PHYTOREMEDIAL POTENTIAL OF Azadirachta indica A. Juss. FOR LEAD CONTAMINATION UNDER SALINE CONDITIONS

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ABSTRACT

Industrial heavy metal contamination is one of the today's burning issues that pollute our soils and water resources. Lead (Pb) is one of the most toxic and hazardous element among them. Azadirachta indica A. Juss., is a multipurpose tree having fast growth rate that can potentially contribute to decontaminate and restore the environment. However, information about Pb-uptake by A. indica under saline conditions is not yet known. A hydroponic study was conducted to assess the salinity level along plant tolerance to lead (Pb) and its accumulation in A. indica. Seedlings were established in the solution culture owing high salinity level (20 d Sm^{-1}) with necessary nutrients and two concentrations of lead i.e. Pb_1 (10 mg L^{-1}) and Pb_2 (20 mg L^{-1}). Results showed reduction in plant height (47% with Pb_2), diameter (57% with Pb_1), root length (41% with Pb_2+salinity) and shoot dry weight (91% with Pb_2+salinity) suggesting the reduced the plant growth as compared to control treatment. Under salinity and Pb stresses, tree grew well with no mortality in all the treatments. Moreover, noticeable variation was found in Pb accumulation among different plant organs such as: roots (21.5 mg kg^{-1} under $Pb_{2)}$ > shoots (7.6 mg kg^{-1} under Pb_2+salinity) > and leaves (5.3 mg kg^{-1} under Pb_2). Based on results it can be concluded that A. indica possesses phytoremedial potential when grown in salinity coupled with lead contamination and translocation of Pb uptake in its leaves.

Keywords: Azadirachta indica, Decontamination, Heavy metal, Lead contamination, Phytoremediation.

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INTRODUCTION

Anthropogenic activities have immensely increased pollution in atmosphere, lithosphere, hydrosphere and biosphere. Increasing levels of various pollutants can result in decreased crop production and deteriorated quality of life. The alarming levels of heavy metal in soil and water have become a major problem in several areas of the world (Lone et al., 2008). The activities like industrial work, mining, automobile, extensive use of pesticides, fertilizer, and sewage sludge add heavy metal pollutants to soil and water medium resulting contaminated environment (Rooney et al., 1999). Heavy metals like Pb, As, Cd, Cr, Cu, Ni, Hg and Zn have been identified in the polluted environment. The type of metal may be different in different locality with regard to source of particular pollutant. Higher levels of heavy metals in soil disturb metabolic activity of plants. Their antagonistic effect may include alteration of physiological and biochemical changes, inhibition of photosynthesis, respiration and slow crop growth leading to damage of major cell organelles and finally plants death (Garbisu & Alkorta, 2001; Schmidt, 2003; Schwartz et al., 2003). Lead (Pb) is the most hazardous and poisonous pollutant that even at low concentration is very precarious to the environmental habitats.

Major anthropogenic activities like mining, burning of coal, smelting of lead ores, effluents of battery industries, automobile gases, finishing process, fertilizers, pesticides, paints and gasoline, additives of gasoline and pigments are the most important lead spreading sources in the environment (Sharma & Dubey, 2005). Soils polluted with Pb decrease crop productivity and yield on agricultural lands (Johnson & Eaton, 1980). A great part of lead from soils freely enters into plant body and get settle in root part. A small proportion is shifted to plant shoot (Patra et al., 2004). According to Lucky & Kenugopal (1997), Pb could be accumulated into plants tissues and organs to become a part of food chain. Furthermore, lead accumulated in other living organisms, causes various disorders in human and animal organs. In both, brain damage, liver damage and kidney disorders are also the consequences of Pb contamination.

McGrath et al., (2002) quoted that Phytoremediation is a green emerging technology to remove pollutants from soil and water by green plants. It is applicable with low cost as compared to other remediation technologies like soil washing which has negative impacts on biological activity, soil structure and fertility. In addition, phytoremediation is an attractive technology as it offers site restoration, preservation of biological activity and sustain soil physical structure and properties. This emerging green technology has been proven a long term in situ remediation technology for bioremediation of soils and water polluted with heavy metals. Appropriate plant species is very important for successful phytoremediation process. Alkorta et al., (2004) and Yang et al. (2009) revealed that growing of suitable agronomic crops could result in entering of Pb in food chain. On the other hand, growing of woody plants or trees exert synergetic impact on food chain because woody portion of trees is not consumed by human or livestock and is mostly used in making wood products. Many species have been declared to check metal tolerance, accumulation and biomass yield in laboratory scale experiments and successfully planted in fields (Mertens et al. 2006; Zhao & McGrath, 2009). Establishment of woody vegetation for amelioration of heavy metal polluted soils is an appealing practice as woody plants have more biomass to give more economic return and securing our food chain system (French et al., 2006).

Azadirachta indica A. Juss. (Meliaceae), locally known as Neem, is found in tropics of Africa, South Asia, India and Pakistan (Qureshi, 2012). Its various parts have been used as a traditional medicine for more than 2000 years due

to its valuable biological as well as anti-inflammatory, antiulcer, antimalarial, antibacterial and antioxidant activities (Biswa et al., 2002; Sunday et al., 2009). Owing to its medicinal properties, this study was designed to find out response of A. indica seedlings under hydroponic conditions as influenced by heavy metal Pb (II) and salinity and finally to explore the translocation of Pb in the above ground parts of A. indica when it is grown under saline conditions.

MATERIALS AND METHODS

Plant Material

Seedlings of Azadirachta indica were grown in sand culture using certified seeds in the nursery. Hoagland solution (5 mMCa $(NO_3)_2$, 0.25 mM KH_2PO_4, 1.25 mM $MgSO_4$, 0.25 mM KCl, 1.75 mM K_2SO_4, 25 M H_3BO_3, 1.25 M $MnSO_4$, 1.25 M $ZnSO_4$, 0.50 M $CuSO_4$, 0.025 M $(NH_4)_6Mo_7O_{24}$, 0.25 mM Fe-EDTA) was prepared according to Hoagland and Arnon (1950) and after germination, uniform sized and healthy seedlings were irrigated with this solution up to 12 weeks. Three months old seedlings were transferred in the container containing nutrient solution and plants were placed on prepared thermo pole sheets in the green house for growth maintaining 6.5 pH on daily basis. Plants were stabilized for 15 days and dead plants were replaced. Seedlings were exposed to nutrient solutions with two concentrations of Pb (i.e. 10 mg L^{-l}, 20 mg L^{-l}) in the form of $Pb(NO_3)_2$. Hoagland solution was changed after every 15 days.

Experiment design and layout

The experiments were laid out in completely randomized design (CRD) with six treatments i.e. Control, Control + Salinity, Pb_1 (Pb=10mgL^{-1}), Pb_2 (Pb=20mgL^{-1}), Pb_1 +Salinity (EC= 20 dSm^{-1}) and Pb_2 + Salinity (EC=20 dSm^{-1}) and three replications and replicate contained three plants so total number of plants (A. indica) used in whole experiment were 54 (6 x 3 x 3).

Data collection

Data was collected using mean height (cm), mean diameter (cm), root length (cm), shoot dry weight (g) and uptake of Pb (mg kg^{-1}) by A. indica. The plant samples (leaves stem and roots) were dried separately on clean plastic sheet and oven-dried at 65-70ºC till constant weight. The dried plant material was ground to make powder through Wiley Mill (I mm size) and was stored in clean plastic bags for chemical analysis.

Chemical analysis

Plant samples were digested using the procedure of US Salinity Laboratory Staff (1954) and analyzed with standard methods. Ground plant material weighing 0.5g was digested in a conical flask with 15 ml di-acid mixture (HNO_3:$HClO_4$, 2:1) using hot plate until turned colorless. After digestion, the flask was left to cool and volume was made 100 ml by adding deionized water and further analysis was carried out with the help of atomic absorption spectrophotometer (Perkin Elmer Analyst 300). Lead concentration was determined by using the method of Rashid (1986).

Statistically analysis

All results were presented as arithmetic means. Data were tested with two-way ANOVA, followed by LSD tests using the Statistix ver. 8.1 statistical packages. The level of significance was set at $P<0.05$.

Bio-Concentration Factor (BCF) was calculated as shown in equation.

$$\mathrm{BCF} = \frac{C}{C}$$

RESULTS

Plant growth

Figure 1 revealed that all growth parameters of A. indica were significant compared to control but all other treatments were non-significant among themselves regarding growth parameters. Similarly, lead (Pb) accumulation in various parts of seedlings (leaves, stem and roots) was found highly significant. Different level of Pb resulted in reduction in mean height and mean diameter compared to control. Data revealed that average values of shoot dry weight and root length in A. indica seedlings were at minimum under Pb_2 + Salinity and Control + Salinity.

The seedlings of A. indica attained maximum in plant height (15.2 cm) under control showing significant difference from all other treatments (Figure 1). However, there was non-significant difference among mean plant height of Pb_1 (11.1 cm), Control + Salinity (10.2 cm), Pb_2 + Salinity (9.5 cm), Pb_1 + Salinity (8.9 cm) and the minimum plant height (8.1 cm) under Pb_2. Similarly, the maximum recorded diameter (0.35 cm) under control condition was statistically significantly different (Figure 2), whereas, there was non-significant difference among all other salinity and heavy metal treatments in plant mean diameters of Pb_1 + Salinity (0.25 cm), Control + Salinity (0.21 cm), Pb_2 + Salinity (0.19 cm), Pb_2 (0.17 cm) and Pb_1 (0.15 cm), respectively.

Regarding root length (Figure 3), maximum root length (15.5 cm) was recorded under Pb_2 treatment which was statistically non-significant to the mean root length of control (12.2 cm) and Pb_1 (11.3 cm) but significant difference from all other treatments like Pb_1 + salinity (10.5 cm), control + Salinity (9.4 cm) and Pb_2 + Salinity (9.2 cm) respectively. It is worth mentioning that root length increment was 27% under Pb_2 that was significantly different than control. Regarding shoot dry weight (Figure 4), maximum shoot dry weight was recorded (3.5 g) under control that was statistically non-significant to Pb_1 (2.5 g) and Pb_2 (2.3 g) but significantly different from all other treatments like control + salinity (1.5 g), Pb_1 + Salinity (0.5 g) and Pb_2 + Salinity (0.3 g), respectively. It is evident from data that maximum plant height was under control which was significantly different from all other treatments. However, there was no statistical difference among mean plant heights of Pb_1, control + salinity, Pb_2+ salinity, Pb_1+ salinity and minimum plant height was under Pb_2, respectively. Our results revealed that heavy metal stress reduced the growth under all other treatments except control. It is because, in control condition, there was no heavy metal stress and in other treatments growth of plants was reduced due to presence of heavy metal and salinity stress.

Pb accumulation in various parts

Regarding lead accumulation in plants, maximum lead accumulation in entire plant was under Pb_2 + Salinity treatment (42.56 mg kg^{-1}) that was significantly different from all other treatments followed by Pb_2 (29.20 mg kg^{-1}), Pb_1 + Salinity (27.23 mg kg^{-1}), Pb_1 (20 mg kg^{-1}), Control + Salinity (0.09 mg kg^{-1}) and Control (0.09 mg kg^{-1}) respectively as shown in Fig. 5. Regarding individual treatment, mean maximum lead accumulation was recorded in leaves (5.3 mg kg^{-1}) under Pb_2 + Salinity that was significantly different from all other treatments (Figure 7). Similarly, it was found that lead concentration in plant leaves for various treatments as descending order Pb_2 (4 mg kg^{-1}), Pb_1 + Salinity (3 mg kg^{-1}), Pb_1 (1.4 mg kg^{-1}), Control + Salinity (0.01 mg kg^{-1}) and Control (0.01 mg kg^{-1}). Maximum lead accumulation was noted in shoots (7.6 mg kg^{-1}) under Pb_2 + Salinity treatment that was significantly different from all other treatments.

Lead accumulation under different treatments in plant shoots was recorded in descending order such as Pb1 + Salinity (5.2 mg kg^{-1}), Pb_2 (3.7 mg kg^{-1}), Pb_1 (2.6 mg kg^{-1}), Control + Salinity (0.04 mg kg^{-1}) and Control (0.02 mg kg^{-1}) respectively, as shown in Figure 8. Maximum lead accumulation was recorded (29.66 mg kg^{-1}) in plant roots that were significantly different from all other treatments. However, lead accumulation in plant roots in descending order was found as Pb2 (21.5 mg kg^{-1}), followed by Pb2 + Salinity (19 mg kg^{-1}), Pb1 (16 mg kg^{-1}), and control (0.06 mg kg^{-1}) respectively as shown in Figure 9.

Bio-Concentration Factor (BCF) values were studied in each treatment as shown in Table 1. BCF values were highest in leaves (0.30 a) under Pb_1 + Salinity, followed by Pb_2+ Salinity (0.26 b), Pb_2

(0.20 b) and Pb_1 (0.14 c), respectively. Similarly, BCF values were highest (0.52 a) under Pb_1 + Salinity, followed by Pb_1 + Salinity (0.38 b), Pb_1 (0.26 c) and Pb_2 (0.18 c) in shoots, respectively. In the rootss, BCF values were highest (1.90 a) under Pb_1 + Salinity, followed by Pb_1 (1.60 b), Pb_2 + Salinity (1.48 c), and Pb_2 (1.07 d), respectively. It has also been observed that BCF values of all plants decreased with the increasing concentration of lead.

DISCUSSION

Salts and metals may deposit in the plant vacuole and may disturb ion balance of whole water relations of the plants (Gupta & Sharma, 1997). Heavy metals not only affect the nutrient homeostatis but also inhibit the photosynthetic apparatus (Jaswant, 1999; Pandit and Kumar, 1999).

As mentioned above, lead accumulation in plants, maximum lead accumulation in entire plant was under Pb_2 + Salinity treatment (42.56 mg kg^{-1}) that was significantly different from all other treatments followed by Pb_2 (29.20 mg kg^{-1}), Pb_1 + Salinity (27.23 mg kg^{-1}), Pb_1 (20 mg kg^{-1}), Control + Salinity (0.09 mg kg^{-1}) and Control (0.09 mg kg^{-1}) respectively. Nawaz et al. (2016) concluded that exposure of plant to the saline growth medium loaded with lead, could modify the pattern of Pb accumulation and its translocation to various parts of plant. In another study, Aktas et al. (2006) reported that application of NaCl could reduce the availability of P leading to excess intake of Zn by the pepper plants. This phenomenon explains why Pb uptake was higher in A. indica under salinity stress.

In this study, lead content (Pb) was deposited in all parts of the plant body, but significantly higher in the roots. The Pb deposition was in the order of root > shoot> leaves (Figure 6). This is in agreement with Rooney et al. (1999) and Nawaz et al. (2016). Rooney et al. (1999) worked on five species of vegetation, grown on lead loaded soils, namely barley, lettuce, rye grass, radish and clover. In all species levels of lead, in roots, increased significantly with increase in soil Pb concentration. Consistently higher concentrations of Pb were detected in the roots of all species in comparison with leaves except for rye grass at smaller concentration of soil Pb. Similar pattern of Pb accumulation was observed by Nawaz et al. (2016) in Eucalyptus camaldulensis seedlings when tested against Pb and salinity stress under hydroponic conditions. Hapke (1991) reported linear relationships between Pb concentration in soils and plants. This study confirmed the present findings that increased in lead concentration and had linear relationship with the uptake in plant.

In the present study, lead contents were significantly affected in leaves, shoots and roots. This is in agreement with Xiong (1997), who reported that seedlings (27 days old) of Sonchus oleraceus growth were significantly affected by the concentration of Pb in leaf, stem, and root. Zheljazkeov et al. (1999) stated that lead concentrations in plants generally depend on its soil concentrations. He further opines that most of the element is generally found in root zone, which supports the general understanding for the mechanism of uptake and translocation of this element with in plants. This is true in case of A. indica of the present study. The present findings revealed that increase in Pb uptake in plant and accumulations in root with increase in substrate Pb level. This was also in agreement with that of Ghani et al. (2010) who reported 2.3-71.3µg/g in tops and 8-11208µg/g in roots of lettuce, exposed for six weeks to 0–50 µg/g of Pb in solution. Present study is also in agreement with those who worked on maize varieties and found their ability to capture Pb from the growth medium and to store it plant roots. Maize plants could be able to shift a small proportion from the stored Pb to foliar portion of the plant (Patra et al., 2004). Sinha et al.

(2006) also supported our findings. Another study conducted by Kumar et al. (1993) also revealed that provision of Pb^{+2} in different concentrations to seedlings of sesame enhanced Pb^{+2} contents in various parts of plant (root, stem and leaves). They suggested that an increase of Pb^{+2} contents in plant had sound fitted correlation with external supply of Pb^{+2}. As for as presence of Pb in roots, stem and leaves of maize plant is concerned, mean value of Pb in plant's parts was significantly increased as compared to control, when it was kept under Pb stress. The lead contents of roots, shoots and leaves of maize varieties increased significantly under the Pb stress when compared to control. Ghani (2010) concluded that higher Pb contents in plant body were only because of higher Pb concentrations in the plant growth medium (soil and water). Plants responded to store more amount of Pb in roots than that of shoots. According to Yang et al. (2000) and Bashmakov et al. (2005), rice seedlings of tolerant variety when exposed to Pb solution generated higher root biomass than that of the sensitive ones because tolerant variety was potentially able to develop extensive (adventitious) root systems.

CONCLUSION

This study was conducted to assess the plant tolerance to lead (Pb) contamination and its accumulation in A. indica. Under salinity and Pb stresses, tree grew well with no mortality in all the treatments. Moreover, there was noticeable variation in Pb accumulation among all plant organs. Based on results it can be concluded that A. indica possesses phytoremedial potential whether grown in salinity coupled with lead contamination and translocation of Pb uptake in its leaves. It can be concluded that A. indica could be a wise selection for remediation of soil contaminated with Pb metal.

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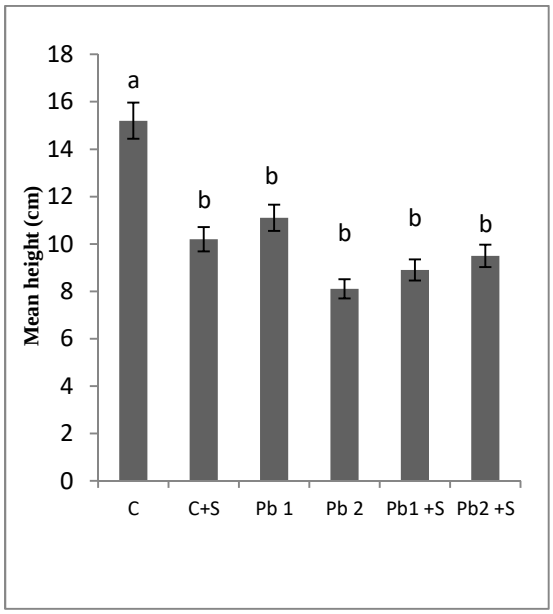

Figure 1. Mean Height (cm) under different treatments

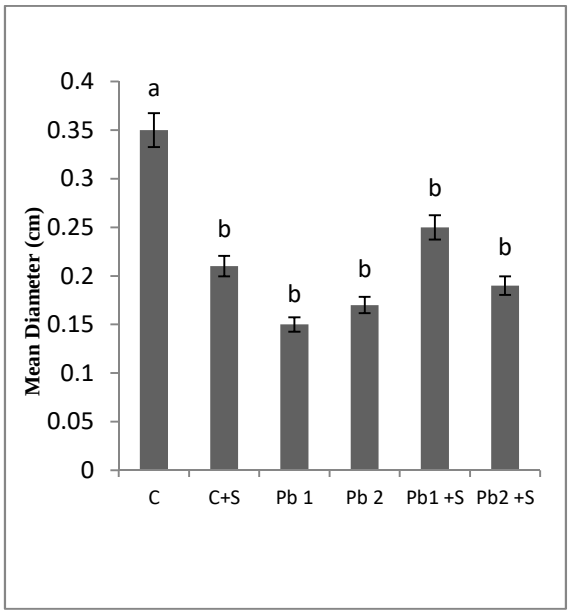

Figure 2. Mean Diameter (cm) under different treatments

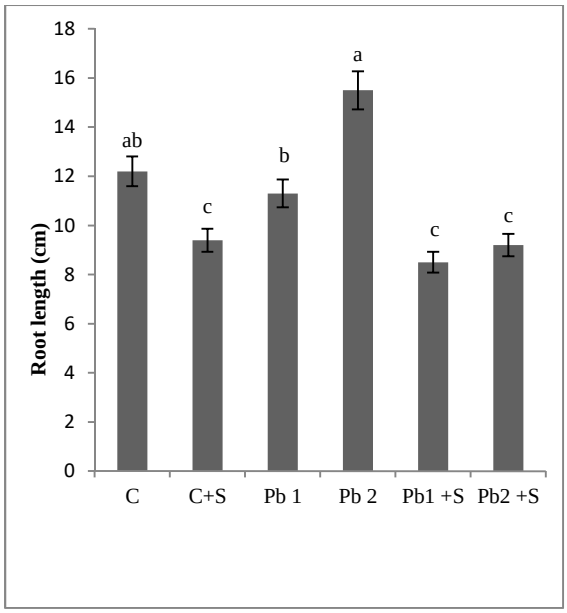

Figure 3. Root length (cm) under different treatments

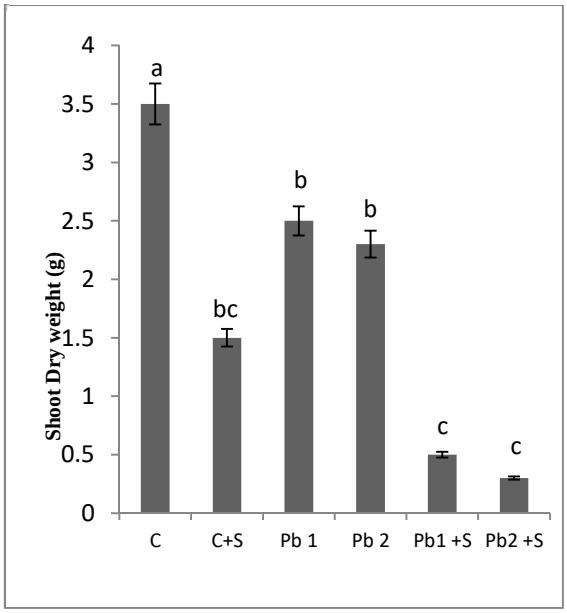

Figure 4. Shoot Dry weight (g) under different treatments

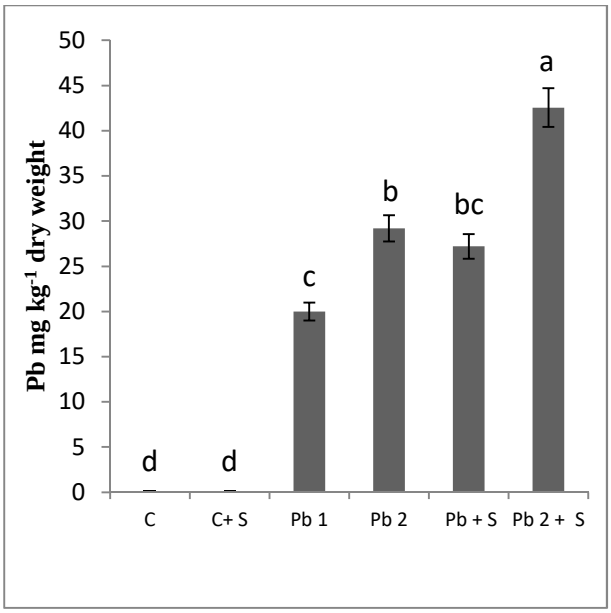

Figure 5. Pb accumulation under different treatments

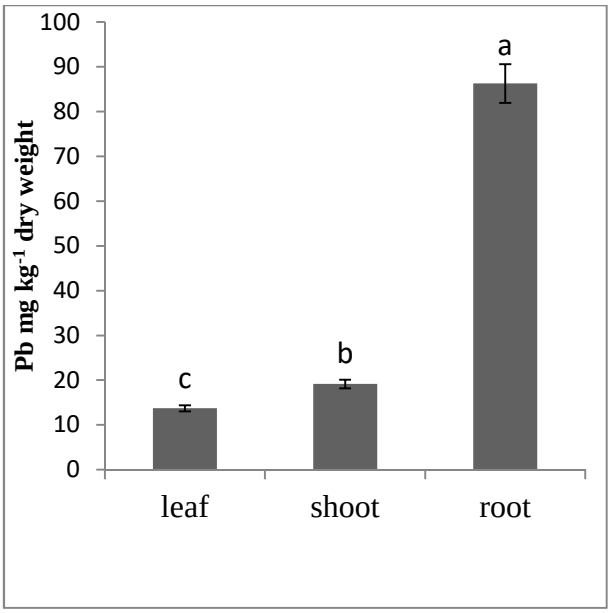

Figure 6. Pb accumulation in different plants parts

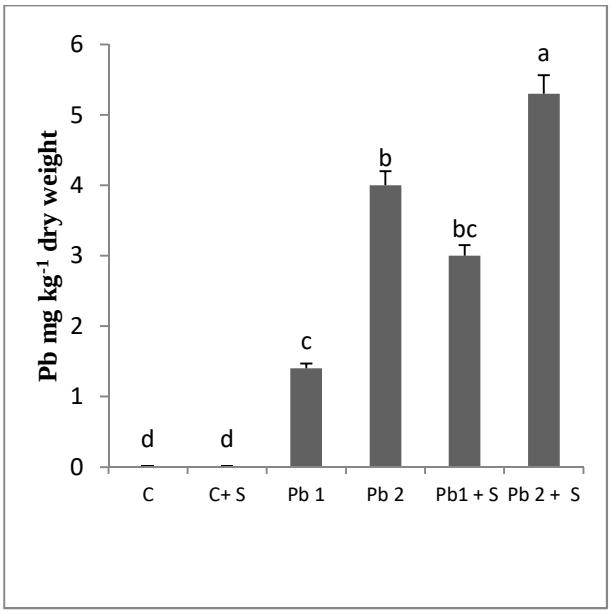

Figure 7. Pb accumulation in leaves under different treatments

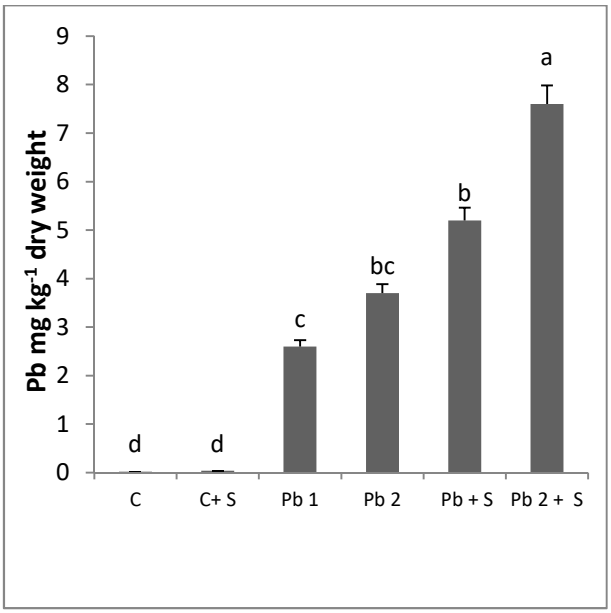

Figure 8. Pb accumulation in shoots under different treatments

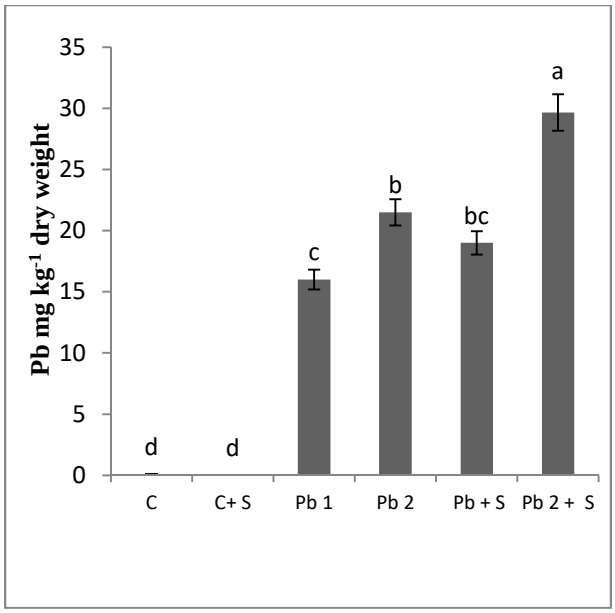

Figure 9: Pb accumulation in roots under different treatments

Table-1. Bio-concentration factor in leaves, shoots and roots under different treatments

Treatment	Leaves	Shoots	Roots
Control	-	-	-
Control + Salinity	-	-	-
Pb1	0.14 c	0.26 c	1.60 b
Pb2	0.20 b	0.18 c	1.07 d
Pb1 +Salinity	0.30 a	0.52 a	1.90 a
Pb2 + Salinity	0.26 b	0.38 b	1.48 c

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