

Temporal variation in the arsenic and metal accumulation in the maritime pine tree grown on contaminated soils

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Abstract The uptake of arsenic and other metals (iron, manganese, copper, zinc, lead, nickel and tungsten) by *Pinus pinaster* Aiton (the maritime pine tree) growing in soils and tailings around an abandoned mine (northern Portugal) was investigated. Aerial parts of *Pinus pinaster* trees were sampled from three substrate areas: a background area, in mine contaminated soils and in tailings. Vegetation material was separated into needles and stems and subdivided into tissues of different maturities (1-, 2-, 3- and 4-years-old). The sampling of the substrate in each area was also undertaken. In general, vegetation concentrations were strongly related to substrate concentrations. The results show that the contents of several elements depend as much on the plant organ as on the age of the tissue. For the researched elements, this species shows a great variability in behavior depending on the age of the organ. The data indicate that the older needles constitute the best samples for use in a conjunct biogeochemical analysis of these elements.

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Introduction

The use of vegetal species as contamination indicators usually takes into account the abilities of these species to accumulate heavy metals and other chemical elements or compounds (e.g., Kabata-Pendias 2010; Wittig 1993). Meanwhile, this accumulation generally does not change linearly with time, and varies either seasonally or with the age of the sampled organs (Kuang et al. 2007; Pratas et al. 2004, 2005). In the first case of variation, the solution to characterizing the variation of the accumulation of compounds lies in the orientation of the sampling campaign for a certain period of time, and in the second case of variation the introduction of the factor “age of the material collected” reveals the need for detailed studies.

During the last two decades, there has been an increase in the study of tree leaves as bioaccumulators of trace elements in the vicinity of industrial plants (e.g., Dmuchowski et al. 2011; Oliva and Mingorance 2006; Rodriguez et al. 2012) and in urban environments (e.g., Baycu et al. 2006; Guéguen et al. 2012; Kord et al. 2010; Liu et al. 2007; Sawidis et al. 2011). In addition, although not as numerous, there have also been studies of tree leaves as bioaccumulators in rural and background areas (e.g., Chiarenzelli et al. 2001; Coşkun 2006). Besides the potential use for biogeochemical prospecting and environmental biomonitoring, those metal-tolerant perennials plants with high biomass and bioproductivity can also be useful in phytostabilization and mine restoration (Hooper and Vitousek 1997; Pratas et al. 2005).

According to Aboal et al. (2004), there is a large amount of information available on the concentrations of trace



elements in the leaves and needles of trees commonly used as biomonitors such as *Pinus sylvestris*, *Picea abies* and *Quercus ilex*. All of these species are common in either northern Europe or southern Europe but are not found at more temperate latitudes; therefore, similar alternative species must be selected to allow studies of this type in these areas. According to Aboal et al. (2004), from among the possible alternatives, *Quercus robur* and *Pinus pinaster* are good candidates because of their distribution; however, to date, biomonitoring studies involving these species have been very limited.

Bargagli (1993) used the needles of *Pinus pinaster* as biomonitors of natural and anthropogenic emissions of mercury (Hg). Aboal et al. (2004) used the needles of *Pinus pinaster* as biomonitors of airborne trace element pollution. *Pinus pinaster* needles have been reported to accumulate aluminum (Al), arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), Hg, manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn). Pratas et al. (2004, 2005) studied the needles and stems of *Pinus pinaster* as the indicators for delineating areas of anomalous soil composition in the biogeochemical prospecting of As, antimony (Sb) and tungsten (W).

In biogeochemical studies, the application of the biological absorption coefficient (BAC) is very common as a bioaccumulation indicator of the chemical elements. The BAC is one of the most important biogeochemical parameters for the verification of the relationships between plants and soil for each element, and Kovalevskii (1979) and Brooks (1983) defined the BAC as the relationship between the concentration of an element in the ashes or in the dry plant material, C_p , and the concentration of the same element in the substrate, C_s , ($BAC = C_p/C_s$). Nagaraju and Karimulla (2002) reported that Perelman classified the values of BAC into five groups (intensive absorption, BAC 10–100; strong absorption, BAC 1–10; intermediate absorption, BAC 0.1–1; weak absorption, BAC 0.01–0.1; and very weak absorption, BAC 0.001–0.01).

The objective of the present study was to evaluate the temporal variation of As and heavy metals (Fe, Mn, Cu, Zn, Pb, Ni and W) in tissues of different ages of maritime pine trees (*Pinus pinaster* Aiton) grown on mine tailings and soils in the area surrounding the abandoned Vale das Gatas mine (N Portugal).

Studied area

The Vale das Gatas mine is located in the district of Vila Real in northern Portugal (Fig. 1). The geological units that emerge in this sector are metasedimentary rocks (Schist-Metagraywacke Complex), Hercynian granites and veinous rocks (mineralized and non-mineralized) (Fig. 1).

The Vale das Gatas granite (VGG) is affected by fracture families that are distributed according to preferential directions. Some of these fractures are filled with quartz, aplite and pegmatite, which support W, tin (Sn) and sulfide mineralizations.

The paragenesis defined for the deposition stages indicated that wolframite is the most plentiful mineral; cassiterite, scheelite, several sulfides (pyrite, chalcopyrite, sphalerite, galena, arsenopyrite, pyrrhotite, stannite, covellite, marcasite), silver (Ag), Pb and bismuth (Bi) sulfosalts and native Bi were also present. The minerals that support mineralization are essentially quartz, fluorite and muscovite.

Topographic elevations in the Vale das Gatas mine area vary between 310 and 815 m. In recent years, the average annual precipitation was 816 mm and the average annual temperature was 13.9 °C. The annual precipitation in the area varied from a minimum of 12.8 mm in August to a maximum of 142 mm in January and the temperature varies between 5.4 °C in January and 23 °C in August. In this region, the characteristic soil units are Leptosols, Cambisols and Anthrosols. The dominant soil unit of the studied area is the Leptosol type, which consists of soils whose principal characteristic is the presence of bedrock at 20 cm below the surface. These soils are divided into a distric (acid) sub-unit associated with the metasedimentary rocks and an umbric (acid and organic-rich) sub-unit associated with granitic rocks.

The Vale das Gatas mine, exploited in the past by the Romans, was in regular operation between 1883 and 1992. The mining operation was conducted underground. Wolframite and scheelite were mined for W, and cassiterite was mined for Sn. However, there has been no mining in the area since 1992, and the mine was abandoned without any environmental remediation. The tailings, which still contain metals, were deposited on the ground. Exposure of these materials to air and water produces acidic water, facilitating the release of contaminants into the surrounding environments (Ávila et al. 2005; Favas 2002; Salgueiro et al. 2008). In this mine site, tailings and acid drainage from the mine galleries (Fig. 2) are the major sources of metallic pollution. The area is crossed by a stream which contributes to the dispersion of contamination.

Materials and methods

Sampling and sample preparation

The samples of *Pinus pinaster* were collected in five sampling areas: three sampling areas outside of the mine influence (A, B and C); in one area (D) in which contamination resulting from the dispersion of the tailings was



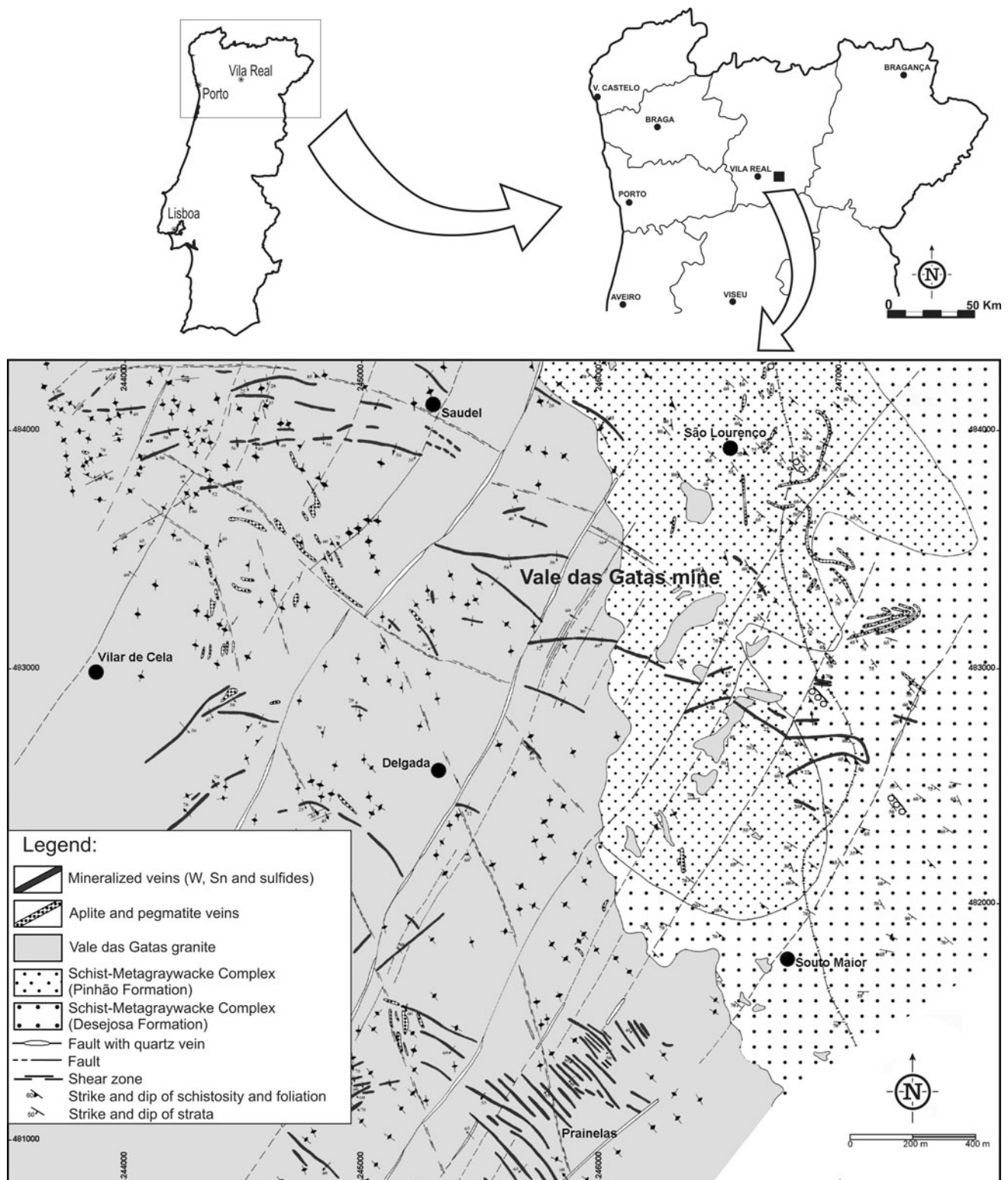


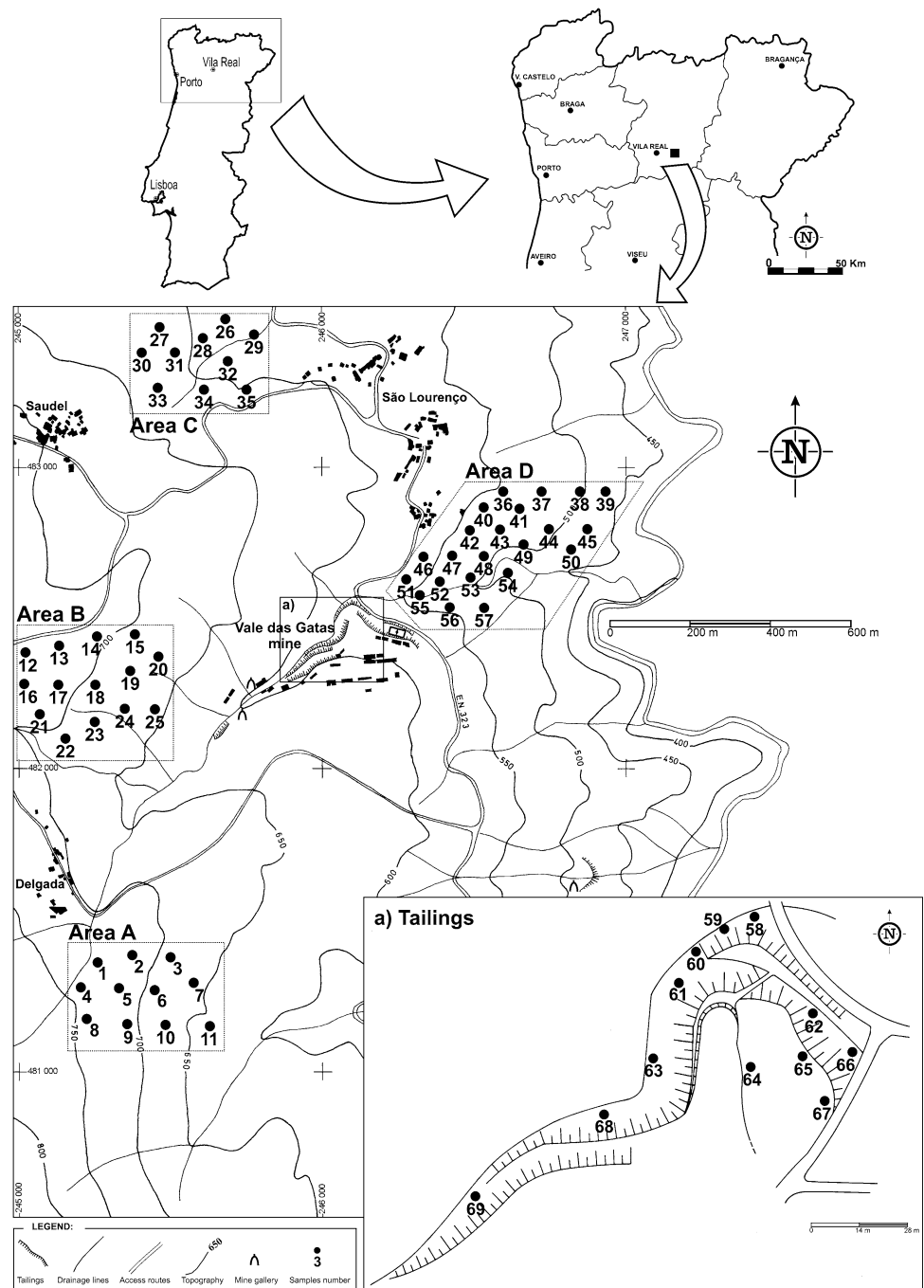
Fig. 1 Location of the Vale das Gatas mine area on the map of Portugal and a simplified geological map of the abandoned Vale das Gatas mine

previously detected (Favas 2002) and in one area with tailings (Fig. 2). The sampling methodology followed the orientations defined by Brooks (1983), Özdemir (2005) and

Sun et al. (2009). The needles, stems and rooting soils of *Pinus pinaster* were collected at the five locations in late July and early August, during the dry season of the year.



Fig. 2 Location of the Vale das Gatas mine area on the map of Portugal and a simplified topographical map of the sampling points (area A: $n = 11$; area B: $n = 14$; area C: $n = 10$; area D: $n = 22$; tailings: $n = 12$)



The pine samples were collected from trees of similar size and about 10–15 years old. Needles and stems were collected from outer branches of the middle canopy at south, west, east, and north directions and then were homogeneously mixed. When the vegetal material was sampled, we separated the different organs (stems and needles) and the tissues by age. We collected stems with 1-, 2-, 3- and 4-years-old and needles with 1-, 2- and 3-years-old. The samples were separately collected into clean cellulose bags

and brought to the laboratory on the same day. Each soil or tailings sample weighs about 5 kg, and consists of a homogenate of four subsample points collected from the rooting zone (0–20 cm depth) at the same four directions with a distance of 80 cm away from the sample tree. The total number of sampling locations was 69 distributed as follows: 11 locations in the area A; 14 locations in the area B; 10 locations in the area C; 22 locations in the area D; and 12 locations in the tailings. This resulted in



69 soil/tailings samples and 69 sampled pine trees. We collected 483 plant tissues (stems and needles with different ages).

In the laboratory, the vegetal material was washed thoroughly, first in running water and then in distilled water, and then dried in a glasshouse at a temperature of 50 °C. When dry, the material was milled into a homogeneous powder. Soil and tailings samples were oven-dried at a constant temperature of 40 °C, manually homogenized and quartered. Two equivalent fractions were obtained from each quartered sample. One fraction was used for the determination of physico-chemical properties, and the other fraction was used for chemical analysis. The samples for chemical analysis were sieved using a 2 mm mesh sieve to remove plant matter, and subsequently screened to pass through a 250 µm screen (Brooks 1983); this fraction was then milled to below 74 µm.

Analytical methods

The main physico-chemical properties of the samples (<2 mm fraction) were characterized at the UTAD Soil Analysis Laboratory (Portugal) using the following methods: the pH was determined in water extracts (1:2.5 v/v); organic carbon was determined with an elemental analyzer (Primacs SCN Analyzer, Skalar); and a conversion factor of 1.724 was used to convert organic carbon (C) into organic matter based on the assumption that organic matter contains 58 % organic C (Nelson and Sommers 1996). The chemical analyses were performed at the Chemical Laboratory at the Department of Earth Sciences, University of Coimbra (Portugal) using current analytical methods. Dried soil/tailings samples (1.0 g) were placed in Teflon crucibles. Ten ml of concentrated nitric acid (HNO₃) was added to the samples, which were then heated in a water bath until dry. Then 5 ml of perchloric acid (HClO₄) and 10 ml of hydrofluoric acid (HF) was added and the evaporation was repeated. The residue was dissolved in 10 ml of hydrochloric acid (HCl) and diluted to 100 ml by adding de-ionized water (Walsh et al. 1997). Heavy metal (Fe, Mn, Cu, Zn, Pb, Ni) concentrations were measured by the atomic absorption spectrophotometry (AAS, Perkin Elmer 2380). To determine the concentrations of As, a hydride generation system (HGS) linked to an atomic absorption device was used.

As for the plant material, dried samples (5.0 g) were placed in porcelain crucibles and reduced to ash in a muffle furnace over 12 h to a maximum temperature of 450 °C. Ash samples were cooled and weighed. Ten ml of HCl was added to the ash and evaporated in an oven. The residue was re-dissolved in 5 ml of HCl and diluted to 25 ml by adding de-ionized water (Brooks 1983; Van Loon 1985). The solution was analyzed for Fe, Mn, Cu, Zn, Pb and Ni by the

AAS. For the analysis of As in plant samples, 2 g were placed in porcelain crucibles with 20 ml of HNO₃ (30 %), which were then heated in a water bath until completely macerated. The residue was dissolved and diluted to 25 ml by adding de-ionized water (Fletcher 1981; Van Loon 1985). The determinations were made by HGS. For the analysis of W in soil/tailings and plant samples the colorimetric method was used. Dried soil samples (0.2 g) were placed in test tubes and were added 1 g of potassium bisulfate. Then the material was calcined in a muffle furnace to a maximum temperature of 570 °C. Ten ml of HCl was added to the samples, which were then heated in a water bath (85 °C). Transferred 5 ml of the supernatant solution to other test tubes with 5 ml of tin chloride (SnCl₂, 20 %) were then heated in a water bath (85 °C) for 10 min. Then 1 ml of dithiol solution was added and heated for at least 6 h. After cooling, 5 ml of petroleum was added and was measured the absorbance at 630 nm (Quin and Brooks 1972). Dried plant samples (2.0 g) were placed in porcelain crucibles, and reduced to ash in a muffle furnace over 12 h to a maximum temperature of 450 °C. Ten ml of SnCl₂ (10 %) was added to the ash, which were then heated in a water bath (85 °C) for 20 min. Then 1 ml of dithiol solution was added and heated for at least 6 h. After cooling, 1 ml of petroleum was added and was measured the absorbance at 630 nm (Quin and Brooks 1972).

The data quality control was performed by inserting triplicate samples into each batch. Analytical precision, defined as the percent relative variation at the 95 % confidence level, ranged from 2 to 6 %, depending on the concentration levels. The detection limits for trace elements in soil samples were 0.025 mg kg⁻¹ for As; 2.5 mg kg⁻¹ for Mn, Cu and Ni; 5 mg kg⁻¹ for Fe and Pb; 0.5 mg kg⁻¹ for Zn; and 0.1 mg kg⁻¹ for W. The detection limits for trace elements in plant samples were 0.005 mg kg⁻¹ for As; 1 mg kg⁻¹ for Fe; 0.5 mg kg⁻¹ for Mn, Cu, Pb and Ni; 0.1 mg kg⁻¹ for Zn; and 0.01 mg kg⁻¹ for W.

Statistical testing

We used principal components analysis (PCA) to interpret the data. A PCA is used to reduce the data dimensionality while preserving the variance–covariance structure of the data. The analysis begins with p random variables X_1, X_2, \dots, X_p , where no assumption of multivariate normality is required. If the principal components are derived from multivariate normal random variables, then they have a noteworthy geometric property. The axes of the constant density ellipsoids correspond to the principal components. In our study, PCA was performed using the AnDad (v. 7.10) software.

The influences of organ age and their interaction on chemical elements distribution were tested with a one-way



analysis of variance (ANOVA) to detect significant differences between means (for $p \leq 0.05$). ANOVA test was performed using the SPSS statistical program (IBM SPSS Statistics 19). When the null hypothesis was rejected, ANOVA was followed by a comparison to identify which factor levels had different means. The separation of means was performed by a post hoc comparison test (LSD-test) at the $p \leq 0.05$ significance level.

Results and discussion

The results of the soil and tailings analysis are presented in Table 1 in terms of averages, contents variations and standard deviations. The basic characteristics (pH and organic matter) of the tested soils and tailings are presented in Table 1. In Tables 2 and 3, the averages, contents variations and standard deviations found in the analyzed samples of *P. pinaster* are presented by sampling areas and organ ages. With the aim of determining the plant-soil relationships, the BAC was determined for each element. The statistical summaries of BAC values are presented in Tables 2 and 3.

Soils and tailings in the Vale das Gatas mine area were acidic and contained a low percentage of organic matter (Table 1). The chemical data (soil/tailings and plant) were analyzed by the PCA. For the soil/tailings samples, the data matrix used in the analysis consisted of 69 samples and 10 variables (pH, organic matter, As, Fe, Mn, Cu, Zn, Pb, Ni and W). The first two principal components explain more than 79 % of the total variance in the data (Fig. 3). The first principal component (axis 1), which explains 56.1 % of the total data variance, defines a pH-organic matter association with positive loadings and a Cu-Mn-W-As-Pb-Zn association with negative loadings. The second component (axis 2), explaining 23.0 % of the total data variance, reveals a Ni-Fe association with positive loadings.

Thus, the data of total element concentrations mainly show two element associations. The Cu-Mn-W-As-Pb-Zn association, which is inversely correlated with pH and organic matter, reflects the presence of mineralised veins in the area. The Ni-Fe association, which reveals the independent behavior that of the first group and that of the pH-organic matter group, maybe exists because of the similar geochemical behaviors and interdependence of these elements (i.e., in geochemical cycles, the behavior of Ni resemble those of Fe) (Adriano 2001).

The PCA highlighted sample “populations”, which are clearly distinct from one another (Fig. 4). Population A consists of samples that can be considered to have geochemical background values. This population is composed by samples from the studied areas A, B and C. Populations B and C reflect geochemical anomalies. Population B consist of all tailings samples and is primarily linked to a

Cu-Mn-W-As-Pb-Zn association. Population C, consisting of the all samples from the studied area D and one sample from area A, is primarily associated with a Ni-Fe association with affinities to other elements such as Mn and Zn.

For the plant samples, the data matrix used in the analysis consisted of 483 samples and 8 variables (As, Fe, Mn, Cu, Zn, Pb, Ni and W). The result of three principal axes, which explained more than 82 % of the total variance, is shown in Fig. 5. Considering the retained axes and given coordinate values, the following variable associations were verified: a group composed of Pb, Zn, W, Fe, As, Cu and in some way, Ni (axis 1); however, Ni is mainly explained by axis 2, as opposed to Mn; in turn, Mn is best explained by axis 3 revealing an independent behavior of the Pb-Zn-W-Fe-As-Cu association.

The PCA also highlighted plant sample “populations”, which are mostly distinct from one another (Fig. 6). Population A is composed of all plant samples from the studied areas A, B and C. This population represents the local biogeochemical background. No significant differences between needles and stems or between tissue ages in this population were calculated. Population B results from the association of the most Ni-Cu contaminated samples. This population is essentially composed by the 1-year-old needles and stems both on the tailings and area D. Population C is composed of the samples situated under the influence of the Pb-Zn-W-Fe-As group. This population is essentially composed by the 2- and 3-years-old needles both on the tailings and area D. Population D, consisting of the remaining samples from tailings and area D (2-, 3- and 4-years-old stems), seems to establish the transition between the B and C populations.

The content variations in vegetal materials were, in general, strongly related to the content variations in soils. It has also been verified that in contaminated locations or tailings, the concentration of metals in plant tissues is high due to the high metal concentrations in the soil. The maritime pine trees growing in the tailings and in the contaminated soils (area D) accumulated the various researched chemical elements in quantities greater than those verified in the plants of the representative areas of the local geochemical background (areas A, B, C), and than those that are typically observed in this species. The observed values also allowed us to verify that the contents of several elements in the vegetal material depend as much on the plant organ as on its age (Fig. 7a, b). In the contaminated sampling locations (tailings and area D), the older needles (2- and 3-years-old) show a tendency to accumulate higher concentrations of As, Fe, Zn, Pb and W. While the Ni and Cu were preferentially accumulated in young needles and stems (1-year-old). This was confirmed by PCA analysis (Fig. 6) and ANOVA analysis (Table 4). In Table 5, the result of post hoc comparison test



Table 1 Statistical summaries of pH, organic matter and total concentrations of studied elements in soils and tailings samples

Parameter	Sampling area	Mean	Minimum	Maximum	Standard deviation
pH	Tailings	3.7	3.5	4.2	0.35
	Area A	5.9	5.6	6.3	0.36
	Area B	5.5	5.2	6.1	0.44
	Area C	4.9	4.5	5.2	0.31
	Area D	4.9	4.7	5.1	0.28
Organic matter (%)	Tailings	0.30	0.26	0.36	0.05
	Area A	2.52	0.31	3.88	1.93
	Area B	4.65	3.13	6.10	1.51
	Area C	4.35	3.31	6.31	1.34
	Area D	1.90	0.49	3.31	1.99
As (mg kg ⁻¹)	Tailings	1,963	541	5,770	2,175
	Area A	37.7	26.7	48.1	10.1
	Area B	47.8	37.4	66.8	16.5
	Area C	56.7	45.5	69.1	11.8
	Area D	128	122	134	8.39
Fe (mg kg ⁻¹)	Tailings	36,483	28,600	46,859	6,689
	Area A	32,816	20,531	60,100	18,613
	Area B	24,355	22,267	26,077	2,049
	Area C	22,684	18,482	30,350	6,720
	Area D	49,298	44,618	53,975	6,608
Mn (mg kg ⁻¹)	Tailings	667	406	898	208
	Area A	243	132	565	214
	Area B	139	119	167	25.6
	Area C	120	103	146	22.6
	Area D	507	457	558	71.2
Cu (mg kg ⁻¹)	Tailings	259	164	352	75.2
	Area A	24.5	11.7	31.0	8.78
	Area B	24.4	23.0	26.7	2.01
	Area C	20.8	12.0	29.0	8.52
	Area D	112	40.0	184	102
Zn (mg kg ⁻¹)	Tailings	302	187	469	103
	Area A	123	107	134	11.3
	Area B	119	73.3	159	43.2
	Area C	85.8	63.1	98.1	19.7
	Area D	275	117	433	224
Pb (mg kg ⁻¹)	Tailings	2,152	584	6,299	2,372
	Area A	85.5	63.0	102	19.6
	Area B	84.4	63.3	103	19.9
	Area C	66.1	55.4	78.0	11.3
	Area D	110	105	115	7.29
Ni (mg kg ⁻¹)	Tailings	21.1	11.6	30.8	6.94
	Area A	32.1	18.2	61.2	19.9
	Area B	23.6	23.1	24.5	0.74
	Area C	22.4	17.8	29.0	5.88
	Area D	54.4	49.8	59.0	6.50
W (mg kg ⁻¹)	Tailings	287	11.0	636	290
	Area A	9.83	5.00	12.0	3.28
	Area B	4.57	2.00	7.09	2.55
	Area C	4.07	3.20	5.10	0.96
	Area D	62.4	59.2	65.6	4.48

(LSD-test) for separation of means of the different ages of needles and stems is reported: the different letters close to the means indicate which ones are significantly different, according to the comparison test. Therefore, there were some significant differences in accumulation of chemical elements depending on the age and type of biological tissue.

Arsenic is not essential for vegetal metabolism, and is highly toxic to plants with a toxicity level of approximately 2 mg kg⁻¹ (Kabata-Pendias 2010). Given this toxicity level, the concentrations observed may be considered high (Tables 2 and 3). The observed values allowed us to verify that the greatest concentrations of As were present in the foliar material, both in the soils, representative of the geochemical background, and in the tailings and contaminated soils. The concentration of this element always increases with the age of the needle, though not as drastically in plants that are not subjected to strong contamination (Fig. 7a). This relationship is more significant when the soils are contaminated by As. In contrast, the concentrations of As in the stems decreased with age (Fig. 7b). This behavior might be related to a plant defense mechanism against high concentrations of this toxic element, which is the translocation of As to the older needles that eventually fall from the plant.

Considering to the plant/soil relations and Perelman's classification, it was verified that all of the woody material and the youngest foliar material were characterized by BAC values (Tables 2 and 3) indicating very weak to weak absorption of As, while the older foliar material (3-years-old) had BAC values indicative of very weak and intermediate absorption with the particular trait that the lowest plant/soil ratios (very weak absorption) were found in the most contaminated area (tailings). Thus, in this species, there is a limiting behavior for very high concentrations of As in the substrate or in the influence of a factor related to soil conditions such as the pH or the presence of Mn and Fe oxides, which have been reported to limit As bioavailability. The bioavailability of As and metals can be affected mainly by the three following factors (e.g., Adriano 2001; Mani et al. 2012): (1) soil capacity (pH, cation exchange capacity, organic matter, amount and type of clay, ion interactions, amount of Fe and Mn oxides and redox potential); (2) plant capacity, which depends on the species, cultivar and age of plant part; and (3) the plant-trace element interactions.

The concentration of Fe in *P. pinaster* needles increased progressively with the age of the organ (Fig. 7a, b). Kabata-Pendias (2010) reported that Dolobovskaya also observed Fe retention by the oldest needles in exemplars of *Pinus sibirica* Turcz (Siberia pine tree) and *P. pinaster*, respectively. The use and retention of Fe in the needles do



Table 2 Statistical summaries of element contents in the needles of *P. pinaster* samples displayed by organ age and sampling area (mg kg^{-1} , values on dry weight basis, DW)

Organ age	Element	Tailings				Area A				Area B				Area C				Area D							
		Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD				
1st year	As	9.09	3.10	9.47	1.80		3.39	0.29	10.8	0.91		3.81	0.20	9.50	0.62		4.52	3.20	6.06	0.96		10.1	5.96	15.1	1.77
	BAC	0.004	0.001	0.009	0.003		0.095	0.006	0.244	0.087		0.057	0.005	0.254	0.099		0.090	0.046	0.114	0.027		0.082	0.045	0.118	0.029
	Fe	110	39.3	181	50.2		55.7	48.6	63.8	5.43		66.3	55.2	78.6	8.29		49.8	45.7	53.5	2.77		140	110	152	15.6
	BAC	0.003	0.001	0.005	0.001		0.002	0.001	0.003	0.001		0.003	0.002	0.005	0.000		0.002	0.001	0.003	0.001		0.003	0.002	0.004	0.000
	Mn	110	44.9	175	46.1		208	153	247	34.5		135	85.6	152	25.1		241	213	269	19.8		135	105	181	26.9
	BAC	0.131	0.050	0.271	0.084		1.124	0.394	1.816	0.550		1.040	0.511	1.137	0.240		1.954	1.842	2.337	0.182		0.295	0.208	0.395	0.071
	Cu	26.1	7.12	45.2	6.51		4.11	2.87	5.13	0.81		4.38	2.62	5.10	0.92		2.69	2.33	3.01	0.24		10.7	6.40	14.1	2.73
	BAC	0.076	0.023	0.159	0.051		0.233	0.093	0.438	0.136		0.190	0.111	0.217	0.039		0.110	0.080	0.225	0.053		0.268	0.035	0.353	0.125
	Zn	188	46.1	330	10.0		26.3	22.4	32.5	3.76		25.0	20.1	29.6	3.36		17.9	17.1	18.4	0.49		35.3	26.9	44.3	6.16
2nd year	BAC	0.647	0.098	1.197	0.420		0.218	0.182	0.242	0.023		0.345	0.157	0.353	0.076		0.234	0.177	0.291	0.055		0.304	0.082	0.325	0.087
	Pb	10.9	9.13	12.6	1.23		4.91	4.78	5.13	0.14		2.74	1.32	4.23	1.03		1.50	1.02	1.97	0.34		9.74	4.12	13.0	3.19
	BAC	0.005	0.001	0.019	0.007		0.064	0.047	0.076	0.013		0.043	0.015	0.049	0.013		0.022	0.018	0.027	0.003		0.084	0.038	0.118	0.029
	Ni	2.88	1.84	3.87	0.72		1.10	0.81	1.44	0.22		1.31	0.75	1.95	0.43		0.99	0.86	1.11	0.09		1.44	0.93	2.60	0.62
	BAC	0.208	0.080	0.335	0.102		0.041	0.013	0.071	0.023		0.055	0.031	0.080	0.017		0.046	0.030	0.062	0.012		0.024	0.017	0.048	0.012
	W	1.17	0.27	2.07	0.44		0.04	0.03	0.05	0.01		0.07	0.03	0.11	0.03		0.09	0.07	0.12	0.02		1.42	0.86	2.01	0.41
	BAC	0.005	0.001	0.106	0.052		0.004	0.003	0.008	0.002		0.034	0.004	0.041	0.011		0.021	0.018	0.028	0.004		0.024	0.014	0.032	0.007
	As	10.8	7.35	12.5	1.13		3.55	0.39	4.36	0.57		4.25	1.50	5.43	0.45		5.01	4.62	7.29	0.98		13.2	4.60	17.6	1.42
	BAC	0.006	0.001	0.014	0.005		0.099	0.008	0.149	0.051		0.064	0.038	0.114	0.030		0.099	0.072	0.160	0.035		0.108	0.034	0.144	0.045
	Fe	254	146	361	75.9		78.7	70.9	89.0	6.61		125	87.6	141	19.8		78.3	69.2	88.3	6.75		278	186	340	55.0
	BAC	0.007	0.004	0.010	0.002		0.003	0.001	0.004	0.001		0.005	0.003	0.006	0.001		0.004	0.002	0.005	0.001		0.005	0.003	0.007	0.001
	Mn	244	201	288	30.9		460	387	595	83.0		406	305	597	106		620	546	693	51.9		154	125	186	21.6
	BAC	0.282	0.243	0.601	0.142		2.302	1.053	3.276	0.921		3.135	1.821	3.565	0.690		5.023	4.748	6.013	0.467		0.338	0.274	0.367	0.037
	Cu	11.5	9.01	13.9	1.73		2.72	2.42	3.29	0.34		2.21	1.60	2.83	0.43		2.11	1.95	2.33	0.14		8.71	5.25	10.7	1.97
	BAC	0.035	0.026	0.070	0.016		0.138	0.081	0.207	0.050		0.097	0.068	0.120	0.019		0.088	0.067	0.175	0.040		0.268	0.047	0.318	0.069
	Zn	217	113	320	17.3		29.8	21.4	35.6	5.20		24.0	19.5	33.6	5.19		26.1	21.4	31.3	3.52		246	186	298	39.7
	BAC	0.546	0.410	1.160	0.269		0.248	0.174	0.332	0.056		0.323	0.151	0.365	0.075		0.332	0.265	0.412	0.054		2.115	0.569	2.597	0.378
	Pb	19.9	12.2	27.6	2.44		4.77	4.31	5.33	0.37		3.20	2.60	3.80	0.42		2.79	2.41	3.13	0.25		21.2	17.6	25.8	2.91
	BAC	0.010	0.002	0.034	0.013		0.063	0.042	0.085	0.016		0.050	0.030	0.062	0.009		0.042	0.040	0.050	0.004		0.185	0.153	0.235	0.029
	Ni	1.18	0.95	1.37	0.15		0.82	0.60	1.10	0.18		0.89	0.63	1.13	0.18		0.92	0.81	1.07	0.09		1.05	0.91	1.19	0.10
	BAC	0.071	0.039	0.082	0.015		0.029	0.013	0.054	0.017		0.038	0.026	0.046	0.007		0.044	0.028	0.060	0.012		0.018	0.017	0.021	0.002
	W	2.35	1.40	3.29	0.47		0.06	0.04	0.07	0.01		0.10	0.05	0.21	0.06		0.08	0.05	0.11	0.02		2.45	2.11	2.79	0.24
	BAC	0.008	0.003	0.213	0.106		0.005	0.003	0.012	0.003		0.050	0.007	0.064	0.016		0.017	0.012	0.025	0.005		0.047	0.036	0.053	0.002



Table 2 continued

Organ age	Element	Tailings				Area A				Area B				Area C				Area D			
		Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
3rd year	As	12.0	7.90	13.9	1.20	4.12	0.70	8.64	0.83	5.43	4.25	6.30	0.73	5.64	4.40	6.10	0.70	14.7	6.22	16.5	1.07
	BAC	0.006	0.001	0.022	0.009	0.114	0.015	0.324	0.115	0.081	0.064	0.161	0.038	0.112	0.086	0.119	0.007	0.121	0.049	0.135	0.033
	Fe	300	214	387	61.4	107	100	115	5.53	251	163	284	45.3	106	103	115	4.55	298	211	375	58.0
	BAC	0.008	0.006	0.010	0.002	0.004	0.002	0.006	0.002	0.011	0.006	0.016	0.002	0.005	0.004	0.006	0.001	0.006	0.004	0.008	0.001
	Mn	410	204	617	146	401	250	545	105	459	413	495	29.1	557	480	635	54.8	157	106	235	46.2
	BAC	0.467	0.247	1.010	0.283	1.951	0.965	2.995	0.852	3.550	2.466	3.867	0.541	4.502	4.349	5.402	0.401	0.343	0.232	0.463	0.087
	Cu	8.41	5.56	11.3	2.04	2.12	1.88	2.58	0.27	2.75	2.10	3.52	0.50	4.39	1.82	6.92	1.80	5.50	4.85	8.71	1.53
	BAC	0.026	0.016	0.051	0.013	0.108	0.063	0.160	0.039	0.119	0.089	0.150	0.023	0.193	0.063	0.367	0.119	0.218	0.043	0.338	0.040
	Zn	377	141	612	16.7	25.9	17.1	33.4	5.80	24.1	18.6	31.2	4.51	27.8	23.8	31.9	2.87	471	386	562	62.2
	BAC	0.908	0.512	2.015	0.533	0.218	0.139	0.312	0.062	0.322	0.148	0.372	0.074	0.376	0.247	0.506	0.109	4.041	1.087	4.513	0.825
	Pb	22.5	13.5	31.5	3.34	5.38	4.80	6.11	0.48	3.76	3.15	3.98	0.31	2.87	2.35	3.40	0.37	24.2	19.6	29.7	3.58
	BAC	0.012	0.002	0.039	0.015	0.069	0.053	0.082	0.011	0.059	0.036	0.092	0.009	0.043	0.041	0.052	0.004	0.211	0.170	0.270	0.036
	Ni	1.08	0.83	1.35	0.18	0.85	0.67	1.17	0.20	1.08	0.98	1.16	0.06	1.39	0.95	1.85	0.32	1.04	0.76	1.22	0.16
	BAC	0.065	0.035	0.071	0.013	0.031	0.011	0.058	0.019	0.047	0.040	0.051	0.003	0.068	0.033	0.104	0.026	0.018	0.014	0.021	0.003
	W	2.37	1.64	3.09	0.41	0.16	0.09	0.29	0.08	0.23	0.16	0.25	0.03	0.17	0.14	0.20	0.02	2.79	2.45	2.98	0.19
	BAC	0.008	0.003	0.0215	0.107	0.014	0.009	0.032	0.010	0.113	0.023	0.126	0.035	0.039	0.028	0.053	0.011	0.041	0.037	0.048	0.004

BAC biological absorption coefficient, concentration in plant/concentration in soil; SD standard deviation



Table 3 Statistical summaries of element contents in the stems of *P. pinaster* samples displayed by organ age and sampling area (mg kg^{-1} , values on dry weight basis, DW)

Organ age	Element	Tailings				Area A				Area B				Area C				Area D			
		Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
1st year	As	8.69	4.28	9.50	1.09	2.67	0.28	3.01	0.12	3.80	2.10	4.40	0.87	4.17	3.53	5.03	0.53	10.1	6.40	14.1	1.72
	BAC	0.004	0.002	0.016	0.007	0.078	0.006	0.113	0.039	0.057	0.054	0.102	0.020	0.083	0.060	0.091	0.011	0.082	0.048	0.116	0.024
	Fe	180	164	195	11.2	48.2	46.4	50.3	1.38	135	56.5	156	38.6	51.9	45.3	58.2	4.55	126	100	169	24.6
	BAC	0.005	0.004	0.006	0.001	0.002	0.001	0.002	0.001	0.006	0.002	0.008	0.002	0.002	0.001	0.003	0.001	0.002	0.002	0.003	0.000
	Mn	90.9	31.8	150	11.7	126	107	162	12.5	147	125	165	14.2	135	130	140	3.72	24.2	16.3	32.5	5.73
	BAC	0.103	0.039	0.224	0.070	0.732	0.190	1.194	0.383	1.133	0.746	1.238	0.185	1.110	0.962	1.309	0.136	0.053	0.032	0.071	0.014
	Cu	70.1	28.5	112	9.41	6.00	4.37	7.15	1.02	6.42	3.40	7.60	1.68	2.71	1.90	3.54	0.58	33.1	21.9	46.4	4.68
	BAC	0.205	0.091	0.427	0.123	0.334	0.141	0.610	0.184	0.279	0.139	0.323	0.072	0.105	0.089	0.225	0.051	0.828	0.119	1.160	0.413
	Zn	159	65.0	253	16.6	27.1	23.0	33.7	4.04	32.8	22.1	38.4	5.94	14.3	12.6	16.5	1.68	50.2	39.5	76.5	13.8
	BAC	0.529	0.139	0.919	0.309	0.223	0.203	0.251	0.018	0.444	0.177	0.482	0.104	0.187	0.146	0.228	0.031	0.429	0.116	0.657	0.226
2nd year	Pb	16.2	15.5	17.0	0.55	6.42	5.34	7.22	0.69	2.55	1.50	3.50	0.71	2.50	1.79	3.19	0.50	11.9	7.62	15.4	1.76
	BAC	0.007	0.003	0.028	0.011	0.083	0.063	0.107	0.017	0.040	0.017	0.051	0.010	0.037	0.032	0.045	0.005	0.103	0.066	0.140	0.026
	Ni	3.61	2.35	4.88	0.60	1.59	1.15	1.86	0.27	2.16	1.83	2.68	0.31	1.62	1.44	1.85	0.15	1.83	1.25	2.52	0.45
	BAC	0.262	0.102	0.422	0.129	0.056	0.019	0.088	0.028	0.091	0.075	0.109	0.012	0.077	0.050	0.104	0.020	0.031	0.021	0.046	0.009
	W	2.12	0.89	3.34	0.37	0.05	0.04	0.06	0.01	0.07	0.04	0.09	0.02	0.05	0.03	0.08	0.02	2.82	1.78	3.15	0.12
	BAC	0.008	0.002	0.193	0.095	0.005	0.004	0.010	0.003	0.034	0.006	0.034	0.010	0.011	0.007	0.016	0.004	0.048	0.029	0.053	0.009
	As	7.53	7.12	9.20	0.80	2.52	2.31	3.97	0.68	3.82	3.10	4.79	0.60	3.92	3.40	4.80	0.50	8.18	7.30	9.10	0.64
	BAC	0.004	0.002	0.014	0.005	0.076	0.052	0.149	0.040	0.057	0.046	0.128	0.030	0.077	0.057	0.087	0.011	0.067	0.057	0.075	0.006
	Fe	400	293	508	76.0	63.1	45.7	73.2	10.7	140	95.6	163	24.5	30.2	27.3	33.5	2.21	391	283	453	61.4
	BAC	0.011	0.008	0.014	0.003	0.002	0.001	0.003	0.001	0.006	0.004	0.006	0.001	0.001	0.001	0.002	0.000	0.007	0.005	0.009	0.002
2nd year	Mn	106	39.4	173	17.3	129	124	138	5.14	115	105	126	7.43	89.1	70.9	107	12.9	30.3	21.5	42.6	7.52
	BAC	0.120	0.048	0.261	0.080	0.726	0.224	1.044	0.340	0.890	0.627	0.969	0.131	0.712	0.688	0.863	0.067	0.066	0.047	0.084	0.014
	Cu	51.7	43.1	60.3	5.11	4.77	4.07	5.18	0.43	4.58	4.03	5.03	0.36	2.21	1.96	2.49	0.19	21.9	18.3	33.1	2.64
	BAC	0.155	0.138	0.315	0.068	0.253	0.131	0.428	0.118	0.200	0.171	0.214	0.018	0.089	0.086	0.183	0.040	0.828	0.245	0.958	0.119
	Zn	270	191	349	15.6	30.0	27.6	34.2	2.56	21.9	15.4	25.6	3.84	7.98	4.99	11.0	2.12	147	95.3	196	13.6
	BAC	0.836	0.408	1.443	0.396	0.251	0.210	0.319	0.042	0.312	0.123	0.346	0.073	0.096	0.079	0.126	0.021	1.263	0.588	1.818	0.184
	Pb	37.9	21.9	53.9	1.31	5.39	4.53	6.57	0.75	3.13	2.61	3.86	0.45	1.78	0.69	2.83	0.76	41.4	33.6	48.6	2.31
	BAC	0.020	0.003	0.065	0.025	0.072	0.044	0.104	0.023	0.048	0.030	0.053	0.008	0.024	0.012	0.036	0.009	0.360	0.292	0.442	0.054
	Ni	2.40	2.26	2.53	0.10	1.02	0.90	1.14	0.08	1.46	1.15	1.63	0.17	0.76	0.58	1.76	0.47	1.88	1.32	2.24	0.34
	BAC	0.153	0.078	0.195	0.043	0.035	0.015	0.056	0.016	0.063	0.047	0.067	0.008	0.032	0.028	0.087	0.023	0.033	0.022	0.041	0.007
	W	1.27	0.58	1.95	0.28	0.12	0.09	0.13	0.01	0.12	0.08	0.14	0.02	0.10	0.07	0.13	0.02	1.33	0.61	1.46	0.24
	BAC	0.005	0.001	0.115	0.057	0.010	0.008	0.024	0.006	0.061	0.011	0.072	0.019	0.021	0.017	0.031	0.006	0.022	0.010	0.025	0.006



Table 3 continued

Organ age	Element	Tailings			Area A			Area B			Area C			Area D							
		Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD				
3rd year	As	6.34	5.19	9.48	0.61	2.02	1.20	3.32	0.76	3.37	1.96	4.20	0.81	3.71	3.42	4.78	0.53	5.70	5.54	6.30	0.29
		BAC	0.003	0.001	0.012	0.004	0.059	0.038	0.124	0.036	0.050	0.029	0.107	0.030	0.073	0.054	0.086	0.012	0.047	0.043	0.052
	Fe	218	208	228	6.98	58.9	51.3	65.1	4.97	248	215	273	20.6	26.6	24.9	28.4	1.24	160	126	196	24.8
		BAC	0.006	0.005	0.008	0.001	0.002	0.001	0.003	0.001	0.011	0.008	0.014	0.001	0.001	0.001	0.001	0.000	0.003	0.002	0.004
	Mn	80.8	24.8	137	19.6	126	98.6	141	16.7	122	114	136	7.94	70.8	58.2	83.5	8.96	19.1	14.7	26.8	4.37
		BAC	0.091	0.030	0.199	0.064	0.752	0.175	1.044	0.379	0.944	0.681	1.028	0.132	0.568	0.564	0.687	0.051	0.042	0.032	0.053
	Cu	27.7	15.5	40.0	4.64	3.65	2.73	4.31	0.58	3.44	3.12	3.95	0.30	1.58	1.14	2.02	0.31	11.9	8.64	22.3	2.21
		BAC	0.082	0.050	0.169	0.043	0.196	0.088	0.334	0.095	0.149	0.129	0.168	0.016	0.062	0.053	0.132	0.030	0.299	0.065	0.316
	Zn	208	141	276	17.8	27.7	22.2	37.1	5.75	24.7	23.4	34.6	4.61	7.21	4.90	9.54	1.64	108	76.5	159	19.6
		BAC	0.650	0.300	1.112	0.315	0.230	0.166	0.301	0.048	0.336	0.155	0.347	0.073	0.088	0.073	0.114	0.017	0.929	0.250	0.997
4th year	Pb	22.6	19.4	25.8	2.25	5.03	4.63	5.66	0.39	4.10	3.67	4.86	0.43	1.50	0.85	2.15	0.46	19.8	16.4	24.6	2.92
		BAC	0.010	0.003	0.039	0.015	0.066	0.047	0.090	0.017	0.065	0.040	0.076	0.010	0.021	0.015	0.028	0.005	0.172	0.143	0.224
	Ni	1.38	1.34	1.43	0.03	0.93	0.80	1.09	0.10	1.33	1.29	1.35	0.02	0.72	0.54	0.91	0.13	1.03	0.96	1.10	0.05
		BAC	0.091	0.045	0.123	0.030	0.032	0.013	0.051	0.015	0.058	0.053	0.062	0.002	0.031	0.030	0.040	0.004	0.017	0.016	0.021
	W	1.01	0.83	1.20	0.13	0.13	0.07	0.19	0.04	0.21	0.17	0.36	0.07	0.06	0.03	0.09	0.02	0.84	0.80	0.96	0.06
		BAC	0.003	0.002	0.092	0.045	0.011	0.006	0.026	0.007	0.106	0.024	0.113	0.031	0.014	0.006	0.022	0.006	0.014	0.013	0.015
	As	5.74	5.37	7.30	0.76	1.78	0.92	3.80	0.07	2.10	1.20	5.10	0.49	3.41	3.18	4.63	0.58	4.54	4.21	6.11	0.75
		BAC	0.002	0.001	0.011	0.004	0.050	0.021	0.142	0.047	0.031	0.018	0.130	0.042	0.067	0.049	0.084	0.013	0.037	0.034	0.048
	Fe	180	158	202	15.6	48.0	34.3	62.1	9.85	147	124	165	14.6	38.2	33.6	44.5	3.88	121	114	146	12.3
		BAC	0.005	0.004	0.006	0.001	0.002	0.001	0.002	0.001	0.007	0.005	0.008	0.001	0.002	0.001	0.002	0.000	0.002	0.002	0.003
Mn	73.4	22.2	126	16.7	114	60.3	149	13.4	111	96.5	129	11.5	67.2	56.6	78.3	7.67	17.0	13.6	25.3	4.35	
	BAC	0.082	0.027	0.180	0.058	0.738	0.107	1.130	0.412	0.856	0.576	0.935	0.134	0.543	0.536	0.650	0.046	0.037	0.030	0.050	0.008
Cu	19.4	11.5	29.1	3.23	3.12	2.92	3.44	0.19	2.63	2.46	2.95	0.18	1.29	1.11	1.44	0.12	8.90	6.45	10.6	1.48	
	BAC	0.057	0.037	0.118	0.029	0.160	0.097	0.249	0.061	0.114	0.098	0.125	0.010	0.052	0.050	0.108	0.024	0.222	0.048	0.261	0.042
Zn	177	119	243	13.7	24.9	21.1	30.9	3.74	22.0	19.4	32.6	5.15	6.84	4.64	8.54	1.40	92.3	86.2	105	6.94	
	BAC	0.551	0.255	0.946	0.274	0.207	0.157	0.251	0.034	0.301	0.138	0.324	0.070	0.088	0.071	0.111	0.016	0.789	0.212	0.840	0.200
Pb	20.4	19.3	23.5	1.57	5.27	4.14	6.23	0.74	3.03	2.45	4.25	0.66	1.91	1.11	2.75	0.58	14.9	12.5	16.9	1.56	
	BAC	0.008	0.004	0.035	0.013	0.069	0.052	0.099	0.020	0.048	0.028	0.049	0.010	0.027	0.020	0.035	0.006	0.129	0.114	0.147	0.013
Ni	1.10	1.01	1.19	0.06	0.80	0.67	0.92	0.09	0.83	0.71	0.95	0.08	0.71	0.54	0.90	0.13	0.79	0.66	0.95	0.10	
	BAC	0.072	0.036	0.103	0.026	0.028	0.011	0.046	0.014	0.036	0.029	0.039	0.004	0.030	0.028	0.039	0.004	0.013	0.011	0.017	0.002
W	1.68	1.42	1.99	0.20	0.50	0.12	1.22	0.14	0.23	0.16	0.32	0.06	0.20	0.08	0.35	0.10	1.35	1.21	1.42	0.08	
	BAC	0.005	0.003	0.053	0.076	0.043	0.010	0.105	0.045	0.113	0.023	0.124	0.034	0.042	0.020	0.069	0.019	0.023	0.020	0.023	0.001

BAC biological absorption coefficient, concentration in plant/concentration in soil; SD standard deviation



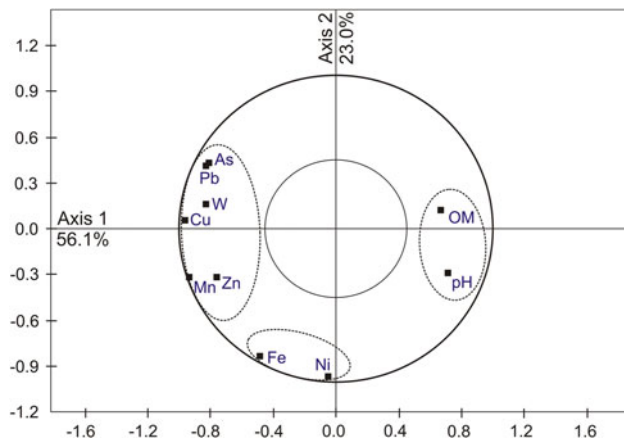


Fig. 3 Projection of the variables in the first factorial plan of the principal components analysis (PCA) (soil/tailings samples)

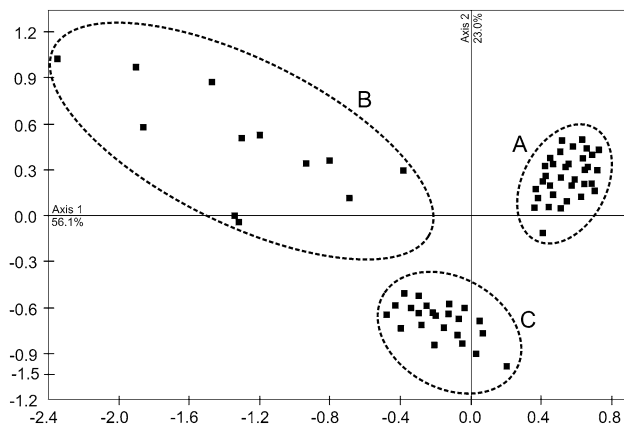


Fig. 4 Projection of the samples in the first factorial plan of the principal components analysis (PCA) (soil/tailings samples)

not seem to be controlled by factors inherent to the level of contamination, given that the retention behaviors were similar in the different sampling areas. Meanwhile, in the woody parts, it seems to be interference from those factors in the bioutilization of this element. Thus, it was verified that the stems of the plants that grew in the tailings and in the contaminated area exhibit a high level of retention of this element in the tissues with 2-years-old, manifesting, from then on, a behavior close to that of the plants growing in the soils, representative of the geochemical background. For Fe, we observed very low plant/soil ratios (Tables 2 and 3), revealing weak to very weak absorption, despite the fact that Fe is an essential nutrient. This behavior, however, seems to be common in soils that are very rich in Fe, and where the geochemical forms are very poorly soluble making the availability of the element very low.

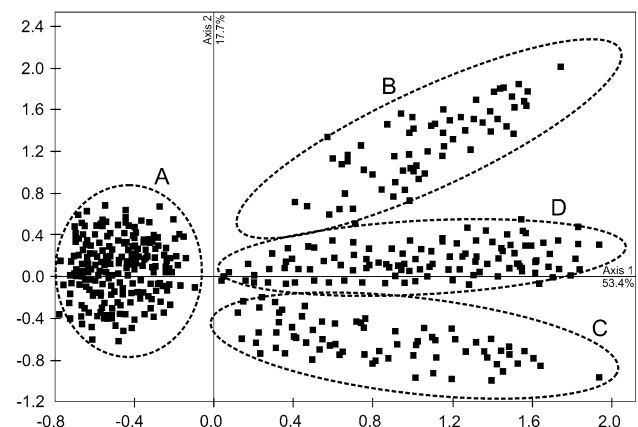


Fig. 6 Projection of the samples in the first factorial plan of the principal components analysis (PCA) (plant samples)

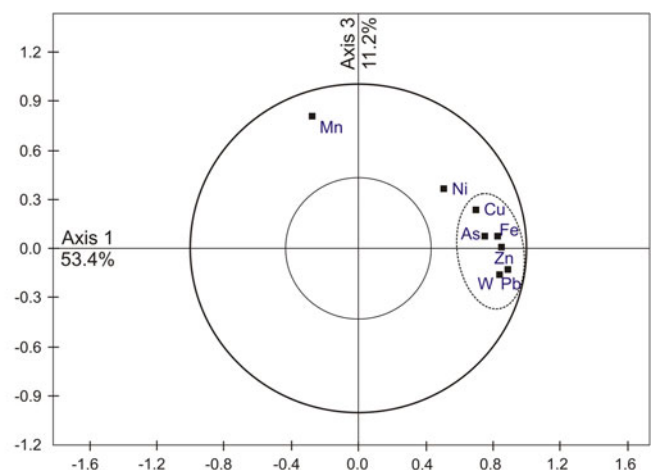
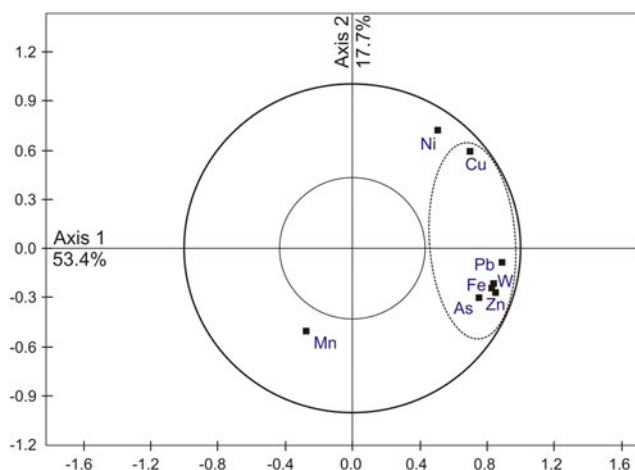


Fig. 5 Projection of the variables in the two first factorial plans of the principal components analysis (PCA) (plant samples)

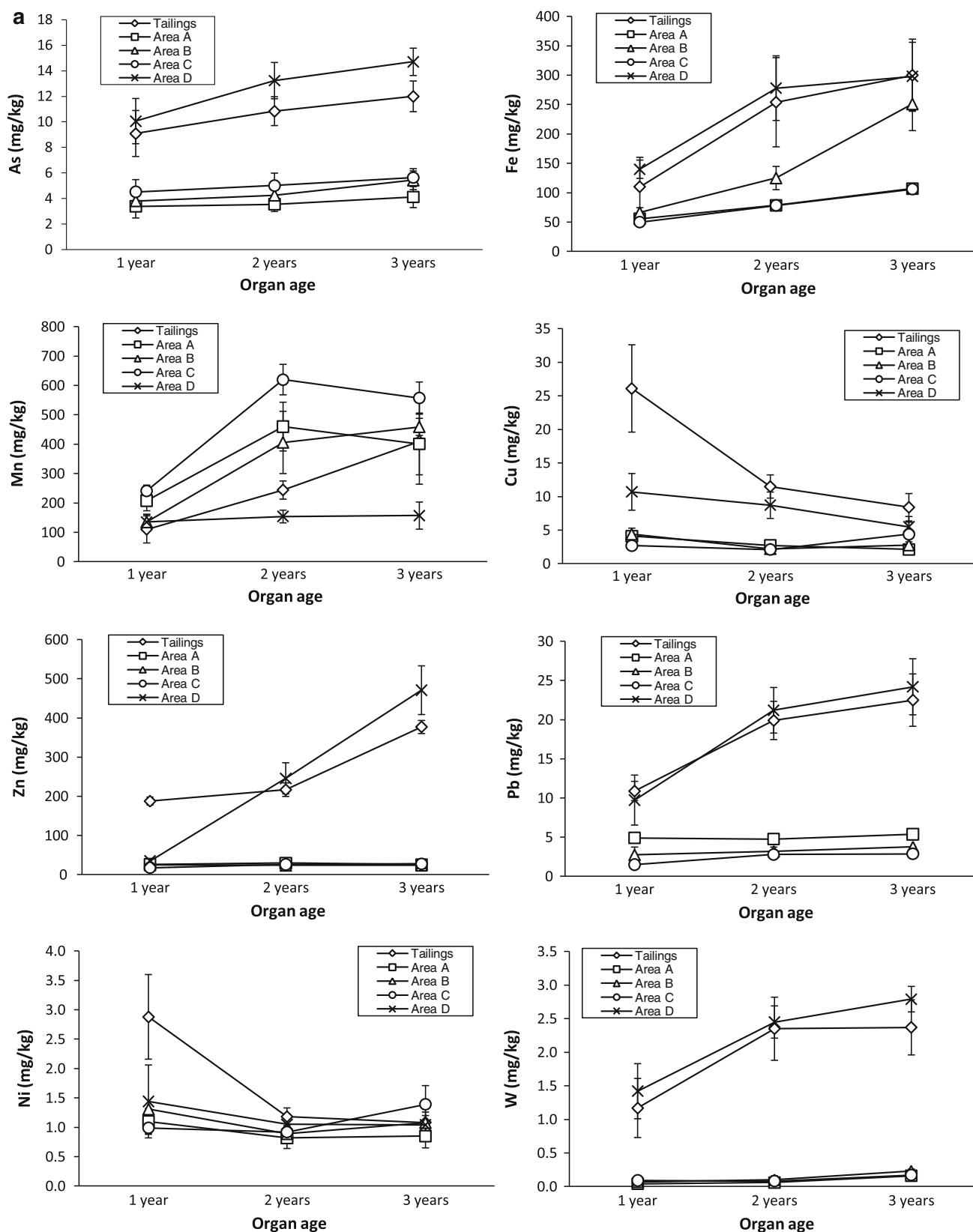


Fig. 7 a Variations in the levels of As, Fe, Mn, Cu, Zn, Pb, Ni and W in the needles of *P. pinaster* samples considering the organ age and the sampling area (mean \pm SD). **b** Variations in the levels of As, Fe,

Mn, Cu, Zn, Pb, Ni and W in the stems of *P. pinaster* samples considering the organ age and the sampling area (mean \pm SD)



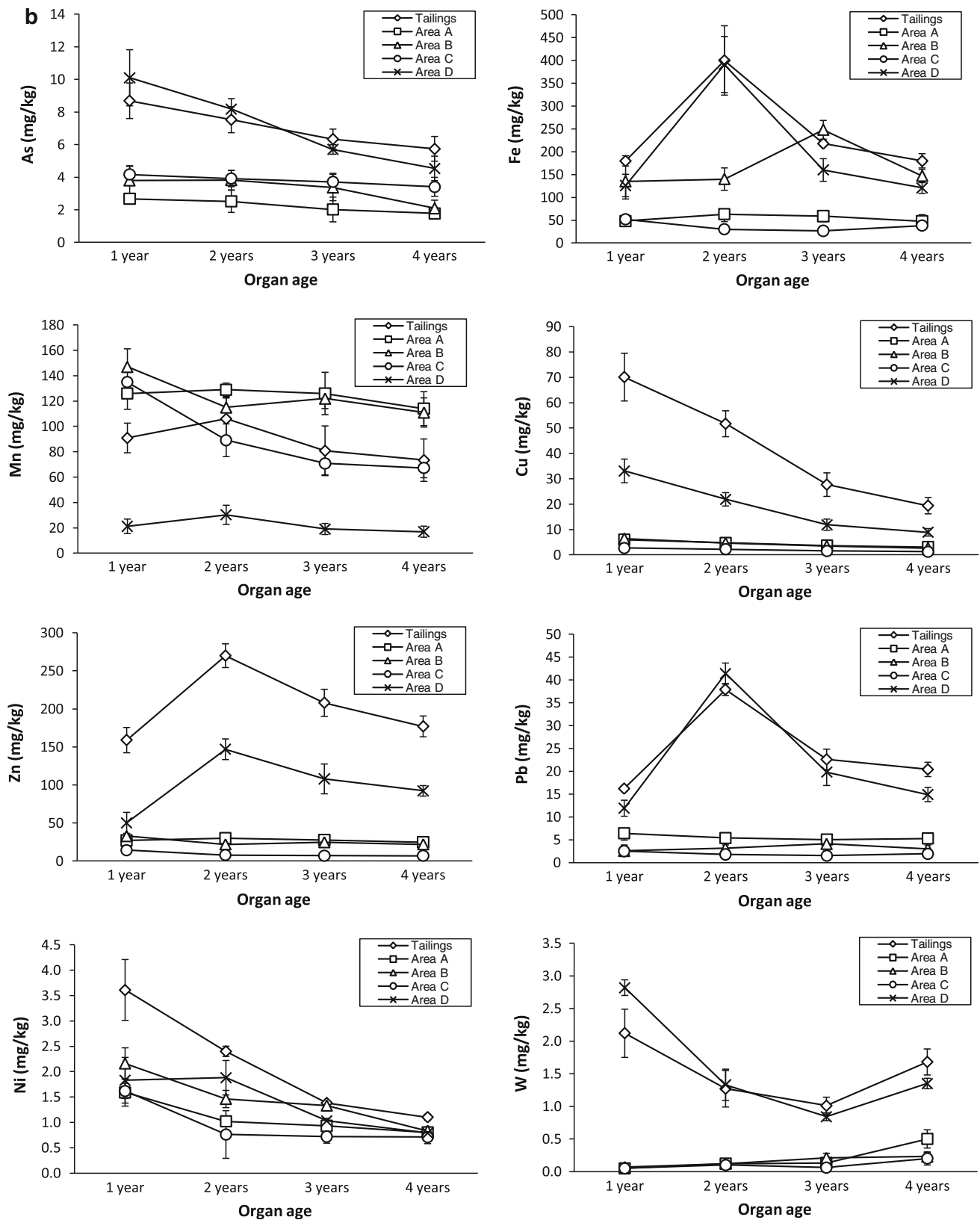


Fig. 7 continued



Table 4 Result of statistical analysis of variance (ANOVA), for test the influences of organ age on chemical elements concentration

Plant organ	Variables	Sum of squares between groups	df	Mean square between groups	Sum of squares within groups	df	Mean square within groups	F	Significant level
Needles	As	174.68	2	87.34	3103.05	204	15.21	5.74	0.004*
	Fe	568706.63	2	284353.31	1360522.59	204	6669.23	42.64	0.000*
	Mn	2422058.05	2	1211029.02	3883559.74	204	19037.06	63.61	0.000*
	Cu	888.71	2	444.35	9177.09	204	44.99	9.88	0.000*
	Zn	535542.47	2	267771.23	4268173.74	204	20922.42	12.80	0.000*
	Pb	1300.32	2	650.16	13441.62	204	65.89	9.87	0.000*
	Ni	14.28	2	7.137	53.32	204	0.26	27.31	0.000*
	W	12.23	2	6.11	224.16	204	1.10	5.563	0.004*
Stems	As	129.76	3	43.25	1504.32	272	5.53	7.82	0.000*
	Fe	410778.41	3	136926.14	2817127.95	272	10357.09	13.22	0.000*
	Mn	29923.97	3	9974.66	507668.62	272	1866.43	5.34	0.001*
	Cu	11023.01	3	3674.34	94888.77	272	348.86	10.53	0.000*
	Zn	56882.56	3	18960.85	1751212.58	272	6438.28	2.95	0.033*
	Pb	3973.57	3	1324.52	35296.78	272	129.77	10.21	0.000*
	Ni	70.68	3	23.559	84.65	272	0.31	75.70	0.000*
	W	10.93	3	3.64	165.87	272	0.61	5.98	0.001*

* Significant difference between the samples ($p \leq 0.05$)**Table 5** Result of post hoc comparison test (LSD-test), for separation of means of the different ages of needles and stems

Plant organ	Variables	1-year-old (n = 69)	2-years-old (n = 69)	3-years-old (n = 69)	4-years-old (n = 69)
Needles	As	5.68 A	6.21 A	7.84 B	–
	Fe	82.45 A	160.20 B	209.81 C	–
	Mn	164.15 A	384.45 B	401.80 B	–
	Cu	9.40 A	5.34 B	4.74 B	–
	Zn	58.85 A	107.28 A	182.48 B	–
	Pb	5.79 A	10.31 B	11.65 B	–
	Ni	1.57 A	0.97 B	1.08 B	–
	W	0.55 A	0.99 B	1.11 B	–
Stems	As	5.49 A	5.30 A	4.37 B	3.81 B
	Fe	105.55 A	200.82 B	142.28 C	107.37 A
	Mn	105.43 A	95.16 AB	85.14 BC	77.81 C
	Cu	23.28 A	17.34 A	9.99 B	7.13 B
	Zn	56.93 A	95.26 B	76.37 AB	65.24 A
	Pb	7.89 A	17.67 B	10.60 A	9.09 A
	Ni	2.18 A	1.53 B	1.08 C	0.85 D
	W	0.96 A	0.55 BC	0.45 B	0.79 AC

The letters A, B, C and D characterize the mean differences. The means significantly different from the others ($p \leq 0.05$) are indicated with a different letter. The two paired letters refer to similitude with more a two groups

The variation in the contents of Mn revealed identical behaviors in the different sampling areas; therefore, the utilization and retention of this element, both in the needles and in the stems, seem to be indifferent to the factors inherent to the contamination. Meanwhile, the plants that grew on the tailings showed a low percentage of retained Mn in growing needles. This fact might be related to several factors, namely the occurrence of Mn in forms that render its difficulty for absorption through the roots (immobile oxides) and a high rate of bioutilization of the

quantities absorbed, given that the Mn is a micronutrient essential for photosynthesis and necessary in chlorophyll synthesis (Nagaraju and Karimulla 2002; Silva and Williams 2001). The needles always showed strong to intense absorption of Mn, and the stems had BAC values indicative of intermediate to strong absorption in almost all of the samples (Tables 2 and 3). This result can also help to explain the low ratios for Fe because according to Nagaraju and Karimulla (2001), when Mn is present in the soil in medium to high concentrations, it can induce deficiencies



in Fe in plants as the result of competition in the absorption and translocation of these elements.

The concentrations of Cu were higher in the younger material, both foliar and woody, with the woody material showing a less marked variation over time. In contrast, in the needles, the concentration was visibly higher in newly formed needles than in the older needles (Fig. 7a, b). This behavior was identical in the different areas mentioned and is likely inherent to vegetal metabolism, corresponding to what was documented by Dolobovskaya for *P. sibirica* (Kabata-Pendias 2010). The samples of different organs and ages had BAC values that indicated weak to intermediate absorption of Cu (Tables 2 and 3). We also verified for all of the exemplars that, in the tailings, although the content of Cu in the substrate was high, the Cu content in the plant was not. This result may be related to the reduced bioavailability of Cu in these soils, reflecting the ability of Cu to be adsorbed and co-precipitated with oxy-hydroxides of Fe, Mn and Al (Adriano 2001).

For Zn, we verified behavior differences according to the sampling area. We observed similar patterns in the plants from the tailings and area D (Fig. 7a, b), verifying that in the needles the concentration of Zn increases with age, while in the stems the concentration increases in the second year and then decreases with age. The behavior in these two areas might reflect the performance of physiological mechanisms attempting to re-establish balance in response to disturbances caused by the presence of toxic elements in excessive quantities. The BAC results allowed us to verify that the absorption of Zn can be classified as intermediate for all of the samples, which had high BAC values, especially the exemplars growing in the tailings (Table 2 and 3). This fact should reflect, on one hand, the high quantities of this element existing in the substrate (Table 1), and on the other hand, the easy utilization of this element by the plants, given that Zn is an element endowed with great mobility in soils with low contents of organic matter (Kabata-Pendias 2010). However, the highest BAC values (strong absorption) were observed in area D, in 2- and 3-years-old needles and in 2-years-old stems.

The variation in the content of Pb also demonstrates some differences in behavior between plants outside and inside the contaminated area. In the plants that grew in the tailings and in area D (Fig. 7a, b), the concentrations of Pb in the needles were shown to increase with age, whereas the stems showed an increase in the concentrations of this metal in the second year and then a decrease with age. For Pb, the values of BAC (Tables 2 and 3) indicated, for all organs and ages, very weak to weak absorption. However, it is necessary to bear in mind that, in this study, only the aerial parts of the plant were sampled, given that there is a general consensus that Pb is mainly accumulated in the roots and is barely translocated to the other parts of the

plant. The exception was the exemplars grown in the contaminated area, for which the BAC values indicated intermediate absorption values.

The variation in the content of Ni was identical to that for Cu, especially in the tailings (Fig. 7a, b) with a decrease with age in the needles and the stems. Thus, in the plants that grow in highly contaminated soils, the translocation of Ni appears to be inhibited, with possible accumulation in the cellular vacuoles, perhaps due to the antagonism between Ni and other trace elements that are present in excess in the plants. For Ni, the BAC values indicate weak to intermediate absorption.

The variations in the contents of W showed differences in behavior between areas outside and inside the contaminated area (Fig. 7a, b). In the areas representative of the geochemical background, the concentrations of W increased with the age, both in the foliar and in the woody material. In contrast, in the tailings and in the contaminated soils, it was verified that there was an increase in the concentration of this metal in woody material with 1-year-old, and a significant decrease after the first year of age with an increase being observed again in the oldest material (4-years-old). The W contents were higher in older needles (2- and 3-years-old). The BAC values for W revealed very weak to weak absorption for all areas.

Those specimens of *P. pinaster* growing in tailings and contaminated soils shows tolerance to imposed stress conditions (metal-contamination and nutrient deficiency) and can fulfill the objectives of stabilization, pollution attenuation and visual improvement. Besides, this species is drought-resistant and has high biomass and bioproductivity. In fact, the constraints related to plant establishment and amendment of the physical–chemical properties of the metalliferous soils depends upon the choice of appropriate plant species. Therefore, the plant community tolerant to toxic trace elements play a major role in remediation of degraded mine soils. Thus, for the Vale das Gatas mine area, this type of approach requires more information about plant communities growing on these soils and tailings to assess their potential for mine restoration and minimization of mining impacts.

Conclusion

The values obtained allowed us to conclude that the content of chemical elements of the needles and stems of maritime pine tree (*P. pinaster*) depend as much on the plant organ as on the age, especially in the most contaminated locations. Thus, in sampling campaigns for biogeochemical studies, it is important to consider both the organ and the age of the sample. Therefore, it is not convenient to use a mix of foliar and woody material in the same



biogeochemical sample. The age of the organ is also important, because for the researched elements (As, Fe, Mn, Cu, Zn, Pb, Ni, W) this species showed a great variability in the accumulation behavior with age. For each particular element that was studied, there is a plant organ and an age that may be more suited for sampling to reflect higher bioaccumulated concentrations. Thus, the 1-year-old needles and stems accumulated higher levels of Ni and Cu. While the older needles accumulated higher levels of As, Fe, Zn, Pb, and W. The 2-years-old stems may also be appropriate samples to detect higher levels of Fe, Zn and Pb.

However, more studies are needed on the plant communities that are growing on these metal-contaminated soils and tailings to evaluate their potential for biogeochemical exploration and environmental biomonitoring, but also for mine stabilization/remediation.

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