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Potentially toxic element contamination in waste rocks, soils and wild flora at the Roşia Montană mining area (Romania)

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Abstract

The relationship between waste rocks and trees growing on the Hop waste-rock dump from the Roşia Montană gold mine (Romania) and also on soils along Roşia river were investigated. Plant samples, consisting in leaves, branches and roots of *Salix* spp., *Popolus tremula*, *Betula pendula*, *Pinus sylvestris* species, and rhizospheric soils of the same species, were analyzed for Zn, Cu and As. Total element concentrations were determined by ICP-AES. Bioaccumulation Factor (BF) and Translocation Factor (TF) were also calculated for the plant data set. Hop waste-rock dump is a highly variable substrate, characterised by stressful conditions for plant growth such as acid pH values and strong As contamination (mean 80 mg kg^{-1}), whereas Cu and Zn contents do not exceed European law limits for industrial sites. Data confirm that *Salix* spp., *B. pendula* and *P. tremula* are able to tolerate such limiting conditions, accumulating Cu and Zn, but excluding As; conversely, *P. sylvestris* acts as a strong excluder species. PTEs were detected also in soils along Roşia river and in relative vegetation (*Alnus glutinosa*), where acid sulphate waters, with pH values as low as 3, continuously flow out from the main mine adit. Concentration of PTEs decreases progressively towards the point of confluence of Roşia river with the Abrud river. *A. glutinosa* acts as a strong excluder species. The results obtained can be the basis to direct further studies in this area where reforms and re-opening of mining are planned.

Key words: Potential Toxic Elements (PTEs); metal-tolerant tree; gold mine; Roşia Montană; Romania.

Introduction

Mining activities are well known for their negative effects on the environment, due to the deposition of large volumes of waste-rocks and tailings on the land surface, which are often composed by sliding materials and represent a source for Potentially Toxic Elements (PTE), mobilization. These elements can have a negative impact on the environment, as they have a long-term persistence and tend to bioaccumulate in the food chain (Lacatasu et al., 1996) becoming harmful to humans and animals (Florea and Busselberg, 2006). The production of Acid Mine Drainage (AMD), as the result of the oxidative dissolution of sulphides and the consequent release of hazardous elements in the environment represents the main pollution concern of mining areas with sulphide-rich residues. AMD affects ecosystems in numerous and interactive ways, whose effects can be categorized as chemical, physical, biological and ecological (Gray, 1997).

A waste rock site at Roşia Montană, the largest gold deposit in Europe, was recently studied for estimating the mineralogical and chemical features of the earth material with a geostatistical approach applied to the data set (Servida et al., 2013).

The investigated East Hop dump consists of heterogeneous material composed by two different rock types, randomly accumulated: the first type consists mainly of dacite fragments and the second one of andesitic breccias. At present, the entire waste rock dump is a source of acid drainage; in terms of AMD features the andesitic breccias materials have ANC > MPA and are therefore able to buffer the production of acidic waters, whereas the dacite fragments materials are characterised by nil ANC (Acid Neutralizing Capacity) and positive MPA (Maximum Potential Acidity) thus resulting an active source of AMD. In general, the concentrations of Cu and Zn into the waste rocks are below the regulatory limits (Carlon, 2007), whereas As concentrations are up to 10 times higher than those imposed by law (Servida et al., 2013).

Plants have a remarkable skill to absorb and accumulate inorganic compounds from the substrate. Some trace elements, such as Cu and Zn, are essential nutrients for plants, becoming environmental pollutants and phytotoxic when they reach threshold concentrations in the substrate (> 300 mg kg^{-1} Zn; 25-40 mg kg^{-1} Cu). On the other hand, arsenic is toxic even at very low concentrations (> 20 mg kg^{-1}, depending on soil properties; Kabata-Pendias, 2001 and references therein).

Tree species are generally not able to adapt to high concentrations of PTE's in soils. However, few metal-tolerant ecotypes belonging to the genetically highly variable Salicaceae family (e.g. *Salix* L. spp. and *Populus* species) became adapted to ecological niches such as nutrient-poor, dry or wet and metal-contaminated environments (Dickinson and references therein, 2000, Stoltz and Greger, 2002; Chang et al., 2005; Unterbrunner et al., 2007). As a consequence, Salicacee represent a rich resource for identification of species with high metal resistance and high or low metal uptake in field (Stoltz and Greger, 2002; Chang et al., 2005;

Unterbrunner et al., 2007). Other common species growing on disturbed areas and mining spoils, as birch (e.g. *Betula pendula* Roth), and pines (e.g. *Pinus sylvestris* L.), are able to grow on these sites thank to the symbiosis with ECM fungi (Kopponen et al., 2001; Adriaensen et al., 2006). Finally, alder species (e.g. *Alnus glutinosa* (L.) Gaertn) are pioneer trees and shrubs highly adaptable to a wide range of ecosystems, improving soil fertility by fixing atmospheric nitrogen in natural and disturbed soils (Hibbs and Cromack, 1990) and establishing symbiosis with arbuscular mycorrhizal fungi (AMF) (Belangera et al., 2011).

Differences in metal uptake and translocation properties of some tree species were observed between plants grown in field and in hydroponic conditions (Stoltz and Greger, 2002), probably due to processes occurring at the soil-root interface. As a matter of fact, some important soil parameters such as pH values, abundance of fine grained fraction (< 0.02 mm), mineral reactivity, organic matter and microorganisms, control trace element content, bioavailability and behaviour (Kabata-Pendias, 2001). The pH value is a very important geochemical variable, as it also affects the plant responses to metal toxicity. The value 6.5 is commonly recognised as the optimum for nutrient availability, corresponding also to a low availability of most PTE, with the exception of As that becomes more mobile at this pH. Also the mineralogical composition of the soil and the relative resistance to weathering of the mineral species affect the mobility of PTEs thus influencing their bioavailability (Bani et al., 2014). These factors become extremely important in highly reactive environments, such as those occurring within sulphide-bearing waste rock dumps, where reaction rates can be exacerbated by the circulation of aggressive acidic solutions (Marescotti et al., 2008; 2010).

Cu, Zn and As contamination affected both the Hop waste-rock dump and the valley of Roşia river, which collects all the mine waters (surficial and underground) of the mine area. These PTEs were investigated by bio-geochemical analyses in waste-rocks, natural soils and plant organs of willow - *Salix* spp., birch - *Betula pendula*, poplar - *Populus tremula*, pine - *Pinus sylvestris* and black alder - *Alnus glutinosa* from the mine site, in order to evaluate the plant-substrate relationships.

Site overview

The Roşia Montană gold mine (Roşia Valley, Romania, N46.29694, E23.12063) is located at an altitude between 770 and 920 m a.s.l., in the Alba county, 16 km east from Campeni village and 128 km south-west from Cluj Napoca. This mine area is one of the largest gold deposits in Europe, located in the Apuseni Mountains of Transylvania, within a historic mining district of about 550 km^2 known as “Golden Quadrilateral”. Since ancient times, Roşia Montană has been well known for its goldbearing ore deposit, hosted in andesites and dacites of Neogene age piercing the pre-volcanic sedimentary basement as breccia pipes (Rosu et al., 2004). They host polymetallic sulphides and Au-Ag-Te mineralisation, present in epithermal veins, mineralised phreatomagmatic breccias and stockworks (Wallier et al., 2006). Underground gold extraction was performed since Roman times, but major open pit extraction began in 1972. Currently the Roşia Montană mine is characterised by the presence of two open pits called Cetate and Carnic ($163x10^6$ and $227x10^6$ tons of material exploited, respectively); in addition, there are more than 100 km of underground tunnels on a vertical span of 300 m, spreading on several adits. This exploitation has generated a large amount of mining wastes and tailings (about $256x10^6$ tons of mining-waste material), piled up on-site into two main waste-rock dumps (Hop and Valea Verde), several minor deposits and two tailing stockpiles. Mine works continued until 2006. The mining area is characterised by extensive AMD processes and heavy metal contamination, which affect also the surrounding streams (Florea et al., 2005). Acid sulphate waters, with pH as low as 3, continuously flow out, with a high flow rate (14.2 l/s; RMGC, 2006), from the lowest gallery ("Sf. Cruci din Orlea"; 714 m a.s.l.), and after a few tens of meters enter the Roşia River, where the pollution effects affect water for many kilometers downstream, until its confluence with Abrud River. A summary of features of the studied area is shown in Figure 1.

Climatic features

The mean annual rainfall in the area is 739 mm, with a minimum during November and February and a peak in July. In addition, the average snowfall is 64 cm y^{-1}, equivalent to about 64 mm y^{-1} of rain, that, added to the rain, gives a total of 803 mm y^{-1}. The average annual temperature is 5.7 °C with a minimum in January (-4.7 °C) and a maximum in August (16.9 °C). These data are referred to Rotunda meteorological station, located 2 km northward of Roşia Montană mine, and they were collected from 1982 to 2005.

Hydrological features

Roşia Montană mining district is located near the headwaters of Corna, Saliste and Roşia streams, all flowing towards Abrud River, a tributary of Aries River. The Corna Valley watershed drains southwest towards the Abrud River just upstream of both Abrud and Sălişte towns; Roşia Valley watersheds flow west to the Abrud River further downstream (Bird et al., 2005).

This area is characterised by moderately steep mountainous terrain ranging from 700 to 1,000 m a.s.l. The main source of groundwater recharge at the mine site is rainwater. The streams gain groundwater as the result of the low permeability of the geologic units and convergent groundwater flow. The average flow rates for the Roşia Valley, Corna Valley and Saliste Valley streams for the period 2001 to 2003 were 0.16, 0.07 and 0.16 m^3s^{-1}, respectively (RMGC, 2006). These watercourses comprise a portion of the Aries watershed and the tributary headwaters that form a part of the hydrographical Danube Basin.

Materials

Hop waste-rock dump

The eastern side of the Hop waste-rock dump

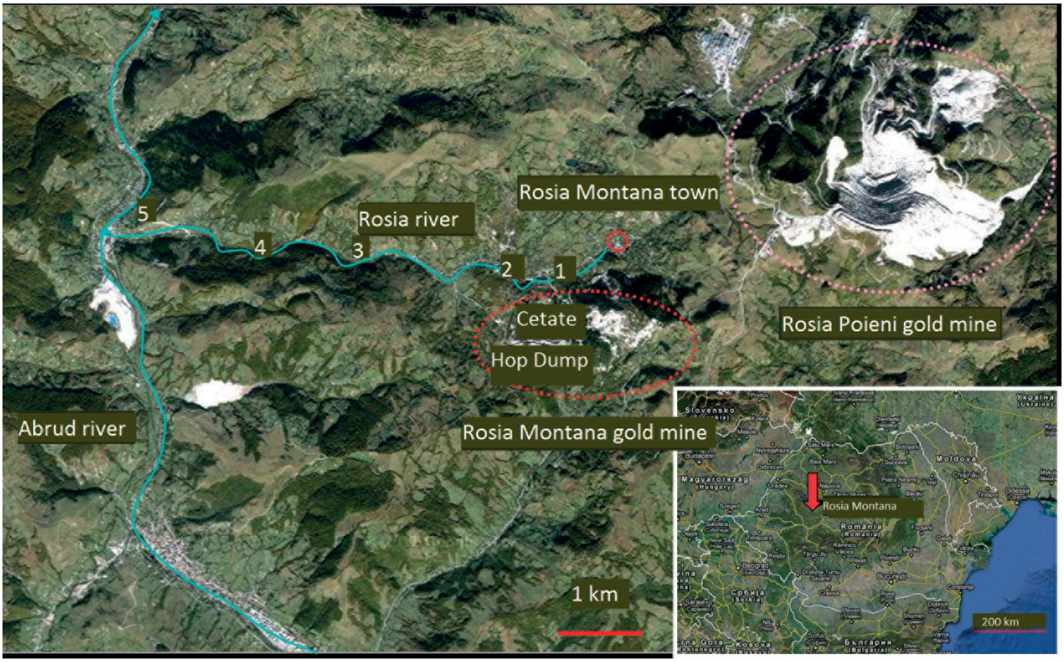

Figure 1. Satellite view of the Roşia Montana mining area. The Hop waste-rock dump is highlighted by the dashed red line together Cetate open pit. Numbers along the course of Roşia river indicate the sites of sampling.

was chosen for this investigation because it is a plain easy-accessible area (2.5 ha), made up by waste-rocks coming from Cetate open pit and piled up between 1998 and 2000. This material is very heterogeneous both for grain size, mineralogy and lithotypes. The clasts of the waste materials are mainly composed by dacite, andesitic breccia and polygenic breccia, with different mineralogy and bulk chemistry composition. These waste-rocks generate AMD, when the balance between the acid producing sulphides (mainly pyrite) and the acid neutralizing carbonates (mainly calcite) is positive; this condition commonly occurs within the dacite-rich part of the dump (Servida et al., 2013).

The dump is colonised by few individuals of pioneer native species, as *Salix* spp., *B. pendula* and *P. tremula* which grow on the central area and on its slope. *P. sylvestris* is also present, but it grows only on the dump edge, near the wood surrounding the area.

Roşia Valley riverbanks

Roşia Valley is characterised by natural riverbanks, colonised mainly by *A. glutinosa* and is surrounded by small villages and crop fields. The valley collects waters from the underground network of tunnels, all hydraulically connected. These latter act as drain channels for the upper part of the valley and discharge to the surface in a point located at an elevation of 714.6 m (i.e. at the adit of the “Sf. Cruci din Orlea” gallery, located about 1 km far from Hop waste-rock dump). In this valley, waste-draining waters represent the main contamination sources of Roşia stream, displaying both a strongly acidic character (pH values 2.7-3.0) and elevated contents of metals (RMGC, 2006). Owing to the high flow rate of mine waters and to the naturally low flow rate of Roşia stream, the dilution factor is low, thus leading to the contamination of the waters downstream the discharge points (Forray and Hallbauer, 2000).

Methods

Sampling (waste rocks, soils and plants)

A sampling of the eastern side of the Hop waste-rock dump was performed, because it is the portion of the dump most exposed to the atmospheric agents, hence the most interested by AMD processes. Moreover, the physical erosion by rain-wash and local landslides continuously expose new surfaces enabling higher chemical weathering to occur.

The sampled material (mainly represented by waste rocks with scarce fraction of natural soil) was performed at 50 cm from topographic surface using a 25 m squared grid, covering an area of about 2.5 ha. About 2 kg of 25 samples were collected for the geochemical characterization of the dump (Servida et al., 2013). Among these, 8 samples were collected in the area colonised by plants in correspondence with more surficial roots. The stratigraphy of the site is not known, therefore the relationship between rock type and vegetation was carried out only by the evidence of sampling. Along the Roşia Valley, up to 5.5 km from the "SF Cruci din Orlea" adit (i.e., up to the confluence of Roşia river into the Abrud river), ten soil samples were collected in 5 sites at different distances from the Roşia river (from 1 to 10 m). Sampling was difficult because the area is intensively degraded. One soil sample, representing the local background value, was sampled 25 m upstream from the "SF Cruci din Orlea" adit in a meadow forage where no evidences of contamination were present.

Waste rocks and soils samples were air-dried for two weeks and then were passed through a 2-cm sieve to obtain a weight of about 250 g at a grain-size < 2 cm; they were then dried at room temperature, mixed, homogenized and sieved through a 2 mm net. The sieved fraction was grounded in an agate jar to obtain a fine powder for subsequent geochemical analyses.

Moreover, plant aboveground (leaves and branches) and belowground (roots) biomass were collected in the same substrate sampling places. For each specimen leaves branches and, when possible, roots were collected.

In particular, on the Hop waste-rock dump were collected: 5 specimen belonging to *Salix* spp. (leaves and branches) growing mainly on dacitic -WR1- (4 samples) but also on andesitic -WR2- (1 sample) waste rocks, 2 samples belonging to *P. tremula* (leaves, branches and roots), 2 samples of *B. pendula* (leaves and branches) growing only on WR1 substrate and one of *P. sylvestris* (leaves) at the edge of the dump.

Along the Roşia Valley, 10 plant specimen of *A. glutinosa* were collected.

All samples were collected in Summer, between late August and early September, during the vegetative season, when metal accumulation in leaves is supposed to be highest (Pulford and Watson, 2003 and references therein; Bani et al., 2014).

Chemical analyses

To remove any soil particles, the plant material was firstly thoroughly washed with tap water, and then cleaned with distilled water. Subsequently, samples were oven-dried at 60 °C for 2 days, homogenized and finally powdered with an electric crusher.

The pH and Electrical Conductivity (EC) of the earth materials were determinate by a WTW Multiline P3 Set after suspending the soil in distilled water (ratio soil:water = 1:2.5).

For PTE (Cu, Zn and As) analysis:

- earth material samples were first grinded < 60 μm and then digested with Aqua Regia (6 ml 30% HCl Merck Suprapur and 2ml 65% HNO_3 Merck Suprapur) in a closed microwave oven (Milestone 1200 Mega);
- plant samples were digested in a 7 ml 65% HNO_3 Merck Suprapur and 3 ml 30% H_2O_2 Merck Suprapur mixture in a closed microwave oven (Milestone 1200 Mega);
- both earth material and plant samples were then accurately filtered and diluted with Milli-Q water before the analysis.

Cu and Zn concentrations were determined by ICP-AES (Jobin Yvon JY24) directly in solution, while As was measured using the hydride method. Calibration for As was done with the standard addition method. Element concentrations were measured in triplicate; the percentage coefficients of relative standard deviation were below 10% and the detection limits were of 1 mg kg^{-1}.

Plant parameters

For all species the Bioaccumulation Factor (BF) and the Translocation Factor (TF) were calculated to identify the biogeochemical behaviour of a plant species. The BF is defined as the ratio between the trace-element concentration in the aboveground plant biomass (leaves) and the one in the soil; its value is > 1 in accumulators and < 1 in excluders. The TF indicates preferential partitioning of metals inside plant organs and is defined as the ratio between metal concentrations in the leaves and in the roots (Kabata-Pendias, 2001). By comparing these two parameters, we can define the ability of different plant species to take up metals from the substrate and transport them to the shoots or stabilise and immobilise them in soils. Excluder plants tend to restrict soil-root and root–shoot transfers and, therefore, do not accumulate metals in their aboveground biomass (Baker, 1981).

Results and discussion

Hop waste-rock dump and strategies of metal uptake by trees

Waste rocks of the dump can be distinguished in two groups showing different composition particularly for pH, EC, AMC parameters and for Zn and As among the PTE (Table 1), according to the different bulk chemistry and mineralogical composition of the waste-rocks (Servida et al., 2013). The area is characterised by low Cu and Zn contents, whose mean concentrations reach the values of 31 and 49 mg kg^{-1} for the dacite-rich waste-rocks (WR1), and 48 and 105 mg kg^{-1} for andesitic breccias-rich waste rocks (WR2), respectively. On the other hand, As average concentration reaches a potentially toxic level of 120 mg kg^{-1} in WR1 samples, with a maximum value of 230 mg kg^{-1} over the maximum European law limit for industrial sites (200 mg kg^{-1}; EU Directive 1998/83/CE). Although acid pH values and strong As contamination (70-230 mg kg^{-1}) represent stressful conditions for plants, the considered tree species growing on Hop waste-rock dump seem to be able to tolerate these limiting conditions, with the exception of *P. sylvestris*.

Table 2 summarises the variation ranges of some chemical parameters of waste rocks in relation with plant species, highlighting that the tree species mainly prefer the acid environment of the dacitic waste rocks, with respect to the slightly alkaline conditions of the andesitic breccias materials.

The different contents of As, Zn and Cu in organs of the considered trees are shown in Figure 2.

On the Hop waste rock dump these trees act as pioneer species, which grow regardless on

Table 1. Geochemical features of the Hop waste-rock dump materials and of natural soils along the Roşia river.

	Cu (mg kg^{-1})	Zn (mg kg^{-1})	As (mg kg^{-1})	pH	EC (µS cm^{-1})	S%	ANC (kg $H_2SO_{4/t}$)	NAPP (kg $H_2SO_{4/t}$)
				HOP DUMP - waste rocks				
WR1 mean n=17	31.36	48.91	121.47	4.37	6047.53	1.16	-4.08	39.71
WR2 mean n=8	48.25	105.23	61.70	8.02	980.25	0.78	80.76	-56.78
				ROSIA VALLEY - soils				
Background	463	107	56	5.8	54			
Site 1	112	263	68	5.8	235			
Site 2	776	627	81	6.5	273			
Site 3	102	207	60	4.6	425			
Site 4	85	179	39	7	203			
Site 5	81	169	36	6.2	167			
mean (n=10)	157.62	236.22	53.84	5.85	240.5			
st dev	218.34	146.01	17.40	0.93	132.36			

WR1 = dacite-rich waste-rocks; WR2= andesite-rich waste-rocks (Servida et al., 2013).

Table 2. Relationship between plant species and geochemical features of substrate on which plants were sampled: data are expressed as range of variation.

Plant species	pH	EC (μS cm^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	As (mg kg^{-1})
			HOP DUMP		
Salix spp	3.0 - 7.7	111 - 1890	12 - 55	10 - 85	73 - 166
B. pendula	4.7 - 7.5	250 - 920	21 - 32	32 - 43	148 - 231
P. tremula	4.7 - 5	110 - 250	18 - 21	10 - 43	112 - 231
P. sylvestris	7.3 - 7.4	1350 - 1960	38 - 41	97 - 91	58 - 77
			ROSIA VALLEY		
A. glutinosa	4.5 - 6.5	54 - 220	46 - 123	107 - 274	31 - 63

substrates characterised by different pH of 3.0-7.7 and different conditions of AMD. Geochemical analyses performed on willow aboveground biomass show that Zn is stored mainly in leaves, where it reaches the highest values of 300-630 mg kg^{-1} (see Table 3 and Figure 2), exceeding toxicity levels of this metal in plants. On the other hand, Cu seems to be preferably stored in branches (17-63 mg kg^{-1}) than in leaves (21-44 mg kg^{-1}). Both results are in accordance with Nissen and Lepp (1997), who observe a general trend of exclusion of Cu by different *Salix* species. Arsenic seems to have no target organs in the aboveground tree biomass.

The relationship between the pH values of the waste rock and the PTE concentrations in leaves are shown in Figure 3: in the leaves of *Salix,* Zn and Cu have a low and fairly constant concentration independent by pH values, for which the species show great variability and adaptability; on the other hand there is a clear correlation between pH and As values. Conversely, As occurring in oxidizing environments (As^{V}) forms stable species that are strongly adsorbed mainly into Fe and Mn oxides, clay minerals and organic matter. Only in environments characterised by pH < 3 and pH > 8 As can become soluble, as reported by Krysiak and Karczewska (2007) in Poland soils of mining and smelting areas. Moreover, some species of the genus *Salix* studied in relation to accumulation and tolerance to metals, have shown a large variability in response to these elements (Watson et al., 1999). Alvarez et al. (2003) indicate that *S. atrocinerea*, growing on dump of an abandoned mine in Galicia (Spain), accumulate Zn but not Cu.

Salix spp. represent the species most able to adapt to these conditions, as known from the literature (Stoltz and Greger, 2002; Chang et al., 2005; Unterbrunner et al., 2007) and confirmed by this study.

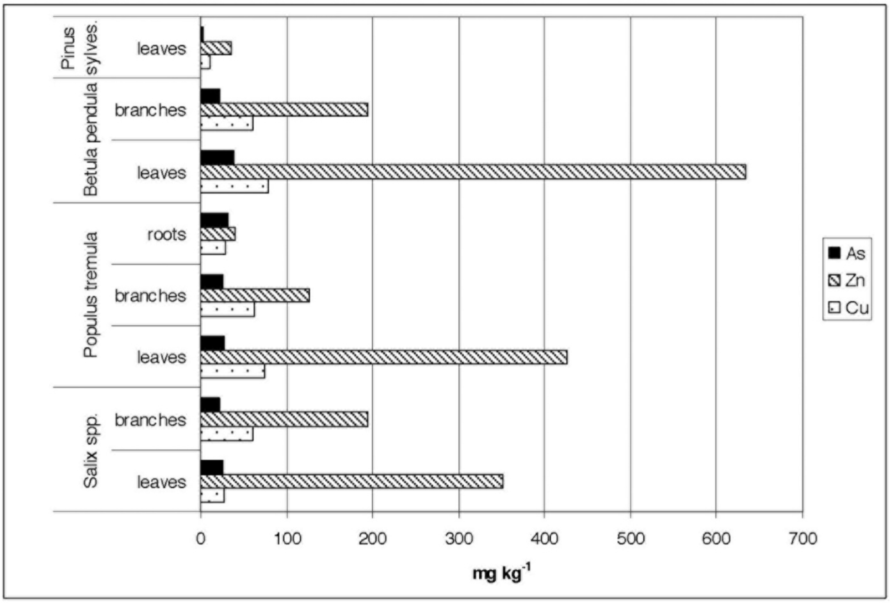

Figure 2. Comparison between the contents of metals in the different organs of the four tree species growing on the Hop dump.

P. tremula and *B. pendula* grow on strong As contaminated substrates, with concentrations as high as 230 mg kg^{-1}: all elements are mainly stored in leaves (Figure 2). In particular, poplar samples come from two different positions of waste dump, characterised by the same lithology (WR1), similar pH values (mean = 4.8) and low EC values. The behaviour of poplars in the two sites is shown in Table 3 and seems to be quite similar. Cu concentrations in leaves and branches are higher than in the roots. According to the Zn affinity with biosphere, the highest concentrations are reached in the leaves, while decrease significantly in the branches, and reach the lowest concentration in the roots. Arsenic contents are low and similar both for aboveground and belowground biomass (Figure 2). *P. tremula* seems to prefer the pH around 5 independent of the content of elements in the soil.

Birch samples come from two sites different for pH values (4.5 and 7.5) and characterised by high As contents (148-231 mg kg^{-1}). As previously seen for the poplar, also in this case the leaves show higher concentrations compared to the branches, for all elements (Figure 2). Moreover, Zn seems sensitive to different pH value of the substrate, because the plants that grows on the acid substrate shows much higher contents in the leaves compared to the sample that grows at neutral pH. However, the small number of samples suggests caution in considering the relations with the pH of the substrate.

B. pendula was found to have a great tolerance

Table 3. PTE concentrations in plant organs, BF and TF values. Data are mean, compared with normal ranges in plants (Kabata-Pendias, 2011). Range of variation is shown in brackets only for samples belonging to *Salix* spp. and *A. glutinosa*, as they are the most numerous.

Sample	Element concentrations (mg kg^{-1})		
	Cu	Zn	As
Salix spp. (n=5)			
leaves	27 (21-45)	351 (302-632)	26 (25-45)
branches	40	299	23
BF	1.4 (0.8-1.9)	15.8 (7.4-39.9)	0.08 (0.06-0.12)
TF (leaves /branches)	1	1.6	1.1
Populus tremula (n=2)			
leaves	75	426	27
branches	63	126	26
roots	29	40	31
BF	3.8	31.5	0.18
TF (leaves/roots)	2.5	12.4	0.9
Betula pendula (n=2)			
leaves	79	643	38
branches	61	194	22
BF	3.2	18.7	0.22
TF (leaves/branches)	1.3	2.9	1.7
Pinus sylvestris (n=1)			
leaves	11	35	2.1
BF	0.3	0.4	0.04
Alnus glutinosa (n=10)			
leaves	19 (15-35)	62 (32-408)	2.5 (1.8-2.6)
roots	43	105	1.4
BF	0.2 (0-0.03)	0.6 (0-2.2)	0.04 (0-0.07)
TF (leaves/roots)	2.5	1.7	0.6
Normal ranges in plants (Kabata-Pendias, 2011)	5-30	27–150	1-1.7

ability for Cu and Zn, showing the highest content in leaves (up to a maximum value of 1,025 mg kg^{-1} for Zn and 90 mg kg^{-1} for Cu) well beyond the limit of toxicity.

All species examined show similar behaviour toward arsenic in relation to the pH of the substrate; in fact the correlation As vs. pH for the nine analysed samples shows a high value of the

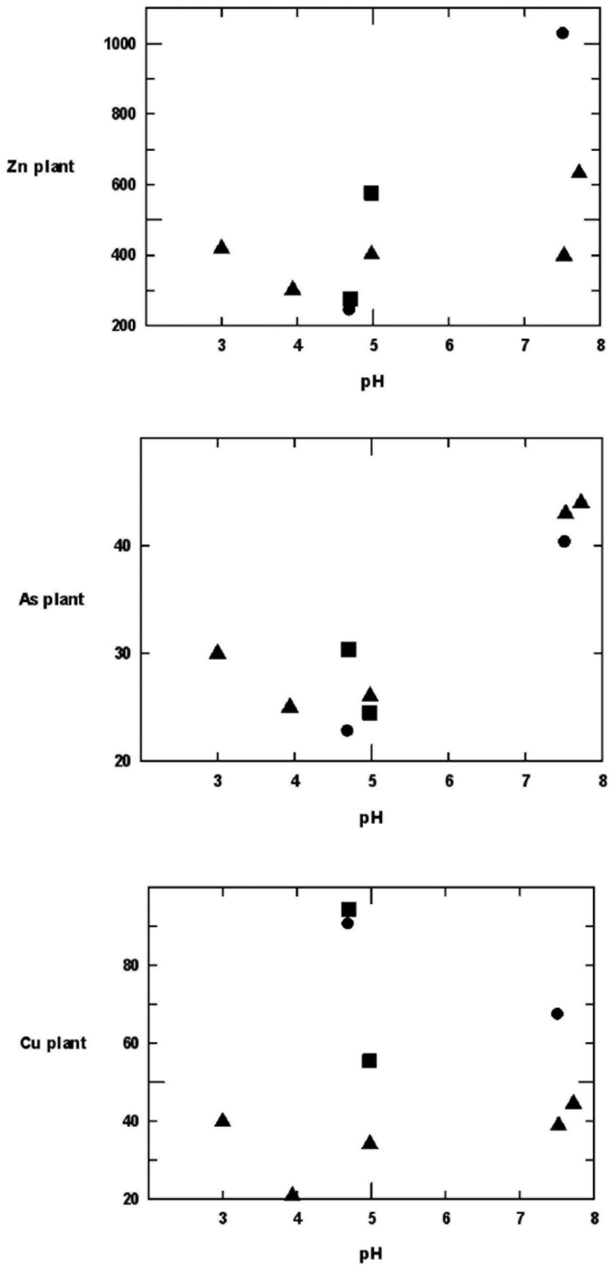

Figure 3. Relationship between pH and PTE contents (mg kg^{-1}) in the studied samples of vegetation growing on the Hop waste-rock dump. Symbols: triangle = *Salix* spp.; square = *Populus*; circle = *Betula*.

correlation coefficient with a high degree of significance ($r = 0.8402$; probability degree = 0.01).

The behaviour of *P. sylvestris* is quite different; it grows on a substrate, located on the edge of the dump, characterised by low As contents (58-77 mg kg^{-1}), neutral pH values (7.3-7.4), high EC values. Therefore, it does not seem to tolerate the severe conditions that characterise the surface of the Hop waste-rock dump. PTE contents in pine are lower compared with other species for Zn, Cu and As as shown in Figure 2. Nevertheless, these preliminary evidences, should be confirmed by further study on a larger sample set. Moreover, literature about the biogeochemistry of Scots pine is scarce: contents of As measured in the same species reported by Ruiz-Chancho et al. (2008) are of the same order of magnitude of Roşia samples, whereas needle contents of *P. sylvestris* at the mine site of Ouche (France), observed by Jana et al. (2012), show very high arsenic concentrations. More abundant are the data regarding *Pinus pinaster* (Freitas et al., 2004; Pratas et al., 2005; Roccotiello et al., 2010; Marescotti et al., 2013), a colonizing species of mine sites, able to completely avoid toxic metals from its organs.

For all species BF and TF were calculated and their mean values are shown in Table 3.

In *Salix* spp., *B. pendula* and *P. tremula,* BF values are > 1 for Cu (1.2–4.5), >> 1 for Zn (range 1.2-56.5) and < 1 for As (0.1-0.6) in all organs. Cu and Zn accumulate especially in leaves, with a maximum bioaccumulation capacity found in *P. tremula*, which shows a maximum BF value of 56.5 for Zn and 4.5 for Cu. In leaves BF values for Zn are one order of magnitude higher than those for Cu, according to the different biological role of these two metals.

In *Salix* spp. the link between the pH values of the waste rock and BF values, is different for the three elements. For Cu, characterised by BF values 0.8-1.9, the highest values correspond to a weakly acidic environment; for Zn, whose BF is high (7.4-39.3), the maximum corresponds to pH = 4.8. Differently for As, where BF is < 0.6, the highest ratios are related with the highest pH values.

P. sylvestris shows a different behaviour: its BFs are << 1 for Cu (mean 0.3) and Zn (mean 0.4) and close to 0 for As. This plant species probably adopts a strong exclusion-strategy, to maintain low levels in its organs; for example, it is known the protection from copper toxic effects on Scots pine by the ectomycorrhizae (Van Tichelen et al., 2001).

These results are consistent with the biological role of Zn, which is an essential micronutrient that can be well tolerated within a limited range of concentrations, whereas As does not have any physiological role and is highly toxic even at very low levels (Kabata-Pendias, 2011). Therefore these data suggest that an exclusion metal-tolerance strategy may work in synergy with physiological detoxification mechanisms, as arsenic contents in all species are higher than normal ranges in plants (Table 3).

TF values can be evaluated according to their typical meaning of transport between the aboveground/belowground plant organs only in the case of poplar. For Cu and Zn, TF values are >> 1, while for As ~ 1: this means that the poplar is able to grow on As-rich substrates and to transfer this element toward aboveground biomass without toxic effects to plant.

In all plant species and for all elements, the bioaccumulation and the translocation values show a clear tendency to transport metals to leaves, according to the behaviour of deciduous accumulator species that employ a PTE detoxification strategy by the Autumn leaves falling (Pulford and Watson, 2003).

Roşia Valley and strategies of metal uptake by trees

Mean PTE contents in soils (Tables 1 and 2) and plant samples (Table 3) along Roşia river are shown. Considering the mean value for all sites the contents appear lower than European law limits for industrial sites (EU directive 1998), reaching an average concentration of 160 mg kg^{-1} for Cu and 230 mg kg^{-1} for Zn. As concentration reaches the mean value of 55 mg kg^{-1} (30-80 mg kg^{-1}). PTE show different contents in soil along the course of the river, the highest values being in the site 2, where samples were collected close to the tunnel adit, corresponding to mine waters downstream, characterised by strong AMD. Concentrations of PTE decrease progressively in sites 3, 4, 5 till the confluence of Roşia river into Abrud river (see Figure 1). Data between metal contents in waters and sediments are presented and widely discussed in Bird et al. (2005); these authors conclude that in the Roşia Montană mining area, which also includes the area considered in the present paper, high metal contents of river sediments are confined within approximately 10 km of point sources.

Along the riverbanks, the alder represents the prevalent tree species, being present in all sampling sites. PTE concentrations in its leaves are comparable to the normal range found in mature leaves of several plant species (Kabata-Pendias, 2011).

Moreover, BF values are always lower than 1 and close to 0 for all elements, and TF values are high and hence PTE are concentrated especially in leaves. As a whole, results show that *A. glutinosa* acts as a strong excluder species.

Conclusions

This study shows that plants species growing on a contaminated site have different strategies respect of PTE as a function of the different characters of the substrate.

Hop waste-rock dump is a highly variable substrate, characterised by stressful conditions for plant growth such as acid pH values and strong As contamination (mean 80 mg kg^{-1}), while Cu and Zn contents appear within European law limits for industrial sites. Soils collected along Roşia Valley characterised by a moderate average PTE content, show a heterogeneous pattern probably influenced by local sources of contamination (i.e. mine waters, small waste-rock dumps).

Salix spp., *B. pendula* and *P. tremula* are able to tolerate such limiting conditions, as also reported in literature. They actively accumulate Cu and Zn in leaves, with BF values significantly high for Zn, although they can not be considered as hyperaccumulators. On the other hand, the same species act as excluders for As, whose concentration in plant organs are lower than in soils, exceeding however toxic values (Kabata-Pendias, 2011). *Salix* spp. represent the species most able to tolerate different environmental conditions and to grow on strong acid substrates, acting as a pioneer species that could be used for revegetation of mine lands and stabilization of dump slopes, even on the area not yet vegetated.

P. sylvestris acts as a strong excluder, avoiding PTE accumulation in its biomass, where As contents are a little bit higher than normal ranges found in most species, and seems suitable for revegetation also on acidic substrates.

Finally, *A. glutinosa,* growing on this substrate, acts as a strong excluder for all elements and could be used in forestation programs along contaminated riverbanks.

The present study could be useful to direct further studies in this area where reforms and re-opening of mining are expected.

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