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# Comparison of Lead Uptake by Four Seedling Species (*Acer cappadocicum*, *Fraxinus excelsior*, *Thuja orientalis* and *Cupressus arizonica*)

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**ABSTRACT** The phytoremediation capability in Pb removal from the contaminated soils by three native seedlings species (*Acer cappadocicum*, *Fraxinus excelsior* and *Thuja orientalis*) and one exotic species (*Cupressus arizonica*) were compared. The seedlings were grown in Pb contaminated soils at 0, 100, 200, 300, 400 and 500 mg kg$^{-1}$ concentrations for 6 months (Mar 21 to Sept. 22, 2015), after which the biomass allocation and Pb accumulation in tissues of root, stem, and leaf were assessed. The results showed that the higher Pb levels (400 and 500 mg kg$^{-1}$ soil) caused significant reduction in growth in all species, but this inhibition was less marked in the two conifer (*T. orientalis* and *C. arizonica*) compared to the two broad-leaf seedlings (*A. cappadocicum* and *F. excelsior*). Pb concentration in different tissues of seedlings increased with its increase in the soil. Further, Pb accumulation in the conifers was twice higher than that of the broad-leave species. Therefore, this study suggests that the two conifer species (*P. orientalis* and *C. arizonica*) can be used for phytoremediation, although further research is needed to make a final decision.

**Key words:** *Biomass, Growth, Lead, Phytoremediation, Seedling*



## 1 INTRODUCTION

Heavy metal pollution, associated with increasing population, expanding industry and urbanization, is one of the main environmental problems in the world and can be extremely dangerous to human health (Gołda and Korzeniowska, 2016). Accumulation of heavy metals in the soil is a serious threat because their toxicity effects on plant biology and soil fauna and may have a high capacity of entering animals and human through the food chain (Pinto *et al*., 2004). Therefore, comprehensive research is necessary for finding the most effective methods for remove or decreasing soil contamination. One of the most effective and economically convenient approaches in removing or decreasing the heavy metal contamination from soil is phytoremediation (Aba-Alkhil and Moftah, 2013), in which plants extract the heavy metals from soil and accumulate them in their organisms.

Being one of the most abundant and widely distributed toxic elements in soil, lead (Pb) adversely affects both terrestrial and aquatic

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ecosystems. Mining, paint residues and various other sources are the main reasons for lead

environmental contamination (Sharma and Dubey, 2005; Yang *et al*., 2016). Pb is not essential for plant metabolism but easily taken up from the soil and its high level can cause serious harm to plant biology mainly by inactivating enzymes and causing physiological injury (Rascio and Navari-Izzo, 2011; Santana *et al*., 2012).

Although some herbaceous plants have been found to be good accumulator of heavy metals and quite suitable for phytoremediation (Dickinson et al., 2009; Lai and Chen, 2009), woody plants have some advantages over herbaceous plants, including deeper roots to reach deeper polluted layers (Komives and Gullner, 2006). Besides, tree roots are reportedly able to reach a rhizosphere zone, which is more amenable to microbial degradation of the contaminants (Al-Surrayal, 2009). Generally, tree species, especially whole family of Salicaceae are considered potential for phytoremediation (Dickinson et al., 2009), while other tolerant species can also be found and used for phytoremediation (Doumentet *al*., 2008).

In Iran, mining operations, increase in industrialization and associated activities have led to soil contamination with Pb (Rahmani *et al*., 2001; Abbaspour*et al*., 2005). Therefore, identification of tolerant tree species among native and exotic species is very important for lead remediation of polluted soils. Several works have been conducted in this regard (e.g. Azampour et al., 2013; Etemadi et al., 2013; Salehi et al., 2014; Mansouri et al. 2015).

The aim of the study was to compare the tolerance of seedling of *Acer cappadocicum*, *Fraxinus excelsior* and *Thuja orientalis* as native species and *Cupressus arizonica* as an exotic species to soil contamination with Pb and the ability of these species to extract Pb, when thinking about their potentiality for phytoremediation.

## 2 MATERIALS AND METHODS

### 2.1 Nursery

Pot experiment was conducted in a nursery located in west of Mazandaran province, approximately 4 km from Nashtarood (36° 42' 42" N and 51° 02' 36.9" E), elevation from sea level: 0). Mean annual rainfall of the area is 1233.3 mm and the lowest and highest mean annual temperature of the region is 30.08 and 8.18 C$^{o}$, respectively.

### 2.2 Plant Material

A total number of 240, one-year-old seedlings of *A. cappadocicum*, *F. excelsior*, *T. orientalis* and *C. arizonica* were planted in individual pots. The pots were filled with the 3-kg of soil, some characteristics of which is provided in Table 1.

**Table 1** Some characteristics of the used soil in the pots

| Soil characteristics | Values |
|---|---|
| Texture | Sand-clay-loam |
| pH | 7.4 |
| Moisture (%) | 39.5 |
| Pb content (mg kg$^{-1}$) | 12.7 |

### 2.3 Lead contamination treatments

At the beginning of experiment, the soil was contaminated with lead in the form of $Pb(NO_3)_2$ at six concentrations of 0, 100, 200, 300, 400 and 500 mg kg$^{-1}$. The pots were labeled to distinguish between treatments and then arranged randomly to eliminate possible bias due to the location within the nursery. The treated seedlings grew under natural environment of the nursery in a six months growing season (from March 21 to September 22, 2015). The experiment was conducted with 10 replicates for each Pb treatment, therefore a total of 60 seedlings were used per species.

**2.4 Growth and biomass measurement**

To estimate seedling growth, collar diameter and height of all the seedlings were recorded with digital caliper and ruler respectively and re-measured at the end of the growing season. At end of the experiment, all seedlings were wholly harvested and separated to leaves, stem and roots. To obtain dry weight of each part, plant organs were oven dried at 70 C$^o$ till constant weight (Bissonnette *et al*., 2010). Finally, dry weight of each seedling was recorded using a digital balance.

**2.5 Measurement of lead in root, stem and leaf**

Lead analysis in plant tissues was performed with atomic absorption thermo electron (ICE 3000 Series AAS). For the sample preparation, 0.5 g of the dried ground plant tissue was digested by 3.5 ml of $H_2SO_4$ converted into ashes in a furnace for 30 minutes at 250 C°. Finally, the ash was dissolved in HCl and lead concentrations were determined (Williams, 1972).

**2.6 Statistical analysis**

The experiment was arranged in a completely randomized design and data of each species was analyzed by one- way ANOVA. All data were statistically analyzed using SPSS 16. Differences among treatments were tested with Duncan at 5% level of significance.

**3 RESULTS**

**3.1 Growth and dry biomass**

**3.1.1 *A. Cappadocicum***

Height and diameter growth of *A. cappadocicum* significantly decreased with increase in Pb concentration (Figure 1, A-B). Dried biomass of leaf and stem significantly decreased with increasing soil Pb concentration, while root dry biomass did not change in response to the treatments. Total seedling biomass gradually declined with increasing soil lead concentration (Figure 1, C-F).

**3.1.2 *F. excelsior***

Height and diameter of *F. excelsior* significantly declined in response to Pb pollution (Figure 2, A-B). The results indicated that *F. excelsior* grown on Pb contaminated soils produced less leaf and stem dry biomass while root biomass was stable in response to different concentrations of Pb, except 400 mg kg$^{-1}$. Total seedling biomass of the species did not change until 300 mg kg$^{-1}$ and then significantly decreased (Figure 2, C-F).

**3.1.3 *T. orientalis***

Generally, Pb contamination led to decreased height and diameter of the species (Figure 3, A-B). Pb contamination also exhibited inhibitory effect on leaf biomass of *T. orientalis* seedlings, while its effect of it on the stem and root biomass was not significant. On the other hand, total dry biomass of the species showed no significant change in response to Pb presence in soil (Figure 3, C-F).

**3.1.4 *C. arizonica***

Diameter of *C. arizonica* seedlings was not affected by Pb contamination and negative effect of the Pb pollution on the height growth of its seedlings was slight (Figure 4, A-B). Stem and root dry biomass of the seedlings did not show significant decrease in response to increasing Pb soil concentration. Although the leaf dry biomass of the species was not affected at concentration 300 mg kg$^{-1}$, higher concentrations of Pb led to decreasing of leaf biomass. Also, total dry biomass of the seedlings didn't decrease till 300 mg kg$^{-1}$ and Pb in high levels led to decreasing of total dry biomass (Figure 4, C-F).

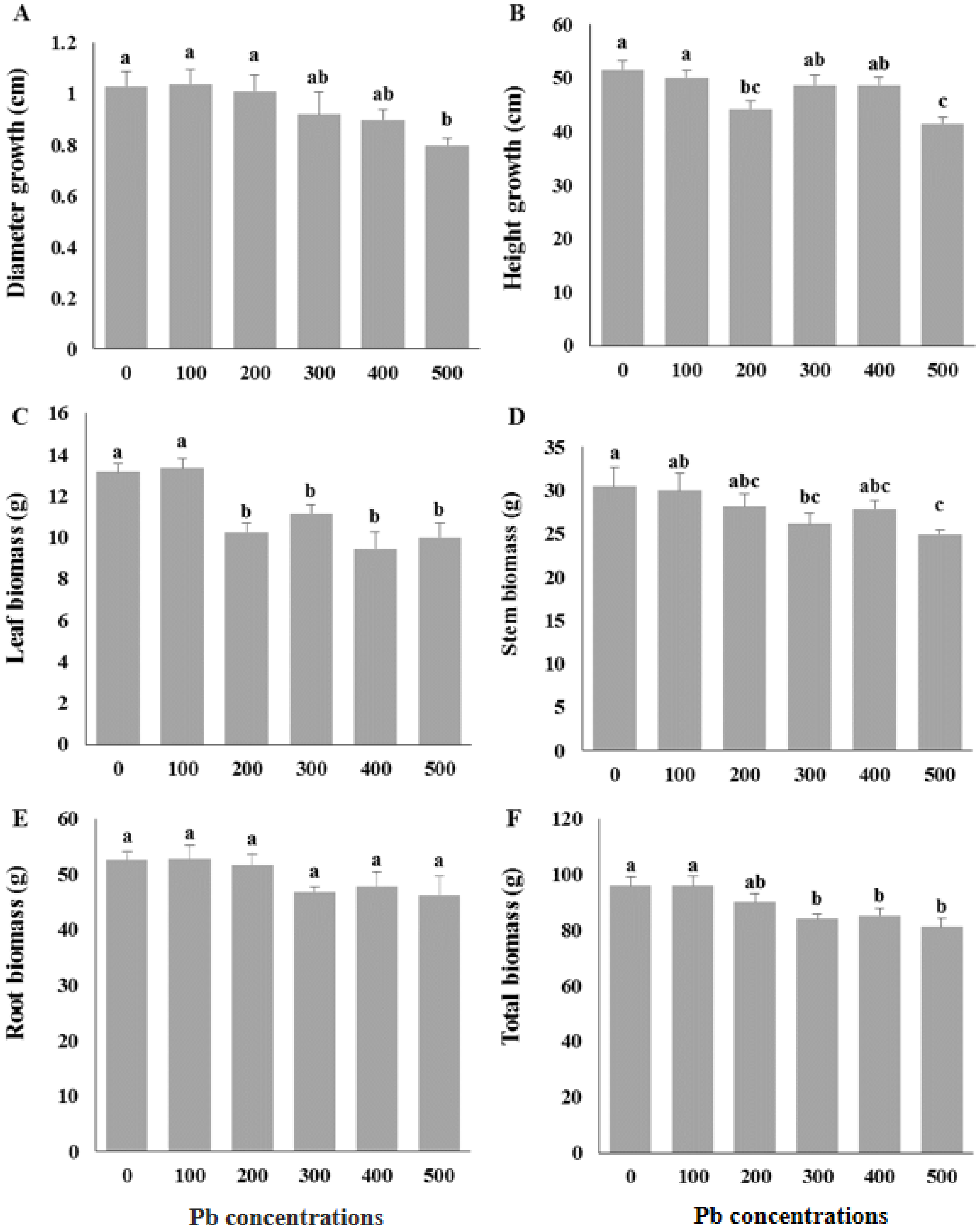


**Figure 1** Effect of Pb contaminations on growth and dry biomass of *A. cappadocicum.*

**Note:** Different letters show significant difference (P<0.05) and same letters show no significant difference (P>0.05)

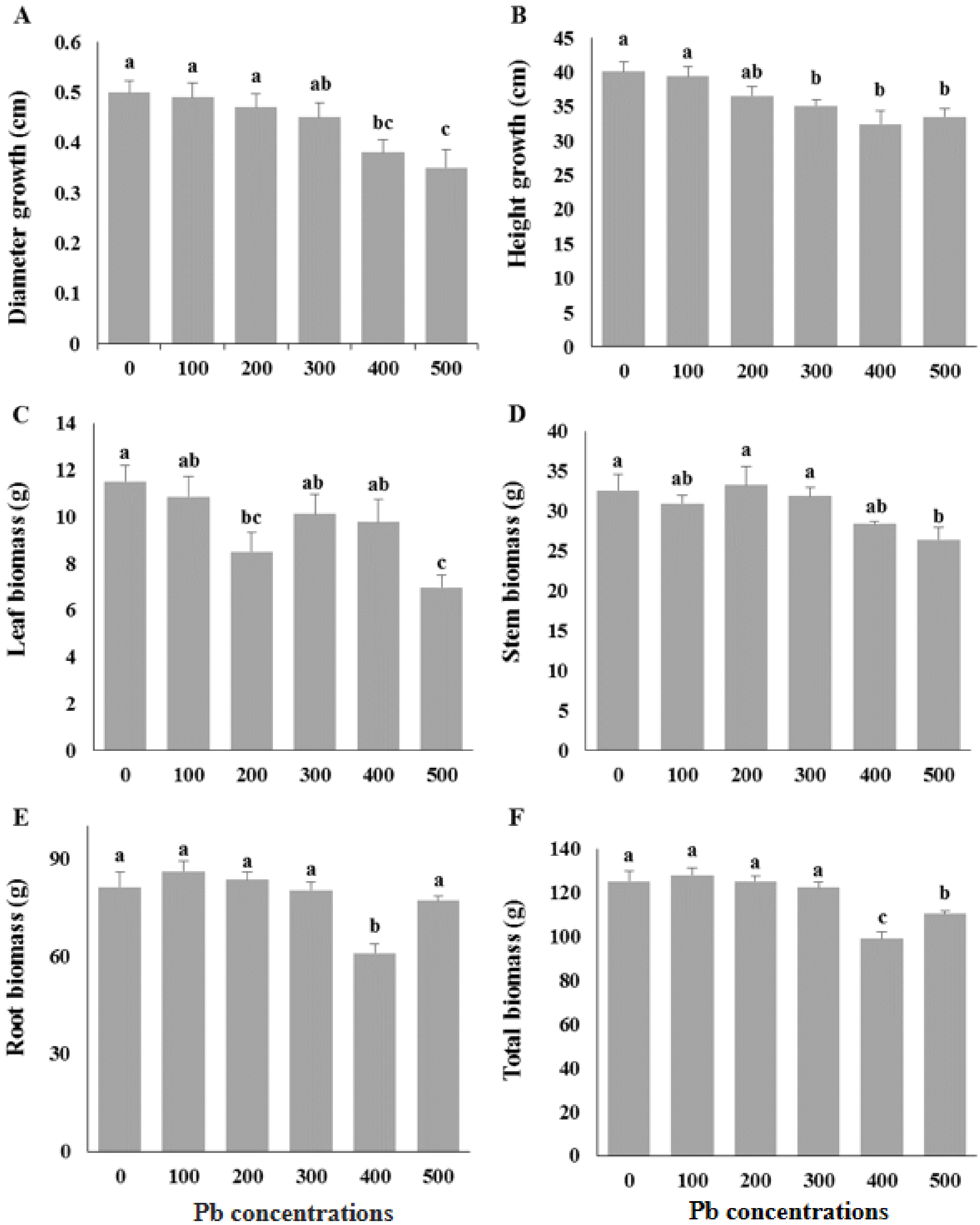


**Figure 2** Effect of Pb contaminations on growth and dry biomass of *F. excelsior.*

**Note:** Different letters show significant difference (P<0.05) and same letters show no significant difference (P>0.05)

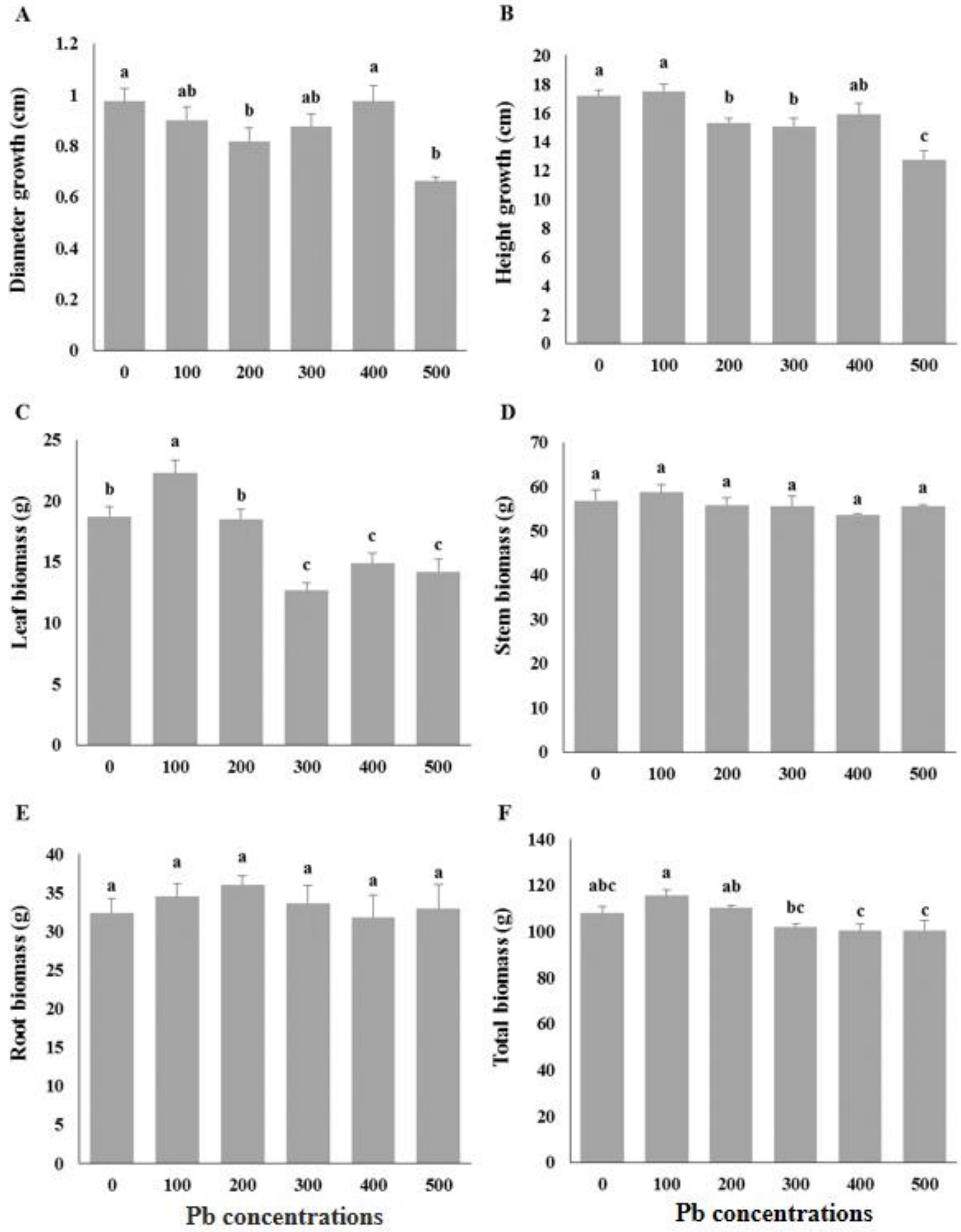


**Figure 3** Effect of Pb contaminations on growth and dry biomass of *T. orientalis.*

**Note:** Different letters show significant difference (P<0.05) and same letters show no significant difference (P>0.05)

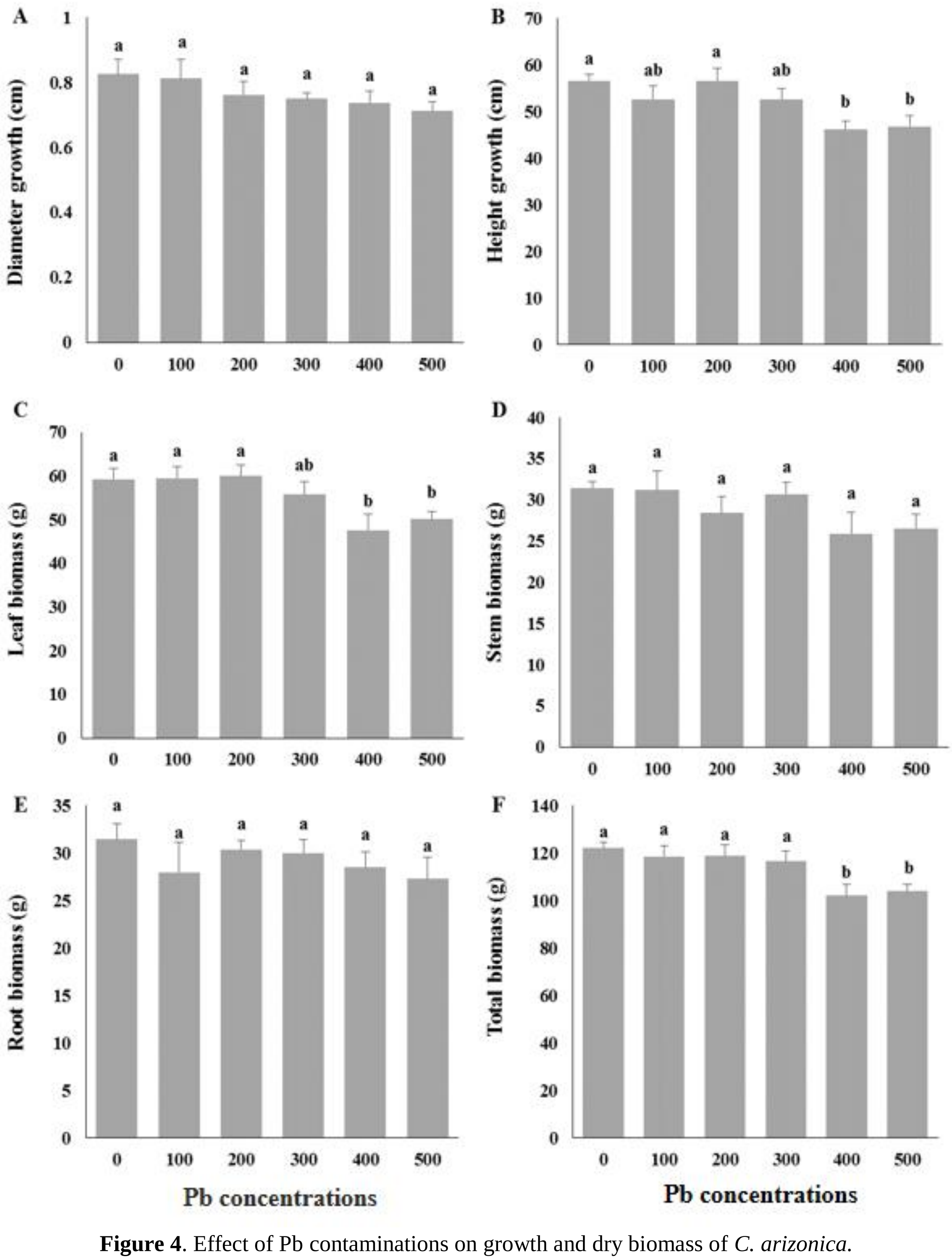


**Figure 4**. Effect of Pb contaminations on growth and dry biomass of *C. arizonica.*

**Note:** Different letters show significant difference ($P<0.05$) and same letters show no significant difference ($P>0.05$)

**3.2 Pb accumulation in plant parts**

The lower mean values of Pb concentrations in different parts of the plant were recorded for control seedlings, which did not receive any extra addition of Pb in the soil. By contrast, at increasing doses of Pb in soil, the concentration of Pb in root, shoot and leaf tissues also increased (Figure 5).

In the case of *A. cappadocicum*, a higher increase in Pb accumulation in the roots and leaf was recorded than in stem when seedlings were exposed to soil with Pb contamination (Figure 5, A). The maximum accumulation of Pb occurred in leaf while the minimum was recorded in its stem (Figure 5, A). Generally, the highest Pb concentration this species was observed at the 400 mg $kg^{-1}$. The Figure 5-A indicates that Pb accumulation in the root of *F. excelsior* was higher than those of leaf and shoot. Generally, the highest Pb concentration in tissues was observed at the highest soil contamination level. Accumulation of Pb in the root of *C. arizonica* was much higher than the other organs (Figure 5, C). Nevertheless, in comparison with its control, accumulation of Pb in leaf increased considerably at increasing Pb concentration. In total, the highest Pb concentration in the seedlings of this species was related to the 400 mg $kg^{-1}$ treatment. In the case of *T. orientalis*, the concentration of Pb in all parts increased progressively as its concentration increased in soil (Figure 5, D). The highest accumulation of Pb in *T. orientalis* was registered at 500 and 400 mg $kg^{-1}$, respectively.

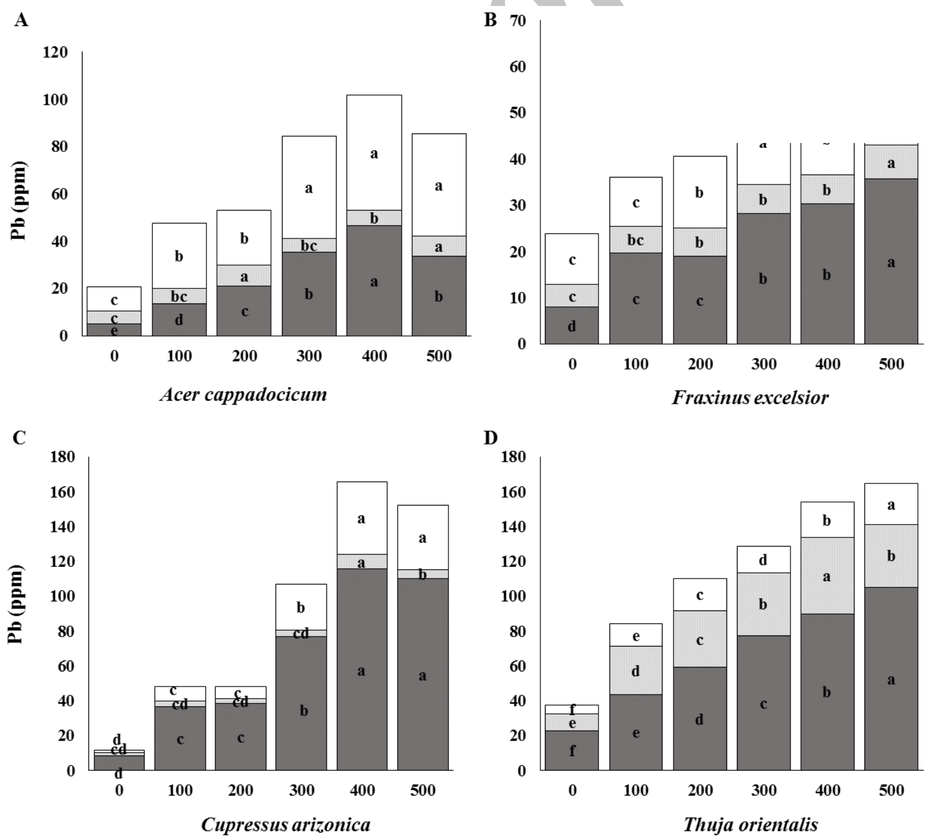

**Figure 5** Comparison of Pb accumulation in the different organs of *A. cappadocicum*, *F. excelsior*, *T. orientalis and C. arizonica* in Pb-contaminated soils

**Note:** Different letters show significant difference (P<0.05) and same letters show no significant difference (P>0.05)

When the seedlings of the four species were subjected to the highest concentration of lead in the soil, *T. orientalis* and *C. arizonica* accumulated more Pb in their organs. The lowest accumulation of this heavy metal was recorded in *F. excelsior* (Figure 6).

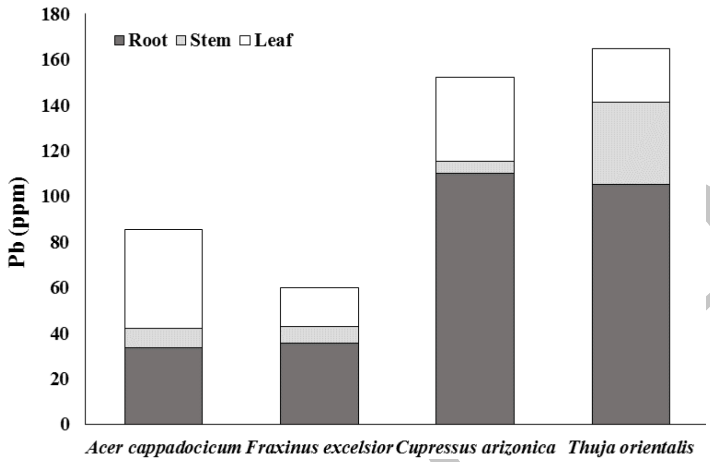


**Figure 6** Capacity of Pb accumulation in different organs in four tree species at concentration of 500 mg kg$^{-1}$

## 4 DISCUSSION

In the current research, we surveyed the growth responses of four species (viz. *A. cappadocicum*, *F. excelsior*, *T. orientalis and C. arizonica*) to lead contamination and found out that each species responded differently. This is in line with the findings of Abdul Qados (2015) on growth responses of three species to lead contamination. The lowest concentration of Pb did not show any significant effects on seedling growth while the highest concentrations (400 and 500 mg kg$^{-1}$) caused variable reductions in growth among the four species. *C. arizonica* was found to be the most tolerant species that even at the highest Pb concentrations showed no negative effect on its diameter growth. It should be noted that at low and medium level of Pb (200 and 300 mg kg$^{-1}$), the negative effects on growth was relatively small in all species, while at the higher Pb levels (400 and 500 mg kg$^{-1}$), reduction of seedlings growth were markedly higher. Moreover, the growth reduction of the seedlings by high Pb levels could be attributed to the suppression of the cells elongation, as the result of irreversible inhibition exerted by the heavy metal on the proton pump responsible for the process (de Souza *et al*., 2012; Hassan *et al*., 2013).

As different organs within a plant respond differently to the presence of heavy metals (Malar *et al*., 2014), biomass is a good integrative indicator of the growth performance in this respect. Therefore, the dry biomass of different organs of the four species in the presence of Pb was surveyed in this work. Roots of the tested species seemed to be more tolerant to Pb pollution than shoots and leaves, since the dry biomass of roots were the least affected organ even at the highest Pb concentration; the highest sensitivity was found in the leaves. Growth disorders and biomass reductions are commonly observed in plants subjected to high metal levels (Panich-Pat *et al*., 2010), but we found that the two conifer species (*T. orientalis and C. arizonica*) were more tolerant than the two broad-leaves species (*A. cappadocicum* and *F. excelsior*) as the dry biomass of the conifers showed slight decrease when subjected to high Pb concentrations. In general, the results indicated more biomass reduction in the presence of the highest Pb concentration. Potential reason explaining the reduced biomass of seedling in the presence of Pb could be ascribed to the reduction in the cells' meristematic activities as the result of disruption of microtubule organization, thereby affecting cell division (Enu *et al.*, 2000). Patil and Umadevi (2014) also suggested that Pb could bind nucleic acids, thus impeding a proper process of DNA replication and, ultimately, affecting cell division.

Lead is not an essential element for plant and its great accumulation in plants can lead to toxic levels. Plant species and even various organs within the same species differ in their ability to accumulate Pb from the soil (El-Gamal and Tantawy, 2010). The results of 6-month growing of four seedling species under different Pb levels clearly indicated that increase in the soil Pb content was associated with its increased accumulation in all parts of the seedlings, but the accumulation capacity of each species was different: maximum accumulation of Pb in *A. cappadocicum* and *F. excelsior* was recorded in their leaves, while the maximum level in *T. orientalis and C. arizonica* (conifer species) was observed in the roots.

In case of *C. arizonica*, Pb was largely stored in roots and to a much lesser extent in the aerial parts, especially the stem. Role of the stem in accumulating Pb was less relevant than the leaf and root tissues, as the minimum accumulation for all the species, except *T. orientalis*, was registered in the stems.

The ability of a plant species to accumulate Pb indicates its ability to extract this heavy metal from the contaminated soil and, therefore, its potentiality for phytoremediation of the contaminated soils (Malar *et al*., 2014). Our findings clearly showed that the ability of the conifers (*T. orientalis and C. arizonica*) in phytoremediation was higher than that of *A. cappadocicum* and *F. excelsior*, because Pb accumulations by the conifers from the highest tested Pb-contaminated soil was almost twice that of the other two species.

The ideal plant species for phytoremediation should be a high biomass-producing species that can both tolerate and accumulate heavy metals (Pulford and Watson, 2003). The high concentrations of Pb the tissues of *T. orientalis and C. arizonica* considered together with their high biomass production suggest that they could be used for remediation of Pb-contaminated soils.

## 5 CONCLUSION

The absence of visual damage and mortality as the result of Pb contamination in the four screened species suggests that all the tested species might have some mechanisms to tolerate Pb, but the experiment at seedling stage demonstrated that conifer species had higher ability for Pb accumulation in their tissues. Surely, toxicity effects of the heavy metal needs to be evaluated for longer growing periods as the response of plants can change throughout growth, so future long-term studies are advised to make a final decision about the phytoremediation capacity of these species.

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# مقایسه توانایی جذب عنصر سرب توسط چهار گونه درختی در مرحله نهال (شیردار، ون، سرو خمره‌ای و سرو نقره‌ای)

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**چکیده** توانایی سه گونه بومی ایران، شامل شیردار (*Acer cappadocicum*)، ون (*Fraxinus excelsior*) و سرو خمره‌ای (*Thuja orientalis*) و یک گونه غیربومی سرو نقره‌ای (*Cupressus arizonica*) از لحاظ گیاه پالایی خاک آلوده به سرب بررسی شد. نهال گونه‌های یاد شده به مدت شش ماه در خاک‌های آغشته به سرب با غلظت‌های (۰، ۱۰۰، ۲۰۰، ۳۰۰، ۴۰۰ و ۵۰۰ میلی‌گرم در کیلوگرم خاک) رشد داده شدند و در نهایت پارامترهای رشد، زی‌توده و سرب تجمع یافته در اندام‌ها سنجش شد. نتایج نشان داد که سرب در غلظت زیاد سبب کاهش رشد و زی‌توده هر چهار گونه شد، ولی در این بین تاثیرات منفی آلودگی سرب بر نهال گونه‌های سرو خمره‌ای و نقره‌ای کمتر بود. با افزایش غلظت سرب در خاک، میزان این عنصر در اندام‌های گیاهی هر چهار گونه افزایش یافت، ولی بیشترین تجمع سرب در گونه‌های سوزنی برگ مشاهده گردید. یافته‌های این پژوهش حاکی از آن است که گونه‌های سرو نقره‌ای و خمره‌ای از لحاظ پالایش فلز سرب موفق‌تر هستند ولی بی‌شک برای تصمیم‌گیری قطعی مطالعات بیشتر نیاز است.

**کلمات کلیدی:** رویش، زی‌توده، گونه درختی، گیاه پالایی، نهال