factor influencing seed germination and seedling growth. Considering to this in the present study, assessed the effect of different concentrations of heavy metal lead on *Vigna radiata* L seedlings and estimate the growth, changes in DNA and protein content.

Method and Materials
Test Plant
Certified seeds of *Vigna radiata* L. were purchased from the local market, Rajkot. Equal sized seeds were screened and washed with tap water for 3-4 times and soaked in distilled water for 2 h. Seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) to prevent any fungal contamination. Seeds were washed with 3-4 times double distilled water immediately before use.

Heavy metals and their different concentrations

The stock solutions of lead (Pb) were prepared at concentrations of 25ppm, 50ppm, 75ppm, and 100ppm by using standard APHA methods. Distilled water was used as a control. The seeds were then allowed to germinate in sterilized Petri dishes on Whatmann filter-paper moistened with 10 ml of selected heavy metal test solution and kept in dark for 36 h. Each Petri dish was contained 30 seeds. The experiment was conducted in a growth room at 20 ± 25ºC for 7 day under white light, Lux 1680 (114240 x1020 photons.m^2.s^{-1}), 12 h photoperiod. For each metal concentration and control groups 10 seedlings were used. The experiment was repeated thrice.

Growth Analysis

Growth is measured in the terms of fresh weight, dry weight, water content and seedling and root length. The length of seedling and root were recorded by using a centimetre scale. For the measurement of fresh and dry weights, freshly harvested seedlings were taken. Freshly harvested seedlings were weighed before and after oven drying to a constant weight at 65ºC for 72 hours. Water content of each stage was determined by difference in fresh and dry weights. Data were taken in 10 replicates and the mean value was calculated with ± standard deviations.

Tolerenc index and phytotoxicity

Tolerance index (TI) was determined as suggested by Iqbal and Rahmati (1992) [12].The phytotoxicity (%) for shoot of 7 days old seedlings were calculated according to the Chou and Lin (1976) [6].

Determination of chlorophyll content

Chlorophyll content was determined according to Arnon (1949) [2] spectrophotometrically. Data were recorded at two wavelength i.e. 645 and 663nm. Data were taken in triplicates and the mean value was calculated with ± standard deviations

DNA Isolation

DNA isolation was performed according to CTAB protocol given by Doyle and Doyle (1987) [8]. DNA was analyzed though 2% agarose gel electrophoresis. The quality and concentration of the DNA were confirmed by measuring optical density 260/280 nm ratio.

Extraction of Protein

Enzymes were extracted from mung bean seedlings at 7 days after germination. The control and treated seedlings were collected and powdered with liquid nitrogen. 1 gram of crushed material was homogenized within 15 ml 0.1M Tris buffer pH 8.0 in a prechilled mortar and pestle. The homogenized was centrifuged at 10,000 g for 15 min at 4ºC and supernatant served as crude protein source.

Protein Estimation

Protein contents were determined according to the method of Lowry *et al*., (1951) [20] using bovine serum albumin as a standard. Calibration rage of BSA was prepared with 100-1000 ug/ml protein.

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli (1970) [17]. All protein samples were prepared in 2X sample buffer. Protein with sample buffer was heated in a boiling water bath for 3 min. Approximately 1mg of reduced protein was loaded into sample well. Protein of control and treated seedlings were loaded onto 10% (W/V) polyacrylamide gel with a vertical electrophoresis unit (Biotek, USA). Electrophoresis was performed at a constant voltage of 50V initially for 1 h and 100V till complete gel run. Supply of current was stopped as soon as the marker dye reached to the lower end of the separating gel. Gel was stained by coomassie brilliant blue R-250, for 1 h and distained overnight.

Results and Discussion

In the present study, we have examined the toxicity of lead on mungbean seedling and measured the different growth parameters and estimated chlorophyll, protein and DNA content. Growth measured by the physical parameters, fresh and dry weights and, water content are shown in Table 1. The fresh weight, dry weight and water content were expressed as mg $seedling^{-1}$. Fresh weight in control seedling was recorded 75.75±1.5mg and then decreased with increasing the concentration of Pb. Similarly, dry weight in control seedling was recorded 6.0±0.3mg which decreased with increasing the concentration of Pb treatment. Water content in control seedling was 69.75±0.2 mg and then decreased in treated plant up to 40.79±0.6 mg in 100 ppm (Table-1). In this work seedling length decreased with increasing the concentrations of lead. The average seedling and root length of mungbean decreased from 23.2-15.0 cm, and 6.5-4.5 cm respectively, due to increased concentration of Pb. Recently heavy metals inhibited seed germination and seedling growth has been reported in wheat (Monpara *et al*., 2018) [22]. Various effects of lead on plant growth have been observed i.e. inhibition of fresh weight, dry weight and length of root and shoot of *Sesamum indicum* (Kumar *et al*., 1993) [16]. In our study, varied concentrations of Pb affected fresh weight of mungbean. The decreased fresh weight of mungbean seedlings might be due to interference of Pb with the physiological processes of the plant (Pereira *et al*., 2013) [27]. Phytotoxicity of seedling was decreased at lower concentration (25 ppm) and increased at higher concentration (100 ppm) Table -1. Tolerance index calculated on the basis ratio of root length ratio of experimental to that of control shows gradual reduction of tolerance index during growth in all treatments. The Tolerance index of seedling was decreased from 90.93 to 73.31 due to treatments with increased concentrations of Pb (Table 1). Similar effects were observed in earlier studies of *Glycine max* (Amin *et al*., 2014) [1] in rice *Oryza sativa* (Panda, 2007) [25] exposed to chromium stress.

The changes in chlorophyll a, chlorophyll b and total chlorophyll content are presented in Table- 1). In this work the content of chlorophyll a, chlorophyll b and total chlorophyll was significantly decreased as the Pb concentrations increased. Control leaf shows 7.4 ± 0.3 and 2.85 ± 0.4 and 10.2±0.5 mg g^{-1} FW chlorophyll a, b and total contents respectively. Similar results have been obtained by many workers working on various crops with metal treatment. Several report have shown that metal stress decrease chlorophyll content in seedling (Nada *et al*., 2007, Gill *et al*., 2012) [23,10]. Under the metal stress, the levels of photosynthetic pigments, Chlorophyll 'a' and Chlorophyll 'b' and Carotenoids decrease as the concentrations of Pb in soil increases (Bhardwaj *et al*., 2009) [5].

DNA content/mg. f.wt of the seedlings has found to be increased at higher concentration of lead. At different concentration of metal treatment of seedling DNA has shown significant variations in comparison to control as expressed in

Table 2. The DNA quality was analyzed by gel electrophoresis and intact bands were observed on 1.0% agarose (Figure 1). The purity and yield of genomic DNA are presented in Table-2. The DNA concentration was control (167 µg/ml) 25ppm (156 µg/ml), 50ppm (138 µg/ml), 75 ppm (191 µg/ml) and 100ppm (389). Dhankhar and Solanki (2011) [7] have reported increase in DNA content with increase in zinc concentration in *Vigna mungo* (L.). Lead toxicity can reduce seed germination and radical growth in plants (Ozyigita *et al*., 2016) [24]. Growth inhibition in plants can be due to inhibition of cell division by inducing chromosomal aberrations (Liu *et al*., 1993; Panda and Choudhury, 2005) [19, 26]. Protein concentration estimated from control and treated seedling is presented in Figure 2. The protein content in seedling was 1.94 mg/ml (control), 1.97 mg/ml (25 ppm), 2.9 mg/ml (50 ppm), 3.67 mg/ml (75 ppm) and (4.58 mg/ml) 100 ppm. In this work the total protein content of seedling was increased with raising the Pb concentration. A similar protein content was increase previously observed with young wheat seedlings (Monpara *et al*., 2018) [22]. The increase in total soluble protein content under heavy metal stress may be related to induce the synthesis of stress proteins (Mishra *et al*., 2006, Lamhamdi *et al*., 2011) [21, 18]. Further the total protein was SDS- PAGE to study the variation in protein profile in both control and treated seedlings (Figure 3). Differential intensity of pattern was observed between control and treated seedlings. The intensity of protein bands was greater in treated seedlings compared to control. Previously, Gomes *et al*., (2012) [11] reported the induction of stress resistant protein in cadmium treated *Caesalpinia peltophoroides*.

Table 1: Effect of different concentrations of Lead on Mungbean seedling growth, Tolerance index, phytotoxicity and Chlorophyll content

Concentration ppm	Fresh Weight (mg)	Dry Weight (mg)	Water Content (mg)	Length (cm)	Length Root (cm)	Phytotoxicity (%)	Tolerance index	chlorophyll a mg/ g FW	chlorophyll b mg/ g FW	chlorophyll Total mg/ g FW
Control	75.75±1.5	6.0±0.3	69.75±0.2	23.2±1.1	6.52±0.2	-	-	7.4±0.8	2.85±0.6	10.2±0.9
25	59.65±1.2	5.1±1.1	54.55±1.2	20.4±1.3	5.55±0.3	12.06	90.93	6.7±0.5	2.23±0.1	9.0±1.1
50	55.50±0.6	4.75±0.5	50.75±0.3	19.8±0.5	5.25±0.4	14.56	85.43	5.5±0.5	2.18±0.1	7.7±0.6
75	52.78±1.1	4.50±1.2	48.28±0.2	17.1±0.5	4.6±0.1	26.29	78.70	3.6±0.1	1.87±0.2	5.4±0.4
100	45.04±0.5	4.25±0.2	40.79±0.6	16.0±0.1	4.52±0.2	30.68	73.31	2.2±0.1	0.89	3.1±0.1

Table 1: DNA Concentration and Purity

Sample	Purity (%)	Concentration (µg/ml)
Control	1.315	167
25ppm	1.523	156
50ppm	1.432	138
75ppm	1.550	191
100ppm	1.621	389

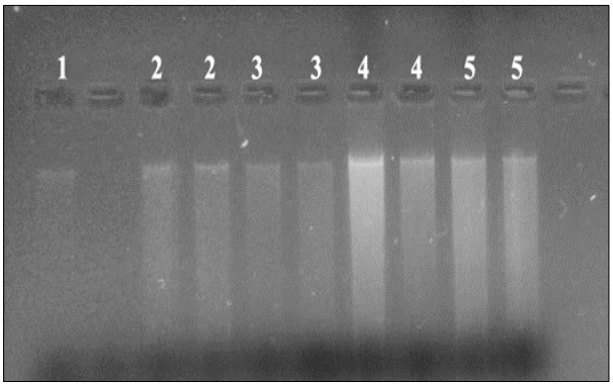

Fig 1: Agarose gelelectrophoresis control and lead treated seedling of genomic DNA lane: (1) control (2) 25ppm (3) 50ppm (4) 75ppm and (5) 100ppm

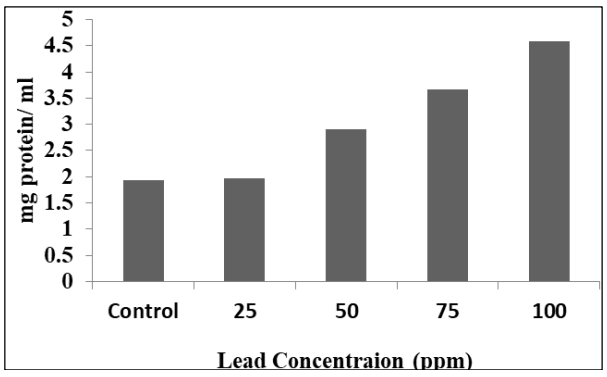

Fig 2: Changes in total protein content in control and treated *Vigna radiata* seedling exposed to lead

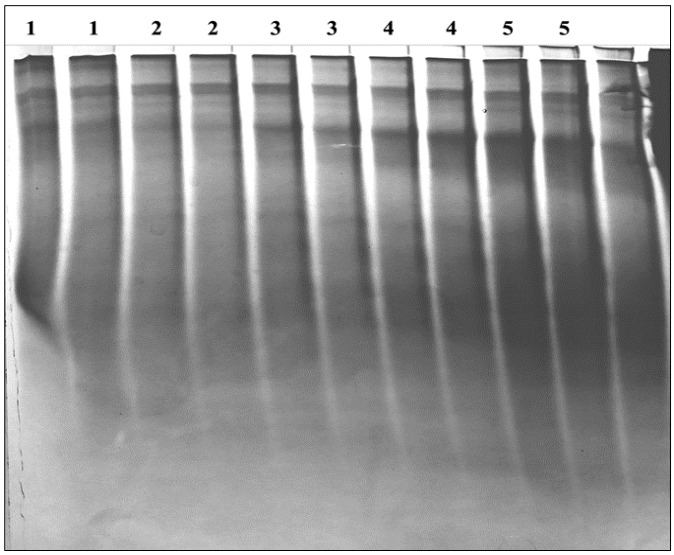

Fig 3: SDS-PAGE profile of control and treated seedlings protein. Lane 1: Control, Lane 2: 25ppm, Lane 3: 50ppm; Lane 4: 75ppm and Lane 5: 100ppm

Conclusions

The present investigation revealed the detrimental effect of heavy metal Pb by showing its toxic impact on seedling growth of *Vigna radiata.* Increase in the concentration led to changes in most of the growth parameters of plants studied during the investigation. High concentrations of Pb treatment is found responsible for decreasing the percentage of tolerance indices in *V. radiata* and that was clearly evident from the inhibition of seedling and root growth. Pb uptakes by the roots and their translocation to shoots at higher concentration might be the cause of drastic reduction in seedling growth and biomass production. The DNA concentration was increased with Pb treatment; this may be due to chromosomal aberrations and abnormal mitosis. Increase in total protein content in seedling with metal treatments suggests that there are some special proteins; they may have active roles in the tolerance of this plant to high

concentrations of lead stress. Lead also inhibits chlorophyll content thus affecting photosynthetic capacity of the plant. However, more research is required at cellular and molecular levels for understanding the mechanism of Pb toxicity in plant.

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