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ORIGINAL PAPER

Assessment of cadmium, zinc and lead contamination in leaf and root of four various species

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Abstract Heavy metals toxicity is a significant problem for ecological, evolutionary, nutritional and environmental reasons. This study was carried out to evaluate the amount of cadmium, zinc and lead absorption in leaf and root of pine, cypress, plantain and ash in Isfahan, Iran, in 2013. For this purpose, three heavy metals (Cd, Pb and Zn) and three sites (heavy traffic, moderate traffic and control) were chosen based on their effects on human health. The results indicated that the highest and lowest lead and cadmium concentrations belonged to heavy traffic site and control site, respectively. Cd in leaf versus Pb in leaf and Cd in root versus Pb in root had the highest correlation coefficient among the traits indicating positive influence of leaf and root on absorbing Cd and Pb from soil, water and air. In all the studied species, the concentration of Pb was higher than that of Cd and Zn. This was certainty due to the vehicle traffic emitting much more lead than cadmium and zinc. In all the studied species, metal concentration in leaves was higher than in roots, which may be due to high concentration of heavy metals in air than in soil. In this study, *Pinus eldarica* Medw. tree was found to be the best species to monitor polluted sites in Isfahan city.

Keywords Air pollution · Correlation coefficient · Heavy metals

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Introduction

Heavy metals from smelting activities have probably affected forest ecosystems since the beginning of metallurgy (Lukina and Nikonov 1995). Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and nickel (Ni) (GWRTAC 1997). Plants growing in a polluted environment can accumulate the toxic metals at high concentration causing serious risk to human health when consumed (Vousta et al. 1996). The accumulation and distribution of heavy metals in plants depend on the environmental factors, such as plant species, element species, chemical and bioavailability, redox, pH, cation exchange capacity, dissolved oxygen, temperature and secretion of roots (Bi et al. 2000; Yu et al. 2000; Su et al. 2000).

Cd is a non-essential element that negatively affects plant growth and development. Cd (8.6 g cm^{-3}) is a widespread heavy metal released into the environment by power stations, heating systems, metalworking industries, waste incinerators, urban traffic, cement factories and as a by-product of phosphate fertilizers (Gupta and Abdullah 2011). Cd is chemically similar to Zn (Chesworth 1991) and may be taken up and transported in plants via similar pathways (Grant et al. 1998). Generally, zinc is an essential element which belongs to Group-II of the periodic table. It acts as a plant nutrient while being toxic at higher concentrations. Since it is assimilated early by plants, it can be highly phytotoxic (Shier 1994; Welch 1995). Growth inhibition is a general phenomenon associated with zinc toxicity (Collins 1981). Lead, a non-redox active metal, is a nonessential element for plants and animals and is considered as one of the hazardous heavy metals polluting of the environment (Salt et al. 1998). Pb-contaminated soils adversely affect various plant processes and can lead to a sharp decrease in crop productivity (Moustakas et al. 1994). The largest contribution of Cd to the plant is in exchangeable form, while residual form is seen to be most prevalent for Pb and Zn (Cheng 2003).

Nabulo et al. (2008) revealed that higher levels of plants trace metals, including Zn, Cu, Pb and Ni, were accumulated in root rather than in rhizome, and the least amount was accumulated in leaf. Their study identified industry as a potential source of trace metal contamination in water, and concluded that the environment pent-up is needed for policy intervention in industrial waste management. Kord et al. (2010) reported that the highest and the lowest metal concentrations were found in the heavy traffic sites and the control site, respectively. However, samples taken from highway sites contained high concentrations of nickel, copper and lead. Their study proved the significant correlations between the heavy metal concentrations in pine needle samples.

In study by Liao and Chang (2004), translocation ability of water hyacinth (*Eichhornia crassipes* Mart. Solms.) was defined by the quantity of Cu, Pb, Cd, Ni and Zn translocated in the plant tissues and was expressed as a root/shoot ratio. The ratio results were in the order of Cu > Pb > Cd > Ni > Zn. The five element concentrations in the roots were 3 to 15 times higher than those in the shoots. The concentrations in the root tissue were found to be in the order: Cu > Zn > Ni > Pb > Cd. The results indicated water hyacinth as a promising candidate for phytoremediation of the wastewater polluted with Cu, Pb, Zn and Cd. In another research, the studied metal ions inhibited germination and root growth of rapeseed seedlings. The toxicity of metal ions decreased in order of Cu > Cr > Hg > Cd > Pb > Ni > Zn. The mentioned results confirmed the differences in metal tolerance of tested rapeseed cultivars (Peško et al. 2011). Kleckerová and Dočekalová (2014) revealed the significant relationship between Cd in leaf versus Cd in root and Pb in leaf versus Pb in root.

This study aimed to investigate the levels of Cd, Zn and Pb pollution in the atmosphere of Isfahan city using *Pinus eldarica* Medw., *Cupressus arizonica* Greene., *Platanus orientalis* and *Fraxinus excelsior* species.

Materials and methods

This study attempted to evaluate the amount of cadmium, zinc and lead absorption in different parts of pine (*Pinus Eldarica* Medw.), cypress (*C. arizonica* Greene.), plantain (*P. orientalis*) and ash (*F. excelsior*) in Isfahan, Iran, (32°39′ and 51°39′E, and 1570 m above sea level) in 2013. In the study area, the average annual precipitation is 120 mm/y, minimum temperature is 3.7 °C in January and maximum temperature is 28.9 °C in August. Moreover, the relative humidity during daytime is relatively high ranging from 30 % in July to 91 % in December.

The city suffers from high traffic density caused by vehicles. The average number of vehicle movements per hour in polluted sites, namely Azadi St. and Ahmadabad St., as well as in control site named Sabahi St., are 540, 338 and 128, respectively. The samples of leaves and surface roots were collected from polluted and control sites in spring. The samples were taken from the trees considering the main wind direction (i.e. the highest wind speed). The samples obtained from each part of the trees were then homogeneously mixed and separately carried in clean cellulose bags to the laboratory on the same day. In laboratory, the samples were carefully washed three times with distilled water to remove adhering particles (Babaoglu et al. 2004). All samples were weighed and then dried in an oven at 70 °C for 48 h. The samples (1 g) were digested with concentrated HNO_3 in a microwave. Heavy metals concentrations were measured by a flame atomic absorption spectrophotometer, PerkinElmer AAS analysis 300 model, with three replicates. The used metal standards were provided from Merck, Germany.

The studied traits were Cd in leaf, Cd in root, Zn in leaf, Zn in root, Pb in leaf and Pb in root. Skewness, kurtosis, homogeneity of variance and normality of the data were tested by Minitab (1998) statistical software. Analysis of variance of factorial experiment based on CRD and means comparison using Duncan's multiple range test at $P < 0.05$ was performed by SAS (2001) and MSTAT-C (1990) softwares.

The phenotypic correlation between variable x and y (r_{xy}) was performed in SAS (2001), and it was estimated following Kwon and Torrie (1964) using Eq. (1):

$$r_{xy} = \frac{\mathrm{Cov}_{xy}}{\sqrt{\left(\mathrm{Var}_x \times \mathrm{Var}_y\right)}} \quad (1)$$

where, Cov_{xy} is covariance between variable x and y, Var_x is variance of x, and Var_y is variance of y. In addition, Excel software was used for charts adjustments as well.

Results and discussion

Results of analysis of variance (ANOVA) showed the significant differences between species for all the traits, indicating presence of genetic diversity among them (Table 1).

Heavy metals concentrations in sampled species are shown in Table 2. The highest amounts of Cd, Zn and Pb concentrations in leaf were related to *P. eldarica* Medw. (6.66, 2.72 and 9.69 ppm, respectively), and the lowest amounts of Cd, Zn and Pb concentrations in leaf belonged to *F. excelsior* species (2.85, 0.95 and 3.67 ppm, respectively) (Table 2). On the other hand, *F. excelsior* species

Table 1 Analysis of variance (ANOVA) of the traits in the studied species

SOV	*df*	Mean square (MS)					
		Cd in leaf	Cd in root	Zn in leaf	Zn in root	Pb in leaf	Pb in root
Species	3	45.88**	8.56**	1.48**	0.34**	117.69**	1.45**
Site	2	137.73**	25.31**	2.94**	1.37**	278.76**	7.27**
Species × site	6	1.54**	2.73**	0.06**	0.04**	5.27**	0.21**
Error	60	0.40	0.19	0.01	0.01	0.86	0.03
CV%		12.63	18.18	8.13	11.19	13.29	9.82

ns not significant, *SOV* source of variation, *df* degree of freedom, *Cd* cadmium, Zn zinc, Pb lead

* and ** Significant at 5 and 1 % levels of probability, respectively

Table 2 Heavy metal contents of four various species collected from different sites in Isfahan city

Treatment	Cd in leaf (mg l^{-1})	Cd in root (mg l^{-1})	Zn in leaf (mg l^{-1})	Zn in root (mg l^{-1})	Pb in leaf (mg l^{-1})	Pb in root (mg l^{-1})
Species						
Pinus eldarica Medw.	6.66 a	2.13 b	2.72 a	0.87 b	9.69 a	3.07 b
Cupressus arizonica	5.52 b	1.78 c	2.25 b	0.73 b	8.02 b	2.59 c
Platanus orientalis	4.91 c	2.30 b	1.79 c	1.26 a	6.51 c	4.74 a
Fraxinus excelsior	2.85 d	3.38 a	0.95 d	1.28 a	3.67 d	4.97 a
Site						
Azadi (heavy traffic)	6.87 a	3.32 a	1.04 c	0.60 c	9.60 a	5.55 a
Ahmadabad (moderate traffic)	5.80 b	2.58 b	2.98 a	1.57 a	8.20 b	4.34 b
Sabahi (control)	2.29 c	1.29 c	1.77 b	0.93 b	3.12 c	1.63 c

Means in each column, followed by similar letter(s) are not significantly different at 5 % probability level, using Duncan's multiple range test

had the highest amounts of Cd, Zn and Pb concentrations in root (3.38, 1.28 and 4.97 ppm, respectively), and *C. arizonica* species had the lowest amounts of Cd, Zn and Pb concentrations in root (1.78, 0.73 and 2.59 ppm, respectively) (Table 2).

The effect of site on all the studied traits was also significant at 1 % probability level, indicating that the concentration (mg/kg) of heavy metals was highly different in various sites (Table 1). The lead and cadmium contents in leaf (9.60 and 6.87 ppm, respectively) and root (5.55 and 3.32 ppm, respectively) were found at high concentrations in heavy traffic site (Azadi St.), whereas moderate traffic site (Ahmadabad St.) contained high concentrations of zinc in leaf and root (2.98 and 1.57 ppm, respectively) (Table 2). On the other hand, control site (Sabahi St.) had the lowest lead and cadmium contents in leaf (3.12 and 2.29 ppm, respectively) and root (1.63 and 1.29 ppm, respectively), and heavy traffic site had the lowest zinc in leaf and root (1.04 and 0.60 ppm, respectively) (Table 2). The mean metal concentration values were arranged in the order: Pb > Cd > Zn. Higher concentration of the studied heavy metals in leaves rather than in roots in all locations illustrated the contribution of significant atmospheric deposition (Kleckerová and Dočekalová 2014).

The effects of species × site interaction were also significant at 1 % probability level for all the studied traits, indicating that there were a significant difference in Cd, Zn and Pb concentrations in leaf and root in all the samples collected from different sites (Table 1). The maximum amounts of Cd and Pb in leaf belonged to *P. eldarica* Medw. species in Azadi St. (8.87 and 13.18 ppm, respectively), and the minimum amounts of Cd and Pb in leaf were related to *F. excelsior* species in Sabahi St. (0.94 and 1.21 ppm, respectively) (Figs. 1, 3). *P. eldarica* Medw. species in Ahmadabad St. had the highest Zn in leaf (4.04 ppm), while *F. excelsior* species in Azadi St. and Sabahi St. had the lowest Zn in leaf (0.59 and 0.68 ppm, respectively) (Fig. 2).

The maximum amounts of Cd and Pb in root belonged to *F. excelsior* species in Azadi St. (5.14 and 7.54 ppm, respectively), and the minimum amounts of Cd and Pb in root were related to *C. arizonica* species in Sabahi St. (1 and 1.27 ppm, respectively) (Figs. 1, 3). *F. excelsior* and *P. orientalis* species in Ahmadabad St. had the highest Zn in root (2.05 and 1.98, ppm), and *C. arizonica* and *P. eldarica* Medw. species in Azadi St. had the lowest Zn in root (0.38 and 0.46 ppm, respectively). Therefore, *P. eldarica* Medw. species could be used to decrease air pollution in Isfahan city.

The results of correlation coefficient showed the significant relationship between most of the studied traits (Table 3). Cd in leaf versus Pb in leaf and Cd in root versus Pb in root had the highest correlation coefficients ($r = 0.99$

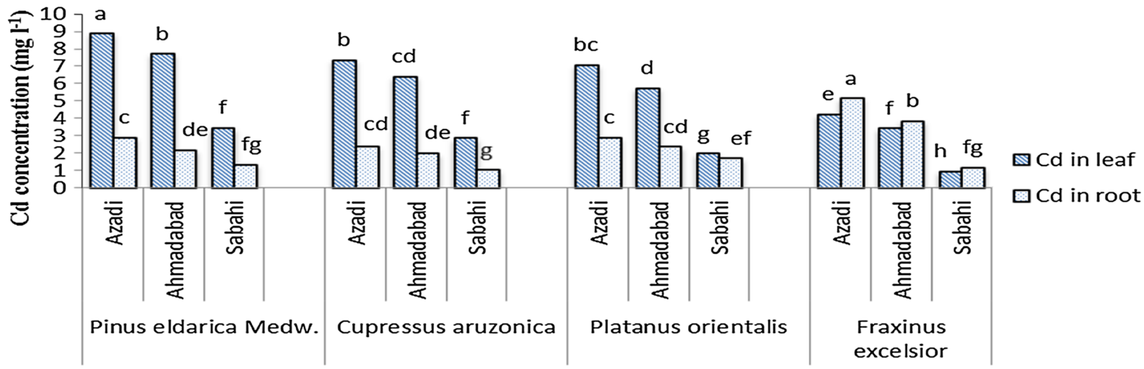

Fig. 1 Cd concentrations of leaf and root in all the species samples collected from different sites

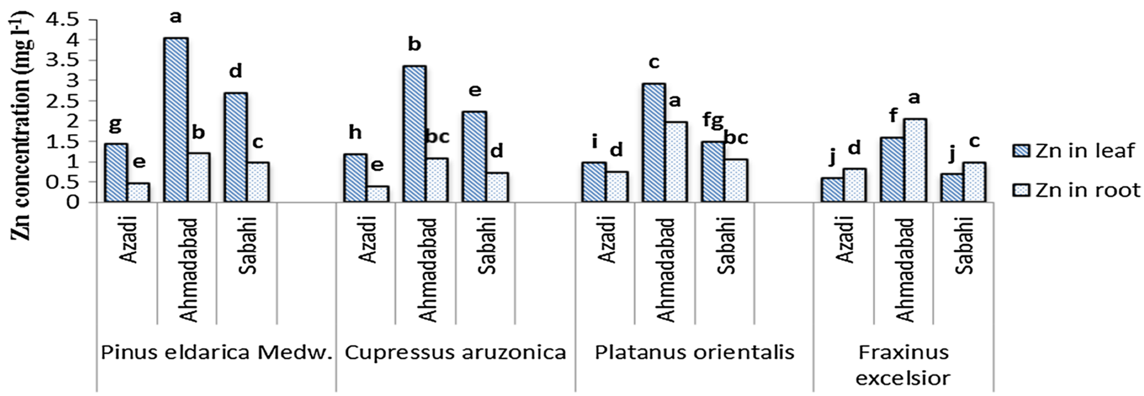

Fig. 2 Zn concentrations of leaf and root in all the species samples collected from different sites

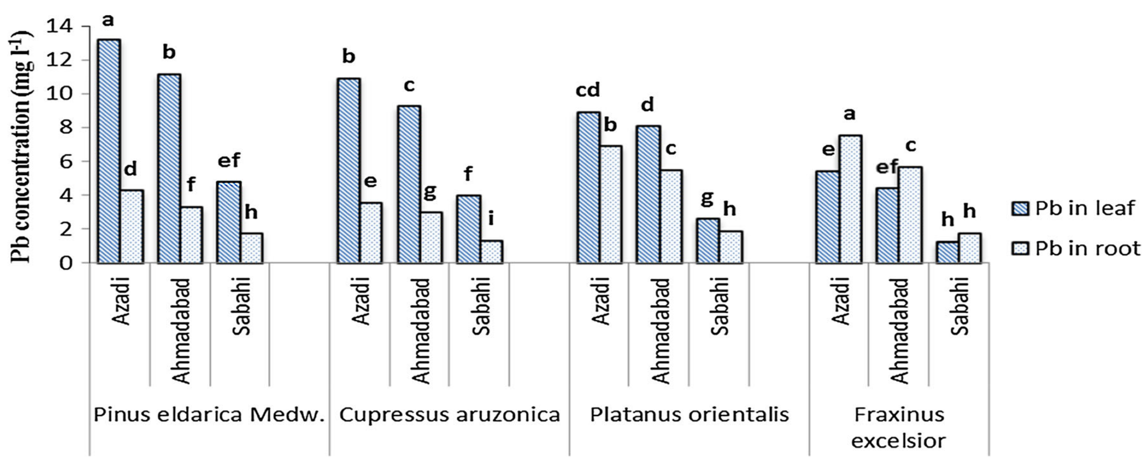

Fig. 3 Pb concentrations of leaf and root in all the species samples collected from different sites

and $r = 0.88$, respectively) among traits, indicating the positive influence of leaf and root on absorbing Cd and Pb from soil, water and air. Roots of plants are the primary contact sites for heavy metal ions. As far as plants are stationary, in those systems which are exposed to frequenting water the total plant body is liable to these ions. Also heavy metals are directly taken into the leaves because of those fragments deposited on the foliar surfaces (Nagajyoti et al. 2010). The correlation coefficients between Zn in root and Cd in leaf, and Cd in root and Pb in leaf were not significant because of the low Zn concentration in root (Table 3).

Heavy metals are thrown out in two forms of elemental and compound which may be organic or inorganic. The various industrial point sources which include former and present missing sites, foundries and smelters, combustion by products and traffic have anthropogenic sources of emission (UNEP/GPA 2004). Cadmium, as one of the

Table 3 Correlation coefficients of metals concentration in leaf and root of the studied four species

Trait	Cd in leaf	Cd in root	Zn in leaf	Zn in root	Pb in leaf	Pb in root
Cd in leaf	–					
Cd in root	0.32**	–				
Zn in leaf	−0.31**	−0.30**	–			
Zn in root	−0.20^{ns}	0.20^{ns}	0.44**	–		
Pb in leaf	0.99**	0.26*	0.35**	−0.21^{ns}	–	
Pb in root	0.49*	0.88**	−0.20^{ns}	0.28*	0.43**	–

ns not significant

* and ** Significant at the 5 and 1 % levels of probability, respectively

heavy metals more mobile in a soil–plant system, is easily taken up by plants, and its essential function has not been known to date (Lehoczky et al. 2000). Cadmium is used in batteries, pigments, fertilizers and detergents, and it is also present in refined petroleum products (Ghosh et al. 2009). This element can be accumulated in plants without causing toxicity symptoms (Lehoczky et al. 1998). The uptake, transport, macro and micro nutrients highly Iron and Zinc interposes into toxicity symptoms (Vollenweider et al. 2006). The regulatory limit of cadmium in agricultural soil is 100 mg/kg soil (Salt et al. 1995). Visible signs of damage include chlorosis and growth inhibition injuries, browning of root tips and fatality are all happen in those plants which grown in soils having high levels of Cd (Mohanpuria et al. 2007; Guo et al. 2008). Ghosh et al. (2009) reported that plant samples of *Brassica rapa* leaves and roots had Cd concentration of exceeding the WHO limit (i.e. >0.3 μg g^{-1}) reported for medicinal plants.

In this study, the level of cadmium in all the studied species decreases with the decrease in traffic density (Fig. 1). Lead is one of the ubiquitously distributed and most abundant toxic element in the soil. It exerts adverse effect on morphology, growth and photosynthetic processes in plants (Nagajyoti et al. 2010). Sources of lead include metal smelting, pigments, lead battery manufacturing and lead contaminated petrol. In soil, lead tightly binds itself to the organic soil particles which may decrease the mobility of lead in most soils and may reduce its uptake by plants (Cooper et al. 1999). Some researchers have reported the close relationship between lead concentrations and traffic intensity (Li et al. 2001; Viard et al. 2004). In the present study, there was a linear correlation between high level of Pb in leaf and heavy traffic at Isfahan city. High metal concentrations in plants are contained in highway roadsides due to the anthropogenic activities and traffic density (Çelik et al. 2005).

Zinc is an essential micronutrient that affects several metabolic processes in plants (Cakmak and Marshner 1993). Major sources of zinc in atmosphere originate from its use in zinc batteries and also its wide use in furniture industries (micro-nutrients). Zinc and Cd are ubiquitous pollutants that tend to occur together at many contaminated sites. While Zn is often phytotoxic, Cd rarely inhibits the plant growth (Pence et al. 2000). Concentrations of Zn found in contaminated soils frequently exceed the limits required to be as a nutrient and may cause phytotoxicity. Zn concentrations in the range of 150–300 mg/kg have been measured in polluted soils (Devries et al. 2002; Warne et al. 2008). High levels of Zn in soil inhibit many metabolic functions of plants, resulting in retarded growth and cell senescence. Zinc toxicity in plants limits the growth of both root and shoot (Fontes and Cox 1998). In this study, the level of zinc in leaf and root of all the plant species was moderate at the control site, indicating that vehicle traffic is a minor emission source for zinc and another zinc source (such as industrial activities) around the site could be blamed.

Conclusion

The results indicated that the maximum and minimum lead and cadmium concentrations were found in the defined heavy traffic and control sites, respectively. The highest correlation coefficient among the traits were related to Cd in leaf vs. Pb in leaf and Cd in root vs. Pb in root, indicating the positive influence of leaf and root in absorbing Cd and Pb from soil, water and air. For all the studied species, the mean metal concentration values were arranged in the order: Pb > Cd > Zn. This could be attributed to the vehicle traffic that emits much more lead than cadmium and zinc. All the studied species had a higher metal concentration in leaves than in roots which could be due to high concentration of heavy metals in air than in soil. *P. eldarica* Medw. tree was found to be the best species to monitor polluted sites in Isfahan city.

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