



Presence, mobility and bioavailability of toxic metal(oids) in soil, vegetation and water around a Pb-Sb recycling factory (Barcelona, Spain)[☆]

S. Mykolenko^a, V. Liedienov^a, M. Kharytonov^a, N. Makieieva^a, T. Kuliush^a, I. Queralt^b, E. Marguí^c, M. Hidalgo^c, G. Pardini^{d,*}, M. Gispert^d

^a Dnipropetrovsk State Agrarian & Economic University 25, Voroshilov Str., Dnipropetrovsk 49027, Ukraine

^b Institute of Environmental Assessment and Water Research, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

^c Department of Chemistry, University of Girona, C/M. Aurèlia Campmany 69, 17003 Girona, Spain

^d Department of Chemical Engineering, Agricultural and Food Technology, University of Girona, Maria Aurèlia Campmany 61, 17003 Girona, Spain

ARTICLE INFO

Article history:

Received 18 July 2017

Received in revised form

14 February 2018

Accepted 18 February 2018

Keywords:

Metal(oid)s

Mobility

Bioavailability

Native plants

Phytoextraction

Phytostabilization

ABSTRACT

The work was conducted to establish contamination from improper disposal of hazardous wastes containing lead (Pb) and antimony (Sb) into nearby soils. Besides other elements in the affected area, the biological role of Sb, its behaviour in the pedosphere and uptake by plants and the food chain was considered. Wastes contained $139532 \pm 9601 \text{ mg kg}^{-1}$ ($\approx 14\%$) Pb and $3645 \pm 194 \text{ mg kg}^{-1}$ ($\approx 0.4\%$) Sb respectively and variability was extremely high at a decimetre scale. Dramatically high concentrations were also found for As, Cd, Cu, Mn, Ni, Sn and Zn. In adjacent natural soils metal(oid)s amounts decreased considerably (Pb $5034 \pm 678 \text{ mg kg}^{-1}$, Sb 112 mg kg^{-1}) though largely exceeded the directives for a given soil use. Metal(oid)s potential mobility was assessed by using $\text{H}_2\text{O} \rightarrow \text{KNO}_3 \rightarrow \text{EDTA}$ sequential extractions, and EDTA extracts showed the highest concentration suggesting stable humus-metal complexes formation. Nevertheless, selected plants showed high absorption potential of the investigated elements. Pb and Sb values for *Dittrichia viscosa* grown in wastes was $899 \pm 627 \text{ mg kg}^{-1}$ and $37 \pm 33 \text{ mg kg}^{-1}$ respectively. The same plant showed $154 \pm 99 \text{ mg kg}^{-1}$ Pb and $8 \pm 4 \text{ mg kg}^{-1}$ Sb in natural soils. *Helichrysum stoechas* had $323 \pm 305 \text{ mg kg}^{-1}$ Pb, and $8 \pm 3 \text{ mg kg}^{-1}$ Sb. *Vitis vinifera* from alongside vineyards contained $129 \pm 88 \text{ mg kg}^{-1}$ Pb and $18 \pm 9 \text{ mg kg}^{-1}$ Sb, indicating ability for metal uptake and warning on metal diffusion through the food chain. The biological absorption coefficient (BAC) and the translocation factor (TF) assigned phytoextraction potential to *Dittrichia viscosa* and *Foeniculum vulgare* and phytostabilization potential to *Helichrysum stoechas*. Dissolved metal (oid)s in the analysed water strongly exceeded the current directive being a direct threat for livings. Data warned against the high contamination of the affected area in all its compartments. Even though native plants growing in metal-contaminated sites may have phytoremediation potential, high risk of metal diffusion may threaten the whole ecosystem.

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1. Introduction

Development of human activities has induced soil

contamination by metals and metalloids since the beginning of industrialization in the 19th century, thereby generating both environmental and ecotoxicological risks (Sylvain et al., 2016). Different industrial activity in Catalonia, Spain and worldwide have strongly contributed to the contamination of extended areas in and around mining and industrial sites, especially when lacking of specific directives for environmental protection. The increasing pressure on the environment and the complexity of regulations require complying with the different aspects of waste management (disposal, treatment, monitoring, remedying, recycling) also taking

[☆] This paper has been recommended for acceptance by Prof. W. Wen-Xiong.

* Corresponding author.

E-mail addresses: svefotini@mail.ru (S. Mykolenko), liedenev.vladyslav@i.ua (V. Liedienov), kharitonov.m.m@dsaeu.dp.ua (M. Kharytonov), makeeva13hope@mail.ru (N. Makieieva), kasiopea0110@gmail.com (T. Kuliush), ignasi.queralt@idaea.csic.es (I. Queralt), eva.margui@udg.edu (E. Marguí), manuela.hidalgo@udg.edu (M. Hidalgo), giovanni.pardini@udg.edu (G. Pardini), maria.gispert@udg.edu (M. Gispert).

into account that 29% of the total waste generated each year is from some type of mining or metal industry activity. Producing considerable amount of residues has caused environmental deterioration in all its meanings: soil pollution and degradation, water quality reduction, biodiversity decrease, and even atmospheric pollution in some moments of the history (Álvarez-Valero et al., 2008). Metal processing industry is a matter of great concern because generate wastes rich in toxic elements. Moreover heavy metals and metalloids may be involved in a series of complex chemical and biological interactions. The most important factors which impact on their mobility are pH, sorbent nature, presence and concentration of organic and inorganic ligands. Furthermore, redox reactions, both biotic and abiotic, are of great importance in controlling the oxidation state and, thus, the mobility and toxicity of many elements. Redox reactions can mobilize or immobilize metals depending on the particular metal species and microenvironments (Violante et al., 2010). Accordingly, soil, water and vegetation may be highly contaminated by heavy metals and metalloids to the point of being a real hazard for human health through the food chain. Most of these activities, whether mining or metal recycling, have been closed or ceased without any action for remediation and strong contamination is still active threatening the surrounding environments.

Both the total metal concentration and the bioavailable fraction present in a specific environmental compartment can be made available within a given time span for uptake by soil fauna and flora or by human ingestion of food (Peijnenburg and Jager, 2003). Hence heavy metals polluted areas pose environmental, safety and economic problems to communities, and also to governments in many countries. At this regard, all these contaminated sites are of paramount interest for the public administrations and the scientific community in order to ascertain the real potential for health risk and to carry out the most adequate methodologies for site remediation. In Catalonia, Spain, DemimeSA was an industry dedicated to the recycling of batteries from automotion and was forcedly ceased by the public authorities due to no accomplishment with metal-rich wastes disposal regulations. Nowadays, after 10 years since the plant closure and lacking of remediation plans, the area presents multimetallic contamination especially of As, Sb and Pb and a high potential for metal/metalloid disperse into the food chain through soil, vegetation and water.

The aims of this research work were (i) to determine the waste and soil physico-chemical characteristics and the total concentrations of As, Cd, Cu, Mn, Ni, Pb, Sb, Sn and Zn in soils, vegetation and water of the area; (ii) to evaluate the heavy metals mobility and bioavailability potential and their extent and (iii) to establish the pollution potential with respect to current legislation in order to get public administration acquainted with forthcoming serious ecological impacts.

2. Material and methods

2.1. Area of study and sampling

The site of previous industrial activities was located in San Llorenç d'Hortons, Barcelona province, Spain, 41° 29' 50"N. 01° 50' 10"E. The factory was emplaced over a hill at 285 m asl and surrounded by vineyards and natural soils downslope on the South-West and North-East direction respectively (Fig. 1). The area affected by metal contamination may be equated to a catchment of approximately 40 000 m² with ellipsoidal shape with an outflow NW-SE direction for runoff water and sediments removed from the overall surface. Discharged sample of water and sediments were periodically collected in order to monitor the metal concentrations released out of the catchment. The mean temperature in this

district is 16 °C and the annual precipitation ranges between 400 and 600 mm (Parera and Safont, 2016).

Sampling was divided into 4 zones: zone 1, the waste landfill; zone 2, the natural soil downslope; zone 3, the natural soil upslope 1 km away from the focus of contamination; zone 4, corresponding to the vineyard adjacent to the focus of contamination. Three soil samples of zone 1 and 2 were sampled to form representative composed specimen identified as W1 and S2. Plants such as *Dittrichia viscosa*, *Foeniculum vulgare* and *Helichrysum stoechas* were also sampled in these two sites. Moreover, due to the strong abundance in zone 1 and 2 of *Dittrichia viscosa*, a known hiper-accumulator plant for Pb, Cd and Sb (Conesa et al., 2011), additional soil samples were collected at the rhizosphere of a representative number of plants and plants were also sampled including root, stem, leaves and inflorescence. Three soil samples were collected to form sample WR1 (waste-rizosphere) for zone 1 and SR2 (soil-rizosphere) for zone 2. Likewise, three soil samples of zone 3 were also sampled forming the composed sample S3. *Dittrichia viscosa*, *Foeniculum vulgare* and *Helichrysum stoechas* were also sampled in this site. Soils under vines (*Vitis vinifera*) of zone 4 bordering the contaminated area were collected to form sample V4. The sampling zones are listed in Fig. 1. Control soil and vegetation samples were also collected in an uncontaminated site with similar geological and pedological characteristics. The soil (obtained by three sampling site) was named S5 and in this site *Dittrichia viscosa*, *Foeniculum vulgare* and *Helichrysum stoechas* were also sampled. All wastes and soils were taken at 0–20 cm depth. Samples were dried at room temperature, sieved at 0–2 mm and stored for subsequent analysis. Likewise, plants were accurately washed to eliminate any waste or soil residues, oven-dried at 40 °C and parts such as root, stem, leaves and flowers (when possible) separated. Successively the different parts were ground with a blender to pass 1 mm sieve and stored for metal (oid)s analysis. Water samples were collected in different points of the catchment and identified as pipeline water, drainage water, creek water 1 (from the main creek) and creek water 2 (from the secondary creek).

2.2. Analytical layout

2.2.1. Soil characterization

Wastes and soil samples were subjected to the following analysis: the texture was determined by using the Robinson's pipette based on the sedimentation of soil particles by gravity to obtain 4 dimensional classes: coarse sand (2000–200 μm), fine sand (200–20 μm), silt (20–2 μm) and clay (<2 μm). Determination of pH was carried out in 1:2.5 (w/v) in water and 0.1 M KCl (Forster, 1995) by using a pHmeter mod. 550 from Eutech Instruments, Singapore. pH values from H₂O refer to free protons in the soil crumb coming into solution, while K⁺ removes protons eventually adsorbed onto soil surfaces; if pH values from KCl solution are much lower than that of water, the amount of protons removed by K⁺ is considerable indicating a forthcoming process of soil acidification as exchangeable sites may be partially proton-saturated. Thus, values of ΔpH ≫ 1 (pH_{H₂O}–pH_{KCl}) may announce acid soil pH and potential mobility of toxic metals (Gäbler, 1997). Electrical conductivity (EC) was estimated in 1:5 (w/v) in water (Forster, 1995) by using a Conductimeter mod. 100 from Eutech Instruments, Singapore. Moreover, when EC values largely exceeded 4 dS m⁻¹ the saturated paste extract method was used (Rhoades et al., 1989). Total calcium carbonate content was determined by the Bernard calcimeter (Forster, 1995). Soil organic carbon (SOC) content determination was carried out by the wet dichromate oxidation method (Schnitzer, 1991). The Scanning Electron Microscope (SEM) Zeiss DSM 960 A (20x a 200000x) connected with RX Bruker Quantax 200 was used for microaggregate observations and



Fig. 1. View of the Demimesa area emplacement few months after the closure. Hazardous waste landfill and the surrounding affected natural and agricultural fields may be noticed. The surface has been divided into 4 zones to evaluate the impact of contamination by metal(oid)s.

Electron Dispersion XRay Fluorescence Analysis (EDXRFA).

2.2.2. Multielemental analysis of waste, soil, vegetation and water samples

Determination of metal and metalloid concentrations (As, Cd, Cu, Ni, Mn, Pb, Sb, Sn and Zn) in wastes, soils, vegetation and water was carried out by Inductively Coupled Plasma (ICP-OES) with an Agilent Technologies 5100 Spectrometer. Instrumental measurements conditions were the following: power – 1200 W, plasma flow – 12 L/min; torch configuration – radial; detector – multi-channel charge transfer; nebulizer – concentric; emission wavelength depending on each selected element As (193.696 nm), Cd (226.502 nm), Cu (327.395 nm), Ni (216.555 nm), Pb (220.353 nm), Sb (217.582 nm), Mn (257.610 nm), Sn (283.998 nm), Zn (202.548 nm). Once defining the wavelengths of the elements to be determined, calibration curves were performed by using standard solution for each individual element prepared at the laboratory from commercial stocks. For each metal the concentration range was measured as a function of the intensity emitted from that given metal.

2.2.2.1. Waste and soil samples. Total metal content determination for wastes and soils were performed through acid digestion by using an Ethos Plus Milestone microwave with HPR-1000/10 S high pressure rotor from Sorisole, (Bergamo, Italy). Digestions were prepared by weighing in a Teflon vessel 0.3 g of sample and adding 7 mL of concentrated HNO₃, 2 mL concentrated HF and 2 mL of H₂O₂ 30%. The vessels were capped and heated following a two-stage digestion program consisting of a first step of 15 min to reach 130 °C and a second step of 15 min to reach 200 °C. Following the matrix decomposition with HF, extracts were additionally treated with 1 g of H₃BO₃ and heated during 15 min at 180 °C to allow the complexation of fluoride. Finally, digests were transferred into a 50 mL flask and brought to volume with purified water (Marguí et al., 2016). Elemental content in digested soil samples was then determined by ICP-OES.

In addition to total metal(oid)s concentration in wastes and soils, the evaluation of potential mobility and bioavailability for biota was carried out by using the sequential extractions method

(Petruzzelli et al., 2008). Sequential extractions of toxic elements are generally used in order to assess the soluble, exchangeable and humus-complexed fractions. Results might advise on the amount of mobile fractions with respect to the total metal amount. Aliquots (1:10 w/v ratio) of waste and soil samples were sequentially extracted first with distilled water then centrifuged and the extract decanted and stored for the analysis of the soluble fraction of metal(oid)s. On the same soil samples the extraction was carried out with 1 M KNO₃ and after centrifugation the extract decanted and stored for the exchangeable fraction of metals. Finally the extraction was carried out on the same samples with 5% EDTA for the complexed fraction as EDTA simulates the organic matter chelating ability towards toxic elements. The sequential extractions were performed during 24 h to observe the reliability of each extractant to metal(oid)s release. Once obtained all the extracts elements concentrations were determined by ICP-OES technique.

2.2.2.2. Vegetation samples. Plants such as *Dittrichia viscosa*, *Foeniculum vulgare* and *Helichrysum stoechas* representing the majority of plant cover were selected in the contaminated area. Aliquots (300 mg) of previously prepared parts of plants, as above mentioned, were placed in a 50 mL beaker with 10 mL of concentrated HNO₃, 2 mL of H₂O₂ 30% and let to react over a heating sand bath at 90 °C during 2 h under extractor fun. After digestion, the acid solutions were filtered in 25 mL flasks and reserved for ICP-OES analysis of the investigated metal(oid)s. Toxic elements may enter soils through several pathways, including runoff and wind deposition from mining and industrial sites and metals may be absorbed by native plants, so that it is paramount knowing the metal absorption capacity of these plants. Metals like Ni, Cu, Mn, and Zn are important to particular biological functions in plants (are catalytically active cofactors in photosynthesis and contribute to the stabilization of proteins) though when they occur in excess can disrupt critical physiological processes and result in toxicity. By contrast, As, Cd, Pb, Sb and Sn do not have biological functions. On the one hand plants may exclude excess bioavailable metal(oid)s (with or without biological functions) at their roots by binding them to organic acids or ligands or storing them within vacuoles where they cannot interfere with important physiological

processes (Baker, 1981). However, certain plants can tolerate and accumulate bioavailable metal(oid)s into underground or above-ground tissues (Unterbrunner et al., 2007) and can be catalogued as potential for phytostabilization or phytoextraction, two processes of phytoremediation. Accordingly, plants able to concentrate metals in the aerial parts (high biological absorption coefficient) are suitable for phytoextraction, while plants with low ability to transfer metals to the aerial parts (low translocation factor) may be adequate for phytostabilization.

At this regard, we determined the biological absorption coefficient (BAC) and the translocation factor (TF) as follows:

$$BAC = \frac{C_{plant}}{C_{soil}}$$

Where C_{plant} is the concentration of a heavy metal in a plant and C_{soil} is the concentration of that heavy metal in the soil.

$$TF = \frac{C_{leaf}}{C_{root}}$$

Where C_{leaf} is the concentration of a heavy metal in the leaves of a given plant and C_{root} is the concentration of that heavy metal in that plant root (Bitterli et al., 2010).

2.2.2.3. Water samples. Water samples collected at the contaminated site area were analysed for soluble metal content by TXRF analysis. For that, an appropriate amount of a $1000 \pm 0.5 \mu\text{g mL}^{-1}$ Rh solution (to reach a final Rh concentration of 10 mg L^{-1}) was added to 1 mL of the target sample for internal standardization. After, the resulting solution was thoroughly homogenized and an aliquot of $10 \mu\text{L}$ was transferred onto a quartz glass sample carrier and dried using an infrared lamp. TXRF analysis of all samples was performed using a benchtop TXRF system (S2 PICOFOX, Bruker AXS Microanalysis GmbH, Berlin, Germany) equipped with a 50 W X-ray tube with a tungsten (W) anode. The characteristic radiation emitted by the elements present in the sample is detected by a silicon drift detector with an active area of 10 mm^2 and a resolution of 147 eV (Mn K α). The measurements were performed working at 50 kV and $1000 \mu\text{A}$ and in air environment. The evaluation of TXRF spectra and calculation of element concentrations on the basis of mass ratio of sample to internal standard was performed using the software (Spectra Plus 5.3, Bruker AXS Microanalysis GmbH, Berlin, Germany) linked to the equipment.

3. Results and discussion

3.1. Soil characteristics

Selected soil properties of the wastes and soils sampled in the contaminated area are showed in Table 1. The textural class was sandy-clay-loam for wastes of zone 1, clay-loam for soils of zone 2, 3 and 4 respectively and sandy-clay-loam for control soil 5. On average, clay increased by 76% in soils 2, 3 and 4 with respect to wastes, which may be relevant in the temporary adsorption of the different metal(oid)s. The control soil (zone 5) also showed a sandy-clay-loam texture. Values of pH ranged from slightly alkaline in zone 1 to moderately alkaline in zone 2 probably indicating that salt seepage may have occurred in downslope soils from the upslope waste landfill. The ΔpH values, i.e. the difference between the pH measured in distilled water and 1 M KCl were always below 0.5 pH units suggesting a high base saturation in all soils mainly by sodium and calcium salts. Effectively, at the time of DemimeSA activity different compounds such as sodium and calcium carbonates were added to the reducing rotating furnace for lead recycling with the

purpose to reduce SO_2 emission by obtaining slags retaining a large part of sulphur as Na_2SO_4 and CaSO_4 . The presence of these ions in wastes and soils may have strongly hindered Pb adsorption by clays as Ca content in soils plays an important role in controlling Pb behaviour (Bradl, 2004). Moreover, field observations in zone 1 and 2 reported the existence of extended surface efflorescence which were supposed to be constituted by Na_2SO_4 and CaSO_4 associated to residual compounds from recycling processes (Sonmez and Kumar, 2008; Morachevskii, 2014). The high abundance of sodium and calcium ions was also reflected in the electrical conductivity (EC) measurements, recording very high values (Table 1). Usually the electrical conductivity of a soil material is measured in distilled water (1:5 soil:solution ratio) but if values overcome 4 dS/m the saturated paste extract method should be used in order to have a more reliable measure of this parameter (Rhoades et al., 1989). In fact, EC values of sample W1 and S2 increased 5.86 and 7.89 times with respect to 1:5 method that is 486% and 689% respectively (Table 1) corroborating that residual salts from roasting operations greatly influenced soil salinization. The sharp increase of EC values might have occurred by the leakage of soluble sodium sulphate from the wastes area to the footslope (zone 2) after continuous overland flow along years. Surprisingly, in samples WR1 and SR2 (wastes and soils sampled at the rhizosphere) the EC values decreased dramatically, probably due to the extremely high variability of salts content in the area of study. SEM observations and EDXRFA analysis (Fig. 2) allowed identifying crystals and elements thus explaining the extremely high salinity of wastes and soils from zone 1 and 2. Electron micrographs of W1 samples showed well developed crystals of Thenardite in a microarray matrix (Fig. 2 A). As expected, the EDXRFA analysis confirmed the presence of the above mentioned salts and the relative abundance of sodium sulphate with respect to calcium sulphate (Fig. 2 B). Results of SEM and EDXRFA were also in agreement with the chemical characteristics of sodium and calcium sulphate. It is known that the chemical solubility of CaSO_4 is 2.40 g L^{-1} (Gangolli, 1999), which means that higher amount in the soil solution is sufficient to cause the precipitation of this compound. Conversely, the solubility of Na_2SO_4 is 195 g L^{-1} , consequently the mechanism of precipitation is largely delayed as the salt needs higher amount to overcome its solubility threshold. This pattern could explain why sodium sulphate was found in largest abundance in the downslope soil of zone 2. The results may indicate that DemimeSA activity was affecting the surrounding environment not only with toxic metals but also with elements able to cause problems of salinity and sodicity, thus further altering the quality of natural soils. It was also assumed that the high salt concentration may have affected the soil organic carbon content at DemimeSA site which was extremely low and, obviously, null in wastes (Table 1). As a consequence, microbial biomass activity in both soils from zone 2 and 3 may have been drastically reduced hindering mineralization and humification processes (Pérez de Mora et al., 2005). Even in the natural soils under scrubs (zone 3), theoretically less susceptible to pollution because farer from the factory, the soil organic carbon was still low. The calcium carbonate content was similar in all wastes and soils (Table 1).

3.2. Total element concentrations in waste and soil samples

Concentrations of metals and metalloids are reported in Table 2 together with the thresholds required by the Catalan Government for different soil use. The analysed elements were extremely high in the zone 1 corresponding to recent and old landfill of hazardous wastes disposed illegally during the DemimeSA activity. Due to the extreme variability of metal content all over the area, the standard error was generally very high. Despite that, the highest metal

Table 1

Physical and chemical parameters of wastes and soils of the study area. Results are the mean of three replications, standard error in parenthesis.

Property/ Waste-Soil	N	CS (%)	FS (%)	SL (%)	CL (%)	pH H ₂ O	pH KCl	EC-1:5 (dS m ⁻¹)	EC-SP (dS m ⁻¹)	CaCO ₃ (%)	SOC (%)
W1	3	21.62 (1.43)	29.87 (1.06)	29.24 (0.62)	19.27 (1.73)	7.41 (0.05)	7.35 (0.09)	12.93 (0.22)	75.80 (3.36)	5.59 (0.62)	n.d.
WR1	3	21.43 (2.55)	30.51 (2.89)	27.73 (2.05)	20.33 (1.89)	7.65 (0.92)	7.39 (0.87)	0.27 (0.06)	1.57 (0.99)	6.06 (0.31)	n.d.
S2	3	15.23 (0.09)	18.96 (4.01)	26.59 (3.29)	39.22 (1.32)	8.39 (0.05)	8.43 (0.05)	15.15 (0.54)	119.63 (1.23)	13.56 (0.89)	0.06 (0.08)
SR2	3	17.32 (2.78)	20.34 (3.15)	27.12 (1.69)	35.22 (2.55)	8.43 (0.03)	7.98 (0.05)	0.14 (0.01)	1.04 (0.07)	9.82 (0.62)	0.14 (0.09)
S3	3	14.38 (1.66)	19.23 (2.08)	30.12 (1.52)	36.27 (3.15)	8.31 (0.08)	7.86 (0.09)	0.24 (0.06)	0.67 (0.15)	7.71 (4.85)	0.51 (0.41)
V4	3	19.45 (2.77)	25.50 (1.97)	26.33 (3.19)	28.72 (1.79)	7.45 (1.12)	7.13 (1.05)	0.47 (0.11)	0.70 (0.13)	7.34 (1.05)	0.89 (0.22)
S5	3	20.45 (3.87)	27.89 (2.52)	24.16 (4.43)	27.50 (2.49)	7.42 (1.56)	7.12 (0.58)	0.33 (0.09)	0.55 (0.11)	5.45 (0.77)	0.50 (0.22)

W1: Waste zone 1; WR1: Waste zone 1 at the rhizosphere under *Dittrichia viscosa*; S2: Soil; SR2: Soil zone 2 at the rhizosphere under *Dittrichia viscosa*; S3: Soil zone 3; V4: Soil from vineyard zone 4; S5: Control soil zone 5; CS: Coarse sand.; FS: Fine sand; SL: Silt; CL: Clay; EC: Electrical conductivity; SP: Saturated paste; SOC: Soil organic carbon; n.d.: Not detectable.

concentrations were confined in the zone 1 with respect to zone 2, 3 and 4. However, results provided the evidence that pollution was extended in natural and cultivated soils with the potential to cause serious ecological impact. Even considering the industrial use of soil of zone 1, the content of Pb and Sb (the two elements on which was based the recycling activity of the ceased factory) exceeded 253 and 122 times respectively in sample W1 and 81 and 102 times in sample WR1 the amount admitted by the Catalan Directive, warning on the great risk of contamination for surrounding environments (Table 2). In the same zone 1, very high concentrations of other toxic metal(oid)s such as As, Cd, Cu, Sn and Zn were found for sample W1, increasing by 4690%, 23%, 271%, 168% and 351% respectively the admitted thresholds. In sample WR1 only As was found to exceed the threshold by 2023% accounting for the persistence of this element in this zone (Table 2). The zones 2, 3 and 4 were considered under the agriculture and forest use of soil and showed different content of metals and metalloids. The samples S2 and SR2 of zone 2, adjacent to zone 1, exceeded the admitted threshold by 100% for Cd, by 1437% for Mn, by 4980% for Pb, and by 1567% for Sb corroborating high dispersal of toxic elements all around. Effectively, in soil of zone 3 the concentration of Cd, Mn and Pb of 3, 321 and 189 mg kg⁻¹ exceeded by 20, 1505 and 215% the toxic elements regulation existing in Catalonia for forest soil use. Likewise, very high concentrations of As (77 mg kg⁻¹), Cd (31 mg kg⁻¹), Pb (7650 mg kg⁻¹) and Sb (18 mg kg⁻¹) were found in the soil under vines (V4) which is located alongside to the ceased factory (Fig. 1) alerting to the potential for health risks generated along many years of Pb and Sb recycling activity. The irregular distribution and amount of metals and metalloids from the main source of pollution supported the assumption of a great spread out by water and wind of the analysed elements and indicated the complexity of establishing the real extent of contamination.

3.3. Potential mobility of the analysed elements assessed by sequential extraction

In order to evaluate the potentially mobile fractions of the investigated metals and metalloids, a method using sequential extractants was used to obtain the soluble fraction (by water extraction), the readily available fraction (by 1 M KNO₃ extraction) which may correspond to the metal fraction presumably adsorbed by clays and the complexed fraction (by using 5% EDTA) as to simulate the chelating effect of organic matter when entraps metals into organic molecules. Extractions were carried out during 24 h to

observe the extraction efficiency with respect to total metal content (Table 3). In general EDTA was more effective in metal extraction indicating that the complexed fraction was much larger with respect to soluble (H₂O extracted) and readily available (KNO₃ extracted) fractions. It is however difficult to explain in which form metals like Pb or Sb may be complexed in a material (the waste) with null content of organic carbon. In Fig. 3 is clearly shown the EDTA capacity to extract the larger amounts of Pb and Sb potentially mobile fractions from the waste of zone 1 (W1), soil of zone 2 (S2) and soil of zone 4 (V4). The summation of metal mobile fractions reported in Table 3 is expressed as per cent of total element concentration of samples W1, S2 and V4. In sample W1 the low percentage of Pb to total may be misleading because of the high concentration (6622.19 mg kg⁻¹) extracted by sequential extraction during 24 h. This amount only represents 4.75% of total Pb in the wastes (W1) but is extremely high and might be available to soil biota. In sample S2 the potentially mobile elements decreased sharply and their percent with respect to total was not of concern with the exception of Sb (Table 3) which still showed a potential mobility around 10–15% of the total amount, viz 112 mg kg⁻¹. The percent to total obtained by sequential extractions of sample V4 was considerably high for all metals with respect to sample W1 and S2. It was postulated that frequent ploughing in vineyards may have removed metals from downward. The high percentage of Cu (56.72% of total) may refer to the contribution of chemical fertilisation during agricultural field work. Though the majority of metals extracted through sequential extractions in zone 1, 2 and 4 did not show high soluble or readily available fractions, metal accumulation and subsequent release to plants and animals may occur, thus threatening the whole ecosystem under study.

3.4. Metal accumulation in native vegetation

Bioavailability of metals and metalloids is a complex phenomenon depending on a cascade of matrix-related, metal-related and species-related issues (Peijnenburg et al., 2003). On the one hand, the purpose to investigate the ability of selected plants such as *Dittrichia viscosa*, *Foeniculum vulgare* and *Helichysum stoechas* to absorb and distribute a given metal in different parts (root, stem, leaf and flower) was addressed to know the bioavailability of metals from wastes and soils to plants. This mechanism may constitute a risk for human health according to the possibility that plant may be fed by herds and might enter into the food chain. On the other hand metal bioavailability is generally used as indicator to establish the

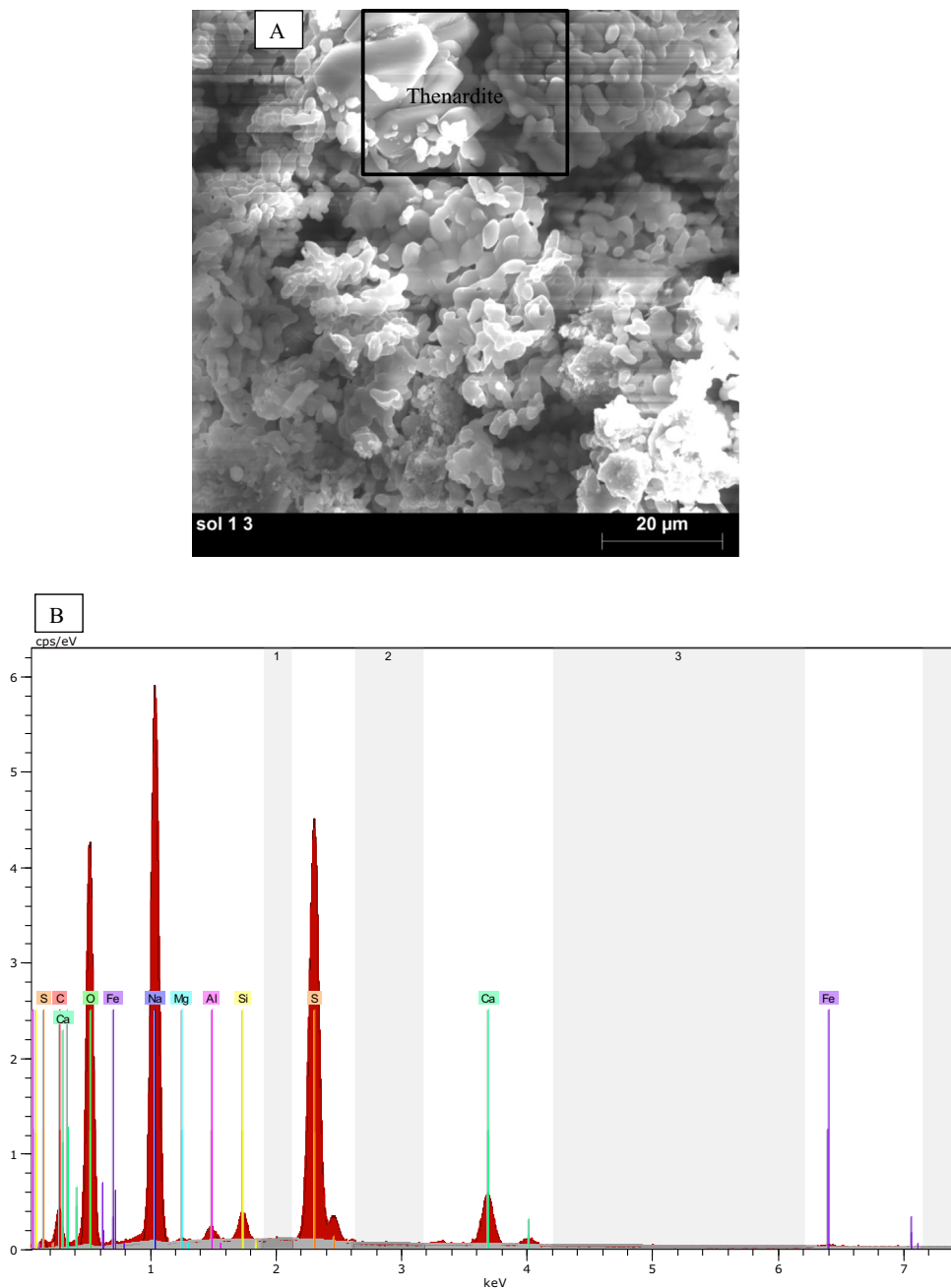


Fig. 2. SEM micrograph of well-shaped Thenardite crystals (A) and EDXRF analysis for the identification of elements forming sodium sulphate (Thenardite) in samples observed by SEM (B). The presence of calcium sulphate (Gypsum) is also assumed. Semiquantitative percentage are as following: C 11.82, O 45.21, Na 24.82, Mg 0.17, Al 0.49, Si 0.74, S 13.11, Ca 3.35, Fe 0.29.

potential of certain plants for natural phytoextraction or phytostabilization (Marchiola et al., 2013). Metal(oid)s concentration was generally very high in the analysed parts of the selected plants specially in zone 1 and 2. A first indication of the metal extent from the contaminated area to the surrounding environment was given by the comparison between the so called in-site control (S3) plants 1 km away from the factory and the ex-site control plants (S5). Plants of in-site control samples (S3), even emplaced far away from the hazardous wastes landfill proved to considerably absorb high amounts of metals, particularly Pb and Sb. For instance, *Helichrysum stoechas* in S3 allowed establishing its strong ability for phytostabilization as the highest values of As, Cd, Cu, Ni, Pb, Sb and

Sn that is 4.57, 5.53, 41.46, 4.98, 663.57, 11.06 and 2.76 mg kg⁻¹ respectively were found in the root and decreased when analysing the aerial parts of these plants. In fact, the concentration of the above elements decreased in the same order by 56%, 85%, 69%, 66%, 75%, 88, 50% and 29% respectively from root to leaves. By contrast, no clear trend was found in metals absorbed by *Dittrichia viscosa* and *Foeniculum vulgare*. The concentrations of the same elements analysed in *Dittrichia viscosa*, *Helichrysum stoechas* and *Foeniculum vulgare* in S5 site (ex-site control) were not of concern, because plants proceeded from an uncontaminated area with similar pedological characteristics. Only Zn showed values ranging from 41 to 100 mg kg⁻¹ along the different parts of plants, but amounts

Table 2

Total element threshold for different soil use as reported by Catalan Directive and total element concentrations found in the wastes and soils under study. Results are the mean of three replications, standard error in parenthesis.

Element	As	Cd	Cu	Mn (mg kg ⁻¹)	Ni	Pb	Sb	Sn	Zn	
Threshold										
Industry	30	55	1000	3000	1000	550	30	1000	1000	
Urban	30	5.5	310	400	470	60	6	1000	650	
Recreational	30	2.5	90	20	45	60	6	50	170	
Agriculture and forest	30	2.5	90	20	45	60	6	50	170	
Samples	N	(mg kg ⁻¹)								
W1	3	2297 (410)	68 (10)	3708 (1020)	1319 (348)	251 (66)	139532 (9601)	3645 (194)	2686 (472)	4514 (2929)
WR1	3	637 (90)	25 (21)	938 (82)	572 (238)	81 (87)	44586 (47074)	3057 (3622)	1107 (873)	812 (695)
S2	3	11 (2)	5 (0.1)	14 (0.7)	277 (33)	16 (1)	5034 (678)	112 (35)	75 (14)	79 (23)
SR2	3	25 (5)	5 (0.6)	33 (3)	338 (37)	17 (0.7)	1063 (167)	88 (14)	24 (2)	66 (5)
S3	3	10 (1.4)	3 (0.4)	18 (3)	321 (26)	15 (2)	189 (26)	1.5 (0.5)	11 (1.1)	68 (21)
V4	3	77 (33)	31 (12)	47 (28)	n.a. –	19 (7)	7650 (923)	18 (7)	10 (2)	129 (45)
S5	3	5 (0.5)	2 (0.5)	24 (7)	14 (3)	21 (5)	34 (11)	0.5 (0.1)	12 (4)	56 (19)

W1: Waste zone 1; WR1: Waste zone 1 at the rhizosphere under *Dittrichia viscosa*; S2: Soil zone 2; SR2: Soil zone 2 at the rhizosphere under *Dittrichia viscosa*; S3: Soil zone 3; V4: Soil from vineyard zone 4; S5: Control soil zone 5; n.a.: Not available.

Table 3

Potential concentrations (mg kg⁻¹) of mobile fractions of metals and metalloids assessed by sequential extraction after 24 h in sample W1, S2 and V4 of zone 1, 2 and 4 respectively. Values are the mean of three replications.

Element	Fractions extracted			Total soil		
	Extractant/ (mg kg ⁻¹)	H ₂ O	KNO ₃	EDTA	Σ fractions concentration	% to total
Sample W1						
As	0.05	0.09	4.98	5.12	2297	0.22
Cd	0.05	0.10	0.91	1.06	68	1.56
Cu	0.04	0.11	95.81	95.96	3708	2.59
Ni	0.05	0.10	2.10	2.25	251	0.90
Pb	1.40	2.79	6618	6622.19	139532	4.75
Sb	0.07	0.07	2.69	2.83	3645	0.08
Sn	0.05	0.11	4.49	4.65	2686	0.17
Zn	0.23	0.59	35.25	36.07	4,14	0.80
Sample S2						
As	0.05	0.10	0.10	0.25	11	2.27
Cd	0.05	0.10	0.10	0.25	5	5.00
Cu	0.05	0.10	0.10	0.25	14	1.79
Ni	0.05	0.10	0.10	0.25	16	1.56
Pb	4.98	10.02	64.99	79.99	5034	1.59
Sb	3.73	7.47	7.47	18.67	112	16.67
Sn	0.33	0.66	0.66	1.65	75	2.20
Zn	0.10	0.20	0.10	0.40	79	0.51
Sample V4						
As	0.04	0.07	3.12	3.23	77	4.19
Cd	0.03	0.08	2.64	2.75	31	8.87
Cu	0.18	8.35	18.13	26.66	47	56.72
Ni	0.06	0.14	6.42	6.62	19	34.80
Pb	0.50	1.81	714.46	716.77	7650	9.36
Sb	0.04	0.08	0.35	0.47	18	2.61
Sn	0.06	0.14	3.45	3.65	10	20.27
Zn	0.34	5.15	12.80	18.28	129	14.17

were considered lower than critical values as suggested by [Broadley et al. \(2007\)](#). These authors inferred that toxicity symptoms usually become visible at concentrations >300 mg Zn kg⁻¹ leaf dry weight. Even though *Helichrysum stoechas* was initially sampled only in

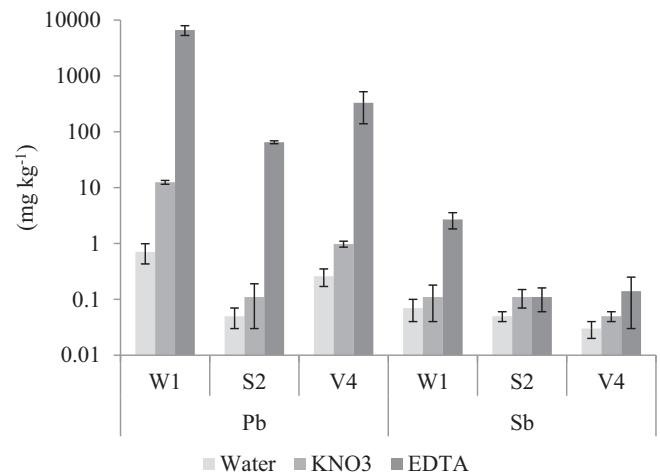


Fig. 3. Lead and antimony concentrations in samples W1, S2 and V4 obtained after H₂O→KNO₃→EDTA sequential extractions of 24h each extractant. Columns and bars represent means and SD of three samples.

zone 2 (S2), results of metals concentrations in this zone are in agreement with those of the same plants in zone 3 (in-site control, S3), indicating a preferential metal absorption by roots with respect to aerial parts. The Pb content in *Helichrysum stoechas* of S2 were 366.24 mg kg⁻¹ in root, 324.86 mg kg⁻¹ in stem, 238.55 mg kg⁻¹ in leaves and 177.33 mg kg⁻¹ in flower. Accordingly, Pb concentration decreased progressively by 11% from root to stem, 27% from stem to leaves and 26% from leaves to flower, i.e. by 52% from root to flower indicating phytostabilization characteristics. Similarly, Sb concentration decreased from 10.76 mg kg⁻¹ in root to 5.11 mg kg⁻¹ in flower, that is by 53% from root to flowers. As above mentioned, similar drop in metal concentration was found in zone S3 where *Helichrysum stoechas* had 89% and 50% less Pb and Sb respectively in leaves than root, strengthening the ability of this plant for metal and metalloids phytostabilization. [Cristina \(2015\)](#) when investigated Cd, Pb and Zn uptake in *Helichrysum stoechas* plants grown in

hazardous wastes and soils at the sphalerite and galena abandoned mine in Osor, Spain, found similar results and pointed out the preferential Pb accumulation in the root of this plant. Samples of *Dittrichia viscosa* from zone 1 (W1) showed high metal concentration of Pb and Sb in the stem (108.40 and 7.42 mg kg⁻¹ respectively) and much higher in leaves (1438.30 and 80.80 mg kg⁻¹ respectively). Jimenez et al. (2011) also reported that *Dittrichia viscosa* grown in contaminated mine-tailings and soils of Sardinia (Italy) contained the highest concentrations of Pb and Zn in the leaves. Effectively, Samples W1 showed 10.10 mg kg⁻¹ of Zn in stem and its concentration increased to 144.17 mg kg⁻¹ in leaves (+1327%). Though the toxic elements distribution in the different parts of the selected plants was not always clear, data pointed out that *Dittrichia viscosa* had phytoextraction ability. When *Dittrichia viscosa* was sampled at the rhizosphere soil (WR1, SR2) and analysed, it was clearly shown that in zone 1 the plant was more able to allocate the highest concentration of As, Cd, Pb and Sb in the stem, reaching a mean of 16.13, 24.13, 1701.80 and 85.63 mg kg⁻¹ respectively. In other field experiments in Sardinia, Buscaroli et al. (2016) suggested that *Dittrichia viscosa* was an interesting species for the application of phytoextraction technologies to reduce heavy metals impact at Montevecchio and Libiola abandoned mine areas. Despite the fact that the dynamics of metal absorption by *Dittrichia viscosa* was somewhat unclear and extremely variable, a steep increase of metal concentration from root to stem and a consequent decrease from stem to leaves and flowers was observed in many of the analysed metals and metalloids. In *Dittrichia viscosa* sampled in zone 1 (WR1), As concentration was 7.93, 16.13, 10.59, and 5.38 mg kg⁻¹ in root, stem, leaves and flower respectively. An increase by 103% from root to stem and a decrease by 34% and 49% from stem to leaves and from leaves to the inflorescence was then recorded. Cd increased by 11% from root (17.41 mg kg⁻¹) to stem (22.35 mg kg⁻¹) and then decreased by 23% and 52% in leaf (19.40 mg kg⁻¹) and flower (16.27 mg kg⁻¹) respectively. The amounts of Pb and Sb were 1088.45 and 19.57 mg kg⁻¹ in root and increased to 1701.80 and 85.63 mg kg⁻¹ in stem (+56% and +338% for Pb and Sb respectively). Mean values of Pb and Sb were 491.86 and 24.42 mg kg⁻¹ in leaves and 319.55 and 14.74 mg kg⁻¹ in flower indicating a decrease of Pb and Sb by 71% and 72% from stem to leaves and by 35% and 39% from leaves to flower respectively. In samples SR2 of zone 2, As, Pb and Sb concentrations were substantially lower in root, stem, leaf and flower of *Dittrichia viscosa* with respect to the same plant samples of zone 1 (WR1). Mean As values of 3.71, 4.50, 2.26 and 2.23 mg kg⁻¹ were recorded in root, stem, leaves and flower, representing -53.35%, -72.10%, -78.66 and -58.56% of samples in WR1. Similarly, mean Pb values in the same parts of plants were 236.31, 336.45, 106.23 and 36.84 mg kg⁻¹, thus decreasing by 78.29%, 80.23%, 78.40% and 88% if compared with WR1 plant concentrations. Yet, Sb mean values had the same pattern with mean values of 12.70, 38.17, 5.40 and 4.85 mg kg⁻¹, i.e. -35%, -55%, -77.89% and -67.10%. With respect to WR1. Moreover, the phytoextraction ability of *Dittrichia viscosa* in zone 2 (SR2) was still evident, as the highest concentration was always recorded in the stem. However, As, Pb and Sb values remained still high though did not change markedly in the different parts of the plant and were always lower in the inflorescence. The potential of *Foeniculum vulgare* for metal adsorption was only checked in stem and leaves in zone 1 and data showed higher amount in stem than in leaves, especially for Pb and Sb values. Mean Pb and Sb concentrations amounted to 496.14 and 15.33 mg kg⁻¹ in stem of this plant and decreased to 88.00 and 5.51 mg kg⁻¹ in leaves, which is 82% and 64% less respectively.

As above mentioned, *Dittrichia viscosa* of Asteraceae is a potential plant for phytoremediation. It is a naturally occurring species and generally spread in contaminated areas. For this reason is often

used as an indicator for soil remediation (Cao et al., 2010; Abreu et al., 2012). Lead and Antimony were preferentially accumulated in the stem of *Dittrichia viscosa* from rhizosphere soil of zone 1 and their concentration drastically decreased in *Dittrichia viscosa* of zone 2 probably because metals are still abundant in the waste area and are irregularly spread by overland flow and wind into the downslope (zone 2) and upslope (zone 3) areas. Despite the mechanism of bioaccumulation of most heavy metals by biota is still widely unknown (Schützendübel and Polle, 2002), *Dittrichia viscosa* has been reported to be highly sensitive to Pb and Sb absorption. Murciego Murciego et al. (2007) established that *Dittrichia viscosa* growing in San Antonio antimony mine (Extremadura, Spain) was able to significantly bioaccumulate these elements with respect to other native plants. Likewise, *Dittrichia viscosa* resulted in high bioaccumulation of Pb in contaminated soils of Sierra Minera and Portman Bay surrounding area (Murcia, Spain), as stated by Perez-Sirvent et al. (2008). A significant regression equation ($r = 0.820$, $p < 0.01$) was found when plotting Sb against Pb concentration accumulated by *Dittrichia viscosa* overall the study area (Fig. 4) suggesting that both elements may undergo the same mechanism of absorption in the different parts of this plant either grown in wastes of zone 1 or soils of zone 2 and probably zone 3.

3.5. Biological absorption coefficient (BAC) and translocation factor (TF)

Metal hyperaccumulators are plants with an unusually high ability for metal(oid)s uptake (Pollard et al., 2014). This characteristic is definitely established through the determination of the biological absorption coefficient (BAC), the ratio of the element concentration in the plant to that in soil (Kabata-Pendias, 2001), and the translocation factor (TF), representing the quotient between the element concentration in the roots and that of leaves in a given plant. BAC was calculated for *Dittrichia viscosa*, *Helichrysum stoechas*, *Foeniculum vulgare* and *Vitis vinifera* whereas TF was determined in the first three native plants because roots of *Vitis vinifera* were not analysed for metals amount (Table 4). The phytostabilization and phytoextraction potential of *Helichrysum stoechas* and *Dittrichia viscosa* respectively has been hypothesized elsewhere in this paper, according to the analysed elements values in these plants though data indicated tremendous variability. The total mean concentration of As, Cd, Cu, Ni, Pb, Sb, Sn and Zn in *Dittrichia viscosa* plant from zone 1 was 10.01 ± 6.04 , 18.27 ± 7.21 , 48.27 ± 22.16 , 3.53 ± 1.32 , 900.54 ± 328.33 , 36.09 ± 17.12 , 3.92 ± 2.22 and 56.99 ± 33.78 respectively, warning on the high

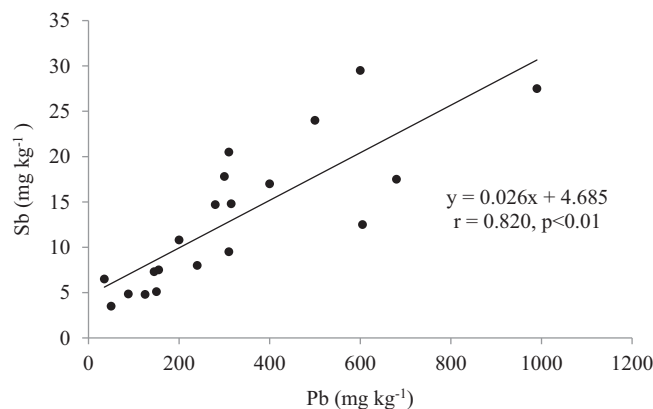


Fig. 4. Linear regression equation depicting the significant correlation between Sb and Pb absorption by different parts of *Dittrichia viscosa* plant.

Table 4

Biological absorption coefficient (BAC) and translocation factor (TF) in the investigated plants in the different sampling zones at the contaminated DemimeSA area.

Element	As	Cd	Cu	Ni	Pb	Sb	Sn	Zn
Zone/Plant	BAC							
Zone 1 - <i>Dittrichia viscosa</i>	0.01	0.27	0.01	0.01	0.01	0.01	0.01	0.02
Zone 2 - <i>Dittrichia viscosa</i>	0.24	3.41	0.81	0.11	0.03	0.07	0.03	0.50
Zone 3 - <i>Dittrichia viscosa</i>	0.07	0.37	1.02	0.09	0.17	1.60	0.16	0.38
Zone 2 - <i>Helichrysum stoechas</i>	0.22	1.14	0.50	0.12	0.06	0.07	0.12	0.47
Zone 3 - <i>Helichrysum stoechas</i>	0.30	0.93	1.26	0.17	1.71	5.19	0.23	0.86
Zone 3 - <i>Foeniculum vulgare</i>	0.15	0.26	0.82	0.13	0.14	2.16	0.36	0.72
Zone 4 - <i>Vitis vinifera</i>	0.22	0.26	0.17	0.37	0.02	0.99	0.98	0.99
	TF							
Zone 1 - <i>Dittrichia viscosa</i>	1.34	0.85	0.75	0.93	0.45	4.37	0.89	1.16
Zone 2 - <i>Dittrichia viscosa</i>	0.61	1.11	0.92	0.93	0.45	0.42	1.18	2.06
Zone 3 - <i>Dittrichia viscosa</i>	0.89	0.59	0.88	0.19	1.13	0.64	0.91	1.35
Zone 2 - <i>Helichrysum stoechas</i>	0.56	0.45	0.43	0.49	0.65	0.58	0.47	0.73
Zone 3 - <i>Helichrysum stoechas</i>	0.44	0.14	0.30	0.24	0.11	0.51	0.71	1.54
Zone 3 - <i>Foeniculum vulgare</i>	5.40	0.56	1.36	4.01	0.91	1.27	2.55	1.12

metal amount that this plant may accumulate in its tissues, independently of extremely low BAC numbers showed in Table 4, representing a low percentage with respect to the total metal concentrations in the wastes of zone 1. The metal hyperaccumulator character of *Dittrichia viscosa* was even clearer when this plant was sampled in zone 2 and analysed for the selected elements (Table 4). Some authors have reported that Cd and Zn were preferentially transported to the aboveground parts of plants and that the bioaccumulation coefficient approached or exceeded the total soil Cd levels (Vogel-Mikuš et al., 2005). The mean Cd concentration in all parts of plants of *Dittrichia viscosa* of zone 1, 2 and 3 was 18.27, 18.85 and 1.12 mg kg⁻¹ respectively, almost exceeding the total Cd concentration in wastes and soils and thus in line with what above mentioned. Moreover, BAC values for *Dittrichia viscosa* of zone 3 were 1.02 and 1.60 for Cu and Sb respectively (Table 4), suggesting that this plant may also be catalogued as hyperaccumulator of these elements. *Helichrysum stoechas* of zone 2 and 3 also showed hyperaccumulation potential with mean concentration of Cd, Pb and Sb of 5.72, 276.45 and 7.47 mg kg⁻¹ and BAC of 1.14, 0.06 and 0.07 from zone 2, and concentration of 2.78, 323.05 and 7.79 mg kg⁻¹ and BAC of 0.93, 1.71 and 5.19 from zone 3 respectively. Once more BAC tends to decrease when the soil is largely and anomaly enriched with Pb and Sb. Particularly for Sb, Pratas et al. (2005) reported Sb values in a range of 0.08–1.74 mg kg⁻¹ for *Helichrysum stoechas* and a BAC value approaching to 5. It was assumed that both *Dittrichia viscosa* and *Helichrysum stoechas* may have a metal(oid)s accumulation threshold and that BAC values are lower or higher depending on the element concentration in the wastes and/or soils. Likewise, *Foeniculum vulgare* and *Vitis Vinifera* showed relatively high BAC values of 2.16 and 0.99 for Antimony (Table 4). Linear regressions were tried by plotting BAC values of *Helichrysum stoechas* towards BAC values of *Dittrichia viscosa* for all metals and metalloids recorded in zone 2 and 3 (Fig. 5). Significant correlation ($r = 0.309$, $p < 0.01$) for zone 2 (Fig. 5A) appointed to a preferential absorption of Cd by both plants whereas Sb would be significantly ($r = 0.869$, $p < 0.01$) more absorbed in zone 3 (Fig. 5B) by the same plants. Besides that, regressions corroborated that BAC may change according to the metal amount in soil and the threshold of absorption capacity of each investigated plant. As reported by Verbruggen et al. (2009), heavy metal tolerance has been developed by plants of totally unrelated taxonomic affinities and it is frequent in Asteraceae like *Dittrichia viscosa* and *Helichrysum stoechas* though the amount of heavy metals taken up by a plant depends mainly on the concentration of heavy metals in the polluted soil and the environmental conditions.

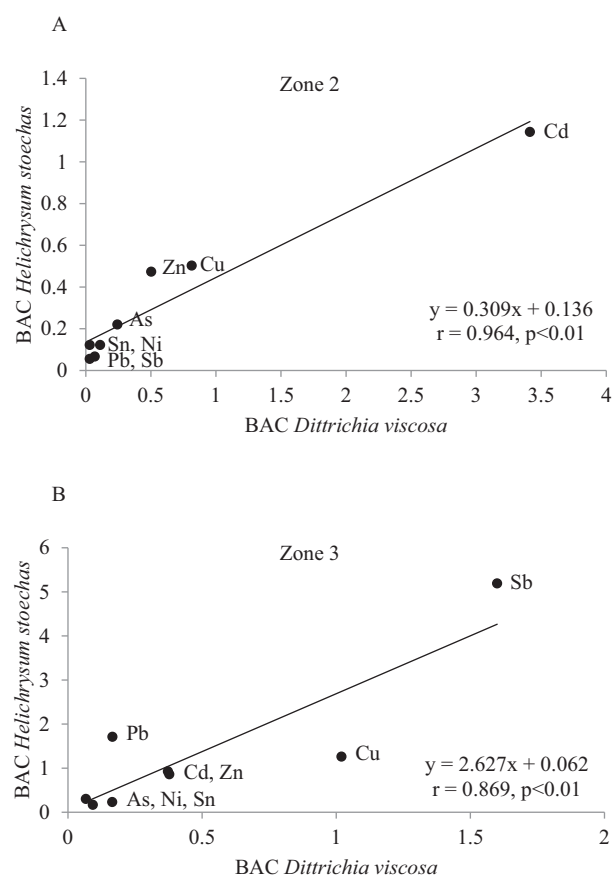


Fig. 5. Linear regression equations and significant correlations between BAC values of *Helichrysum stoechas* and *Dittrichia viscosa* of zone 2 (Fig. 5A) and zone 3 (Fig. 5B) for all the investigated elements.

According to Krämer (2010) environmental conditions may influence the plant absorption dynamics. Metallophytes (plants sensitive to metal absorption) can thus be either excluders of heavy metals or they can be accumulators. Some plants are hyperaccumulators, however, only for a single or few specific heavy metals such as *Thlaspi caerulescens* which can hyperaccumulate Zn and Cd, but not Pb (Robinson et al., 1998; Broadley et al., 2007). Bert et al. (2002) inferred that hyperaccumulators of Pb do not seem to exist (or are rare) due to the fact that Pb is extremely immobile and

is not easily accumulated by plants. Results of Table 4 indicate however that *Helichrysum stoechas* may act as Pb accumulator as it showed a BAC of 1.71 highlighting its potential for phytoremediation of Pb contaminated soils. Of considerable concern was the BAC numbers for *Vitis vinifera* (Table 4) showing values of 0.99, 0.98 and 0.99 for Sb, Sn and Zn respectively, indicating metal absorption into plant tissues. However, no data are available on the content of these elements in grapes or wines.

In most plants, heavy metals are predominantly accumulated in roots. The leaf/root or shoot/root ratio is generally below unity in most plants but not in metallophytes. In some plants, the concentration of heavy metals in shoots and leaves can be particularly high and the partitioning of heavy metals between shoots and roots may differ from one metallophyte to the next and with each individual heavy metal (Krämer, 2010). Data resulting from the calculation of the translocation factor (TF) shown in Table 4 report the leaf/root ratio sometimes may be misleading because the higher concentration of a given element in the aboveground tissues is often found in the shoot or stem. Surprisingly, TF values for *Foeniculum vulgare* largely exceeded the unity and showed a great potential as hyperaccumulator of As, Cu, Ni, Sb, Sn and Zn. Only TF value for Pb was close to unity and for Cd to half unity. There is scanty bibliography on the heavy metals and metalloids accumulation capacity of *Foeniculum vulgare* in natural environments though special attention should be taken as its use is spread as medicinal plant and in many regions it represents a primary source of the health care (Stanojkovic-sebica et al., 2015). Simulation experiments carried out under controlled conditions resulted in high absorption of Cd, Cu, Ni and Pb by *Foeniculum vulgare* (Ziaratin and Hosseini, 2014), but no references were found of heavy metals uptake under natural conditions. Likewise, As and Sb uptake by *Foeniculum vulgare* is not well documented. This plant should therefore be considered for phytoremediation technology as also its BAC values were high for Cu and Zn and especially for Sb (Table 4). The potential for phytostabilization of *Helichrysum stoechas* and phytoextraction of *Dittrichia viscosa* is shown in Fig. 6 where TF values for Pb and Sb are drawn to the ratios stem/root (s/r), leaf/root (l/f) and flower/root (f/r) for the two plants. As it may be observed, TF values of *Helichrysum stoechas* for both Pb and Sb are mainly under the unity, which might reveal its potential for phytostabilization (Fig. 6A and C). By contrast, TF values of *Dittrichia viscosa* (Fig. 6B and D) largely exceeded the unity and might account for the ability of this plant to concentrate Pb and Sb in their aerial parts. Independently on this, both plants progressively decreased Pb and Sb amount from the stem to the leaves and flowers probably indicating some self-regulation. In fact, TF for Pb was 27% and 26% lower in leaf/root and flower/root than in stem/root of *Helichrysum stoechas* of zone 2 (S2), and 69% and 45% lower in *Helichrysum stoechas* of zone 3 (S3). In addition, TF for Pb decreased by 75% from zone 2 to zone 3 (Fig. 6A). A similar trend was recorded in *Dittrichia viscosa* where TF for Pb decreased in the same order by 71% and 36% in zone 1 and by 55% and 65% in zone 2. The average decrease of TF from zone 1 to zone 2 for *Dittrichia viscosa* was 31% (Fig. 6B). Dahmani-Muller et al. (2000) reported similar findings for *Cardaminopsis halleri* when uptaking Zn, Cd, Pb, and Cu in different parts of the plants grown in a metal smelter. However, they also found that concentrations of these metals were 3–8 times greater in brown leaves than in green leaves, suggesting a second strategy, i.e. detoxification mechanism by leaf fall. The decrease of TF for Antimony in *Helichrysum stoechas* and *Dittrichia viscosa* followed a similar pattern strengthening their phytostabilization and phytoextraction role respectively. As both plants are seed resprouters, it was assumed they may use some strategy to avoid high metal concentrations in parts involved in seed production (Fig. 6).

Heavy metal tolerance is common in many plant species and

may not surprise that they use different strategies to cope with the excess of heavy metals. Slomka (2011) reported that *Viola tricolor* secretes metals into hairs of the leaves and stems and discard them when overloaded. The same author stated that *Minuartia verna* has the ability to continuously forming new maiden leaves essentially free of heavy metals. These leaves are used to deposit heavy metals during the vegetation period and then fall off when overloaded with heavy metals. It is therefore evident that plants may use different strategies of autoregulation with respect to heavy metals excess and further studies are needed to ascertain the metal concentration in *Dittrichia viscosa*, *Helichrysum stoechas*, *Foeniculum vulgare* and other plants related with food and beverage production like *Vitis Vinifera* during their growing period.

3.6. Total metal and metalloids concentrations in water samples

Different waters were sampled in the contaminated areas during the sampling campaign. Even though this work did not consider the metal content monitoring in water around the contaminated area, results in Table 5 show that even this ecological compartment may be affected by the dispersion of toxic elements. In fact, Br concentration in pipeline and drainage water resulted considerably higher (16.43 and 15.02 mg L⁻¹) with respect to natural water values of 0.01–0.02 mg L⁻¹ reported in literature (Kabata-Pendias, 2001). On global average, Br content in soil of 1.6 mg kg⁻¹ is considered normal, with exception of volcanic soils or soils affected by evaporitic processes. As above mentioned, average Br values of 0.01 and 0.02 mg L⁻¹ are generally reported in clean natural water. However, according to WHO (WHO, 2004) drinking water should be in a range of 0.01–10 µg L⁻¹ though values up to 293 µg L⁻¹ has been found in water particularly rich of bromide. Enhanced bromide concentrations in stream water occur in southern Portugal over sediments of marine origin, where slow-flowing groundwater with long residence time picks up a high proportion of easily dissolved ions, and climatic conditions favour evapotranspiration, increasing salts in solution. An isolated anomalous value in stream water of north-central Spain (7.90 mg L⁻¹), in the Ebro basin, can be ascribed to evaporitic lithology with marl, clay and gypsum.

Lead also resulted in considerable concentration in the four analysed waters (Table 5) if compared with the values (0.5–100 µg L⁻¹) reported by WHO for drinking water (WHO, 2004). A recent study in Ontario (Canada) found that the average concentration of lead in water actually consumed over a 1-week sampling period was in the range 1.1–30.7 µg L⁻¹, with a median level of 4.8 µg L⁻¹. In the analysed water of DemimeSA area Pb reached maximum values of 2620 µg L⁻¹. Even though may be misleading to compare this water to drinking water, such amount may be taken into account due to the possibility to contaminate adjacent streams and water used for agriculture or potabilization. Sherlock and Quinn (1986) reported high amounts of Pb in waters of Scotland where 40% of the samples exceeded potabilization threshold fixed in 100 µg L⁻¹ (these water were known as plum-bosolvent) but were still 26 times lower than those of the investigated area of this work.

Antimony concentration in the analysed waters was also found to be higher with respect to the EU Directive and normal values found in natural water. Concentrations of antimony in groundwater and surface water normally range from 0.1 to 0.2 µg L⁻¹ (Bowen, 1979). The highest Sb water content documented in natural environments was found in the Xikuangshan antimony mining and smelting area, China (Liu et al., 2010), where Sb values in water in a range of 0.33–11.4 µg L⁻¹ were two to three orders of magnitude higher than the typical concentration of dissolved Sb in unpolluted rivers at that studied area. As above mentioned, the results of Table 5 reported Sb values ranging from 840 to 2060 µg L⁻¹ warning

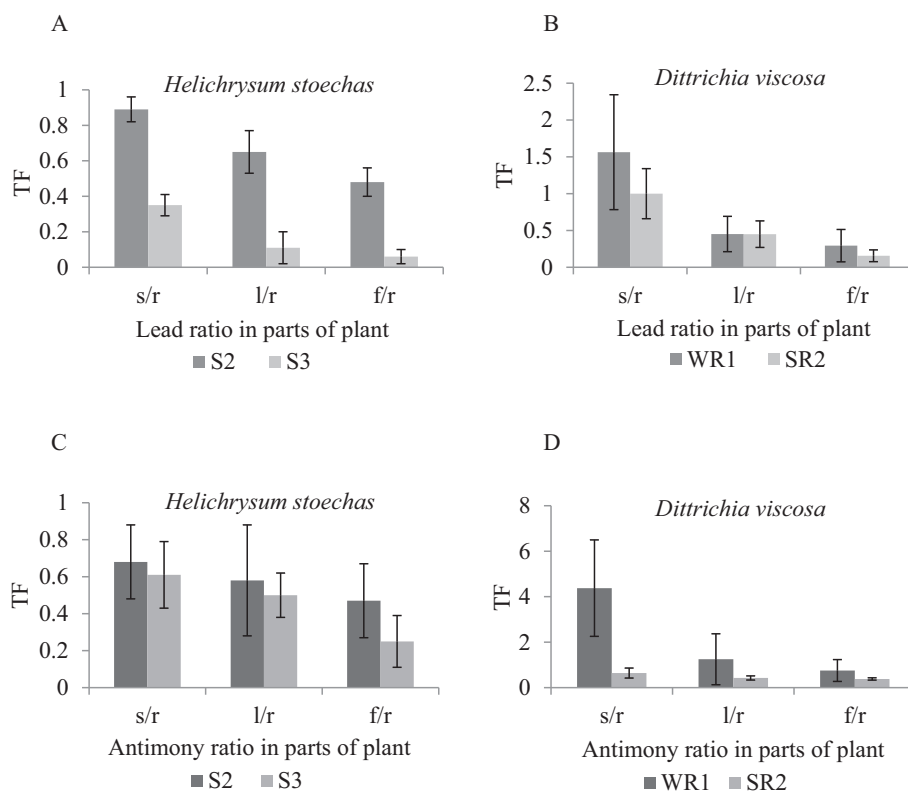


Fig. 6. Translocation factors (TF) values depicting the stem/root (s/r), (Fig. 5B) for all the investigated elements leaf/root (l/r) and flower/root (f/r) ratios for Pb and Sb absorbed by *Helichrysum stoechas* and *Dittrichia viscosa* from samples WR1, SR2, S2 and S3. Values validate the phytostabilization ability of *Helichrysum stoechas* and phyextraction ability of *Dittrichia viscosa*. Columns and bars represent means and standard deviation of three samples.

Table 5

Total metal and metalloids concentration in water samples collected in the contaminated area of DemimeSA. Standard error in parenthesis.

Element	Br	Ca	K	Mn	Ni	Pb	Sb	Se	Sr	Zn
Water	mg L ⁻¹									
Directive ^a	0.01	n.a.	n.a.	0.05	0.02	0.01	0.005	0.01	n.a.	n.a.
Pipeline water	16.43 (1.83)	114.46 (21.35)	16.21 (0.07)	6.66 (0.47)	1.42 (0.32)	2.62 (0.29)	0.84 (0.11)	0.34 (0.09)	1.99 (0.33)	2.75 (0.46)
Drainage water	15.02 (0.19)	119.62 (3.97)	9.27 (1.77)	5.54 (0.67)	0.80 (0.32)	1.78 (0.17)	0.86 (0.58)	0.38 (0.12)	2.31 (0.54)	1.98 (0.25)
Creek water 1	0.19 (0.04)	139.99 (20.22)	5.37 (0.61)	0.62 (0.43)	0.28 (0.01)	0.92 (0.17)	1.93 (0.38)	n.d.	0.46 (0.06)	0.14 (0.07)
Creek water 2	0.23 (0.01)	176.16 (2.06)	5.60 (0.52)	1.20 (0.09)	0.40 (0.11)	1.14 (0.06)	2.06 (0.15)	n.d.	0.55 (0.08)	0.20 (0.04)

n.a.: Not available; n.d.: Not detectable.

^a EU Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.

on the strong impact on ecosystem and emphasizing the importance of water monitoring around the DemimeSA investigated area. As antimony is not likely to occur at significantly higher concentrations in natural waters, except in those areas affected by industrial or mining activity, high attention should be deserved to investigate the diffusion of this element not only into plants but also into animals.

4. Conclusions

The investigations devoted to assess the degree of contamination of wastes, soils, vegetation and water at the DemimeSA area have proved that all the analysed compartments present anomalous content of metals and metalloids which largely exceed the current regulations for different use of soil.

The wastes and soils collected in different zone of the contaminated area resulted strongly enriched with metals and metalloids and its concentrations were very irregularly spread all over the surface and were extended at least 1 km away in any direction. The three plant species selected to check their metals and metalloids absorption capacity responded satisfactorily and made it possible to allocate *Dittrichia viscosa* a phytoextraction potential and *Helichrysum stoechas* a phytostabilization potential for the analysed elements. The biological absorption coefficient (BAC) revealed also a clear preferential absorption of Cd and Sb by both *Dittrichia viscosa* and *Helichrysum stoechas* in zone 2 and 3 respectively. Though their role in the application of light clean-up technology in heavy metals polluted areas were known, data reported in this work are of relevance because of the extended presence of toxic elements in the study area. In addition, *Foeniculum vulgare* was appointed as a

strong phytoextractor for As, Cu, Ni, Pb, Sb, Sn and Zn as showed by translocation factor (TF) values. The potential of this plant, belonging to Apiaceae family, for toxic elements retention has been studied at laboratory scale but its behaviour in natural environments is not well documented and its results may be of reference for similar heavy metal(oid)s polluted sites. The irregularity of metal deposition by rainfall and wind has limited an accurate statistical analysis though results may be of importance because of reporting natural scale values and orienting on the ability of native plants to temporarily cut the diffusion of toxic elements into the surrounding inhabited environment. Further studies should however be undertaken in order to add additional information on the fate of these elements and to program sound remediation.

Acknowledgments

This work has been performed in collaboration with the State Agrarian University of Dnepropetrovsk (Ukraine). The Erasmus+ Program K107 is also acknowledged for allowing Ukranian PhD student mobility and participation in the research activity devoted to the assessment of contamination at the DemimeSA area.

References

- Abreu, M.M., Santos, E.S., Ferreira, M., Magalhaes, M.C.F., 2012. *Cistus salvifolius* a promising species for mine wastes remediation. *J. Geochem. Explor.* 113, 86–93.
- Álvarez-Valero, R., Pérez-López, R., Matos, J., Capitán, M.A., Nieto, J.M., Sáez, R., Delgado, J., Caraballo, M., 2008. Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. *Environ. Geol.* 55, 1797–1809.
- Baker, A.J.M., 1981. Accumulators and excluders—strategies in the response of plants to heavy metals. *J. Plant Nutr.* 3, 643–654.
- Bert, V., Bonnin, I., Saumitou-Laprade, P., De Laguérie, P., Petit, D., 2002. Do Arabidopsis halleri from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? *New Phytol.* 155, 47–57.
- Bitterli, C., Bañuelos, G.S., Schulin, R., 2010. Use of transfer factors to characterize uptake of selenium by plants. *J. Geochem. Explor.* 107, 206–216.
- Bowen, H.J.M., 1979. *Environmental Chemistry of the Elements*. Academic Press, London.
- Bradl, H.B., 2004. Adsorption of heavy metal ions on soils and soils constituents. *J. Colloid Interface Sci.* 277, 1–18.
- Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I., Lux, A., 2007. Zinc in plants. *New Phytol.* 173, 677–702.
- Buscaroli, A., Zannoni, D., Menichetti, M., Dinelli, E., 2016. Assessment of metal accumulation capacity of *Dittrichia viscosa* (L.) Greuter in two different Italian mine areas for contaminated soils remediation. *J. Geochem. Explor.* <https://doi.org/10.1016/j.gexplo.2016.10.001>.
- Cao, A., Carucci, A., Lai, T., Bacchetta, G., Casti, M., 2010. Use of native species and biodegradable chelating agents in the phytoremediation of abandoned mining areas. In: 4th European Bioremediation Conference, ID001-Pp 1–4, Cagliari, Italy.
- Conesa, H.M., Maria-Cervantes, A., Álvarez-Rogel, J., González-Alcaraz, M.N., 2011. Influence of soil properties on trace element availability and plant accumulation in a Mediterranean salt marsh polluted by mining wastes: implications for phytomanagement. *Sci. Total Environ.* 409, 4470–4479.
- Cristina, A., 2015. Applicazione di tecniche di fitorimediazione per la stabilizzazione/estrazione di Cd, Pb, Zn dai suoli dell'ex area mineraria "Les Mines d'Osor" in Spagna. Tesi di laurea Università degli Studi di Napoli "Federico II" Corso di Laurea per l'Ambiente e il Territorio, Napoli, p. 151.
- Dahmani-Muller, H., van Oort, F., Gélie, B., Balabane, M., 2000. Strategies of heavy metal uptake by three plant species growing in a metal smelter. *Environ. Pollut.* 109, 231–238.
- Forster, J., 1995. Soil sampling, handling, storage and analysis. In: Alef, K., Nannipieri, P. (Eds.), *Methods in Soil Microbiology and Biochemistry*. Academic Press, London, pp. 49–122.
- Gäbler, H.-E., 1997. Mobility of heavy metals as a function of pH of samples from an overbank sediment profile contaminated by mining activity. *J. Geochem. Explor.* 58, 185–194.
- Gangolli, S., 1999. *The Dictionary of Substances and Their Effects*. Royal Society of Chemistry, London.
- Jimenez, A., Bacchetta, G., Casti, M., Navarro, F.B., Lallena, A.M., Fernandez-Ondono, E., 2011. Potential use in phytoremediation of three plant species growing on contaminated mine-tailing soils in Sardinia. *Ecol. Eng.* 37, 392–398.
- Kabata-Pendias, A., 2001. *Trace Elements in Soils and Plants*, third ed. CRC Press, Boca Raton.
- Krämer, U., 2010. Metal hyperaccumulation in plants. *Annu. Rev. Plant Biol.* 61, 517–534.
- Liu, F., Le, C., McKnight-Whitford, A., Xia, Y., Wu, F., Elswick, E., Johnson, C.C., Zhu, C., 2010. Antimony speciation and contamination of waters in the Xikuangshan antimony mining and smelting area, China. *Environ. Geochem. Health* 32, 401–413.
- Marchiola, L., Fellet, G., Boscuttia, F., Montella, C., Mozzi, R., 2013. Gentle remediation at the former "Pertusola Sud" zinc smelter: evaluation of native species for phytoremediation purposes. *Ecol. Eng.* 53, 343–353.
- Marguí, E., Iglesias, M., Camps, F., Sala, L., Hidalgo, M., 2016. Long-term use of biosolids as organic fertilizers in agricultural soils: potentially toxic elements occurrence and mobility. *Environ. Sci. Pollut. Control Ser.* 23, 4454–4464.
- Morachevskii, A.G., 2014. Physicochemical studies of utilization of lead batteries. *Russ. J. Appl. Chem.* 87, 241–257.
- Murciego Murciego, A., García Sánchez, A., Rodríguez González, M.A., Pinilla Gil, E., Toro Gordillo, C., Cabezas Fernández, J., Buyolo Triguero, T., 2007. Antimony distribution and mobility in topsoils and plants (*Cytisus striatus*, *Cistus ladanifer* and *Dittrichia viscosa*) from polluted Sb-mining areas in Extremadura (Spain). *Environ. Pollut.* 145, 15–21.
- Parera, R., Safont, F., 2016. Caracterització edafoclimàtica d'una finca agrícola situada en San Llorenç d'Hortons (Alt Penedès). T.F.G. Universitat Politècnica de Catalunya. Escola Superior d'Agricultura de Barcelona, Barcelona, p. 80.
- Peijnenburg, W.J.G.M., Jager, T., 2003. Monitoring approaches to assess bio-accessibility and bioavailability of metals: matrix issues. *Ecotoxicol. Environ. Saf.* 56, 63–77.
- Pérez de Mora, A., Ortega-Calvo, J.J., Cabrera, F., Madejón, E., 2005. Changes in enzyme activities and microbial biomass after "in situ" remediation of heavy metal-contaminated soil. *Appl. Soil Ecol.* 28, 125–137.
- Perez-Sirvent, C., Martinez-Sanchez, M.J., Garcia-Lorenzo, M.L., Bech, J., 2008. Uptake of Cd and Pb by natural vegetation in soils polluted by mining activities. *Fresenius Environ. Bull.* 17, 1666–1671.
- Petruzzelli, G., Szymura, I., Lubrano, L., Pezzarossa, B., 2008. Chemical speciation of heavy metals in different size fractions of compost from solid urban wastes. *Environ. Technol. Lett.* 10, 521–526.
- Pollard, A.J., Reeves, R.D., Baker, A.J.M., 2014. Facultative hyperaccumulation of heavy metals and metalloids. *Plant Sci.* 217, 8–17.
- 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. *J. Geochem. Explor.* 85, 99–107.
- Rhoades, J.D., Manteght, N.A., Shouse, P.J., Alves, W.J., 1989. Soil electrical conductivity and soil salinity: new formulations and calibrations. *Soil Sci. Soc. Am. J.* 53, 433–439.
- Robinson, B.H., Leblanc, M., Brooks, R.R., Kirkman, J.H., Gregg, P.E.H., 1998. The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. *Plant Soil* 203, 47–56.
- Schnitzer, M., 1991. Soil organic matter. The next 75 years. *Soil Sci.* 151, 41–58.
- Schützendübel, A., Polle, A., 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *J. Exp. Bot.* 53, 1351–1365.
- Sherlock, J.C., Quinn, M.J., 1986. Relationship between blood lead concentrations and dietary lead intake in infants: the Glasgow duplicate diet study 1979–1980. *Food Addit. Contam.* 3, 167–176.
- Slomka, A., 2011. Violets of the section *Melanium*, their colonization by arbuscular mycorrhizal fungi and their occurrence on heavy metal heaps. *J. Plant Physiol.* 168, 1191–1199.
- Sonmez, M.S., Kumar, R.V., 2008. Leaching of waste battery paste components. Part 2: leaching and desulphurisation of PbSO₄ by citric acid and sodium citrate solution. *Hydrometallurgy* 95, 82–86.
- Stanojkovic-sebica, A., Pivica, R., Josica, D., Dinica, D., Stanojkovic, A., 2015. Heavy metals content in selected medicinal plants commonly used as components for herbal formulations. *J. Agric. Sci.* 21, 317–325.
- Sylvain, B., Motelica-Heino, M., Miard, F., Joussein, E., Soubrand, M., Bourgerie, S., Morabito, D., 2016. Phytostabilization of As, Sb and Pb by two willow species (*S. viminalis* and *S. purpurea*) on former mine technosols. *Catena* 136, 44–52.
- Unterbrunner, R., Puschenreiter, M., Sommer, P., Wieshammer, G., Tlustoš, P., Zupan, M., Wenzel, W.W., 2007. Heavy metal accumulation in trees growing on contaminated sites in Central Europe. *Environ. Pollut.* 148, 107–114.
- Verbruggen, N., Hermans, C., Schat, H., 2009. Molecular mechanisms of metal hyperaccumulation in plants. *New Phytol.* 181, 759–776.
- Violante, A., Cozzolino, V., Perelomov, L., Caporale, A.G., Pigna, M., 2010. Mobility and bioavailability of heavy metals and metalloids in soil environments. *J. Soil Sci. Plant Nutr.* 10, 268–292.
- Vogel-Mikuš, K., Drobné, D., Regvar, M., 2005. Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. *Environ. Pollut.* 2, 233–242.
- WHO, 2004. *Guidelines for Drinking Water Quality*, fourth ed. World Health Organization, Geneva.
- Ziaratin, P., Hosseini, A., 2014. The effect of fennel (*Foeniculum vulgare*) on potential phytoremediation of Pelargonium. *Int. J. Plant, Anim. Environ. Sci.* 4, 439–445.