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Science of the Total Environment

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## Accumulation of heavy metals in phytoliths from reeds growing on mining environments in Southern Europe

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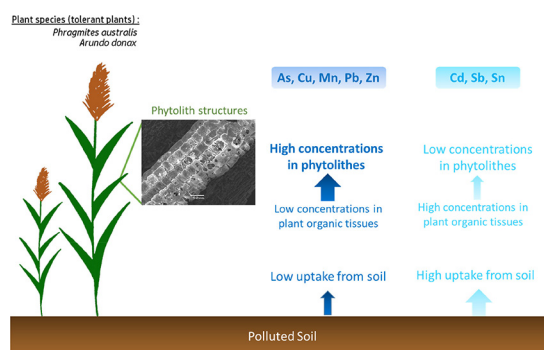
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### HIGHLIGHTS

- Reeds are tolerant to high metal concentrations in soils.
- As, Cu, Mn, Pb and Zn are preferentially bioaccumulated in phytoliths.
- Cd, Sb and Sn are concentrated in reeds' organic tissues in mining areas.
- Insights are suggested for further efficient phytoremediation processes.
- Phytolith composition could become a paleo environmental proxy of past activities.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 25 September 2019

Received in revised form 15 November 2019

Accepted 16 November 2019

Available online xxxx

Editor: Filip M.G. Tack

#### Keywords:

Phytoliths

Heavy metals (HM)

Bioaccumulation

Environment contamination

### ABSTRACT

In Southern Europe, soil contamination by heavy metals (HM) due to mining and industrial activities is a long-known problem. Yet, despite soils being widely contaminated through decades, some plants are still able to grow. Some of these plants, like giant reed (*Arundo donax*) or common reed (*Phragmites australis*) are capable of accumulating substantial amount of HM. These reeds also contain small silica structures in their shoots, called phytoliths. However, the role of phytoliths in reducing stress caused by these HM remains unknown. The aim of this work is then to determine if phytoliths represent a preferential structure for the bioaccumulation of HM in plants. Therefore, plants from mining-contaminated sites in Spain and France were sampled and HM concentrations in total plant shoots were compared to those in phytoliths for eight metal(oid)s: As, Cd, Cu, Mn, Pb, Sb, Sn and Zn. Results show that *Arundo donax* and *Phragmites australis* tend to accumulate Cd, Sb and Sn but limit the uptake of As, Cu, Mn, Pb and Zn in plant shoots despite that the concentration of these HM in soil is quite high. Therefore, reeds appear as tolerant to high metal concentrations in soils, and phytoliths are identified as preferential structures for encapsulation of As, Cu, Mn, Pb and Zn, while Cd, Sb and Sn were mainly found to be accumulated in organic tissues rather than in phytoliths.

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## 1. Introduction

Silicon is the second most abundant element in Earth's crust; thus it is involved in many chemical reactions (Anderson, 1983). Whereas its role in inorganic reactions has been known for a long time, and only over the past two decades the importance of the biogenic cycle of silicon in terrestrial environments has been recognized (Conley, 2002; Derry et al., 2005; Meunier et al., 1999). Further, Si is known for being able to alleviate metal stress in plants in many ways (Adrees et al., 2015; Rizwan et al., 2016). This element can immobilize heavy metals in the growth environment (by precipitation or chelation) (Kidd et al., 2001; Liang et al., 2007; Ma et al., 1997; Neumann et al., 1997); increase the plant's antioxidant defense system (Ali et al., 2015; Farooq et al., 2013); modify the expressed genes (Kim et al., 2014; Li et al., 2014) or alter the plant's structure such as root length (Keller et al., 2015; Vaculík et al., 2009).

From soil solutions, dissolved silicic acid can precipitate in the aerial tissues of some plants as micrometric opal particles called phytoliths (Piperno, 1987, 2006) which can present a wide range of different forms (Conley, 2002; Liu et al., 2013; Shakoob et al., 2016). While the role of phytoliths in the cycling of major elements like Si (but also C and nutrients) has been recognized (Bartoli, 1981, 1983; Piperno, 1987), their contribution to heavy metals (HM) bioaccumulation in plant tissues remains unclear.

However, as HM are nowadays of high interest in environment concerns, the potential role of phytoliths in HM sequestration and immobilization in plants has to be better investigated. Indeed, since the industrial revolution, anthropogenic activities have led to the emission and diffusion of HM in different compartments of the environment (Duffus, 2002; Nriagu and Pacyna, 1988). These HM are able to enter the food chain through contaminated soils, water sources and air deposits (Fytianos et al., 2001). They cannot be chemically or biologically degraded, and therefore represent a threat to soils because they may prevent them from delivering their ecosystem services (Madejón et al., 2018; Rodríguez Martín et al., 2014). Among the HM, the most dangerous are As, Cd, Cr, Hg and Pb because of their extensive use, toxicity and widespread distribution in the environment (Baird and Cann, 2012). Others also present a risk for environment and human health such as Cu (Tokar et al., 2013), Mn (Emsley, 2011) or Zn (Eisler, 1993).

In Southern Europe, one of HM main source in soil is the mining industry, which is expected to grow in the coming years (Elshkaki et al., 2016; Patiño Douce, 2016) due to energy transition increasing demand for primary resources. Actually, HM can be largely accumulated in tailings (from mining wastes), which are highly vulnerable to water and wind erosion, as reported by Conesa and Faz (2011) in their study concerning the Cartagena-La Unión mining district (Murcia region, SE Spain), one of the most notable sites of geochemical pollution and geotechnical instability in Spain's abandoned mining heritage. The same results were reported by Munoz et al. (2003) in Saint-Salvy's mine and by Khaska et al. (2015) in Salsigne's mine, both in south of France. The surface water running through mining sites are strongly enriched in heavy metals, as reported by Viers et al. (2018) in the Huelva province, in Spain. Therefore, HM can move through soils, join surface water systems and then be transferred to plants during mineral nutrition.

Despite that the mining sites listed above are heavily contaminated, some plants are still able to grow in these HM-enriched environments. For example, in the Cartagena-La Unión mining district, several studies reported the presence of plants presenting relative tolerance to high HM concentrations, and reaching 30,000, 10,000 and 1100 mg kg<sup>-1</sup> for Pb, Zn and As, respectively (Álvarez-Rogel et al., 2004; Conesa et al., 2006; García et al., 2003; Schreck et al., 2019). Among these plants we find reeds, such as *Phragmites australis* (common reed) or *Arundo donax* (giant reed). These two species are well known for their capacity to produce phytoliths (Liu et al., 2013; Ramsey et al., 2016; Shakoob et al., 2016) and therefore can be used as model species to reveal the phytoliths' capacity to accumulate HM.

To our knowledge, elemental composition of phytoliths is poorly documented in the literature. Although phytoliths are often considered as a "trap" for organic matter and nutrients e.g. K, P and Fe (Li et al., 2013; Parr and Sullivan, 2005; Nguyen et al., 2015; Song et al., 2012; Song et al., 2015), few data concern their capacity to accumulate the HM. Buján (2013) studied several Ericaceae species and suggested that phytoliths can alleviate metal stress in silicon-enriched plants, in relation to geochemical conditions of the area. Recently, two studies performed in Viet Nam (Nguyen et al., 2019; Tran et al., 2019) highlighted that rice phytoliths can encapsulate the lead and thus become possible pollutant source in paddy soils, whereas grass phytoliths growing in area of Cu-fungicides application can store sizeable amount of Cu. In the latter case, Cu is encapsulated within the silica body of the phytolith (Tran et al., 2019).

The aim of the present work is to better quantify the role of phytoliths in HM transfer through the soil-plant continuum and HM bioaccumulation in highly contaminated environments. For this, we analyzed HM concentrations in phytoliths from two species of reeds growing in 4 sites in France and Spain, highly contaminated due to mining-extraction activities. The concentrations of HM from phytoliths and plants growing in contaminated sites were compared to those from non-contaminated sites. Enrichment factors were then calculated to determine if HM are preferentially concentrated in phytolith structures relative to the plant shoots and soils. Finally, the role of phytoliths in HM recycling and release during organic matter degradation in the litter horizon is discussed, as their contribution in HM mobility during organic matter degradation in the litter horizon remains unclear.

## 2. Materials and methods

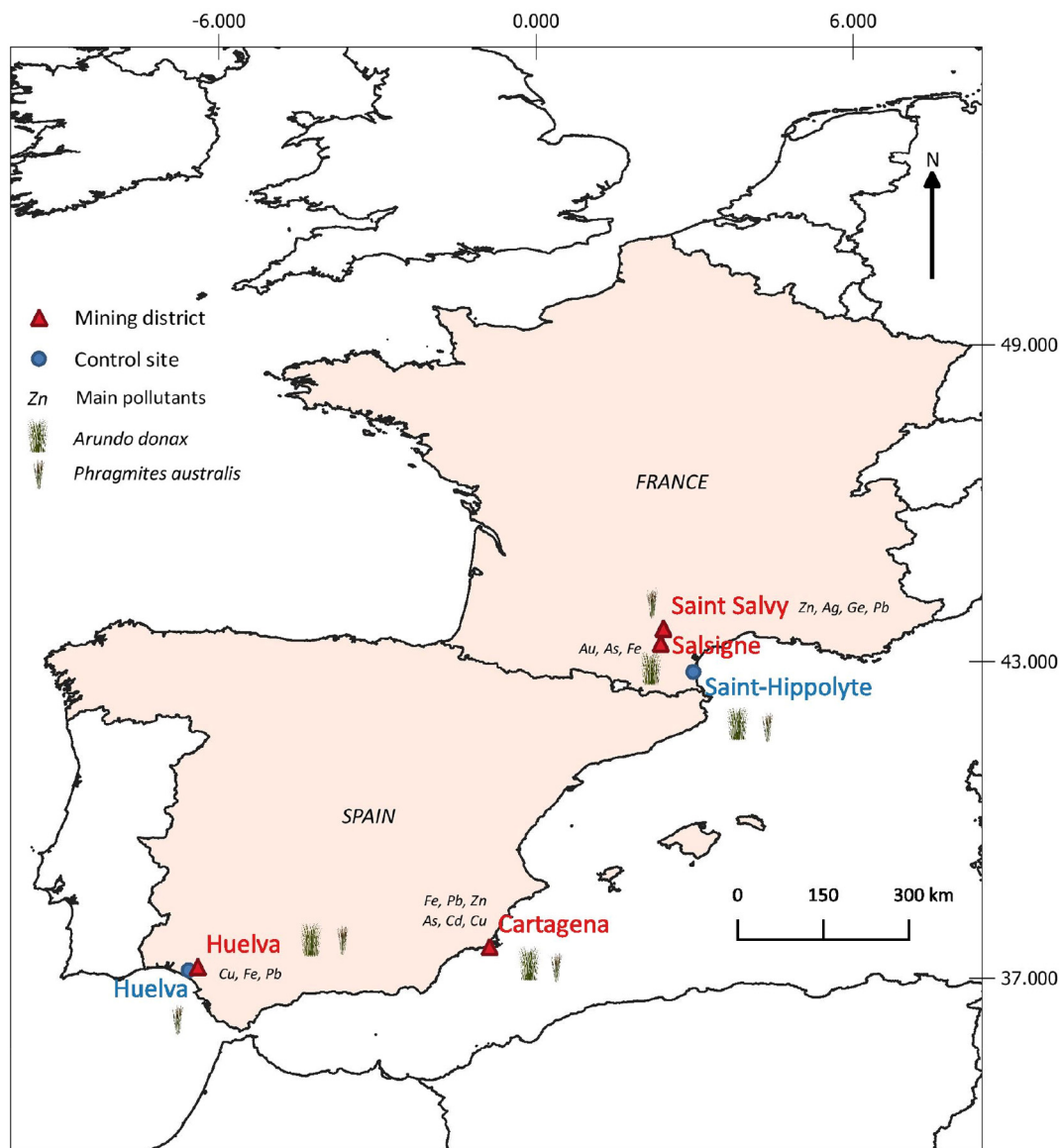
### 2.1. Study area and sampling strategy

Four polluted sites from mining districts were studied: two in Spain and two in France. One control site was also chosen in France, and one control zone unimpacted by mining activities in Spain (Fig. 1). A total of 11 plant samples were taken along with their associated soils (Table 1). Two plant species of reed were studied (*Phragmites Australis* and *Arundo Donax*) and collected on each site depending of their natural abundance.

**Cartagena-La Unión (Spain)** is located in the southeast of the Iberian Peninsula. It corresponds to a mining district covering an area of 50 km<sup>2</sup>. The district has been an important mining area for >2000 years, and ceased its activity in 1991 (Conesa et al., 2006; Rodríguez Martín et al., 2014). The main ore deposits contain iron, lead and zinc as major metal components (Oen et al., 1975) and are distributed between 48 tailings dumpsites covering 160 km<sup>2</sup> (Orozco et al., 1993; Ortega et al., 1993). These mine tailings contain high concentrations of HM, such as Pb (>30,000 mg kg<sup>-1</sup>), Zn (about 10,000 mg kg<sup>-1</sup>) and As (about 1000 mg kg<sup>-1</sup>) as reported by Conesa et al. (2006) but also Schreck et al. (2019). In this area, some experimental zones (Santa Antonieta and Las Matildes - in which soil and plant's samplings were performed) were specifically treated by addition of organic amendments in order to test the efficiency of various remediation procedures.

**Huelva province (Spain)** is located in the Iberian Pyritic Belt, in SW Spain. The region is one of the most important in Europe in regard to the mining of pyrites and sulfides of metals (Cu, Fe, Pb) (Martín et al., 1998). The extraction of pyrites began during the Roman period and reached a peak in the middle of the 19th century. Finally, a chemical industry complex was installed at the Odiel's estuary in the middle of 1960s, including factories of fertilization and Cu extraction (Martín et al., 1998). Sampled sites were located about 20 km south of the mines. Some samples of soil and reeds (*Phragmites australis*) were also taken in non-impacted zone (Cartaya), as a control site for Spanish sampling.

**Salsigne (France)** is located in the southern foothills of the Montagne Noire in Southern France. The mine deposits are mostly



**Fig. 1.** Location of the studied sites in Southern Europe. The different sites are represented by a red triangle for mining sites and a blue dot for control sites. Main elements (metalloids) present in soils according to the type of ores exploited are also reported for each polluted area. Two species of reeds were collected at different sites shown by corresponding symbols. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

composed of Au, As and Fe (Khaska et al., 2015). The mining activity began in the nineteenth century for Fe, then in 1908 Au and As were exploited. The open pit and mine were closed in 1998 and 2004 respectively (Khaska et al., 2015). According to the same author, several pools of As contamination of stream waters, alluvial aquifers and vegetables were detected in the Orbiel Valley, the Orbiel being the river flowing near the mining site, in a waste storage zone.

**Saint-Salvy de la Balme (France)** is a mining-district located at the southwestern end of the Massif Central, in France. The ore was composed of sphalerite and galena, and 450,000 tons of Zn, Ag, Ge and Pb were produced between 1975 and 1994, along with 4 million tons of waste (Munoz et al., 2003). After the mine was closed, dumps (covering 2 ha and 40 m thick) were covered with clayey material and sowed with grass.

**Saint-Hippolyte (France)** was chosen as a control site for French sampling, it hosts a 7 ha wetland (La Sagnette), protected since 2012 and belonging to the *Natura 2000* network. Soils are mostly made of sand and silt from dredging (Syndicat RIVAGE, 2013).

## 2.2. Soils characterization

Different physico-chemical parameters were assessed: pH, soil texture, organic matter (OM) content estimate and cationic exchange capacity (CEC).

Soil pH was determined via suspending 5 g of soil in 25 mL of Milli-Q ultrapure water, after agitation and using a pH-meter (*inoLab® pH 7110* by WTW). A qualitative determination of the particle sizes expressed as soil texture was performed in the field and using the triangular diagram of the basic soil textural classes according to USDA particle sizes. Organic matter content was estimated directly in the field, and linked to soil management and agricultural or environmental practices. Finally, the Cation Exchange Capacity (CEC) was measured using the cobaltihexamine method. This method was chosen for its efficiency to measure CEC at soil pH (Aran et al., 2008). About 40 mL of a cobaltihexamine solution ( $0.0193 \text{ mol} \cdot \text{L}^{-1}$ ) were added to 2 g of soil in a polypropylene tube. After agitation (60 min at 350 rpm), the solution was filtered ( $0.20 \mu\text{m}$ ) and the concentration of cobaltihexamine was determined by spectrophotometry (*Varian's Cary® 50*).

**Table 1**  
Plant and soil samples description.

Type of site	Location	Type	Species	ID	Coordinates (WGS 84, decimal)		Site
					Latitude	Longitude	
Polluted sites	Cartagena – La Unión – Spain	Plant	<i>Phragmites australis</i>	CRT-2	37.59434	−0.88193	Avenque Tailing
		Plant	<i>Arundo donax</i>	CRT-5	37.63155	−0.85485	Las Matildes (rehabilitated site, agricultural zone)
		Plant	<i>Arundo donax</i>	CRT-7	37.58003	−0.87684	Playa de Gorguel (downstream of Avenque)
		Plant	<i>Phragmites australis</i>	CRT-9	37.59415	−0.88610	Santa Antonieta (rehabilitated site)
		Soil	–	CRT-A	37.59434	−0.88193	Avenque Tailing
		Soil	–	CRT-B	37.63155	−0.85485	Las Matildes (rehabilitated site, agricultural zone)
		Soil	–	CRT-C	37.58003	−0.87684	Playa de Gorguel (Downstream of Avenque)
	Huelva – Spain	Soil	–	CRT-D	37.59415	−0.88610	Santa Antonieta (rehabilitated site)
		Plant	<i>Arundo donax</i>	HUE-1	37.215397	−6.402272	Riverbed of the Tinto river
		Plant	<i>Phragmites australis</i>	HUE-4	37.223297	−6.583839	Riverbed of the Odriel river
		Soil	–	HUE-A	37.215397	−6.402272	Riverbed of the Tinto river
		Soil	–	HUE-C	37.223297	−6.583839	Riverbed of the Odriel river
	Salsigne – France	Plant	<i>Arundo donax</i>	SAL-1	43.317764	2.388060	Riverbed of the Orbiel river/Pont du Limousis
		Soil	–	SAL-A	43.317764	2.388060	Riverbed of the Orbiel river/Pont du Limousis
Saint-Salvy de la Balme – France	Plant	<i>Phragmites australis</i>	SVY-1	43.625362	2.408043	Waste tailing	
	Soil	–	SVY-A	43.625362	2.408043	Waste tailing	
	Soil	–	HUE-3	37.19954	−7.43348	Cartaya (unimpacted by mining activities)	
Control sites	Huelva – Spain	Soil	–	HUE-B	37.19954	−7.43348	Cartaya (unimpacted by mining activities)
		Plant	<i>Phragmites australis</i>	HIP-1	42.802915	2.973004	Near Sals' lagoon
	St Hippolyte – France	Plant	<i>Arundo donax</i>	HIP-2	42.802915	2.973004	Near Sals' lagoon
		Soil	–	HIP-A	42.802915	2.973004	Near Sals' lagoon

### 2.3. Extraction of phytoliths from plant shoots

Sufficient amounts of phytoliths were extracted from plant material by dry ashing (Parr et al., 2001a, 2001b) which allows complete removal of organic matter. First, 10–20 g of plant material was rinsed with Milli-Q ultrapure water; dried in an oven at 50 °C and cut into 1–2 cm pieces. Then, the material was heated in a porcelain crucible at 700 °C for 12 h to remove organic matter by combustion. The ashes were then removed from crucibles and transferred to polypropylene tubes (0.2 g per tube). Then 10 mL of 10% HCl were added to each tube; which were put in a water bath at 70 °C for 20 min. After that, tubes were centrifuged at 4500 rpm for 5 min, and the supernatant is disposed. The remaining material was then rinsed with Milli-Q ultrapure water and centrifuged at 4500 rpm for 5 min, and the supernatant was discarded. Next, 10 mL of 15% H<sub>2</sub>O<sub>2</sub> was put into each tube and heated in water bath at 70 °C for 20 min. After that, tubes were centrifuged at 4500 rpm for 5 min, and the supernatant was discarded. Finally, the remaining material was rinsed with Milli-Q ultrapure water via repeated centrifugation at 4500 rpm during 5 min, and the supernatant was discarded. Tubes with remaining sediment were then put in an oven and dried at 50 °C.

Note that dry ashing of plant biomass for the phytolith extraction was proven to be the most efficient technique for obtaining organic-free phytoliths of bamboo (Frayse et al., 2006b) but also horsetail, larch, elm and fern (Frayse et al., 2009). In these works, we demonstrated that the use of dry ashing following by through acid wash removed the totality of organic matter linked. This conclusion was based on combination of state-of-the-art spectroscopic techniques such as the differential thermal gravimetry (DTG) analysis which should that extracted exhibited the same weight loss as amorphous silica which corresponds to the removal of non-structural water but no traces of organic matter. Similarly, the diffuse reflectance infrared Fourier transformed (DRIFT) spectroscopy evidenced full disappearance of the bands of aliphatic carbon groups after thermal treatment (Frayse et al., 2006a, 2006b). In the present study, we did not detect any traces of organic carbon within the phytoliths using ESM EDS analysis (see below) while our previous analyses of grass phytoliths obtained by this technique did not reveal any organic C within the analytical resolution (0.1%) of the C-S Horiba analyzer Alisson. Finally, thorough methodological work of Parr et al. (2001a, 2001b) demonstrated that phytoliths pattern dimensions and curvatures obtained via dry (500 °C) and wet (boiling) extractions are statistically identical and

there is no detectable evidence of morphological impact of these methods.

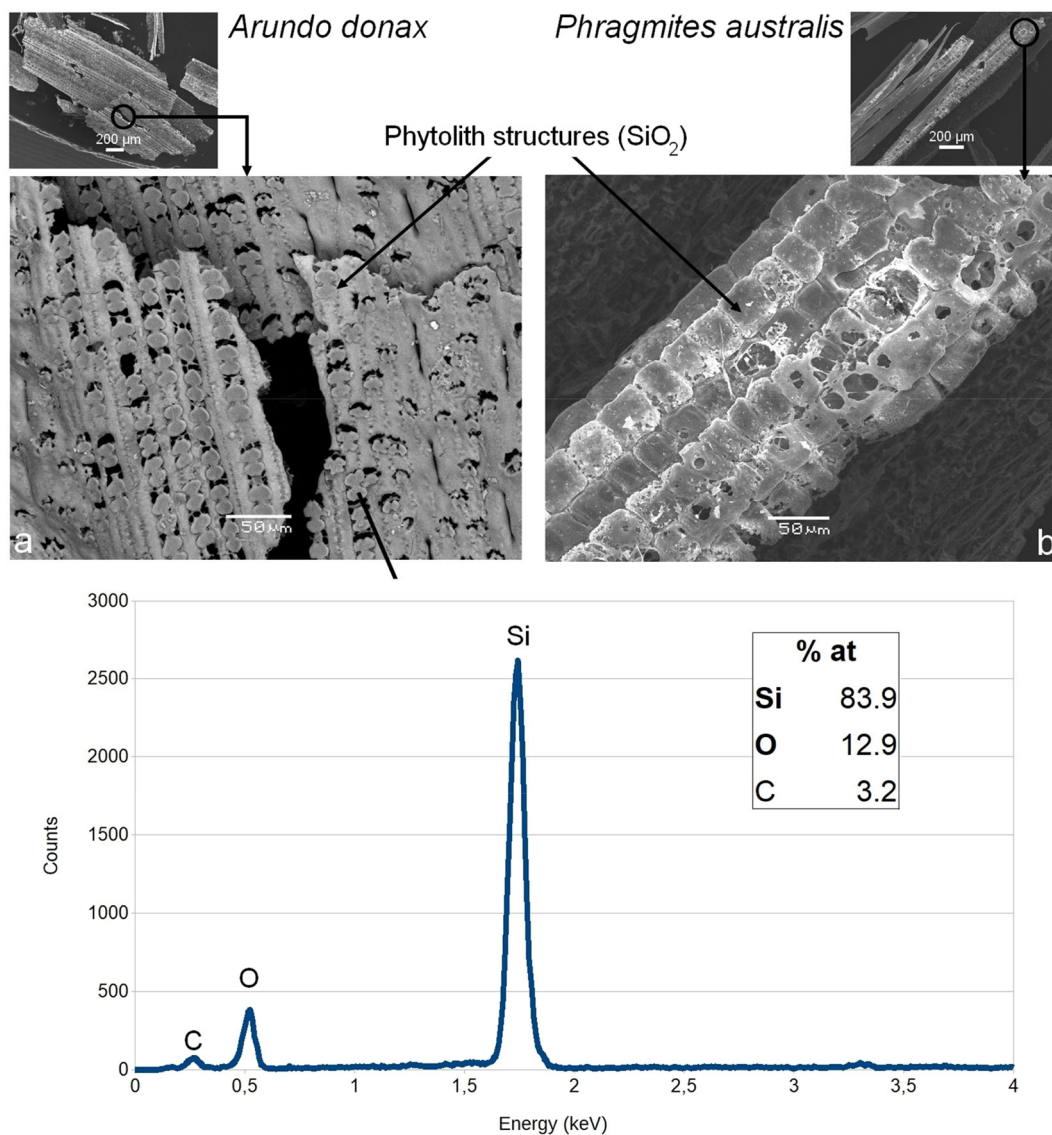
### 2.4. SEM-EDX observations

Phytoliths from both *Arundo donax* and *Phragmites australis* were observed using a SEM-EDX (Fig. 2), to assess the efficiency of the extraction step by checking the chemical composition of the extracted tissues, as well as to explore the morphology of the phytolith structures, as previously reported by various authors (Shakoor et al., 2016; Nguyen et al., 2019; Tran et al., 2019). Environmental SEM observations were performed using a Jeol JSM 6360LV (Jeol®) instrument equipped with EDX Detector to investigate the efficiency of the extraction step, the phytolith morphology, and potential elemental distribution on the plant surface. Before observation analysis, the different phytolith samples were dried and fixed on a carbon substrate without any further preparation before analysis. The apparatus was operated in low-vacuum mode (~133 Pa) at 20 kV.

### 2.5. Elemental concentration determination

The acid digestion of shoot samples (100 mg) and phytoliths (around 70 mg) involved 0.2 mL of Suprapur HF, 8 mL of bi-distilled 15 N HNO<sub>3</sub>, and 1 mL of H<sub>2</sub>O<sub>2</sub>, added together in closed Teflon vials in a class 10,000 clean room in the GET laboratory (Géosciences Environnement Toulouse, France). Acid digestion was performed in CEM® Discover microwave. Blank tests indicated that the level of contamination induced by the acid digestion procedure was negligible, <2% of the sample element of interest. Samples were then let to evaporate to dryness, and then diluted in 15 mL of 2% HNO<sub>3</sub>. They were diluted again by a factor of 2000. Indium was used for an internal calibration. The certified reference materiel “Apple leaves” (NIST® SRM® 1515) was used to check the validity and reproducibility of each analysis. The same procedure was applied for soil samples, using “San Joaquin soil” (NIST® SRM® 2709a) as certified reference material. Note that wet digestion using a HF attack in a microwave is the most efficient method of full digestion of both mineral and organic matrices for TE analyses, as demonstrated in former studies of peat soils, vegetation and river suspended matter (Stepanova et al., 2015; Viers et al., 2013; Krickov et al., 2020). In this regard, we used HF attack with evaporation step to remove all Si prior trace metal analysis for consistency with





**Fig. 2.** SEM-EDX observations of phytolith structures. a. Phytolith assemblage from *Arundo donax* (HUE-1 sample), BSE image (backscatter electron). The associated spectrum shows the composition of a phytolith. b. Phytolith assemblage from *Phragmites australis* (SVY sample), SEI image (secondary electron image). The composition of phytoliths from *Arundo donax* and *Phragmites australis* are similar.

majority of previous analyses of plant material. However, there are alternative techniques such as 1%  $\text{Na}_2\text{CO}_3$  leaching following by  $\text{HNO}_3$  digestion that also allows efficient extraction of metals occluded within plant phytoliths (i.e., Tran et al., 2019; Nguyen et al., 2019).

Concentrations of HM were obtained by measurement on a quadrupole ICP-MS (Agilent 7500®) in the GET laboratory. Quantification limits are of 2 ppb for Cu and inferior to 0.2 ppb for all other elements (As, Cd, Mn, Pb, Sb, Sn and Zn). Uncertainties for soils analysis ranged from 1 to 10% for As, Cu, Mn, Pb, Sb and Zn, and from 1 to 20% for Cd and Sn. For plants and phytoliths analysis, uncertainties ranged from 0.1 to 5% for all elements. The agreement of certified sample analyses with recommended concentrations was better than 78% for considered elements.

Finally, in order to better assess the bioaccumulation potential of plants, via HM uptake by roots from soil, enrichment factors ( $EF_{\text{plant}}$ ) were calculated as the ratio between HM concentrations in plant shoots and HM concentrations in soil:

$$EF_{\text{plant}} = \frac{[HM]_{\text{plantshoots}}}{[HM]_{\text{soil}}}$$

where [HM] corresponds to the concentration of HM in the respective reservoirs. Values of  $EF_{\text{plant}} > 1$  suggest a significant bioaccumulation of HM in plant shoots.

Moreover, another enrichment factor ( $EF_{\text{phytoliths}}$ ) was calculated to estimate the importance of the partitioning of HM between the whole plants and their phytolith's structures:

$$EF_{\text{phytoliths}} = \frac{[HM]_{\text{phytoliths}}}{[HM]_{\text{plantshoots}}}$$

where [HM] corresponds to the concentration of HM in the respective reservoirs. Values of  $EF_{\text{phytoliths}} > 1$  suggest a significant bioaccumulation of HM in phytoliths.

## 2.6. Statistical analyses

Statistical treatment allowed rigorous comparison of metal concentrations in the whole plant and the phytoliths. This included a non-parametric Kruskal-Wallis test, suitable for low number of samples as in this work. This test was run for each element, both full set of data and solely for "polluted" samples.

**Table 2**  
Physico-chemical parameters (pH, texture, OM content estimate, CEC) and heavy metal concentrations (mean  $\pm$  SE, in  $\text{mg kg}^{-1}$ ) of the studied soils. All samples were analyzed in triplicates for heavy metal concentrations.

Type of sample	Location	Site	Sample name	pH	Soil texture	OM content estimate	CEC (cmol/kg)	Elemental content ( $\text{mg kg}^{-1}$ )								
								As	Cd	Cu	Mn	Pb	Sb	Sn	Zn	
Polluted sites	Cartagena – Spain	Avenque Tailing	CRT-A	5.18	Loamy sand	Low	1.79	973 $\pm$ 37	18.7 $\pm$ 6.2	181 $\pm$ 4	1798 $\pm$ 13	14770 $\pm$ 659	121 $\pm$ 9	30.1 $\pm$ 7.5	10345 $\pm$ 337	
		Las Matildes	CRT-B	5.10	Silty clay	Medium	4.30	569 $\pm$ 37	6.98 $\pm$ 0.36	233 $\pm$ 5	3742 $\pm$ 22	36019 $\pm$ 801	197 $\pm$ 14	41.4 $\pm$ 6.9	4352 $\pm$ 12	
		Playa de Gorguel	CRT-C	6.02	Sand	Low	1.74	1390 $\pm$ 99	13.6 $\pm$ 1.8	146 $\pm$ 3	2434 $\pm$ 2	7066 $\pm$ 263	175 $\pm$ 12	23.7 $\pm$ 5.3	5836 $\pm$ 84	
		Santa Antonieta	CRT-D	6.93	Silty clay	High	7.03	257 $\pm$ 30	5.81 $\pm$ 1.29	49.3 $\pm$ 2.5	2160 $\pm$ 10	1028 $\pm$ 53	45.8 $\pm$ 3.3	18.1 $\pm$ 2.4	2641 $\pm$ 55	
	Huelva – Spain	Niebla / Rio Tinto	HUE-A	2.82	Loamy sand	Medium	0.96	669 $\pm$ 2	0.28 $\pm$ 0.01	168 $\pm$ 1	127 $\pm$ 1	1035 $\pm$ 6	113 $\pm$ 1	31.7 $\pm$ 0.2	109 $\pm$ 1	
		Gibraleon / Rio Odriel	HUE-C	3.94	Loamy sand	Medium	2.15	264 $\pm$ 1	0.61 $\pm$ 0.01	567 $\pm$ 4	543 $\pm$ 1	460 $\pm$ 3	27.5 $\pm$ 0.1	8.18 $\pm$ 0.13	294 $\pm$ 3	
	Salsigne – France	Orbiel / Pont du Limousis	SAL-A	7.91	Loamy sand	Medium	6.46	325 $\pm$ 2	0.43 $\pm$ 0.01	56.3 $\pm$ 0.4	33.4 $\pm$ 0.6	52.2 $\pm$ 0.1	2.70 $\pm$ 0.04	3.38 $\pm$ 0.03	90.3 $\pm$ 0.1	
	Saint-Salvy – France	Waste tailing	SVY-A	8.01	Silty clay	Medium	n.a	161 $\pm$ 1	29.4 $\pm$ 0.2	69.0 $\pm$ 0.3	5209 $\pm$ 25	847 $\pm$ 2	30.6 $\pm$ 0.1	5.46 $\pm$ 0.04	14202 $\pm$ 45	
	Control samples	Huelva – Spain	Cartaya	HUE-B	6.78	Silty clay	Medium	8.29	10.8 $\pm$ 0.1	0.12 $\pm$ 0.01	47.0 $\pm$ 0.6	353 $\pm$ 4	49.0 $\pm$ 0.7	2.97 $\pm$ 0.02	2.50 $\pm$ 0.04	128 $\pm$ 2
			St Hippolyte – France	La Sagnette	HIP-A	7.53	Loamy sand	Low	12.5	5.43 $\pm$ 0.1	0.17 $\pm$ 0.01	16.2 $\pm$ 0.1	188 $\pm$ 1	19.1 $\pm$ 0.1	0.76 $\pm$ 0.01	2.20 $\pm$ 0.07

Notes: CEC = cationic exchange capacity; OM = organic matters; n.a. = not analyzed.

### 3. Results

#### 3.1. Phytolith extraction and morphological observations

The extraction step was used to quantify the percentage of phytoliths in plant shoots (in total dry weight, DW). Phytoliths were found to represent 2.57% DW ( $\pm 0.50$ ) for *Arundo donax* and 3.53% DW ( $\pm 1.10$ ) for *Phragmites australis*.

SEM-EDX observations of reed samples show the presence of phytolith structures in *Arundo donax* (Fig. 2a) and *Phragmites australis* (Fig. 2b). The EDX spectra confirms the phytolith composition ( $\text{SiO}_2 \cdot \text{XH}_2\text{O}$ ) and allows the validation of the extraction procedure (Fig. 2). Also, even if changes in morphology have been reported in the literature under heat treatment (Nguyen et al., 2019; Tran et al., 2019), the morphologies of observed phytoliths (bilobate for *Arundo donax*; rectangular for *Phragmites australis*) are similar with those already observed by Shakoore et al. (2016) on phytoliths located on sites unimpacted by pollution. It shows that neither metal(oid) pollution from our sites nor the extraction procedure (and especially ashing at high temperature) have changed phytoliths' morphology. This is consistent with results of thorough methodological work of Parr et al. (2001b) who demonstrated that phytoliths pattern dimensions and curvatures obtained via dry (500 °C) and wet (boiling) extractions are statistically identical and there is no detectable evidence of morphological impact of these methods.

#### 3.2. Soil physico-chemical properties

Results of soil physico-chemical parameters (pH, texture, OM estimate and CEC) are presented in Table 2. The pH values range from 2.82 to 8.01, showing the diversity of sampling sites. Soils from polluted sites in Spain (Cartagena and Huelva) have pH values ranging 2.82 to 6.93. In France, soils are less acidic as the pH ranged between 7.91 and 8.01. In control sites, pH ranged from 6.78 to 7.53. The soil granulometry is variable according to the considered soils but coarse texture is often encountered in this kind of mining waste environment (sand and loamy sand to silty clay). The organic matter contents are quite low to medium.

Values of CEC are quite low for all soils, ranging from 0.96 to 12.5  $\text{cmol} \cdot \text{kg}^{-1}$  (1  $\text{cmol} \cdot \text{kg}^{-1}$  = 0.01  $\text{mol} \cdot \text{kg}^{-1}$ ). Polluted soils have CEC values ranging from 0.96 to 7.03  $\text{cmol} \cdot \text{kg}^{-1}$ , whereas soils from control sites have higher CEC values, ranging 8.3 to 12.5  $\text{cmol} \cdot \text{kg}^{-1}$ .

#### 3.3. Heavy metals concentrations in soils

Concentrations of HM in soils (Table 2) are different between the regions and the various investigated sites inside each area.

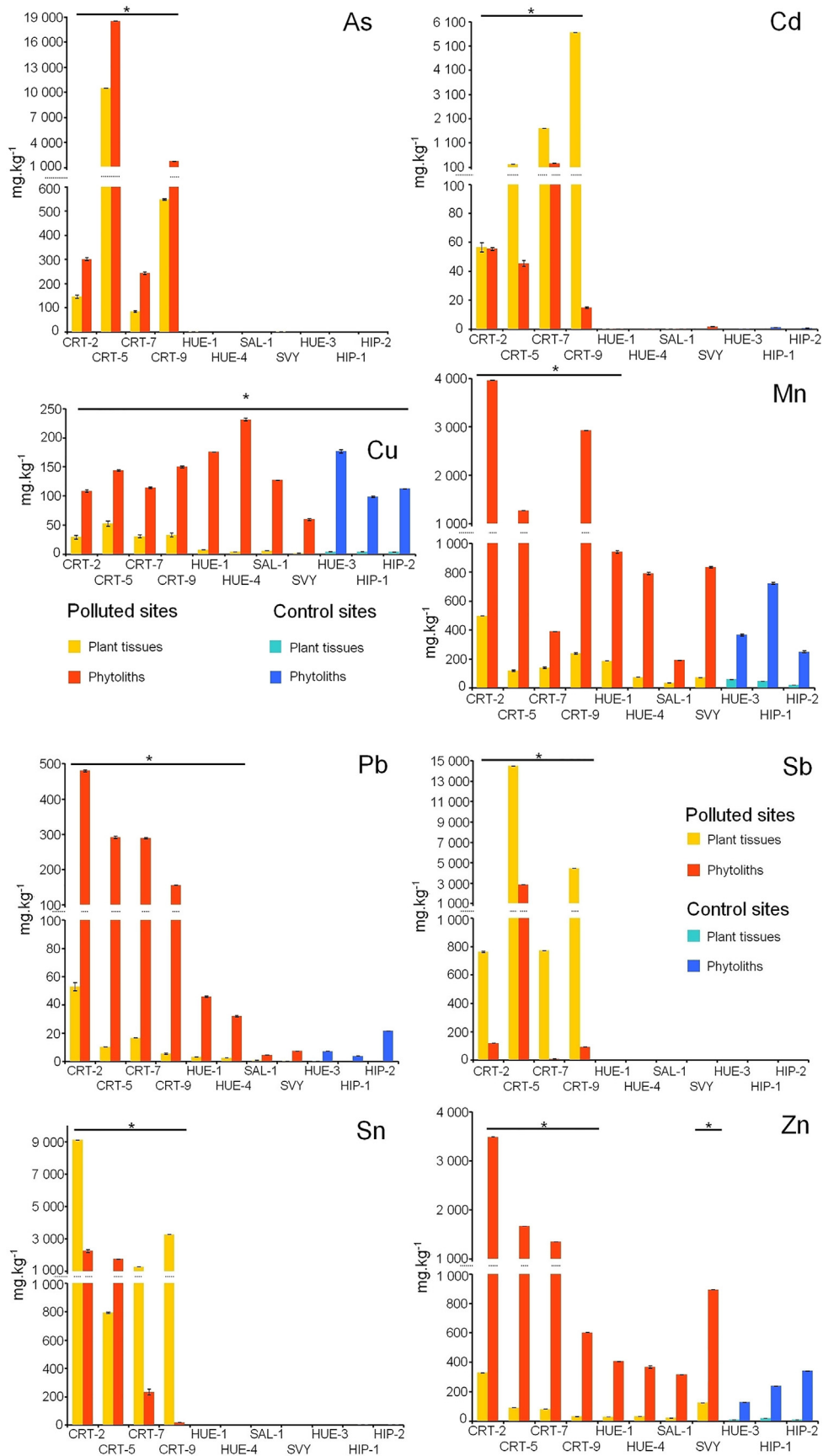
**Arsenic** concentrations range from 161 to 1390  $\text{mg kg}^{-1}$  on polluted sites, and are inferior to 10.8  $\text{mg kg}^{-1}$  on control sites. **Cadmium** concentrations are inferior to 0.6  $\text{mg kg}^{-1}$  everywhere except in Cartagena, where they vary from 5.8 to 18.7  $\text{mg kg}^{-1}$  and in Saint-Salvy, where Cd concentration reaches 29.5  $\text{mg kg}^{-1}$ . **Copper** concentrations range from 49 to 567  $\text{mg kg}^{-1}$  on polluted sites, and are between 47 and 16  $\text{mg kg}^{-1}$  in control sites. **Manganese** concentrations range from 1798 to 5209  $\text{mg kg}^{-1}$  in Cartagena and Saint Salvy, and from 33 to 543  $\text{mg kg}^{-1}$  on other polluted sites. On control sites Mn concentrations range from 188 to 353  $\text{mg kg}^{-1}$ . **Lead** concentrations vary widely from site to site. On polluted sites, values range from 460 to 36,000  $\text{mg kg}^{-1}$  except in Salsigne where Pb concentration has been measured at 52  $\text{mg kg}^{-1}$ . On control sites, concentrations range from 19 to 49  $\text{mg kg}^{-1}$ . **Antimony** concentrations vary the same way as Pb concentrations. On polluted sites values range from 28 to 197  $\text{mg kg}^{-1}$ , except in Salsigne (2.7  $\text{mg kg}^{-1}$ ). On control sites, Sb concentrations are lower than 3  $\text{mg kg}^{-1}$ . **Tin** concentrations range from 3.4 to 41.4  $\text{mg kg}^{-1}$  on polluted sites, and from 2.2 to 2.5  $\text{mg kg}^{-1}$  on control sites. Finally, **zinc** concentrations are broadly different from site to site. In Cartagena and Saint-Salvy they range from 2641 to 14,203  $\text{mg kg}^{-1}$ . On other polluted sites (Huelva and Salsigne) they range from 90 to 294  $\text{mg kg}^{-1}$ . Zinc concentrations on control sites were measured at 53 and 128  $\text{mg kg}^{-1}$ .

#### 3.4. Heavy metals concentrations in plant shoots and phytoliths

Heavy metals concentrations in plant shoots and in phytoliths are illustrated in Fig. 3 and the raw data are listed in Appendixes A and B, respectively.

##### 3.4.1. Heavy metals in plants

The highest **arsenic** concentrations are found in plants from Cartagena: they range from 85 to 10,500  $\text{mg kg}^{-1}$ . In other sites (polluted and control sites), As concentrations in plants are found to be lower than 1  $\text{mg kg}^{-1}$  or undetectable. The same observation is true for **Cd**, **Sb** and **Sn** concentrations: these elements have highest concentrations ( $>1 \text{ mg kg}^{-1}$ ) in plant shoots in Cartagena. There, Cd concentrations range from 56 to 5650  $\text{mg kg}^{-1}$ ; Sb concentrations from 762 to 14,500  $\text{mg kg}^{-1}$ ; and Sn concentrations from 794 to 9110 ppm. **Copper**



**Fig. 3.** Heavy metals (HM) concentrations (as a mean of 3 replicates  $\pm$  SE expressed in  $\text{mg kg}^{-1}$ ) according to the studied sites in plant tissues and phytoliths. Yellow corresponds to HM concentrations in plant tissues from polluted sites; orange to HM concentrations in phytoliths from polluted sites; light blue to HM concentrations in plant tissues from control sites; dark blue to HM concentrations in phytoliths from control sites. The asterisk (\*) indicates a significant difference between HM concentrations in plant tissues and in phytoliths (Kruskal-Wallis test,  $p < 0.05$ ). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

concentrations in plant shoots range from 29 to 52 mg kg<sup>-1</sup> in Cartagena, and from 1.7 to 7.0 on other polluted sites. On control sites, Cu concentrations remain in a very narrow range between 3.4 and 3.5 mg kg<sup>-1</sup>. The same observations are made for **Mn** and **Pb** concentrations. Manganese concentrations range from 120 to 497 mg kg<sup>-1</sup> in Cartagena, and from 33 to 187 mg kg<sup>-1</sup> in other polluted sites. On control sites, Mn concentrations vary between 21.6 and 58.5 mg kg<sup>-1</sup>. Lead concentrations range from 5.6 to 53 mg kg<sup>-1</sup> in Cartagena, and from 0.28 to 3.2 mg kg<sup>-1</sup> on other polluted sites. On control sites, Pb concentrations are lower than 0.36 mg kg<sup>-1</sup>. **Zinc** concentrations range from 21 to 329 mg kg<sup>-1</sup> on polluted sites, and from 7.8 to 18.8 mg kg<sup>-1</sup> on control sites.

#### 3.4.2. Heavy metals in phytoliths

In phytoliths, HM concentrations are significantly higher than those measured in aerial plant tissues for Cu, Mn, Pb and Zn (Kruskal-Wallis test; p-value < 0.05) and also substantially higher for As (not significant but a tendency is widely observed, Fig. 3). By contrast, these HM concentrations in phytoliths (in comparison to total plant shoots) are lower for Cd, Sb and Sn (Kruskal-Wallis test; p-value < 0.10). **Arsenic**, **Cd**, **Sb** and **Sn** concentrations are higher in phytoliths from Cartagena than in other sites, ranging from 243 to 18,520 mg kg<sup>-1</sup>, 15 and 259 mg kg<sup>-1</sup>, 8 and 2826 mg kg<sup>-1</sup>, and 19 and 2260 mg kg<sup>-1</sup>, respectively. **Copper** concentrations are relatively homogeneous, ranging from 60 to 231 mg kg<sup>-1</sup> in phytoliths from plants grown on polluted sites and from 98 to 176 mg kg<sup>-1</sup> on control sites. **Manganese** concentrations in phytoliths range from 391 to 3956 ppm in Cartagena, and from 191 to 939 ppm in other polluted sites. On control sites, Mn concentrations in phytoliths vary between 250 and 723 mg kg<sup>-1</sup>. **Lead** concentrations in phytoliths vary between 156 and 480 mg kg<sup>-1</sup> in plants from Cartagena, and between 4.4 and 46.9 mg kg<sup>-1</sup> in reeds from other polluted sites. Phytoliths from control sites have Pb concentrations ranging from 3.9 to 21.6 mg kg<sup>-1</sup>. **Zinc** concentrations in phytoliths range from 892 to 3489 mg kg<sup>-1</sup> in plants from Cartagena and Saint-Salvy, and from 316 to 406 mg kg<sup>-1</sup> in reeds from other polluted sites (Huelva and Salsigne). On control sites, Zn concentrations in phytoliths vary between 129 and 341 mg kg<sup>-1</sup>.

## 4. Discussion

### 4.1. Distribution of contaminants in soil environment

First of all, relatively low OM contents, coarse texture and CEC values measured in soil samples for different sites are in accordance with literature for similar metal-contaminated soils (Aihemaiti et al., 2018; García-Carmona et al., 2019; Rodríguez et al., 2009). This suggests that these mining-affected soils have low fertility, few exchange complexes, and therefore quite low ability to hold cations. These factors facilitate metal lixiviation, which is further accelerated by the overly acidic character of soils.

High concentrations of As, Cd, Cu, Pb and Zn were observed in Cartagena soils, consistent with results of Conesa et al. (2006) and Blondet et al. (2019). Besides, elevated concentrations of Mn, Sb and Sn were measured in these soils. All observed HM concentrations are hundred to thousand times superior to the geochemical background levels reported in the region (Alcolea Rubio, 2015). In the Huelva province, generally high concentrations in As, Cu, Pb, Sb and Sn in soils were observed, in accordance with the type of ores exploited in the region (Martín et al., 1998). In the Salsigne's soil, high concentrations of As have been measured, as also reported by Khaska et al. (2015). In Saint-Salvy, As, Cd, Mn and Pb were found in great concentrations, along with Sb and Zn in smaller proportions, consistent with the list of elements produced by the mine (Munoz et al., 2003). Finally, for both control sites (Huelva and Saint-Hippolyte) concentrations measured for all elements of interest were always lower or similar to geochemical

background levels reported in French soils (Baize, 2000; Darmendrail et al., 2000).

Therefore, all soils - except the control ones - are strongly contaminated in HM, as follows from the total HM concentrations in these soil samples. This observation is fully consistent with the past mining activities already described in these regions. Moreover, because these soils also have low capacity to hold heavy metals due to their low CEC, coarse texture and low OM content, these elements become mobile and potentially phytoavailable, and could then be transferred to plants and bioaccumulated in their shoots.

### 4.2. Heavy metals uptake and bioaccumulation in plants

Previous studies already reported that *Phragmites australis* and *Arundo donax* are able to accumulate HM in their shoots, roots, stems, and more specifically in their leaves. For example, Bonanno (2012, 2013), working on biomonitoring of trace element contamination in water and sediments, demonstrated that both species can accumulate HM, and showed a correlation between Cu and Zn concentrations in plants (*Arundo donax*) and sediments for Cu and Zn. Cicero-Fernández et al. (2017) also reported the ability of *Phragmites australis* to accumulate HM. It was recently shown that both species strongly accumulate As and Pb (up to 8000 mg kg<sup>-1</sup> and 1000 mg kg<sup>-1</sup>, respectively), but preferentially in roots (Castaldi et al., 2018).

As described in Section 3.3, HM concentration in plant shoots vary depending on the considered site and the element. Plants exhibit widely different HM concentrations, whereas HM concentrations in their respective soils are similar, suggesting potential influences of other parameters such as soil characteristics or metal(oid) speciation.

The values of enrichment factor EF<sub>plant</sub> are reported for each heavy metal and for each combination plant species/investigated soil in Table 3. From results presented in Table 3, it is possible to distinguish two groups of metal(oid)s. First group is composed of As, Cu, Mn, Pb and Zn. For these five elements, EF<sub>plant</sub> are <1, except 2 samples of plant/soil in Cartagena (for As), and one couple plant-soil in Huelva for Mn. Low values of EF for these elements indicate that *Arundo donax* and *Phragmites australis* are capable to grow in highly contaminated soils via limiting the uptake of As, Cu, Mn, Pb and Zn. Despite low values of EF<sub>plant</sub> in Cartagena plant and soil, the concentrations of these elements in plants of this region are higher than in other sites, even though HM concentrations in soils of Cartagena are not very different from the other sites. This suggests the role of other factors, such as soil characteristics and metal speciation in the environment, governing metal(oid)s uptake by plants in this region. For example, a concentration of 10,479 mg kg<sup>-1</sup> of As in plant shoots was measured in the site of Las Matildes, Cartagena. This high As concentration in plant shoots could be explained here by the addition of organic amendments on soil which increase metal(oid)s uptake by plants, as already reported for As by Yang et al. (2012) and Castaldi et al. (2018) for these two plant species. Overall, high concentrations found in plant shoots from Cartagena sites are in accordance with other studies on *Arundo donax* and *Phragmites australis* in regions similarly impacted by anthropogenic activities worldwide (Castaldi et al., 2018; Conesa et al., 2014; Liu et al., 2019; Minkina et al., 2018).

Another group of HM listed in Table 3 is composed of Cd, Sb and Sn. These elements have a very high EF<sub>plant</sub> in Cartagena, from 3 to 973, which shows that *Arundo donax* and *Phragmites australis* are able to efficiently accumulate Cd, Sb and Sn in their aerial tissues. Moreover, as a result, these reeds could be considered as tolerant to Cd, Sb and Sn. However, we observe a different behavior in Huelva (for Sb and Sn) and in Saint-Salvy (for Cd and Sb). Despite relatively high HM concentrations in soils, these elements are not detected in plant shoots, or at very small concentrations. This suggests that, under particular conditions, reeds are able to prevent Cd, Sb and Sn uptake and accumulation in shoots tissues. Among these conditions, there could be metal speciation or redox state in soil, modulating their bioavailability for plants.



**Table 3**  
Enrichment factor (EF<sub>plant</sub>) of heavy metals for each combination plant species/investigated soil.

Type of sample	Location	Site	Species	As	Cd	Cu	Mn	Pb	Sb	Sn	Zn	
Polluted sites	Cartagena – Spain	Avenque tailing	<i>Phragmites australis</i>	0.15	3.02	0.16	0.28	<0.01	6.32	303	0.03	
		Las Matildes	<i>Arundo donax</i>	18.4	30.2	0.23	0.03	<0.01	73.6	19.2	0.02	
		Playa de Gorguel	<i>Arundo donax</i>	0.06	125	0.21	0.06	<0.01	4.41	54.0	0.01	
		Santa Antonieta	<i>Phragmites australis</i>	2.13	974	0.67	0.11	0.01	96.5	181	0.01	
	Huelva – Spain	Niebla/Rio Tinto	<i>Arundo donax</i>	<0.01	-	0.04	1.46	<0.01	n.d.	<0.01	0.26	
		Gibraleon/Rio Odiel	<i>Phragmites australis</i>	<0.01	-	0.01	0.13	0.01	n.d.	-	0.11	
	Salsigne – France	Orbiel/Pont du Limousis	<i>Arundo donax</i>	<0.01	-	0.10	1.0	-	-	-	-	
	Saint-Salvy – France	Waste tailing	<i>Phragmites australis</i>	0.01	n.d.	0.02	0.01	<0.01	n.d.	-	0.01	
	Control samples	Huelva – Spain	Cartaya	<i>Phragmites australis</i>	-	-	0.07	0.17	0.01	-	-	-
		St Hippolyte – France	La Sagnette	<i>Phragmites australis</i>	-	-	-	0.25	-	-	-	-
<i>Arundo donax</i>				-	-	-	0.12	-	-	-	-	

Notes: “EF<sub>plant</sub>” corresponds to the ration between heavy metal (HM) content in plant tissues and HM content in soil; “n.d.” means that the element was not detected in a significant quantity in plant tissues; “-” means that the EF<sub>plant</sub> calculated was not significant, i.e. HM content in soil is inferior to the limit of detection.

4.3. Preferential bioaccumulation of HM in phytoliths

In our study on reed plants, the values of EF<sub>phyto</sub> are reported for each HM in Table 4.

The first group composed of As, Cu, Mn, Pb and Zn presents relatively high EF<sub>phyto</sub> values (between 2 and 28). arsenic and Cu presents lower EF<sub>phyto</sub> values, ranging from 1.8 to 3.8, compared to Mn, Pb and Zn for which the EF<sub>phyto</sub> values ranged from 2.8 (Mn) to 28 (Pb). Phytoliths of reeds strongly concentrate Pb, similar to results of Nguyen et al. (2019) on rice shoots in Viet Nam. These results suggest that, despite reeds being able to limit the uptake of As, Cu, Mn, Pb and Zn (see Section 4.2), efficient sequestration of these elements in phytoliths is observed in case of polluted soils. This is consistent with results of Buján (2013) on Ericaceae species from polluted soils which strongly concentrate Mn, Zn, Cu, Pb and As in their phytoliths.

In this regard, it is worth noting that, although phytoliths account for only about 3% of dry weight (DW) of the reed biomass, a more than one order of magnitude difference in Zn, Pb and Mn concentrations in plant shoots and phytoliths makes phytoliths a significant compartment of metal accumulation, presumably via encapsulation. For example, about 45% of total Zn bioaccumulated in reed shoots is sequestered in their phytoliths (see Fig. 3 and Table 4).

In the case of Cu, the tendency of bioaccumulation in plant structures is less clear. Indeed, Cu seems to be encapsulated in phytolith structures (Fig. 3), as already observed by Tran et al. (2019) in grass from orange growing area. However, we observe similar concentrations of Cu in phytoliths for all species on polluted and control sites, although the concentrations of this element in soils were rather different. This observation has already been reported by Blotevogel et al. (2019) on different vineyard soils, where Cu concentrations were different in each soil

sample but quite similar in all grapevine leaves studied. The other group of HM composed of Cd, Sb and Sn, which exhibited EF<sub>phytoliths</sub> values inferior to 1, except one site in Cartagena for Sn. Contrary to HM of the first group, these elements are not preferentially stored in phytoliths, but rather accumulated in plant shoots, where they were found at high concentrations (see Section 4.2).

Overall, the HM behavior and their potential sequestration via encapsulation in the phytolith structures are in accordance with first results of Buján (2013) and most recent data of Nguyen et al. (2019) and Tran et al. (2019). The identity of plant species and specific chemical reactions of HM co-precipitation with amorphous silica during phytoliths formation in plant cells are presumably the main factor controlling the degree of phytoliths enrichment in HM. However, further systematic studies of various families of plants coupled with relevant soil analyses are needed to reveal physico-chemical, physiological, and genetic mechanisms of HM incorporation into phytoliths structures during plant life cycle.

4.4. Role of phytoliths during litter mineralization process and element recycling in polluted zones

Our study highlights the potential role of phytoliths in HM recycling at the earth surface. It follows that, once taken up by plants, various HM can be preferentially bio-accumulated either in organic or mineral (phytoliths) part of the reed tissues. For this, HM have to cross the gradients of pH, oxygen and ionic strengths, and interact with different types of chelates present in plant sap solution (Avellan et al., 2017). Later, when plants enter the senescence, metals and metalloids present within plant tissues are released during organic matter mineralization via bio-physico-chemical processes at the soil surface. As such, the

**Table 4**  
Enrichment factor (EF<sub>phyto</sub>) of heavy metals for each combination plant species/investigated soil.

Type of sample	Location	Site	Species	As	Cd	Cu	Mn	Pb	Sb	Sn	Zn	
Polluted sites	Cartagena – Spain	Avenque tailing	<i>Phragmites australis</i>	2.06	0.98	3.75	7.96	9.08	0.16	0.25	10.6	
		Las Matildes	<i>Arundo donax</i>	1.77	0.21	2.74	10.7	28.2	0.20	2.22	18.2	
		Playa de Gorguel	<i>Arundo donax</i>	2.87	0.15	3.76	2.81	17.3	0.01	0.18	16.5	
		Santa Antonieta	<i>Phragmites australis</i>	3.19	<0.01	-	12.3	27.9	0.02	0.01	-	
	Huelva – Spain	Niebla/Rio Tinto	<i>Arundo donax</i>	-	-	-	5.03	-	-	-	-	
		Gibraleon/Rio Odiel	<i>Phragmites australis</i>	-	-	-	10.8	-	-	-	-	
	Salsigne – France	Orbiel/Pont du Limousis	<i>Arundo donax</i>	-	-	-	-	-	-	-	-	
	Saint-Salvy – France	Waste tailing	<i>Phragmites australis</i>	-	-	-	11.7	-	-	-	7.17	
	Control samples	Huelva – Spain	Cartaya	<i>Phragmites australis</i>	-	-	-	-	-	-	-	-
				<i>Phragmites australis</i>	-	-	-	-	-	-	-	-
St Hippolyte – France		La Sagnette	<i>Arundo donax</i>	-	-	-	-	-	-	-		

Notes: “EF<sub>phyto</sub>” corresponds to the ration between heavy metal (HM) content in phytoliths and HM content in plant tissues. “-” means that the EF<sub>phyto</sub> calculated was not significant, i.e. HM content in plant tissues is inferior to the limit of detection or considered as similar to the background level.

vegetation may exhibit significant impact on HM recycling and biogeochemical cycles (Roulier et al., 2018), via changing element distribution between organic and inorganic pools. The latter point has important implications to the long-term stability of sequestered metals, as already suggested by Tran et al. (2019) in the case of Cu encapsulation in the grass phytoliths, prior its return to the soil. For example, in the case of CaCO<sub>3</sub> biominerals produced by tobacco, metals as Zn and Cd are both present as Ca substituents in calcite and vaterite, whereas the organic complexes play rather minor role (Sarret et al., 2006; Isaure et al., 2010).

In this regard, phytoliths can be considered as irreversible sink that allows the sequestration of HM and decreases their potential bioavailability in soil after litter mineralization. For this reason, phytoliths are largely employed in archaeological and paleo-environmental research to track vegetation reconstruction and paleoecology, due to their high robustness and capacity to be preserved under conditions that would destroy organic tissues (Harvey and Fuller, 2005; Parr and Sullivan, 2005; Jenkins, 2009; Rashid et al., 2019). Thus, the present study demonstrates strategic potential of reed phytoliths to be used as proxies of modern and paleo soil contamination events. We therefore call for concerted efforts to address the contribution of phytoliths in HM release during organic matter degradation in the litter horizon. Specifically, via selecting plants that have various capacities to sequester HM in phytoliths, new insights on soil decontamination and phytoremediation can be acquired.

## 5. Conclusion

Results demonstrated that *Arundo donax* and *Phragmites australis* are able to accumulate HM in their shoots. Cadmium, Sb and Sn are stored in great quantities in plant aerial tissues, whereas the phytoliths extracted from reeds exhibited high concentrations of As, Cu, Mn, Pb and Zn. Therefore, phytoliths could play a significant role in reducing metal stress caused by these metal(oid)s, allowing plants to grow on highly metal-contaminated soils. Specific mechanisms involving passive or active pathways of HM uptake and bioaccumulation in plant shoots can be hypothesized but remain largely unclear. Further investigations are needed using for example in situ synchrotron-based spectroscopy to better understand the speciation and localization of different metal(oid)s in plant tissues, especially in phytoliths. Discovering how HM are sequestered in reed tissues will help to understand how the plant copes with metal toxicity, and if this sequestration in the form of a biomineral (phytolith) is durable. Such information would provide new insights for the selection of most efficient plants for phytoremediation processes in contaminated sites and has implication for the use of phytoliths as paleo environmental proxies.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors would like to thank Rémi Freyrier for his help getting some results for HM concentrations in soils, Thierry Aigouy for his help with the SEM-EDX observations and Alice Gandara and Enzo Civallero for extraction protocol improvements. This work was carried out as part of the SOIL TAKE CARE SOE1/P4/F0023 project through the European Regional Development Fund (ERDF), under the Interreg Sudoe Program.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.135595>.

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