



Mercury species accumulation and distribution in *Typha domingensis* under real field conditions (Almadén, Spain)

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Abstract

Monomethylmercury (MeHg) is one of the most toxic and the most commonly occurring organomercury compound and the wetlands are one of the main areas of generation of this Hg form. Concretely, it is in the macrophyte root system where better conditions are given for its generation. However, the knowledge of absorption and subsequent distribution of mercury (Hg) and monomethylmercury in aquatic plants is still limited. Mercury mining district such as Almadén (Ciudad Real, Spain) is a natural laboratory where different rivers flow and the species *Typha domingensis* Pers. is a common macrophyte which grows in their riverbanks. The aim of the present work is to apply a recently developed method specially designed to analyze Hg species in plant tissues to the different fractions of *T. domingensis* under real field conditions and to study the accumulation and distribution of Hg species (inorganic Hg and MeHg) within the plant. The results proved that whatever Hg species has preference to be accumulated in the belowground fractions and demonstrated a high efficiency in the accumulation of MeHg.

Keywords Mercury species · Sediment · *Typha domingensis* · Bioaccumulation factors · Distribution · Field conditions

Introduction

Mercury (Hg) is one of the most toxic and widespread trace metal in the world (Adriano 2001). In addition of natural sources of Hg, some human activities, such as mining and smelting, chlor-alkali production, fossil fuel burning, or pesticide manufacturing, have greatly favored its release into the environment (Wang et al. 2004). Within the organic Hg species, dimethylmercury is the most toxic compound followed by monomethylmercury (MeHg) which is the most common

organomercury form in the environment with a high capacity of bioaccumulation and biomagnifying in the food web (Braza et al. 2000; Mergler et al. 2007). The production of this compound is related to the presence of sulfate-reducing bacteria (and other methylating microorganisms) and anoxic conditions (Choi and Bartha 1994). Thus, the wetlands are one of the main areas of generation of MeHg (O'Driscoll et al. 2005). However, the studies carried out on Hg speciation in aquatic environments have been mostly focused on accumulation in animals, with scarce works in plants, although it is in the root system where better conditions are given for the generation of MeHg. Some of these conditions are the release of labile carbon from root exudates or the regeneration of metabolic substrates at the oxygenated rhizosphere-bulk sediment interface which stimulate sulfate-reducing bacterial activity (Canário et al. 2007; Cosio et al. 2014; Windham-Myers et al. 2009). In this way, the plants are one of the first organisms that are exposed to this compound.

The use of macrophytes in order to reduce, remove, or immobilize pollutants from water and sediments has been spreading throughout the last decades due to the fact that is a sustainable, environmental friendly, and low-cost technology (Rai 2009). In order to know the behavior of this kind of plants and their potential use in phytoremediation, it is necessary to

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carry out field trials in addition to laboratory tests because the metal effects in a real scenario may differ from those observed under laboratory controlled conditions. However, there is a lack of studies specifically focused on Hg under field conditions. Millán et al. (2014) and Lominchar et al. (2015) carried out studies about total Hg accumulation in *Typha domingensis* Pers. and concluded that it could be used as a bio-monitor as well as a phytoextraction technology in areas affected by Hg pollution. Simon and Boudou (2001) reported that other aquatic plants such as *Spartina* sp. and *Elodea canadensis* have the ability to absorb organic Hg and transform it into an inorganic form, meanwhile Gothberg and Greger (2006) and Greger and Dabrowska (2010) reported a contrary phenomenon, the MeHg could be formed in macrophytes. Due to the controversy, the scarce works, and the fact that the root system of macrophytes can be conveyor of Hg from the sediments to the food chain (Cosio et al. 2014; Miretzky et al. 2004; Simon and Boudou 2001), more studies focused on macrophytes are required in order to determine whether there is methylation, demethylation, or reduction of Hg inside it and what mechanisms are involved. A first step in order to reach this aim is to study the speciation of this element.

Currently, there are different methods used for analyzing MeHg in plants but they require a sequence of several time-consuming steps. Moreover, these methods are usually selective for MeHg and do not allow the simultaneous determination of other Hg species. In a previous study, we developed a fast and sensitive method for the simultaneous determination of Hg species, specifically designed for plant tissue (Jiménez-Moreno et al. 2018). In that work, MeHg spiked and non-spiked CRMs certified for total Hg were used. Furthermore, the optimization and adaptation of Hg species extraction using *T. domingensis* leaves were performed. The present work goes one step further, and its aim is the application of this method to the different fractions of *T. domingensis* Pers. (wetland's plant) grown under real field conditions in an area with high-Hg concentrations in order to know the accumulation and distribution of Hg species (inorganic Hg and MeHg) within this aquatic plant. In addition, the method precision on each wild plant fraction (leaves, rhizomes, roots, and root cores) was also studied.

Material and methods

Study area and samples collection

The present study was carried out on the Valdeazogues River basin (Fig. 1) within the Almadén mining district (Ciudad Real, Spain). This area is recognized as the largest Hg reservoir in the world, and its exploitation has been developed over 2000 years, being finished at the beginning of twenty-first century.

As can be seen in Fig. 1, three sampling points were selected. In these areas, as well as throughout the district, plants of

Typha domingensis Pers. grow naturally in the riverbanks. One of the sampling points was located at Azogado stream (AZ-1), which course collects the waters from the Almadén mine (one of the most important Hg mine in the district) and its metallurgical complex. And the other two were placed in the Valdeazogues River, the first one (VZ-1) was located before the confluence of Azogado stream with Valdeazogues River and the second one (VZ-2) was located after this confluence and close to the old railway station of Chillón that was used to load and transport cinnabar ore in the past.

In each location, five plant samples were collected into plastic bags in June 2013. Moreover, sediment samples under each selected plants were collected into Teflon bottles of 250 mL previously washed in a nitric acid solution (5% v:v) during 24 h and then rinsed thoroughly with Milli-Q water. All samples were stored in cooler until their analysis in the laboratory.

Sample analyses

Sediment samples were freeze-dried in a LyoQuest – 55 system (Telstar), applying a program with an initial ultra-fast freezing from room temperature to $-50\text{ }^{\circ}\text{C}$ sustained for 2 h, followed by a sustained temperature of $-50\text{ }^{\circ}\text{C}$ and vacuum at 0.2 mbar for 24 h. After that, they were sieved to the fine fraction $< 2\text{ mm}$ and ground in agate mortar (RM 200, Retsch). Collected plants were divided into four fractions: leaves, rhizomes, roots, and root cores. This last part was considered as the joining point of rhizome, roots, and leaves and therefore, the transition between belowground part and aerial part. After the division, each plant tissue was rinsed several times with distilled water in ultrasonic bath (Ultrasons-H, Selecta), freeze-dried and ground with an electric mill (A 11, IKA).

Mercury species in sediments were extracted and quantified according to the procedure developed by Berzas Nevado et al. (2008). For that, about 1 g of each sediment sample was extracted with 10 mL of 6 N HNO_3 using a closed-vessel microwave (Milestone Ethos Plus) applying a ramp of 5 min from room temperature to $80\text{ }^{\circ}\text{C}$ and kept it for $80\text{ }^{\circ}\text{C}$ for 5 min. The quantification was carried out by gas chromatography (Shimadzu, model GC 2010) coupled to atomic fluorescence detection (Millenium Merlin model 10.025) commonly named as GC-pyro-AFS.

For Hg speciation analysis in plants, the novel analytical method specifically for the mercury speciation in plants developed by Jiménez-Moreno et al. (2018) was employed. In this case, 0.2 g of each plant fraction was extracted with 10 mL of 6 N HNO_3 in the same microwave system used for Hg speciation in sediments. The temperature program applied was a ramp of 10 min from room temperature to $80\text{ }^{\circ}\text{C}$, followed by a sustained temperature of $80\text{ }^{\circ}\text{C}$ for 10 min. After that, simultaneous Hg species determination was carried out by derivatization with sodium tetraethylborate and injection into GC-pyro-AFS. An additional pre-concentration step by

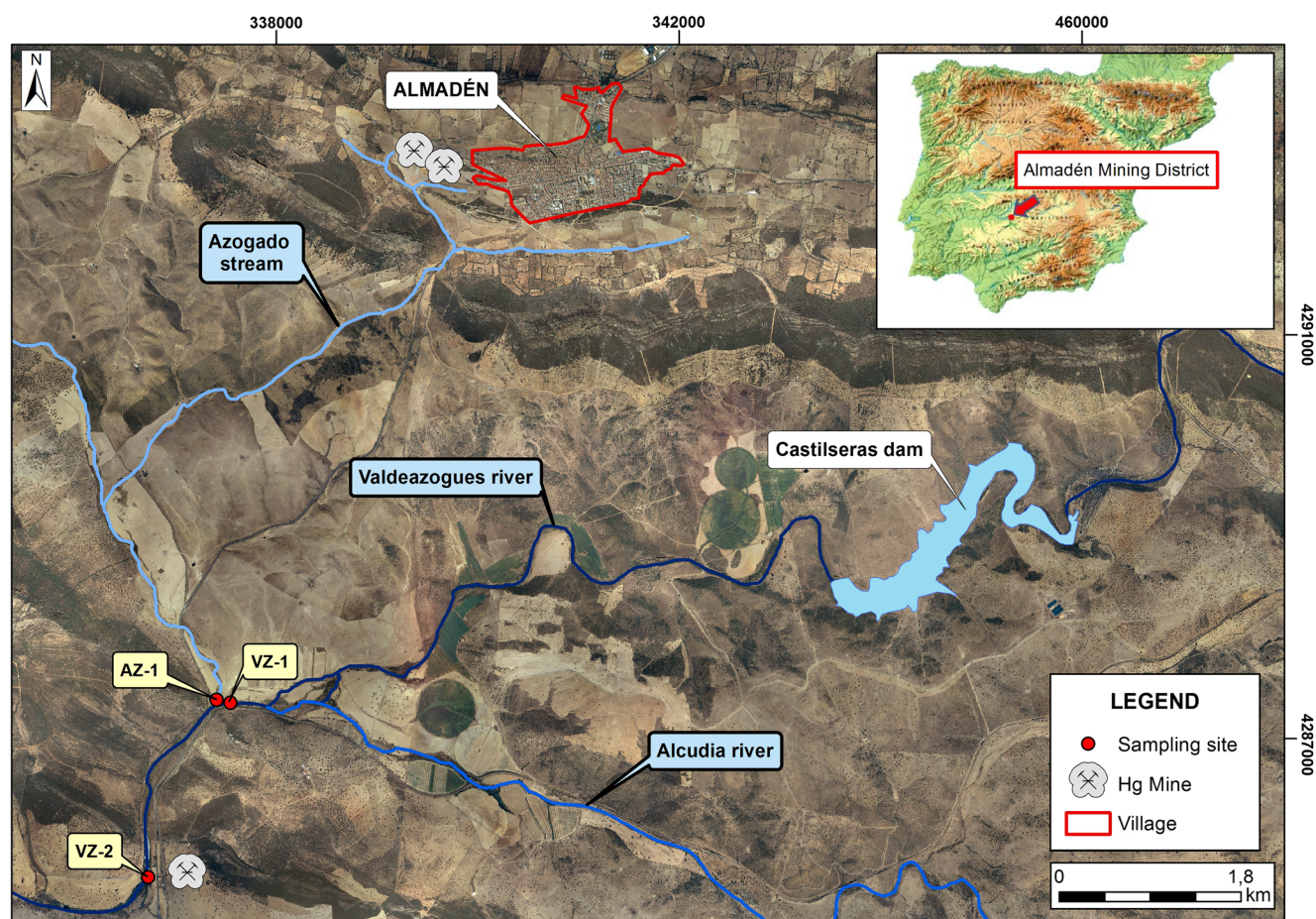


Fig. 1 Sampling sites in Almadén mining district

evaporation under a nitrogen stream of $120\text{--}150\text{ mL min}^{-1}$ during 10 min was also used. Detection limits were 0.7 and 0.8 ng g^{-1} for inorganic Hg (IHg) and MeHg, respectively. The total Hg concentration was calculated as sum of species for both sediment and plants.

Furthermore, certificate reference materials (CRM) were analyzed to test the accuracy of both methods. In this case, IAEA-405 (polluted marine sediment) and ERM-CC580 (estuarine sediment) were the CRMs employed for the sediment analyses and spiked BCR-060 (Aquatic plant), BCR-482 (liken), and NCS ZC73027 (rice) for plants. Certified and spiked concentration did not differ significantly from measured concentration according to a t test with a level of significance of 95%. All the extractions of sediments, plant fractions, and CRMs were performed in duplicate, and their analyses were developed in triplicate.

Statistical analyses

The Shapiro-Wilk's test was performed to determine the Normal distribution of the data set while the Levene test was carried out to check the homoscedasticity of values. In every case of the present study, there was no normality assumption

and sample size was small, therefore nonparametric tests were performed. Kruskal-Wallis test was used to compare medians of k independent samples with more than two categories (Hg in the different plant fractions; the relative standard deviations (RSD) of the plant fraction Hg measurements and RSD of the different Hg species in order to study the method variability; Hg in the sediments of the different sampling points). Mann-Whitney test was used between pairs of the above conditions (two independent samples). The significant level was calculated as follows: $0.01/k$ where k is the number of pair combinations to compare.

Results and discussion

Hg and MeHg in sediments

The average concentrations of IHg and MeHg in the sediments collected in each sampling point are shown in Table 1. According to the results, the lowest IHg concentration was measured in the sampling point VZ-1, while the highest was recorded in AZ-1 as a consequence of the material transported from the Almadén mine, its metallurgical complex and the mining tailing. Thus, the confluence between

Table 1 Concentrations of MeHg (ng g^{-1}) and inorganic Hg ($\mu\text{g g}^{-1}$) in sediments. Me. = median. $\bar{x}$ = mean. SD = standard deviation. Concentrations obtained from the analysis of five different samples at each sampling point

Sampling point	MeHg (ng g^{-1})			IHg ($\mu\text{g g}^{-1}$)			% MeHg
	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	
VZ-1	8.6	8.7	0.5	39.8	39.7	2.1	0.021
VZ-2	11.8	11.7	0.8	422	421	38	0.003
AZ-1	22.8	23.1	1.5	734	724	39	0.003

Azogado stream and Valdeazogues River produces a clear influence in the last one, increasing ten times the Hg concentration in VZ-2 in relation to VZ-1. It would be expected a high-Hg concentration in VZ-1 because previously to that location, the river has crossed a large area of the mining district. However, the existence of some meanders, backwaters, and especially the presence of the Castilseras dam upstream of this sampling point favor that the dragged materials precipitate. This behavior was also observed in previous works (García-Ordiales et al. 2014; Lominchar et al. 2015).

Regarding to MeHg, the tendency was very similar to IHg, with the highest concentration measured at the sampling point AZ-1, which was twice higher than the other two sampling sites. In all cases, the percentage of MeHg in relation to total Hg was very low, being less than 0.03% for VZ-1 and 0.003% for VZ-2 and AZ-1.

A previous study carried out by Berzas Nevado et al. (2009), where sediment samples were taken in the Almadén mining district in 2006 showed that the MeHg and total Hg concentrations in an area close to point VZ-1 were $8.6 \pm 0.7 \text{ ng g}^{-1}$ and $22 \pm 1 \mu\text{g g}^{-1}$, respectively, which are close to the values of the present work although the percentage of MeHg to total Hg was higher (0.04%). However, the concentration of MeHg obtained in other location near to VZ-2 reached a value of $880 \pm 24 \text{ ng g}^{-1}$ (1.2%) which was 38 times higher than the value of 2013, while the concentration of total Hg was six times lower. These results demonstrated that the production of MeHg in dynamic locations like rivers can be very changeable along the time. In addition to sediment transport, there are many factors such as temperature, pH, organic carbon, salinity, redox potential, or the presence of plants that are not always stable over time and can affect the generation of MeHg (Barkay et al. 1997; Canário et al. 2007; Gentès et al. 2013).

Moreover, the MeHg concentrations and the percentages of MeHg to total Hg obtained in the present study were also similar to those recorded in other aquatic environments around the world that are also affected by Hg mining, such as sediments from southwestern Alaska, Idrija Hg mine (Slovenia), or Palawan Hg mine (Philippines) (Gray et al. 2000; Gray et al. 2003; Hines et al. 2000; Horvat et al. 2004) or paddy soils dedicated to rice cultivation in different mining regions of China (Horvat et al. 2003; Meng et al. 2010, 2014). However, the percentages of MeHg to total Hg in other areas affected by Hg mining, such as Monte Amiata (Italy) or Hg

mines of California (USA), were 30 times higher as average than the present study ones (Davis et al. 2008; Rimondi et al. 2012; Ryuba 2003).

Mercury speciation in the different plant fractions

Total Hg in leaves (IHg + MeHg) did not surpass neither the limits of hyperaccumulation ($10 \mu\text{g g}^{-1}$) nor the threshold of toxicity ($1\text{--}3 \mu\text{g g}^{-1}$) for that metal (Kabata-Pendias 2011; Lasat 2003).

The concentrations of Hg species in each plant fraction are shown in Fig. 2 and Table 2. As it can be observed, the highest concentrations of IHg were measured in the belowground

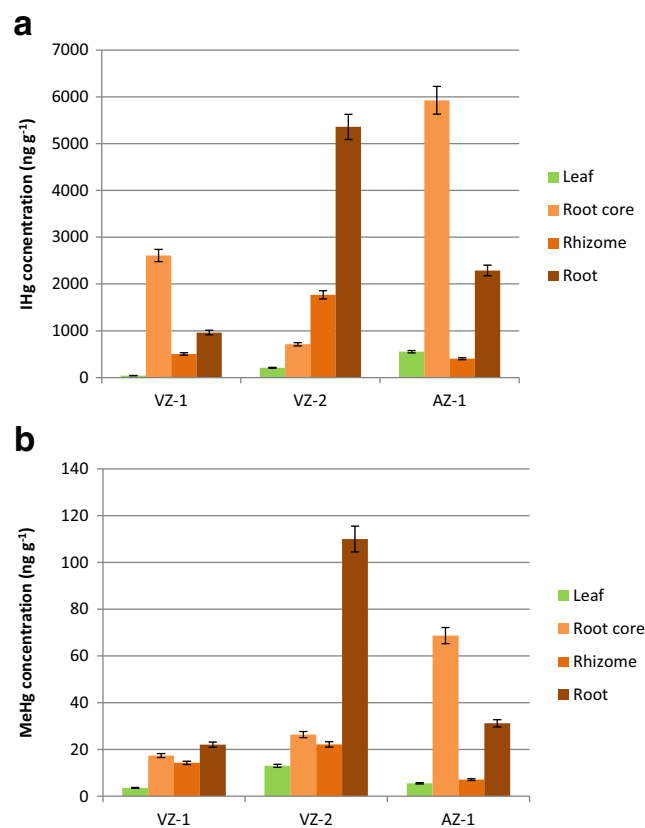


Fig. 2 **a** Distribution of inorganic Hg in the different *T. domingensis* fractions collected in different locations along the Valdeazogues River basin (Ciudad Real, Spain). **b** Distribution of MeHg in the different fractions of *T. domingensis* collected in different locations along the Valdeazogues River basin (Ciudad Real, Spain). The values are medians $\pm$ error bars: 95% confidence interval

Table 2 MeHg and IHg concentrations (ng g^{-1}) and percentages of MeHg with respect to total Hg in the different fractions of *Typha domingensis* samples collected in Almadén mining district in 2013. Me = median. $\bar{x}$ = mean. SD = standard deviation. Concentrations obtained from the analysis of five different samples at each sampling point

Sampling point	Leaves								Root cores						Rhizomes						Roots															
	MeHg (ng g ⁻¹)				IHg (ng g ⁻¹)				% MeHg				MeHg (ng g ⁻¹)				IHg (ng g ⁻¹)				% MeHg				MeHg (ng g ⁻¹)				IHg (ng g ⁻¹)				% MeHg			
	Me.		$\bar{x}$		SD		$\bar{x}$		SD		Me.		$\bar{x}$		SD		Me.		$\bar{x}$		SD		Me.		$\bar{x}$		SD		Me.		$\bar{x}$		SD			
	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD	Me.	$\bar{x}$	SD			
VZ-1	3.6	3.6	0.3	42	47	16	7.6		17.4	17.7	2.0	2608	2617	281	0.7		14.3	14.7	6.1	505	532	44	2.8		22.1	18.5	3.9	962	960	25	1.9					
VZ-2	13.0	13.7	2.0	209	209	8.2	6.6		26.4	25.5	4.6	711	711	32	3.6		22.2	21.0	4.2	1767	1781	163	1.2		110	113	41	5358	5361	253	2.1					
AZ-1	5.5	5.4	1.1	552	555	27	1.0		68.7	70.7	4.8	5927	5905	45	1.2		7.1	6.8	0.5	403	417	52	1.6		31.2	31.7	1.8	2288	2225	369	1.4					

fractions of *T. domingensis*, being this pattern also found for total Hg in other studies with *T. domingensis* and *T. latifolius* (Anning et al. 2013; Lominchar et al. 2015; Willis et al. 2010). In the case of MeHg, similar preference to be accumulated in the belowground fractions was observed, and it is also common to other macrophyte species such as *Elodea nuttallii* (Regier et al. 2013), *Vaccinium myrtillus*, *Calamagrostis villosa*, and *Deschampsia flexuosa* (Schwesig and Krebs 2003).

The MeHg portion of total Hg in leaves, root cores, rhizomes, and roots ranged from 1.0 to 7.6%, 0.7 to 3.6%, 1.2 to 2.8%, and 1.4 to 2.1%, respectively. Comparing these results with other plants, it can be found that the MeHg percentages of leaf and root obtained in the present work were higher than those measured by Meng et al. (2010) in rice crops growing in a mercury mining area. In addition, the MeHg ratios in leaf of *Typha* were higher than those obtained in species of *Sarcocornia fruticosa*, *Halimione portulacoides*, and *Spartina maritima* which were collected in a contaminated salt marsh (Canário et al. 2010). However, Regier et al. (2013) reported percentages of MeHg in shoots of *E. nuttallii* (submerged freshwater plant species) up to 28%. It is known that metal accumulation in emergent aquatic macrophytes like *T. domingensis* is usually lower than submerged or floating species (Albers and Camardese 1993).

The reproducibility was studied between the Hg species (MeHg and IHg) and between the different fractions of the wild plants. In order to calculate the method precision for both Hg species measured in the different fractions of the plant and because of the different averages, it was calculated the relative standard deviations (RSD) as percentage of the typical deviation corresponding to the average (Table 2). The statistical results showed that it was not possible to establish significant differences among the RSD of the different parts of the plant for both Hg species. The variability of the method applied to the different fractions of the plant was homogeneous ($H(3) = 1.45, p > 0.05$). Thus, the different fractions (different matrix) did not seem to interfere in the analysis method.

The translocation factor (TF), which is the ratio between the concentration in aerial part and roots, was lower than 1 for both Hg species (Table 3). This factor confirmed the excluder behavior of this plant with a restricted transport of Hg and MeHg from the roots to the leaves (Baker 1981). This response is very common in plants, making it possible to protect

Table 3 Translocation factor of each Hg species

Sampling point	TF	
	MeHg	IHg
VZ-1	0.194	0.049
VZ-2	0.121	0.039
AZ-1	0.170	0.250

Table 4 Bioaccumulation factors of MeHg and IHg in each fraction of *T. domingensis*

Plant fraction	BAF					
	VZ-1		VZ-2		AZ-1	
	MeHg	IHg	MeHg	IHg	MeHg	IHg
Leaf	0.4	0.001	1.2	0.000	0.2	0.001
Root core	2.0	0.066	2.2	0.002	3.1	0.008
Rhizome	1.7	0.013	1.8	0.004	0.3	0.001
Root	2.1	0.024	9.7	0.013	1.4	0.003

themselves from the adverse effects of Hg in photosynthetic processes such as the inhibition of the photosystem II (Bernier et al. 1993; Kupper et al. 1996). In the present study, and taking into account the Hg species, the TF of MeHg was higher than TF of IHg. Furthermore, in the case of MeHg, TF was similar in all cases, while the TF of IHg seemed to depend on other factors like Hg concentration in the sediment of every sampling point. Lominchar et al. (2015) calculated TF of total Hg for *T. domingensis* sampled in Valdeazogues River in 2010 and 2011. Those values were similar to the TF of IHg calculated in the present work.

Furthermore, in order to know the efficiency of *T. domingensis* to uptake and accumulate IHg and MeHg in their tissues, bioaccumulation factor (BAF) was calculated as the ratio between Hg species concentration in each fraction and the respective sediment (Yoon et al. 2006). According to Table 4, the IHg-BAF value was lower than 1 in all cases and the leaves had the lowest IHg-BAF value. However, the MeHg-BAF was higher than 1 in most cases, which represents the existence of a tendency to accumulate this compound within the plant (Zhang et al. 2010; Meng et al. 2010). MeHg in belowground fractions in areas with high-Hg concentrations is generally higher than those found in surrounding sediments. Further studies focused on *T. domingensis* need to carry out in order to determine what processes and mechanisms take place inside it.

Conclusions

In the present study, the sediment samples collected around the confluence of Azogado Stream with Valdeazogues River in the Almadén mining district showed that the Azogado Stream produces a high influence into the Valdeazogues River.

The precision of the new method for Hg speciation analysis developed by Jiménez-Moreno et al. (2018) did not vary among the different fractions of the plant. Thus, the developed method is applicable to every fraction of the *Typha domingensis* Pers. grown under real field conditions. Regarding the distribution of Hg species in samples of

T. domingensis from Almadén mining district, the results showed a preference to be accumulated in the belowground fractions with a low translocation to aerial part. Furthermore, BAF values demonstrated a high efficiency in the accumulation of MeHg.

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