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Accumulation of As, Cd and selected trace elements in tubers of *Scirpus maritimus* L. from Doñana marshes (South Spain)

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Abstract

The collapse of a pyrite-mining, tailing dam on 1998 contaminated an area of 4286 ha along the Agrio and Guadiamar river valleys in southern Spain. Over 2700 ha of the Doñana marshes, an important wintering area for wetland European birds, were contaminated. This study reports analyses of the tubers of *Scirpus maritimus* (an important food for greylag geese, *Anser anser*) collected in 2000 in the "Entremuros" (spill-affected area) and in nearby unaffected Doñana marshes (control areas). In the spill-affected area mean tuber tissue concentrations of Cd (0.25 mg kg^{-1}) and Zn (61 mg kg^{-1}) were greater than in those tubers from the control area (0.02 mg kg^{-1} for Cd, and 22 mg kg^{-1} for Zn); values of Cd and Zn in "Entremuros" (samples collected two years after the mine spill) were much smaller than those reported only a few months after the accident. Trace elements (As, Fe, Mn and Tl, and to a lesser extent Cd and Pb) showed a preferential accumulation in the outer skin of tubers. Surprisingly, concentrations of As and Fe were greater in tubers from some marsh sites not affected by the mine-spill than in tubers from the "Entremuros". We suggest that relic river channels within the Doñana marshes may be contaminated by trace elements from historic mining activities. An exhaustive study of macrophytes and other plant species in this area is recommended to identify potential risks to wildlife.

Keywords: *Bolboschoenus maritimus*; Heavy metals; Aznalcóllar; Mine spill; Macrophytes; Greylag goose; Wetlands

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1. Introduction

In hydrogeologic sedimentary environments, potentially toxic metals and metalloids may be mobilized in solution as free cations (e.g., Cu^{2+}, Zn^{2+}), or ionic complexes (e.g., $H_2AsO_3^-$, $H_2AsO_4^-$). High levels of these bioavailable cations, anions and/or anionic complexes of heavy metals/metalloids may pose risks to ecosystem health (Siegel, 2002).

The failure (in April 1998) of a tailings pond dam at the pyrite mine of Aznalcóllar (Seville, SW Spain) released about 4.5×10^6 m^3 of spill, comprising acidic water, fine divided metal sulphides (mainly pyrite) and materials used in the float on process for ore treatment. This affected more than 4200 ha along the Agrio and Guadiamar river valleys. Grimalt and Macpherson (1999) give a general description of this event and its environmental impact.

The spill mud accumulated for a distance of 40 km down river; while the polluted water continued for additional 20 km until it was stopped by several transverse dikes built in the channelled "Entremuros" (meaning "between-dikes") area (Fig. 1). About 2×10^6 m^3 of polluted water accumulated there. This water was treated and removed to the Guadalquivir main channel before autumn 1998 (Grimalt et al., 1999), but the Entremuros sediments remained polluted.

The mine accident had a major ecological impact because the Guadiamar river discharges into the marshes

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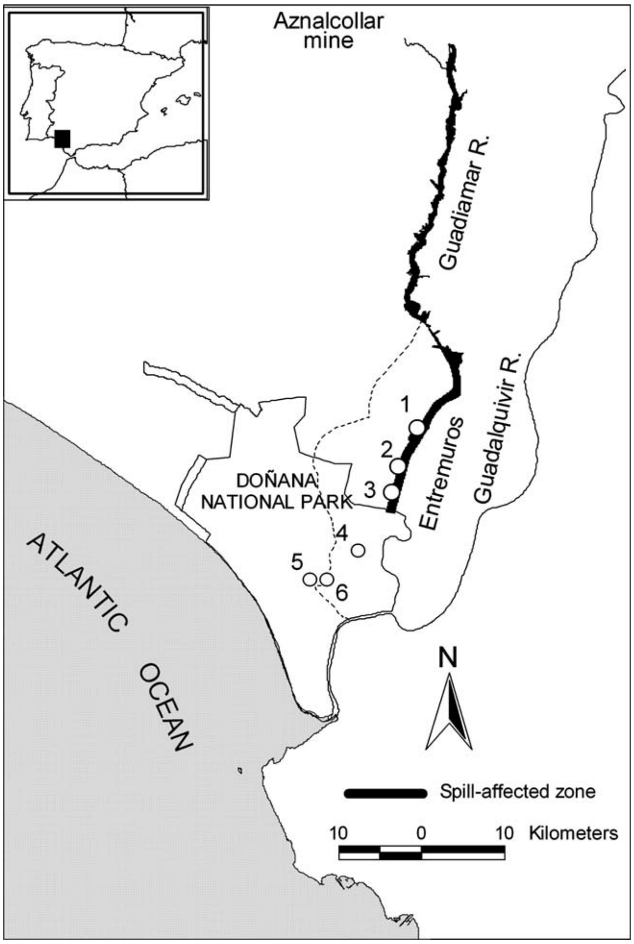

Fig. 1. Map with the spill-affected zone along the Guadiamar river, and location of the sampling sites (circles) of *Scirpus maritimus* tubers: sites 1, 2 and 3 are in the affected "Entremuros"; while sites 4 ("Caño Travieso"), 5 ("Arroyo de la Madre") and 6 ("Caño Guadiamar") are in the unaffected marshes of Doñana National Park. The dotted line indicates the Guadiamar relic channel entering the marshes.

of Doñana National Park, which is a wintering area for many European water birds. Greylag geese (*Anser anser*) feed on the tubers of *Scirpus maritimus,* a very common marsh plant in the "Entremuros" area (Amat, 1995; Taggart et al., 2005).

This paper deals with trace element concentrations in *S. maritimus* tubers collected in the spill-affected area of "Entremuros" compared to those from surrounding, non-affected marshes of the Doñana National Park. In addition, the trace elements content of tubers are compared with those measured a few months after the mine accident to evaluate the temporal pollution trend.

2. Material and methods

2.1. Site and plant description, and sampling procedures

The study area is located in the lower course of the Guadiamar river, and adjacent marshes of the Guadalquivir delta (SW Spain). In this area, called "Entremuros", the Guadiamar river has been channelled between two dikes (Fig. 1). The climate is of Mediterranean type, with an average annual rainfall of 550 mm, mild rainy winters and warm dry summers.

S. maritimus L., syn. of *Bolboschoenus maritimus* (Asch.) Palla (Cyperaceae), is a rhizomatous emergent macrophyte widely distributed in shallow brackish water bodies of temperate regions (Valdés et al., 1987). Previous studies have shown that *S. maritimus* is one of the major components of the perennial vegetation in the temporary Doñana marshes (García et al., 1993; Espinar et al., 2002). This forms dense populations with maximum biomass values of about 2.3 kg m^{-2} dry weight, with tubers mass accounting for 40–50% of the total (Espinar, 2004).

Tubers sprout at the beginning of the wet season (autumn); shoots emerge from the water in late autumn, flowers and fruits are formed through out the spring, and when the marshes dry up in summer, the dormant tubers are buried in the sediment. The tuber is the main reserve of carbohydrates (starch) for plant growth; for example, tuber size has been proven to be related to the capacity of new shoots to emerge from water during prolonged flooding conditions (Clevering et al., 1995). Tubers of *S. maritimus* contribute a high proportion of the primary production of the Doñana marshes, and constitute an important food for wintering geese (Amat et al., 1991; Amat, 1995).

Sampling of *S. maritimus* tubers and associated soil/sediment was carried out in summer 2000, in two areas: the spill-affected "Entremuros" part of the Guadiamar river, and an adjacent non-affected marsh (Fig. 1). In each area, three sites were randomly selected. In "Entremuros" (37°2′35″ N 6°16′44″ W, 37°4′13″ N 6°16′15″ W, and 37°6′48″ N 6°14′46″ W), the three sites were located along the channel. In the Doñana marsh, the three sites were at "Arroyo de la Madre" (36°56′44″ N 6°23′2″ W), "Caño Guadiamar" (36°56′48″ N 6°21′40″ W) and "Caño Travieso" (36°58′44″ N 6°19′18″ W) (Fig. 1). General soil characteristics of the two study areas are shown in Tables 1 and 2.

Table 1
Results of the analyses of soil samples (mean ± SE, $N = 9$) from three sites of Doñana marshes and three sites from the spill-affected "Entremuros" channel

Variable	Doñana marshes	Entremuros
pH (H_2O)	7.7 ± 0.02	$7.9 \pm 0.06^{*}$
EC (dS m^{-1})	13.4 ± 0.6	$9.4 \pm 1.1^{**}$
$CaCO_3$ (g kg^{-1})	134 ± 9	125 ± 13
OC (g kg^{-1})	3.4 ± 0.3	$1.9 \pm 0.2^{***}$
Sand (g kg^{-1})	30 ± 2	36 ± 9
Silt (g kg^{-1})	310 ± 6	310 ± 9
Clay (g kg^{-1})	670 ± 7	670 ± 8
N (g kg^{-1})	0.37 ± 0.02	$0.12 \pm 0.01^{***}$
P (mg kg^{-1})	16 ± 0.9	$19 \pm 1.1^{*}$
K (mg kg^{-1})	740 ± 31	$599 \pm 19^{**}$

EC means electrical conductivity, and OC means organic carbon.
Significance levels are indicated: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Table 2
Total and EDTA-extractable concentration (mg kg^{-1}) of S and trace elements of the soils (mean ± SE, $N = 9$)

Element	Doñana marshes	Entremuros
Total		
S	3400 ± 112	3500 ± 500
As	11.8 ± 0.5	18.1 ± 1.2**
Cd	1.45 ± 0.06	3.29 ± 0.22*
Cu	47 ± 2.7	61 ± 8.4
Fe	38 856 ± 773	40 844 ± 926
Mn	745 ± 11	1522 ± 63**
Pb	38 ± 2.6	45 ± 4.5
Zn	104 ± 7	718 ± 95***
EDTA-extractable		
As	0.19 ± 0.04	0.10 ± 0.03
Cd	0.16 ± 0.11	0.95 ± 0.20*
Cu	23 ± 8	22 ± 5
Fe	231 ± 12	197 ± 31
Mn	113 ± 4	161 ± 9
Pb	16 ± 1	17 ± 2
Zn	21 ± 12	148 ± 26*

Significance levels in the comparison between spill-affected and non-affected soils are indicated: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

At each study site, plant tubers were collected from three different stands of *S. maritimus*, each stand at least 10 m from its neighbour. Once in the laboratory, three subsamples were processed: (A) "Washed-tubers" were washed (for 20 s approximately) with a solution of phosphate-free detergent, then with a 0.1 N HCl solution and finally with distilled water. (B) "Inner tissue" subsample was washed and peeled before analysis. (C) "Unwashed-tubers" were analysed with soil adhering to their surfaces, to investigate the possible toxic impact of trace elements through their consumption as food (e.g., Brekken and Steinnes, 2004). The results obtained for unwashed tubers are only indicative of the potential amounts of trace elements that can be ingested by geese. All samples were oven dried at 70 °C, ground and passed through a 500 μm stainless-steel sieve.

A soil or sediment sample (0–25 cm depth) was taken from each sampled *Scirpus*-stand. These were oven-dried at 40 °C, crushed to pass through a 2 mm sieve, then ground to <60 μm prior to S and total trace element determinations.

2.2. Analytical methods

Tuber samples were analysed for N by Kjeldahl digestion. Mineral nutrients (P, K, S, Ca and Mg) and trace elements (As, Cd, Cu, Fe, Mn, Pb, Tl and Zn) were extracted by wet oxidation with concentrated HNO_3 under pressure in a microwave digester. Three consecutive steps (5 min. each) of power (250 W, 450 W and 600 W) were applied. Analysis of mineral nutrients, Fe and Mn in the extracts was performed by ICP-OES (inductively coupled plasma spectrophotometry; Thermo Jarrell Ash Corporation) after dilution to volume of 50 ml with water of 18 MΩ deionised quality. Analysis of trace elements was performed by ICP-MS (inductively coupled plasma-mass spectroscopy; Perkin Elmer, Sciex-Elan 5000), using an internal standard (Rh) and multielement standard solutions for calibration, with exception of the "Unwashed-tubers" subsamples (with higher values) that were analysed by ICP-OES. The accuracy of the analytical method was assessed by carrying out analyses of three BCR (Community Bureau of Reference) reference samples: BCR 62 (Olive leaves), CRM 279 (Sea lettuce) and CRM 281 (Ryegrass) (see reference and experimental values in Madejón et al., 2006a).

Soil samples were analysed for sulphur and trace elements by ICP-OES, after digesting the samples (<60 μm) with a mixture of conc. HNO_3 and HCl (1:3 v/v "aqua regia") for two 9 min. periods at 600 and 350 W respectively, in a microwave digester; these values are referred to as "total" concentration. "Available" concentration of trace elements was estimated by extracting the samples (<2 mm) with 0.05 M EDTA solution at pH 7.0, followed by ICP-OES analysis of the extracts. Concentrations of elements in plant and soil samples are given on a dry weight basis.

2.3. Statistical analyses

The differences between the concentrations of trace elements in the tubers from the spill-affected "Entremuros" channel, and those in the Doñana marsh (control) were assessed by the Student *t*-test. Data normality and homocedasticity were tested prior to analysis; when necessary, variables were transformed logarithmically. If, after transformation, the data did not have a normal distribution, the non-parametric Mann–Whitney U test was used for group comparisons. For elements with high within-area heterogeneity, such As in Doñana marshes, a subsequent ANOVA test was carried out, considering the sub-site (three per area) as the source of variation. All statistical analyses were carried out using SPSS 10.0 for Windows.

3. Results and discussion

3.1. Soil pollution

In general, the spill-affected soils had greater (total) concentrations of more mobile elements Cd (×2.5) and Zn (×7) than the control soils, this was also true for As and Mn (Table 2). When considering the available fraction, only Cd and Zn showed significant differences (Table 2).

The soil pollution in this lower part of the Guadiamar river, mainly affected by the acid waters, was lower than in the upper part, where soils were affected directly by the sludge. For example, closer to the source of the spill, soil total As contents were 95 times greater than in control soils and soil total Pb content 45 times greater (Madejón et al., 2002, 2004).

3.2. Composition of *S. maritimus* tubers

3.2.1. Macronutrients

The tubers of *S. maritimus* are reserve organs, rich in carbohydrates but poor in protein and mineral nutrients (Amat et al., 1991); for example in "Entremuros" they had 14 g kg^{-1} N, 4 g kg^{-1} K and 2.5 g kg^{-1} P (Table 3). According to recommendations for animal nutrition (e.g., Georgievskii, 1982a), K was within the range of 2–6 g kg^{-1}, required for most poultry, but P was below 8 g kg^{-1}, required for adult geese.

Calcium concentration was relatively low in the tubers: only about 0.4 g kg^{-1} in the inner tissues, and up to 5 g kg^{-1} when analysed with adhered soil, as consumed by geese (Table 3). These values are much lower than the adult goose requirement of 16 g kg^{-1} Ca (Georgievskii, 1982a). There was some difference between areas, (washed) tubers from the Doñana marshes had higher Ca values than from Entremuros (Table 1).

The concentration of Mg in unwashed tubers, up to 2.4 g kg^{-1}, was greater than poultry require (0.3–0.6 g kg^{-1}; Georgievskii, 1982a). Sodium concentration in tubers was generally adequate. The Na requirements for most poultry are 1.5 g kg^{-1}, but should not exceed the range 3–4 g kg^{-1} (Georgievskii, 1982a).

3.2.2. Micronutrients

In general, micronutrient concentrations were greater in tubers from "Entremuros" than from the Doñana marshes, with exception of Fe and Mn (in washed tubers). In the case of Mn, this pattern contrasts with the lower Mn concentrations in soils of Doñana marshes (Table 2).

Unwashed tubers had greater concentrations of micronutrients than washed tubers, especially in these from "Entremuros" (e.g., 9.7 times for Fe, and 3 times for Mn). Some of these tuber differences (especially for Fe and Zn) did not reflect the elemental ratios found in soils. These discrepancies seem to indicate that other variables, apart of soil composition, are influencing micronutrient content of the tubers.

The concentration of Zn in the tubers of *S. maritimus* from the spill-affected area was greater than in those from control marshes (Table 3), reflecting soil Zn pollution (Table 2). Zinc was identified as one of the main pollutants from the Aznalcóllar mine spill and several studies have found accumulation in plants (e. g., Madejón et al., 2002, 2003, 2004, 2006a). However, there was a temporal trend of reduced Zn accumulation in *S. maritimus* tubers. Soon after the mine spill (two months later, in June 1998), Meharg et al. (1999) reported a mean Zn concentration of 1390 mg kg^{-1}, with an extreme value of 23 731 mg kg^{-1}, for tubers of *S. maritimus* collected in "Entremuros"; while in this study (with tubers collected two years later) there were much lower Zn concentrations, up to 180 mg kg^{-1}. This significant reduction can be explained because tubers collected by Meharg et al. (1999) were exposed directly to acid waters, rich in Zn and other heavy metals and metalloids, while 2 years after the accident (this study), the area had stabilized and growth conditions for *Scirpus* changed.

The nutritional level of Zn in tubers from the unaffected marshes (mean value of 22 mg kg^{-1} for washed tubers) was smaller than the required nutritional range for poultry (30–70 mg kg^{-1}; Georgievskii, 1982b). On the other hand, the polluted tubers from "Entremuros" had a maximum Zn

Table 3
Concentration of macronutrients (g kg^{-1}), micronutrients (mg kg^{-1}) and non-essential (NE) trace elements (mg kg^{-1}) in tubers of *Scirpus maritimus* collected in the Doñana marshes (controls) and in the spill-affected "Entremuros", (mean ± SE, $N = 9$)

Element	Tubers (washed)		Inner tissues of the tubers (washed)		Tubers plus soil (unwashed)	
	Doñana marshes	"Entremuros"	Doñana marshes	"Entremuros"	Doñana marshes	"Entremuros"
Macronutrients						
N	9.8 ± 0.60	14.7 ± 1.06***	10.6 ± 0.81	14.4 ± 1.92	8.2 ± 0.5	13.6 ± 0.9***
P	1.5 ± 0.07	2.0 ± 0.11***	1.9 ± 0.11	2.5 ± 0.31	1.7 ± 0.1	2.1 ± 0.2
K	2.9 ± 0.17	3.3 ± 0.23	4.2 ± 0.43	4.0 ± 0.38	4.3 ± 0.3	4.4 ± 0.3
S	0.9 ± 0.05	0.9 ± 0.04	0.67 ± 0.02	0.75 ± 0.06	0.8 ± 0.04	1.1 ± 0.04***
Ca	1.9 ± 0.23	1.0 ± 0.11***	0.45 ± 0.07	0.43 ± 0.13	3.2 ± 0.5	4.9 ± 0.7
Mg	1.0 ± 0.06	1.0 ± 0.08	0.65 ± 0.02	0.80 ± 0.09	1.3 ± 0.1	2.4 ± 0.5**
Na	0.25 ± 0.03	0.21 ± 0.03	0.11 ± 0.02	0.12 ± 0.04	2.9 ± 0.3	2.7 ± 0.4
Micronutrients						
Cu	8.93 ± 0.72	13.0 ± 0.88**	6.60 ± 0.59	10.2 ± 1.11**	9.7 ± 0.71	18.0 ± 1.12***
Fe	1003 ± 261	165 ± 45.6***	74.5 ± 19.4	53.2 ± 7.63	1247 ± 221	1603 ± 375
Mn	65.7 ± 5.54	42.0 ± 4.43**	13.9 ± 1.26	20.9 ± 3.13	76.7 ± 6.0	126 ± 24
Zn	21.7 ± 0.74	61.3 ± 5.38***	20.4 ± 0.90	47.2 ± 5.04***	13.5 ± 0.82	125 ± 13.5***
NE trace elements						
As	3.17 ± 0.74	0.97 ± 0.15***	0.40 ± 0.11	0.19 ± 0.03	3.39 ± 0.54	2.08 ± 0.31*
Cd	0.02 ± 0.003	0.25 ± 0.08***	0.008 ± 0.002	0.11 ± 0.03***	0.23 ± 0.08	0.62 ± 0.09**
Pb	1.38 ± 0.16	1.15 ± 0.16	0.27 ± 0.03	0.36 ± 0.07	6.83 ± 3.24	6.10 ± 2.25
Tl	0.02 ± 0.002	0.03 ± 0.006	0.010 ± 0.001	0.014 ± 0.004	–	–

Significance levels between the two areas are indicated: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

value of 180 mg kg^{-1}, close to the recommended threshold level (<178 mg kg^{-1}) to prevent marginal sublethal effects, but lower than the level (<2000) to prevent the death of chicks and ducklings (Meharg et al., 1999).

Differences in Cu content of tubers were similar to that found for Zn, with a consistently higher concentration in the spill-affected area (Table 3), despite differences in soil pollution not being significant (Table 2). The Cu concentration in tubers from Doñana marshes (10 mg kg^{-1}) was within the required nutritional range for most poultry (3–11 mg kg^{-1}), while in the spill-affected area, tubers contained up to 26 mg kg^{-1} of Cu (Georgievskii, 1982b).

The pattern of Fe concentration in tubers was contrary to those shown for Zn and Cu; with much higher values in the control marshes than in the spill-affected "Entremuros" (1003 vs. 165 mg kg^{-1} in washed tubers; Table 3), despite non-significant differences between soils (Table 2). The ANOVA test by site showed significant differences ($F = 4.63$, $p = 0.01$); the tubers from one site located in an abandoned channel ("Caño Guadiamar") had the highest Fe concentration (mean of 1699 mg kg^{-1}), greater than all other sites in the Doñana marshes (mean of 656 mg kg^{-1}) (Fig. 2).

The Fe concentration in both areas is higher than the nutritional requirements for poultry (50–80 mg kg^{-1}), but the maximum observed value (3000 mg kg^{-1}) is above the tolerance threshold (1000 mg kg^{-1} of Fe^{2+} for chicken; Chaney, 1989), and may induce toxic effects. In addition, tuber surfaces may accumulate As-rich iron plaque due to release and diffusion through soil of O_2 released by plant roots, with a further oxidation and precipitation of reduced Fe, providing sorption/co-precipitation sites for As in the soil solution (Blute et al., 2004; Taggart et al., 2005). However, potential toxicity of Fe-rich tubers is only found at one site within the Doñana marshes, probably indicating past pollution originating from mining activities, drained through the now not functional "Caño Guadiamar".

Tubers (washed samples) from the Doñana marshes also had higher Mn concentration than those from Entremuros (Table 3), and all of them were within the required nutritional range for poultry (30–70 mg kg^{-1}; Georgievskii, 1982a).

3.2.3. Non-essential trace elements

Concentration of trace elements were similar (in the case of Pb and Tl), greater (for As), or smaller (for Cd) in tubers from the Doñana marshes than those from Entremuros. Unwashed tubers had greater concentrations than washed tubers, with a maximum increase (11.5 fold) for Cd in tubers from the Doñana marshes.

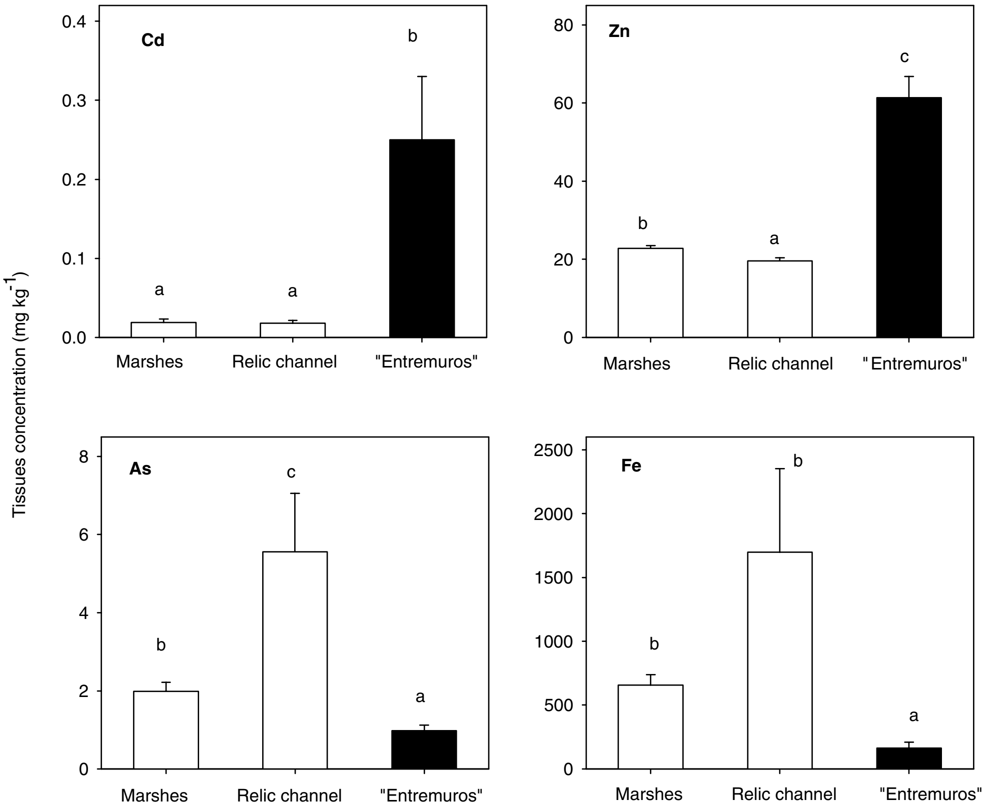

Fig. 2. Concentration of the "spill elements" Cd and Zn (above), and the "cryptic pollution elements" Fe and As (below), in (washed) tubers of *Scirpus maritimus*. The tubers from spill-affected "Entremuros" sites ($N = 9$; black bar) are compared with those from the Doñana marshes ("Arroyo de la Madre" and "Caño Travieso" sites, $N = 6$; white bar), and those with cryptic pollution in a relic Guadiamar channel ("Caño Guadiamar", $N = 3$; white bar). Mean and S.E. bars are indicated; bars with the same letter do not differ significantly by Tukey test ($p < 0.05$).

Cadmium together with Zn, strongly accumulated in plants polluted by the Aznalcóllar mine spill (e.g., Madejón et al., 2002, 2003, 2004, 2006a). However, there was a temporal reduction in tuber Cd concentration, from the high values reported 2 months after the spill (mean 3.6 mg kg^{-1} and a maximum value of 18.2 mg kg^{-1}; Meharg et al., 1999) down to a maximum of 0.9 mg kg^{-1} (for unwashed tubers) reported here 2 years later. Despite the 1998 Cd values exceeding the maximum level of 0.5 mg kg^{-1} tolerated by livestock (Chaney, 1989 , but see Beyer, 2000), 2 years later Cd values in tubers were of low or negligible toxicity.

Arsenic concentration in *S. maritimus* tubers showed a striking pattern, with higher values found in the Doñana marshes (Table 3), despite higher As contents in the "Entremuros" sediments (Table 2). The ANOVA test showed a significant difference in tuber As concentration by site ($F = 7.02$, $p = 0.003$); maximum As values, as in the case of Fe, were found in tubers from the abandoned waterway "Caño Guadiamar" (mean 5.5 mg kg^{-1}), while tubers from other Doñana sites had lower As contents (mean 2.0 mg kg^{-1}), and were not significantly different than those from the spill-affected "Entremuros" (mean of 1.0 mg kg^{-1}) (Fig. 2). The association of As with a root/tuber iron plaque (as mentioned above) may pose an ecotoxicological risk for herbivores (Taggart et al., 2005).

The high concentration of As in the mine sludge (up to 4000 mg kg^{-1}, Cabrera et al., 1999), caused some social alarm due to its known toxicity and some accumulation in plants has been documented (Madejón et al., 2002). However, the metal-rich waters entering the channelled "Entremuros" region contained relatively little As in solution (Pain et al., 1998); although some As was deposited with As-rich suspended particulates (Taggart et al., 2005). Despite the presence of 18 mg kg^{-1} As in the "Entremuros" sediment (Table 2), *S. maritimus* tubers only accumulated about 1.0 mg kg^{-1} (Table 3), very similar levels to those recorded 2 years earlier (1.1 mg kg^{-1} in 1998; Taggart et al., 2005).

In this study we have detected some "outlier" values of As in *S. maritimus* tubers, which may have a significant ecological impact. Some tubers from the Doñana marshes, considered as "natural", non-affected controls, have much higher As concentrations than those in the spill-affected "Entremuros". We suggest that the sampling site selected in the marshes, the "Caño Guadiamar", has a significant residual contamination which may be attributed to historic mining activities in the region discharging into the Doñana marshes through this abandoned, "natural" Guadiamar channel. Previous studies to the mine accident have documented that waters of Guadiamar river, entering the Doñana National Park, were characterised by high heavy metal contents, in special during flood events, due to the transport of heavy metal-rich solids precipitated upstream (Cabrera et al., 1987; Arambarri et al., 1996).

Although soil As pollution was not detected in total or extractable fractions (Table 2), *S. maritimus* tubers acted as "biosensors", showing up that cryptic environmental pattern (see Madejón et al., 2006b). Because tubers are a main source of food for wintering geese, the spatial distribution and intensity levels of this As pollution in the Doñana marshes should be investigated.

Other trace elements, such as Pb and Tl, did not show significant differences between spill-affected "Entremuros" and Doñana marshes (Table 3). Maximum values of Pb in tubers, (up to 24.6 mg kg^{-1} in unwashed tubers from the "Caño Guadiamar" site) were smaller than the maximum levels tolerated by chicken (30 mg kg^{-1}; Chaney, 1989). Thallium concentrations of the tubers from both areas were very low (range of 0.01–0.07 mg kg^{-1}), following the general trend reported for other spill-affected plants (Madejón et al., 2002, 2003, 2004); with the exception of the unusual high Tl concentration (ca. 50 mg kg^{-1}) in the flowers of the herbaceous crucifer *Hirschfeldia incana* (Madejón et al., 2005).

4. Conclusions

Two years after the Aznalcóllar mine spill, soils and sediments of "Entremuros", affected by the acid waters, had a relatively low level of pollution. In soils Cd and Zn were the main pollutants in both total and EDTA-extractable fractions. However, with exception of Cd, concentrations of As, Pb, Cu, Zn and Tl in *S. maritimus* tubers were well below toxic limits for birds reported in the literature. A temporal (from 1998 to 2000) reduction in concentrations of Cd and Zn in tubers was also demonstrated, reflecting a progressive normalization of this important wintering area for greylag geese. However, Cd concentrations in unwashed tubers are still greater than the toxic limit of 0.5 mg kg^{-1} established for most animals, which makes further monitoring of this metal in plants of "Entremuros" area advisable.

Surprisingly, As and Fe had some anomalous high values in tubers for one site, "Caño Guadiamar", in the Doñana marshes (considered to be a non-affected control). We suggest that cryptic pollution may respond to the influence of old, abandoned channels of the Guadiamar river, which have historically discharged sediments enriched with metals originating from past mining activities, upstream in the drainage basin. The possible ecological impact of this As pollution on wintering geese and other animals should be investigated.

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