RESEARCH ARTICLE

Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L.

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Abstract
Background Cuttings and seedlings of *Jatropha curcas* L. were exposed to different regimes of lead (Pb) stress as Pb $(NO_3)_2$ at 0 (CK), 0.5, 1, 2, 3, and 4 mM kg^{-1} soil.
Objectives The effect of Pb treatment on the root length, tolerance index, photosynthetic pigments, photosynthetic activity, lipid peroxidation, and antioxidant enzyme was studied in a greenhouse pot experiment.
Results The results showed that root lengths and tolerance index decreased with increase of Pb concentration, but tolerance index of cuttings was always lower than those of the seedlings. For cuttings, Pb treatment had a stimulating effect on chlorophyll content, carotenoid content, and superoxide dismutase (SOD) activity at low concentration and an inhibitory effect at higher concentration. For seedlings, SOD activity increased with increasing Pb concentration. In both seedlings and cuttings, Pb caused inhibition of leaf growth and photosynthesis, and induced the membrane damage which was more obvious in the cuttings. In comparison with the control, the dynamic tendency of catalase and perxidase activities in the leaves of Pb-stressed plants all ascended, and then declined.
Discussion The increase in enzyme activities demonstrated that seedlings were more tolerant to Pb stress than cuttings. These results also indicate that the antioxidant system may play an important role in eliminating or alleviating the toxicity of Pb in *J. curcas* seedlings and cuttings. The accumulation of Pb increased in a concentration-dependent manner; however, its translocation from root to shoot was low. The cuttings accumulated significantly higher Pb in roots than seedlings.

1 Introduction

At present, the demand for fossil fuels is increasing very rapidly, and it is estimated that the remaining reserves of fossil fuel will be exhausted by 2020 (Kumar and Kumar 1984). Rapidly increasing energy demand has become a serious challenge both in developed and developing countries. Exploitation of renewable energy and sustainable energy is one of the effective solutions to this problem. Development of renewable energy can not only contribute to the energy supply but also achieve economic and environmental benefits (Wang 2005). More and more attention has been paid to bioenergy developed from energy plants in recent years. Among the various renewable energy choices, seed oil crops have a potential for meeting the increasing requirements of petroleum and its products. *Jatropha curcas*, a multipurpose, drought-resistant, perennial plant belonging to *Euphorbiaceae* family is gaining lot of importance for the production of biodiesel. *J. curcas*, commonly known as physic nut, is today recognized as a petro substitute (Zhang et al. 2008; Kumar and Sharma 2008). It is an important shrub commonly found in tropical and partially subtropical areas, as a commercial crop or on the boundaries as a hedge to protect fields from grazing

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animals and to prevent erosion (Kumar and Sharma 2008; Dehgan and Webster 1979).

With rapid development in industry all around the world since the twentieth century, the heavy metal concentrations in agricultural soil increased rapidly in many areas around the world (Kabata and Pendias 1984). Heavy metals are important environmental pollutants, and many of them are toxic even at very low concentrations. Lead (Pb) is not an essential nutrient for plants, and it is one of the hazardous heavy metal pollutants of the environment that originates from various sources like mining and smelting activities, burning of coal, effluents from storage battery industries, automobile exhausts, pesticides, and from additives in pigments and gasoline as well as from the disposal of municipal sewage sludge enriched with Pb (Eick et al. 1999). Pb pollution of the environment is of major ecological concern due to its impact on human health through the food chain and its high persistence in the environment (Piechalak et al. 2003). Responses of plants to Pb exposure include decrease in root elongation and biomass (Fargasova 2001), accelerated leaf senescence (Siedlecka and Krupa 2002), inhibition of chlorophyll biosynthesis, inhibition of seed germination, a wide range of adverse effects on growth and metabolism of plants (Moustakas et al. 1994), interfere with nutrient uptake, influence the net photosynthetic rate and respiration, and alternate permeability of cell membrane (Sharma and Dubey 2005). Pb can also alternate the activity and quantity of the key enzyme of various metabolic pathways such as those of the photosynthetic Calvin cycle (Stevens et al. 1997), nitrogen metabolism (Kumar and Dubey 1999), and sugar metabolism (Verma and Dubey 2001).

Toxic levels of heavy metal affect a variety of processes in plants. One of the major consequences is the enhanced production of reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, and hydrogen peroxide (H_2O_2) (Verma and Dubey 2003; Souguir et al. 2011). ROS can cause oxidative damage to the biomolecules when produced in larger amounts leading to cell membrane peroxidation, loss of ions, protein hydrolysis, and even DNA strand breakage. Plants' defenses to metal toxicity may constitute different strategies. First is the avoidance of metal entry into the cell via exclusion or binding of metal to cell wall. For Pb, binding to cell wall is one of the major mechanisms of detoxification (Antosiewicz and Wierzbicka 1999). Second, plants have developed an anti-oxidative system, including low-molecular-mass antioxidants as well as anti-oxidative enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidase (POD, EC 1.11.1.7) (Cakmak and Horst 1991).

The plants growing in a medium or soil containing considerable amounts of Pb will accumulate the metal in all parts of the plant body, but at a higher level in roots than in shoots (Kumar et al. 1993; Lonardo et al. 2011). Stefanov et al. (1995) reported that the accumulation of Pb occurs mainly in the leaves. Pb is apparently not translocated into the reproductive tissues. Dicots have been reported to accumulate significantly higher concentrations of Pb in the roots than did monocots (Huang and Cunningham 1996). The uptake, transport, and accumulation of Pb by plants are strongly governed by soil and plant factors, and they differ significantly with plant species (Zhang et al. 1998). The uptake of Pb by tolerant plants was usually less than non-tolerant plants (Liu et al. 2004). Non-tolerant species absorb relatively high amounts of metals, and the extent of this is partly dependent on the efficiency with which the root system can limit the transport of metal ions to the shoot.

J. curcas grows readily from seeds or cuttings. The objectives of this study were to investigate the effect of Pb on plant growth, physiology, uptake, and translocation of Pb in seedlings and cuttings of *J. curcas*. Moreover, comparative responses of cuttings and seedlings of *J. curcas* to Pb stress were identified. This investigation could highlight a better understanding of the biological mechanisms adopted by the seedlings and cuttings in response to physiologically toxic concentration of Pb. This information could be useful for scale cultivation of *J. curcas*.

2 Materials and methods

2.1 Plant materials and Pb treatments

Mature *J. curcas* seeds were collected from 3-year-old trees within the same clone, from Suining, Sichuan Province, China. They were sown in the field 20 days after germination; plants with uniform sizes were selected and transplanted into earthen pots (20×18 cm) containing air-dried red soil and farmyard manure in 3:1 proportion. One plant was in one pot. The soft branches of *J. curcas* were cut down from the above same clone trees, and a total of 150 cuttings were collected. The average height and diameter of the cuttings was 24.6 cm and 6.1 mm, respectively. Cuttings were immersed briefly in a solution of fungicide to avoid fungal infection and kept under a shade for 15 min in open air before they were planted in the earthen pots (20×18 cm) on 2 May 2010. All seedlings and cuttings planted in the pots were placed in a greenhouse. They were irrigated at 5-day intervals.

One month later, 96 uniform-sized seedlings and cuttings were selected respectively for Pb treatment. Pb nitrate solution was added in the pots to obtain the Pb concentrations of 0 (CK), 0.5, 1, 2, 3, and 4 mM kg^{-1} soils. Pots were arranged in a randomized complete experimental block design with four replications. Both control and treated pots were irrigated daily with tap water carefully to avoid leach out of solution from treated pots.

2.2 Growth parameters

Sixty days after metal exposure, the root lengths (mean of three longest roots in a plant) of cuttings and seedlings of *J. curcas* were measured (centimeter plant) for every Pb treatment. Tolerance index (TI) for roots was calculated according to Wilkins's equation (Wilkins 1957) where ML_{Pb} and ML_C represented the mean length of longest roots in Pb treatments and controls, respectively: TI (percentage)=$ML_{Pb}/ML_C \times 100$. In each replicate, the third leaf from the top of plants was used for the determination of leaf area. Leaf area was determined using a leaf area meter (LI-3100, LI-COR Company, USA).

2.3 Determination of photosynthetic pigments and MDA contents

The upper second fully expanded leaves were sampled for analysis of photosynthetic pigments, malondialdehyde (MDA), total soluble protein, and the oxidative stress on the 60th day. For estimation of photosynthetic pigments, the plant material (0.1 g) was ground in chilled 80% acetone in the dark. After centrifugation at 10,000×g for 10 min at 4°C, absorbance of the supernatant was taken at 470,646 and 663 nm. The content of chlorophylls was estimated by the method of Lichtenthaler (1987) and that of carotenoid content by using the formula given by Duxbury and Yentsch (1956).

Lipid peroxidation was determined by estimation of the MDA content following Heath and Packer (1968) with slight modification. Plant material (0.5 g) was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000×g for 5 min. For every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% thiobarbituric acid was added. The mixture was heated at 95°C for 30 min and then cooled quickly on ice bath. The resulting mixture was centrifuged at 10,000×g for 15 min, and the absorbance of the supernatant was taken at 532 and 600 nm. The nonspecific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1}.

2.4 Determination of total soluble proteins and activities of antioxidant enzymes

In order to assay protein content and enzyme activity, fresh leaves (0.5 g) were ground with liquid nitrogen and homogenized in 5 ml of 50 mmol sodium phosphate buffer (pH 7.0) including 0.5 mmol EDTA and 0.15 mol NaCl. The homogenate was centrifuged at 12,000×g for 10 min at 4°C, and the supernatant was used for protein determination and enzyme assays. Total soluble protein content was determined by the method of Bradford (1976) using BSA as a standard.

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium as described by Beauchamp and Fridovich (1971). POD activity was estimated by the method of Li (2000). CAT activity was estimated by the method of Aebi and Bergmeyer (1983).

2.5 Determination of photosynthesis

Different parameters of photosynthesis such as net photosynthetic rate (Pn), stomatal conductance (Cond), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were determined after the plants were treated with different Pb concentrations for 60 days. In each treatment, four plants were selected randomly, and the third leaf from the top of each plant was used for determining photosynthesis by an infrared analyzer (LI-6400 System, LI-COR Company, USA). Average values of four plants were considered as one treatment.

2.6 Determination of Pb content

After harvesting, leaves and roots were extensively washed with distilled water and then oven-dried at 80°C to constant weight. The oven-dried samples were ground through 200 meshes. The Pb concentrations of the samples were determined with atomic absorption spectroscopy (Perkin Elmer 2100, Germany) following $HNO_3^-/HClO_4$ (4:1) digestion procedures (Fang 1991).

2.7 Statistical analyses

All data were processed by statistical package SPSS (version 11.5). Values reported here are means of four replicates. Data were tested at significant levels of $P<0.05$ using one-way ANOVA. Graphical work was carried out using Origin software 8.0.

3 Results

3.1 Effects of Pb on plant leaf area, root length, and tolerance index

Visible changes were observed in the appearance of the above-ground parts of plants treated with Pb. Typical symptoms of Pb toxicities to cuttings were developed after Pb treatments, especially for 4 mM Pb treatments in cuttings, where obvious yellow and dark brown dots were seen on leaves. Yellow and dark brown dots were not observed at 4 mM Pb treatments for seedlings. Leaf

morphological parameters such as leaf area also decreased significantly ($P<0.05$) as compared to the control except for 0.5 mM Pb stress. Pb treatments reduced the leaf areas of cuttings and seedlings in higher Pb concentration; leaf area of cuttings is more sensitive to Pb treatment compared to that of seedlings (Fig. 1).

In comparison to the control plants, the appearance of the roots of the Pb-exposed cuttings and seedlings changed significantly. The color of the roots changed gradually under the influence of Pb, from creamy white to dark brown. The elongation growth of roots was slowed down, and the number of hair roots decreased. Dose-dependent decrease in root length was found both in cuttings and seedlings (Fig. 2a). However, roots of cuttings are more sensitive to Pb treatment compared to that of seedlings. Pb treatment at 0.5 mM significantly decreased the length of cuttings, while there was no significant decrease in seedlings. The TI values of the examined plants were significantly different (Fig. 2b) in Pb stress. TI values of cuttings were always lower than those of seedlings. The cuttings exhibit the highest sensitivity to Pb (TI=39.2%).

3.2 Effect of Pb on photosynthetic pigments and MDA contents

The leaves of cuttings and seedlings were significantly affected by Pb stress, which resulted in a decline of chlorophyll content (Fig. 3). The chlorophyll b content of cuttings and seedlings decreased significantly with increasing Pb concentration. A similar change pattern was seen in chlorophyll a and (a + b) contents. The chlorophyll b content in cuttings peaked at 0.5 mM. Additionally, the chlorophyll (a + b) content of cuttings decreased about 29.9%, while this decrease was around 37.5% for seedlings
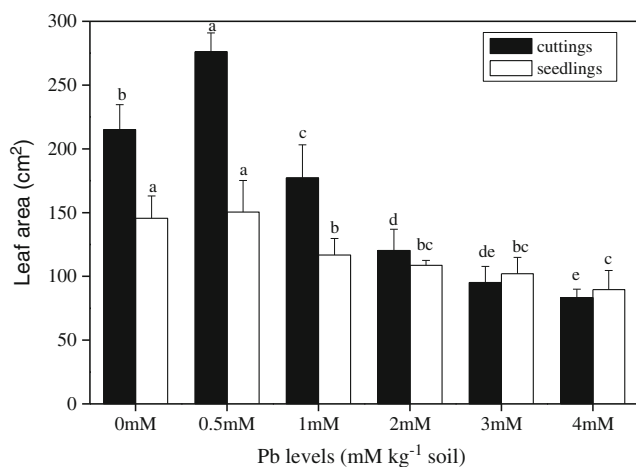

Fig. 1 Effect of Pb concentrations on leaf area in cuttings and seedlings of *Jatropha curcas* L. treated with different concentrations of Pb. Significant differences from controls ($p\leq0.05$) are marked with *different letters*

at 4 mM Pb level compared to the control (Fig. 3d). The highest carotenoid content was measured in 0.5 mM Pb stress for cuttings and then decreased significantly with increasing Pb stress. However, the differences in carotenoid contents of cuttings and seedlings diminished at a highly toxic Pb level. Carotenoid content of cuttings and seedlings decreased, 31.9% and 23.6%, respectively, compared to the control at a highly toxic Pb level (Fig. 3c).

The MDA contents of cuttings and seedlings were found to have significant differences. The MDA content of cuttings increased with increasing Pb stress. The MDA content was found to be relatively more in cuttings than that in seedlings in control. Additionally, the MDA contents of cuttings increased about 100.91%, while this increased to 108.81% for seedlings at a highly toxic Pb level compared to the control (Fig. 4b).

3.3 Effect of Pb on protein contents and antioxidant enzyme activity

The leaves of cuttings and seedlings were significantly affected by Pb (Fig. 4a). For cuttings, protein content of leaves decreased with increasing Pb stress. The protein content increased and then decreased in leaves of seedlings; the protein peaked at 1 mM Pb stress and was still significantly higher than the controls at 4 mM. The protein content was found to be relatively more in leaves of seedlings than that of cuttings under Pb stress.

At 4 mM Pb concentration, SOD activity decreased for the cuttings, whereas it continued to increase in the seedlings (Fig. 5a). For seedlings, there was no significant difference in the activity of SOD at 0.5 mM and 1 mM Pb concentrations compared to the controls; activity of SOD was maximum at 4 mM Pb stress. For cuttings, activity of SOD was maximum at 3 mM Pb followed by a decrease with increase in Pb concentration. SOD activity of cuttings increased about 142.5%, while this increase was around 70.9% for seedlings at Pb stress compared to the control.

POD activity in seedlings peaked at 3 mM Pb and increased approximately 2.26 times compared to controls. POD activity in cuttings peaked at 1 mM Pb and decreased to control leaves at 4 mM Pb. POD activity was lower in cuttings than that in seedlings. In cuttings and seedlings, POD activity showed an increase at lower concentration and a decline at higher concentration (Fig. 5b).

CAT activity showed an increase at lower Pb concentration and a decline at higher Pb concentration. CAT activity in seedlings peaked at 3 mM Pb and decreased to control level at 4 mM Pb (Fig. 5c). For cuttings, CAT activity peaked at 2 mM Pb and was still significantly higher than the controls at 4 mM Pb. CAT activity was lower in cuttings than that in seedlings. And seedlings had quite high CAT activity in leaves when exposed to heavy metal Pb stress.

Fig. 2 Effect of Pb concentrations on root length (a) and on tolerance index (b) in cuttings and seedlings of *Jatropha curcas* L. treated with different concentrations of Pb. Significant differences from controls ($p \leq 0.05$) are marked with *different letters*
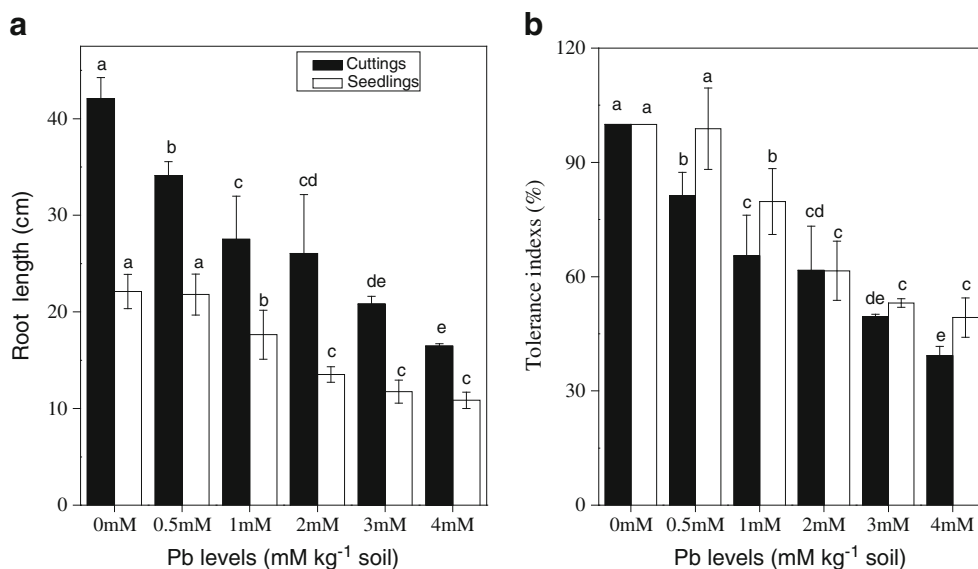
3.4 Effect of Pb on photosynthesis

The response of different parameters of photosynthesis to Pb toxicity is shown in Fig. 6a–d. The results showed that the elevated Pb caused a decrease in the Pn in the *J. curcas* leaves. This was accompanied with a decrease of Cond and Tr (Fig. 6b, d). In seedlings and cuttings, Pn showed a similar trend of steep decrease as noted for Cond and Tr; Pn, Cond, and Tr were found to be relatively more in leaves of cuttings than in leaves of seedlings under lower Pb concentration, but an opposite result is seen under higher Pb concentration. In seedlings and cuttings on the other

hand, the differences between treated and control plants for the Ci were significant ($P < 0.05$).

3.5 Metal Pb concentrations in plant tissues

There were significant differences between treatments in plant Pb content. The magnitude of increase in the Pb concentration of both plants was found to be dependent on soil Pb concentration (Fig. 7a, b). Furthermore, the Pb concentration was relatively more in roots than in leaves. At higher Pb stress (4 mM), there was a 62 times increase in roots and 33 times increase in leaves of cuttings, whereas

Fig. 3 Effect of Pb concentrations on chlorophyll a content (a), chlorophyll b content (b), carotenoid content (c), and on chlorophyll (a+b) content (d) in cuttings and seedlings of *Jatropha curcas* L. treated with different concentrations of Pb. Significant differences from controls ($p \leq 0.05$) are marked with *different letters*
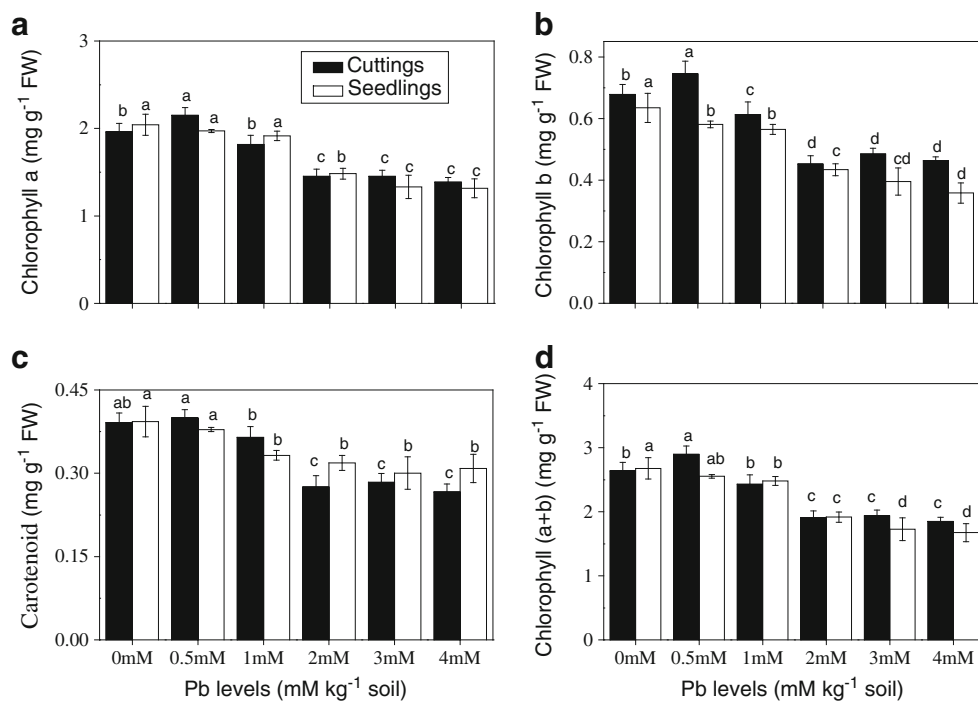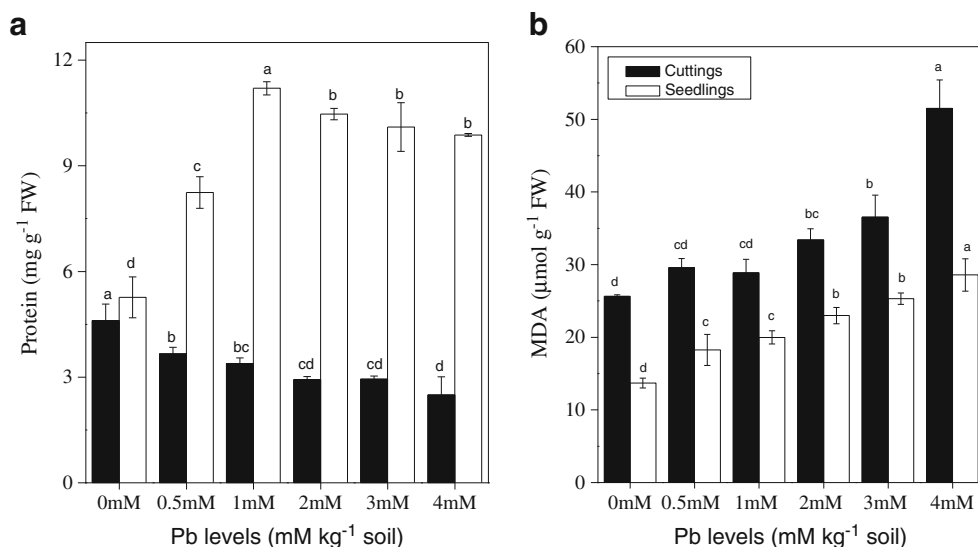
Fig. 4 Effect of Pb concentrations on protein content (**a**) and on MDA content (**b**) in cuttings and seedlings of *Jatropha curcas* L. treated with different concentrations of Pb. Significant differences from controls ($p \leq 0.05$) are marked with *different letters*

a 453 times increase in roots and 14 times increase in leaves of seedlings were observed when compared with their respective controls.

4 Discussion

4.1 Effect of Pb on leaf area, root length growth, and tolerance index

Leaves are considered as one of the most important plant organs due to their role in capturing light and making food via photosynthesis. A number of studies have reported inhibited plant growth under Pb toxicity (Gabara and Goaszewska 1992; Piechalak et al. 2003). Similarly, in the present study, a sharp decrease in the leaf area, root length, and growth of cuttings was noted, which is in agreement with the decrease in plant leaf area and root length reported in various other studies (Tanyolac et al. 2007). On the basis of these results, our findings suggested that an elevated Pb concentration can inhibit the normal growth and development of cuttings and seedling of *J. curcas*. On the other hand, the absence of Pb toxicity on leaf area and root length reflects obvious tolerance of seedlings to higher Pb

Fig. 5 Antioxidant enzyme activities of cuttings and seedlings of *Jatropha curcas* L. exposed to excess Pb. **a** SOD activity, **b** POD activity, **c** CAT activity. Significant differences from controls ($p \leq 0.05$) are marked with *different letters*
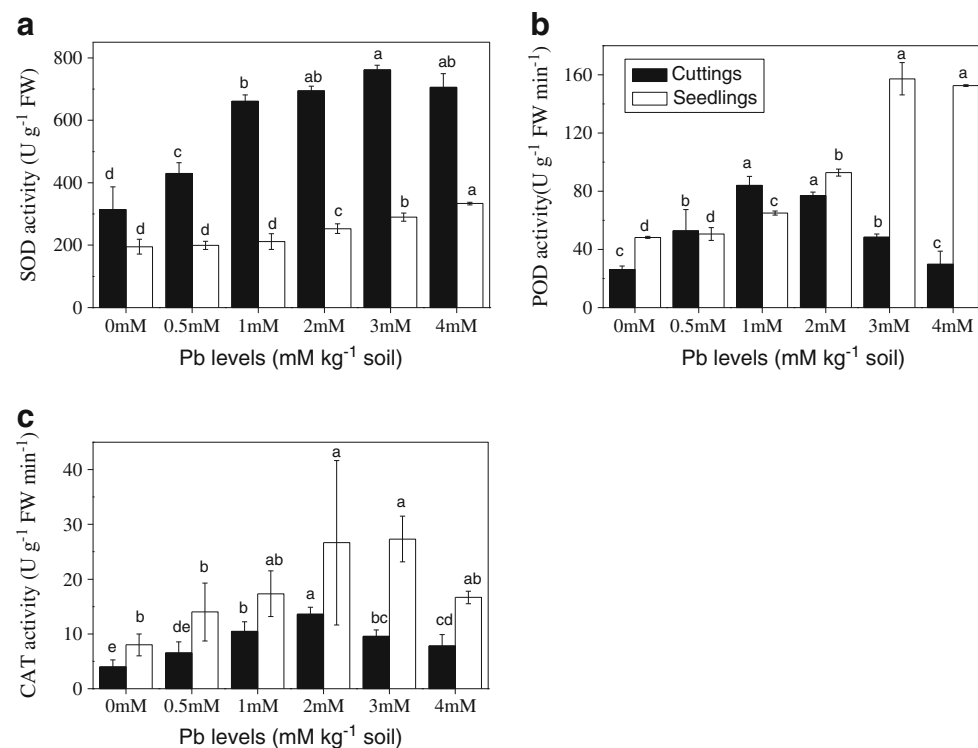
Fig. 6 **a** Net photosynthetic rate (*Pn*), **b** stomatal conductance (*Cond*), **c** intercellular CO_2 concentration(*Ci*), and **d** transpiration rate(Tr) of the seedlings and cuttings of *Jatropha curcas* L. grown under different Pb levels. *Data points* and *error bars* represent mean ± SD of four replicates. *Different letters* indicate significant differences (*P*<0.05) among the treatments and CK
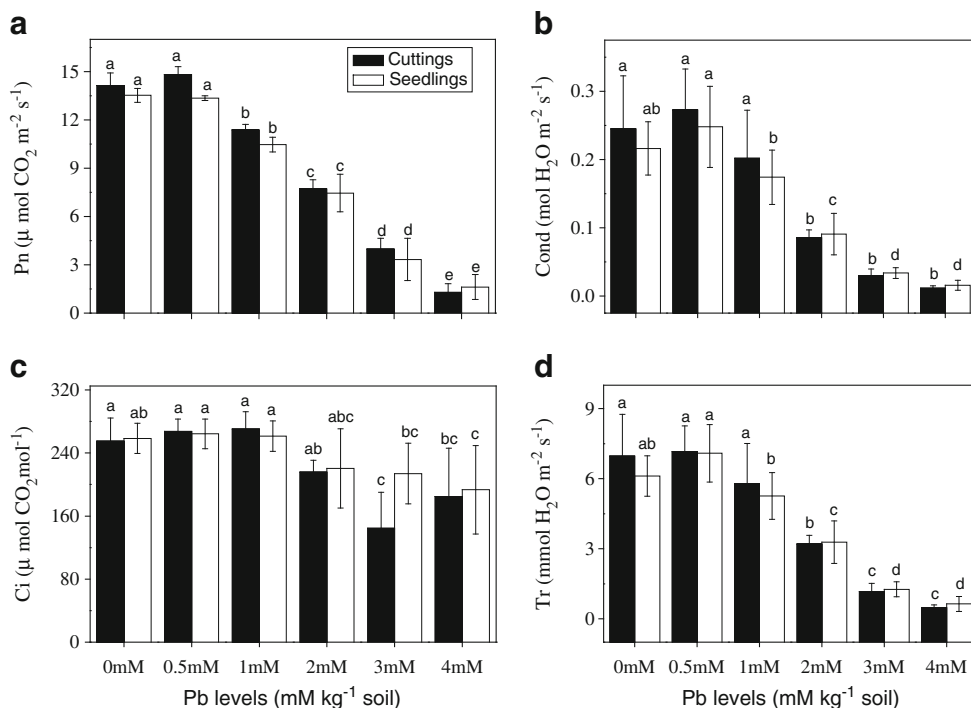

concentrations in the growth medium. Differences between TI values indicated also that seedlings have the highest resistance among the two plants and have the fastest initiation of the detoxicative system (Figs.1 and 2).

4.2 Effect of Pb on Photosynthetic pigments and MDA contents

Heavy metals inhibit chlorophyll and carotenoid biosynthesis and retards the incorporation of these pigments in photosystems. On the other hand, the decrease in net photosynthesis

as a consequence of reduced absorption of essential mineral nutrients is an indirect reason for plant chlorosis (Vazquez et al. 1987). In this study, at higher Pb concentration, the chlorophyll and carotenoid content decreased with the increasing concentrations of externally supplied Pb in cuttings and seedlings (Fig. 3). This reduction in chlorophyll and carotenoid content of cuttings and seedlings under high concentration of Pb stress can be regarded as a specific response of the plants to metal stress, which resulted in chlorophyll degradation and inhibition of photosynthesis (Gajewska et al. 2006); it was probably caused by interaction

Fig. 7 Pb concentrations in the leaf (**a**) and roots (**b**) of cuttings and seedlings of *Jatropha curcas* L. grown under different levels of Pb for 60 days
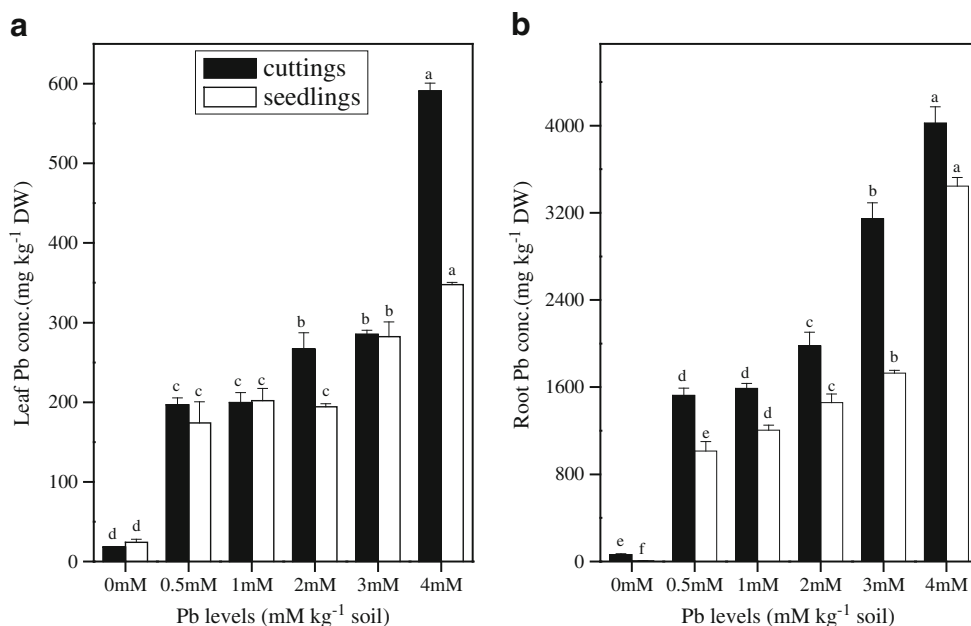
of Pb to –SH group of enzymes of chlorophyll biosynthesis as well as lipid peroxidation-mediated degradation as indicated by Singh et al. (2006). A similar type of result was reported by Tanyolac et al. (2007) with maize (*Zea mays* L.) stressed by Cu.

MDA is the final product of peroxidation of membrane lipids and accumulates when the plants are subjected to oxidative stress. Therefore, MDA level is routinely used as an index of lipid peroxidation under stress conditions. In the present study, MDA content increased significantly in leaves of cuttings and seedlings. On the other hand, the MDA content in leaves of seedlings at any concentration was lower than that in cuttings. Generally, free radical generation and membrane damage would be low in tolerant plants and thereby formation of lower levels of MDA content. Therefore, in this study, a relatively lower MDA content in seedlings to Pb stress may support its tolerant nature.

4.3 Effect of Pb on protein contents and antioxidant enzyme activity

Metal stress can act at different sites to inhibit a large number of enzymes having functional sulfhydryl groups. It results in the deleterious effect in the normal protein form by disrupting the pathways and protein synthesis (Nagoor 1999). In this study, lower Pb stress induced the decline in soluble protein contents in cuttings, but a significant increase in soluble protein contents was seen in seedlings (Fig. 4a). Induction in protein content is possible due to induction of stress proteins (Srivastava et al. 2005) under lower metal exposure. These stress proteins may constitute various antioxidant enzymes and also some heat shock proteins (Srivastava et al. 2005; Prassad 1996). Therefore, the seedlings' tolerance to oxidative stress was higher than the cuttings. This reduction may be due to degradation by proteases (Romero-Puertas et al. 2002; Prassad 1996). Many studies showed that the protein content of plants was decreased by Pb accumulation (Mohan and Hosetti 1997).

Heavy metals induce oxidative stress by generation of superoxide radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen, collectively termed ROS (Devi and Prasad 1998). ROS can rapidly attack all types of biomolecules such as nucleic acids, proteins, lipids, and amino acids (Luna et al. 1994), leading to irreparable metabolic dysfunction and cell death. To cope and repair the damage caused by ROS, plants have evolved complex antioxidant systems.

SOD is a metalloenzyme present in various cellular compartments, functioning at the first step of ROS generation, i.e., superoxide formation, superoxide radicals can act as a precursor to other ROS (Alscher et al. 2002). SOD dismutates two superoxide radicals to H_2O_2 and oxygen and thus maintains superoxide radicals in a steady-state level. In this study, for cuttings, the decline in SOD activity at 4 mM indicated that the oxygen scavenging function of SOD was impaired. For seedlings, SOD activity was increased with increase Pb stress concentration and peaked at 4 mM Pb concentrations. This increase in SOD activity could possibly be the result of both a direct effect of heavy metal ions and an indirect effect mediated via an increase in levels of O_2 (Chongpraditnum et al. 1992). SOD activity of seedlings peaked at higher metal concentrations than those of cuttings, suggesting that this increase in SOD has better protection against oxidant damage.

POD catalyzes H_2O_2-dependent oxidation of substrate. POD activity is also considered a useful biomarker for sublethal metal toxicity in examined plant species. Previous studies in other plants have reported increases, decreases, and no changes in POD activity in response to heavy metal exposure (Shaw 1995). In our study, the results presented show increased activities of POD activity at lower Pb concentrations and a decline with increase in Pb concentration in cuttings and seedlings. Previous studies in metal-tolerant plant species have reported that POD activity was found to be sufficiently high to enable the plants to protect themselves against oxidative stress (Tanyolac et al. 2007). In our study, seedlings were able to maintain high levels of POD activity at higher Pb stress. Moreover, POD participating in lignin biosynthesis can build up a physical barrier against toxic heavy metals. Therefore, this also indicates that seedlings maybe more efficient in avoiding damage from heavy metals than cuttings.

CAT is a universally present oxidoreductase that decomposes H_2O_2 to water and molecular oxygen, and it is one of the key enzymes involved in the removal of toxic peroxides. In the present study, CAT activities in cuttings and seedlings significantly increased at lower Pb concentrations, while at higher Pb concentrations, it was decreased. Increase in CAT activity can be explained by an increase in its substrate, i.e., to maintain the level of H_2O_2 as an adaptive mechanism of the plants (Reddy et al. 2005). Decline observed at higher concentration of Pb might be attributed to inactivation of enzyme by ROS, decrease in synthesis of enzyme, or change in assembly of its subunits (Verma and Dubey 2003). In our study, CAT activity was higher in seedlings than in cuttings under any Pb concentration. The higher CAT activities in seedlings indicate that its H_2O_2 scavenging mechanism is more effective than in cuttings.

4.4 Effect of Pb on leaf photosynthesis

It is widely accepted that photosystem (PS) I electron transport is less sensitive to metal ion inhibition in contrast to PS II. In our study, Pb-induced reduction in Pn of plants was probably caused by stomatal closure since Pb-treated

plants were accompanied by a lower stomatal conductance as well as transpiration rate especially those growing at higher levels of Pb (Fig. 6). On the other hand, a sharp reduction in the Pn of Pb-treated plants could also be expected from the severely damaged chloroplast internal organization of the thylakoid membranes of the stroma and grana which were dissolved and not visible after Pb exposure. Khudsar et al. (2004) observed similar results in *Artemisia annua*. The parallel change of Pn and Cond in peanut leaves reinforces evidence that the changes in Pn could be mainly attributed to the changes in Cond (Jones 1992).

4.5 Effect on Pb concentrations in plant tissue

Plant absorption and accumulation of Pb have been reported in roots, stems, and leaves, etc. which increase with increasing the exogenous Pb levels. It has been reported that Pb is unevenly distributed in roots, where different root tissues act as barriers to apoplastic and symplastic Pb transport, and hence, Pb transport to shoot gets restricted (Trivedi and Erdei 1992) as shown in the present study. At lower Pb concentration, the Pb accumulation was almost the same for cuttings and seedlings, but with the increase in external Pb concentration (4 mM), the leaves of cuttings absorbed a higher amount of Pb than those of seedlings (Fig. 7a). The cuttings seem to possess a stronger ability to uptake Pb and transport to leaves than seedlings. Based on comparative studies of metal concentration in plant parts, Baker and Walker (1990) suggested that uptake, translocation, and accumulation mechanisms differed for various heavy metals and for the species. Ouzounidou et al. (1994) observed that at high external metal concentrations, sensitive plants contained more metal in their shoots than the tolerant plants.

5 Conclusions

These results suggest that Pb induces oxidative stress in seedlings and cuttings and that elevated activity of antioxidative enzymes could serve as important components of antioxidative defense mechanism against oxidative injury. There were significant differences between cuttings and seedlings of *J. curcas* in terms of Pb accumulation, responses of antioxidant enzymes, and photochemical activities when exposed to Pb toxicity. Under Pb stress, seedlings showed lower Pb accumulation and MDA in leaves but higher root tolerance index, protein content, and higher antioxidant enzyme than cuttings. The lower MDA content and Pb accumulation and higher POD and CAT activities in seedlings suggested the tolerance capacity of this plant types to protect the plant from oxidative damage. Conclusively, *J. curcas* seedlings can be successfully grown in Pb-polluted soils.

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