Research article

Bioaccumulation of thallium and other trace metals in *Biscutella laevigata* nearby a decommissioned zinc-lead mine (Northeastern Italian Alps)

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ABSTRACT

The mineral body exploited in Salafossa (Eastern Dolomites) was one of the largest lead/zinc-containing mineral deposits in Europe. Both metals were mainly present as sulphides (*sphalerite*, ZnS and *galena*, PbS). Mining activity started around 1550, but it was only around 1960 that the richest veins of the minerals were discovered. The mine closed in 1985, and concentrations of several trace metals, such as thallium (Tl), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn), were detected in the soils and plant samples (*Biscutella laevigata* L.) that were collected from eighteen sites selected outside the mine. *B. laevigata* is a *pseudometallophyta* species, and it often grows near mining areas where the soil's metal concentrations are significantly higher than those of soil with a natural geochemical background.

The total metal concentrations in the plant tissue (roots and leaves of *Biscutella laevigata*) and in the soil samples — both bulk-soil and the *B. laevigata* root system (rhizo-soil) — were determined through Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The metal extractability and leachability of the soil samples were estimated using soil extractions with DTPA (Diethylene-triaminepentaacetic acid). In addition, metal mobility caused by rainwater runoff was estimated by using a leaching test with a dilute solution of H_2SO_4 and HNO_3.

The results showed that metals were present in a chemical form available for uptake by the plants' roots. In fact, high concentrations of the metals were also found in the plant tissue (roots and leaves) of *B. laevigata*, and these concentrations were higher than those whose soils present natural geochemical background levels in the corresponding rhizo-soil. Thus, *B. laevigata* has shown a marked ability to bioaccumulate trace metals, especially Tl and, to a lesser extent, Zn, Pb, Fe and Mn, and it can influence metal mobility in the rhizo-soil.

To assess the uptake and translocation processes of the trace metals, resulting in their bio-accumulation, two different indices were calculated: the *enrichment factor in roots* (EFr), as the ratio between the metal concentration in belowground biomass and in the respective rhizo-soil, and the *translocation factor* (TF), as the ratio between the metal concentration in the leaves and the corresponding roots. For both indices, values > 1 denoted enrichment of the metal in the roots or its trans-location to the upper tissues. The results showed that EFr and TF were considerably high only for Tl, reaching a maximum value of 60 for EFr and 11.6 for TF. Conversely, the other investigated metals did not show significant bioaccumulation (EFr < 1), and they showed TF > 1 only at a few sites.

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1. Introduction

Mining activity and mineral processing are generally considered to be the major anthropogenic sources of trace metals and other contaminants in the environment. Soil contamination by trace

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metals, derivative of stored or abandoned tailings and raw materials, is considered to be a worldwide environmental concern (Callender, 2004; Garcia et al., 2008; Álvarez-Ayuso et al., 2013; Monterroso et al., 2014; Stefanowicz et al., 2014). High concentrations of trace metals in soils can be result of natural geochemical processes, but human activities can significantly influence and exacerbate these processes. In addition, soil contamination represents an issue of global interest because of the possibility of these elements being transferred to the hydrosphere, atmosphere and biosphere, thus representing a potential hazard to human health (Concas et al., 2006; Bačeva et al., 2014; Courtin-Nomade et al., 2016). As a consequence, the mobility and leachability of contaminants in soil, including trace metals, is of great importance when considering their uptake by vegetation and animals (Wierzbicka et al., 2004; Xiao et al., 2004a; Nannoni et al., 2011).

In spite of the fact that they are highly impacted environments, abandoned mine areas can still provide good ecological niches for rare plant species which are able to survive and grow in the presence of high concentrations of trace metals in soil (Batty, 2005). There are a few plant species that possess specific physiological mechanisms for detoxification that exclude uptake, promote toleration or prevent the accumulation of high concentrations of trace metals (Baker and Brooks, 1989; Dinelli and Lombini, 1996; Callahan et al., 2006). These plants are known as *hyperaccumulators* (Callahan et al., 2006), including *Noccaea caerulescens*, which has been widely studied due to its propensity for accumulating and tolerating high concentrations of Zn, Cd and Ni in soils (Kabata Pendias, 2011).

Biscutella laevigata is a trace metal-hyperaccumulating species, especially of Tl, growing in regions where soil metal concentrations are significantly higher than those of soils with natural geochemical backgrounds (LaCoste et al., 1999; Sheoran et al., 2009; Fellet et al., 2012; Pošćić et al., 2013, 2015). Tl is frequently associated with sulphide mineralization, and is usually found to substitute for Pb in *galena* (LaCoste et al., 1999). The ability of *B. laevigata* to accumulate Tl is particularly significant at abandoned mine sites, such as Salafossa, where the sulphide chemical alteration processes aid in the release of Tl. When present in an aqueous solution, Tl may further be subject to adsorption on the surface of the soil and sediments particles, such as in the presence of the oxides and hydroxides of Fe and Mn (Vink, 2003; Lin and Nriagu, 2009; Xiong, 2009).

This species is able to grow on both metal-contaminated and uncontaminated soils, so it can also be defined as a *pseudometallophyte*. Differences between waste-heap and mountain populations have been demonstrated; in addition to the morphological differences which are related to the different habitats, different levels of tolerance and accumulation of Zn, Pb and Cd have also been found. For instance, a higher tolerance has been demonstrated in the *B. laevigata* waste-heap population in comparison to the mountain population in several studies (Wierzbicka and Pielichowska, 2004; Pošćić et al., 2015).

Through the application of simple indices of enrichment, bioconcentration and translocation (Lombini et al., 1998; Escarré et al., 2011; Fellet et al., 2012; Pošćić et al., 2013), *B. laevigata* has been used as an indicator of trace metals contamination and in the estimation of their bioaccumulation. Generally, for *hyperaccumulators*, such as *B. laevigata*, these indices reach values greater than one, denoting a metal enrichment in the root system or its translocation to the aboveground tissues (McGreath and Zhao, 2003).

This paper presents part of the results of an interdisciplinary study aimed to assess the environmental impact of the potentially toxic elements around the abandoned mine of Salafossa, located in the Northeastern Italian Alps. Several components were investigated, such as mine drainage, abandoned tailings, soils and riverine

sediments affected by mined materials, residues of "sink and float" treatments of sulphide mineralization and general vegetation (Pavoni et al., 2014). Mine drainage waters have been shown to be highly enriched in Zn and, more importantly, Tl (at rates up to 15 times higher than the limit fixed by U.S. EPA), which are spread into the Piave River. Since Tl has been recognized as one of the most toxic trace elements for humans (Zhang et al., 1998; Kazantzis, 2000; Xiao et al., 2004b, 2007; Peter and Viraraghavan, 2005), there is great concern about the potential risk of the harmful effects due to Tl exposure on the local population living downstream from the mine if they have come into contact with contaminated spring waters (Campanella et al., 2016).

As a result of these concerns, the main aim of this work was then to verify not only the trace metal bulk contents and "bioavailability" in the soils, but also their accumulation in *B. laevigata*, one of the most widespread species in the area, in order to determine if and where the accumulation capacity depends on the metal availability in the soils by considering the differences between vegetated and not vegetated soil samples. Since the same plant has also been tested for phytoremediation, a technique was adopted with the objective of removing trace metals and other contaminants from soils or waters capable of exploiting the extractive capacity of the plants (Anderson et al., 1999; Jadia and Fulekar, 2009; LaCoste et al., 1999; Zhao et al., 2003; Sheoran et al., 2009). Such results may be of help in exploring the opportunity of using this species in different regions of the study area in order to decontaminate the soils and to mitigate the impact of Tl in the surface environment.

2. Study area

The general area of the study is located in the eastern Dolomites belonging to the Alps, within the Veneto region (Northeastern Italy) and along the Piave River (Fig. 1). The region is characterized by an alpine climate with cool summers and cold snowy winters. Spring and autumn are fresh, windy and rainy.

The Salafossa mining area was one of the largest Pb/Zn-containing mineral deposits in Europe. Both metals were mainly present as sulphides: *sphalerite* (ZnS) and *galena* (PbS). The mineral deposit belongs to a unique ore body included in the Ladinian-Carnian Dolomites of the Lower Triassic (Assereto et al., 1977).

Mining activity started around 1550, but it was only around 1960 when the richest veins of the minerals were discovered. From the Salafossa mineral deposits, a little more than 11 million tons of *tout-venant* (raw material), with an average content of 0.9% for Pb and 4.7% for Zn, were extracted. On the whole, 92,000 tons of Pb and 482,000 of Zn were recovered from the mineral deposit (Zas Friz, 1999). The mining activity continued until 1985, the year of its final closure.

The external area of the mine is characterized by the presence of heterogeneous deposits constituted by the remains of excavated rock mixed with mineralized tailings coming from the various mineral extraction processes and ore weathering products (mainly Fe hydroxides). Although there was a specific dumping area during the mining activity, the waste material was usually either dropped on the mine slopes or used for the construction of the access road to the dumping area, which is located at a higher altitude.

As a consequence, the accumulation of large volumes of waste-rock dump material has led to widespread heterogeneous trace metal contamination around the external area of the mine. In addition to Zn and Pb, coming from the alteration of *sphalerite* and *galena*, respectively, there are other elements such as Tl, Fe and Mn, which may be present as minor constituents of the sulphides (Cook et al., 2009; Álvarez-Ayuso et al., 2013). The high concentrations of trace metals in the substrate may affect the vegetation growth; in fact, the deposit area, the entrances to the mine tunnels and the
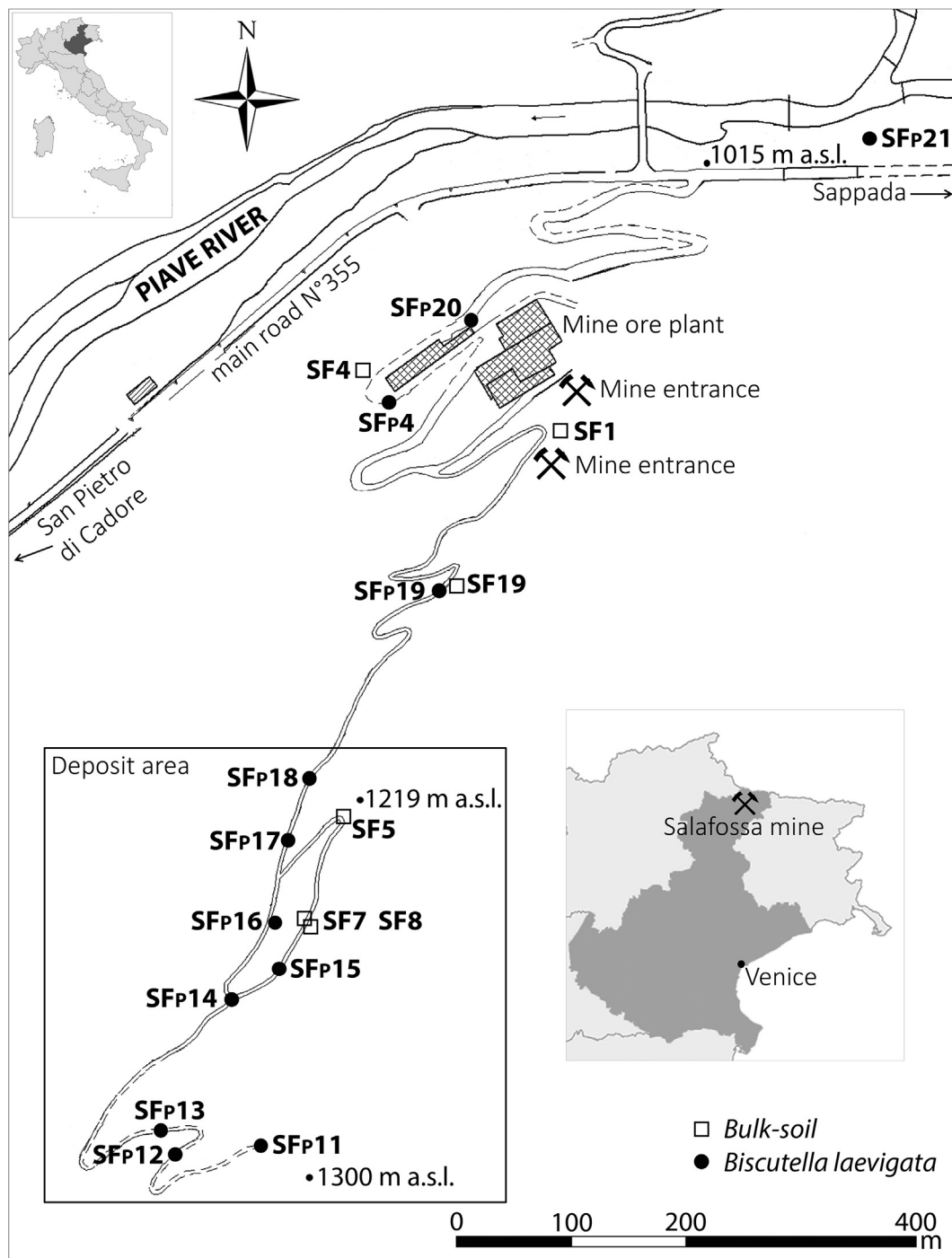
Fig. 1. Index map of the Salafossa mining area with the location of the sampling points where plants and the corresponding rhizo-soil were collected.

zones surrounding the buildings where the processes for the minerals extraction took place are devoid of vegetation. However, some plant species, including *B. laevigata*, are still able to thrive in the presence of waste contaminated by trace metals.

3. Materials and methods

3.1. Field work and sample preparation

Soil samples and individuals of *B. laevigata* plants growing on the external area of the Salafossa mine were collected in the autumn of 2014. Eighteen sampling points were chosen by considering the most representative areas and by taking into account the location of the minerals extraction processes (Fig. 1). Twelve plant tissue samples (including roots and leaves) as well as the related surface horizon soil from the rizosphere of each plant (considered hereafter as rhizo-soil) along with six bulk-soil samples were analyzed for trace metal concentrations and to test the variability of metal uptake by the plants.

Soil samples (both bulk-soil and rhizo-soil) and plant samples were dried at room temperature for two days. Plant tissue samples were washed with distilled water and then with deionized water

(Milli-Q, $\rho = 18.2$ MΩ cm^{-1}) in order to remove any soil particles that may have interfered with the chemical analysis. The roots and leaves were dried again at room temperature for two days, frozen, freeze-dried (CoolSafe 55-4 SCANVAC) and finely ground. The plant tissue samples were then acid-digested, using HNO$_3$ (69.5% w/w) and H$_2$O$_2$ (30% w/w) in an open-vessel system (PerkinElmer SPB 100-12) (Pansu and Gautheyrou, 2006; Colombo, 2002) and subjected to increasing temperatures of up to 120 °C.

Both bulk-soil and rhizo-soil samples were sieved to <2 mm, finely ground and acid-digested through a total dissolution in a closed microwave system (Milestone, MLS 1200) using *aqua regia* (a mixture of HNO$_3$ and HCl (37% w/w), 1:3) and HF (48% w/w) according to the USEPA 3052 method. The mineralization was performed through two heating steps. In the second step, H$_3$BO$_3$ was added in order to buffer the excess of HF. The extracts obtained from the mineralization process were filtered through paper filters (8 μm, Whatman), diluted up to a volume of 50 mL by adding Milli-Q water and filtered again with syringe filters (0.45 μm, GHP Acrodisc 25 mm) before analysis.

3.2. Bioavailability and leaching

The leachable fraction of the same metals was assessed by using an extracting solution of triethanolamine (TEA, 0.1 M), diethylenetriaminepentaacetic acid (DTPA, 0.005 M) and CaCl$_2$·2H$_2$O (0.01 M). A DTPA soil test developed for neutral to alkaline soils and considered to be most suitable for calcareous soils was used, and in the case of our rhizo-soil samples, high pH values (8.37—8.73) were presented (Lindsay and Norwell, 1978; Hammer and Keller, 2002; Fellet et al., 2012). HCl was added in order to adjust the pH to 7.3 ± 0.05 and the solution was diluted up to a volume of 1 L by adding Milli-Q water.

With respect to the extraction process, an aliquot of each rhizo-soil sample was placed in a conical flask and the extraction solution was added and stirred on a magnetic plate for 2 h. Subsequently, the suspensions were filtered by gravity through paper filters (0.45 μm, Whatman), filtered again through glass fibre filters (0.45 μm) and diluted up to a volume of 100 mL by adding Milli-Q water.

The metal bioavailable percentages were calculated according to the following formula (1), considering the ratio between the concentration of the metal extracted and the total metal concentration in the rhizo-soil:

$$\%_{bioavailable} = ([Me]_{extracted}/[Me]_{total})*100 \tag{1}$$

In addition, a leaching test was performed in order to assess the metal mobility within the soils (both for the bulk-soils and rhizo-soils) under the effects of rainwater runoff. According to the USEPA 1312 method (USEPA, 1994), the rhizo-soil samples were subjected to soil extraction with a pH 4.2 ± 0.05 solution of H$_2$SO$_4$ and HNO$_3$ for 18 ± 2 h (Gray, 2003; Marguí et al., 2004). Following the extraction process, the material in the extractor vessels was separated into its liquid and solid components through the same filtering process explaned above. In order to evaluate the mobility of each metal, a leaching percentage was calculated similarly to the above reported formula (1), but by considering the ratio between the leached concentration and the total concentration in the soil.

3.3. Analytical measurements

The concentrations of Ca, Mg, K, Zn, Pb, Fe, Mn, As, Cu and Cd were determined in all of the specimens by means of Inductively Coupled Plasma − Optical Emission Spectrometry (ICP-OES) using an Optima 8000 ICP-OES Spectrometer (PerkinElmer, USA)

equipped with an S10 Autosampler (PerkinElmer, USA). The measurements were conducted using calibration curves obtained by dilution (range: 0—100 mg L^{-1}) of the standard solutions for ICP-OES analyses (Sigma-Aldrich, USA). Samples exceeding this range were diluted with milliQ water. The precision of the measurements as relative standard deviations for the analysis were always less than 5%.

The Tl concentrations were determined through Electro-Thermal Atomic Absorption Spectrometry (ETAAS) with Zeeman background correction. A Thermo M series AA spectrometer equipped with a GF95Z Zeeman Furnace and a FS95 Furnace Autosampler (Thermo Electron Corporation, Cambridge, UK) was used for the analyses. A solution of Mg(NO$_3$)$_2$ (0.5% w/v) was used as a matrix modifier for Co and Cr. Samples were analyzed by measuring them against the standard solutions for instrumental calibration (range: 0—100 μg L^{-1}). The precision of the measurements as relative standard deviations (RSD%) for the analysis was always less than 5%. The Limits of Detection (LOD) are listed in the Supporting Information (Table S1).

4. Results and discussion

4.1. Bulk-soil and rhizo-soil characterization

The bulk-soil samples showed very high total concentrations of Zn (1351—33,489 mg kg^{-1}) and Pb (146.5—9284 mg kg^{-1}), whose presence is connected to the mineral extraction processes of *sphalerite* and *galena* respectively. In addition, other trace metals, which may be present as minor constituents of the sulphides, were present in significant concentrations: Fe (0.7—14.7%), Mn (247—1081 mg kg^{-1}) and Tl (<LOD — 17 mg kg^{-1}) (Table S2).

In contrast, the DTPA extractable concentrations were generally lower than the total concentrations. The ranges of the extracted concentrations were as follows: 46.7—595.3 mg kg^{-1} of Zn, 2.3—470.1 mg kg^{-1} of Pb, 2.6—16.5 mg kg^{-1} of Fe, 1.1—9.5 mg kg^{-1} of Mn and < LOD — 6.3 mg kg^{-1} of Tl (Table 1).

X-Ray Diffraction (XRD) analyses were performed on the bulk-soil samples for mineralogical characterization in order to support the geochemical data. In addition to *sphalerite* and *galena*, the main sulphides of interest for the extraction activity, the mineralogical analysis revealed the presence of pyrite (FeS$_2$) and other minerals. Dolomite (CaMg(CO$_3$)$_2$) and quartz (SiO$_2$) reflect the lithological characteristics of the study area, while hematite (Fe$_2$O$_3$), goethite (FeOOH) and sulphur (S) are results of the sulphide oxidation processes.

Generally, lower concentrations of the same metals were found in the rhizo-soil samples, except for Tl, which had a higher total concentration in the rhizo-soil samples than in the bulk-soil samples. The rhizo-soil samples were characterized by the following ranges for total and extracted concentrations, respectively: 127.6—18,297 mg kg^{-1} and 12.2—753.8 mg kg^{-1} of Zn, 12.7—3144 mg kg^{-1} and 2.0—231.5 mg kg^{-1} of Pb, 0.1—3.0% and 1.8—114 mg kg^{-1} of Fe, 172.5—586 mg kg^{-1} and 0.9—57.2 mg kg^{-1} of Mn, 1.0—102.8 mg kg^{-1} and < LOD — 2.0 mg kg^{-1} of Tl (Table S2 and Table 1).

The DTPA-extractable concentrations, which indicate potential leachability, confirmed the presence of the elements in chemical forms that may be available for uptake by the plants' roots. The results displayed notable bioavailable percentages for Zn (4.1—20.7%), Pb (1.1—83.7%) and Tl (0.4—16.1%).

On a logarithmic scale, the relationship between total metal content and the extractable fraction in the soil samples (both bulk-soil and rhizo-soil) was described by a linear function in the case of Zn ($R^2 = 0.93$, P < 0.001, n = 12 and $R^2 = 0.86$, 0.001 < P < 0.01, n = 6 in the rhizo-soil and bulk-soil samples, respectively), Tl ($R^2 = 0.70$,

Table 1
Bioavailable concentrations of Ca, Fe, Mg, Mn, K, As, Pb, Cu, Cd, Zn and Tl, expressed as mg kg^{-1}, in the bulk-soil and rhizo-soil samples.

Bioavailable concentration − DTPA												
	Sample ID	Ca	Fe	Mg	Mn	K	As	Pb	Cu	Cd	Zn	Tl
BULK-SOIL	SF1	3285	3.8	455.2	3.8	124.9	< LOD	470.1	< LOD	< LOD	595.3	4.8
	SF4	819.3	2.6	218.2	1.1	67.0	< LOD	2.3	1.7	2.4	461.5	0.2
	SF5	973.5	11.5	183.5	6.9	26.8	< LOD	3.3	0.6	0.3	199.6	6.3
	SF7	1038	13.3	254.1	9.5	29.6	< LOD	21.0	0.4	0.3	341.9	0.7
	SF8	1191	16.4	332.9	4.0	28.9	< LOD	45.3	< LOD	< LOD	46.7	0.8
	SF19	1047	16.5	294.4	7.1	217.0	< LOD	49.8	1.2	0.4	154.3	< LOD
RHIZO-SOIL	SFP 4	1210	19.2	763.8	0.9	136.9	< LOD	34.7	0.3	0.8	657.9	0.5
	SFP 11	1455	14.8	373.9	3.3	44.2	< LOD	2.0	1.7	< LOD	12.2	2.0
	SFP 12	1348	13.9	330.7	8.5	46.0	< LOD	16.9	0.2	< LOD	31.9	0.1
	SFP 13	1332	10.0	787.9	26.2	107.5	< LOD	40.8	0.5	0.3	92.4	0.0
	SFP 14	1420	7.1	389.3	7.1	62.4	< LOD	7.2	0.6	< LOD	71.0	0.1
	SFP 15	1475	114.0	478.6	57.0	120.7	< LOD	100.3	1.0	0.5	157.6	1.8
	SFP 16	1316	16.2	331.1	7.6	37.0	< LOD	33.7	0.5	< LOD	115.6	2.1
	SFP 17	1340	4.8	463.5	1.7	35.8	< LOD	41.2	1.8	0.6	682.4	1.3
	SFP 18	1349	19.9	422.6	9.0	40.7	< LOD	231.5	0.7	0.6	435.9	0.7
	SFP 19	1522	32.5	585.7	10.7	99.6	< LOD	26.6	0.5	0.3	89.5	0.0
	SFP 20	1107	1.8	274.1	1.3	147.8	< LOD	35.4	1.4	2.0	753.8	0.1
	SFP 21	1542	35.2	431.5	57.2	249.8	< LOD	22.1	1.1	0.2	81.0	0.5

P < 0.001, n = 12 and R^2 = 0.44, P < 0.10, n = 6 in the rhizo-soil and bulk-soil samples, respectively) and Pb (R^2 = 0.47, 0.01 < P < 0.05, n = 12 in the rhizo-soil samples). The relationships were generally weaker for the bulk-soil samples, and there was no correlation (R^2 = 0.004, P > 0.10, n = 6) between the total Pb concentration and the leachable fraction of this element in the bulk-soil samples (Fig. 2). The mobility of Pb, which was lower than that of Zn and Tl, can explain the absence of the relationship between the total metal concentration and the leachable fraction in the bulk-soil samples. On the contrary, the total and leachable fractions were correlated in the rhizo-soil samples, even if the R^2 value was not significantly high (R^2 = 0.47). The higher Pb mobility in the rhizo-soil samples could be due to the presence of *B. laevigata* which might have promoted the release of Pb from the soil minerals in the soil solution through the rhizosphere acidification process.

In consideration of Tl, the relationship between the total and extracted concentrations was weaker for the bulk-soil samples (R^2 = 0.44) than for the rhizo-soil samples (R^2 = 0.70). *B. laevigata*, being a Tl *hyperaccumulator*, tends to promote its uptake and accumulation and, as a consequence, the rhizo-soil samples, influenced by the presence of the plant, showed a more significant relationship between the total and leachable fractions.

4.2. Plant tissue characterization

The plant tissue samples of *B. laevigata* showed high concentrations of several trace metals (Table S3), which were present in the following ranges in the roots and leaves, respectively: 60.5–1025 mg kg^{-1} and 31.1–2535 mg kg^{-1} of Zn, < LOD − 191.7 mg kg^{-1} and < LOD − 41.1 mg kg^{-1} of Pb, 50.0–392.5 mg kg^{-1} and 61.4–306.4 mg kg^{-1} of Fe, 10.9–69.2 mg kg^{-1} and 18.7–100.5 of Mn, 1.4–1267 mg kg^{-1} and 7.2–4657 mg kg^{-1} of Tl.

The presence of *B. laevigata* can affect trace metal leachability in the rhizo-soil, inducing their release from the soil minerals. Therefore, the leachable fraction must not be considered as bioavailability in *sensu strictu* but instead as the metal percentage still present in the rhizo-soil in a leachable form suitable for uptake by the root system.

Zinc and Pb, the main elements extracted from the mine, were obtained from both the rhizo-soils and plant tissue samples of *B. laevigata*, reaching the maximum values of 18,297 and 3144 mg kg^{-1} in the rhizo-soil samples (SF$_P$20), 1025 mg kg^{-1} (SF$_P$4) and 192 mg kg^{-1} (SF$_P$20) in the roots and 2532 mg kg^{-1} and

41.1 mg kg^{-1} in the leaves (SF$_P$4), respectively (Fig. 3). It should be noted that this evidence is not new for the Alpine region, since high concentrations of Zn and Pb were also found in the *B. laevigata* collected from contaminated soils near the Zn/Pb mining site of Raibl (NE Italy) (Fellet et al., 2012). Here, concentrations of Zn and Pb fell within the following ranges: 2302–42,513 mg kg^{-1} and 448–69,387 mg kg^{-1} in mining soils, 34.7–5692 mg kg^{-1} and n.d. − 1939 mg kg^{-1} in roots and 20.2–2980 mg kg^{-1} and n.d. − 874 mg kg^{-1} in shoots, respectively.

The high amounts of Zn and Pb are definitely related to *sphalerite* and *galena* processing. Zn was present both in the roots and in the leaves of each plant sample, testifying not only to the uptake by the root system but also to the translocation from the roots to the leaves. This element is essential for the synthesis of amino-acids and auxin, and thus its translocation to the epigeal parts of the plant is clearly required (Lombini et al., 1998; Escarré et al., 2011; Fellet et al., 2012; Álvarez-Ayuso et al., 2013). The ranges of the concentrations of Zn, Pb and Tl in the soil samples and plant tissues samples were compared with those from other Zn/Pb mining sites found in literature (Table 2). Generally, the soil samples of the mining sites considered, including this study area, were characterized by extremely high concentrations of Zn and Pb. With respect to Tl, the soil samples showed significant values at Raibl, with the maximum value of 4549 mg kg^{-1}, whereas at the other mining sites, such as Salafossa and "Les Aviniéres" (France), the concentrations were lower. In spite of these values, our plant tissue samples were characterized by high Tl concentrations.

Only in some samples (SF$_P$4, SF$_P$15, SF$_P$16, SF$_P$17, SF$_P$18, SF$_P$19 and SF$_P$20) was Pb present at a higher rate than LOD in the plant tissue (roots and leaves). Lead tends to be partitioned into the roots, but the rhizo-soils are generally characterized by relatively high amounts of this metal, both as total concentrations and in the bioavailable form (Fig. 3). Lead, when present as a sulphide, is characterized by low solubility and, consequently, low mobility. For some samples, this evidence and the metal leachability in the rhizo-soil seemed to be contradictory. A possible explanation could be that Pb may be present as a carbonate (cerussite, PbCO$_3$) and not as a sulphide. Cerussite is typically associated with oxidation zones which involve Pb deposits, and it is derived from the chemical weathering of *galena* through porewaters rich in carbonic acid. The ionic form of Pb (Pb^{2+}) is rapidly immobilized from aqueous calcite solutions (CaCO$_3$) and Pb precipitate as cerussite on the surface of calcite (Gamsjäger et al., 1984).
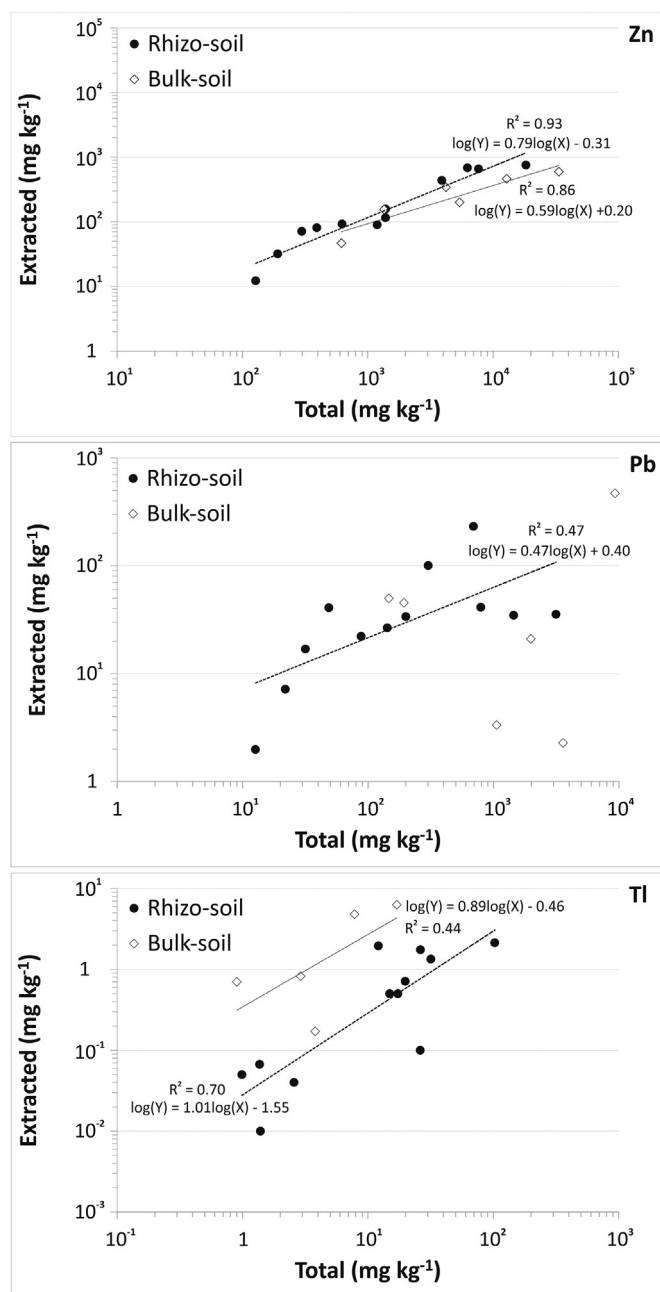
Fig. 2. Relationships between total and DTPA-extracted concentrations of Zn, Pb and Tl measured in the bulk-soil and the rhizo-soil samples.

Moreover, it is important to consider the influence of *B. laevigata*, which can cause *galena* alteration through the rhizosphere acidification process. The release of H^+ ions by the plants' roots and the resulting pH decrease may have induced the formation of cerussite, which in turn is vulnerable to dissolution processes, releasing Pb in the ionic form (Pb^{2+}), which is more mobile and, consequently, more bioavailable.

Extremely high concentrations of Tl were observed in the roots and leaves of *B. laevigata*, and these concentrations were higher than those in the rhizo-soil. The maximum concentrations were reached in the SF_P16 sample with 102.8 mg kg^{-1}, 1267 mg kg^{-1} and 4657 mg kg^{-1} in the rhizo-soils, roots and leaves, respectively (Fig. 3). Although Tl concentrations were lower than those reported by other studies (Anderson et al., 1999; Fellet et al., 2012), they still

exceeded the hyperaccumulation threshold limit of 20 mg kg^{-1} for critical toxicity (Krämer, 2010).

Rhizo-soil depletion of Tl, as evidenced by the low percentages of the leachable fraction for some samples, may have been due to the influence of *B. laevigata* rather than to a metal absence in the rhizo-soils. This species, favouring Tl uptake, could have influenced the distribution of this element in the rhizo-soil. In fact, the enrichment of Tl in the leaves may indicate the efficiency of the uptake and translocation processes, although some differences can be related to the peculiar features of the substrate at each sampling point.

The Tl and other trace metals distributions in the external mine area were extremely heterogeneous; the Tl concentration in the rhizo-soils ranged from values close to LOD (0.99 mg kg^{-1} in SF_P12) to very high concentrations (102.8 mg kg^{-1} in SF_P16). This extreme variability was due not only to the heterogeneous distribution of raw materials but also to the presence of *B. laevigata*, which may have affected their presence in the rhizo-soil through the plants' ability to bioaccumulate the metals.

In order to effectively evaluate metal hyperaccumulation into *B. laevigata* tissue, the total metal concentrations of Zn, Pb and Tl in the rhizo-soil were compared to the corresponding concentrations in the leaves (Fig. 4A). In the case of Zn and Pb, the metal concentrations in the rhizo-soil and the leaves were not significantly correlated ($R^2 = 0.18$, $P > 0.10$, $n = 12$ for Zn; $R^2 = 0.0005$, $P > 0.10$, $n = 4$ for Pb; $R^2 = 0.56$, $0.001 < P < 0.01$, $n = 12$ for Tl). With respect to Zn, the absence of such a relationship could be attributed to the chemical form of this element in the rhizo-soil samples; due to the very high Zn concentration in the rhizo-soil samples, this element might have been present mainly as a sulphide.

Nonetheless, the trend of Tl was different than that of either Zn or Pb, showing a higher value of R^2, which supports the idea that *B. laevigata* prefers the absorption, and the consequent accumulation, of this particular element. The Tl uptake and translocation processes were confirmed by the highly significant relationship ($R^2 = 0.93$, $P < 0.001$, $n = 12$) between the metal concentration in the roots and that in the leaves of the *B. laevigata* samples. The same relationship with highly significant results also occured in the case of Zn ($R^2 = 0.69$, $P < 0.01$, $n = 12$), revealing that this element was also present in a chemical form suitable for roots uptake and the consequent translocation to the upper tissues (Fig. 4B).

The *B. laevigata* samples were also characterized by relatively high concentrations of Fe and Mn, which were present both in the soils (both bulk-soil and rhizo-soil samples) and in the plant tissue (roots and leaves). In the absence of acid mine drainage because of the neutralization potential of carbonates, the geochemical behaviour of Fe, which is similar to that of Mn, is governed by oxidation-reduction processes under the influence of physico-chemical conditions (Kabata-Pendias, 2011), mainly in terms of the redox potential (Eh) and pH. Oxidative conditions with alkaline pH and reduction processes with acid conditions promote the precipitation of Fe or its dissolution, respectively (Kabata-Pendias, 2011).

Generally, the uptake and translocation processes are interfered with oxidative conditions, precipitation processes and competition of Fe^{2+} or Mn^{2+} ions with other metal cations (Kabata-Pendias, 2011). In this study, the presence of Fe and Mn in the bulk-soils, the rhizo-soils and in the tissues of the *B. laevigata* samples could have been due to the sulphide oxidation processes, which promote the mobilization of Fe and Mn compounds.

The leachable fractions of these metals were low compared to their total concentration in the rhizo-soil. However, the mobile fractions were suitably exploited by the plant, through both the bioaccumulation in the roots and the following translocation to the aboveground biomass as explained above (Fig. 3).
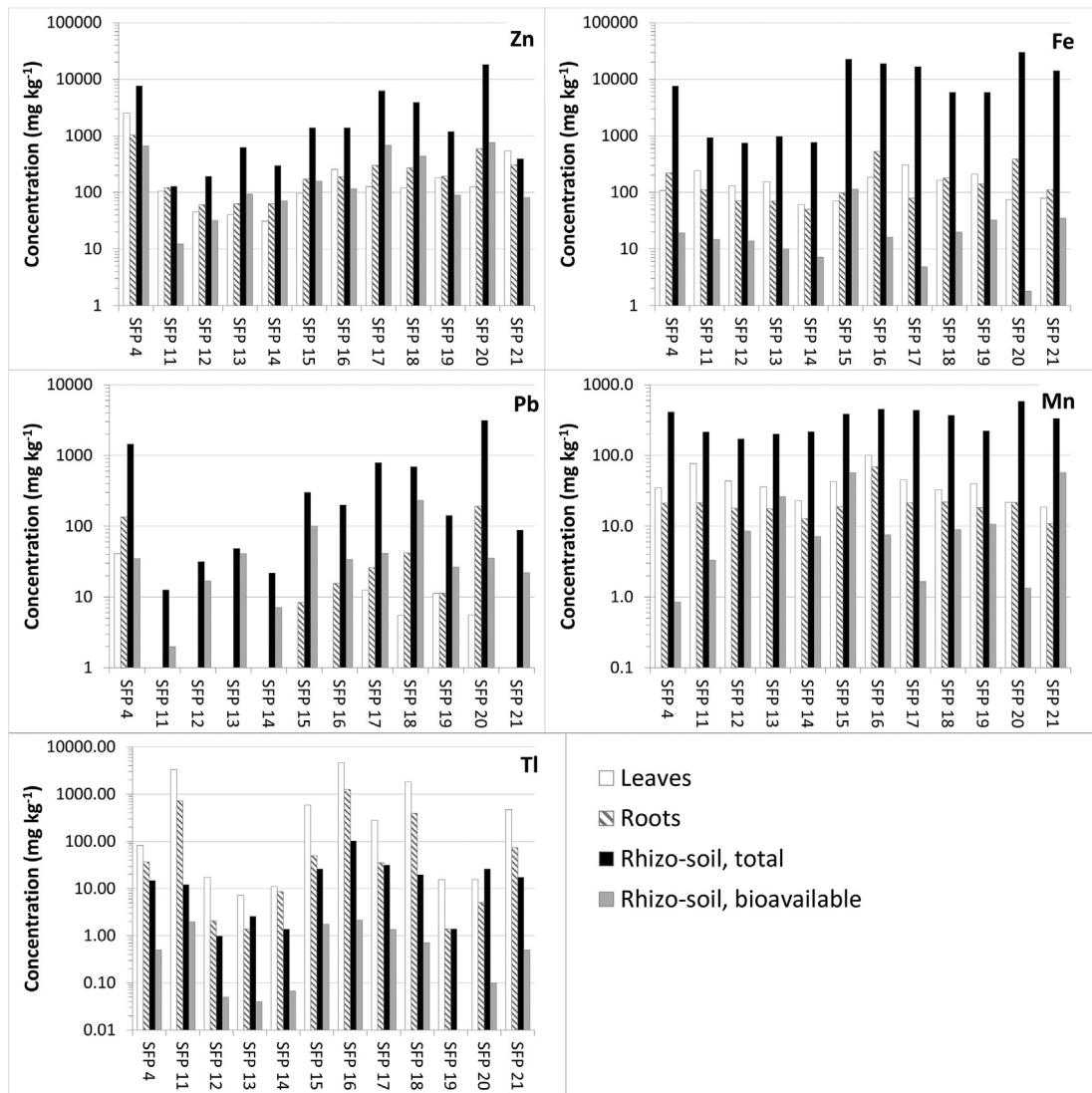
Fig. 3. Bar charts of total and bioavailable (DTPA) concentrations of Zinc, Lead, Thallium, Iron and Manganese in rhizo-soil samples and plant tissue (roots and leaves) of *B. laevigata*.

4.3. Leaching test

Soil and water contamination by trace metals originating from mining activities is one of the most important issues of environmental concern today. Leaching tests are usually employed to assess or predict the environmental effects of these potentially toxic concentrations on the environment (Rauret, 1998; Marguí et al., 2004; Santos et al., 2015). A leaching test was applied to the soil samples (both the bulk-soils and rhizo-soils) in order to simulate the conditions for metal mobility in situations of rainwater runoff. The leached metal concentrations are reported in Table 3. The mobility of each metal was evaluated by considering the leaching percentages (Table S4), which were lower than one for all of the elements considered, except for Tl and K. Regarding the rhizo-soil samples, the maximum values of 11% and 29% for Tl and K, respectively, were reached in SF$_P$11.

Overall, the leaching percentages were higher for K compared to Tl, indicating a higher mobility of this element from the soil matrix. Its absorption by plants is enhanced by its biological role as a macronutrient and does not depend on its availability in soils; instead, it is controlled by specific physiologic preferences.

B. laevigata, which is a Tl *hyperaccumulator*, prefers its assimilation and bioaccumulation in the tissues. This absorption is also favoured by the ionic substitution between Tl$^+$ and K$^+$, due to their similarities in terms of charge and ionic radius (Kabata-Pendias, 2011).

Generally, the rhizo-soil samples were characterized by higher K concentrations rather than those of Tl and, as a result, the corresponding leaching percentages for K are the highest. The more significant leaching of K can be explained by two considerations. On the one hand, *B. laevigata*, a Tl *hyperaccumulator*, prefers its assimilation and bioaccumulation in the tissues, as mentioned above. On the other hand, the ionic substitution which occurs between Tl$^+$ and K$^+$ can promote more significant Tl uptake by the root system.

Similar to the rates for the rhizo-soils, the leaching percentages are significantly high only for Tl and K in the bulk-soil samples, reaching the maximum values of 99.8% and 16.2% for Tl and K, respectively, in SF1 (Table S4). In this case, the leaching percentages were higher for Tl, contrary to the results of the rhizo-soil samples. The difference between the leaching percentages of Tl and K in the rhizo-soil and bulk-soil samples can be explained by the influence of *B. laevigata* on the Tl mobility in the soil solution. In the bulk-soil samples, the Tl mobility was not influenced by the presence of the

Table 2
Range of concentrations of Zn, Pb and Tl (mg kg^{-1}) in plant tissue and soil samples collected in analogous mining sites as reported in literature.

Location	Zn	Pb	Tl	Reference
Salafossa, Italy (S)	616—33,489	147—9284	< LOD—17.0	This work
Salafossa, Italy (R)	60.5—1024	< LOD—192	1.39—1267	
Salafossa, Italy (L)	31.1—2532	< LOD—41.1	7.19—4657	
Salafossa, Italy (TP)	47.0—1776	< LOD—98.7	4.3—2960	
Mine of Rubias, Spain (S)	340—52,000	46—6100		Monterroso et al. (2014)
Silesia-Crocow Upland, Poland (S)	394—70,435	94—23,006	6—51	Stefanowicz et al. (2014)
Él Losar del Barco village, Spain (S)	104—4085		0,550—1,13	Álvarez-Ayuso et al. (2013)
Raibl, Italy (S)	2302—42,513	448—69,387	150—4549	Fellet et al. (2012)
Raibl, Italy (R)	34.7—5692	n.d.—1939	n.d.—9984	
St. Laurent le Minier, France (S)	39,364	34,289	3.5	Escarré et al. (2011)
St. Laurent le Minier, France (R)	3871	5997	< LOD	
Kosovka Mitrovica, Kosovo (S)	86.1—1553	53,4—5536		Nannoni et al. (2011)
Olkusz, Poland (S)	501—5150	177—2300	< LOD—19	Cabala et al. (2009)
San Quintìn mine, Spain (S)	470—20,911	1243—93,901		Rodrıguez et al. (2009)
Boleslaw, Poland (R)			0.01	Wierzbicka et al. (2004)
Boleslaw, Poland (S)			39	
Les Aviniéres, France (L)			244—308	Anderson et al. (1999)
Les Aviniéres, France (TP)			20—15,199	
Les Aviniéres, France (S)			28	Leblanc et al. (1999)
Les Aviniéres, France (L)			214—308	
Gailitz, Austra (L)			295—495	
St. Laurent le Minier, France (TP)	2290	191	504	LaCoste et al. (1999)
St. Laurent le Minier, France (S)	56,900	14,600	25	
Mount Prinzera, Italy (R)	69			Lombini et al. (1998)
Mount Prinzera, Italy (TP)	53			

S = soil; R = roots; L = leaves; TP = total plant.
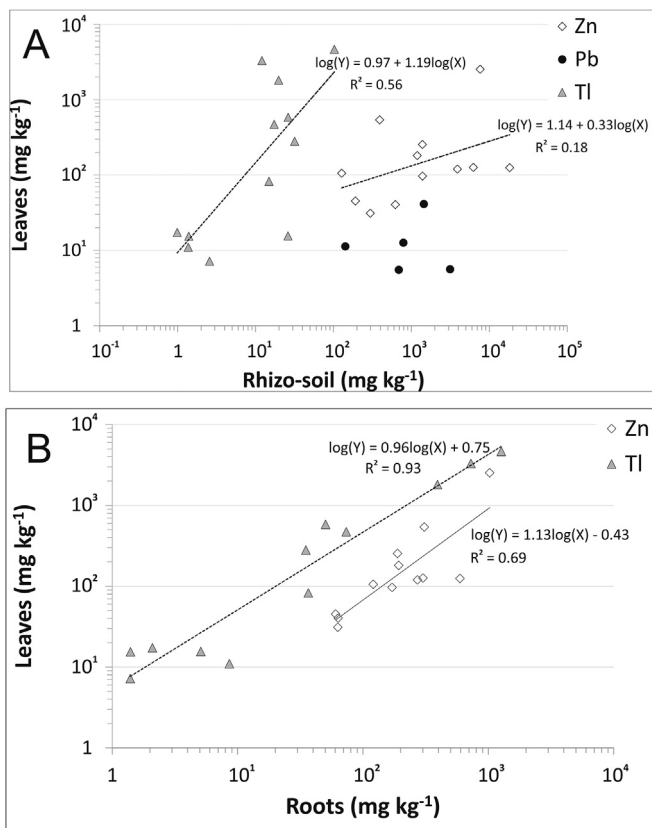
Fig. 4. A) Relationships between total metal concentrations of Zn, Pb and Tl measured in the rhizo-soil and in the leaves of *B. laevigata* samples. B) Relationships between metal concentrations of Zn and Tl in roots and leaves of *B. laevigata* samples.

plant, and the rainwater runoff might have promoted its release from the soil minerals into the soil solution.

4.4. Plant-soil relationships

High concentrations of trace metals in soils (bulk-soil and rhizo-soil) and the tissues of the *B. laevigata* samples confirm soil contamination at the abandoned mine site of Salafossa. Fig. 5 shows the relationships between the total plant concentrations of Zn, Pb and Tl and the extractable fractions of the same metals in the corresponding rhizo-soil samples. The correlations, described by a linear function, were highly significant ($R^2 = 0.81$, $P < 0.001$, $n = 12$) only in the case of Tl, testifying to its preferential uptake by and accumulation in the *B. laevigata* tissues. The uptake process results were also evident in the case of Zn, even if the relationship between the metal concentrations in the plant tissue and its extractable fraction in the rhizo-soil was weaker ($R^2 = 0.37$, $0.01 < P < 0.05$, $n = 12$) than the corresponding rates for Tl. Regarding Pb, there was no significant correlation ($R^2 = 0.04$, $P > 0.10$, $n = 7$) between the metal concentrations in the total plant and in the corresponding rhizo-soil.

To assess the uptake and translocation processes of the trace metals resulting in their bioaccumulation, two different indices were calculated: the *enrichment factor in roots* (EFr) and the *translocation factor* (TF) (Zhao et al., 2003; Petranich, 2010-2011; Fellet et al., 2012) (Table 4).

The EFr, as the ratio between metal concentration in below-ground biomass and total concentration of the same metal in the respective rhizo-soil, allows for the evaluation of the metal uptake by the root system (2).

$$EF_r = [Me]_{roots}/[Me]_{rhizo-soil} \tag{2}$$

On the other hand, the metal translocation processes from roots to the upper tissues can be determined by the TF, as the ratio between metal concentration in the leaves and in the corresponding roots (3).

$$TF = [Me]_{leaves}/[Me]_{roots} \tag{3}$$

For both indices, values > 1 denote whether there was

Table 3
Leached metal concentrations (mg kg^{-1}) in the rhizo-soil and bulk-soil samples (LOD: Limit of Detection).

| Leaching test	Sample ID	Ca	Fe	Mg	Mn	K	As	Pb	Cu	Cd	Zn	Tl
RHIZO-SOIL	SFP 4	237.3	0.4	298.9	0.5	227.9	< LOD	< LOD	< LOD	< LOD	0.5	0.2
	SFP 11	216.0	1.3	136.7	0.5	158.1	< LOD	< LOD	< LOD	< LOD	< LOD	1.3
	SFP 12	257.3	1.1	148.8	0.7	147.5	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	SFP 13	352.0	0.8	389.8	0.6	222.0	< LOD	< LOD	< LOD	< LOD	0.5	< LOD
	SFP 14	231.1	1.2	145.9	0.6	97.3	< LOD	< LOD	< LOD	< LOD	0.9	< LOD
	SFP 15	416.4	1.4	218.8	0.7	151.2	< LOD	< LOD	< LOD	< LOD	0.6	1.0
	SFP 16	177.5	0.5	108.8	0.6	79.5	< LOD	< LOD	0.4	< LOD	1.8	1.5
	SFP 17	201.6	< LOD	201.2	0.5	89.0	< LOD	< LOD	< LOD	< LOD	0.9	0.6
	SFP 18	223.0	0.5	165.9	0.5	82.1	< LOD	< LOD	< LOD	< LOD	1.0	0.3
	SFP 19	209.3	0.4	226.4	0.5	277.2	< LOD	< LOD	< LOD	< LOD	0.4	< LOD
	SFP 20	419.8	1.3	169.8	0.8	343.3	< LOD	< LOD	0.8	< LOD	8.2	0.3
	SFP 21	462.1	1.1	193.0	0.9	396.8	< LOD	< LOD	< LOD	< LOD	0.7	0.3
BULK-SOIL	SF1B	3114.2	< LOD	457.2	0.7	203.3	< LOD	< LOD	< LOD	< LOD	4.9	7.8
	SF4B	232.2	1.1	139.7	0.9	169.0	< LOD	< LOD	0.4	< LOD	14.3	0.2
	SF5	195.0	< LOD	89.5	0.5	94.4	< LOD	< LOD	< LOD	< LOD	2.4	4.6
	SF7	175.7	< LOD	109.2	0.5	97.1	< LOD	< LOD	< LOD	< LOD	1.0	0.5
	SF8	174.0	< LOD	133.1	0.5	97.6	< LOD	< LOD	< LOD	< LOD	< LOD	1.1
	SF19	184.7	< LOD	95.8	0.7	199.0	< LOD	< LOD	< LOD	< LOD	1.5	< LOD
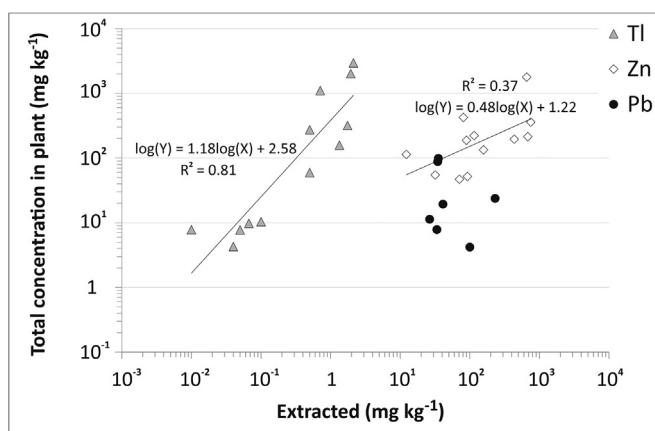
Fig. 5. Uptake of Tl, Zn and Pb by *B. laevigata* as a function of the extractable fraction of the same metals in the rhizo-soil samples. Total plant concentration has been calculated considering plant tissue (roots and leaves) biomass and metal concentrations.

Table 4
EFr and TF values (n.d.: non determined).

	Enrichment factor in roots — EF$_r$					Translocation factor — TF				
Sample ID	Zn	Pb	Tl	Fe	Mn	Zn	Pb	Tl	Fe	Mn
SFP 4	0.13	0.09	2.46	0.03	0.05	2.47	0.31	2.25	0.49	1.65
SFP 11	0.95	n.d.	59.93	0.12	0.10	0.88	n.d.	4.55	2.17	3.57
SFP 12	0.32	n.d.	2.12	0.09	0.10	0.75	n.d.	8.28	1.85	2.43
SFP 13	0.10	n.d.	0.54	0.07	0.09	0.64	n.d.	5.16	2.21	2.04
SFP 14	0.21	n.d.	6.27	0.07	0.06	0.49	n.d.	1.28	1.23	1.81
SFP 15	0.12	0.03	1.92	0.00	0.05	0.57	n.d.	11.60	0.71	2.24
SFP 16	0.14	0.08	12.33	0.03	0.15	1.35	n.d.	3.67	0.35	1.45
SFP 17	0.05	0.03	1.10	n.d.	0.05	0.42	0.48	8.00	3.90	2.10
SFP 18	0.07	0.06	19.80	0.03	0.06	0.44	0.13	4.62	0.91	1.49
SFP 19	0.16	0.08	1.01	0.02	0.08	0.94	1.00	11.03	1.49	2.20
SFP 20	0.03	0.06	0.19	0.01	0.04	0.21	0.03	3.06	0.19	1.00
SFP 21	0.78	n.d.	4.24	0.01	0.03	1.76	n.d.	6.41	0.71	1.72

enrichment of the metal in the roots (active accumulation) or its translocation to the upper tissues.

The results showed that EFr and TF were considerably high only for Tl, reaching a maximum value of 59.93 (SF$_P$11) for EFr and 11.60 for TF (SF$_P$15) (Table 4). Conversely, the other investigated metals did not show significant bioaccumulation in the roots (EFr<1), while TF reached values greater than one only at a few sites.

Trace metal bioaccumulation by vegetation depends on two main factors: the metal mobility in soil and the uptake ability of the metal's bioavailable forms by the root system. At the abandoned mine site of Salafossa, the influence of both factors was observed: Tl was present in a bioavailable form, although not for all samples, and *B. laevigata*, favouring Tl uptake, was able to promote its release from the corresponding rhizo-soil. The marked Tl uptake, confirmed by the high concentrations found in the roots and leaves and the elevated values of EFr and TF, not only highlighted the effective ability of *B. laevigata* to accumulate this element in its tissues, but it can also explain the deficiency of a bioavailable form of Tl in some rhizo-soil samples. Samples from the deposit area were characterized by higher values of EFr and TF rather than those located nearby the ore plant and the main entrance of the mine (Fig. S1).

From a general point of view, this situation is due to the extreme heterogeneity of trace metal distribution, which depends on the characteristics of each sampling point. In fact, among samples collected from the deposit area, it is possible to identify those characterized by the highest values of EFr (59.93 in SF$_P$11, 19.80 in SF$_P$18 and 12.33 in SF$_P$16) but also those with the lowest values (0.54 in SF$_P$13, 1.10 in SF$_P$17 and 1.92 in SF$_P$15). Moreover, it has been observed that some samples from the deposit area were characterized by low values of EFr (1.92 in SF$_P$15 and 1.10 in SF$_P$17) and high values of TF (11.60 in SF$_P$15 and 8.00 in SF$_P$17) at the same time. High TF values denote Tl enrichment in the aboveground biomass. This aspect justifies the low concentration of Tl in the rhizo-soils and roots of some samples due to the marked ability of *B. laevigata* to bioaccumulate and translocate this element in the leaves, rather than indicating a Tl deficiency in the soil itself (Lombini et al., 1998; Krämer, 2010; Fellet et al., 2012).

The Raibl lead/zinc mining site (Julian Alps, Italy) represents a situation comparable to that of the Salafossa mine. The presence of *B. laevigata* and its ability to accumulate Tl were confirmed by the authors, although they used two different indices to assess the uptake and translocation processes of the trace metals. The *bioconcentration factor* (BF, or shoot-to-soil concentration ratio) and the *translocation factor* (TF, shoot-to-root concentration ratio) reached maximum values of 27.3 and 15.4, respectively, indicating Tl accumulation in the *B. laevigata* tissues (Fellet et al., 2012).

5. Conclusions

The extraction procedure using the DTPA solution has highlighted the presence of trace metals in a chemical form that is bioavailable for uptake by the plant root system of *B. laevigata* in a decommissioned mining area. The trace metal mobility, especially for Tl, was also confirmed by the leaching test performed on the soil samples (both for bulk-soil and rhizo-soil). In addition, important concentrations of Tl and other trace metals (Zn, Pb, Fe and Mn), although the latter to a lesser extent, were found in the plant tissue (roots and leaves) at a higher rate than in the rhizo-soil samples. This evidence indicates that part of the metals were not only available in the soil of the mining area but they have also been significantly absorbed by plants.

Therefore, the most important outcome of this study is the confirmed ability of *B. laevigata* to bioaccumulate Tl and to translocate the element in its aerial part. Consequently, *B. laevigata* is a good indicator of the trace metal bioavailability, especially of Tl, not only in the study area but in all those areas where mineral bodies of sulphides are cultivated. It is apparent that this species can accumulate very high concentrations of Tl and Zn in its tissues, albeit at higher rates in the leaves than in the roots, which means that there is significant translocation and bioconcentration, two important characteristics for the feasibility of this clean-up method (McGreath and Zhao, 2003). However, some restrictions on the large-scale application of such method could be implemented due to the limited plant biomass produced, which cannot be considered as important for maintaining good efficiency. Regardless, the use of this plant species as a monitoring tool to evaluate the extension of the contamination seems to be a promising preliminary approach in environmental studies on abandoned mining areas.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2016.07.022.

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