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 hyrcanum, 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 aesthetic solution (Cao & Ma, 2002; Mulligan, et al., 2001). However
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...
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\textsubscript{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\textsubscript{2}O\textsubscript{2} at 125\textdegreeC on a heater until the digestion mixture was evaporated (Khattak & Jabeen, 2012). 0.1M HNO\textsubscript{3} was added to digestion residue and the extract was filtered through Wattman filter paper No 42. The solution was diluted with HNO\textsubscript{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 - 931.780 for 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)

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

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

<table>
<thead>
<tr>
<th>Sampling stations</th>
<th>Heavy metal (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr</td>
</tr>
<tr>
<td>SS1</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Min</td>
</tr>
<tr>
<td></td>
<td>Max</td>
</tr>
<tr>
<td>SS2</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Min</td>
</tr>
<tr>
<td>SS3</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Min</td>
</tr>
<tr>
<td>SS4</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Min</td>
</tr>
<tr>
<td></td>
<td>Max</td>
</tr>
<tr>
<td>SS5</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Min</td>
</tr>
<tr>
<td></td>
<td>Max</td>
</tr>
<tr>
<td>SS6</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Min</td>
</tr>
<tr>
<td></td>
<td>Max</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Cr</th>
<th>Mn</th>
<th>Fe</th>
<th>Co</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square</td>
<td>7.474</td>
<td>35.980</td>
<td>30.828</td>
<td>28.826</td>
<td>30.619</td>
<td>34.691</td>
<td>103.120</td>
<td>90.586</td>
<td>86.368</td>
</tr>
<tr>
<td>df</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Asymp. Sig.</td>
<td>0.188</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tree species</th>
<th>Cr (mg/kg)</th>
<th>Mn (mg/kg)</th>
<th>Fe (mg/kg)</th>
<th>Co (mg/kg)</th>
<th>Ni (mg/kg)</th>
<th>Cu (mg/kg)</th>
<th>Zn (mg/kg)</th>
<th>Cd (mg/kg)</th>
<th>Pb (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer hyrcanum</em></td>
<td>Mean 1.325</td>
<td>68.158</td>
<td>156.143</td>
<td>2.160</td>
<td>1.470</td>
<td>39.616</td>
<td>223.658</td>
<td>3.183</td>
<td>280.925</td>
</tr>
<tr>
<td></td>
<td>Min 0.001</td>
<td>45.650</td>
<td>137.050</td>
<td>0.001</td>
<td>0.001</td>
<td>10.220</td>
<td>17.950</td>
<td>0.001</td>
<td>11.050</td>
</tr>
<tr>
<td></td>
<td>Max 3.650</td>
<td>102.700</td>
<td>218.520</td>
<td>5.680</td>
<td>4.310</td>
<td>87.830</td>
<td>322.100</td>
<td>9.550</td>
<td>441.400</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Populus nigra</em></td>
<td>Mean 0.861</td>
<td>147.125</td>
<td>110.568</td>
<td>1.555</td>
<td>0.635</td>
<td>27.391</td>
<td>291.750</td>
<td>4.166</td>
<td>255.658</td>
</tr>
<tr>
<td></td>
<td>Min 0.001</td>
<td>93.350</td>
<td>97.120</td>
<td>0.001</td>
<td>0.001</td>
<td>9.920</td>
<td>223.550</td>
<td>2.750</td>
<td>68.400</td>
</tr>
<tr>
<td><em>Ulmus pumila</em></td>
<td>Mean 0.950</td>
<td>49.800</td>
<td>164.001</td>
<td>2.335</td>
<td>1.378</td>
<td>83.483</td>
<td>242.508</td>
<td>2.508</td>
<td>315.808</td>
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<tr>
<td></td>
<td>Min 0.001</td>
<td>41.900</td>
<td>140.920</td>
<td>0.001</td>
<td>0.001</td>
<td>9.230</td>
<td>107.400</td>
<td>0.800</td>
<td>187.800</td>
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<tr>
<td></td>
<td>Max 3.000</td>
<td>59.850</td>
<td>185.100</td>
<td>5.930</td>
<td>3.710</td>
<td>372.430</td>
<td>419.000</td>
<td>4.600</td>
<td>423.850</td>
</tr>
<tr>
<td><em>Robinia pseudoacacia</em></td>
<td>Mean 0.583</td>
<td>31.391</td>
<td>125.860</td>
<td>0.175</td>
<td>0.002</td>
<td>39.416</td>
<td>113.283</td>
<td>0.650</td>
<td>201.950</td>
</tr>
<tr>
<td></td>
<td>Min 0.001</td>
<td>23.850</td>
<td>91.670</td>
<td>0.001</td>
<td>0.001</td>
<td>4.020</td>
<td>62.200</td>
<td>0.001</td>
<td>67.600</td>
</tr>
<tr>
<td></td>
<td>Max 2.900</td>
<td>53.050</td>
<td>197.600</td>
<td>0.450</td>
<td>0.010</td>
<td>194.130</td>
<td>202.050</td>
<td>1.900</td>
<td>501.750</td>
</tr>
<tr>
<td><em>Fraxinus excelsior</em></td>
<td>Mean 0.334</td>
<td>49.558</td>
<td>95.235</td>
<td>1.943</td>
<td>1.162</td>
<td>37.616</td>
<td>82.216</td>
<td>0.292</td>
<td>162.483</td>
</tr>
<tr>
<td></td>
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<td>79.750</td>
<td>0.001</td>
<td>0.001</td>
<td>9.470</td>
<td>62.700</td>
<td>0.001</td>
<td>71.200</td>
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<tr>
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<td>Max 1.150</td>
<td>75.700</td>
<td>114.120</td>
<td>5.430</td>
<td>3.810</td>
<td>83.680</td>
<td>101.200</td>
<td>0.800</td>
<td>246.500</td>
</tr>
<tr>
<td><em>Cupressus sempervirens var</em></td>
<td>Mean 2.100</td>
<td>66.020</td>
<td>217.282</td>
<td>1.376</td>
<td>0.582</td>
<td>41.366</td>
<td>120.660</td>
<td>3.070</td>
<td>1346.680</td>
</tr>
<tr>
<td><em>arizonica</em></td>
<td>Min 0.001</td>
<td>12.750</td>
<td>104.170</td>
<td>0.001</td>
<td>0.001</td>
<td>11.520</td>
<td>75.900</td>
<td>0.001</td>
<td>17.300</td>
</tr>
<tr>
<td></td>
<td>Max 5.350</td>
<td>240.150</td>
<td>356.950</td>
<td>6.480</td>
<td>2.910</td>
<td>93.080</td>
<td>223.550</td>
<td>6.500</td>
<td>3354.550</td>
</tr>
<tr>
<td><em>Thuja orientalis</em></td>
<td>Mean 0.358</td>
<td>33.000</td>
<td>308.652</td>
<td>2.963</td>
<td>1.643</td>
<td>31.448</td>
<td>271.266</td>
<td>4.138</td>
<td>817.311</td>
</tr>
<tr>
<td></td>
<td>Min 0.001</td>
<td>24.400</td>
<td>200.820</td>
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<td>0.001</td>
<td>1.380</td>
<td>98.400</td>
<td>2.350</td>
<td>215.950</td>
</tr>
<tr>
<td></td>
<td>Max 1.300</td>
<td>39.100</td>
<td>465.820</td>
<td>7.130</td>
<td>4.760</td>
<td>130.280</td>
<td>445.750</td>
<td>7.350</td>
<td>1718.350</td>
</tr>
<tr>
<td><em>Salix alba</em></td>
<td>Mean 0.542</td>
<td>56.633</td>
<td>124.436</td>
<td>1.155</td>
<td>0.460</td>
<td>37.178</td>
<td>214.450</td>
<td>0.916</td>
<td>91.408</td>
</tr>
<tr>
<td></td>
<td>Min 0.001</td>
<td>13.500</td>
<td>105.550</td>
<td>0.001</td>
<td>0.001</td>
<td>7.170</td>
<td>104.650</td>
<td>0.001</td>
<td>27.300</td>
</tr>
<tr>
<td></td>
<td>Max 1.950</td>
<td>120.200</td>
<td>155.380</td>
<td>4.930</td>
<td>2.760</td>
<td>85.230</td>
<td>379.150</td>
<td>3.300</td>
<td>213.500</td>
</tr>
</tbody>
</table>
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:**

**Fe:**

**Zn:**

**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:**

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

<table>
<thead>
<tr>
<th>Heavy metal (mg/kg)</th>
<th>Cr</th>
<th>Mn</th>
<th>Fe</th>
<th>Co</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square</td>
<td>df</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>24.366</td>
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<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Asymp. Sig.</td>
<td></td>
<td>0.646</td>
<td><strong>0.001</strong></td>
<td><strong>0.000</strong></td>
<td>0.392</td>
<td>0.444</td>
<td>0.703</td>
<td><strong>0.001</strong></td>
<td><strong>0.000</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tree species</th>
<th>Heavy metal (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ref.</td>
</tr>
<tr>
<td><em>Acer hyracanum</em></td>
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<td><em>Ulmus pumila</em></td>
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<td><em>Robinia pseudoacacia</em></td>
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</tr>
<tr>
<td>2</td>
<td>-</td>
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<tr>
<td>3</td>
<td>-</td>
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<tr>
<td><em>Fraxinus excelsior</em></td>
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<tr>
<td><em>Cupressus sempervirens var arizonica</em></td>
<td>1</td>
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<tr>
<td><em>Thuja orientalis</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Salix alba</em></td>
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<tr>
<td><em>Platanus orientalis</em></td>
<td>4</td>
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<tr>
<td><em>Platanus sp.</em></td>
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</tr>
<tr>
<td><em>Pinus Eldarica Medw</em></td>
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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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REFERENCES


Screening for accumulator plants


