Research article

Metal bioaccumulation in spontaneously grown aquatic macrophytes in Fe-rich substrates of a passive treatment plant for acid mine drainage

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ABSTRACT

Some plants may thrive in polluted environments, accumulating high concentrations of metal/loids in their organs. This study investigates for the first time the bioaccumulation and translocation of metal/loids in *Typha domingensis* spontaneously grown in extremely Fe-rich substrates (38–44% of Fe_2O_3) from different components of an acid mine drainage disperse alkaline substrate passive treatment. Most metal/loids were predominantly accumulated in the roots over the aerial parts of the plant, with concentrations of 0.66–9.5% of Fe, 0.02%–0.18% of Al, 55–2589 mg kg^{-1} of Mg, 51–116 mg kg^{-1} of Zn, 17–173 mg kg^{-1} of Cu, and 5.2–50 mg kg^{-1} of Pb. Bioconcentration factors were mostly below 1 for metal/loids in the studied aneas (e.g. 0.03–0.47 for Cu, 0.10–0.73 for Zn, 0.04–0.28 for As, 0.07–0.55 for Pb, 0.27–055 for Cd, 0.24–0.80 for Ni), which evidences that *T. domingensis* behaves as an excluder species in these substrates. Translocation factors were below 1 for most elements (e.g. 0.01–0.42 for As, 0.06–0.50 for Pb, 0.24–0.65 for Cd, and 0.10–0.56 for Sb), except for Mn, Ni and in some cases for Tl, Cu and Zn, which indicates limited transfer of metals between plant tissues. Mineralogical and geochemical substrate properties are pointed out as the main factors responsible for the lower bioconcentration and translocation of potentially toxic elements. In addition, the oxidizing conditions existent in the pore water-root system may also limit the mobility of metals from Fe oxides and hydroxysulfates, the main component of the substrate. The formation of a Fe plaque inside the roots may also limit the transfer of metals to the aerial parts. The spontaneous occurrence of *T. domingensis* in the substrates of the acid mine drainage passive treatments is an environmental indicator of the efficiency of the system and could be used as a complementary polishing step, given the strong tolerance of this plants to high concentrations of metal/loids.

1. Introduction

Aquatic plants in natural systems are commonly exposed to low concentrations of metals and metalloids (metal/loids). Some of them, such as Fe, Cu, Mn or Zn, act as essential nutrients although at elevated concentrations may have toxic effects (Kabata-Pendias, 2000). Other metal/loids such as As, Cd, or Pb, in turn, do not play significant biological roles and are potentially toxic, even at very low concentrations (Bonanno and Cirelli, 2017). For instance, these elements have been classified as Priority Substances for their persistence in the environment, bioaccumulation capacity and related toxicity to exposed organisms (Decision 2455/2001/EC). Easily mobile carcinogenic As could be

leached down to ground waters, posing a risk when reaching the drinking water system. Cadmium, whose solubility strongly depends on Eh-pH conditions, has been reported to produce kidney damage, decreased calcium levels in bones and neuroendocrine disruption effects, among others. Lead, the most ubiquitous toxic metal in the environment, has been widely recognised to affect nervous systems. In addition, Al, As, Ba, Bi, Cd, Cr(VI), Pb, Li, Mn, Hg, Se, Te and Tl have been recognised as having neurotoxic potential in animals and humans (Andersen et al., 2000; Kabata-Pendias, 2000). Nonetheless, some specific plants may thrive in polluted environments, even when exposed to high concentrations of metal/loids. For instance, macrophytes, highly tolerant to elevated metal/loid concentrations, can modify their internal

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root morphology to grow in polluted water bodies (Hadad et al., 2011). Presenting fast growth and considerable biomass, these plants can accumulate high metal/loid concentrations in their tissues (Deng et al., 2004; Vymazal, 2011), which make them suitable for phytoremediation. In recent years, numerous studies have been performed to study the growth and tolerance of macrophytes to high levels of metal/loids (Bonanno and Cirelli, 2017; Bonanno et al., 2018; Suñe et al., 2007).

Formed after the oxidation of sufides, acid mine drainage (AMD) is a relevant problem for water pollution worldwide, characterised by highly acidic and metal-rich effluents (Akcil and Koldas, 2006). Remediation of these waters can be done by active and passive technologies, with the first requiring energy consumption, maintenance and continuous addition of chemicals, and are commonly used in operating mines. However, passive treatment systems (PTS) use renewable energies such as gravity, microbial metabolism, photosynthesis and simple geochemical reactions, requiring scarce maintenance work, and are therefore particularly suitable for abandoned mines (Johnson and Hallberg, 2005). Among passive technologies, the dispersed alkaline substrates (DAS) have turned out to be highly effective to treat extremely acidic and metal-rich mine waters in derelict mines. This technology has been successfully implemented at the Iberian Pyrite Belt (IPB, SW Spain) (Ayora et al., 2013; Orden et al., 2021), where the main rivers (Odiel and Tinto) draining this area represent a worldwide example of AMD pollution (Nieto et al., 2013). Thus, the AMD (average pH < 3 and electrical conductivity (EC) > 3 mS/cm) gravitationally runs through reactive tanks (filled with alkaline materials) and decantation ponds, leading to the removal of acidity and trace metal/loids (e.g. Fe, Al, Cu, Zn, As, Cr, Mo, V, Cd, Pb), which are accumulated in the different steps of the PTS by precipitation/sorption processes. As a result, an extremely Fe-rich substrate (and other metal/loids) is accumulated in these treatment steps. After some months of operation of two settled DAS PTS, the spontaneous cattail colonisation of some DAS steps was observed (Supplementary Material, Fig. S1). The predominant species was *Typha domingensis*, a plant commonly found in flooded environments within a high tolerance range of water salinity, which can grow in flooded soils with pH values ranging from 5.5 to 8, being, therefore, an indicator of alkaline conditions (Sesin et al., 2021). In this study, a high-density population of plants was observed in continuously flooded substrates. Perfectly adapted to polluted environments if nutrients are available and showing variable heights (1–3 m), this species has been reported as the dominant macrophyte in a wetland constructed for the treatment of metallurgical effluents (Maine et al., 2009). The growth of these emergent macrophytes has been previously reported in other PTS, and they are even included in AMD treatment systems as a final step to polish the water quality due to its high metal tolerance (Cutright et al., 2012; Dan et al., 2017; Singh and Chakraborty, 2020; Vymazal, 2011). Despite the increased number of studies dealing with metal bioaccumulation, there is no information on metal/loid bioaccumulation in cattails in such Fe-rich substrates, like those found in the IPB-DAS treatment systems. Considering the existence of numerous similar cases of this type of polluted site worldwide, this information could help to fill the gap in knowledge about the bioaccumulation and translocation capacity of these plants in extremely metal-polluted environments. In addition, restoration measures must be urgently performed at the IPB to achieve good status of rivers, lakes and groundwater required by the European Commission (EC) Water Framework Directive (e.g. pH > 6.0). Implementation of several DAS-treatment systems would contribute to fulfil the EC regulation by 2027. Thus, spontaneously grown southern cattails, widely distributed in the province, not only will contribute to the retention of potentially toxic elements, but also are an inexpensive way to reduce and stabilize metal pollution.

2. Methodology

2.1. Study sites

A full description of DAS technology and the evolution of AMD during treatment can be seen in Ayora et al. (2013) and Orden et al. (2021). Briefly, the DAS system is composed of a natural Fe-oxidizing lagoon (NFOL, Macías et al., 2012a), which enhances the oxidation of Fe(II) to Fe(III) (Fig. S2). After that, the water is channelled into successive reactive tanks filled with the limestone-DAS reactive mixture, causing the nearly total depletion of trivalent metals (i.e. Fe and Al) and significant removal of divalent metals (e.g. Cu, Zn, Cd, Ni, Co and Mn) and metalloids (e.g. As, V and Sb) (Fig. S2). When AMD hydrochemistry is dominated by divalent metals, a MgO-DAS final step would be necessary to accomplish the depuration process (Macías et al., 2012b). All reactive tanks are serially connected to settling ponds to enhance the sedimentation of the precipitated minerals.

To study metal bioaccumulation in emergent macrophytes (i.e. *T. domingensis*) growing in different steps of the DAS system, five different individuals were collected from different settling ponds (MC5, MC7 and ME6) and a MgO-DAS reactive tank (MC-RT3) of the DAS PTS of Mina Concepción (MC) and Mina Esperanza (ME) (Fig. S2). In the case of the reactive tank, only one individual cattail could be collected without damaging the plant due to the large compartment depth. In addition, another five cattail individuals were collected from a small water reservoir not affected by mining waters, located at 8 and 10 km from Esperanza and Concepción PTSs, respectively. These samples were used as background regional freshwater reservoir (PC) to study the influence of the pollution level in the substrate on the metal bioaccumulation capacity of aquatic plants.

2.2. Sample processing and analysis

Cattail samples were collected in separate sterile plastic bags and immediately transported to the laboratory, where they were cut into separated roots, stems and leaves, washed carefully with tap water to remove the material attached to the plant surfaces and then rinsed with deionized water. Later, subsamples were sonicated separately for 1 h at 30 °C using a POWER SONIC 510 ultrasonic device to completely remove mineral particles. Afterwards, plant samples were oven-dried at 30 °C for 6 days until constant weight. In addition, substrate soil samples were collected in each sampling point using a plastic shovel, dried at 30 °C until constant weight, and sieved below 2 mm for analytical purposes. Subsequently, samples (i.e. biomass and soil) chemical determinations were performed by ICP-OES and TQ-ICP-MS after acid digestion at the certified MSALABS laboratories (Langley, Canada). Plant samples were dried and macerated to 1 mm before performing four-acid digestion (HF + HNO_3+HCl + $HClO_4$) in a microwave, while solid samples were processed using $LiBO_2$, except for the determination of Ag, As and Pb, where four-acid digestion (HF + HNO_3+HCL + $HClO_4$) was also performed. The quality of the analysis was verified by the measurement of certified materials (i.e. OREAS24b, SRM 1573a), with all elements below 5% of certified values. Blanks and duplicates were also measured in each analytical sequence. Solid samples collected in each substrate were also mineralogically characterised by X-ray diffraction (XRD) at the R&D Central Services of the University of Huelva using a Bruker D8 Advance X-ray diffractometer with Cu Kα monochromatic radiation. XRD settings were fixed at 20 mA, 40 kV, and a step size of 2° 2θ/min (3–65°).

Apart from plants and soil samples, Temperature, pH, electrical conductivity (EC) and redox potential (ORP) of the supernatant waters were measured in situ, using a portable HACH SensION + MM150 multi-parameter probe previously calibrated with certified buffer solutions. Measured ORP values were referenced to the standard hydrogen electrode (Eh) according to Nordstrom and Wilde (1998). In addition, supernatant water samples were collected in each sampling point, filtered

through 0.2 μm Millipore filters, acidulated with ultrapure HNO_3 and kept refrigerated (4 °C) until chemical analyses by ICP-MS and ICP-OES at the R&D Central Services of the University of Huelva.

2.3. Data treatment

The bioaccumulation of metals and the translocation from subaerial to aerial parts of the plants were studied by determining the bio-concentration (BCF) and translocation factors (TF). The BCF expresses the phytoaccumulation ability of plants, defined as the relationship between total metal content in plant tissues (C_p, mg kg^{-1}) and total metal content in the top layer of soil (C_s, mg kg^{-1}), according to Eq (1) (Dan et al., 2017):

$$BCF = C_p/C_s \qquad \text{Eq 1}$$

The TF defines the ability of plants to translocate metals from the subaerial (roots) to the aerial parts of a plant (i.e. steams and leaves). It is calculated according to Eq (2) (Dan et al., 2017):

$$TF = C_a/C_r \qquad \text{Eq 2}$$

where C_a is the metal concentration (mg kg^{-1}) in the aerial parts of the plant, while C_r is the metal concentration (mg kg^{-1}) in the roots.

3. Results

3.1. Substrate characterisation

Plant roots take up the nutrients and trace elements from the substrate where they grow up; therefore, their characterisation is of paramount importance. The studied substrates from PTS are characterised by their high Fe content, with concentrations of Fe_2O_3 ranging from 38 to 44% (Table 1). The neutralization process in the DAS systems causes the precipitation of Fe, which is subsequently settled in the settling ponds of both sites (Fig. S2). The substrate in the freshwater reservoir (PC) shows noticeably lower values of Fe (4.8% of Fe_2O_3, Table 1). In this latter case, the content in Al was higher (18% of Al_2O_3) than that observed in the PTS components (MC5, MC7, MC-RT3 and ME6; from 1.7 to 8.0% of Al_2O_3; Table 1) due to the abundance of aluminosilicates in natural sediments. In the reactive tank and setting ponds, Al may come

Table 1
Chemical composition of solid substrates studied from Mina Concepción (MC5, MC7 and MC-RT3), Mina Esperanza (ME6) and freshwater reservoir control site (PC) (See Figs. S1 and S2).

	LoQ	MC5	MC7	MC-RT3	ME6	PC
	%					
Al_2O_3	0.01	8.0	3.3	3.5	1.7	18
CaO	0.01	5.5	5.1	4.2	1.3	0.41
Fe_2O_3	0.01	37	38	44	44	4.8
K_2O	0.01	0.22	0.24	0.08	0.03	2.7
MgO	0.01	0.25	1.5	0.35	0.14	0.73
MnO	0.01	0.42	0.79	0.60	<LoQ	0.07
Na_2O	0.01	0.09	0.05	0.04	0.03	0.82
P_2O_5	0.01	0.06	0.06	0.05	<LoQ	0.14
SO_3	0.01	3.4	3.3	0.90	4.2	0.22
TiO_2	0.01	0.05	0.06	0.01	<LoQ	0.93
Trace Elements (mg kg^{-1})						
As	0.20	153	166	41	5.1	25
Cd	0.02	2.2	2.9	0.99	0.27	0.51
Cu	0.20	2326	1349	951	910	196
Ni	0.20	5.8	9.1	8.4	1.0	40
Pb	0.50	171	167	52	19	78
Sb	0.50	15	11	3.4	0.50	7.4
Se	1.0	2.0	2.0	<LoQ	<LoQ	<LoQ
Tl	0.02	1.3	0.85	0.22	0.15	0.76
V	10	<LoQ	14	16	<LoQ	125
Zn	2.0	1152	1144	1265	420	201

LoQ: limit of quantification. See the text for explanation.

exclusively from the removal of dissolved Al at increased pH values. The content of SO_3 in the PTS components was higher than that observed in the freshwater reservoir, with values ranging from 3.3 to 4.2%. However, SO_3 in MC-RT3 showed values below 1.0%, which was in the range of that of a freshwater reservoir (PC; Table 1). The substrates of PTS components also exhibited higher levels of Ca (from 1.3 to 5.5% of CaO) than that of a freshwater reservoir (PC, 0.41% of CaO); the high levels of Ca in PTSs are inherited from the reactive material used in the neutralization process. However, the similar concentration of MgO found in all substrates is striking (below 1.0% of MgO except for MC7 with 1.5; Table 1), especially in MC-RT3, which was originally filled with MgO to provide alkalinity to the AMD treatment. However, this MgO original content was exhausted by AMD neutralization. The contents of other major elements of soils, such as Na, K, Mn, P or Ti exhibited values below 1.0% in all substrates, except for K in the PC sample, which showed a 2.7% of K_2O content (Table 1).

Regarding trace element concentrations, the substrates of the PTS components exhibited high concentrations of Cu, Zn, Pb and As and lower quantities of other trace metal/loids, such as Cd, Ni, V, Sb or Tl (Table 1). Among the PTS components studied, the settling pond of the Mina Esperanza PTS (ME6) showed noticeably lower trace metal/loid concentrations than those observed in the rest of the PTS components of Mina Concepción (Table 1). However, the most striking result was the anomalously high concentrations of some trace metal/loids observed in the substrate of the freshwater reservoir selected as the regional background, in some cases exceeding those observed in the PTS components, which were remarkably more polluted substrates. For instance, concentrations of around 200 mg kg^{-1} of Cu and Zn, 125 mg kg^{-1} of V, 78 mg kg^{-1} of Pb, 40 mg kg^{-1} of Ni and 25 mg kg^{-1} of As were determined in the substrate of the reservoir. Although this water body is situated outside the mining area and does not receive direct inputs from AMD, it is located very close to the road connecting the Riotinto sulfide mine to the Huelva port. Therefore, it is reasonable to think that these anomalously high concentrations could be attributed to the deposition of mineral particles from trucks transporting the mineral concentrate by the nearby high road. In this sense, the soil chemical composition (Table 1) fits well with the mineralogical information obtained. Although the substrates of the PTS components were poorly crystalline, the XRD patterns indicate the occurrence of quartz (SiO_2), gypsum ($CaSO_4 \cdot 2H_2O$), goethite (FeOOH) and Na-jarosite ($NaFe_3(SO_4)_2(OH)_6$, with Na also replaced by Ag and Pb in some samples. The presence of Fe oxides (i.e. goethite) and hydroxysulfates (i.e. jarosite) explains the high Fe contents observed in the PTS substrates. These minerals have a great capacity to scavenge trace metals (Acero et al., 2006), which may be the reason for the elevated concentrations of these elements observed in the PTS substrates (Table 1). On the other hand, the substrate of the freshwater reservoir contained abundant minerals with a higher crystallinity, such as quartz and micas, although the presence of minor amorphous phases cannot be discarded.Cattails thrive in circum-neutral environments, as evidenced by the pH values observed in both the PTS components and the reservoir water (pH 6.0 to 7.0) where *Typhas* appeared, except in MC5, where waters had a pH of 4.9. Cattails may also take up nutrients from the water overlying the substrate. Table S1 shows the hydrochemical composition of the substrate's supernatant waters. Redox potential values ranged from 235 mV in ME6 to 446 mV in MC5, with intermediate values for the rest of the water samples, denoting oxidizing conditions. Most water bodies showed good quality, with low values of EC (from 250 to 356 μS cm^{-1}) and very low concentrations of sulfate and metals (generally below 0.10 mg L^{-1}; Table S2), which evidences the good performance of DAS systems. Nonetheless, ME6 waters exhibited relatively high EC values (1954 μS cm^{-1}; Table S2) and elevated concentrations of sulfate (2105 mg kg^{-1}), Fe (208 mg kg^{-1}), Ca (527 mg kg^{-1}), Mg (173 mg kg^{-1}), Mn (3.7 mg kg^{-1}) or Zn (1.3 mg kg^{-1}). These elevated concentrations in ME6 may be related to the operation periods of the different PTS, while Mina Esperanza PTS began operation in 2014, Mina Concepción began in 2016. Thus, alkaline

materials may have been exhausted in Mina Esperanza, reducing the efficiency of the treatment, whereas Mina Concepción appears to have some remnants, maintaining the efficiency of the system. In addition, the pollution degree in the Mina Esperanza inflow (Orden et al., 2021) is higher than in Mina Concepción (Martínez et al., 2019).

3.2. Metal/loid content in plant tissues

The metal/loid content accumulated in roots, stems and leaves of individuals collected in each sampling point (n = 5, except in RT3) can be seen in Table S2. Roots were, by far, the tissues with a greater bio-accumulation capacity for most metal/loids. Due to the high Fe content in substrates, roots exhibited high concentrations of Fe, with values ranging from 0.66 to 9.5% (Fig. 1), with the maximum value reached in ME6. Despite exhibiting a Fe content in PC an order of magnitude lower than in PTS components, Fe concentrations in PC roots (1.1%) were within the range observed in PTS. The content in Al was noticeably lower than that of Fe, with values ranging from 0.02% in RT3 to 0.18% in MC5 (Fig. 1). Once again, Al contents in the roots of individuals of the PC site were in the same order as in PTS. Regarding Mn, strikingly elevated concentrations were found in MC-RT3 (average of 2589 mg kg^{-1}), noticeably higher than those observed in the rest of the cattail roots (from 9.5 to 975 mg kg^{-1}). As previously stated, Fe minerals commonly host significant amounts of trace metal/loids, such as As, Pb, Sb, Cu, Zn, Tl, Cd, Ni or V, which may be available for plants. Some of these trace metal/loids were preferentially accumulated in roots from Fe-rich substrates of the PTS. For example, the roots accumulated elevated concentrations of Cu (up to 173 mg kg^{-1}), Zn (up to 116 mg kg^{-1}) and Tl (up to 3.6 mg kg^{-1}) in contrast to the lower values found in cattail roots growing in the freshwater reservoir substrate (i.e. average values of 60 mg kg^{-1} of Zn, 44 mg kg^{-1} of Cu and 0.06 mg kg^{-1} of Tl; Fig. 1). However, the roots from the PC site exhibited similar or even higher contents for some trace metal/loids than the roots studied in the PTS samples. For instance, the concentration of As and Pb in the PTS roots ranged from 1.2 to 46 mg kg^{-1} and 5.2–44 mg kg^{-1}, respectively (Fig. 2 and Table S2), while those in the PC were 27 and 39 mg kg^{-1}, respectively. Likewise, significantly higher concentrations were observed in PC roots for Ni (4.7 mg kg^{-1}), V (4.2 mg kg^{-1}), Cd (2.1 mg kg^{-1}) and Sb (1.8 mg kg^{-1}), than those observed in PTS roots (Fig. 2 and Table S2).

The concentrations for most metal/loids in the aerial parts of the plants, in both the PTS and PC samples, were noticeably lower than those observed in the roots (Figs. 1 and 2). Thus, contents of 0.01–0.04% of Fe, 37–70 mg kg^{-1} of Zn, 12–40 mg kg^{-1} of Cu, 0.86–2.4 mg kg^{-1} of Pb, 0.1–1.9 mg kg^{-1} of Tl, 0.10–0.48 mg kg^{-1} of As and 0.10–0.41 mg kg^{-1} of Cd were measured in the stems of cattails. Conversely, some metals, such as Ni and Mn, exhibited similar concentrations in stems than in roots: 1.3–4.6 mg kg^{-1} of Ni and 174–830 mg kg^{-1} of Mn (Figs. 1 and 2), although in this latter case, higher values were observed in MC7 and MC–RT3 roots. The decreasing tendency of accumulation from subaerial to aerial parts of the plant was also observed in leaves for Zn (21–35 mg kg^{-1}), Cu (6.3–16 mg kg^{-1}), Ni (0.92–2.4 mg kg^{-1}), Pb (0.68–1.3 mg kg^{-1}), Tl (0.10–0.62 mg kg^{-1}), As (0.10–0.30 mg kg^{-1}) and Cd (0.10–0.19 mg kg^{-1}). However, in the case of Fe and Mn, a slightly higher accumulation was observed in leaves (0.02–0.06% and 348–1541 mg kg^{-1}, respectively) for stems. In the case of Al, Sb and V, their contents in stems and leaves were remarkably low, indicating a poor transfer from the subaerial to aerial parts of the plant. Comparing the concentrations of metal/loids in both PTS and freshwater reservoir aerial parts, it is striking that cattails growing in the reservoir accumulated similar concentrations than those growing in PTS substrates, and even higher concentrations were observed for Cd, Ni and Mn (Figs. 1 and 2) in the freshwater reservoir plants.

3.3. Bioconcentration and translocation factors

Table 2 shows the BCF in the different systems studied, with values over 1 (BCF > 1) indicating strong metal/loid absorption by plants. BCF values were mostly below 1 for most metal/loids in the individuals studied (Table 2). BCF values significantly above 1 were found in ME6 for Tl (38), As (7.9), Mn (7.5) and Ni (5.0) and in MC5 and ME6 for Tl and Pb (Table 2). This was also relevant to the strong metal/loid extraction capability of the freshwater reservoir plants (PC) from the surrounding substrate, with BCF values of 5.3 for Cd, 5.0 for Mn and 1.1 for As. In addition, these plants showed higher BCF values for Cu, Zn, Cd and Fe than those in PTS components (Table 2).

Values of TF were below 1 for most elements. However, there were some exceptions, such as Ni and Mn, with most values above 1 (from 1.4 to 2.1 and from 0.50 to 8.4, respectively), Tl for MC5 (2.3) and MC-RT3 (1.0) and Cu and Zn for MC-RT3 and PC (1.1–1.3 and 1.1–1.4,
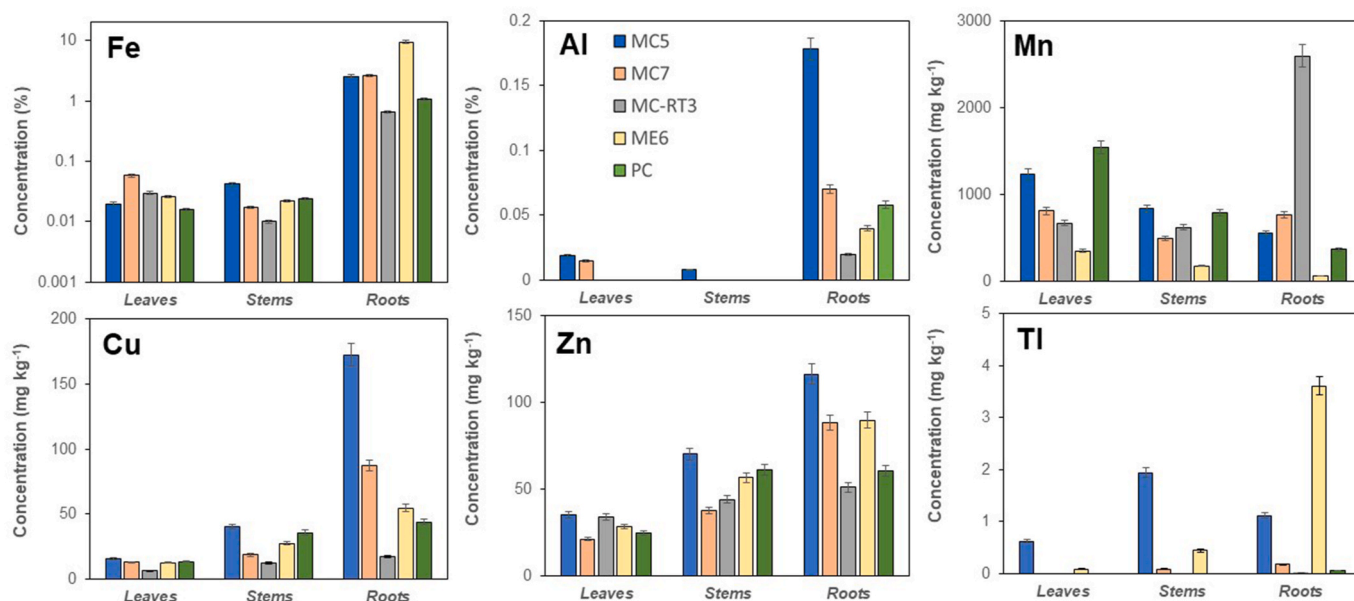

Fig. 1. Average accumulation of Fe, Al, Mn, Cu, Zn and Tl in different plant tissues (i.e. leaves, stems and roots) of collected cattails (*Typha domingensis*) for each sampling site (n = 5, except for MC-RT3) *2-column fitting images.*
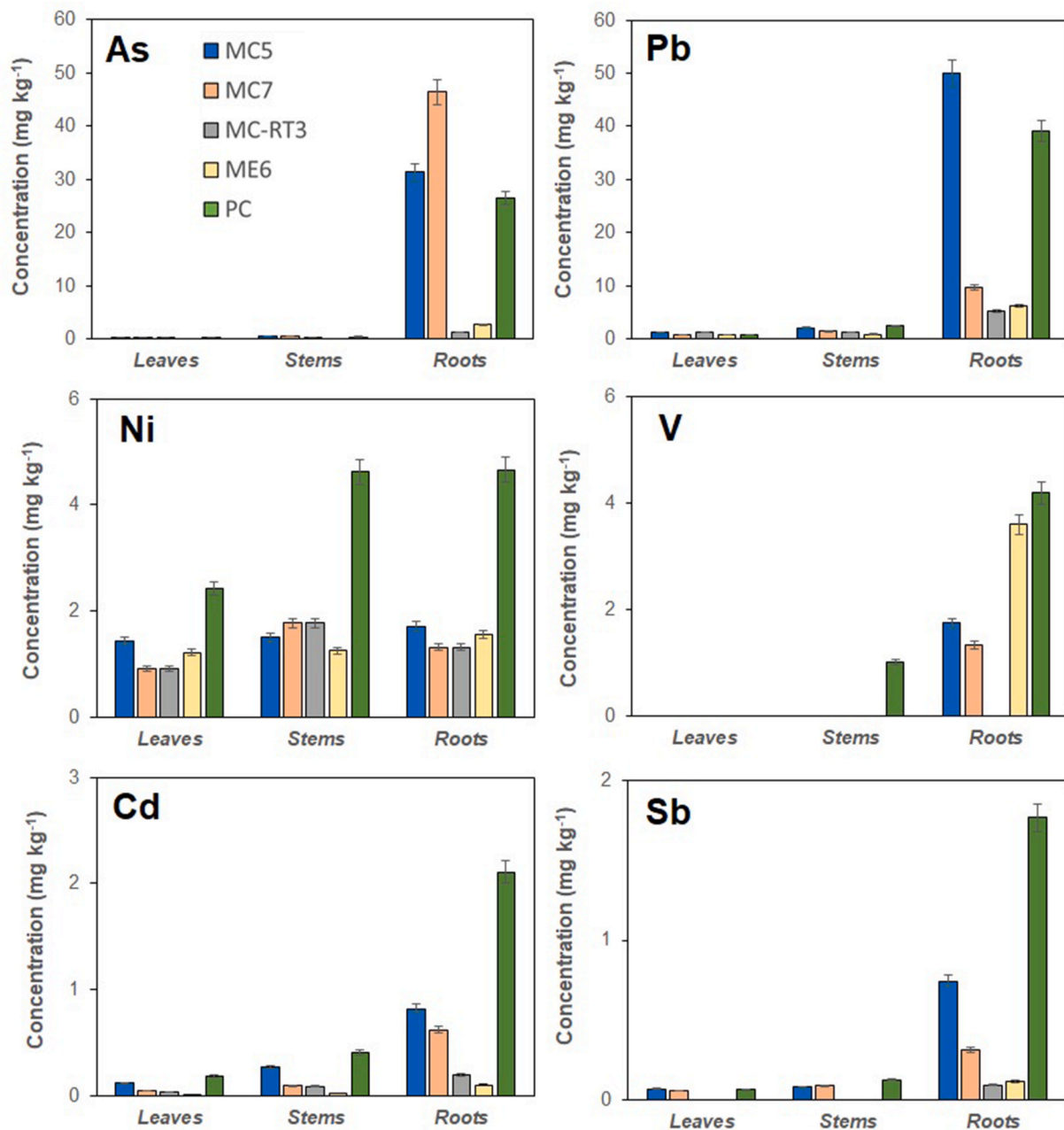
Fig. 2. Average accumulation of As, Pb, Ni, V, Cd and Sb in different plant tissues (i.e. leaves, stems and roots) of collected cattails (*Typha domingensis*) for each sampling site (n = 5, except for RT3) *Single-column fitting images.*

respectively) (Table 2).

4. Discussion

4.1. Bioaccumulation capacity of plants

In general terms, the accumulation tendency for most metal/loids (Zn, Cu, Pb, Tl, As, Sb, V and Cd) in studied cattails was roots > stems > leaves. These significantly higher metal/loid concentrations in subaerial tissues than aerial ones have been widely reported in the literature, suggesting the exclusion of metals from aerial tissues of plants as a metal tolerance strategy (Vymazal, 2011; Compaore et al., 2020). However, in the case of Fe, Al and Mn (Fig. 2), a preferential accumulation in leaves over stems was observed (roots > leaves > stems), while Ni showed similar concentrations in stems and roots (roots ~ stems > leaves) (Fig. 2). This fact might be associated with the greater capacity of plant

to uptake micronutrients (e.g. Fe and Mn) compared to other non-essential elements included in the potentially toxic elements group, such as As, Ni or Pb (Kabata-Pendias and Szteke, 2015). A decreasing pattern of metal/loid bioaccumulation from subaerial to aerial parts (root > stem > leaf) for As, Cd, Cr, Cu, Mn, Ni, Pb and Zn has been previously reported in *T. domingensis* growing in a trace-metal/loids-polluted coastal area (Table S3; Bonanno et al., 2018). Compared to those results observed in cattail individuals growing in the PTS substrates, noticeably higher values for As (average of 22 mg kg^{-1}), Cu (77 mg kg^{-1}), Mn (863 mg kg^{-1}) and Pb (22 mg kg^{-1}) were observed (Table S2). However, slightly lower concentrations were observed in roots for some other metals, such as Zn, Cd and Ni (81 mg kg^{-1}, 0.77 mg kg^{-1} and 2.0 mg kg^{-1}, respectively). For aerial parts, the concentrations reported by Bonanno et al. (2018) were similar or even higher for most metal/loids than those reported in this study (Tables S2 and S3). Only concentrations of Cu and Mn in aerial parts of cattails growing in the PTS

Table 2

Values of the bioconcentration factor and Translocation Factor (relation between metal concentration in the aerial parts of the plant and in the subaerial parts of the plant (i.e. roots)) for metal/loids studied in the different PTS components and the freshwater reservoir; values above 1 are shown in bold.

	Bioconcentration Factor (BCF)					Translocation Factor (TF)				
	MC5	MC7	MC-RT3	ME6	PC	MC5	MC7	MC-RT3	ME6	PC
Mn	0.80	0.34	0.83	**7.5**	**5.0**	**3.8**	**1.7**	0.50	**8.4**	**6.4**
Fe	0.10	0.10	0.03	0.31	0.33	0.02	0.03	0.06	0.01	0.04
Al	0.03	0.03	0.01	0.01	0.00	0.15	0.21	0.50	0.10	0.17
Cu	0.10	0.09	0.04	0.10	0.47	0.32	0.36	**1.1**	0.78	**1.1**
Zn	0.19	0.13	0.10	0.39	0.73	0.90	0.67	**1.5**	0.95	**1.4**
As	0.21	0.28	0.04	**7.9**	**1.1**	0.02	0.02	0.42	0.01	0.02
Pb	0.31	0.07	0.15	**1.1**	0.55	0.06	0.22	0.50	0.08	0.08
Tl	**2.8**	0.31	0.09	**38**	0.08	**2.3**	0.52	**1.0**	0.30	0.32
Cd	0.55	0.27	0.33	0.53	**5.3**	0.48	0.24	0.65	0.36	0.28
Sb	0.06	0.04	0.03	0.78	0.26	0.19	0.45	0.56	0.13	0.10
Ni	0.80	0.44	0.24	**5.0**	0.29	**1.7**	**2.1**	**1.5**	**1.4**	**1.5**

BCF; the relation between the total metal/loids content in plant tissues and the solid substrate.

TF; relation between metal concentration in the aerial parts of the plant and in the subaerial parts of the plant (i.e. roots).

were higher than those found in the same species growing in coastal wetlands polluted by trace metal/loids (Bonanno et al., 2018). On the other hand, Zn, Cu and Ni root concentrations of 340 mg kg^{-1}, 50 mg kg^{-1} and 55 mg kg^{-1}, respectively, have been reported, and leaf concentrations of 215 mg kg^{-1}, 30 mg kg^{-1} and 40 mg kg^{-1}, respectively, have been reported in *T. latifolia* growing in a stream affected by sewage effluents (Sasmaz et al., 2008). The higher values observed by these authors for Zn compared to this study (Table S3), despite having a noticeably lower substrate concentration of this element (70 mg kg^{-1}) than in the PTS substrates (420–1265 mg kg^{-1}; Table 1). For the same species growing in an anthropogenically-impacted river in Iran (Haghnazar et al., 2021; Table S3),higher concentrations in roots for Zn, Ni, Cu and Pb than those observed in this study were reported, but lower values for As and Mn were found.

A more accurate assessment of metal/loids concentrations in halophytes growing in AMD-impacted sites can be performed. In this context, a study based on metal accumulation in cattails growing in a subsurface-flow constructed wetland, a PTS commonly applied in coal mining, showed a preferential metal accumulation in roots compared to stems and leaves, especially for Fe and Al, with concentrations of 7952–10702 mg kg^{-1} and 308–492 mg kg^{-1}, respectively (Singh and Chakraborty, 2020). Once again, these concentrations are remarkably lower than those observed in the roots of cattails growing in DAS treatments of the IPB (average value of 3.3% of Fe and 0.07% of Al; Table S1). However, Fe concentrations in shoots reported by these authors were similar (<2000 mg kg^{-1}) to those found in the aerial parts of cattails in the IPB-PTS components (average of 0.02–0.03% of Fe). Therefore, there seems to exist a limitation for Fe to be translocated to aerial parts of the plant in extremely Fe-rich substrates. The same finding was observed in *T. latifolia* growing in an AMD-impacted site (Cutright et al., 2012). However, values for Mn in leaves and stems of cattails were noticeably lower (60–117 mg kg^{-1} and 26–64 mg kg^{-1}, respectively) than those observed in our study (579–919 mg kg^{-1}).

High accumulation of Fe in roots can be related to the reduction of Fe (III) to Fe(II) in the rhizosphere due to microorganism activity (Lurthy et al., 2021). On the other hand, the previously reported complexation of Fe with organic acids released by some aquatic macrophytes might explain the limitation of transport of this element to the subaerial parts of plants (Bienfait, 1988; Carranza-Álvarez and Alonso-Castro, 2008).

4.2. Metal/loid bioaccumulation and translocation

Plants uptake metals by tissues through different processes, such as absorption, chelation, ionic exchange, translocation to aerial parts and precipitation induced by root exudates (Kabata-Pendias, 2000; Maine et al., 2004; Suñe et al., 2007). The capacity of plants to uptake metal/loids can be estimated by the BCF, which relates the metal/loid concentration in the plant tissues with that in the substrate. BCF values (Table 2) indicate that *T. domingensis* behaves as an excluder species in these characteristic substrates, as most values were generally below 1. Comparing these results with those reported in the literature (Bonanno et al., 2018), it stood out that the *T. domingensis* had higher Cd (1.4) than the plants collected in this study but lower Mn (0.22) and Ni (0.14) (Table S3). In turn, the bioconcentration capacity of this plant for As (0.18), Cu (0.14), Pb (0.09) and Zn (0.28) was within the range observed in cattails collected in this study. Higher BCF values for Fe (1.5–2.4), Mn (1.8–2.3), Al (0.66–1.1) and Ni (0.28–0.66) in *T. latifolia* growing in a wetland system aimed to treat AMD were also found by Singh and Chakraborty (2020). These values are noticeably higher for Fe than those reported in our study (0.03–0.33) despite the higher Fe concentrations observed in the substrate (38–44% of Fe$_2$O$_3$ in PTS substrates and 4.8% in PC; Table 1). In addition, the strong absorption (BCF> 1) observed in cattails for Pb, Cd, Zn, and Cu highlights that *T. latifolia* may be a phytoaccumulator of Pb, Cd, Zn and Cu (Haghnazar et al., 2021). Even though these differences in trace elements bioconcentration may be related to different species of cattails (Sawidis et al., 1995), no significant differences in bioaccumulation between cattails such as *T. domingensis*, *T. latifolia* and *T. angustifolia* have been reported (Bonanno and Cirelli, 2017). In this sense, it is worth mentioning that these three *Typha* species are commonly present in the region of study with slightly different ecological functioning, but occupying the same niche (WCSP, 2023).

The translocation factors (TF), which represent the ratio of metal/loid concentration in the subaerial and aerial parts, provides an insight into the internal metal/loid transport in plants. Values of TF > 1 indicate the translocation of metal/loids from the root to the aerial parts of the plants. In this study, metal/loids are preferentially retained in root tissues since TF values were below 1 for most elements, except for Ni, Mn, Cu and Zn for some of the sampling sites (Table 2). This finding partially agrees with those of Kabata-Pendias (2000), who claimed that the transport of trace elements among plant organs depends on the electrochemical variables of elements, with moderate mobility for Mn, Ni, Cd and Zn, and low mobility due to strong retention in roots for Co, Cu, Pb and Fe. Other reported results have shown a selective metal uptake of Fe < Mn < Al for *T. latifolia* growing in metal-rich substrates from AMD in Ohio (USA), with TF values of 3.7 for Al, 2.0 for Mn and only 0.20 for Fe (Cutright et al., 2012). The same authors also reported lower TF values in *T. latifolia* for Fe (of 0.11–0.26), Mn (1.6–2.7) and Ni (0.06–0.09) but higher TF values for Al (0.64–1.1) than those observed in this study. *Typha* species have also been catalogued as excluder species for Cu and Pb by showing BCF and TF values < 1 in wetlands affected by mining effluents (Nabuyanda et al., 2022). Similarly, ineffective metal/loid translocation in *T. latifolia* with TF < 1 for most metal/loids, except for Zn, has been found in indigenous aquatic

macrophytes of the Bahmanshir River (Haghnazar et al., 2021). The very low values of TF for Ni (0.19) observed by these authors (Table S3) are also striking, like others (Azizi et al., 2020; Compaore et al., 2020; Singh and Chakraborty, 2020) compared to the results obtained in this study. The major factor controlling the availability of metals in our study seems to be related to the mineralogical and geochemical properties of the substrate, unlike in Bonanno et al. (2018), who pointed out plant species and elements as the main controllers of translocation from the substrate to roots.

4.3. Influence of the substrate on the metal bioaccumulation and translocation

As previously mentioned, the low BCF and TF values observed for *T. domingensis* in this study, despite the high metal/oid concentrations observed in the substrate, point at this species as an important metal excluder. This capacity has been previously reported by Klink et al. (2013), among others, and attributed to a well-developed metal tolerance strategy characterised by a high root retention capacity. Enhanced metal/loid concentrations in the substrate may lead to increasing values of bioconcentration. For example, Bonanno et al. (2018) reported sediment concentrations of $970-1100$ mg kg^{-1} of Mn, $315-410$ mg kg^{-1} of Zn, $112-145$ mg kg^{-1} of Cu, $59-68$ mg kg^{-1} of Pb, $36-44$ mg kg^{-1} of Ni, $9.7-14$ mg kg^{-1} of As and $0.64-0.96$ mg kg^{-1} of Cd. These concentrations are noticeably lower than those reported in this study (Table 1), but the BCF values obtained by these authors are mostly similar. Likewise, other cases of cattails growing in less polluted substrates show similar or even higher BCF values than those reported in this study. In this context, mineralogy may play an important role in metal/loid uptake since metals associated with certain mineral fractions, such as carbonate, Fe and Mn oxyhydroxides, organic matter, or sulfide bounds, are less easily available for rooted macrophytes, which preferentially take up elements in their ionic form (Jackson, 1998). Deng et al. (2004) reported a positive correlation between concentrations of Pb and Cu in subaerial and aerial tissues in plants with the substrate ionic-exchangeable fraction (i.e. DTPA-extraction). Thus, the presence of ionic-exchangeable fractions in DAS substrates is scarce, since they are dominated by jarosite, a Fe hydroxysulfate, from which trace elements are not easily available for plants, although they can be released by mineral transformation into goethite with time (Acero et al., 2006). Roots and some associated microorganisms have a great capacity for the mobilisation of trace elements bound by certain soil constituents due to the production of organic compounds, which release these elements from firmly bound minerals (Kabata-Pendias, 2011). The low mobility of metals from the Fe oxides and hydroxysulfates, which mainly composed the studied substrates, would explain the lower BCF observed in this study compared with other previously mentioned works.

Other factors, such as metal speciation or mineral crystallinity, may also play an important role in metal uptake. It is assumed that the reduction step is obligatory in the root uptake of Fe (Carranza-Álvarez and Alonso-Castro, 2008; Chaney et al., 1997). Thus, substrates with a predominance of Fe(II) minerals would hold more bioavailable metals than those with a predominance of Fe(III) minerals, such as those from this study. Thus, the higher Fe bioconcentration observed by Singh and Chakraborty (2020) may be related to differences in Fe availability on the mineralogical assemblage, considering the artificial nature of these substrates used for the remediation of coal mine soils. Whereas the DAS substrate was mainly formed by jarosite and to a lesser extent goethite, that reported by Singh and Chakraborty (2020) included goethite and Fe sulfide (i.e. pyrite). Redox conditions may also play an important role in metal bioavailability. In strongly reduced sediments (Eh < -150 mV), S predominates as sulfide and forms insoluble metal precipitates, while in strongly oxidised sediments (Eh $> +200$ mV), S may predominate as sulfates and Fe and Mn as oxides. Both metal sulfides and oxides are good metal/oids scavengers, reducing their mobility. Therefore, the higher mobility of metals may take place at intermediate Eh values. At

the PTS, supernatant waters Eh values ranged from 235 mV in ME6 to 446 mV in MC5 (Table S1), with intermediate values for the rest of the waters. These values could be considered oxidant conditions, also limiting the mobility of metals from the substrate. In addition, Fe concentrations in roots of the cattails growing in non-AMD affected substrates (PC, 4.8% of Fe$_2$O$_3$) were within the same range as those from the DAS substrates (38–44% of Fe$_2$O$_3$), with predominantly higher BCF and TF values (Table 2). The lower crystallinity of Fe minerals in PTS substrates over those of freshwaters does not explain the limited Fe transfer from the substrate to the PTS plants. In this context, the low TF observed in this study may be related to the formation of Fe plaques. Previous studies showed that cattails may retain Fe in the roots, preferentially as a root plaque (Bose et al., 2008); however, the relationship between this Fe root plaque, metal sorption and translocation is not yet completely understood (Cutright et al., 2012). It is generally believed that the plaque may act as a reservoir during soil-nutrient deficiencies (Hossain et al., 2009) or as a chemical barrier to reduce the toxicity of certain potentially toxic elements (Jiang et al., 2009; Karathanasis and Johnson, 2003). Furthermore, these authors reported that Mn is not effectively sorbed by the plaque and is consequently translocated to the aerial parts of the plant, while Al is also accumulated in the roots. However, when dealing with such huge concentrations of trace metals, the Fe plaque may become saturated, and the root may be exposed to higher metal concentrations (Kabata-Pendias, 2001). Considering the TF values observed in this work and those found in the literature, it seems that the plaque may have not been saturated in the studied soils.

Some uptake by above-sediment plant parts is also possible due to the availability of dissolved metal species in the overlying water column (Cardwell et al., 2002). In this case, the concentrations of metal/loids in these waters may control the uptake by plants. This fact was specifically observed in *T. domingensis*, which was capable of accumulating metal ions preferentially from wastewater rather than from sediments (Hegazy et al., 2011). Mufarrege et al. (2014) studied the bioaccumulation of *T. domingensis* exposed to concentrated solutions of Cr, Ni and Zn, indicating that metals were not only accumulated by sediment and roots but were also taken up by the leaves in direct contact with the solution. In the case of ME6, BCF > 1 was observed despite having lower concentrations in the solid substrate (Table 1). These plants were exposed to higher dissolved metal concentrations in the overlying waters), which point to these waters as an optional uptake route for cattails growing in Fe-rich substrates. Nonetheless, the direct uptake by leaves in contact with waters may be of minor importance considering the TF observed in ME6 (Table 2), with respect to the other substrates with lower dissolved concentrations.

5. Conclusions

This study evaluated the concentration and transfer of metal/loids in cattails spontaneously growing in the substrates of different components of two passive treatment systems for treating extremely acidic and metal-rich waters at the IPB. Plants bioconcentrated considerable Fe, Al, Zn, Cu and Pb, with lesser amounts of As, Cd, Ni or Tl, which can be attributed to the plants' ability to discern between essential and non-essential (potentially toxic) element uptake. Among tissues, most metal/loids were predominantly accumulated in the roots instead of the aerial parts of the plant. However, there were some exceptions, such as Mn and Ni, and to a lesser extent Tl, Cu and Zn, with high concentrations observed in stems and leaves. Mineralogical and geochemical properties of the substrate may limit the accumulation of metal/loids in the plant tissues. The studied substrate was composed mainly of Fe oxides and hydroxisulfates with a high capacity to retain metals, leading to a low availability for plants. Considering the results obtained in this study, the occurrence of *T. domingensis* individuals in the substrates of the DAS-PTS is a reliable environmental indicator of the efficiency of the PTS and could also be used as a polishing step in the AMD global treatment strategy worldwide.

The IPB is one of the largest metallogenic provinces, with intense mining activity developed since pre-Roman times. However, this contamination by sulfide mining is a worldwide problem affecting other countries, such as China, France, Germany, Australia, Sweden and the USA. These countries may assume serious environmental liabilities, especially in derelict mines. The implementation of PTS may help to mitigate or reduce this pollution. The demonstrated availability of spontaneously grown cattail in these PTS systems not only will contribute to the retention of potentially toxic elements, but it is also a cheap way to stabilize metal-contaminated substrates. In this context, the accumulation of metals of economic interest by these plants in the PTS should be studied. For instance, phytoremediation for the extraction of REE in plants is already being applied at large-scale in countries like China, reporting high removal yields. Considering the high concentrations of REE in AMD waters entering the PTS, the retention of REE by cattails could be a promising source of economic metals.

CRediT authorship contribution statement

M. Dolores Basallote: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Virginia Zarco:** Methodology, Investigation, Visualization, Writing – review & editing. **Francisco Macías:** Conceptualization, Methodology, Investigation, Validation, Supervision, Writing – review & editing. **Carlos R. Cánovas:** Project administration, Funding acquisition, Writing – original draft, Writing – review & editing. **Pablo J. Hidalgo:** Conceptualization, Investigation, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jenvman.2023.118495.

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