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Influence of NaCl-salinity on Pb-uptake behavior and growth of River Red gum tree (*Eucalyptus camaldulensis* Dehnh.)

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Abstract: Lead (Pb) is one of the most toxic and persistent elements, with no known biological role. High concentrations of lead enter saline soils through anthropogenic activities and industrial waste. The decontamination of Pb polluted sites through phytoremediation, especially by using woody plants, is an attractive approach. *Eucalyptus camaldulensis* is a good accumulator of Pb and a recommended tree species for reclamation of saline soils. However, there is very limited knowledge about the Pb-uptake behavior of *Eucalyptus camaldulensis* under saline conditions. The objective of this study was to examine the simultaneous impact of NaCl salinity and Pb stress on the phytoremedial potential and growth behavior of *E. camaldulensis*. Six-week-old *Eucalyptus camaldulensis* plants with uniform morphological features were transferred into hydroponic conditions, and they were exposed to the NaCl salinity stress at 200 mM and Pb stress at 10 mg L^{-1} and 20 mg L^{-1} (Pb1 and Pb2, respectively). The plants showed a 100% survival rate in all treatments. All the morphological parameters (plant height, plant diameter, and shoot dry weight) for *E. camaldulensis* were significantly affected when exposed to Pb or Pb + NaCl-salinity, with the exception of root length which was 36% higher in Pb2 as compared to the control. The Pb uptake (mg Pb $plant^{-1}$) was much higher (49.3) under Pb2 + NaCl-salinity, as compared to the control (1.2) and other treatments, which shows that saline conditions increased the Pb-uptake in *E. camaldulensis*. The order of deposition of Pb (mg Pb kg^{-1} of dry weight) in plant parts was root (103.4) >> shoot (17.5) > leaves (13.6). Similarly, the bioconcentration factor (BCF) of the roots was 4 to 6 times higher than shoot BCF.

Key words: *Eucalyptus camaldulensis,* contaminated soils, heavy metal, NaCl-salinity, Pb uptake, phytoremediation

1. Introduction

Anthropogenic activities such as mining, industrial processing, sewage sludge management, and agricultural practices result in the annual release of huge amounts of pollutant metals (Pb, Cu, Ni, Cr, Cd, etc.) that enter soil and water systems (Singh et al., 2003; Boyd, 2010). In most developing countries, metal-bearing wastewaters are disposed of through traditional unpaved drains, and then they are used for crop irrigation purposes (Nasir et al., 2012; Hidri et al., 2014), which has resulted in the development of heavy-metal–polluted soils (Chabukdhara and Nema, 2013; Yao et al., 2014). In Pakistan, heavy metal pollution in soil due to wastewater irrigation has been reported by various authors (Mushtaq and Khan, 2010; Iqbal et al., 2011a; Nasir et al., 2012). Phytoremediation is a cheap, ecofriendly, and cost-effective approach, compared to physical and chemical approaches, to decontaminate polluted soils (Salt et al., 1995, 1998; Waranusantigul et al., 2011). Moreover, the use of woody vegetation has advantages compared to ephemeral/annual plants because it has greater biomass, higher economic value, and there is less risk of pollutants entering the food chain (Pulford and Watson, 2003; Peng et al., 2012). However, finding the appropriate site-specific tree species and developing a better understanding of the biological processes of heavy metal absorption, translocation, accumulation, phytotoxicity symptoms, and tolerance mechanisms are desired in research into improving this existing technology (Gomes et al., 2012).

The presence of large amounts of lead (Pb) in soils and waters is primarily due to anthropogenic activities such as use of Pb-based paints, shotgun pellets, pesticides, and gasoline, because it occurs naturally in small amounts within the earth's crust (Arshad et al., 2008; Gupta et

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al., 2013). Lead is one of the most toxic and persistent elements with no known biological role (Foulkes, 2000), and lead exposure in human beings can result in brain and kidney problems as well as gastrointestinal diseases (Jarup, 2003). Long-term lead exposure can even result in failure of the central nervous system and the liver (Gupta et al., 2013). Lead is one of the great phytotoxic elements as well, and recent studies have shown that the cytoplasm is the primary site of Pb toxicity (Kopittke et al., 2011). The negative effects of lead on the morphological and physiological characteristics of plants have been widely reported (Gupta et al., 2009; Huang et al., 2012).

The phytoremediation of heavy-metal–polluted soils can become troublesome when it is coupled with salinity, as in the irrigated tracts of semiarid regions of Pakistan (Raziuddin et al., 2011). Apart from Pakistan, about one-third of the world's irrigated lands, and half of those in the semiarid and coastal regions, are moderately or severely affected by salinity (Rawat and Banerjee, 1998). In Pb-polluted saline soils, plants have to tolerate the toxicity of Pb as well as the major abiotic stress of salinity; hence, all the higher vascular plants are unable to survive and grow in these harsh conditions (Van der Moezel et al., 1988). The use of tolerant plants that are suitable for degraded lands and for Pb-phytoremediation purposes is recommended, because these plants are better in terms of survival, growth, and reproduction than nontolerant plants (Gupta et al., 2013).

The *Eucalyptus camaldulensis* Dehnh. is highly recommended to combat salinity, because it can tolerate high levels of NaCl-salinity as compared to other tree species (Marcar, 1993; Akilan et al., 1997; Van der Moezel et al., 1988). Salinity up to a level of 160 mM NaCl did not affect the survival of *E. camaldulensis*; however, it can affect plant growth and dry-matter production negatively (Rawat and Banerjee, 1998; Grieve et al., 1999). The *Eucalyptus* species was introduced to Pakistan in the 19th century from Australia due to its ornamental importance and evergreen nature (Iqbal et al., 2011b). Since the early 20th century, following intensive evaluation of its wood qualities and growth behavior, *Eucalyptus camaldulensis* Dehnh. has become a very popular tree species in Pakistan (Qureshi et al., 1993; Mahmood et al., 2003). This species has been planted successfully for the afforestation of marginal lands (saline and waterlogged) as well as farmlands due to its ability to produce good economic returns in a short time (Akhter et al., 2005). This species also has great potential for use in phytoremediation and/or phytostabilization, because a major portion of the heavy metal is accumulated in roots, with very low transport rates from root to shoot (Gomes et al., 2012; Peng et al., 2012). It was reported that *E. camaldulensis* is a good Pb-accumulator; however, higher concentrations of Pb (≥20 mg/L) in soil solution can adversely affect its growth without any threat to the survival of the plant (Waranusantigul et al., 2011).

In light of previous studies, it is evident that *E. camaldulensis* Dehnh. is appropriate to grow in saline soils as well as in Pb-contaminated soils in Pakistan; however, the morphological and ecophysiological response of *E. camaldulensis* in Pb-contaminated saline soils is not yet known. Therefore, the present study was designed to evaluate the Pb- tolerance and accumulation behavior of *E. camaldulensis* under NaCl-saline conditions. The first objective of this study was to analyze the Pb-phytoextraction capacity and morphological-cum-physiological response of *E. camaldulensis* Dehnh. to different Pb concentrations. The second objective was to analyze how Pb-phytoextraction behavior of *E. camaldulensis* is modified under NaCl-based salinity. To the best of our knowledge, this is the first study to determine the simultaneous impact of NaCl-salinity and Pb stress on the Pb-uptake behavior of *E. camaldulensis*.

2. Materials and methods

2.1. Hydroponic experiment

Certified seeds of *Eucalyptus camaldulensis* Dehnh. from a monogenetic provenance were acquired from the Punjab Forestry Research Institute (PFRI), Gatwala, Faisalabad, Pakistan. Seedlings were raised from the last week of February 2013 to the first week of March 2013 on moist sand with the application of Hoagland's solution (Hoagland and Arnon, 1950): 5 mM $Ca(NO_3)_2$, 1.75 mM K_2SO_4, 1.25 mM $MgSO_4$, 0.25 mM KH_2PO_4, 0.25 mM KCl, 25 µM H_3BO_3, 1.25 µM $MnSO_4$, 1.25 µM $ZnSO_4$, 0.50 µM $CuSO_4$, 0.025 µM $(NH_4)_6Mo_7O_{24}$, and 0.25 mM Fe-EDTA. The experiment was carried out in the experimental area of the Department of Forestry, Range Management and Wildlife and in the greenhouse of the Institute of Soil Science and Environmental Sciences, University of Agriculture, Faisalabad (31°26′N, 73°06′E; 184.4 m). Data about climatic conditions during the seedling raising period are presented in Table 1 and were collected from the Agricultural Meteorology Cell, Department of Crop Physiology, University of Agriculture, Faisalabad, located some meters away from experimental area. Six-week-old, uniform sized seedlings were transferred into 50 L capacity plastic tubs covered with sterilized plastic sheets under greenhouse conditions (humidity: ~50%, temperature: 20–31 °C, and photon flux: 6000–9000 lux). Tubs were filled with Hoagland's solution, and their pH was set at 5.5 ± 0.1 using KOH or HCl. Deionized (Na^+ and Cl^- free) water was used to make Hoagland's solution. After an additional growth period of 2 weeks, two different concentrations of lead (Pb) were added to the nutrient solution in the form of $Pb(NO_3)_2$: 10 mg Pb L^{-1} (Pb1) and 20 mg Pb L^{-1} (Pb2). The two Pb concentrations were chosen considering the

Table 1. Climatic data during *E. camaldulensis* seedling growth in the nursery.

Month	Temperature (°C)		Relative humidity	Sunshine (h)	Rainfall (mm)	Wind speed (km/h)
	Range	Average				
February	8.3–19.2	13.8	81%	5.2	55	4.6
March	13–27	20	61.2%	8.6	1.3	5.4
April	19.7–33.5	26.6	36.7%	8.9	21.6	6.2

WHO-set limit for Pb in irrigation water (5 mg Pb L^{-1}) as well as the level of Pb concentration found at some sites in Pakistan (Iqbal et al., 2011a). A stress of 200 mM NaCl-salinity was applied to the desired plants in hydroponics by dissolving appropriate quantities of chemically pure NaCl salts in nutrient solution, as described by Rawat and Banerjee (1998). The hydroponic media were continuously aerated and replaced weekly.

2.2. Treatments

There were a total of six treatments in the experiment: a combination of 3 levels of Pb (0, 10, and 20 mg Pb L^{-1}; Pb0, Pb1, and Pb2, respectively) and 2 levels of NaCl concentration (0 and 200 mM NaCl; NaCl0 and NaCl1, respectively). Three replicates for each treatment and three seedlings for each replicate were employed during our study.

2.3. Measurements

After 6 weeks of treatment, the photosynthetic rate and stomatal conductance of seedlings were measured using a portable photosynthetic system. The measurements were taken between 1000 and 1100 hours and were repeated to record 5 observations for each leaf sample. The plants were harvested after 7 weeks of exposure to different treatments. Individual plant height, diameter, and root length were measured, and visual observations were made. The plants were separated into root and shoot fractions, placed in paper bags, and dried in an oven at 70 °C to a constant weight to determine shoot and root dry weights.

2.4. Chemical analysis

Dried plant samples were ground into powder, sieved at 1 mm, and 0.5 g of the plant samples were digested in 10 mL of strong acid solution ($HNO_3/HClO_4$, 3:1, v v^{-1}). Then Pb concentrations in the digestions were determined using an atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) in the Hi-Tech Lab of the University of Agriculture, Faisalabad, Pakistan, following standard conditions and using a highly sterilized glass apparatus and highly purified deionized water. Calibrated standard was prepared from commercially available stock solution (AppliChem) in the form of aqueous solution. To study Pb bioconcentration behavior, the bioconcentration factor (BCF) was calculated by dividing the Pb-concentration in plant tissue (mg g^{-1} DW) at harvest by the metal concentration in the solution, as calculated by Sharma and Agrawal (2006) and Gomes et al. (2012).

2.5. Statistical analysis

Collected data were analyzed statistically by using analysis of variance (ANOVA) in completely randomized design (CRD) and least significant difference method (LSD) in SPSS for Windows. The level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Plant growth and visual observation

All *E. camaldulensis* plants survived the NaCl-salinity stress at 200 mM NaCl and Pb stresses at 10 mg L^{-1} and 20 mg L^{-1}. However, more luxurious growth was observed in the control treatment (in the absence of salinity and/or Pb), and the leaves were thin and yellowish with brown spots when NaCl-salinity stress was coupled with Pb stress.

The experimental results related to effects of two Pb concentrations and NaCl-salinity on the morphological characteristics (plant height, diameter, root length, root/shoot ratio, and shoot and root dry weights) are presented in Figure 1. It was found that plant height (30.7 cm) and plant diameter (0.5 cm) were significantly higher in the Pb0-NaCl0 (control) treatment, and both growth parameters were negatively affected by NaCl-salinity and Pb stress (Figures 1a and 1b). In the absence of NaCl-salinity, two concentrations of Pb in the nutrient solution (Pb1 + NaCl0 and Pb2 + NaCl0) resulted in a 32.5% and 52.1% reduction in plant height in *E. camaldulensis*, respectively, as compared to the control (Figure 1a). Root length of *E. camaldulensis* plants was adversely affected by NaCl-salinity, and minimum root length (19.8 cm) was found in the presence of NaCl-salinity (Figure 1c), but application of Pb resulted in the enhanced growth of roots. At 20 mg Pb L^{-1} (Pb2 + NaCl0), root length was about 33% more as compared to Pb1 + NaCl0 (10 mg Pb L^{-1}) and 36% higher than the control. The maximum root-to-shoot ratio was observed in the Pb2 + NaCl0 treatment (2.22), and the minimum (0.78) was observed in Pb0 + NaCl0 (control) (Figure 1d). Figures 1e and 1f demonstrate that shoot dry weight was highest in Pb0 + NaCl0 (6.1 g $plant^{-1}$), while root dry weight was highest in Pb2 + NaCl0 (4.8 g $plant^{-1}$).

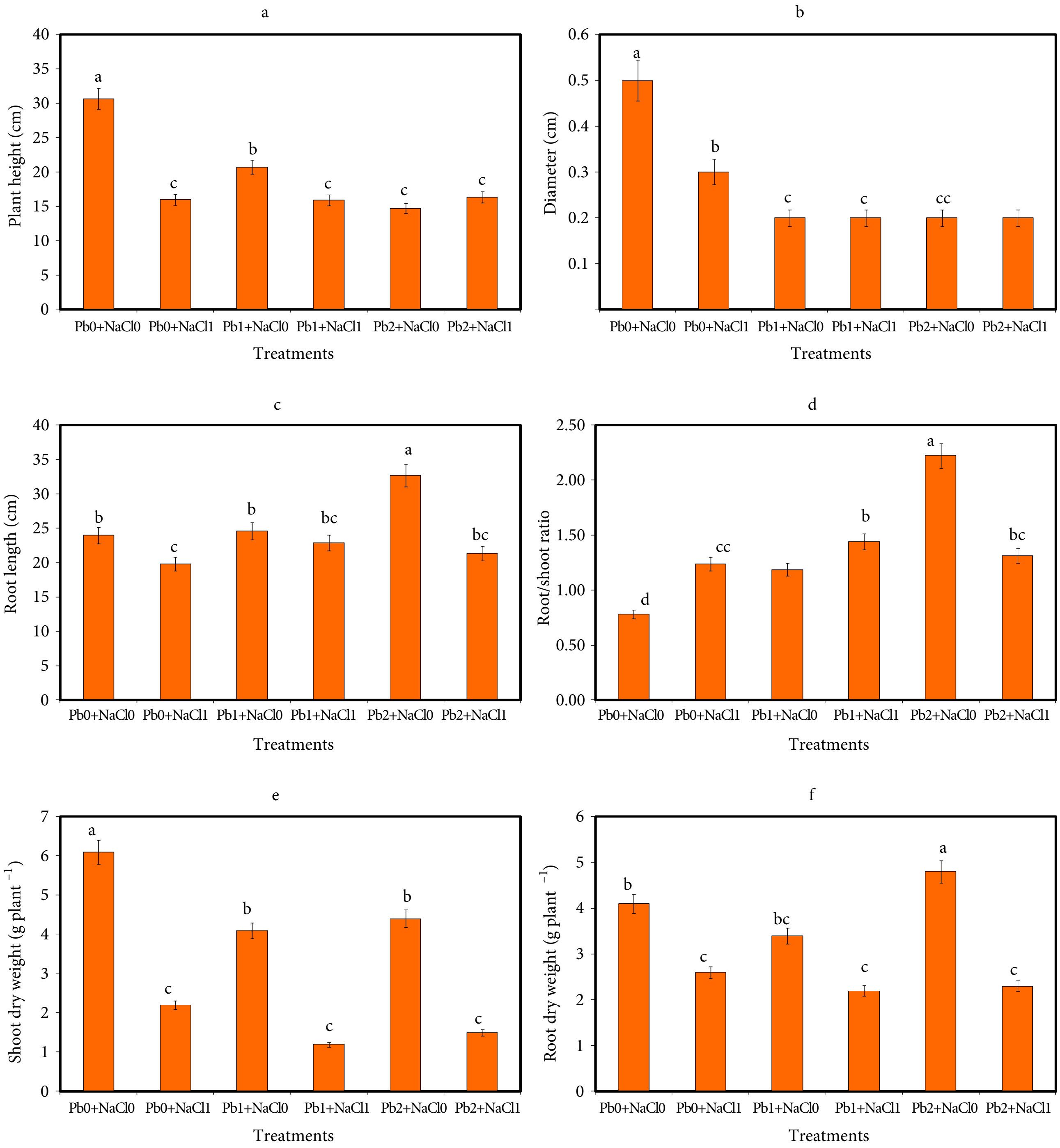

Figure 1. Effect of different Pb concentrations and NaCl-salinity on morphological characteristics of *E. camaldulensis*. Here, Pb0 and NaCl0 designate control, NaCl1 is salinity at 200 mM NaCl, Pb1 is 10 mg Pb L^{-1}, and Pb2 is 20 mg Pb L^{-1}.

3.2. Pb contents, accumulation, and bioconcentration factor

Pb concentration in plant parts (root, shoot, and leaves) is largely influenced by Pb concentration in the nutrient solution as well as salinity (Figure 2). With the increase in the Pb concentration from 10 mg L^{-1} in Pb1 + NaCl0 to 20 mg L^{-1} in Pb2 + NaCl0, the Pb uptake and accumulation increased in the root portion, but this did not significantly affect the transport of Pb from roots to shoots and leaves (Figures 2a–2c). The NaCl-salinity treatment (NaCl1) significantly modified Pb uptake behavior in roots and also affected the transport of Pb from roots to leaves; however,

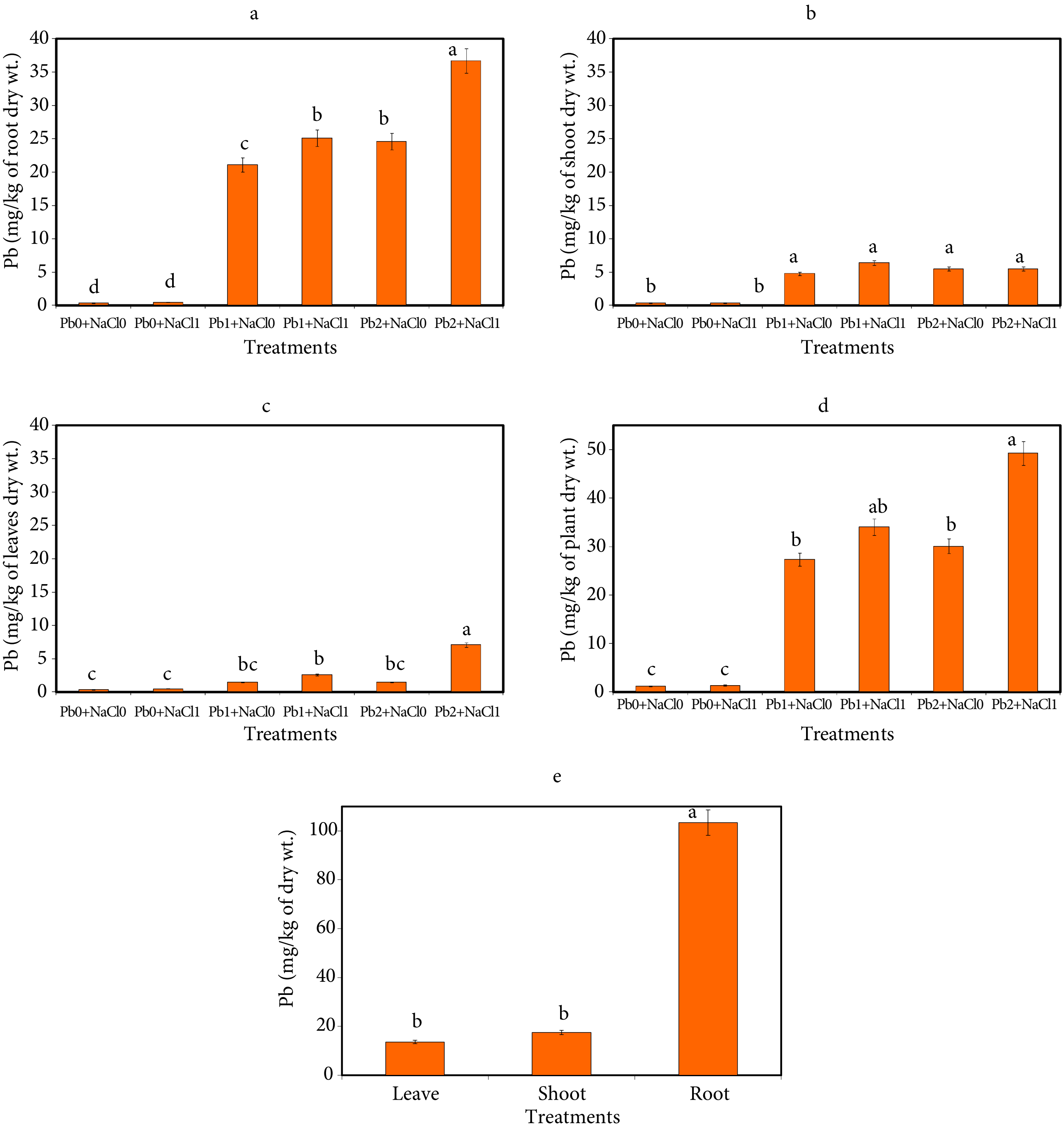

Figure 2. Pb-uptake behavior of *E. camaldulensis*. a; root, b; shoot, c; leaves, d; whole plant, e; different plant parts. Here, Pb0 and NaCl0 designate control, NaCl1 is salinity at 200 mM NaCl, Pb1 is 10 mg Pb L^{-1}, and Pb2 is 20 mg Pb L^{-1}.

no significant difference was found for Pb content in shoots. The Pb accumulation in roots increased by 49% at 20 mg Pb L^{-1} and 200 mM NaCl (Pb2 + NaCl1) as compared to the Pb2 + NaCl0 treatment. Similarly, the lead content was 373% greater in leaves under the Pb2 + NaCl1 treatment as compared to the Pb2 + NaCl0 treatment (Figure 2c). Total Pb uptake by the plant (mg Pb $plant^{-1}$) was also greatest in Pb2 + NaCl1 (49.3) as compared to Pb0 + NaCl0 (1.2), Pb0 + NaCl1 (1.4), Pb1 + NaCl0 (27.4), Pb2 + NaCl0 (30.1), and Pb1 + NaCl1 (34.1) (Figure 2d). The Pb concentration gradient was as follows: root > shoots ≥ leaves (Figure 2e). A maximum Pb uptake (mg Pb kg^{-1} of dry weight) of about 103.4 was found in the root portion of the plants and was significantly higher than in the shoots (17.5) and leaves (13.6). The bioconcentration factor (BCF) of roots was 4 to 6 times higher than the BCF of shoots (Table 2). The BCF shoot and BCF root at 10 mg Pb L^{-1} were higher (0.48 and 2.11, respectively) as compared to 20 mg Pb L^{-1} (0.275 and 1.23, respectively). NaCl-salinity treatment at 10 mg Pb L^{-1} increased shoot BCF by 33% and root BCF by 19% as compared to 10 mg Pb L^{-1} treatment.

3.3. Photosynthesis and stomatal conductance

The photosynthesis rate and stomatal conductance were distinctly affected by Pb concentrations (10 mg Pb L^{-1} and 20 mg Pb L^{-1}) and NaCl-salinity (200 mM NaCl) as represented in Table 2. The presence of stress, either Pb or salinity, increased the photosynthetic rate and stomatal conductance. The highest photosynthetic rate and stomatal conductance (3.5 and 425, respectively) were observed when Pb stress at 20 mg L^{-1} was coupled with NaCl-salinity stress at 200 mM (Table 2).

4. Discussion

The results showed that survival of the *Eucalyptus camaldulensis* plants was not affected by high NaCl salinity or higher levels of Pb in nutrient solutions. These results are in agreement with previous studies (Marcar, 1993; Rawat and Banerjee, 1998; Van der Moezel et al., 1988; Gomes et al., 2012; Peng et al., 2012) that have shown that *E. camaldulensis* tree species can be successfully grown on salt-affected soils as well as Pb-polluted soils. However, significant biomass reduction was observed at 200 mM NaCl and in the presence of Pb. It is reported that *E. camaldulensis* is tolerant of NaCl-salinity, but NaCl-salinity above 200 mM can affect the biomass production of *E. camaldulensis* plants (Rawat and Banerjee, 1998). The decoloration of leaves and the appearance of various symptoms on the leaves could be the result of deficiencies in nutrients that are essential for physiological functioning of plants (Barcelo and Poschenrieder, 1990) or due to Pb phytotoxicity (Sharma and Dubey, 2005). The results for root/shoot ratio showed that the application of Pb resulted in enhanced root growth and reduced shoot growth; NaCl-salinity negatively affected root growth as well shoot growth (Figure 1d). However, the negative effects of NaCl-salinity on shoot growth were higher than on root growth. The increase in root length in the presence of higher lead concentrations may be due to the increased production of roots, which are needed to absorb more of the available nutrients in stressful environments (Jarup, 2003; Peng et al., 2012).

In general, *E. camaldulensis* was a good accumulator of Pb, and it accumulates significantly higher proportions of lead in the root portion than in the shoots or leaves; that means Pb is stabilized in roots of *E. camaldulensis* as in other woody plants (Pulford and Watson, 2003; Gomes et al., 2012). Lower concentrations of Pb in shoots or leaves indicate the poor ability of *E. camaldulensis* tree species to translocate Pb from roots to shoots (Waranusantigul et al., 2011). This phenomenon, coupled with low utilization of this plant as human food, makes it very safe to use *E. camaldulensis* for phytoremediation purposes.

NaCl-salinity, when coupled with Pb stress, modified the accumulation and translocation behavior of the plants. Increased absorption of Pb in the presence of salinity may be due to increased mobility of Pb in nutrient solution (Acosta et al., 2011) or the increased permeability of tree roots (Wang et al., 2006). However, an in-depth physiological study is required to investigate the demonstrated response of *E. camaldulensis* in the presence of salinity and Pb concentrations.

The observed photosynthetic characteristics and stomatal conductance indicate that salinity and Pb stress stimulated the photosynthetic rate and stomatal conductance, which is contrary to some findings (Tattini et al., 1995; Raziuddin et al., 2011) but in agreement with

Table 2. Effects of different Pb concentrations and NaCl-salinity levels on bioconcentration factor (BCF), photosynthetic rate, and stomatal conductance.

Pb concentrations mg L^{-1}	NaCl- salinity mM	BCF shoot	BCF root	Photosynthetic rate (μM^{-2} s^{-1})	Stomatal conductance (mol^{-2} s^{-1})
0	0	-	-	1.5 c	280 c
0	200	-	-	1.8 c	310 c
10	0	0.48 b	2.11 b	2.0 c	315 c
10	200	0.64 a	2.51 a	2.1c	300 c
20	0	0.275 c	1.23 c	3.0 b	350 b
20	200	0.275 c	1.84 bc	3.5 a	425 a

Values having the same superscript (a, b, c, d, e) are not significantly different.

others (Chavan and Karadge, 1986; Rawat and Banerjee, 1998). The increase in the above-mentioned characteristics may be due to an increase in CO_2 absorption per unit leaf area due to salinity stress (Rawat and Banerjee, 1998).

In conclusion, *Eucalyptus camaldulensis* is fast growing tree that has been increasingly adopted by Pakistani farmers due to high income returns in short rotations of 3–5 years. This study has shown that this tree can be successfully grown in saline and Pb-polluted soils, and the efficiency of *E. camaldulensis* as a phytostabilizer of Pb increases in NaCl-saline soils. However, a detailed ecophysiological field study is needed to explain the increased absorption of Pb under NaCl-saline conditions.

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References

Acosta J, Jansen B, Kalbitz K, Faz A, Martinez-Martinez S (2011). Salinity increases mobility of heavy metals in soils. Chemosphere 85: 1318-1324.

Akhter J, Mahmood K, Tasneem M, Malik K, Naqvi M, Hussain F, Serraj R (2005). Water-use efficiency and carbon isotope discrimination of *Acacia ampliceps* and *Eucalyptus camaldulensis* at different soil moisture regimes under semi-arid conditions. Biol Plantarum 49: 269-272.

Akilan K, Farrell R, Bell D, Marshall J (1997). Responses of clonal river red gum (*Eucalyptus camaldulensis*) to waterlogging by fresh and salt water. Anim Produc Sci 37: 243-248.

Arshad M, Silvestre J, Pinelli E, Kallerhoff J, Kaemmerer M, Tarigo A, Shahid M, Guiresse M, Pradere P, Dumat C (2008). A field study of lead phytoextraction by various scented *Pelargonium* cultivars. Chemosphere 71: 2187-2192.

Barcelo J, Poschenrieder C (1990). Plant water relations as affected by heavy metal stress: a review. J Plant Nutr 13: 1-37.

Boyd RS (2010). Heavy metal pollutants and chemical ecology: exploring new frontiers. J Chem Ecol 36: 46-58.

Chabukdhara M, Nema AK (2013). Heavy metals assessment in urban soil around industrial clusters in Ghaziabad, India: probabilistic health risk approach. Ecotox Environ Safe 87: 57-64.

Chavan PD, Karadge B (1986). Growth, mineral nutrition, organic constituents and rate of photosynthesis in *Sesbania grandiflora* L. grown under saline conditions. Plant Soil 93: 395-404.

Foulkes E (2000). Transport of toxic heavy metals across cell membranes. P Soc Exp Biol Med 223: 234-240.

Gomes M, Marques T, Carneiro M, Soares AM (2012). Anatomical characteristics and nutrient uptake and distribution associated with the Cd-phytoremediation capacity of *Eucalyptus camaldulenses* Dehnh. J Soil Sci Plant Nutr 12: 481-496.

Grieve C, Guzy M, Poss J, Shannon M (1999). Screening eucalyptus clones for salt tolerance. HortScience 34: 867-870.

Gupta D, Nicoloso F, Schetinger M, Rossato L, Pereira L, Castro G, Srivastava S, Tripathi R (2009). Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. J Hazard Mater 172: 479-484.

Gupta D, Huang H, Corpas F (2013). Lead tolerance in plants: strategies for phytoremediation. Environ Sci Pollut R 20: 2150-2161.

Hidri Y, Fourti O, Eturki S, Jedidi N, Charef A, Hassen A (2014). Effects of 15-year application of municipal wastewater on microbial biomass, fecal pollution indicators, and heavy metals in a Tunisian calcareous soil. J Soils Sediments 14: 155-163.

Hoagland DR, Arnon DI (1950). The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station, California, USA.

Huang H, Gupta D, Tian S, Yang XE, Li T (2012). Lead tolerance and physiological adaptation mechanism in roots of accumulating and non-accumulating ecotypes of *Sedum alfredii*. Environ Sci Pollut R 19: 1640-1651.

Iqbal MA, Chaudhary MN, Zaib S, Imran M, Ali K, Iqbal A (2011a). Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb) in agricultural soils and spring seasonal plants, irrigated by industrial waste water. J Environ Technol Manag 2: 1-9.

Iqbal Z, Akhtar M, Qureshi TM, Akhter J, Ahmad R (2011b). Variation in composition and yield of foliage oil of *Eucalyptus polybractea*. J Chem Soc Pak 33: 183-187.

Jarup L (2003). Hazards of heavy metal contamination. Brit Med Bull 68: 167-182.

Kopittke PM, Kinraide TB, Wang P, Blamey FPC, Reichman SM, Menzies NW (2011). Alleviation of Cu and Pb rhizotoxicities in cowpea (*Vigna unguiculata*) as related to ion activities at root-cell plasma membrane surface. Environ Sci Technol 45: 4966-4973.

Mahmood K, Marcar N, Naqvi M, Arnold R, Crawford D, Iqbal S, Aken K (2003). Genetic variation in *Eucalyptus camaldulensis* Dehnh. for growth and stem straightness in a provenance-family trial on saltland in Pakistan. Forest Ecol Manag 176: 405-416.

Marcar N (1993). Waterlogging modifies growth, water use and ion concentrations in seedlings of salt-treated *Eucalyptus camaldulensis, E. tereticornis, E. robusta and E. globulus*. Aust J Plant Physiol 20: 1-13.

Mushtaq N, Khan KS (2010). Heavy metals contamination of soils in response to wastewater irrigation in Rawalpindi region. Pak J Agric Sci 47: 215-224.

Nasir A, Arslan C, Khan MA, Nazir N, Awan UK, Ali MA, Waqas U (2012). Industrial waste water management in district Gujranwala of Pakistan—current status and future suggestions. Pak J Agric Sci 49: 79-85.

Peng X, Yang B, Deng D, Dong J, Chen Z (2012). Lead tolerance and accumulation in three cultivars of *Eucalyptus urophylla X E. grandis*: implication for phytoremediation. Environ Earth Sci 67: 1515-1520.

Pulford I, Watson C (2003). Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29: 529-540.

Qureshi R, Nawaz S, Mahmood T (1993). Performance of selected tree species under saline-sodic field conditions in Pakistan. In: Lieth H, Al Masoom, AA, editors. Towards the rational use of high salinity tolerant plants. Berlin, Germany: Springer, pp. 259-269.

Rawat J, Banerjee S (1998). The influence of salinity on growth, biomass production and photosynthesis of *Eucalyptus camaldulensis* Dehnh. and *Dalbergia sissoo* Roxb. seedlings. Plant Soil 205: 163-169.

Raziuddin, Farhatullah, Hassan G, Akmal M, Shah S, Fida M, Shafi M, Bakht J, Zhou W (2011). Effects of cadmium and salinity on growth and photosynthesis parameters of Brassica species. Pak J Bot 43: 333-340.

Salt DE, Blaylock M, Kumar NP, Dushenkov V, Ensley BD, Chet I, Raskin I (1995). Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nat Biotechnol 13: 468-474.

Salt DE, Smith R, Raskin I (1998). Phytoremediation. Annu Rev Plant Biol 49: 643-668.

Sharma P, Dubey RS (2005). Lead toxicity in plants. Braz J Plant Physiol 17: 35-52.

Sharma RK, Agrawal M (2006). Single and combined effects of cadmium and zinc on carrots: uptake and bioaccumulation. J Plant Nutr 29: 1791-1804.

Singh O, Labana S, Pandey G, Budhiraja R, Jain R (2003). Phytoremediation: an overview of metallic ion decontamination from soil. Appl Microbiol Biot 61: 405-412.

Tattini M, Gucci R, Coradeschi MA, Ponzio C, Everard JD (1995). Growth, gas exchange and ion content in *Olea europaea* plants during salinity stress and subsequent relief. Physiol Plantarum 95: 203-210.

Van der Moezel P, Watson L, Pearce-Pinto G, Bell D (1988). The response of six eucalyptus species and *Casuarina obesa* to the combined effect of salinity and waterlogging. Aust J Plant Physiol 15: 465-474.

Wang S, Guo S, Li J, Hu X, Jiao Y (2006). Effects of salt stress on the root growth and leaf water use efficiency of cucumber seedlings. Ying Yong Sheng Tai Xue Bao 17: 1883-1888.

Waranusantigul P, Lee H, Kruatrachue M, Pokethitiyook P, Auesukaree C (2011). Isolation and characterization of lead-tolerant *Ochrobactrum intermedium* and its role in enhancing lead accumulation by *Eucalyptus camaldulensis*. Chemosphere 85: 584-590.

Yao H, Lu J, Yuan X, Wu J, Zhao J, Yu X, Zhou Y (2014). Concentrations, bioavailability, and spatial distribution of soil heavy metals in a long-term wastewater irrigation area in North China. CLEAN - Soil, Air, Water 42: 331-338.