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EDTA-INDUCED IMPROVEMENT IN GROWTH AND WATER RELATIONS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) PLANTS GROWN IN LEAD CONTAMINATED MEDIUM

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Abstract

A sand culture experiment was carried out to examine whether EDTA applied through the rooting medium could mitigate the injurious effects of Pb on growth and water relations in two sunflower hybrids (H-33 and H- 64A93). Eight Pb and EDTA levels i.e., 0, 1 and 2 m*M* $Pb(NO_3)_2$, 1 and 1.5 m*M* EDTA and the combinations of Pb and EDTA as 1 + 1, 1+ 1.5 and 2 + 1.5 [m*M* Pb $(NO_3)_2$ + EDTA, respectively] with three replications were maintained during the course of study. Water relations parameters i.e., leaf water potential (Ψ_W), solute potential (Ψ_S), turgor potential (Ψ_P) and relative water contents (RWC) were estimated after 40 days of exposure to Pb & EDTA level and thereafter plants were harvested and their shoot and root dry weights recorded. Applications of either Pb or EDTA decreased shoot and root dry mass of both sunflower hybrids. However, application of 1 + 1 m*M* Pb + EDTA was effective in maintaining higher shoot dry weight, but for the root dry weight, 1 + 1.5 m*M* Pb + EDTA treatment was more effective than the others. The sunflower hybrid H-33 performed better than H-64A93 under Pb, EDTA and Pb + EDTA treatments. Relative water contents (RWC); Ψ_W, Ψ_S, and Ψ_P decreased with increase in Pb or EDTA levels, however, addition of EDTA to the Pb contaminated medium was found to be beneficial in increasing the RWC and Ψ_P necessary for sustaining plant growth and productivity under normal and stress conditions. Sunflower hybrid H-33 maintained higher Ψ_P and RWC than those of H-64A93. Overall, addition of EDTA to Pb contaminated medium was found beneficial in improving the plant water relations and growth in the two sunflower hybrids used in this study.

Introduction

Like many other heavy metals, lead (Pb) is as a potent environmental pollutant. Lead toxicity has become very important due to its great concern for human health (Juberg *et al*., 1997; Liu *et al*., 2007; Rossi, 2008; Healey, 2009; Murata *et al*., 2009). The main source of Pb is automobile exhausts in urban areas which contribute substantially to the atmospheric pollution and plants growing near highways are affected more by the Pb pollution than in other localities. Sewage sludge enriched with Pb and other metals is regularly discharged on to the fields and garden soils due to increasing trends in urbanization (Paivoke, 2002; Pirzada *et al*., 2009). Excessive Pb exposure can cause mental retardation and behavioral disorder. Its exposure in human beings can occur through multiple pathways i.e., through inhalation of air, intake of water, soil or dust, as it is emitted in the environment from vehicles and automobiles. It can also enter the food chain via plants (Shafiq *et al.*, 2008). For plants, although Pb is not considered as an essential element, its absorption and accumulation in different parts takes place frequently and its accumulation increases with increase in exogenous Pb levels (Singh *et al*., 1998; Zhu *et al.*, 2007). Once entered in plant, it detrimentally influences plant growth resulting in reduced leaf area (Reddy *et al.*, 2005; Islam *et al.*, 2008) and it also inhibits activities of many enzymes (Javed & Saher, 1987; Verma & Dubey, 2003; Reddy *et al.*, 2005). Guard cells are generally smaller in size in plants treated with Pb. Lead lowers the level of compounds that are associated with maintenance of cell turgor and cell wall plasticity and thus lowers the water potential within the cell leading to stomatal closure (Bazzaz *et al*., 1974; Sharma & Dubey, 2005).

Phytoremediation, i.e., utilization of plants in order to remediate heavy metal contaminated soils, has received considerable attention (Raskin *et al*., 1994; McGrath *et al*., 2002; Mataka *et al.*, 2006; Haung *et al.*, 2008). In phytoremediation, phytoextraction seems to be the most promising technique and has attracted attention due to its low cost on implementation and environment-friendly behavior (Salt *et al*., 1995; McGrath *et al.*, 2002; Singer *et al.*, 2007). Two modes for the phytoextraction of metals are currently under use: use of hyperaccumulator plants having high metal accumulating capacity (Brown *et al*., 1994; Kumar *et al*., 1995; Singer *et al.*, 2007) and the utilization of high biomass producing plants with a chemically enhanced method of phytoextraction (Salt *et al*., 1995; Hernández-Allica *et al.*, 2008). The success of phytoextraction is based on biomass production, heavy metal concentration in plant tissues, and bioavailability of heavy metals in the rooting medium (McGrath, 1998; Hernández-Allica *et al.*, 2008). Ethylene diaminetetraacetic acid (EDTA) is often found to be the most effective chelating agent (Blaylock *et al*., 1997; Haung *et al.*, 2008), which considerably enhances the accumulation of metals in the above ground parts of plants because it develops a metal-chelate complex which enhances its mobility within the plant by increasing its transport from roots to aerial parts (Turgut *et al*., 2004; Zhuang *et al.*, 2007).

Sunflower is a high biomass producing and stress tolerant crop and it can accumulate significant amount of Pb when applied in combination with chelating agents such as EDTA (Huang & Cunningham, 1996; Blaylock *et al.*, 1997; Azhar *et al.*, 2006; Krystofova *et al.*, 2009). So, sunflower can be used for metal remediation (Navari-Izzo & Quartacci, 2001; Niu *et al.*, 2007). Thus, the present investigation was undertaken with a premier objective to appraise the influence of Pb, EDTA and Pb + EDTA on biomass production and water relation parameters of sunflower plants grown in sand culture.

Materials and Methods

A sand culture experiment was conducted in earthen pots measuring 27 cm in diameter and 24 cm depth. Fine and washed river sand was filled in 48 pots. To sow sunflower seeds in pots, five holes (about 2 cm deep at equal distances) were made in each pot. One seed was placed in each hole which was then covered with a small amount of wet sand. After one week, the plants were thinned to three uniform size plants.

After germination plants in pots were irrigated with 2 L of 0.5 strength Hoagland's nutrient solution at one week interval up to the completion of the experiment. Thirty day-old plants were subjected to eight different treatments of Pb, EDTA or both. Eight treatment were control, 1 m*M* Pb $(NO_3)_2$, 2 m*M* Pb $(NO_3)_2$, 1 m*M* EDTA, 1.5 m*M* EDTA, 1 m*M* Pb $(NO_3)_2$ + 1 m*M* EDTA, 1 m*M* Pb $(NO_3)_2$ + 1.5 m*M* EDTA, and 2 m*M* Pb $(NO_3)_2$ + 1.5 m*M* EDTA in distilled deionized water. Half strength Hoagland solution was applied on the following day of all treatment solutions applied. This was done to avoid the precipitation of Pb with sulfate and phosphate present in the nutrient solution. The experiment was set up in a completely randomized design with a factorial arrangement in three replicates.

Water relations parameters i.e., water, osmotic and turgor potentials as well as relative water contents in leaves were determined at the vegetative stage i.e., when the plants were 40 day-old. Leaf water potential (Ψ_W) of the third from the top of each plant from each treatment was determined using a pressure chamber (Scholander type) between 8.00 to 10.00 a.m. The same leaf which was used for water potential estimation was frozen at -20^{o}C for one week for osmotic potential (Ψ_S) determination. The leaf sap was extracted from the frozen leaf samples and the sap so extracted was directly used for the determination of osmotic potential using a vapor pressure osmometer (Wescor-5500). The difference between osmotic potential (Ψ_S) and water potential (Ψ_W) values represented leaf turgor potential.

For the determination of relative water contents (RWC), leaf samples were excised from the plants before dawn, their fresh mass (Fw) taken and placed in distilled water in the dark for 24 h for their re-hydration to take place. The following morning, leaf turgid weight (Tw) was recorded and then the samples were dried in an oven at 65 ^{o}C for 48 h and dry weight (Dw) determined. RWC was calculated as under:

$$RWC = [(Fw - Tw)/(Fw - Dw)] \times 100$$

After recording all these measurements, the plants were harvested. Plant roots, after removing carefully from the sand, were washed thoroughly in distilled water. After separating the plants into shoots and roots, their fresh weights were recorded and then placed in an oven at 65 ^{o}C for 72 h to record their dry weights.

Statistical analysis: Data of all variables were subjected to the analysis of variance technique using the STATISTICA computer program. The graphs were plotted using the Microsoft Excel program. The significant differences among the mean values were assessed using the least significance difference test (Steel *et al.*, 1997).

Results

Plant growth: Application of Pb, EDTA or in combination decreased shoot dry weight of both sunflower hybrids (Fig. 1). The data showed that both hybrids performed similarly under all the treatments of Pb and EDTA and showed a decreasing trend with increase in Pb, EDTA and Pb + EDTA concentrations in the solution except at 2 + 1.5 m*M* Pb + EDTA where the reverse was true for H-64A93. However, the combined treatment (1 + 1 m*M* Pb + EDTA) was found very effective in maintaining high shoot dry weight in H-33. Overall, H-33 showed higher shoot dry weight than that of H-64A93 in all treatments.

Lead and EDTA treatments differed significantly with regard to root dry weight (Fig. 2) of the two sunflower hybrids. It decreased with the application of Pb or EDTA alone, however, the addition of EDTA in the Pb contaminated medium significantly improved the root dry weight in both hybrids. A similar trend was observed in both hybrids except at 2 + 1.5 m*M* treatment in H-33 and at 1 + 1.5 m*M* in H-64A93. However, H-33 maintained higher root dry weight than H-64A93 at different levels of Pb and EDTA and the treatment level 1 + 1.5 m*M* Pb + EDTA in H-33 improved root dry weight more than that of other two levels.

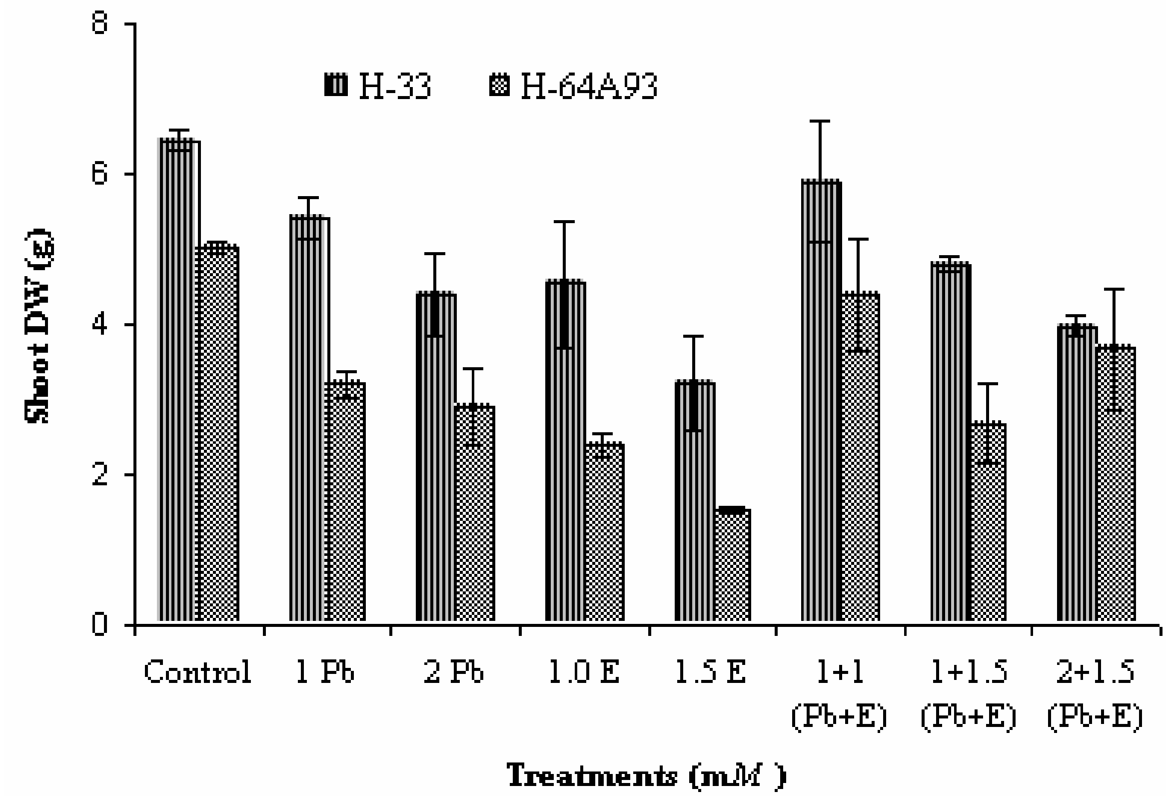

Fig. 1. Shoot dry weight of two sunflower hybrids as affected by Pb and EDTA treatments.
Note: Pb = Lead; E= EDTA; Pb + E = Lead + EDTA

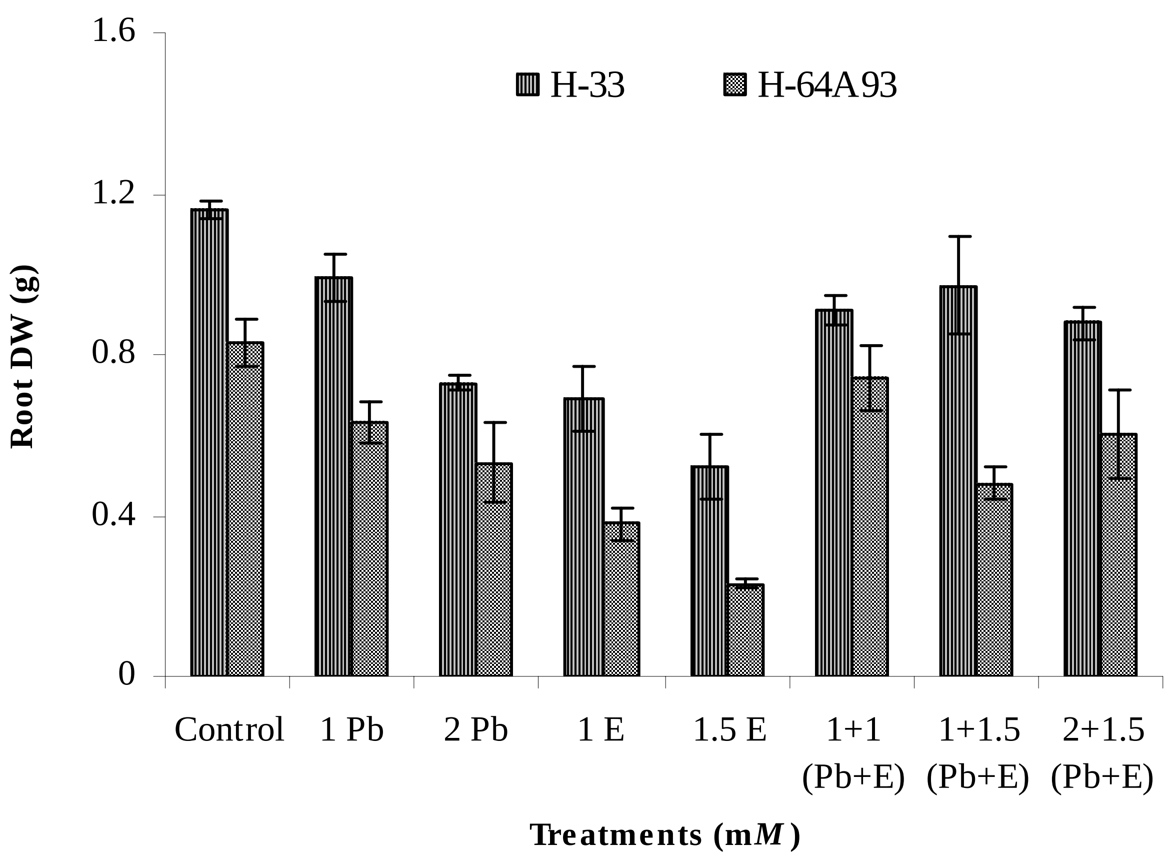

Fig. 2. Root dry weight of two sunflower hybrids as affected by Pb and EDTA treatments.
Note: Pb = Lead E= EDTA; Pb + E = Lead + EDTA

Water relations: It is inferred from Fig. 3 that Pb and EDTA treatments differed significantly in their effectiveness on relative water contents (RWC) which decreased with increase in Pb levels. Severe reduction in RWC was noted in case of Pb treatment in H-33, while the reverse was true in H-64A93. Application of EDTA alone had an increasing trend in RWC in H-33, while it was reverse in H-64A93. The treatments involving combination of Pb and EDTA maintained higher RWC and among those the levels 1 + 1 and 1 + 1.5 m*M* Pb + EDTA improved significantly the RWC in H-33.

Leaf water potential (Ψ_w) of sunflower hybrids was significantly affected by different Pb, EDTA or Pb + EDTA treatments (Fig. 4). A marked reduction in leaf water potential was observed with the application of Pb alone, but EDTA showed a slight reduction particularly at higher levels of EDTA. The combined application of Pb + EDTA enhanced Ψ_w to some extent. However, H-33 maintained higher Ψ_w than that in H-64A93 under all treatments except the higher three levels.

The effects of Pb, EDTA and Pb + EDTA on leaf osmotic potential (Ψ_s) in the two sunflower hybrids are summarized in Fig. 5. Lead and EDTA treatments differed significantly in their effects on Ψ_s and the osmotic potential decreased slightly with increase in Pb and EDTA levels. The same was true under the treatments involving combination of Pb + EDTA.

Leaf turgor potential (Ψ_P) in both sunflower hybrids was affected with Pb, EDTA and Pb + EDTA treatments (Fig. 6). Application of Pb in the growth medium adversely affected Ψ_P because turgor potential decreased with increase in treatment levels, while EDTA showed non-significant effect on this water relation parameter. However, H-33 maintained higher Ψ_P than that in H-64A93 in all the treatments except the highest treatment where the reverse was true.

Discussion

Presence of Pb in the growth medium adversely affected the plant growth by reducing biomass in both hybrids. It is possible that due to the uptake of Pb, plant metabolism might have been severely affected which ultimately led to reduce plant growth as a whole (Greeman *et al*., 2001). It is now well evident that the photosynthetic activities, stomatal conductance, transpiration and enzymatic activities etc. all are hindered by the excessive uptake of Pb (Azhar *et al.*, 2006; Islam *et al.*, 2008; Farooqi *et al.*, 2009). Obviously, reduced production of photosynthates in plants leads to reduced biomass production and hence reduced growth (Kalita & Sharma, 1995; Sanchez *et al*., 1999; Liu *et al*., 2000). In the present study, maximum decline in shoot and root biomass was found at the highest level of Pb, but it was lower than 50% (Figs. 1 & 2). This showed that sunflower has a genetic potential to tolerate heavy metal toxicity which could be enhanced by the application of EDTA (Ruley *et al*., 2006). Addition of EDTA to the growth medium was found to be effective in lessening the adverse effects of Pb on growth. This might have been the reason why the plants treated with Pb + EDTA maintained higher biomass than the plants grown under either Pb or EDTA separately. It has been reported that Pb present in the growth substrate is injurious for plant growth (Manju *et al*., 2000; Koul, 2001; Kabir *et al*., 2008; Rehman & Iqbal, 2008) and addition of EDTA to the Pb containing medium is effective in promoting plant growth. However, hybrid H-33 was better than Hybrid 64A93, because the former was superior to the latter in maintaining root growth considerably high. This may have been one of the strategies of this hybrid to tolerate the Pb toxicity.

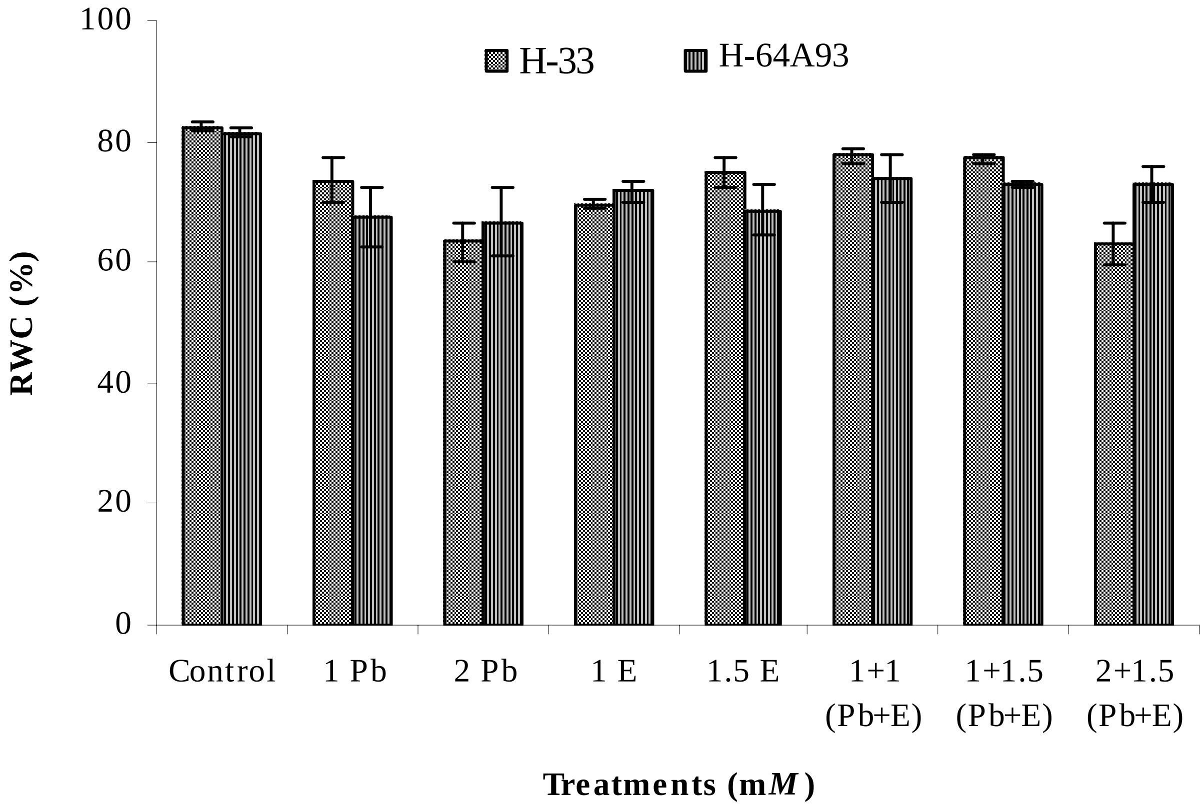

Fig. 3. Relative water contents of two sunflower hybrids as affected by Pb and EDTA treatments.
Note: Pb = Lead; E= EDTA; Pb+E = Lead+EDTA

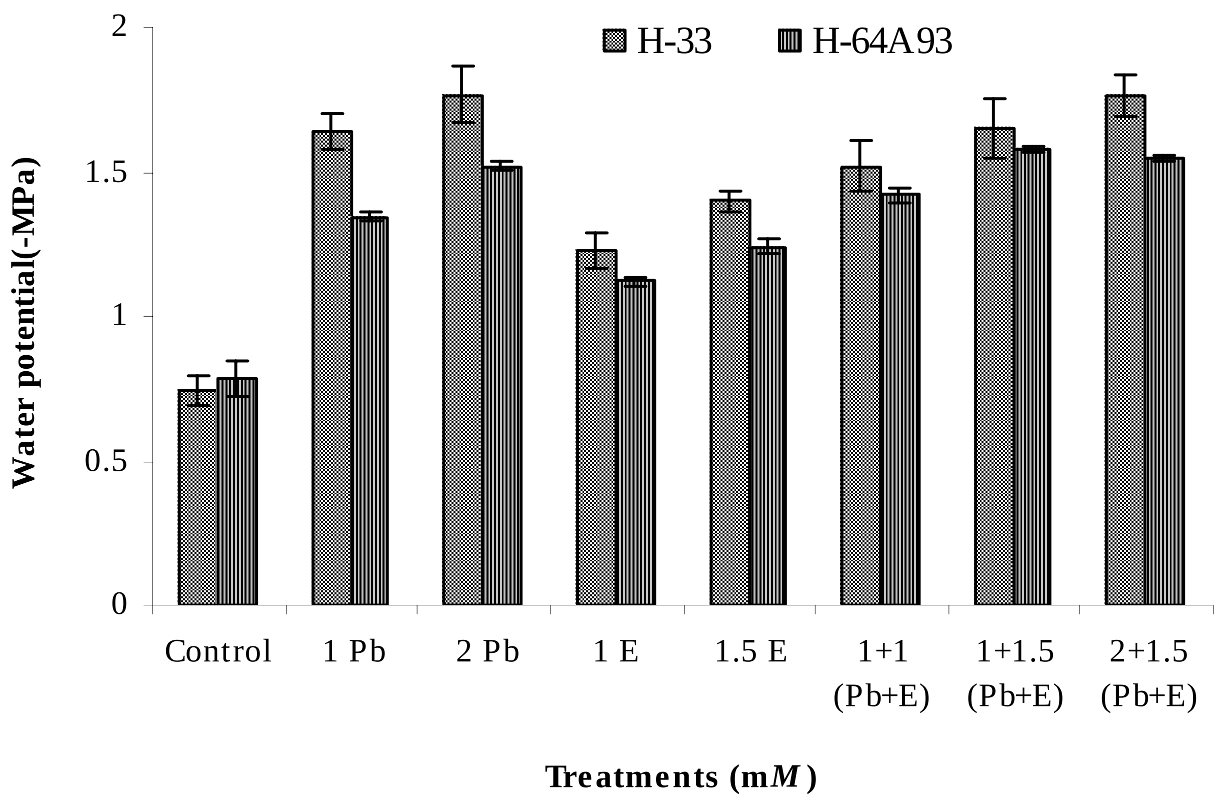

Fig. 4. Leaf water potential of two sunflower hybrids as affected by Pb and EDTA treatments.
Note: Pb = Lead ; E= EDTA ; Pb + E = Lead + EDTA

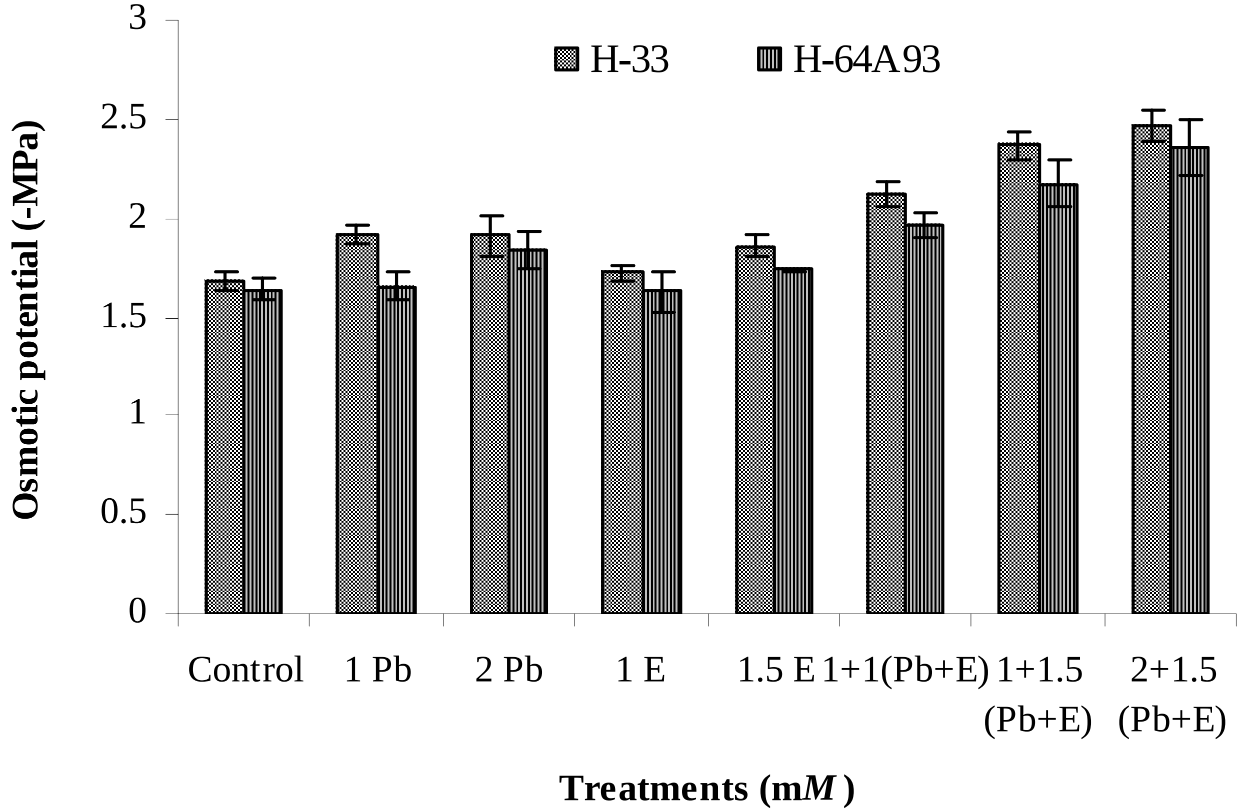

Fig. 5. Leaf osmotic potential of two sunflower hybrids as affected by Pb and EDTA treatments.
Note: Pb = Lead ; E= EDTA ; Pb+E = Lead+EDTA

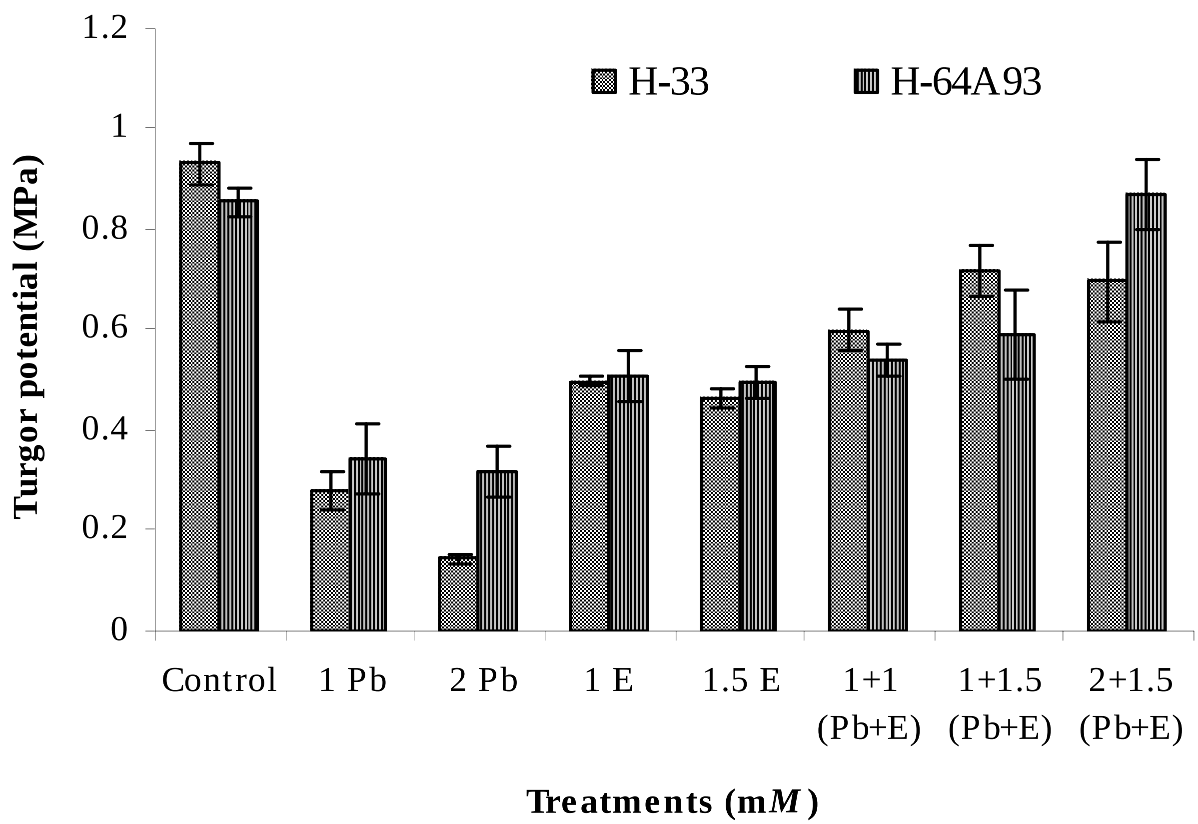

Fig. 6. Leaf turgor potential of two sunflower hybrids as affected by Pb and EDTA treatments.
Note: Pb = Lead ; E= EDTA ; Pb + E = Lead + EDTA

It is evident from present study that contamination of either Pb or EDTA in the growth medium reduced RWC, Ψ_w, Ψ_S and Ψ_P and both hybrids showed reduction in all these parameters. However, addition of EDTA to the growth medium containing Pb increased RWC and Ψ_P in sunflower plants and helped the plants to osmotically adjust themselves by reducing Ψ_W and Ψ_s. The present studies also indicated that Pb contamination had similar effect to that of salt stress on water relations, because all water relation parameters were reduced significantly due to metal stress, quite analogous to what has been reported in case of salt stress (Ashraf, 2004). The reduction in osmotic potential may be due to the accumulation of salts/osmolytes or osmoprotectants, which are beneficial in adjusting the plants to environmental conditions and in alleviating the adverse effects of heavy metals or other stresses (Kamenova-Yuchimenko *et al.*, 1995; Ashraf & Foolad, 2007). In this study, Pb tolerant hybrid (H-33) had higher RWC and turgor potential and lower Ψ_W and $\Psi_{S,}$ which might have effectively maintained the photosynthetic rate to produce high biomass under metal stress.

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