



## Heavy metals accumulation in willows growing on Spolic Technosols from the abandoned Imperina Valley mine in Italy

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### ABSTRACT

Human activities such as metals mining and milling operations provide one of the most important sources of contamination in the environment. Abandoned mines can be an important source of toxic elements. The threat of heavy metal pollution posed by mine soils generally concerns more than one metal.

The aim of this study was to assess total concentration of six potentially toxic metals (Cd, Cr, Cu, Pb, Zn and Fe) in the soil and plant samples of three dominant willow species (*Salix purpurea* L., *Salix caprea* L. and *Salix eleagnos* Scop.) collected from abandoned mixed sulphide mine dumps (Imperina Valley, North-east Italy). Results demonstrate that metal concentrations in soils are in general above the Italian average limits and they are also significantly (except Cr), as compared with controls ( $p \leq 0.05$ ), with averages of 2.12 mg Cd kg<sup>-1</sup>, 2267 mg Cu kg<sup>-1</sup>, 9552 mg Pb kg<sup>-1</sup>, 1243 mg Zn kg<sup>-1</sup> and 299,973 mg Fe kg<sup>-1</sup>.

The phytoremediation ability of selected *Salix* species for heavy metals was estimated. The results have revealed significant differences among willow species ( $p \leq 0.05$ ) regardless of the species selected. The transfer factor and bioaccumulation coefficient of selected metals varied among plant species and from different sites. Some of the investigated species have potential for soil stabilization and extraction of heavy metals. The results indicate that there is an increasing need for further research projects mainly focused on the mechanisms whereby such willows are able to survive in contaminated soils.

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### 1. Introduction

Over the past decades, human activities such as metal mining and milling operations have been recognized as one of the most important sources of contamination in the environment, along with mine and mill waste water (Jung, 2001; Navarro et al., 2008). Heavy metal contamination has been one serious problem in the vicinity of abandoned mine sites due to the discharge and dispersion of mine-waste materials into the ecosystem (Jung and Thornton, 1996). These heavy metals have a potential to contaminate soil and water (Haque et al., 2008; Lim et al., 2008).

Extraction of metals from sulphide minerals usually results in large amounts of waste materials which often contain elevated concentrations of potentially harmful metals such as Cu, Zn, Cd, and Pb (Jian-Min et al., 2007; Lee et al., 2001). The degree of heavy metal contamination around mines varies depending upon geochemical characteristics and degree of mineralization of the tailing (Navarro et al., 2008). Yun-Guo et al. (2006) reported that abandoned mining

sites represent significant sources of metal pollutants in water and soils and a threat to the ecosystem. These metals can be transported, dispersed and accumulated in plants and then passed through the food chain to human beings as the final consumer.

There are several former mixed sulphide (mainly Cu, Fe, Pb, Zn) mining sites in Italy. Soils and plants growing on contaminated mine sites have been studied to determine the ability of these plants to accumulate metals and to grow on mine waste in the perspective of an ecological restoration of sites (Bini, 2012; Fontana et al., 2010; Wahsha et al., 2012). Recently, preliminary studies in the Department of Environmental Sciences of Ca' Foscari University of Venice have focused on the toxicity and influence of heavy metals in contaminated soils and in both edible and wild plants (Fontana et al., 2011; Wahsha et al., 2011).

However, such remediation technique strategies such as immobilization or extraction by physico-chemical techniques are too expensive and generally impractical (Zabludowska et al., 2009). Therefore, there is an increasing request to develop *in situ* low-cost technologies to have effective surface metal stabilization. The use of wild flora can be a useful tool, since there are some native plant species that can colonize parts of these polluted sites (Conesa et al., 2006). Recently, review articles on phytoremediation of metal enriched mine waste have been

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published (Mendez and Maier, 2008; Mukhopadhyay and Maiti, 2010), which highlight the use of plants to reduce, remove, degrade, or immobilize environmental pollutants.

In the last few years many studies have focused on the potential use of trees as a suitable vegetation cover for phytoremediation (French et al., 2006; Jensen et al., 2009). A very suitable tree for use in phytoremediation is willow (Landberg and Greger, 2002). Pulford and Watson (2003) detailed the phytoremediation potential of willow in heavy metal contaminated areas. Willows have not been included in the group of hyperaccumulators of heavy metals, but on the other hand they provide potential bioindicator of pollution (Mleczeek et al., 2009). However, metal concentrations in willows depend on species, growth performance, root density, distribution within the soil profile and sampling period (Chehregani, et al., 2009). Moreover, willow has been recently recognized as a good accumulator of heavy metals (Meers et al., 2007).

The aim of this study was to assess total concentration of six potentially toxic metals (Cd, Cr, Cu, Pb, Zn and Fe) in the soil and plant samples of three dominant willow species (*Salix purpurea* L., *Salix caprea* L. and *Salix eleagnos* Scop.) collected from abandoned mixed sulphide mine dumps (Imperina Valley, North-east Italy), in order to propose these plants for phytoremediation plans.

## 2. Materials and methods

### 2.1. Study area

The area of consideration in this study is an abandoned dumped mine site placed in the mountain region of Belluno (North-east Italy) with an altitude ranging between 543 m and 990 m above sea level, and oriented in the SW–NE direction along the Imperina creek valley (Fig. 1). The geological substrate consists of dolomite rocks (Dolomia Principale, Upper Triassic) on the right side of the valley and metamorphic basement (Pre-Permian) on the left side, while at the bottom, in unconformity with the previous geological formations, the calcareous–arenaceous complex of Werfen (Upper Permian–Lower Triassic) outcrops (Campana et al., 2007; Giordano, 2008). The vegetation cover is mainly composed of mixed forests (*Abies alba* Mill., *Picea abies* (L.) H. Karst., 1881, *Fagus sylvatica* L. and *Ostrya carpinifolia* Scop.), with clearances where herbaceous and shrubby vegetation prevail over the arboreal one. Part of the territory lies within the National Park of the Belluno Dolomites. The mineralized area, which is located along the tectonic contact between the metamorphic basement and the dolomite rocks, is a deposit of mixed

sulphides, composed primarily of cupriferous pyrite, pyrite and chalcopyrite, with minor amounts of other metallic minerals (Frizzo and Ferrara, 1994).

Copper and sulphur were the main products extracted. Since the beginning of the 15th century, and until the final closure in 1962, copper was extracted and processed directly *in situ* through roasting, a method with a severe impact on the area due to acid rain formation and intensive wood cutting (Campana et al., 2007), that left bare soils. Since that time, vegetation cover was naturally established, pedogenetic processes started again, and a new soil type – Spolic Technosol (WRB, 2006) – began to form.

### 2.2. Field survey, sampling and analysis

According to the procedures described by Hood and Jones (1997) and Margesin and Schinner (2005), five sites were selected (Fig. 1) according to homologous geological, morphological and pedological conditions, vegetation coverage and anthropogenic impact and the same pedoclimate conditions. Soil pits were open and described following national guidelines (Costantini, 2007). All locations were sampled for topsoil and plants in the period between spring and summer 2010.

Soil samples were collected from the upper horizon at a depth of 0–30 cm. Each soil sample was a composite of 5–7 subsamples collected in a given sector (4 m<sup>2</sup>). Samples were mixed, packed and transported to the laboratory. According to the procedures described by the manual of the Italian Ministry of Agriculture and Forestry published in 2000 and 1994, the soil samples were air-dried and sieved to 2 mm and the following parameters were determined: pH, carbonates, organic carbon, cation exchange capacity and soil particle size distribution.

At least five samples of selected willow species (at the early vegetative phase and normal morphological appearance) were sampled at each site with their corresponding soil clod (same pedoclimate). Samples were placed in plastic bags not completely closed with a nonmetallic closure, to allow gas exchange, and transported to the laboratory. Willow species were classified according to Pignatti (1982) as the following: *S. purpurea* L., *S. caprea* L., and *S. eleagnos* Scop. Plant samples were rinsed gently with tap and distilled water to remove the adhering soil, then were divided into leaves, stems and roots, dried in ventilated oven at 80 °C for two days. Dried tissues were grinded in an agate mill (<100 µm). For the analysis of pseudo-total metal content, 0.2 g of soil samples was subjected to a complete digestion in the microwave (model 1600-Ethos, Milestone)

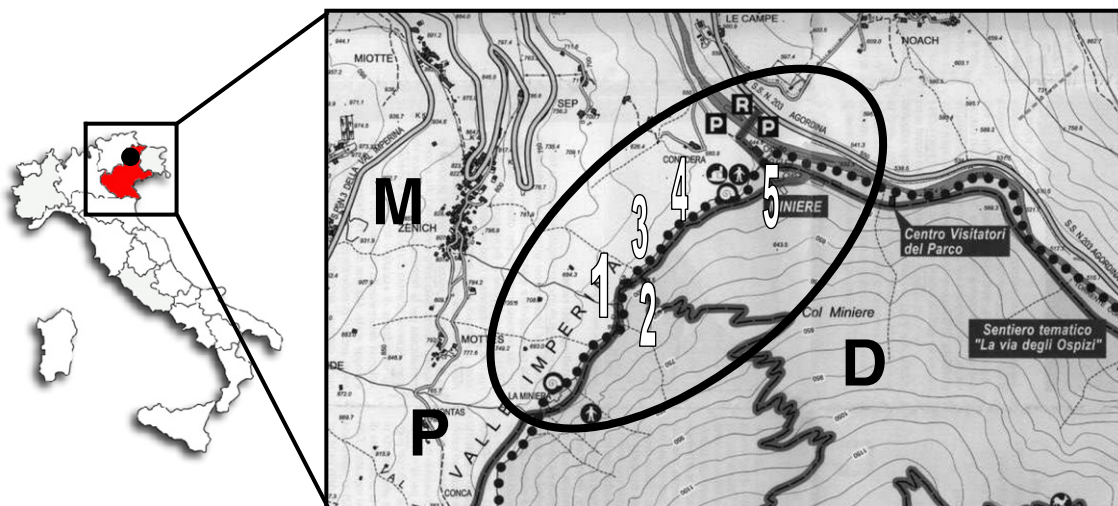


Fig. 1. Location of the studied area and sampling sites of Imperina Valley. M = metamorphic basement, P = phyllite, D = dolomite.

**Table 1**Selected chemophysical properties of the studied soils. All data expressed as mean values  $\pm$  S.D (n=3).

Sampling site	pH		<sup>a</sup> CEC ( $\text{cmol}_c \text{ kg}^{-1}$ )	Organic carbon ( $\text{g kg}^{-1}$ )	CaCO <sub>3</sub> ( $\text{g kg}^{-1}$ )	Particle size distribution %			Texture
	In water	In KCl				Sand	Silt	Clay	
1	5.30 $\pm$ 0.20	3.47 $\pm$ 0.20	15.2 $\pm$ 0.50	7 $\pm$ 0.30	<sup>b</sup> NM	35	48	17	Loam
2	8.00 $\pm$ 0.35	<sup>b</sup> NM	21.1 $\pm$ 1.00	16 $\pm$ 0.10	204 $\pm$ 9	40	47	13	Loam
3	7.10 $\pm$ 0.20	<sup>b</sup> NM	11.0 $\pm$ 0.60	10 $\pm$ 0.70	264 $\pm$ 14	65	20	15	Sandy loam
4	4.92 $\pm$ 0.41	3.56 $\pm$ 0.14	10.6 $\pm$ 1.40	7 $\pm$ 0.40	<sup>b</sup> NM	45	50	5	Silt loam
5	7.53 $\pm$ 0.30	<sup>b</sup> NM	21.3 $\pm$ 0.70	35 $\pm$ 0.40	175 $\pm$ 18	75	20	5	Sandy loam

<sup>a</sup> Cation exchange capacity (CEC).<sup>b</sup> NM = not measured.

in closed containers made of Teflon. The breakdown was performed in 5 mL of *aqua regia*, while 0.5 g of plant samples was digested in an acid mixture of 5 mL 65% HNO<sub>3</sub> and 3 mL 30% H<sub>2</sub>O<sub>2</sub> in open vessels on the hot plate, followed by filtration with filter cellulose Whatman n. 42. After digestion, both soil and plant samples were analyzed by flame atomic absorption spectrometry according to the method reported by Margesin and Schinner (2005).

### 2.3. Metal accumulation efficiency

To evaluate the metal accumulation efficiency in plants, the bioaccumulation factor (BCF) and translocation factor (TF) were calculated. BCF is defined as the ratio of metal concentration in the roots to that in soil and the TF is the ratio of metal concentration in the shoots to the roots (Malik et al., 2010). The definition of metal hyperaccumulation has to take in consideration not only the metal concentration in the above ground biomass, but also the metal concentration in the soil (Malik et al., 2010). Both BCF and TF have to be considered while evaluating whether a particular plant is a metal hyperaccumulator. Therefore, plants with both BCF and TF greater than one have the potential to be used in phytoextraction. Besides, plants with bioconcentration factor greater than one and translocation factor less than one have the potential for phytostabilization. A hyperaccumulator plant should have BCF > 1 or TF > 1, as well as total concentration of Cu, Co, Cr or Pb > 1000 mg kg<sup>-1</sup>, or of Fe, Mn or Zn > 10,000 mg kg<sup>-1</sup> in the aerial parts (Kabata-Pendias, 2011).

### 2.4. Statistical analysis

Statistical analysis was based on ANOVA and is presented as means  $\pm$  S.D. The statistical significance was declared when p value was equal to or less than 0.05. Statistical analyses were performed

using Sigma Stat statistical software version 3.5 for the calculation of ANOVA and KNIME<sup>tech</sup> software for the linear correlation coefficient.

## 3. Results and discussion

### 3.1. Chemical and physical analyses

Selected soil variables (pH, CEC, organic carbon content, total carbonate, soil particle size distribution and soil texture) were analyzed in all soil samples and a summary of these analyses is given in Table 1.

Many characteristics of the studied soils vary; for example, the pH values oscillate from about 5 to nearly 8, due to the nature of the soil parent material; the highly acidic pH value found in site 4 soil is probably due to weathering oxidation of iron sulphides (pyrite and chalcopyrite) in the soil and substrate, consistent with the findings reported by Bini (2012). *In situ* pH measurements between approximately 7.5 and 8.5 indicate the presence of carbonates as secondary copper mineral phases (malachite, azurite).

Another parameter in Table 1 is the CEC of the soil, which depends, besides organic matter, on the percentage of clay and on the clay mineralogy. The CEC is also closely related to soil texture; our results have shown generally low values of CEC for soil samples collected from sites 1, 3 and 4. Soil from site 5 was above the expected value 21.3  $\text{cmol}_c \text{ kg}^{-1}$ . In contrast, soils from sites 2 and 6 were within the normal range. The distribution of carbonates can vary greatly with the soil particles and one way of distinguishing the differences is in the location of the effervescence around the small insulated particles such as nodules (Pansu and Gautheyrou, 2006). Our results for soil calcium carbonate content ranged from 18% (site 5) to 27% (site 3). Moreover, the organic carbon content is generally low, with exception of soil from site 5. The soil texture is loamy (sites 1 and 2), sandy-loam (sites 3 and 5) or silt loam (site 4), and the soils from

**Table 2**Concentration of metals in soils of Imperina Valley. Cd, Cr, Cu, Pb, Zn and Fe are expressed as mg kg<sup>-1</sup>. All the values are mean of five replicates  $\pm$  S.D.

Sampling site	Cd	Cr	Cu	Pb	Zn	Fe
1	1.4 $\pm$ 1.2 i	11 $\pm$ 1 i	3511 $\pm$ 19 i	20,977 $\pm$ 69 i	1722 $\pm$ 24 i	491,263 $\pm$ 250 i
2	0.85 $\pm$ 0.5 i	31 $\pm$ 3 i	2822 $\pm$ 40 i	14,147 $\pm$ 95 i	1096 $\pm$ 11 i	320,437 $\pm$ 178 i
3	<DL <sup>d</sup>	113 $\pm$ 5 i	491 $\pm$ 28 i	196 $\pm$ 44 i	490 $\pm$ 6 i	61,087 $\pm$ 95 j
4	4.35 $\pm$ 1.1 i	14 $\pm$ 2 i	4098 $\pm$ 36 i	12,124 $\pm$ 56 i	2513 $\pm$ 13 i	578,632 $\pm$ 229 i
5	5.14 $\pm$ 0.7 i	81 $\pm$ 3 i	411 $\pm$ 42 i	314 $\pm$ 51 i	394 $\pm$ 10 i	48,446 $\pm$ 307 j
It. av <sup>1, a</sup>	0.53	100	51	21	89	37,000
Int. av <sup>2, a</sup>	0.30	200	20	10	50	–
E.V <sup>3, a</sup>	5	100	100	100	250	–
R.L <sup>4, b</sup>	–	150	120	200	150	–
C.S.T.C <sup>5, c</sup>	3–8	75–100	60–125	100–400	70–400	1000*

<sup>1</sup>Italian average, <sup>2</sup>International average, <sup>3</sup>Excessive values, <sup>4</sup>Residential limits, <sup>5</sup>Critical soil total concentration, <sup>a</sup>Reference average values (source: Angelone and Bini, 1992), <sup>b</sup>Threshold limits in the Italian legislation (D.L. 152/2006, Annex 5), <sup>c</sup>Certified reference material, critical soil total concentration (C.S.T.C) is the range of values above which toxicity is considered to be possible (source: Alloway, 1995), <sup>d</sup>Less than the detection limit. \*Source: Kabata-Pendias, 2011.

The letter symbol following  $\pm$  S.D within the same column indicates if there is a significant difference or not when compared to controls in site 6. i indicates significant difference at p < 0.05 and j indicates no significant difference according to ANOVA.

**Table 3**

Linear correlation coefficient calculated on the concentrations of metals in soils. Indicates correlation is significant at the 0.05 level (2-tailed).

	Cd	Cr	Cu	Pb	Zn	Fe
Cd	1					
Cr	0.201	1				
Cu	-0.209	-0.847	1			
Pb	-0.515	-0.816	0.867	1		
Zn	-0.248	-0.604	0.688	0.616	1	
Fe	-0.003	-0.754	0.933	0.734	0.440	1

all sites are classified as Spolic Technosols according to the World Reference Base for Soil (WRB, 2006).

### 3.2. Heavy metals accumulation in soils

Table 2 summarizes the results of the average of the total concentrations of Cd, Cr, Cu, Pb, Zn and Fe in the soils tested. Comparing the values found with those of reference levels, according to Angelone and Bini (1992), the total concentrations of most of the investigated metals in the soil samples were significantly higher (ANOVA  $p < 0.05$ ), and almost above the toxicity threshold according to the Italian legislation (D.L. 152/2006).

The area is almost not contaminated by Cr except site 3 which has a concentration above toxicity threshold, as reported by Wahsha et al. (2012), as it was  $113 \pm 5 \text{ mg kg}^{-1}$ . Instead, there is a contamination by Zn, Cu, Pb and Fe, which present very high concentrations, particularly at sites affected by intense mining activities (e.g. sites 1, 3, 5) and ore processing (site 4).

As shown in Table 3, there is a linear positive correlation between Pb, Cu, Zn and Fe (Cu/Pb 0.867; Pb/Zn 0.616; Cu/Zn 0.688; Cu/Fe 0.933, significant at  $p < 0.05$ ). This is consistent with their calcophilous behavior, since these metals tend to form compounds with sulphur, as chalcopyrite ( $\text{CuFeS}_2$ ), sphalerite ( $\text{ZnS}$ ) and galena ( $\text{PbS}$ ), commonly

found in the Imperina Valley ore deposits (Frizzo and Ferrara, 1994). Chromium is negatively correlated with Cu (-0.847), Pb (-0.816), Zn (-0.604) and Fe (-0.754). Conversely, Fe indicates a significant positive correlation with Pb (Fe/Pb 0.734). Furthermore, Fe it is not significantly correlated with Cd. Therefore, it is likely that most of the iron in soils of the study area derives from the alteration of pyrite and chalcopyrite mineralization. The same type of metal combination occurring in the mineralization of Imperina Valley is found in soil. This means that no element of the mineralization has been removed in a special way, since the factors of pedogenesis have only acted for some decades in the areas affected by ore processing, and thus the soil chemical characteristics still seem to be close to those of the parent material, as it was found by Bini et al. (2004) in mine soils of Tuscany.

### 3.3. Heavy metals accumulation in plants

The definition of metal hyperaccumulation has to take in consideration not only the metal concentration in the above ground biomass, but also the metal concentration in the soil (Malik et al., 2010). Both BCF and TF have to be considered while evaluating whether a particular plant is a metal hyperaccumulator. Therefore, plants with both BCF and TF greater than one have the potential to be used in phytoextraction. Besides, plants with bioconcentration factor greater than one and translocation factor less than one have the potential for phytostabilization. A hyperaccumulator plant should have  $\text{BCF} > 1$  or  $\text{TF} > 1$ , as well as total concentration of Cu, Co, Cr or Pb  $> 1000 \text{ mg kg}^{-1}$ , or of Fe, Mn or Zn  $> 10,000 \text{ mg kg}^{-1}$  in the aerial parts (Kabata-Pendias, 2011). The concentrations of heavy metals in willow species of Imperina Valley are presented in Table 4. Willow plants (genus *Salix*) accumulated significant quantities of heavy metals in both leaves and roots, irrespective of the species and the obtained results are in agreement with data from literature (Meers et al., 2007; Sander and Ericsson, 1998). None of the willow plants collected during sampling showed visible signs of toxicity. Willows are able to tolerate and accumulate Cd in the root system in agreement with Landberg and Greger (2002) and Landberg et al.

**Table 4**

Concentration of heavy metals in *Salix* spp. ( $\text{mg kg}^{-1}$  dry weight). All the values are mean of five replicates  $\pm$  S.D.

Site	Plant		Cd	Cr	Cu	Pb	Zn	Fe
1	<i>S. purpurea</i>	L <sup>a</sup>	4.2 ± 1.4	3.51 ± 0.13	30.1 ± 0.2	29.1 ± 0.9	192 ± 11	731 ± 17
		S <sup>b</sup>	3.9 ± 0.1	2.40 ± 0.30	11.1 ± 0.2	<DL <sup>c</sup>	146 ± 12	93 ± 21
		R <sup>d</sup>	6.1 ± 0.3	2.92 ± 0.58	32.7 ± 0.4	11.3 ± 1.17	112 ± 10	250 ± 16
1	<i>S. eleagnos</i>	L <sup>a</sup>	<DL <sup>c</sup>	4.51 ± 0.09	30.0 ± 1.0	29.1 ± 3.4	433 ± 20	512 ± 6
		S <sup>b</sup>	1.1 ± 0.2	1.44 ± 0.13	22.0 ± 3.0	4.9 ± 4.1	283 ± 11	100 ± 31
		R <sup>d</sup>	2.5 ± 0.5	4.20 ± 0.17	43.0 ± 5.0	37.2 ± 2.1	118 ± 21	459 ± 7
2	<i>S. eleagnos</i>	L <sup>a</sup>	<DL <sup>c</sup>	5.32 ± 1.3	34.8 ± 1.8	39 ± 4	467 ± 8	490 ± 22
		S <sup>b</sup>	<DL <sup>c</sup>	3.11 ± 3.5	22.7 ± 7.0	13 ± 3	263 ± 5	83 ± 7
		R <sup>d</sup>	3.2 ± 0.3	5.10 ± 1.3	40.4 ± 5.0	42.9 ± 1	272 ± 6	400 ± 3
3	<i>S. purpurea</i>	L <sup>a</sup>	<DL <sup>c</sup>	3.31 ± 1.43	25.0 ± 0.8	25.6 ± 1.2	189 ± 8	700 ± 31
		S <sup>b</sup>	<DL <sup>c</sup>	2.69 ± 0.90	11.1 ± 0.6	9.1 ± 0.3	86 ± 1	112 ± 20
		R <sup>d</sup>	<DL <sup>c</sup>	3.11 ± 0.58	17.3 ± 0.1	<DL <sup>c</sup>	110 ± 3	319 ± 22
3	<i>S. eleagnos</i>	L <sup>a</sup>	<DL <sup>c</sup>	3.29 ± 3.1	29.0 ± 3.4	46.1 ± 6.0	500 ± 1	479 ± 9
		S <sup>b</sup>	<DL <sup>c</sup>	1.54 ± 2.00	19.0 ± 4.2	12.4 ± 1.6	146 ± 6	81 ± 11
		R <sup>d</sup>	<DL <sup>c</sup>	4.15 ± 1.41	40.1 ± 1.0	66.7 ± 2.1	291 ± 7	351 ± 6
4	<i>S. purpurea</i>	L <sup>a</sup>	1.5 ± 0.7	3.60 ± 0.69	28.8 ± 1.9	22.1 ± 7	312 ± 41	626 ± 7
		S <sup>b</sup>	0.18 ± 0.1	2.01 ± 1.7	11.0 ± 0.7	<DL <sup>c</sup>	<DL <sup>c</sup>	39 ± 3
		R <sup>d</sup>	5.4 ± 0.9	2.91 ± 1.43	20.0 ± 1.0	<DL <sup>c</sup>	66 ± 3	183 ± 1
4	<i>S. caprea</i>	L <sup>a</sup>	1.9 ± 0.2	2.71 ± 0.63	32.0 ± 0.2	127 ± 5	211 ± 5	959 ± 8
		S <sup>b</sup>	<DL <sup>c</sup>	4.50 ± 0.73	14.0 ± 1.8	84.4 ± 81	210 ± 2	169 ± 4
		R <sup>d</sup>	5.9 ± 1.6	2.41 ± 1.0	33.7 ± 1.3	519 ± 11	211 ± 7	532 ± 7
5	<i>S. eleagnos</i>	L <sup>a</sup>	3.4 ± 1.3	2.10 ± 1.31	31.7 ± 1.3	31.5 ± 3.4	581 ± 4	611 ± 7
		S <sup>b</sup>	0.21 ± 0.3	2.22 ± 0.64	33.2 ± 5.8	14 ± 5.1	450 ± 8	88 ± 6
		R <sup>d</sup>	3.8 ± 0.9	2.09 ± 0.95	121.0 ± 1.0	37.3 ± 2.1	311 ± 3	368 ± 9
5	<i>S. caprea</i>	L <sup>a</sup>	1.6 ± 1.4	3.95 ± 2.3	35.5 ± 11	176.6 ± 8	389 ± 8	843 ± 2
		S <sup>b</sup>	<DL <sup>c</sup>	4.56 ± 1.33	<DL <sup>c</sup>	127 ± 7	<DL <sup>c</sup>	201 ± 6
		R <sup>d</sup>	2.4 ± 0.6	3.79 ± 2.08	46.4 ± 4.3	519 ± 10	148 ± 21	710 ± 14

<sup>a</sup> Leaves.

<sup>b</sup> Stem.

<sup>c</sup> Below detection limit.

<sup>d</sup> Root.

(2011). Chromium presents concentrations below the phytotoxicity threshold reported by Kabata-Pendias (2004); this is consistent with concentration levels recorded in the soil (Table 2). Iron concentrations in willow plants showed a large range of variation: between 39 and 959 mg kg<sup>-1</sup>. However, this metal is not considered toxic unless it is present at very high concentration in the soil (above 1000 mg kg<sup>-1</sup> according to Kabata-Pendias, 2011). Copper concentrations in both leaves and roots of willow are above the toxicity threshold (Kabata-Pendias, 2004). Willows proved to have the ability to accumulate Pb in roots more than in the aerial parts, and in the leaves more than in the stems, with the exception of *S. purpurea*, where Pb is accumulated in leaves. Regarding Zn, our results show that the highest concentrations are recorded in *Salix* leaves, and decrease gradually from stems to roots, counteracting the Cd concentration trend. Zinc concentrations in *Salix* exceed the toxicity level recommended by Kabata-Pendias (2004).

It is noteworthy to point out, however, that willows' ability to accumulate heavy metals in different parts is independent of the species; rather, it depends on local factors as soil and pedoclimate (particularly temperature, aeration and water content) and on plant physiology and aging (Wahsha et al., 2012). Moreover, a counteracting behavior of essential and toxic heavy metals is likely to occur as a barrier effect of the roots (Fontana et al., 2010).

The calculation of translocation factors highlights that willows translocate and accumulate metals in the aerial parts, in particular Cu (*S. purpurea* TF<sub>Cu</sub> = 4.72), Pb (*S. purpurea* TF<sub>Pb</sub> = 3.42), Zn (*S. caprea* TF<sub>Zn</sub> = 3.48), Fe (*S. purpurea* TF<sub>Fe</sub> = 1.44), and Cr (*S. purpurea* TF<sub>Cr</sub> = 1.24). Most of the plant species had BCF less than one and TF more than one, although the concentration of heavy metals remained below 1000 mg kg<sup>-1</sup>. In general, metal concentrations in plants vary with plant species; plant uptake of heavy metals from soil may occur either passively with the mass flow of water into the roots, or through active transport from root cells (Kabata-Pendias, 2004; Mun et al., 2008).

In this study, the metal translocation ability, combined with rapid growth and a higher biomass than herbaceous plants, qualifies willows as good candidates for phytoremediation of polluted soils (Bini, 2007). Since most of the studied willows were capable to uptake and translocate more than one metal from roots to shoots and, based on highest TF value, they can be used for phytoextraction. On the other hand, Cd shows very low translocation factors in all investigated plants, and proved to be blocked in the roots, since it is known to be unessential to plants, thereby suggesting some exclusion strategy by plants (Vandecasteele et al., 2002).

#### 4. Conclusion

Anthropic influence related to mining activity in soils of the studied area is evident. Soils in the mining site are highly contaminated by heavy metals, mainly Cu, Zn, Pb and Fe. The metal content in willows showed relatively high concentrations of these elements. The results of this study indicated that there is an increasing need for further research on the mechanisms whereby such plants are able to survive in contaminated soils. Furthermore, studies should aim to determine the growth performance, biomass production and metal accumulation of these species in metal contaminated soils for their better management and conservation.

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