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# Selective uptake of major and trace elements in *Erica andevalensis*, an endemic species to extreme habitats in the Iberian Pyrite Belt

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**Abstract**

To assess the ecophysiological traits and the phytoremediation potential of the endemic heather *Erica andevalensis*, we determined the concentrations of major and trace elements in different plant parts and in rizosphere soils from Riotinto mining district (Huelva, Spain). The results showed that *E. andevalensis* may grow on substrates with very high As, Cu, Fe and Pb concentrations (up to 4114, 1050, 71900 and 15614 μg/g dry weight, respectively), very low availability of macro- and micronutrients and with pH values ranging from 3.3 to 4.9. In these harsh edaphic conditions *E. andevalensis* selectively absorbed and translocated essential nutrients and excludes potentially phytotoxic elements, which were accumulated in the root epidermis. The concentrations of major and trace elements in *E. andevalensis* aerial parts from the Riotinto mining district were in the normal range for plants; likewise other *Erica* species it accumulated Mn and only in a very polluted site we measured leaf concentrations of As and Pb within the excessive or toxic limits for plants. Differently from previous studies, which emphasized the soil pH and bioavailability of phytotoxic elements as the main stress factors, this study showed that in the Riotinto region, *E. andevalensis* can tolerate wide range of pH and toxic element concentrations; the harshest environments colonized by monospecific patches of this species were characterized above all by very low availability of nutrients. The extraordinary capability to adapt to these extreme habitats made *E. andevalensis* a priority species to promote the phytostabilization and the development of a self-sustaining vegetative cover on Riotinto mine tailings.

**Key words**: mine tailing; Riotinto; tolerant plants; *Erica andevalensis*; rhizosphere soils

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## Introduction

The Iberian Pyrite Belt (IPB), one of the largest metallic sulfide deposits in the world and massive ore bodies in the Riotinto district (Huelva province, SW Spain), has been exploited for about 5000 years (Ruiz et al., 1998; Davis et al., 2000). As a result of mining and smelting activities and intensive deforestation, large portions of the region have undergone disruption of the landscape and lack vegetation. The Tinto River, which originates at the Peña de Hierro and drains parts of these areas, has very acidic waters (mean pH 2.3; Lopez-Archilla et al., 2001) with high concentrations of Fe, Zn, Cu, Pb, Ag, As, Bi, Au and Sn. In the last decade considerable effort have been made to assess the impact of past mining and smelting activities in the Tinto River fluvial and estuarine ecosystems (Hudson-Edwards et al., 1999; Davis et al., 2000; Braungardt et al., 2003). This extreme environment is of great astrobiological interest as it is considered a terrestrial analogue of the Martian surface (Amils et al., 2007). In fact, only acidophilic microorganisms can survive in the Tinto waters and they are possible metabolic relics from the early evolution of life on Earth (Fernandez-Remolar et al., 2008).

The dry, coarse and unstable tailings from abandoned mining and smelting plants in the Riotinto mining district constitute an environment that is as hostile as the river waters. Most substrates are coarse, unstable and dry; they have very low concentrations of organic matter and nutrients and the acidic environment enhances the bioavailability of potentially toxic elements (Hudson-Edwards et al., 1999). Despite these extreme environmental conditions, *Erica andevalensis* an endemic species to the IPB (Cabezudo and Rivera, 1980), may colonize mine tailings and the bank sediments of rivers such as the Tinto. In the Riotinto region this species grows in monospecific patches

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(in the most hostile habitats) or in association with *Erica australis* and few other plant species (Aparicio, 1999). The eco-physiology of this vulnerable endemic species, which was classified as endangered by the Andalusian Regional Government, is largely unknown (Abreu et al., 2008; Turnau et al., 2007). Thus, we began multidisciplinary studies to characterize biological and environmental factors affecting the survival and the distribution of *E. andevalensis* in the Riotinto mining district (Rossini Oliva et al., 2009a, 2009b). This knowledge is necessary for the species protection and conservation and to evaluate its potential use in the phytoremediation of sulfide mine environments (Prasad et al., 2010).

Previous studies on *E. andevalensis* considered above all environmental aspects rather than its nutritional status. Furthermore, available data on its chemical composition and distribution throughout the IPB are rather controversial. In the São Domingos mine (Portugal) for instance, this species does not grow outside the São Domingos river bank areas (periodically flooded, and with pH ranging from 3.0 to 4.0), and it looks like it does not cohabit with *E. australis* (which grows in soils with pH values > 3.5; Abreu et al., 2008). Both species can grow in soils highly contaminated with Pb, As and Sb and concentrations of most trace elements in their aerial tissues are in the normal range for plants. However, some plant samples from more contaminated soils have Mn, Pb and As concentrations within the excessive or toxic limits for plants; even in non-contaminated soils, both species behave as Mn-accumulators and Al-tolerant species (Abreu et al., 2008). In general Ericaceae have intrinsic ability to tolerate high levels of Al (Yang et al., 1996) and Mn (Markert, 1996). In the Riotinto region, *E. andevalensis* and *E. australis* may grow intermixed with other plant species in wet river bank areas where they form the *Junco rugosi-Ericetum andevalensis* community. The two species may also colonize coarse and very dry mine tailings to form the *Ericetum australi-andevalensis* community (Cabezudo et al., 1989). According to Soldevilla et al. (1992), *E. andevalensis* is the only angiosperm species adapted to extreme environmental conditions (substrates with pH < 2.5, high heavy metal concentrations and strong impoverishment in nutrient), and in the Riotinto region the plant tissues accumulate Mn, Fe and Cu. However, a recent study on *E. andevalensis* from the same area by Rodríguez et al. (2007) concluded that element concentrations in plant tissues are not excessive. Asensi et al. (1999) studied Cu uptake in *E. andevalensis* and stated that this species cannot be used in biogeochemical surveys because its Cu content does not reflect that in the soil. In this context, the main objectives of this study were: (1) to determine the typical concentrations of major and trace elements in different parts of *E. andevalensis* growing in monospecific and mixed (*E. andevalensis-E. australis*) communities; (2) to evaluate the spatial and temporal variations in major and trace element concentrations in plant parts and the possible relationships with physico-chemical features of supporting substrates; and (3) to recognize the potential role of *E. andevalensis* in phytostabilization and re-vegetation of the Riotinto mining area.

## 1 Experimental

### 1.1 Study area

Plant and soil samples were collected in March and September 2007 from four sites in the Riotinto mining area (Fig. 1). The regional geology consists mostly of volcano-sedimentary complexes, which host IPB sulfide mineralizations (above all pyrite, chalcopyrite and other Cu ores; sphalerite and accessory galena and arsenopyrite). The first mining and smelting activity in the district was developed by the Iberians and Tartessians (about 3000 B.C.), and the mines are now closing down due to economic reasons (Chopin and Alloway, 2007). A large proportion of the region, which has produced a total of about 1600 million metric tons of material (Davis et al., 2000), is currently occupied by open pits, tailing deposits and other mining wastes. Most soils are shallow, poorly developed and contain large amounts of coarse debris. The area has a Mediterranean climate with a mean annual rainfall of 750 mm and mean annual temperature of 18°C. Most rainfall occurs during autumn and winter (mean 70 mm/month), and summers are very hot and dry. Areas affected by past mining and smelting activities are devoid of vegetation or contain patches of simple plant communities dominated by *E. andevalensis* or by mixed communities of *E. andevalensis* and *E. australis*.

### 1.2 Sampling and sample pre-treatments

*Erica andevalensis* was sampled at four sites with different substrata and environmental characteristics: (1) Zaranda (UTM 29S 4173808/713542, altitude 390 m a.s.l.), an area not directly affected by past mining and smelting activities, in which some environmental recovery measures such as terrace-planting of *Pinus pinea* have been undertaken; (2) Nerva (UTM 29S 4175471/715131, 335 m a.s.l.), with mining and smelting waste piles that are unstable, dry and covered by only a few patches of vegetation; (3) Peña de Hierro (UTM 29S 417341/0715652, 533 m a.s.l.), in which the areas interested by environmental remediation procedures and re-vegetation attempts; (4) the

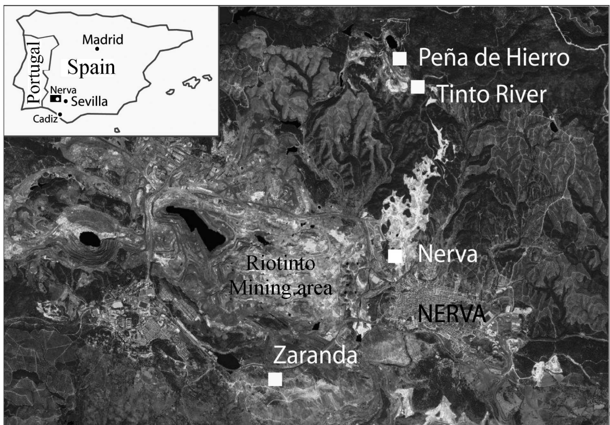


**Fig. 1** Study area and sampling locations (Nerva, Peña de Hierro, Tinto River, Zaranda).

Tinto River headwater (UTM 29S 715829/177713, 407 m a.s.l.) from the vegetated bank where *E. andevalensis* seems to thrive best.

Within each site (surface area of about 100 $m^2$), representative samples of topsoil (0–10 cm) and plants were collected from two different sub-sites: one with monospecific patches of *E. andevalensis*; another where specimens of *E. andevalenis* grown side by side with *E. australis* and other plant specis (i.e., *Nerium oleander*, *Cistus ladanifer*, *C. monspeliensis*, *C. salvifolius*, *C. populifolius*, *Halimium ocymoides*). An exception to this scheme was in the Tinto River sampling site, where *E. andevalensis* did not grow in monospecific stands. In each sub-site, plant sampling was obtained from at least five *E. andevalensis* plants. Leaves of the same age and stage of development were selected in the laboratory; live roots were carefully separated from soil, cleaned and then divided into two sub-samples: coarse roots (the main roots with a diameter ranging from 5 to 13 mm) and fine roots (diameter < 5 mm). Samples of rhizosphere soil were pull together from each plant (to a depth of about 20 cm) by detaching inorganic particles from roots (Baudoin et al., 2002). Samples of leaves, stems and large and fine roots were washed with freshwater, rinsed with deionised water, dried overnight at 70°C, and powdered homogenously. The bark was detached from large roots to be analyzed separately from internal tissues (cortex and vascular system). Both topsoils and rhizosphere soils were air-dried and carefully inspected to remove larger organic and inorganic debris; then, samples were sieved through a 2-mm mesh and stored for subsequent analysis.

### 1.3 Analysis and quality control

Rhizosphere soils and plant samples were digested with concentrated $HNO_3$ in closed teflon vessels at high pressure (90 bars; in a microwave oven with stepwise power application). The mineralized and diluted solution was analyzed through Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES Optima 530ODV, PerkinElmer, USA) for Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Pb, S and Zn. Flow Injection-ICP-AES for As and Atomic Absorption Spectrophotometry (Analyst 700, PerkinElmer, USA) with graphite furnace for Cd and Pb. Element concentrations were determined by the method of standard additions and are expressed on μg/g dry weight (dw) basis. Procedural blanks were usually below the detection limit. The accuracy of digestion and analytical procedures was checked by routine determination of element concentrations in standard reference materials (SRM No 1572 and 1573) from the National Institute of Standards and Technology (Gaithersburg, USA). The analytical recoveries from the certified values ranged from 86% to 93% for Al, from 91% to 103% for plant macronutrients (Ca, K, Mg, P and S) and from 90% to 96% for trace elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn).

Topsoils were analysed for: (1) pH by potentiometer in 1:2.5 ($W/V$) soil/water extracts; (2) electrical conductivity (EC) in 1:2.5 ($W/V$) soil/water extracts with a conductimeter XS510; (3) cation exchange capacity (CEC) by a method based on the triethylenetetramine–Cu complex (Meier and Kahr, 1999); (4) the organic matter (OM), derived from the organic C content (modified Walkley and Black method; Mingorance et al., 2007); (5) total N determined by elemental analyzer (Truemac-CNS, LECO, USA); (6) available P, performed by the Bray-P method (Bray and Kurtz, 1945); (7) the texture, determined by the Robinson pipette method combined with sieving.

### 1.4 Data analysis

Major and trace element dataset was tested for normality with the Shapiro-Wilk's test ($p > 0.05$) and for homogeneity of variance with the Levene's test ($p > 0.05$); data not respecting normality assumptions were log-transformed. Differences among groups were tested by analysis of variance, using the Sheffé test for post-hoc comparisons. Principal component analysis was applied for data reduction of elemental concentrations in rhizosphere soils and in leaves of *E. andevalensis*. The principal components included in the model had eigenvalues greater than 1. Interpretation of principal components was done in terms of factor coordinates (correlation between element data and factor axes) and relative contribution of different groups of data to the variance of factor axes. Significance of separation of groups of soils and plants by specific principal components was tested by the analysis of variance applied to the corresponding factor scores. All the statistical analyses were performed by Statistica (StatSoft Inc., USA) software program and probability level was set to $p < 0.05$ or $p < 0.01$.

A Translocation Coefficient (the quantitative ratio between element concentrations in plant leaves and fine roots of the same plant; TC) and a Bioaccumulation Coefficient (the ratio between element concentrations in leaves and the rhizopsheric soil; BC) were calculated to recognize the preferential partitioning of elements to the leaves (TC values > 1) and their accumulation in leaves (BC values > 1).

## 2 Results

### 2.1 Topsoil and rhizosphere soil

Physical and chemical features of topsoils from the four study areas are summarized in Table 1. All samples were coarse-textured, very acidic, had low nutrient contents and C/N ratios ranging from 19.0 to 65.4. Soils from Zarandas had the highest pH (about 4.80) and very low EC values; those from the Tinto River banks and mixed patches at Nerva showed the highest OM content. Although differences were not statistically significant, most soil samples with monospecific communities of *E. andevalensis* had lower nutrients than samples supporting mixed (*E. andevalensis*/*E. australis*) communities (Table 1).

Soils detached from *E. andevalensis* roots had pH values in the same range of those measured on topsoils (from 3.30 ± 0.05 in samples from the Tinto River bank to 4.74 ± 0.16 in those collected at Zaranda) of the same site and pH values did not vary significantly between subsites with monospecific or mixed *Erica* communities. All

**Table 1** Physical-chemical characterization of superficial soils ($n$ = 32) in four sites at Riotinto in monospecific (mono) or mixed *E. andevalensis* patches

| Site | Habitat | pH | EC (μS/cm) | CEC (meq/100 g) | OM (%) | Clay (%) | Silt (%) | Sand (%) | P (%) | N (%) | C (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Nerva | Mono | 3.50 ± 0.32 | 300 ± 78.0 | 28.8 ± 1.35 | 2.43 ± 0.82 | 24.4 ± 1.77 | 35.2 ± 2.87 | 40.8 ± 1.11 | 0.32 ± 0.12 | 0.04 ± 0.02 | 1.41 ± 0.47 |
| | Mixed | 3.57 ± 0.13 | 290 ± 152 | 52.3 ± 19.5 | 19.2 ± 9.91 | 13.9 ± 1.46 | 28.5 ± 2.59 | 57.7 ± 2.97 | 1.16 ± 0.76 | 0.17 ± 0.04 | 11.2 ± 5.75 |
| Peña de Hierro | Mono | 3.34 ± 0.03 | 221 ± 36.3 | 19.6 ± 9.74 | 0.79 ± 0.35 | 18.0 ± 9.69 | 23.0 ± 4.93 | 58.9 ± 6.61 | 0.09 ± 0.02 | 0.02 ± 0.02 | 0.46 ±0.20 |
| | Mixed | 3.45 ± 0.21 | 192 ± 89.6 | 19.1 ± 4.11 | 1.89 ± 0.75 | 15.3 ± 5.47 | 27.8 ± 4.12 | 56.9 ± 6.97 | 0.14 ± 0.04 | 0.03 ± 0.02 | 1.09 ± 0.44 |
| Tinto River | Mixed | 3.50 ± 0.83 | 635 ± 483 | 24.1 ± 9.06 | 4.56 ± 2.81 | 15.0 ± 4.22 | 22.2 ± 6.03 | 62.8 ± 10.1 | 0.61± 0.73 | 0.12 ± 0.10 | 2.65 ± 1.63 |
| Zaranda | Mono | 4.84 ± 0.17 | 32.3 ± 6.68 | 21.2 ± 2.76 | 0.77 ± 0.31 | 19.7 ± 2.15 | 26.0 ± 7.56 | 54.3 ± 9.27 | 1.82 ± 0.33 | 0.01 ± 0.01 | 0.45 ± 0.18 |
| | Mixed | 4.79 ± 0.28 | 66.3 ± 46.9 | 23.2 ± 4.85 | 0.75 ± 0.22 | 21.9 ± 5.05 | 31.0 ± 5.03 | 47.3 ± 7.93 | 2.64 ± 0.93 | 0.02 ± 0.01 | 0.38 ± 0.13 |

EC: electrical conductivity of the saturated extract; CEC: cation exchange capacity; OM: organic matter.

samples of rhizosphere soils were characterized by very low concentrations of functional elements (Ca, K, Mg, Mn, Na and P; Table 2) and their average Ca content was up to 2 order of magnitude lower than average concentrations in European topsoils (Salminen et al., 2005). Soils from Peña de Hierro and the Tinto River had the lowest Ca and Mn concentrations and the highest K concentrations in the area, with an extreme K/Ca ratio > 22.

Rhizosphere soils from Nerva, Peña de Hierro and the Tinto River had significantly higher ($p < 0.05$) As, Cd, Cr, Fe, Pb and S concentrations than samples from Zaranda. At Nerva, As, Cu, and Pb concentrations (3445 ± 789; 1334 ± 113; 13,467 ± 2013 μg/g dw, respectively) in soils from monospecific *E. andevalensis* patches were statistically significant higher ($p < 0.01$) than those in samples from mixed *Erica* communities (1202 ± 345; 765 ± 345; 4690 ± 687 μg/g, respectively).

Principal components analysis provided a good representation of the original variance (90.4%) in the rhizosphere soil dataset. Three factors with eigenvalue > 1 were included in the model: the first (PC1) highly correlated (loadings > 0.7) with elements originating from metallic sulfide deposits (55.9% of the original variance); PC2 correlated with Ca, Mn, Na and K (27.4%), while PC3 accounted for a residual amount of variance (7.1%). By plotting the relative factor coordinates in the first two vector space (Fig. 2), soils from Nerva accounted for the highest relative contribution to the variance of the first factor axis (in total 43%) and those from Peña de Hierro provided the highest contribution to the second axis variance (38%).

## 2.2 Element composition of *E. andevalensis* leaves, stems and roots

The results of analytical determinations in different *E. andevalensis* parts showed that the elemental composition of leaves, stems and the inner tissues of coarse roots were

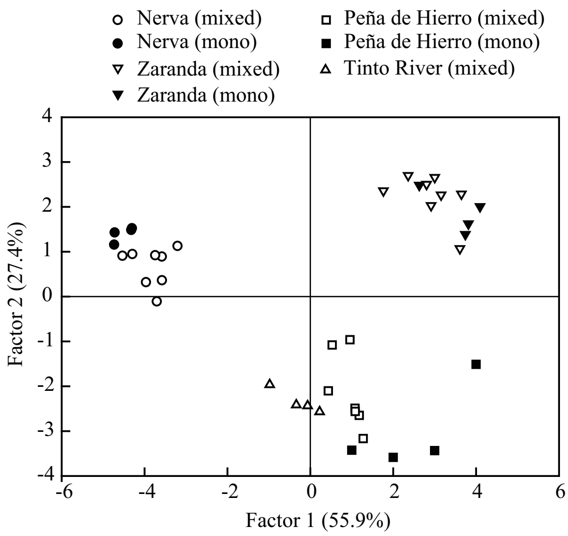


**Fig. 2** Projection of original data in the vector space defined by the principal component analysis of rhizosphere soils from Riotinto.

**Table 2** Element concentrations in rhizosphere soil from *E. andevalensis*

| | Nerva ($n$) | Peña de Hierro | Tinto River | Zaranda | European topsoils baseline |
|---|---|---|---|---|---|
| Al (%) | 1.83 ± 0.49a (1.30–2.46) | 2.30 ± 0.34a (1.87–2.95) | 3.13 ± 0.38b (2.58–3.44) | 3.04 ± 0.68b (2.36–4.05) | 10.5 ± 4.82 (0.37–26.7) |
| As (μg/g) | 2323 ± 1325a (934–4114) | 158 ± 47.1b (116–250) | 128 ± 35.5b (106–181) | 71.9 ± 21.5c (47.1–109) | 11.6 ± 20.1 (0.32–282) |
| Ca (μg/g) | 894 ± 320a (528–1339) | 312 ± 72.8b (210–401) | 327 ± 106b (220–473) | 884 ± 150a (698–1129) | 35,400 ± 72,600 (260–47,700) |
| Cd (μg/g) | 4.79 ± 0.87a (3.72–6.27) | 3.21 ± 0.78b (1.98–4.48) | 4.10 ± 0.96ab (2.85–5.17) | 0.69 ± 0.39c (0.38–1.57) | 0.28 ± 0.71 (<0.01–14.1) |
| Cr (μg/g) | 106 ± 17.2a (84.3–138) | 56.1 ± 26.3b (9.70–76.8) | 80.4 ± 25.6ab (42.2–95.3) | 13.0 ± 4.11c (8.20–21.4) | 94.8 ± 285 (<3.0–6230) |
| Cu (μg/g) | 1050 ± 319a (641–1432) | 54.9 ± 11.4b (41.7–72.5) | 66.7 ± 4.00b (62.6–72.1) | 106 ± 18.6c (86.0–144) | 17.3 ± 19.0 (0.81–256) |
| Fe (%) | 7.19 ± 1.90a (5.73–10.8) | 5.56 ± 0.91a (4.88–7.67) | 6.80 ± 1.59a (5.68–9.08) | 1.20 ± 0.18b (0.88–1.42) | 3.80 ± 2.34 (0.16–22.3) |
| K (μg/g) | 4241 ± 816a (3263–5359) | 7299 ± 1108b (5314–9188) | 7107 ± 377b (6669–7468) | 5112 ± 1308c (3831–7584) | 20,200 ± 9540 (260–61,300) |
| Mg (μg/g) | 1577 ± 457a (1026–2428) | 754 ± 259b (434–1161) | 1898 ± 577a (1502–2749) | 890 ± 127b (688–1121) | 11,800 ± 11,730 (<100–24,600) |
| Mn (μg/g) | 150 ± 22.3a (112–177) | 20.4 ± 8.30b (11.3–34.6) | 60.4 ± 9.61c (53.4–74.4) | 105 ± 21.7d (72.3–135) | 524 ± 540 (<10–6480) |
| Na (μg/g) | 409 ± 143a (256–640) | 524 ± 55.6a (455–621) | 875 ± 46.5b (815–922) | 106 ± 14.6c (88.8–134) | 11,500 ± 9490 (400–44,500) |
| Ni (μg/g) | 4.16 ± 2.02a (1.68–7.53) | 0.52 ± 0.51b (0.10–1.50) | 1.29 ± 0.84b (0.59–2.41) | 1.20 ± 0.80b (0.10–2.60) | 30.7 ± 124 (<2.0–2560) |
| P (μg/g) | 636 ± 117a (479–853) | 117 ± 64.7b (57.7–230) | 144 ± 48.5b (113–217) | 83.1 ± 13.6b (66.6–107) | 1500 ± 1160 (110–13,200) |
| Pb (μg/g) | 9079 ± 4894a (3971–15,614) | 209 ± 27.5b (188–265) | 146 ± 23.2b (104–159) | 83.3 ± 11.8c (67.3–101) | 23.9 ± 50.2 (<3–886) |
| S (μg/g) | 9556 ± 1691a (7281–12,428) | 4264 ± 746b (3611–5715) | 6419 ± 730a (5682–7210) | 164 ± 16.3c (142–190) | 437 ± 3890 (<50–11,200) |
| Zn (μg/g) | 159 ± 38.7a (121–240) | 37.9 ± 8.08b (23.2–47.9) | 89.9 ± 10.8c (79.1–104) | 30.2 ± 3.82b (25.3–36.2) | 60.9 ± 115 (4.00–2270) |

Data are expressed as mean (± standard deviation) and range. Topsoils (2–20 cm) geochemical baselines are reported for comparisons (Salminen et al., 2005). Different letters indicate significant differences between groups (ANOVA, post-hoc Sheffé test applied to log-normalized data, $p < 0.05$).

**Table 3** Element concentrations (μg/g dw) in leaves, stems, fine and coarse roots of *E. andevalensis* ($n$ = 24) in March and September 2007

| | Root | | | Stem | Leaf | TC | BC |
|---|---|---|---|---|---|---|---|
| | Fine | Coarse (cortex) | Coarse (inner tissue) | | | | |
| Al | 2608 ± 1349<br>(798–7011) | 3414 ± 1747<br>(1456–7866) | 250 ± 95.8<br>(98.9–437) | 248 ± 91.3<br>(107–384) | 231 ± 66.9<br>(145–426) | 0.09 | 0.02 |
| As | 17.8 ±27.2<br>(2.04–125) | 31.0 ± 59.7<br>(1.44–225) | 2.18 ± 3.14<br>(0.13–11.0) | 1.86 ± 1.79<br>(0.32–5.55) | 4.08 ± 5.71<br>(0.07–24.1) | 0.23 | 0.01 |
| Ca | 2371 ± 1820<br>(634–9544) | 2568 ± 1349<br>(986–5167) | 1598 ±884<br>(397–3415) | 2125 ± 986<br>(986–3813) | 5140 ± 1507<br>(2898–9335) | 2.16 | 11.6 |
| Cd | 0.25 ± 0.19<br>(0.04–0.86) | 0.29 ± 0.31<br>(0.07–1.25) | 0.11 ± 0.14<br>(0.01–0.49) | 0.02 ± 0.02<br>(0.01–0.07) | 0.01 ± 0.01<br>(0.01–0.03) | 0.04 | 0.01 |
| Cr | 4.68 ± 2.31<br>(1.67–12.0) | 3.29 ± 2.01<br>(1.09–7.26) | 1.55 ± 0.88<br>(0.58–3.47) | 5.80 ± 2.59<br>(2.04–11.7) | 4.27 ± 2.99<br>(0.44–16.2) | 0.91 | 0.15 |
| Cu | 292 ± 281<br>(25.4–1033) | 333 ± 354<br>(15.6–1046) | 9.98 ± 5.46<br>(3.26–21.8) | 17.6 ± 14.3<br>(4.56–58.0) | 5.43 ± 1.78<br>(2.55–10.2) | 0.02 | 0.01 |
| Fe | 3560 ± 3545<br>(251–15,109) | 3572 ± 4564<br>(258–17,350) | 166 ± 98.4<br>(45.3–453) | 460 ± 328<br>(96.2–1354) | 237 ± 96.1<br>(96.9–541) | 0.07 | 0.01 |
| K | 2199 ± 1126<br>(212–5130) | 1514 ± 756<br>(702–3501) | 1578 ± 683<br>(569–2639) | 2300 ± 841<br>(1302–4602) | 2962 ± 528<br>(2222–4192) | 1.35 | 0.49 |
| Mg | 803 ± 239<br>(340–1246) | 670 ± 182<br>(333–1061) | 520 ± 203<br>(223–949) | 811 ± 340<br>(419–1622) | 2806 ± 763<br>(1834–5220) | 3.49 | 2.72 |
| Mn | 701 ± 239<br>(171–1185) | 619 ± 369<br>(174–1285) | 687 ± 485<br>(344–1197) | 1809 ± 1010<br>(295–2897) | 1839 ± 773<br>(302–2935) | 2.62 | 15.6 |
| Na | 409 ± 216<br>(93.2–962) | 203 ± 97.4<br>(58.8–426) | 253 ± 124<br>(103–575) | 405 ± 257<br>(47.0–943) | 481 ± 333<br>(121–1848) | 1.18 | 1.29 |
| Ni | 3.48 ± 1.40<br>(1.04–6.61) | 3.64 ± 2.02<br>(1.05–6.39) | 2.19 ± 1.24<br>(0.91–4.77) | 3.47 ± 2.80<br>(1.16–12.8) | 3.10 ± 1.72<br>(0.90–8.66) | 0.89 | 2.23 |
| P | 390 ± 189<br>(82.3–806) | 222 ± 117<br>(104–533) | 194 ± 53.8<br>(87.8–313) | 391 ± 146<br>(179–633) | 549 ± 219<br>(272–1035) | 1.41 | 4.21 |
| Pb | 352 ± 734<br>(26.0–3202) | 359 ± 535<br>(24.6–1807) | 17.0 ± 23.4<br>(2.00–79.8) | 56.1 ± 83.7<br>(3.79–291) | 7.30 ± 9.78<br>(0.50–36.9) | 0.02 | 0.01 |
| S | 1022 ± 992<br>(271–5069) | 756 ± 505<br>(288–2319) | 480 ± 175<br>(197–862) | 930 ± 424<br>(413–1931) | 2759 ± 1180<br>(985–5933) | 2.70 | 2.90 |
| Zn | 45.7 ± 32.2<br>(10.4–115) | 56.5 ± 46.6<br>(6.76–139) | 19.5 ± 13.2<br>(5.50–50.4) | 10.4 ± 7.59<br>(2.23–27.5) | 16.5 ± 8.76<br>(1.65–45.0) | 0.36 | 0.34 |

Traslocation coefficient (TC) = $C_{\text{element,leaf}}/C_{\text{element,fine root}}$; bioaccumulation coefficient (BC) = $C_{\text{element,leaf}}/C_{\text{element,rhizosoil}}$.

much less variable than those in rhizosphere soil, fine roots and the cortex of coarse roots (Table 3). Although root samples were carefully washed before the analysis, the high concentrations of Al and other typical elements in metallic sulfides of the IPB (As, Cd, Cu, Fe and Pb) indicate that soil particles adsorbed to fine roots and the cortex of coarse roots probably contributed to average values summarized in Table 3. Anyhow, in all samples the TC and BC values for As, Cd, Cu and Pb were < 0.23 and < 0.01, respectively (Table 3). On the contrary, *E. andevalensis* could absorb and translocate Ca, K, Mg, Mn, Na, Ni, P and S to the aerial parts (average BC values > 10 for Ca and Mn). Among the analyzed plant parts the leaves resulted the most responsive in term of differential accumulation of elements and Fig. 3 gives a comprehensive representation of spatial and seasonal variations of major and trace elements in *E. andevalensis* leaves from the four sampling areas. In general, samples collected in September had higher As and Pb and lower Al, Ca, Mg and Mn concentrations. At Nerva the leaves accumulated significantly higher As and Pb concentrations than leaves from the other sampling sites and the content of these elements in samples from monospecific *E. andevalensis* patches was significantly higher ($p < 0.01$) than in those from mixed *Erica* communities. Multivariate data analysis applied to the leaf dataset showed four principal components which represent the 66.1% of the original variance: PC1 was highly correlated (loadings ⩾ 0.7) with Ca, Mg, Mn and Zn; PC2 with As (0.89) and Pb (0.88); PC3 with Fe (0.79); and PC4 with Cr (0.87) and Ni (0.79). Potassium and Na had a high loading in PC3 (0.64 and 0.60, respectively), while Al showed an elevated cross-loading with PC1 (0.64) and a moderate cross-loading with PC3 (0.53). The partitioning of data structure according to the two most relevant components separated samples collected at Nerva from those from the other sampling areas and, among leaves collected at Nerva, significantly distinguished those from monospecific and mixed *Erica* habitats ($p < 0.01$).

## 3 Discussion

Previous studies on vegetated soils from the Iberian Pyrite Belt (Freitas et al., 2004; Pratas et al., 2005; Chopin and Alloway, 2007; Rodríguez et al., 2007; Abreu et al., 2008) highlighted the role of low pH values and high concentrations of phytotoxic elements such as Cu, Pb, As or Sb as the main stress factors for plants. Our results show that another major hindrance to plant colonization and survival in the Riotinto mining district is the low and/or imbalanced availability of essential elements. Although in the abandoned São Domingos mine (Portugal) *E. andevalensis* grows exclusively in São Domingos River banks (soil pH ranging from 3.0 and 4.0), and it does not cohabit with *E. australis* (which grows in soils at pH > 3.5; Abreu et al., 2008), in the Riotinto mining district *E. andevalensis*

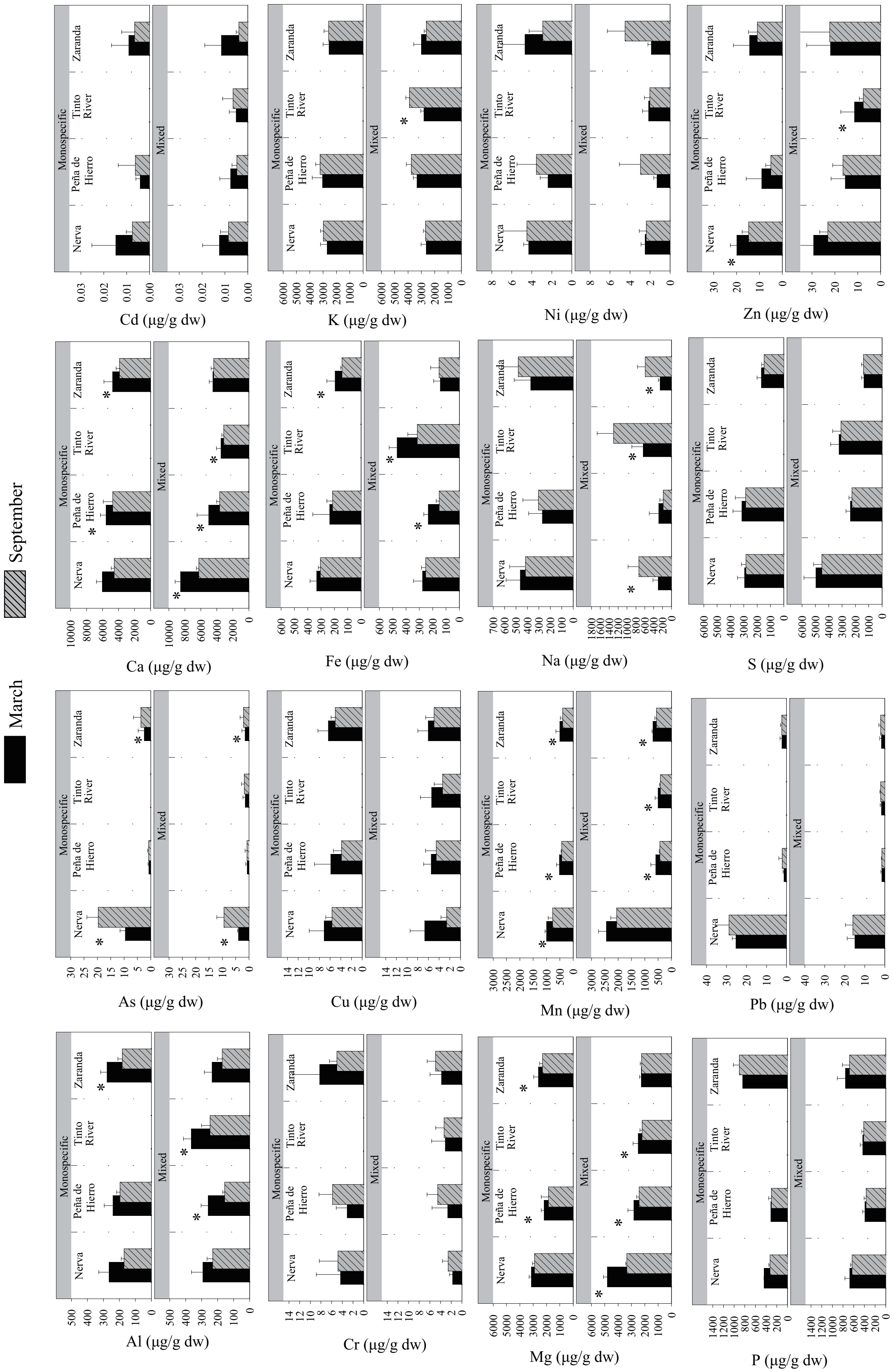


**Fig. 3** Major and trace element concentrations in leaves of *E. andevalensis* from mining areas of Riotinto collected in March and September 2007 in monospecific and mixed patches. * indicate statistical differences by the period of sampling ($p < 0.01$).

forms monospecific or mixed communities in the banks of watercourses and in dry and unstable mine tailings. Our results also show that the species can tolerate wider ranges of pH (indicatively from 3.0 to 5.0) and toxic element concentrations (e.g., from 47 to 4114 μg/g dry wt for As and from 67 to 15,614 μg/g dry wt for Pb).

Metalliferous soils are by nature very heterogeneous and the excavation and processing of minerals increases spatial variations in soil composition and properties. A geochemical survey in soils of the same region (Chopin et al., 2003) reported Al, As, Cu, Fe, Mn, Pb and Zn concentrations in the same range as those of this study and the authors observed that in contrast to other mines and smelters of similar size, trace element concentrations at Riotinto and Tharsis were only high in the immediate vicinity (up to maximum of 2 km) of mines and smelters. We found that in the Riotinto region the As, Cu and Pb concentrations at the root-soil interface can vary up to one order of magnitude (Table 2) and maximum values at Nerva, can be one or two orders of magnitude higher than those usually considered toxic for plants (Kabata-Pendias and Pendias, 1984). Using a multivariate approach, contamination by Pb and other elements was the most predominant factor explaining variance of the soil data, and at the sampling area most affected by past mining activities (Nerva), this factor significantly differentiated between soils of monospecific and mixed habitats (ANOVA, $p < 0.05$). However, in our study areas, probably, the soil pH and total concentrations of potentially toxic elements are not the main factors affecting the spatial distribution of (monospecific or mixed) *Erica* communities. In fact, at Zaranda, *E. andevalensis* does not grow intermixed with *E. australis* in soils with pH values $\geqslant$ 4.8, and As, Cd, Fe and Pb concentrations among the lowest measured in the four study areas. Although further research will be necessary to identify physiological and environmental factors making *E. andevalensis* the only vascular plant that can colonize the harshest environments in the Riotinto mining district, among soil features considered in this study, the adaptation to very low nutrients seems much more important than that to low pH values or high concentrations of potentially toxic elements. In general, substrates supporting monospecific patches of *E. andevalensis* have lower organic C, N, and P (Table 1) and multivariate analysis showed that the low concentrations of Ca and Mn in rhizosphere soil are the second most relevant factor, explaining the variance of major and trace elements in the Riotinto substrates.

Despite the variability and extreme values of trace element concentrations in rhizosphere soils, the acidity and very low concentrations of Ca and organic matter which could increase the element biovailability, the chemical composition of inner root tissues and aerial parts of *E. andevalensis* were in the same range of those reported for *E. australis* samples collected in a nature reserve of Monfragüe in SW Spain (Paton et al., 1999), in the Cantabrian Mountains in NW Spain (Alonso and García-Olalla, 1997) or in other *Erica* species from Mediterranean reference areas (Peñuelas et al., 2001). When average values in Fig. 3 are compared with literature data, much higher concentrations and within the excessive or toxic limits for plants were only found for As and Pb in inner root tissues, stems and leaves from monospecific patches of *E. andevalensis* at Nerva.

In spite of very dry and poor soils of the Riotinto region, *E. andevalensis* did not show nutrient unbalance and major element ratios, frequently used to evaluate the nutrient balance in crops, were generally optimal (only the K/Mg and K/Ca ratios were in certain sites, lower than those usually reported for crops) (Tisdale et al., 1993). Fungi forming ericoid mycorrhizal infections in the hair roots of Ericaceae, undoubtedly enhance the absorption of essential macro- and micronutrients and probably sequester excess toxic elements in the root bark and rhizosphere soil. Although total Mn concentrations in all substrates were very low, *E. andevalensis* has an extraordinary capability to accumulate this element (up to 2936 μg/g in some samples from Nerva). It is known that Ericaceae and Theaceae are Mn bioaccumulators (Schüürmann and Markert, 1998) and previous studies in the IPB (Turnau et al., 2007; Rodríguez et al., 2007; Abreu et al., 2008) reported the Mn bioaccumulation in *E. andevalensis* leaves. Although Soldevilla et al. (1992) reported in this species the bioaccumulation of other elements such as Al, Cu and Fe; these results were probably affected by element contributions from soil particles adsorbed onto analyzed plant samples. In fact, our survey shows that Al, Cu and Fe mainly accumulate at the interface between inner root tissues and the external medium. In all plant samples they are translocated in limited amounts and leaf concentrations of Cu, likewise those of Cr, Ni, P and S, did not show spatial or seasonal variations. Concentrations of the other elements (Fig. 3) in leaves from different sampling areas, sites or seasons showed some statistically significant variations. However, further sampling and analyses will be necessary for a more reliable assessment of these variations and to identify environmental and physiological factors determining these variations.

## 4 Conclusions

Pyrite mine tailings in the Riotinto district are acidic, dry, coarse-textured, unstable, strongly impoverished in macro- and micronutrients essential to the plant metabolism and contain very high concentrations of As, Cu, Fe and Pb. *Erica andevalensis* has an extraordinary capability to tolerate the harshest edaphic conditions in this region through the selective absorption of essential elements and the accumulation of phytotoxic elements in the root barks (i.e. at the interface between internal tissues and the external medium). In agreement with the results of some recent studies, the concentrations of major and trace elements in *E. andevalensis* aerial parts from the Riotinto mining district are in the normal range for plants. The species accumulates Mn and only in leaves of very polluted sites the concentrations of As and Pb are within the excessive or toxic limits for plants. However, differently from previous studies, which emphasized the low pH values and high concentrations of As, Cu, Pb or Sb in soils as the main stress factors, this work shows that in the Riotinto area *E. andevalensis* can tolerate wide range of pH and toxic element concentrations. The harshest environments colonized by monospecific *E. andevalensis* communities are characterized by a very low bioavailability of nutrients. It is just the capability to adapt to these extreme conditions that makes *E. andevalensis* a very interesting species to promote the phytostabilization and the development of a self-sustaining vegetative cover in Riotinto mine tailings.

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## References

Abreu M M, Tavares M T, Batista M J, 2008. Potential use of *Erica andevalensis* and *Erica australis* in phytoremediation of sulphide mine environments: São Domingos, Portugal. *Journal of Geochemical Exploration*. DOI: 10.1016/j.gexplo.2007.04.007.

Alonso I, García-Olalla C, 1997. Mineral composition of soils and vegetation from six mountain grassland communities in northern Spain. *Journal of the Science of Food and Agriculture*, 73: 200–206.

Amils R, Gonzáles-Toril E, Fernández-Remolar D, Gómez F, Aguilera Á, Rodríguez N et al., 2007. Extreme environments as Mars terrestrial analogs: the Rio Tinto case. *Planetary and Space Science*, 55: 370–381.

Aparicio A, 1999. *Erica andevalensis* Cabezudo & Rivera. In: Libro Rojo de la Flora Silvestre Amenazada de Andalucía, Tomo I: Especies en Peligro de Estincion (Blanca G, Cabezudo B, Hernández-Bermejo J E, Herrera C M et al., eds.). Consejería de Medio Ambiente, Sevilla, Spain. 119–122.

Asensi A, Bennet F, Brooks R R, Robinson B, Stewart R, 1999. Copper uptake studies on *Erica andevalensis*, a metal-tolerant plant from southwestern Spain. *Communication in Soil Science and Plant Analysis*, 30: 1615–1624.

Baudoin E, Benizri E, Guckert A, 2002. Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. *Applied Soil Ecology*, 19: 135–145.

Bray R H, Kurtz L T, 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, 59: 39–45.

Braungardt C B, Achterberg E P, Elbaz-Poulichet F M, Morley N H, 2003. Metal geochemistry in a mine-polluted estuarine system in Spain. *Applied Geochemistry*, 18: 1757–1771.

Cabezudo B, Rivera J, 1980. Notas taxonómicas y corológicas sobre la Flora de Andalucía occidental, 2: *Erica andevalensis* Cabezudo & Rivera sp. nov. *Lagascalia*, 9: 223–226.

Cabezudo B, Nieto Caldera J M, Pérez Latorre A V, 1989. *Junco rugosi-Ericetum andevalensis* ass. nova. *Acta Botanica Malacitana*, 14: 294–296.

Chopin E I B, Alloway B J, 2007. Trace element partitioning and soil particle characterization around mining and smelting areas at Tharsis, Riotinto and Huelva, SW Spain. *Science of the Total Environment*, 373: 488–500.

Chopin E I B, Black S, Hodson M E, Coleman M L, Alloway B J, 2003. A preliminary investigation into mining and smelting impacts on trace element concentrations in the soils and vegetation around Tharsis, SW Spain. *Minerological Magazine*, 67: 279–88.

Davis R A, Welty A T, Borrego J, Morales J A, Pendon J G, Ryan J G, 2000. Rio Tinto estuary (Spain): 5000 years of pollution. *Environmental Geology*, 39: 1107–1116.

Fernandez-Remolar D C, Gomez F, Prieto-Ballesteros O, Schelble R T, Rodriguez N, Amils R, 2008. Some ecological mechanisms to generate habitability in planetary subsurface areas by chemolithotrophic communities: The Rio Tinto subsurface ecosystem as a model system. *Astrobiology*, 8: 157–173.

Freitas H, Prasad M N V, Pratas J, 2004. Plant community tolerant to trace elements growing on the degraded soils of São Domingos mine in the south east of Portugal: environmental implications. *Environment International*, 30: 65–72.

Hudson-Edwards K A, Schell C, Macklin M G, 1999. Mineralogy and geochemistry of alluvium contaminated by metal mining in the Rio Tinto area, southwest Spain. *Applied Geochemistry*, 14: 1015–1030.

Kabata-Pendias A, Pendias H, 1984. Trace Elements in Soils and Plants. CRC Press, Boca Raton, USA.

Lopez-Archilla A I, Marin I, Amils R, 2001. Microbial community composition and ecology of an acidic aquatic environment: The Tinto River, Spain. *Microbial Ecology*, 41: 20–35.

Markert B A, 1996. Instrumental Element and Multi-element Analysis of Plant Samples Methods and Applications. Wiley and Sons, Chichester, USA.

Meier L P M, Kahr G, 1999. Determination of the cation exchange capacity (CEC) of clay minerals using the complexes of copper(II) ion with triethylenetetramine and tetraethylenepentamine. *Clays and Clay Minerals*, 47: 386–388.

Mingorance M D, Barahona E, Fernandez-Galvez J, 2007. Guidelines for improving organic carbon recovery by the wet oxidation method. *Chemosphere*, 68: 409–413.

Mitchell D T, Gibson B R, 2006. Ericoid mycorrhizal association: ability to adapt to a broad range of habitats. *Mycology*, 20: 2–9.

Paton D, Nuñez-Trujillo J, Diaz M A, Muñoz A, 1999. Assessment of browsing biomass, nutritive value and carrying capacity of shrublands for red deer (*Cervus elaphus* L.) management in Monfragüe Natural Park (SW Spain). *Journal of Arid Environments*, 42: 137–147.

Peñuelas J, Filella I, Tognetti R, 2001. Leaf mineral concentrations of *Erica arborea*, *Juniperus communis* and *Myrtus communis* growing in the proximity of natural $CO_2$ spring. *Global Change Biology*, 7: 291–301.

Prasad M N V, Freitas H, Fraenzle S, Wuenschmann S, Markert B, 2010. Knowledge explosion in phytotechnologies for environmental solutions. *Environmental Pollution,* 158: 18–23.

Pratas J, Prasad M N V, Freitas H, Conde L, 2005. Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. *Journal of Geochemical Exploration*, 85: 99–107.

Rodríguez N, Amils R, Jiménez-Ballesta R, Rufo L, de la Fuente V, 2007. Heavy metal content in *Erica andevalensis*: an endemic plant from the extreme acidic environment of Tinto River and its soils. *Arid Land Research and Management*, 21: 51–65.

Rossini Oliva S R, Leidi E O, Valdes B, 2009a. Germination responses of *Erica andevalensis* to different chemical and physical treatments. *Ecological Research*, 24: 655–661.

Rossini Oliva, S R, Valdes B, Leidi E O, 2009b. Accumulation and *in vivo* tissue distribution of pollutant elements in *Erica andevalensis*. *Science of the Total Environment*, 407: 1929–1936.

Ruiz F, Gonzales-Regalado M L, Borrego J, Morales J A, Pendon J G, Munoz J M, 1998. Stratigraphic sequence, elemental concentrations and heavy metal pollution in Holocene sediments from the Tinto-Odiel Estuary, southern-western Spain. *Environmental Geology*, 34: 270–278.

Salminen R, Batista M J, Bidovec M, Demetriades A, De Vivo B, De Vos W et al., 2005. FOREGS Geochemical Atlas of Europe, Part 1: Background Information, Methodology and Maps. Geological Survey of Finland, Espoo, Finland.

Schüürmann G, Markert B A, 1998. Ecotoxicology: Ecological Fundamentals, Chemical Exposure, and Biological Effects. John Wiley-Interscience Series, New York, USA.

Soldevilla M, Maranón T, Cabrera F, 1992. Heavy metal content in soil and plants from a pyrite mining area in southwest Spain. *Communication in Soil and Plant Analysis*, 23: 1301–1319.

Tisdale S L, Nelson W L, Beatin J D, Havlin J L, 1993. Soil Fertility and Fertilizers. Macmillan Publ, New York, USA.

Turnau K, Henriques F S, Anielska T, Renker C, Buscot F, 2007. Metal uptake and detoxification mechanisms in *Erica andevalensis* growing in a pyrite mine tailing. *Environmental and Experimental Botany*, 61: 117–123.

Yang W Q, Goulart B L, Demchak K, 1996. The effect of aluminum and media on the growth of mycorrhizal and nonmycorrhizal highbush blueberry plantlets. *Plant and Soil*, 183: 301–308.