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Technical Note

The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA_3 and IAA) and EDTA alone and in combinations

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ABSTRACT

This investigation was made to examine the role of gibberellic acid (GA_3), indole-3-acetic acid (IAA) and EDTA in improving phytoextraction of the Pb and plant growth on Pb added soil. GA_3, IAA and EDTA were applied separately and in combinations. GA_3 and IAA were applied as foliar spray and seed soaking. EDTA was applied in single and split doses. Analysis of the Pb in different parts of plant was carried out using atomic absorption/flame spectrophotometer. EDTA significantly reduced the plant growth and dry biomass, whereas GA_3 and IAA foliar spray increased it significantly when compared with control (only Pb added soil). In combined treatments of EDTA + GA_3 and EDTA + IAA, the growth and biomass was restored, which shows that GA_3 and IAA did compensate the negative effect of EDTA on plant growth. The separate treatments of EDTA, GA_3 and IAA increased the Pb uptake and translocation significantly moreover in combine treatments, synergistic effect was found and remarkable increase in Pb uptake and translocation into shoot was observed. EDTA increased the Pb uptake but declined the biomass; subsequently the total Pb accumulation was decreased in plant. The maximum total Pb was found in combined treatment of EDTA + GA_3. These findings suggest more investigation to find a combination of GA_3 with a very low concentration of EDTA, as in high concentration it causes soil and ground water pollution.

1. Introduction

Metal contaminated soil is particularly challenging to restore. Pb is one of the heavy metals of concern. Its contamination originates mainly from smelting and mining processes, agriculture (pesticides), industrial wastes and urban activities (paint and additives in petroleum) (Marchiol et al., 2004). Lead not only affects plant growth and productivity but also enters into the food chain causing health hazards to man and animals (Wierzbicka and Antosiewicz, 1993). Unlike organic compounds, metals cannot be degraded, and soil clean up usually requires their removal (Lasat, 2002). Most of the conventional methods include excavation and land fill, thermal treatment, acid leaching, and electro-reclamation. However, because of the low efficiency, high cost, and large destruction of soil fertility and structure, these methods are ineffective in agricultural settings (Jing et al., 2007). Various biological processes are being used to decontaminate the soil. One effective and promising process is phytoremediation, which proposes the use of plants to extract, sequester and detoxify pollutants (Jing et al., 2007). Phytoremediation comprises of phytoextraction, phytovolatilization, rhizofiltration and phytostabilization (Chaney et al., 1997).

The advantages of phytoremediation include low cost, preservation of natural soil properties, and reliance on solar energy to power the remediation (Zhuang et al., 2007). The success of phytoremediation depends on the bioavailability of the metal and the ability of the plant to accumulate the metals. Plants with high metal accumulating capacity often have a slow growth rate and produce limited amounts of biomass on metals contaminated soil (Denton, 2007). Plant growth regulators like auxins and gibberellins are organic substances that regulate intracellular processes to improve plant growth. The major problem in metal phytoextraction efficiency is the metal immobilization in soil. Chemical treatments have been used to overcome this problem and the most promising application of this technology is for the remediation of Pb-contaminated soils using EDTA (Blaylock, 2000). Plant growth regulators increase the plant growth and biomass (Tassi et al., 2008), and EDTA enhances the Pb bioavailability in the soil but the higher mobilization of metal due to EDTA application reduces the plant growth which ultimately decreases the phytoextraction efficiency of the plant (Bruno-Fernando et al., 2007). Therefore, this

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study was conducted with objective to investigate the plant growth regulators (gibberellic acid GA_3 and indole-3-acetic acid IAA) and EDTA either alone or in combinations (synergism) to find out their role in: (1) improving the growth and biomass of the plant on Pb contaminated soil; (2) increasing the Pb accumulation in the plant; and (3) improving the phytoextraction.

2. Materials and methods

2.1. Soil and seeds preparation for pot experiments

Soil was prepared by mixing clay, sand and humus in the ratio of 2:1:1 respectively, and dried in the sun light. The dry soil was artificially polluted by lead nitrate. 800 mg $Pb(NO_3)_2$ (Sigma Aldrich, St. Louis, MO) kg^{-1} soil were added and mixed well for 3 d. Each plastic pot (18 cm × 24 cm) was filled with 2.5 kg soil (soil pH 6.2 ± 0.3 $n = 5$) and watered before 24 h of sowing the seeds. Ten seeds of maize (*Zea mays* L. *cv*. Pioneer-33556, from National Agriculture Research Centre Islamabad, Pakistan) in each pot were sown. Three replicate pots were used for each treatment, arranged in a completely random design. After germination three healthy seedlings were selected in each pot and the remaining were eradicated. Plants were grown in a green house under natural light conditions with day/night temperatures of 30/15 °C. Pots were watered twice a week, each time single pot received 500 mL water (tap water) according to water holding capacity of soil (sufficient to saturate the soil without leaching). No additional fertilizers were applied to the soils during experiment. Plants were harvested after 60 d treatments, washed with tape water and separated into roots, stems and leaves. The roots were further washed with a solution containing 5 mM Tris HCl pH 6.0 and 5 mM EDTA, and then rinsed with distilled water in order to remove surface bound metal ions (Genrich et al., 2000). Plants were grown in the greenhouse of Quaid-i-Azam University, Islamabad, Pakistan.

2.2. Treatments done during pot experiments

The following treatments were made during experiment. EDTA split doses + Pb (T1), EDTA single dose + Pb (T2), GA_3 foliar spray + Pb (T3), IAA foliar spray + Pb (T4), GA_3 seed soaking + Pb (T5), IAA seed soaking + Pb (T6), GA_3 foliar spray + EDTA + Pb (T7), IAA foliar spray + EDTA + Pb (T8), Control without Pb (C) and Control with Pb (C1). A control without Pb (C) was compared with C1 for Pb effect on the plant growth and biomass. Whilst C1 was compared with all treatments for Pb phytoextraction (+Pb = 800 mg $Pb(NO_3)_2$ kg^{-1} soil).

2.2.1. Exogenous application of gibberellic acid (GA_3) and indole-3-acetic acid (IAA)

Stock solutions (0.1 M) each of IAA and GA_3 (Sigma Aldrich) were prepared by dissolving required amount of each IAA and GA_3 in 100 µL of 95% EtOH and the required volume was raised with distilled water. Subsequent dilution was done to prepare 10^{-6} M aqueous solutions of each IAA and GA_3 from stock solutions. 3% (v/v) of non-ionic surfactant Agral (Syngenta) was added to final volume of each IAA and GA_3 solutions. Two modes of applications were used for each of IAA and GA_3, seeds dip and foliar spray. Seeds were dipped for 12 h prior to sowing, whilst foliar spraying involved three sprays at intervals of 15 d with the first spray 15 d after sowing.

2.2.2. Ethylenediaminetetraacetic acid (EDTA) addition into soil

Aqueous solutions of EDTA (Sigma Aldrich) were applied in two ways, single dose and split doses. In single dose treatment, 400 mg kg^{-1} soil of EDTA was applied once whilst in split doses, total of 400 mg kg^{-1} soil was applied in four split doses each dose of 100 mg kg^{-1} soil on weakly bases. Single dose and first split dose was given after 25 d of sowing and then split doses were consecutively given after 32, 39 and 46 d. Aqueous solutions of EDTA were made according to the water holding capacity of soil.

2.2.3. Combined additions of EDTA and IAA or GA3

Each IAA and GA_3 at concentration of 10^{-6} M solution were applied as foliar spray at 15 d interval in combination with EDTA applied in four split doses on weekly bases, each dose consisted of 100 mg kg^{-1} soil.

2.3. Determination of plant height, root length and dry biomass

Plant height and root length were measured with a centimeter rule (Model: DK-436, Danking Enterprise Ltd., Taiwan) from the root and shoot joint to the apices. Different parts (roots, stems and leaves) of plants were dried in oven at 80 °C for 48 h and then dry biomass of roots and shoot (stem + leaves) was noted.

2.4. Analysis of Pb in plant tissues

The oven dried samples of roots, stems and leaves were ground into a fine powder using a commercial blender and stored in polyethylene bags of small size until use for acid digestion. Perchloric-acid digestion method was used for acid digestion (Allen, 1974). All replicate plants were analyzed in the same way and from one replicate plant each 0.25 g powder of roots, stem and leaves were taken in 50 mL flask and 6.5 mL of mixed acid solution i.e. nitric acid, sulfuric acid and perchloric acid (Sigma Aldrich) in the ratio of 5:1:0.5 was added and digested on an electric hot plats until the white fumes came out from the flasks. Thereafter the digested samples were transferred into 50 mL volumetric flasks and the volume was made up to 50 mL with distilled water. The samples were filtered and then filtrates were analyzed for Pb contents in the roots, stem and leaves by shimadzu AA-670 atomic absorption/flame spectrophotometer (Kyoto, Japan). Working standard solutions of Pb were prepared from the stock standard solutions containing 1000 mg kg^{-1} of element in 2 N nitric acid. The total Pb accumulation in entire plant was calculated as Pb in plant (µg g^{-1} DW) × biomass of entire plant (g DW).

2.5. Statistical analysis

The mean value of the three plants in each pot was measured first, and then the mean of each three replicate pots was analyzed (total of nine plants were used for each treatment, mean of mean was analyzed). Data were evaluated statistically by ANOVA and comparison of mean values was done by using Tukey's honestly significant difference test at $p \leqslant 0.05$. The Minitab15 Statistical Software was used for the analysis (Minitab Inc., USA).

3. Results and discussion

3.1. Effect of Pb on the germination rate

The Pb did not decrease the germination rate of maize seeds and the young seedlings did not exhibit any visible toxic symptoms. Lopez et al. (2009) reported that seed germination rate of alfalfa was not reduced by Pb. Similarly, Chen et al. (2004) examined seeds from 10 different plants species and reported germination without any toxic effect of Pb at concentration of 860 mg kg^{-1}soil. In the present study, 800 mg $Pb(NO_3)_2$ kg^{-1} soil was used which is equivalent to 500 mg Pb kg^{-1} soil.

3.2. Effect of treatments on the root length and the plant height

The root length and plant height under different treatments is presented in Fig. 1a and b. The Pb significantly reduced the root length (Figs. 1a and 2) and plant height (Fig. 1b) when control C (without Pb) was compared with C1 (with Pb). Similar reduction in root size by Pb has been reported by Lopez et al. (2009). C1 (Pb only) was compared with all other treatments to find the effect of the treatments on root length (Fig. 1a) and plant height (Fig. 1b) on Pb added soil. The effect of the foliar spray of GA_3 (T3) and IAA (T4) was similar and showed significant increase in root length and plant height when compared to C1. Seed soaking treatments of GA_3 or IAA (T5 and T6) showed similar results like that of C1. EDTA treatments (T1 and T2) reduced the root length and plant height significantly when compared with C1. Whilst EDTA in combination with foliar spray of GA_3 (T7) and IAA (T8) showed significant increase in plant height (Fig. 1b) but the root length was not increased when compared with C1 (Fig. 1a). These findings indicate that EDTA reduces and GA_3 and IAA increase the root length and plant height on Pb contaminated soil. Mohd and Shivendra (2008) reported significant reduction in growth with Pb + EDTA. The growth promoting hormones increase the absorptive area of roots and hence increase the uptake of water and nutrients to improve plant growth (Glinka, 1980).

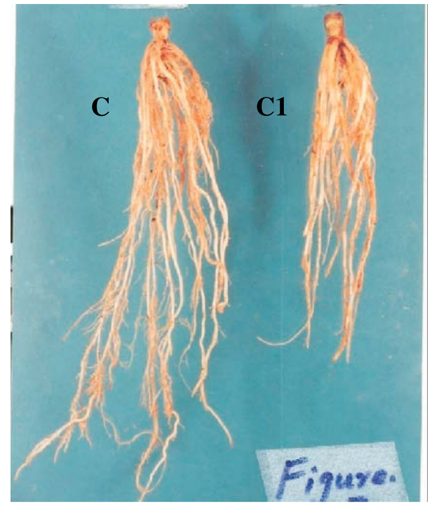

Fig. 2. Effect of the Pb on root length. C (control without Pb), C1 (only Pb added soil).

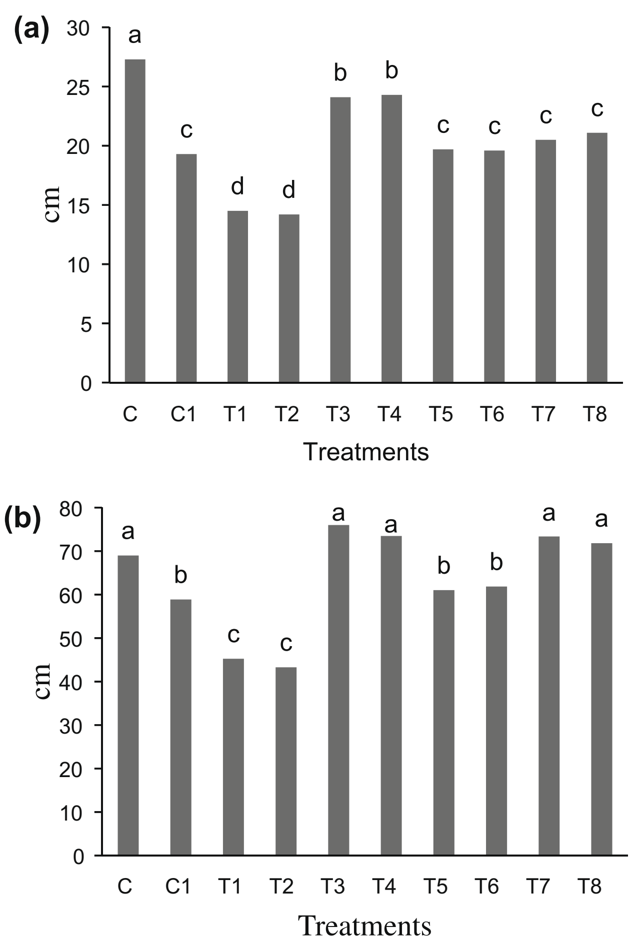

Fig. 1. Effect of different treatments on plant growth. (a) Root length, (b) plant height. Different letters indicate significantly different values (Tukey's honestly significant difference test at $p \leqslant 0.05$). Control without Pb (C), with Pb only (C1), EDTA split doses + Pb (T1), EDTA single dose + Pb (T2), GA3 (FS) + Pb (T3), and IAA (FS) + Pb (T4), GA3 (SS) + Pb (T5), IAA (SS) + Pb (T6), GA3 (FS) + EDTA + Pb (T7), and IAA (FS) + EDTA + Pb (T8). FS (foliar spray), and SS (seed soaking).

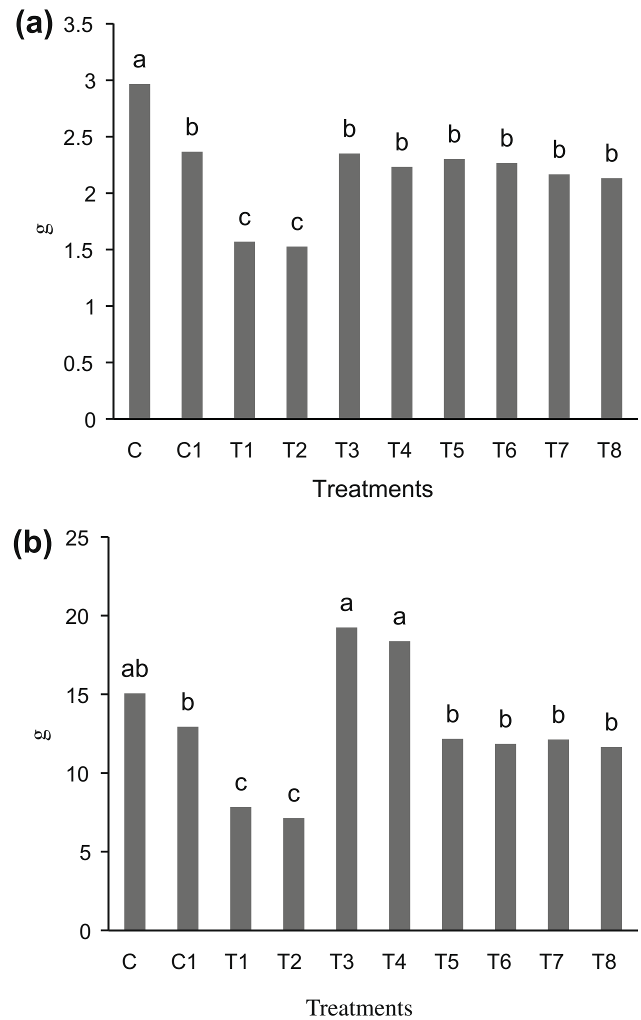

Fig. 3. Effect of different treatments on dry biomass. (a) Root biomass, (b) shoot biomass. Different letters indicate significantly different values (Tukey's honestly significant difference test at $p \leqslant 0.05$).

3.3. Effect of treatments on the dry biomass of roots and shoots

Data of root and shoot dry biomass are presented in Fig. 3a and b. The Pb significantly reduced the root biomass; the shoot biomass was reduced but statistically not significant when C (without Pb) was compared with C1 (with Pb) in Fig. 3a and b respectively. Heavy metals generally decrease plant dry matter (John et al., 2009). The EDTA treatments (T1 and T2) significantly reduced the root (Fig. 3a) as well as the shoot (Fig. 3b) dry biomass when compared with C1. All of the other treatments of GA_3 and IAA either alone or in combination with EDTA, showed similar root biomass and no difference was found when compared with C1 (Fig. 3a). In Fig. 3b, the response of the GA_3 and IAA foliar spray (T3 and T4) is similar and showed the significant increase in shoot dry biomass when compared with C1. These findings reveal that EDTA decreases the root and shoot dry biomass of the plant grown on Pb added soil, while GA_3 and IAA increase the biomass of plant even if applied in combination with EDTA (T7 and T8). These negative effects of EDTA might be possibly due to increase in high mobility of metals in soil solution (Lou et al., 2007; Epelde et al., 2008). Increase in plant biomass by GA_3 and IAA might be due to stimulation of cell division, shoot initiation and enhancement of plant resistance to metal stress by plant growth regulators (Tassi et al., 2008).

3.4. The role of different treatments in Pb uptake and translocation

The lead concentration ($\mu g\ g^{-1}$ dry weight) in different parts of plant is shown in Fig. 4a–c. EDTA either in split doses (T1) or in single dose (T2) increased Pb uptake significantly in roots and its translocation into stem and leaves, Similarly, the foliar spray of GA_3 (T3) and IAA (T4) also showed significant increase in Pb uptake in roots and its translocation into stem and leaves when compared to C1 in Fig. 4a, b and c, respectively. GA_3 or IAA in combination with EDTA (T7 and T8) produced a synergistic effect on Pb uptake and translocation, and showed significant increase of Pb in root (Fig. 4a), stem (Fig. 4b) and leaves (Fig. 4c) when compared with C1. The combination of either GA_3 + EDTA (T7) or IAA + EDTA (T8) showed significant effect on translocation of Pb into leaves and the translocation ratio was about 400 times higher when compared with C1 (Fig. 4c). GA_3 + EDTA (T7) was superior to IAA + EDTA (T8) in translocation into stem or leaves, whilst T8 (IAA + EDTA) was better than T7 (GA_3 + EDTA) in accumulation of Pb in roots. It shows that GA_3 is superior to IAA for Pb translocation into shoot. Wang et al. (2007) reported significant increase in Pb content in maize roots when applied with exogenous IAA. The observed increase in the Pb accumulation in the stems and leaves could be related to the increase in the transpiration ratio by the exogenous plant growth regulators, which subsequently could translocate more nutrients and contaminants (Zhou et al., 2007; Tassi et al., 2008). The GA_3 and IAA seed soaking treatments (T5 and T6) showed no effect on Pb uptake or translocation. The split doses treatment of EDTA (T1) was found better than EDTA in single dose (T2) for Pb uptake and translocation.

3.5. Total Pb accumulation in entire plant and its percent distribution in roots vs. shoot

Fig. 5a shows the total Pb accumulation by the whole plant and its percent distribution in root/shoot is shown in Fig. 5b. EDTA either in split dose (T1) or in single dose (T2) increased the total Pb significantly when compared with C1 (Pb only), but T1 (split dose) was superior to T2 (single dose) and significant difference was observed between these two treatments (Fig. 5a). These findings confirms the findings of Reinhard et al. (2008) who found, the split doses of EDTA better than in single dose for Pb accumulation.

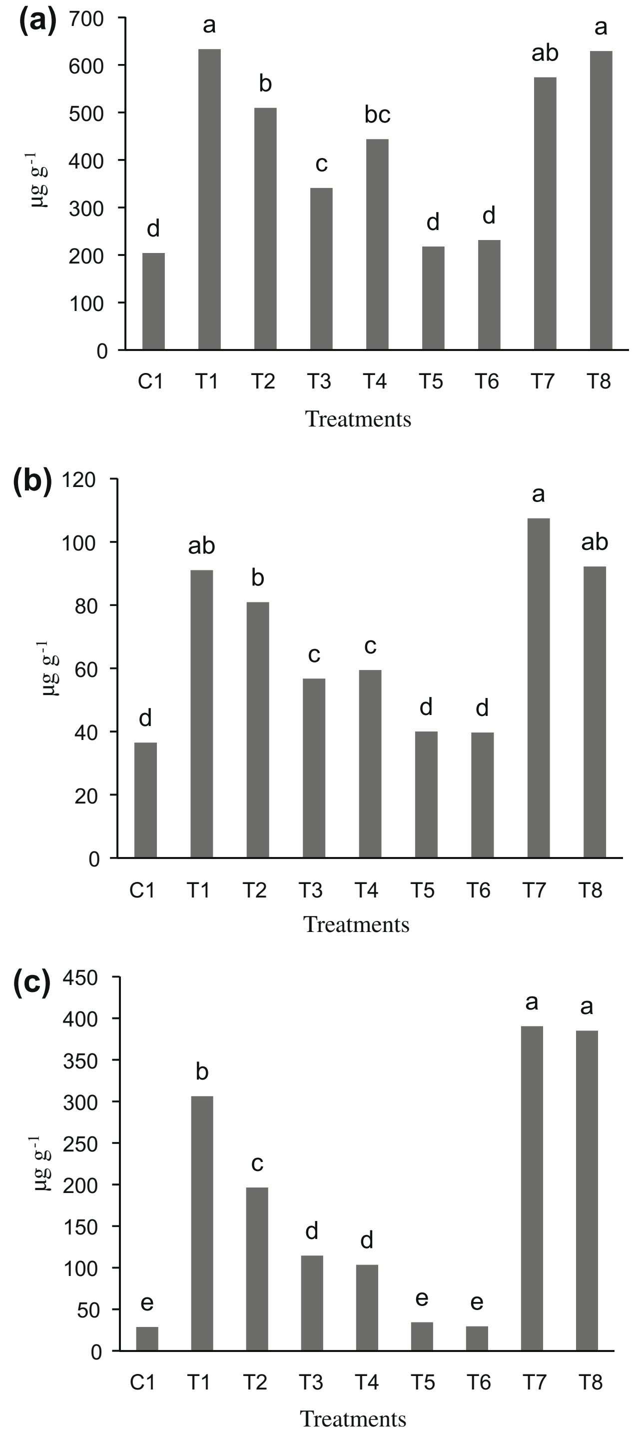

Fig. 4. Lead (Pb) accumulation in plant. (a) Pb in roots, (b) Pb in stem, (c) Pb in leaves. Different letters indicate significantly different values (Tukey's honestly significant difference test at $p \leqslant 0.05$). Control without Pb (C), with Pb only (C1), EDTA split doses + Pb (T1), EDTA single dose + Pb (T2), GA3 (FS) + Pb (T3), IAA (FS) + Pb (T4), GA3 (SS) + Pb (T5), IAA (SS) + Pb (T6), GA3 (FS) + EDTA + Pb (T7), and IAA (FS) + EDTA + Pb (T8). FS (foliar spray), and SS (seed soaking).

The foliar spray of GA_3 (T3) and IAA (T4) increased the total Pb accumulation in plant significantly when compared with C1 (Fig. 5b). The increase in total Pb accumulation by T3 and T4 was found higher than that shown by T2 (EDTA in single dose, high concentration). This reduction in total Pb accumulation by EDTA (T2) might be due to the reduction in biomass of the plant as shown in Fig. 3a and b and similarly the increase in total Pb accumulation

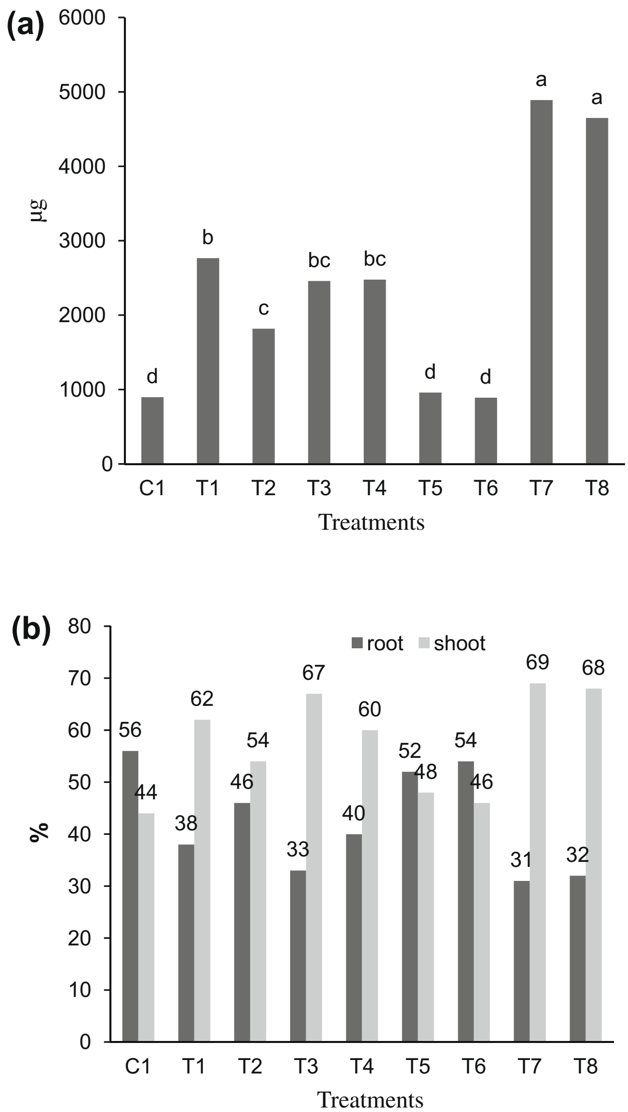

Fig. 5. Accumulation and distribution of Pb in entire plant. (a) Total Pb accumulation in entire plant, (b) percent (%) distribution of total Pb in roots vs. shoot. Different letters indicate significantly different values (Tukey's honestly significant difference test at $p \leqslant 0.05$).

by T3 (GA_3 foliar spray) and T4 (IAA foliar spray) might be due to the higher biomass. EDTA enhances Pb translocation into shoots, but reduces growth rate and biomass (Bruno-Fernando et al., 2007; Epelde et al., 2008; Mohd and Shivendra, 2008). The observed increase in Pb accumulation by GA_3 and IAA treatments could be related to the increase in transpiration rate by the exogenous plant growth regulators, which could subsequently translocate more nutrients and contaminants (Zhou et al., 2007; Tassi et al., 2008).

The EDTA in combinations with GA_3 (T7) and IAA (T8) showed highly significant increase in total Pb accumulation by plant (Fig. 5a) along with higher percentage of translocation into shoot as shown in Fig. 5b. The EDTA facilitates the metals uptake and translocation (Wang et al., 2007). The plant growth regulators enhance the growth rate, biomass (Tassi et al., 2008) and transpiration rate (Wu et al., 1999) and also could enhance metal stress resistance in plant (Mohd and Shivendra, 2008). These factors in combination (synergism) might be involved in the amazingly improved phytoextraction of Pb by T7 and T8.

4. Conclusions

These finding showed that increase in Pb accumulation was associated with as increase in dry biomass by foliar spray of GA_3 and IAA treatments. Translocation ratio of the Pb into shoot was increased by EDTA but dry biomass was declined, subsequently reduced the total Pb phytoaccumulation. The EDTA in combination with GA_3 or IAA dramatically increased the Pb accumulation in plant. The GA_3 role was found better than IAA in Pb translocation into shoot. These findings encourage the use of GA_3 along with lower concentration of EDTA rather than EDTA in high concentration. Here is suggested more investigation to find an optimum combination of GA_3 along with a very low concentration of EDTA, which in high concentration causes severe soil and ground water pollution.

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