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Research Article

Ecophysiological Studies of Lead Stress on *Vigna mungo* L. Seedlings

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Abstract

In the present study of eco physiological effects of lead nitrate was observed by taking a local cultivated cereal crop *Vigna mungo* (L). The germination data showed that there is a negative impact of concentration of lead nitrate on the germination of seeds. There is decrease in seed germination at higher concentration in comparison to control. Root and shoot growth of seedling was effected when exposed to high concentration of lead nitrate. Roots were more affected and much reduced than shoots. Morphologically they look different from normal roots by their size and shapes. Effect of different concentration of lead nitrate was visible in different pigment concentration of leaves. Lead toxicity inhibits chlorophyll formation which visually showed in degeneration of green pigments in shoots exposed to different concentration of lead nitrate. With the increase in concentration of the toxicant, the exposed seedlings showed a decline in chl-a, chl-b, total chl, carotenoid and pheophytin content in shoots. This was a clear indication that the fall in the growth rate, pigment content had direct impact on photosynthesis. Conclusively, our results show that lead at higher concentration decreases seed germination, chl-a, chl-b, total chl, carotenoid and pheophytin contents in the mung seedlings.

Keywords: Mung; seedlings; pigments; Lead; growth

Introduction

The most common heavy metals contaminants are lead (Pb), cadmium (Cd), chromium(Cr), copper(Cu), mercury(Hg), nickel(Ni) and zinc(Zn) due to the awareness of the pessimistic effect of ecological pollution, everyone is appropriately aware about innovative methods for preventing pollution of the environment including soil.

Among the heavy metals, $Pb(NO_3)_2$ is a very toxic metal in all ecosystems, the source of $Pb(NO_3)_2$ is natural weathering processes. The main sources of $Pb(NO_3)_2$ pollution are exhaust fumes of automobiles, chimneys of factories, lead effluent from storage battery industries, mining and finishing operation of fertilizers pesticides and additives in pigment and gasoline. The lead occurs in soils in the form of insoluble and soluble salts. It strongly binds to organic soil particle which may decrease the mobility of lead in most soils and reduce uptake by plant.

Lead is one of the most comprehensively dispersed heavy metals and is incredible toxic to all ecosystems. In complete plant, lead can affect photosynthesis at the stomata level, mesophyll cell, pigment content and light and dark reactions. It interferes with nutritional elements of seedlings and plants consequently causing deficiencies or horrible ion release within the plant as well as growth humiliation, and

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sometimes disable the creation of quality food products and animal feeds. Moreover $Pb(NO_3)_2$ was showed to be able to alleviate lead toxicity in crop plants.

Ewais (1997) revealed the effect of cadmium, nickel and lead on growth, chlorophyll content. These three heavy metals inhibited the shoot growth but were less suppressive to root growth. They also lowered leaf chlorophyll content. The heavy metal additions to soil increased their contents in both roots and shoots, several times more in roots than in shoots.

At high concentrations, all heavy metals have strong toxic effects and are regarded as environmental pollutants (Nedelkoska and Doran, 2000; Chehregani *et al*., 2005). Heavy metals are potentially toxic for plants: phytotoxicity results in chlorosis, weak plant growth, yield depression, and may even be accompanied by reduced nutrient uptake, disorders in plant metabolism and, in leguminous plants, a reduced ability to fixate molecular nitrogen (Bazzaz *et al*., 1974). Jain *et al*., (1990) studied the heavy metal lead and zinc uptake by two aquatic plants i.e. *Azolla pinnata* and *Lemna minor* from metal enriched solutions and found that one of them acts as a micronutrient for plant growth at lower concentration while other can inhibit plant growth. Mishra and Choudhuri (1997) have reported the differential effects of lead and mercury on inhibition of germination of seeds of two rice cultivars. Lead (Pb) is one of the most abundant toxic metal in the earth's crust.

Decrease in dry weight might be due to accumulation of certain nutrients, reduction in photosynthesis and chlorophyll a synthesis as suggested for cowpea by Joshi *et al.,* (1999). Chlorophyll content, in general, gradually decreased with increase in lead concentration as compared to its control. The growth and metabolism of black gram was adversely affected when the plants were exposed to different concentrations of lead. Lead stress causes multiple direct and indirect effects on plant growth and metabolism and also alters some physiological processes (Diaz *et al.*, 2001). Jiraporn *et al.*, (2005) observed the toxicity and accumulation of Lead and chromium in *Hydrocotyle umbellate*.

Hussain *et al*. (2006) observed the effect of lead and chromium on growth, photosynthetic pigments and yield component in mash bean (*Vigna mungo* L.). Application of both lead and chromium caused a significant reduction in all growth parameters as compared with that of control. Ghani (2010) reported the effect of lead toxicity on growth, chlorophyll and lead (Pb) contents of two varieties of maize (*Zea mays*). Exposure of maize varieties to excess Pb resulted in a significant root growth inhibition though shoot growth remained less affected. The results of chlorophyll analysis indicated that the highly toxic Lead level affected photochemical efficiency in Neelam variety, while no significant effect was observed in the Desi variety. Hamid *et al*. (2010) revealed that the physiological responses of *Phaseolus vulgaris* to different lead concentrations. Increasing lead acetate levels lead to several disruptions of *Phaseolus vulgaris* plants, which are reflected by reductions of protein, chlorophyll, carbohydrate DNA and RNA content. However, phenolic content of plants were increasing with increasing levels of heavy metal lead. The effect of lead toxicity was more pronounced at 100 ppm as compared to 25 and 50ppm lead concentration.

Shu *et al*. (2012) studied the effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. and the results showed that root lengths decreased with increase of Lead concentration. In both seedlings and cuttings, Lead caused inhibition of leaf growth and photosynthesis.

Bhatti *et al*. (2013) reported that the effect of heavy metal lead (Pb) stress of different concentration on wheat (*Triticum aestivum* L.) and it was founded that the Lead reduced the morphological parameters such as shoot/root length, shoot fresh/dry weights, number of tillers. Lead stress also decreases the photosynthetic pigments such as chl a, chl b. So Lead as a heavy metal has detrimental effect on wheat growth and development.

The present work is one such attempt just to monitor the change in *Vigna mungo* L. Seeds and seedling in response to lead nitrate.

Material and Methods

Selection of Plant Material

The test organism for the present study is *Vigna mungo* L), Variety:-B3-8-8 Prasad Black gram. The seeds of *Vigna mungo* (L.) was collected from OUAT, Ankushpur. Healthy seeds of uniform sizes were used.

Vigna mungo L. is a perennial legume of family Fabaceae and is a short duration variety. It is grown mainly as a pure kharif mono crop with green gram, sorghum etc. Black gram is grown during June to July and harvested in the month of October-November. The seedlings are soft, fibrous, hard, and fragile and can be grinded easily. The seeds of this variety showed high percent of germination both in fields and in laboratory conditions. Hence, this variety is chosen as experimental material.

Test Chemical

Lead nitrate $Pb(No_3)_2$ was used as the test chemical which was a guaranteed reagent from Merck limited, India. First stock solution of 1000mg/l was prepared by dissolving 1gm of test chemical in 1 liter of distilled water. Different concentration of the metal were prepared by using distilled water as the solvent from the above stock solution, different concentration of solution of 200,400,600,800,1000mg/l prepared by proportional dilution with distilled water which is used for various treatments.

Study of Seed Viability

The seeds were soaked with distilled water or different concentrations of test solutions from 12hr-24hrs. After a scheduled period of exposure, the seeds were washed with tap water, followed by distilled water and their viability was tested according to the procedure of Moore (1969). The result was expressed as % of mortality of seed grain.

Germination Studies

The seeds of *Vigna mungo* L. showed 90% germination during September-December for germination studies sterilized Borosilicate glass petriplates were used for the study. For germination studies, Borosil (R), petridishes were sterilized in autoclave. The black gram seeds were surface sterilized with 5% solution of liquid detergent for 10-15 minutes then the seeds were thoroughly washed with tap water. The sterilized seeds (with 90% alcohol and $Hgcl_2$) were arranged equal space on the periphery of sterilized petridishes lined with filter paper. Sterilized absorbent cotton and blotting paper were added to it. Seeds were soaked for 24h in the desired concentrations, 15 numbers of seeds were sown in each petridish at uniform distance in all the sets. The blotting papers were made to wet at regular intervals with distilled water and respective concentration of test chemicals (200,400,600,800,1000)mg/l. The petridishes were incubated in the dark at room temp (32 degree celcius). The emergence of radical or plumule was considered as an index of germination. Seeds were allowed to germinate. Better sprouted and healthy seedlings of 10 days old were used as experimental material. Care was taken to avoid drying and over flooding of test chemical in petridishes.

Selection of Effective Concentration

Pilot experiment indicates that the metal exerts both promoting and retarding effect on the germination and seedling growth of the plant and it was essential to select a limited number of concentration for further experiments. Thus five concentrations of 200, 400, 600, 800, 1000 mg/l were selected. The data obtained from the germination and seedling growth experiments were used for screening.

Morphological Studies

The growth of plant was evaluated by measuring the shoot and root length of seedlings on 10th day. 15 cm scale was used for the measurement of the shoot and root length.

Estimation of Chlorophyll

The fresh samples of shoot material of the 10^{th} day old seedlings were collected. Care was taken for separation of each control and treated samples. A known quantity of about 50mg of samples of weighed shoot material was taken in a mortar and pestle with 80% acetone, ground thoroughly and centrifuged. The supernatant was collected in a test tube. The residue was again treated with 80% acetone again centrifuged and the two supernatants were pooled together and volume was made to 10ml. The absorbance of each extract was determined in a spectrophotometer at wavelengths of 475, 645,663,665,660 nm of wave length.

The total chlorophyll (chl-a, chl-b) content was measured by recording the absorbance of the extract at 645 and 663nm wavelength and the values were calculated by using the formula given by Arnon (1949). The carotenoid content measured by recording the absorbance of the extract at 475nm and the values were calculated by using the formula given by Arnon (1949).

Results

The maximum germination was observed in control (100%) and with the increase in the concentration of $Pb(No_3)_2$ the % germination of seeds decreased. The root length also decreased with the increased level of $Pb(No_3)_2$. The maximum root length was observed at untreated plant when compared to treated plants. The shoot length decreased with increased level of $Pb(No_3)_2$ concentration. The maximum shoot length observed in control.

The results of the study are given in Fig. 1-4.

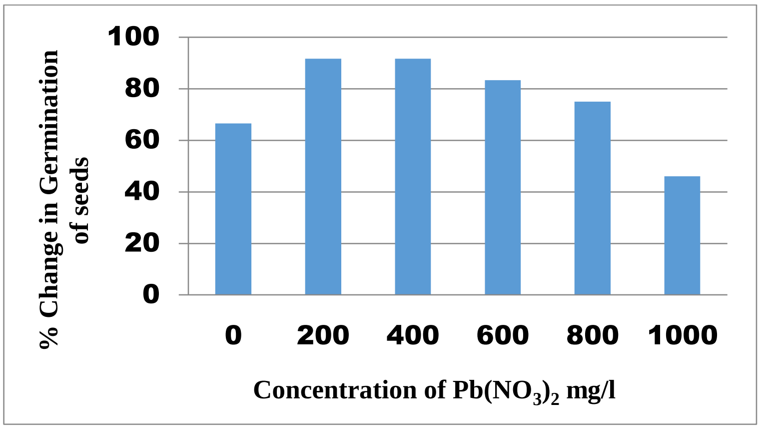


Fig. 1: Effect of different concentrations of lead nitrate on germination of 48 hrs old seedlings of Vigna mungo L. with respect to control

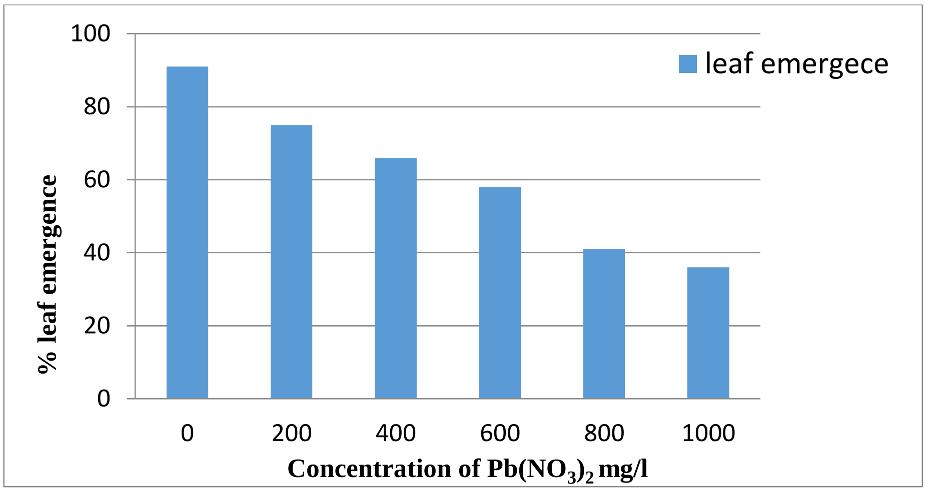


Fig. 2: Effect of different concentrations of Lead Nitrate on Leaf emergence of *Vigna mungo* L. seedling with respect to control

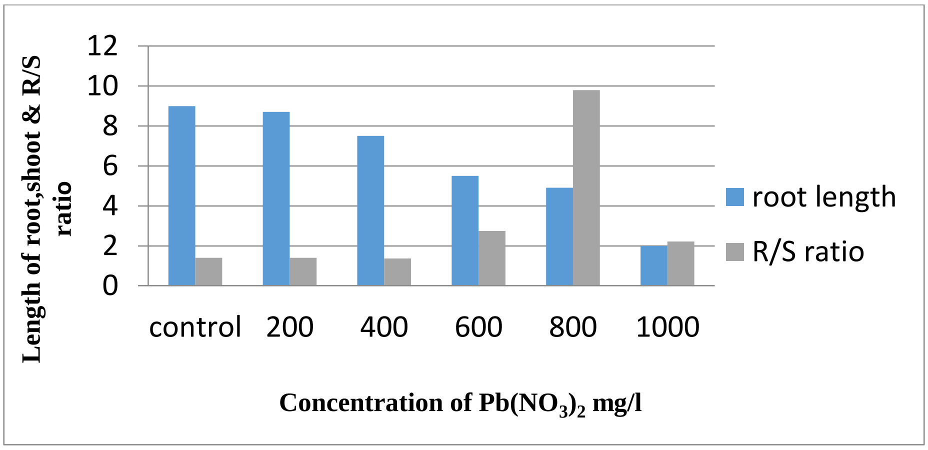
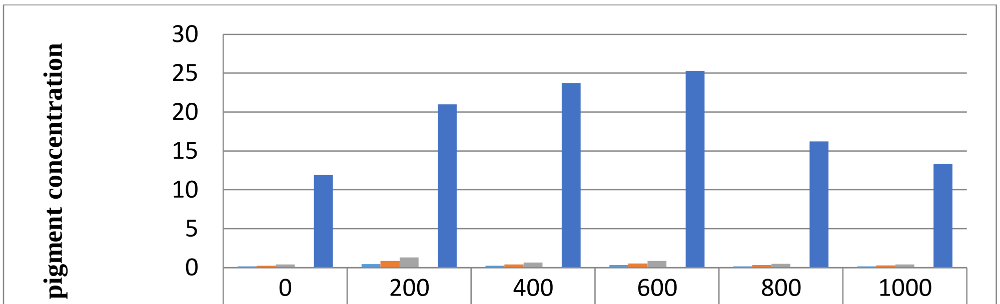


Fig. 3: Effect of different concentrations of $Pb(NO_3)_2$ on Ten days old *Vigna mungo* L. seedlings on root and shoot length.



	0	200	400	600	800	1000
chl.a	0.168	0.46	0.23	0.328	0.157	0.137
chl.b	0.218	0.838	0.419	0.514	0.314	0.277
total chl.	0.39	1.298	0.649	0.842	0.471	0.415
Carotenoid	0.006	0.023	0.013	0.014	0.09	0.004
Phaeophytin	11.91	20.97	23.75	25.29	16.23	13.34

Fig. 4: Effect of Lead Nitrate on the pigment contents mg/l of *Vigna mungo* L. of Ten days old seedling

Discussion

The growth and metabolism of Black gram was adversely affected when the plants were exposed to different concentrations of lead. Lead stress causes multiple direct and indirect effects on plant growth and metabolism and also alters some physiological process (Diaz et al 2001).

Plants height is decreased with increase in lead concentration. This is due to decrease in mitotic frequency and lead accumulation in cell wall components especially pectic substances and hemicelluloses (Tomar *et al* 2000). Lead was also reported to retard cell division and differentiation and also reduce their elongation thus affect the plant growth and development (Kastori *et al* 1993). Decrease in fresh weight of plants might be due to accumulation of certain nutrients, reduction in photosynthesis and chlorophyll-a synthesis as suggested for cowpea by Joshi *et al* (1999). Chlorophyll content, in general, gradually decreased with increase in lead concentration as compared to its control.

Chlorophyll biosynthesis is inhibited by heavy metals, particularly by inhibiting amino levulinic acid dehydrogenase and proto chlorophyllidereductase. Sulphydryl interaction of these enzymes was proposed as mechanism of this inhibition. Carotenoid content of 10 days old seedlings progressively declined with the increase in lead level. This reduction might be due to interference of lead in pigment metabolism as reported. The heavy metals pollution space of the environment is on the increase at a very fast rate globally. In this context to assess the polluting effect of Lead on different parts of the plants has become of absolute importance According to Pollacco (1977) heavy metals decreased chlorophyll content in *A. procera* leaves with increasing concentrations of metals. Reduction in chlorophyll content may be due to the interference of all the metals with chlorophyll synthesis and fat metabolism, inhibiting root shoot growth, photosynthesis, nutrient uptake, leaf area, biomass etc.

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