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PhytoremeDiation of Heavy Metals Contaminated Environments: Screening for Native Accumulator Plants in Zanjan-Iran

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ABSTRACT: Environmental pollution with heavy metals is a global struggle. Phytoremediation is an effective and low-cost technology for refinement of polluted soils. This research was conducted in Zanjan province (located in North West Iran) where metallurgical industries are developed quickly. In this study, based on the heavy metals contamination of soil in the studied area six sampling sites were selected taking into account the industrial distributions as well as the low/high traffic congestions. Leaves from eight tree species namely: *Populus nigra, Ulmus pumila, Fraxinus excelsior, Robinia pseudoacacia, Acer hyracanum, Salix alba, Thuja orientalis, and Cupressus sempervirens var arizonica*, were sampled and analyzed by ICP-OES (Spectro Genesis) for their heavy metal contents (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb). Results showed that heavy metals in stations close to the lead and zinc smelting industrial complexes are much higher than average showing a high correlation with their respective metal concentrations in soils. This clearly indicates that heavy metal contents in tree leaves in the studied area are solely related to industrial activities notably National Iranian Lead and Zinc (NILZ) as well as Zinc Specialized Industrial Complex (ZSIC) companies. Based on the results, the studied native plants accumulate different metals selectively and *Populus nigra* was found to be the best accumulator plant for Mn, Zn and Cd, *Thuja orientalis*, as the best phytoextractor for Fe, and *Cupressus sempervirens var arizonica* is the best species among the studied native plants for accumulation of Pb.

Key words: Heavy metals, Phytoremediation, Lead and Zinc industries, Zanjan province

INTRODUCTION

During the past decade, considerable concern has been expressed by environmental scientists over the increasing levels of a range of toxic elements in the environment. Among the most potentially hazardous are the so-called ‘heavy metals’, a term applied to cover a range of elements. These elements have atomic weights between 63.546 to 200.590 and a specific gravity greater than 4.0 i.e. at least 5 times that of water. They are toxic, none biodegradable and have biomagnified characteristics (Lepp, 1975; Adepoju-Bello, *et al.*, 2009; Momodu & Anyakora, 2010).

Environmental pollution with heavy metals is a global disaster that is related to human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and melting operations (Igwe & Abia, 2006). Among the pollutants, heavy metals are normally accumulated in plant tissues at various concentrations without showing toxicity symptoms (Yasar, *et al.*, 2010). High stability of these metals in water, soil, and even in animals poses a major threat to human health and ecological environment (Hashemi, *et al.*, 2012). Numerous efforts have been undertaken recently to find methods of removing heavy metals from soil, such as phytoremediation (Igwe & Abia, 2006), Soil washing (Dermont, *et al.*, 2008), Nano materials (Zhang, 2003), remediation with bacteria (Valls & Lorenzo, 2002), electrical force and heat (Xia & Chen, 1997; Jiang, *et al.*, 2000). Many of these technologies are costly, (as for example excavation of contaminated materials and their chemical/physical treatment) or do not achieve a long-term nor esthetic solution (Cao & Ma, 2002; Mulligan, *et al.*, 2001). However

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phytoremediation technology among them can provide a cost-effective, long-lasting and esthetic solution for remediation of contaminated sites (Ma, *et al.*, 2001). Phytoremediation is defined as the use of plants to remove pollutants from the environment or to render them harmless (Salt, *et al.*, 1998). It is a technology of purging soils and water systems from toxic heavy metals (Clements, *et al.*, 2002). The potential use of trees as a suitable vegetation cover for heavy metal-contaminated land has received increasing attention over the last decade (Aronsson & Perttu, 1994; Glimmerveen, 1996). Trees have been suggested as a low-cost, sustainable and ecologically sound solution to the remediation of heavy metal-contaminated land (Dickinson, 2000). The lack of reported toxicity symptoms in trees indicates that their tolerance mechanisms may allow them to withstand higher heavy metal concentrations than agricultural crops (Riddell-Black, 1993).

The Angoran area in Zanjan province, located in North West Iran, has a large metalliferrous site and has been considered a traditional mining region having large reserves of lead and zinc. Related industrial activities were caused and the risk of metals contamination of soils, plants, and surface/ground water resources was increased in Zanjan province (Chehregani *et al.*, 2009). Many related industries are developed in this province, notably, National Iranian Lead and Zinc Company (NILZ) which is one of the largest of its kind in Middle East with a large market capitalization. Zinc Specialized Industrial Complex (ZSIC) covering fifty eight zinc production units is another important industrial complex developed in the studied area with a current annual consumption of about one million tons of raw ore and a production of 0.19 million tons of Zn.

The heavy metal contaminations of soil around these industrial complexes encouraged assessment of the heavy metals remediation by means of their extraction by native plants. In this study, the presence and concentrations of seven heavy metals (Fe, Co, Ni, Cu, Zn, Cd and Pb) in plants leave samples collected from the NILZ Company, ZSIC complex and their neighborhoods plus samples from two parks in Zanjan city were determined using inductive coupled plasma-optical emission spectroscopy (ICP-OES). This permitted ascertaining 1) the assessment of the heavy metal pollution in studied area 2) the recognition of native accumulator species for phytoremediation. This study was carried out in summer 2013 in the Environmental Science Research Laboratory, university of Zanjan, Zanjan-Iran. To the best of our knowledge the present work is the first report on the determination of heavy metals in native trees growing in the studied area.

MATERIALS & METHODS

Zanjan province (located in North West Iran), has a large metalliferrous site and has been considered as a traditional mining region since antiquity. There are still large reserves of lead and zinc in the area. Both mines and smelting units within the province present a risk of contamination of soils, plants, and surface/groundwater resources through dissemination of particles carrying metals by wind action and/or by runoff from the tailings (Chehregani, *et al.*, 2009). The research was focused on the environmental impacts of NILZ and ZSIC companies (Zamani, *et al.*, 2012).

NILZ company (36° 662 N, 48° 482 E) located within Bonab Industrial Estate (BIE), about 12 km east of Zanjan city. This company was established in 1992, with a current consumption of about 300,000 tons of raw ore and an annual production of 55000 tons of Pb and Zn (Parizanganeh, *et al.*, 2010). The plant is situated over an aquifer, which is the only source of fresh water available in the area, supplying a part of drinking water to Zanjan citizens and its neighboring areas as well as water used for agricultural and industrial consumptions. The tailings from BIE, estimated to be about 2.5 million tons, contain a variety of toxic elements, notably Pb, Zn, and Cd (Zamani, Yaftian, & Parizanganeh, 2012). The ZSIC (36° 662 N, 48° 482 E) was established in 1996. The tailings from the industrial complex, estimated to be about 2.5 million tons by now, contain a variety of toxic elements. They are damped in the vicinity of the complex and are exposed to wind and rains, contributing to soil, plant, surface and ground water contamination. Fig. 1 illustrates the location of the study area and the sampling stations.

127 leave samples from eight native plant species, i.e. *Populus nigra, Ulmus pumila, Fraxinus excelsior, Robinia pseudoacacia, Acer hyracanum, Salix alba, Thuja orientalis, and Cupressus sempervirens var arizonica*, were collected from NILZ (SS2), ZSIC (SS6), their surroundings (SS1 and SS5) and two locations within the Zanjan city with high (SS4) and low traffic congestions(SS3) (Fig. 1). These species are widely distributed in the study area and can survive under a wide range of temperature and grow in almost any type of soils in the studied area. Tree leaves were sampled from June to September 2013. The samples were collected in brown paper bags and transferred to environmental science research laboratory of environmental science department in university of Zanjan for sample preparation and analysis. In the laboratory, leaves were washed with distilled water and

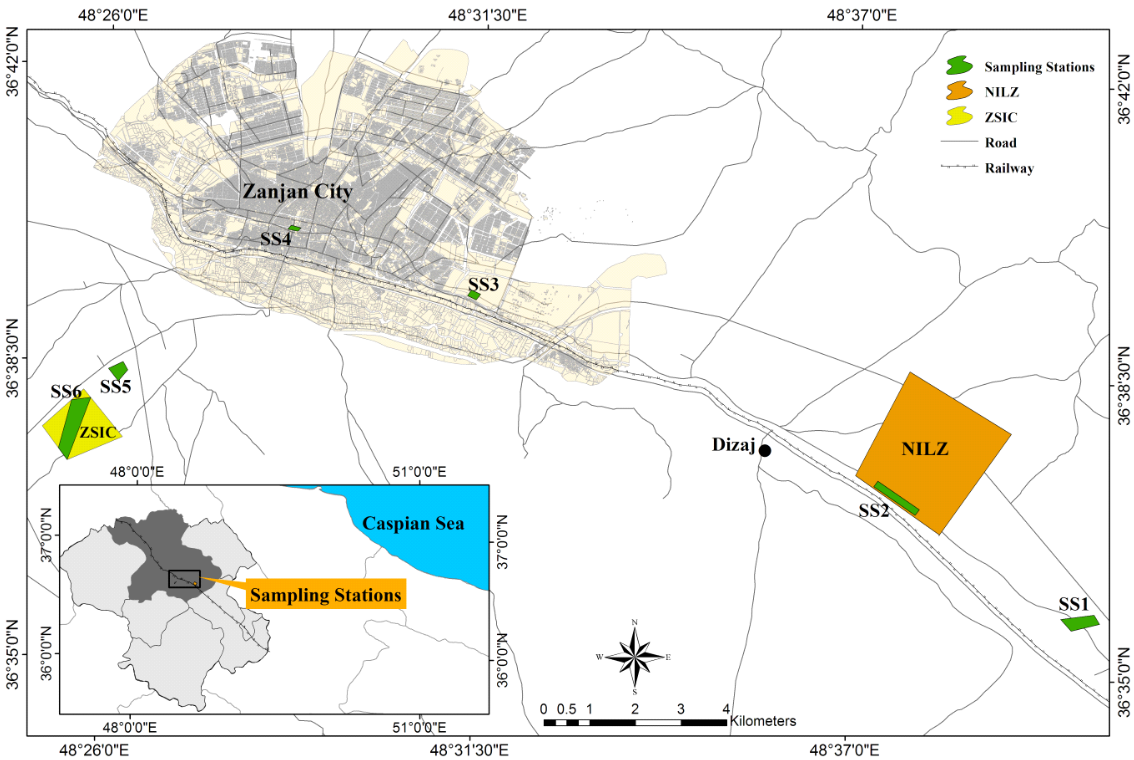

Fig. 1. Location map of the study area indicating sampling stations

dried in oven for 48 hours at 60°C. Rinsing was done for removing atmospheric deposition (Serbula *et al.*, 2012; Ugolini *et al.*, 2013). However, distinguishing the origin of metal concentrations within internal tissues either by uptake from the leaf surface or the absorption from the soil by roots is very difficult. Indeed, the concentration of contaminants within leaf samples could depend on the mobility of the metal within the soil-plant system through the transpiration stream and phloem flux (Ugolini *et al.*, 2013). Dried leaves were grounded into fine powder. For acid mineralization of plant tissues, 10ml of HNO_3 were added to 1.0 g dry weight (DW) of tissue and was kept at room temperature for 24 hours. Digestion of samples was performed by adding 2ml of H_2O_2 at 125 æ%C on a heater until the digestion mixture was evaporated (Khattak & Jabeen, 2012). 0.1M HNO_3 was added to digestion residue and the extract was filtered through Wattman filter paper No 42. The solution was diluted with HNO_3 0.1M to 50 ml. The heavy metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) in the plant extracts were then analyzed by ICP-OES (Spectro Genesis). 127 leaf samples and 10 blanks were used and analyzed in this study. Three analytical replicates were measured for each sample.

Statistical analyses were performed by using SPSS (IBM SPSS Statistics 21) software for Windows version. The analysis of experimental data was carried out by one-way ANOVA and Kruskal-Wallis tests (Einax, et al, 1997; Miller & Miller, 2005). All significance statements reported in this study are at the $P < 0.05$ level.

RESULT & DISCUSSION

Table 1 show descriptive statistics of heavy metal content for all trees in the studied area. The amounts of metals (mg of Heavy metal in kg of dry weight of plant leaf) were found in the range 0.001-5.950 for Cr; 11.950-260.650 for Mn; 39.470 - 532.200 for Fe; 0.001 - 9.100 for Co; 0.001 - 7.100 for Ni; 0.001 -931.780for Cu; 0.001 - 2111.750 for Zn; 0.001 - 51.600 for Cd and 0.001 -3354.550 for Pb. The order of metal contents in plant leaf based on their mean values varies as: Zn (258.786) > Pb (218.525) > Fe (189.373) > Mn (60.661) > Cu (41.556) > Cd (3.693) > Co (1.596) > Ni (0.819) > Cr (0.639).

Descriptive statistics of heavy metal contents in the six studied stations are given in Table 2. The results reveal that the metal contents in the samples selected from within the industrial's borders (SS2, SS5 and SS6) are significantly higher than those from the other area (Fig. 2). The one-way ANOVA and Kruskal-Wallis tests allow testing the significant difference of the means. Kolomogorov smirnov test shows that the distribution of heavy metals isn't normal. Therefore Kruskal-Wallis

Table 1. Descriptive statistics of heavy metal content in sampled leaf trees (mg/kg)

Heavy metal	N	Minimum	Maximum	Mean	Std. Deviation
Cr	127	0.001	5.950	0.639	1.189
Mn	127	11.950	260.650	60.661	47.588
Fe	127	39.470	532.200	189.373	111.524
Co	127	0.001	9.100	1.596	2.250
Ni	127	0.001	7.100	0.819	1.519
Cu	127	0.001	931.780	41.556	93.037
Zn	127	0.001	2111.750	258.876	392.849
Cd	127	0.001	51.600	3.693	7.787
Pb	127	0.001	3354.550	218.525	423.732

Table 2.Comparison of heavy metal content in sampled leaf trees among sampling stations

Sampling stations		Heavy metal (mg/kg)								
		Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
SS1	Mean	1.547	56.484	142.566	2.373	1.250	131.470	53.323	0.179	8.638
	Min	0.001	16.700	39.470	0.001	0.001	0.001	0.001	0.001	0.001
	Max	5.950	114.800	474.620	6.530	4.660	931.780	165.750	1.250	26.750
SS2	Mean	0.826	60.862	170.435	1.790	0.967	41.561	201.038	2.458	438.772
	Min	0.001	12.750	79.750	0.001	0.001	1.380	17.950	0.001	11.050
	Max	5.350	240.150	465.820	7.130	4.760	372.430	445.750	9.550	3354.550
SS3	Mean	0.183	36.042	131.907	0.342	0.050	11.538	13.729	0.001	18.484
	Min	0.001	11.950	48.400	0.001	0.001	4.720	0.001	0.001	5.000
	Max	1.950	72.400	417.750	1.000	0.500	21.370	63.950	0.001	39.450
SS4	Mean	0.367	32.555	236.288	0.280	0.009	12.325	15.664	0.001	14.080
	Min	0.001	17.500	88.700	0.050	0.001	2.970	0.001	0.001	5.000
	Max	1.550	59.900	459.450	0.650	0.150	21.570	50.400	0.001	35.550
SS5	Mean	0.314	105.486	237.094	2.752	1.539	20.022	702.654	13.461	141.455
	Min	0.001	35.650	113.800	0.650	0.001	10.420	230.300	0.550	19.900
	Max	1.000	260.650	464.050	9.100	7.100	32.220	2111.750	51.600	454.350
SS6	Mean	0.456	84.116	294.233	2.361	1.154	76.545	993.505	11.261	283.433
	Min	0.001	65.450	166.400	1.250	0.090	60.920	5740.000	6.000	121.600
	Max	1.300	114.250	532.200	4.350	2.250	111.320	1871.300	22.500	608.800

test, a non-parametric method, was used to compare the presence of heavy metals among sampling stations. This test shows a statistically significant difference among the sampling locations for studied heavy metals content in sampled tree leaves (Table 3).

Table 4 demonstrates the statistical description of heavy metal contents in the eight studied tree species in the station SS2. This station was selected for the comparison of heavy metal's content among different types of trees because of an overall higher content of all studied metals and that in this stations all tree species exist and are sampled.

The results (table 4) show that the mean heavy metal content in the studied tree species is different. Therefore Kruskal-Wallis test was used for the comparison of the presence of heavy metals among trees. Statistically significant differences are observed among sampled tree leaves for metals Fe, Zn, Cd and Pb. However the concentrations of Cr, Co, Ni and Cu in different plant species are the same (Table 5).

Previous investigations have also documented that the leaves of Robinia pseudoacacia can be used as a bioindicator of heavy metals contamination (Çelik, *et al.*, 2005 and Serbula, 2012). Çelik also found higher levels of some heavy metals in samples collected from industrial sites. Table 6 briefly highlights some previous investigations screening for accumulator plants in different parts of the globe.

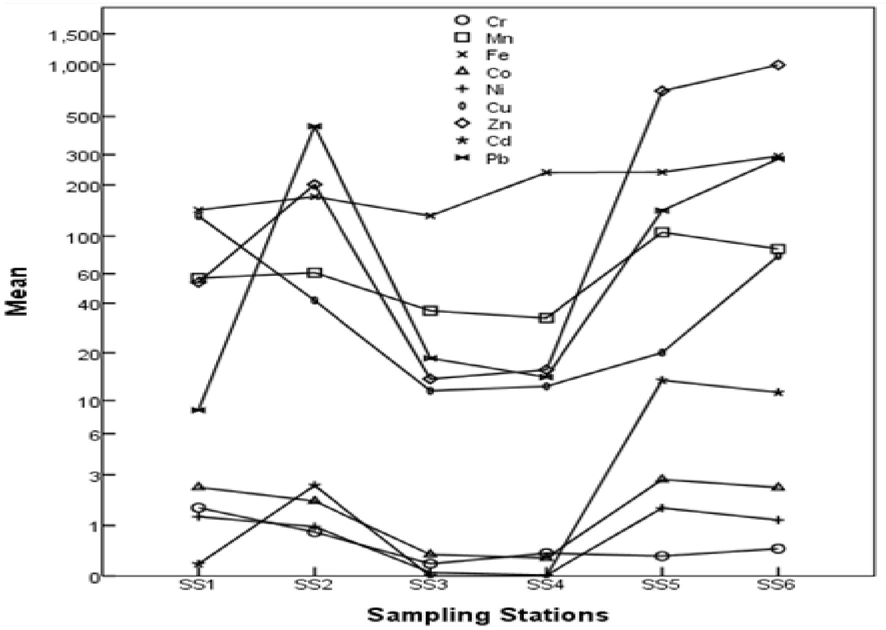

Fig. 2. Mean concentration of heavy metals (mg/kg) in sampling stations

Table 3. Comparison of heavy metal content in sampled leaf trees among different sampling stations using Kruskal Wallis test

	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Chi-Square	7.474	35.980	30.828	28.826	30.619	34.691	103.120	90.586	86.368
df	5	5	5	5	5	5	5	5	5
Asymp. Sig.	0.188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

df=degree of freedom

Table 4.Comparison of heavy metal content among different tree species in station SS2

Tree species		Heavy metal (mg/kg)								
		Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Acer hyracanum	Mean	1.325	68.158	156.143	2.160	1.470	39.616	223.658	3.183	280.925
	Min	0.001	45.650	137.050	0.001	0.001	10.220	17.950	0.001	11.050
	Max	3.650	102.700	218.520	5.680	4.310	87.830	322.100	9.550	441.400
Populus nigra	Mean	0.861	147.125	110.568	1.555	0.635	27.391	291.750	4.166	255.658
	Min	0.001	93.350	97.120	0.001	0.001	9.920	223.550	2.750	68.400
	Max	2.050	180.450	148.770	6.230	3.810	93.380	374.250	5.550	445.850
Ulmus pumila	Mean	0.950	49.800	164.001	2.335	1.378	83.483	242.508	2.508	315.808
	Min	0.001	41.900	140.920	0.001	0.001	9.230	107.400	0.800	187.800
	Max	3.000	59.850	185.100	5.930	3.710	372.430	419.000	4.600	423.850
Robinia pseudoacacia	Mean	0.583	31.391	125.860	0.175	0.002	39.416	113.283	0.650	201.950
	Min	0.001	23.850	91.670	0.001	0.001	4.020	62.200	0.001	67.600
	Max	2.900	53.050	197.600	0.450	0.010	194.130	202.050	1.900	501.750
Fraxinus excelsior	Mean	0.334	49.558	95.235	1.943	1.162	37.616	82.216	0.292	162.483
	Min	0.001	29.950	79.750	0.001	0.001	9.470	62.700	0.001	71.200
	Max	1.150	75.700	114.120	5.430	3.810	83.680	101.200	0.800	246.500
Cupressus sempervirens var arizonica	Mean	2.100	66.020	217.282	1.376	0.582	41.366	120.660	3.070	1346.680
	Min	0.001	12.750	104.170	0.001	0.001	11.520	75.900	0.001	17.300
	Max	5.350	240.150	356.950	6.480	2.910	93.080	223.550	6.500	3354.550
Thuja orientalis	Mean	0.358	33.000	308.652	2.963	1.643	31.448	271.266	4.138	817.311
	Min	0.001	24.400	200.820	0.001	0.001	1.380	98.400	2.350	215.950
	Max	1.300	39.100	465.820	7.130	4.760	130.280	445.750	7.350	1718.350
Salix alba	Mean	0.542	56.633	124.436	1.155	0.460	37.178	214.450	0.916	91.408
	Min	0.001	13.500	105.550	0.001	0.001	7.170	104.650	0.001	27.300
	Max	1.950	120.200	155.380	4.980	2.760	85.230	379.150	3.300	213.500

The order of tree species based on their mean values for each individual heavy metal in mg per kg of dry sample content varies as:

Mn:	*Populus nigra* (147.125) > *Acer hyracanum* (68.158) > *Cupressus sempervirens var arizonica* (66.020) > *Salix alba* (56.633) > *Ulmus pumila* (49.800) > *Fraxinus excelsior* (49.558) > *Thuja orientalis*, (33.000) > *Robinia pseudoacacia* (31.391)
Fe:	*Thuja orientalis*, (308.652) > *Cupressus sempervirens var arizonica* (217.282) > *Ulmus pumila* (164.001) > *Acer hyracanum* (156.143) > *Robinia pseudoacacia* (125.860) > *Salix alba* (124.436) > *Populus nigra* (110.568) > *Fraxinus excelsior* (95.235)
Zn:	*Populus nigra* (291.750) > *Thuja orientalis*, (271.266) > *Ulmus pumila* (242.508) > *Acer hyracanum* (223.658) > *Salix alba* (214.450) > *Cupressus sempervirens var arizonica* (120.660) > *Robinia pseudoacacia* (113.283) > *Fraxinus excelsior* (82.216)
Cd:	*Populus nigra* (4.166) > *Thuja orientalis*, (4.138) > *Acer hyracanum* (3.183) > *Cupressus sempervirens var arizonica* (3.070) > *Ulmus pumila* (2.508) > *Salix alba* (0.916) > *Robinia pseudoacacia* (0.650) > *Fraxinus excelsior* (0.292)
Pb:	*Cupressus sempervirens var arizonica* (1346.680) > *Thuja orientalis*, (817.311) > *Ulmus pumila* (315.803) > *Acer hyracanum* (280.925) > *Populus nigra* (255.658) > *Robinia pseudoacacia* (201.950) > *Fraxinus excelsior* (162.483) > *Salix alba* (91.408)

Table 5. Comparison of heavy metal content among different sampled tree species in station SS1 using Kruskal Wallis test

	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Chi-Square	5.112	24.366	36.102	7.360	6.854	4.648	23.658	27.044	24.006
df	7	7	7	7	7	7	7	7	7
Asymp. Sig.	0.646	**0.001**	**0.000**	0.392	0.444	0.703	**0.001**	**0.000**	**0.001**

Table 6. Investigations screening for accumulator plants in different parts of the globe

Tree species	Heavy metal (mg/kg)									
	Ref.	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Acer hyracanum	1	3.650	102.700	218.520	5.680	4.310	87.830	322.100	9.550	441.400
Populus nigra	1	2.050	180.450	148.770	6.230	3.810	93.380	374.250	5.550	445.850
Ulmus pumila	1	3.000	59.850	185.100	5.930	3.710	372.430	419.000	4.600	423.850
Robinia pseudoacacia	1	2.900	53.050	197.600	0.450	0.010	194.130	202.050	1.900	501.750
	2	-	349.200	3087.000	-	-	20.810	89.910	3.700	206.200
	3	-	-	-	-	-	236.700	100.300	-	31.20
Fraxinus excelsior	1	1.150	75.700	114.120	5.430	3.810	83.680	101.200	0.800	246.500
Cupressus sempervirens var arizonica	1	5.350	240.150	356.950	6.480	2.910	93.080	223.550	6.500	3354.550
Thuja orientalis	1	1.300	39.100	465.820	7.130	4.760	130.280	445.750	7.350	1718.350
Salix alba	1	1.950	120.200	155.380	4.980	2.760	85.230	379.150	3.300	213.500
Platanus orientalis	4	2.400	-	-	0.3	4.200	14.500	76.800	-	4.500
Platanus sp.	5	0.661	-	319.411	-	-	25.391	-	-	13.748
Pinus sp.	5		-	-	-	-	-	-	-	14.447
Pinus Eldarica Medw	6	3.970	-	-	-	16.700	15.430	24.160	-	62.300

Sources: Present work (1), Çelik, *et al.*, 2005 (2), Serbula., 2012 (3), Pourkhabbaz *et al.*, 2010(4), Sawidis *et al.*, 2011(5), Kord *et al.*, 2010 (6).

CONCLUSION

Results of this research work indicates that the very high concentrations of heavy metals in stations SS2 and SS6 located inside the industrial complexes (NILZ & ZSIC) are certainly anthropogenic and industrial activity is the main cause of soil/plant pollution. The concentrations of certain related heavy metals used in these industries (Zn, Cd and Pb) decreases sharply in both soil/plants by increasing distance from the industrial complexes.

Based on the results, the best native accumulator plant for Mn, Zn and Cd is *Populus nigra*. *Thuja orientalis* is the best accumulator plant for Fe, and *Cupressus sempervirens var arizonica* is the best species for Pb accumulation. We suggest these species, as an effective native metal accumulator, for phytoremediation of heavy metals from polluted soils.

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