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Assessing the Legacy of Red Mud Pollution in a Shallow Freshwater Lake: Arsenic Accumulation and Speciation in Macrophytes

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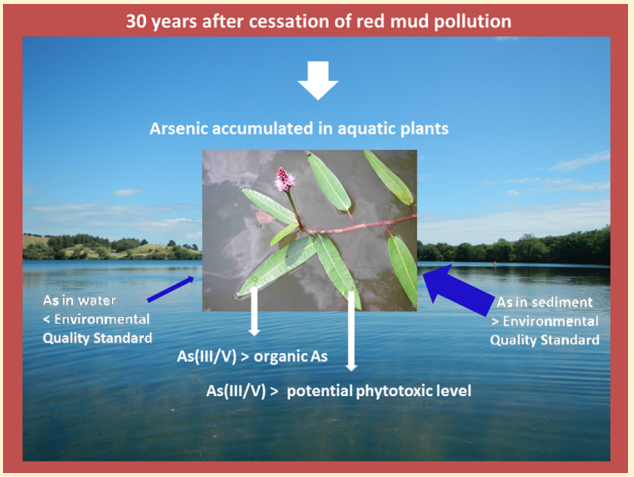
Supporting Information

ABSTRACT: Little is known about long-term ecological responses in lakes following red mud pollution. Among red mud contaminants, arsenic (As) is of considerable concern. Determination of the species of As accumulated in aquatic organisms provides important information about the biogeochemical cycling of the element and transfer through the aquatic food-web to higher organisms. We used coupled ion chromatography and inductively coupled plasma mass spectrometry (ICP-MS) to assess As speciation in tissues of five macrophyte taxa in Kinghorn Loch, U.K., 30 years following the diversion of red mud pollution from the lake. Toxic inorganic As was the dominant species in the studied macrophytes, with As species concentrations varying with macrophyte taxon and tissue type. The highest As content measured in roots of *Persicaria amphibia* (L.) Gray (87.2 mg kg^{-1}) greatly exceeded the 3−10 mg kg^{-1} range suggested as a potential phytotoxic level. Accumulation of toxic As species by plants suggested toxicological risk to higher organisms known to utilize macrophytes as a food source.



INTRODUCTION

The estimated global production of red mud, a byproduct of alumina production, is ∼120 million t a^{-1}.[1] In October 2010, failure of a containment reservoir in Ajka, Western Hungary, resulted in the release of ∼1 million m^3 of red mud waste, contaminating the Marcal River (catchment area 3078 km^2) and entering the Danube River.[2] The Hungarian red mud spill raised concerns about inappropriate storage of red mud and the impact of the waste on the receiving environment. However, little is known about the geochemical behavior of red mud in freshwaters[2] or of the short- and long-term ecological impacts and likelihood of recovery following its release into the environment. This is conspicuous given that many of its chemical constituents are redox sensitive and so likely to persist in aquatic depositional environments where they can represent an environmental and human health risk.

Red mud is highly alkaline due to the addition of sodium hydroxide (NaOH) during the production process and contains metal oxides and elevated concentrations of a range of minor trace elements[3−5] that can be toxic to aquatic organisms. Initial research after the Ajka accident focused on the short-term impact of the pollution on freshwaters. Mayes et al.[6] reported elevated concentrations of some contaminants, e.g., arsenic (As), vanadium (V), chromium (Cr), and nickel (Ni), in fluvial sediment downstream of the spill. The association of these elements mainly with residual phases suggests limited potential for their mobilization in the environment. However, in depositional zones, release of these elements may be expected.[6] Furthermore, river sediments contaminated with constituents of red mud can have pronounced toxic effects, for example, causing reduced bioluminescence of *Vibrio fischeri* and growth of *Lemna minor* and *Sinapis alba*.[2] In aquatic ecosystems with short residence times, the long-term effects of pollution might be spatially limited to depositional zones, allowing system recovery.[2] However, the residence time of pollutants in higher

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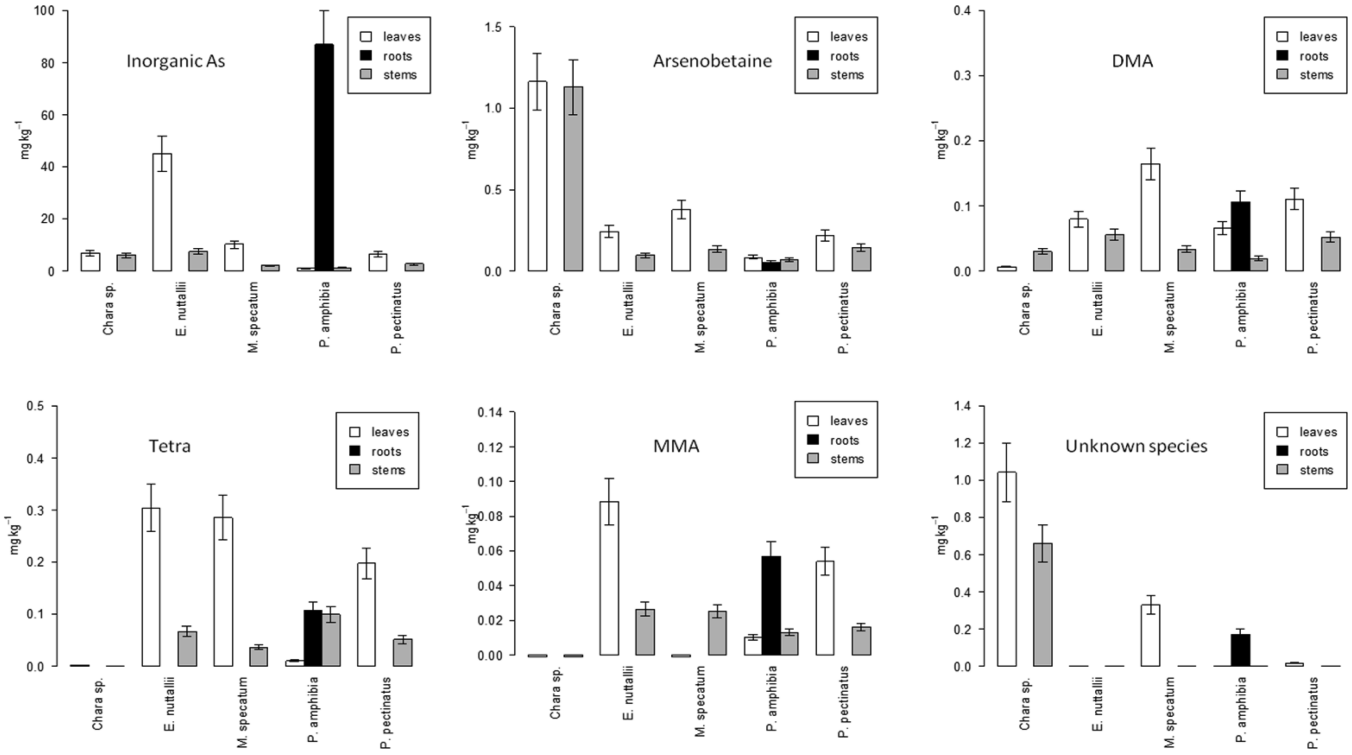

Figure 1. Concentrations of As species in tissues of five macrophyte species in Kinghorn Loch in July 2013. Root tisues were only analyzed for *P. amphibia*. Bars represent the mean of 3 samples from each plant tissue and error bars standard deviation of the mean. Note the different *y*-axis scales.

retention time aquatic ecosystems can exceed those in fluvial systems.[7,8] Therefore, long-term monitoring of the red mud impact on aquatic life is particularly important in lakes that can have potentially longer time-scales of recovery.

Among red mud contaminants, As is of considerable concern. It is a toxic element,[9] with high concentrations in the aquatic environment posing an environmental and human health risk in many countries, e.g., in Pakistan, Bangladesh, India, Taiwan,[10] Japan,[11] China,[12] and the U.S.A.[13] Arsenic concentrations in contaminated groundwater were reported up to 1 mg L^{-1} in some areas of Bangladesh and 3 mg L^{-1} in Vietnam,[14] while an As concentration of 792 mg kg^{-1} was measured in the surface sediments of Lake Biwa, Japan.[11] In macrophytes, uptake of As can reduce phosphorus (P), nitrogen (N), potassium (K), chlorophyll *a* and protein content in tissues and, due to the chemical similarity of As to P, can interfere with some biochemical reactions.[15]

Concentrations of contaminants accumulated by plants can be higher than in water, but lower than in bed sediments.[16] The bioavailability of trace elements to plants can be regulated by a range of site specific factors including their concentration in the environment, trace element speciation, exposure time, and absorption mechanism.[16] For example, uptake of the inorganic As species, arsenate, occurs through phosphate uptake pathways and has been shown to be negatively correlated with phosphate uptake by the macrophyte *Spirodela polyrhiza* L.[9] In addition, the capacity of aquatic plants to sequester and accumulate metals is affected by many factors, such as plant growth rate, biomass accumulation, and affinity for metal uptake.[15] Previous studies have shown that As bioaccumulation varies among macrophyte species and among aquatic plant tissues.[15−18]

Arsenic can occur in inorganic and organic chemical forms, or species, which are associated with different levels of toxicity.[19] For example, the inorganic species arsenite (As(III)) is more toxic than organic arsenobetaine to plants and animals.[19] The uptake mechanism[20] and level of accumulation in plants[19,21] also vary between As species. Significant differences between concentrations of different As species have been reported in rice (*Oryza sativa* L.), with the predominant form being inorganic As.[21,22]

Determination of As speciation in plants is important for assessing the plant As toxicity to consumers at higher levels of the food chain.[23−25] Most previous research on As species in plants has focused on crops and As speciation in plants in the aquatic environment has not been extensively studied, with most of the existing knowledge based on controlled laboratory experiments.[9,18,20] Consequently, little is known about variation of individual As species between tissues of aquatic plants. Inorganic and organic As species have been determined previously in whole macrophytes,[9,19,26] but concentrations of As species have not been examined in different plant tissues. Determination of the species of As accumulated in macrophytes allows for more accurate assessment of the environmental risk that increases with the occurrence of inorganic As and is particularly important at sites where fish and waterfowl are used for human consumption.

We assessed the impact of As on five macrophyte taxa—three submerged species (*Potamogeton pectinatus* L., *Elodea nuttallii* (Planch) H. St. John, *Myriophyllum spicatum* L.), one rooted, floating-leaved species (*Persicaria amphibia* (L.) Gray), and one multicellular algae (*Chara* spp.) in Kinghorn Loch 30 years after the diversion of red mud leachate. Arsenic speciation analysis was applied to quantify inorganic As (arsenite, As(III) and arsenate, As(V)) and four organic As species: arsenobetaine, dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), and tetramethylarsonium ions (Tetra). On the basis of the results of previous studies on total As accumulation

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in macrophytes,[15−17] As speciation in rice[21] and terrestrial plants[14] and As species uptake by macrophytes in controlled laboratory experiments,[9,18,20] the following hypotheses were tested: (1) macrophytes in Kinghorn Loch accumulate higher concentrations of inorganic compared to organic As species; (2) accumulation of As species varies between above-sediment tissues (leaves and stems) of macrophytes and among macrophyte taxa; and (3) accumulation of As species in *P. amphibia* is greatest in the roots of the plant compared to the above-sediment tissues. This work provides the first comprehensive assessment of As accumulation and speciation in aquatic macrophytes following red mud pollution.

MATERIALS AND METHODS

Study Site. Kinghorn Loch is a small lake of surface area 11.3 ha, mean depth 4.5 m and maximum depth 12.8 m, situated in Fife, Scotland (56°10′N; 3°11′W). The lake received highly alkaline leachate from a nearby red mud landfill site from 1947 to 1983 when the discharge was diverted. Caustic liquor reaching the lake consisted of a highly alkaline solution of NaOH and carbonate, with a mean pH of 12.1. It contained high concentrations of dissolved aluminum (Al; 137 mg L^{-1}), As (3.56 mg L^{-1}), vanadium (V; 5.3 mg L^{-1}), phosphate (PO_4—P; 2.95 mg L^{-1}), sulfate (SO_4—S; 154 mg L^{-1}), and chloride (Cl; 67.8 mg L^{-1}). Red mud solids, released to the loch sporadically, accounted for most of the elevated input of iron (Fe).[4] The physicochemical pathways of pollutant transport within the bed sediments of the loch have been discussed in detail by Edwards (1985).[4] Long-term monitoring of water chemistry of the loch was initiated in 1980 by the Forth River Purification Board and continued by the Scottish Environment Protection Agency (SEPA). The pollution led to intense algal blooms, fish kills, and severe reduction of zooplankton, macroinvertebrate, and macrophyte species diversity and biomass.[4] By 1985, two years after the diversion of the leachate from the lake, a collapse in the phytoplankton activity, development of zooplankton, and an increase in abundance of benthic macroinvertebrates (although no increase in species richness) were observed.[4] Bioaccumulation studies of the plankton and macroinvertebrate populations in 1985 indicated no significant As bioaccumulation in Kinghorn Loch.[4]

A pilot survey was conducted in 2013 to assess metal concentrations in surface waters and bed sediments.[27] Total As concentrations in lake surface water and bottom water (1 cm above sediment; sampled July 2013; Site 1; Figure 1) were 14.9 μg L^{-1} and 14.8 μg L^{-1}, respectively, and did not exceed Standards for Protection of Aquatic Life in the U.K. (50 μg L^{-1}).[28]

Concentrations of As in lake surface sediment at Site 1 (87 mg kg^{-1}) and mean As concentration from 6 sites across the lake (160 mg kg^{-1})[27] in July 2013 exceeded the Canadian Sediment Quality Guidelines for Protection of Aquatic Life, which predicts toxicity from sediment concentrations ≥17 mg kg^{-1} of As.[29] We confirmed As release from bed sediments under reducing redox conditions in Kinghorn Loch in a laboratory intact-core incubation study.[27]

Macrophyte Sampling. Macrophyte sampling was conducted on 18 July 2013 when whole plants were collected by hand and using a double-headed rake from a boat at three sites in Kinghorn Loch (56°40′26″N; 3°11′34″W (Site 1); 56°40′18″N; 3°11′33″W (Site 2); 56°40′22″N; 3°11′16″W (Site 3); and Supporting Information, SI, Figure S1). The sites were chosen to represent areas at different distances (approximately 100, 250, and 430 m for Sites 1, 2, and 3, respectively) and directions from the NW part of the lake, where the pollution entered the waterbody. Between three and ten plants per taxa (depending on the species specific volume of fresh plant material) were collected at each site. Samples represented the five most abundant macrophyte taxa in Kinghorn Loch—*P. amphibia*, *P. pectinatus*, *E. nuttallii*, *M. spicatum*, and *Chara* spp.—as determined by a full lake survey conducted on 18 July 2013 following the procedure outlined in Gunn et al.[30]

Sample Preparation. The macrophytes were stored in polyethylene bags at 4 °C until processing in the laboratory within 24 h. The plant tissues were placed into bags containing distilled water and manually shaken, repeatedly, to remove sediment, macroinvertebrates, and epiphytic biofilms. We acknowledge that this approach may not have removed entirely epiphytic organisms, although others have reported near complete removal of this community following similar treatments, as well as plant tissue damage under severe agitation.[31] To test for any variation in As accumulation in the different parts of macrophytes, *P. pectinatus*, *E. nuttallii*, and *M. spicatum* samples were divided into leaves and stems, and samples of *P. amphibia*, the only collected species with well-developed roots, into leaves, stems and roots. Axes and branches of the multicellular algae *Chara* spp. were also separated and are referred to as stems and leaves, respectively, for the purpose of statistical analysis. Samples of each macrophyte tissue type for each species were combined by site from a number of individual plants to obtain enough dry material for As analyses. Samples were then oven-dried at 80 °C and ground using a Retsch mixer mill MM200.

Chemical Analysis. Three replicate samples of each tissue type from each macrophyte species were analyzed. Approximately 100 ± 5 mg of each powdered sample was digested with 1% nitric acid using a microwave digester (CEM Mars 6 1800W). Arsenic speciation was determined by ion chromatography (Thermo Dionex IC5000 Ion Chromatograph) coupled to inductively coupled plasma mass spectrometry (Thermo Scientific iCap Q ICP-MS). Arsenic species in the solutions were identified by comparing their retention times with those of standards including inorganic As (As(III) and As(V)), arsenite, DMA, MMA, and Tetra and quantified by calibration curves with peak areas. Species with retention times not matching any of the standards were classified as unknown species. Total identified As species were calculated as the sum of identified inorganic and organic As species. The detection limit for all As species was 0.001 mg kg^{-1}. The As species concentrations measured in duplicate analyses of the Certified Reference Material (CRM; NIST 1568b Rice flour) conducted in the same manner were within 25% of the certified values. The certified and measured values of As species in the CRM are listed in Table 1. The CRM did not contain arsenobetaine and Tetra. The method for determination of As speciation is described in detail in the SI.

Statistical Analyses. The means of three replicate samples were used for all statistical analyses. Concentrations of different As species in *P. amphibia* tissues (leaves and stems) were analyzed together with data for the other macrophytes species to examine across species variation in the above-sediment parts of macrophytes, and separately (concentrations in leaves, stems, and roots) to test for differences between As concentrations in below- and above-sediment parts of *P. amphibia*.

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Table 1. Certified Values and Means (n = 2) of As Species Concentrations (mg kg^{-1} Dry Weight) Measured in the CRM (NIST 1568b Rice Flour)

		measured concentration	
arsenic species	certified value	mean	s.d.
arsenobetaine		<LOD	
DMA	0.180	0.216	0.003
tetra		<LOD	
MMA	0.0116	0.014	0.000
inorganic As	0.092	0.110	0.020

A one-sided two sample t-test was used to test whether the mean concentration of inorganic As was greater than the mean concentration of total organic As in the above-sediment tissues of macrophytes and also whether below-sediment surface tissues of *P. amphibia* contained higher concentrations of As species than above-sediment tissues.

Mixed effect modeling was conducted to examine variations in concentrations of each As species across macrophyte species and tissue type (within shoots). Root samples were not included in these models, as they were only available for one species. Model validation was conducted on the final models. Homogeneity of variance was assessed by plotting residuals versus fitted values and the normality assumption was evaluated by plotting theoretical quantiles versus standardized residuals (Q−Q plots). In models with all macrophyte species, several response variables (i.e., inorganic As, arsenobetaine, total organic and the sum of all identified As species) were $\log_{10}$ transformed to meet homogeneity and normality assumptions. The random effect part of the model allowed for variations among sites and incorporated a split plot design of the data. The analysis started with the model containing both nominal fixed explanatory variables (i.e., macrophyte species and macrophyte tissue) and the two-way interaction between these two terms. The model selection process followed the procedure described in Zuur et al.[32] Maximum likelihood ratio (ML) tests were performed, using the ANOVA function, to select the best-fit model by comparing AIC values and to determine the significance (P < 0.05) of dropped terms. The final model was rerun using restricted maximum likelihood (REML). Statistical analyses were conducted using R version 3.0.1,[33] using the "nlme" package[34] for the mixed modeling.

RESULTS

Difference between Inorganic and Organic As Concentrations Across Macrophyte Taxa. Figure 1 shows variations in As species concentrations across the five macrophyte taxa and different plant tissues of each of the taxa. The above-sediment parts of the five macrophyte species in samples combined across sampling sites contained significantly higher concentrations of inorganic As species than total organic As, with mean values of 8.80 mg kg^{-1} d.w. (s.d. 13.8) and 0.56 mg kg^{-1} d.w. (s.d. 0.44), respectively (Table 2). Higher concentrations of inorganic As were measured compared to total organic As concentrations in both tissues of all macrophytes species (Figure 1, Table S1). *P. amphibia* also contained significantly higher concentrations of inorganic As than total organic As, with mean values of 40.0 mg kg^{-1} d.w. (s.d. 65.7) for inorganic and 0.25 mg kg^{-1} d.w. (s.d. 0.11) for organic forms (Table 2), respectively, calculated as the arithmetic mean of above and below-sediment surface tissues As concentrations.

Differences in As Species Accumulation in Above-Sediment Macrophyte Tissues. The lowest mean concentrations of inorganic As in the above sediment-surface tissues were measured in the leaves and stems of *P. amphibia* (0.76 mg kg^{-1} ± 0.14 and 1.07 mg kg^{-1} ± 0.29, respectively) and the highest in leaves of *E. nuttallii* (45.1 mg kg^{-1} ± 20.7; Figure 1, Table S1). Two organic As species (MMA and Tetra) were below the limit of detection in all tissue types of *Chara* spp., with MMA also not detectable in leaves of *M. spicatum*. Mean concentrations of the four organic As species ranged from 0.01 mg kg^{-1} d.w. (DMA in *Chara sp.* branches, MMA in both tissues of *P. amphibia*, Tetra in *P.amphibia* leaves) to 1.16 mg kg^{-1} (arsenobetaine in *Chara* spp. branches). Unknown As species were present in the leaves of *P. pectinatus* and *M. spicatum* and in both tissues of *Chara* spp., with the highest mean concentration (1.04 mg kg^{-1} ± 0.47) measured in the latter species.

The model selection process indicated a model with plant tissue, plant species and the interaction term included as an optimal one for inorganic As, arsenobetaine, MMA, Tetra, total organic As, and all identified As species (Table S2). Due to a lack of a significant effect, the interaction term was omitted from the model for unknown As species. None of the explanatory variables was found to have a significant association with plant DMA concentration.

E. nuttallii had significantly higher (estimate: 1.79, S.E.: 0.27, t: 6.67, P: < 0.001) and *P. amphibia* significantly lower (estimate: −2.22, S.E.: 0.27, t: −8.27, P: < 0.001) concentrations of inorganic As than *Chara* spp., while there was no significant difference in concentration between *P. pectinatus* and *M. spicatum* and the latter species (Table S3). The results also indicated that *E. nuttallii*, *M. spicatum*, and *P. pectinatus* accumulated lower (estimate: −1.75, S.E.: 0.21, t: −8.39, P: < 0.001; estimate: −1.49, S.E.: 0.21, t: −7.16, P: < 0.001; estimate: −0.80, S.E.: 0.21, t: −3.83, P: 0.003, respectively) concentrations of inorganic As in stems than in leaves, while in *P. amphibia* higher inorganic As (estimate: 0.48, S.E.: 0.21, t: 2.32, P: 0.043) was measured in stems compared to leaves. There was no significant difference between concentrations of this As species in leaves and stems of *Chara* spp.

Compared to *Chara* spp., the other four macrophyte taxa accumulated significantly lower (*E. nuttallii*, estimate: −1.49, S.E.: 0.38, t: −3.91, P: 0.005; *M. spicatum*, estimate: −1.23, S.E.: 0.38, t: −3.23, P: 0.012; *P. pectinatus*, estimate: −1.60, S.E.:

Table 2. Results of One-Sided t-Test Analysis to Assess if Inorganic As Concentration Is Greater than Total Organic As Concentration (mg kg^{-1}) Measured in Tissues of Five Macrophyte Taxa and in *P. amphibia* in Kinghorn Loch in July 2013

	total inorganic As		total organic As				
	mean	s.d.	mean	s.d.	t-value	df	p-values
all macrophyte taxa (above sediment tissues)	8.80	13.8	0.56	0.44	3.2607	29.06	0.001
P. amphibia (leaves, stems, roots)	40.0	65.7	0.25	0.11	2.0072	10.00	0.036

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Table 3. Results of One-Sided *t*-Test to Assess if Mean Concentrations of As Species in Below-Sediment Surface Tissues Are Greater than Means of As Species (mg kg^{-1}) Measured in Above-Sediment Tissues of *P. amphibia* in Kinghorn Loch in July 2013

	below-sediment surface tissue		above-sediment tissues				
	mean	s.d.	mean	s.d.	*t*-value	df	*P*-value
inorganic As	86.9	75.8	0.91	0.27	2.5372	4.000	0.032
arsenobetaine	0.05	0.04	0.07	0.02	−0.8661	6.291	0.791
DMA	0.11	0.04	0.04	0.03	3.2193	7.007	0.007
MMA	0.06	0.03	0.01	0.00	3.5631	4.156	0.011
tetra	0.11	0.07	0.07	0.08	0.8955	8.923	0.197
total organic As	0.32	0.12	0.19	0.07	2.1963	6.213	0.035
total identified As	87.2	75.9	1.11	0.33	2.5377	4.000	0.032
unknown As species	0.17	0.22	0.00	0.00	−1.7466	4.000	0.922

0.38, *t*: −4.20, *P*: 0.003; *P. amphibia*, estimate: −2.73, S.E.: 0.38, *t*: −7.19, *P*: < 0.001) concentrations of arsenobetaine (Table S3). The arsenobetaine concentrations measured in stems of *E. nuttallii* and *M. spicatum* were lower (estimate: −0.92, S.E.: 0.38, *t*: −2.44, *P*: 0.035; estimate: −0.87, S.E.: 0.38, *t*: −2.29, *P*: 0.045, respectively) than in the leaves, while there were no significant differences in the other three species between the concentrations in the leaves and stems.

MMA concentrations in *P. pectinatus* and *E. nuttallii* were significantly higher (estimate: 0.055, S.E.: 0.02, *t*: 3.59, *P*: 0.007; estimate: 0.09, S.E.: 0.02, *t*: 5.83, *P*: < 0.001, respectively) than in *Chara* spp. and MMA in stems of *E. nuttallii* was significantly lower (estimate: −0.06, S.E.: 0.02, *t*: −2.98, *P*: 0.014) compared to MMA in leaves of this species (Table S3). There was no significant difference between MMA in stems and leaves of *Chara* spp.

The model for Tetra indicated no significant difference in concentration between *P. amphibia* and *Chara* spp. (Table S3). The Tetra concentrations in the other three species were higher (*E. nuttallii*, estimate: 0.30, S.E.: 0.03, *t*: 9.07, *P*: < 0.001; *M. spicatum*, estimate: 0.28, S.E.: 0.03, *t*: 8.51, *P*: < 0.001; *P. pectinatus*, estimate: 0.19, S.E.: 0.03, *t*: 5.85, *P*: < 0.001) than in *Chara* spp., with concentrations in the stems of these species significantly lower (*E. nuttallii*, estimate: −0.24, S.E.: 0.04, *t*: −5.34, *P*: < 0.001; *M. spicatum*, estimate: −0.25, S.E.: 0.04, *t*: −5.62, *P*: < 0.001; *P. pectinatus*, estimate: −0.14, S.E.: 0.04, *t*: −3.27, *P*: 0.008) than in the leaves. In contrast, Tetra in *P. amphibia* was significantly lower (estimate: 0.12, S.E.: 0.04, *t*: 2.62, *P*: 0.026) in leaves compared to stems.

The model showed that the four macrophyte species had lower total organic As concentrations than *Chara* spp., but the difference was only significant (estimate: −1.93, S.E.: 0.27, *t*: −7.22, *P*: < 0.001) in *P. amphibia*, *E. nuttallii*, *M. spicatum*, and *P. pectinatus*. For these species, the concentrations of total organic As in stems was significantly lower than observed in leaves (Table S3).

Two species had significantly different total identified As concentrations in comparison with *Chara* spp.—*E. nuttallii* and *P. amphibia*—which accumulated higher (estimate: 1.65, S.E.: 0.28, *t*: 5.99, *P*: < 0.001) and lower (estimate: −2.19, S.E.: 0.28, *t*: −7.96, *P*: < 0.001) concentrations, respectively (Table S3). Total identified As was significantly lower in stems of *E. nuttallii*, *M. spicatum*, and *P. pectinatus* (estimate: −1.74, S.E.: 0.20, *t*: −8.77, *P*: < 0.001; estimate: −1.48, S.E.: 0.20, *t*: −7.44, *P*: < 0.001; estimate: −0.80, S.E.: 0.20, *t*: −4.05, *P*: 0.002, respectively) compared to leaves and significantly higher (estimate: 0.47, S.E.: 0.20, *t*: 2.37, *P*: 0.039) in stems than leaves of *P. amphibia*.

Chara spp. contained significantly higher concentrations of unidentified As species than the other four macrophyte taxa (*E. nuttallii*, estimate: −0.85, S.E.: 0.15, *t*: −5.59, *P*: < 0.001; *M. spicatum*, estimate: −0.69, S.E.: 0.15, *t*: −4.52, *P*: 0.002; *P. pectinatus*, estimate: −0.84, S.E.: 0.15, *t*: −5.54, *P*: < 0.001; *P. amphibia*, estimate: −0.85, S.E.: 0.15, *t*: −5.59, *P*: < 0.001), with significantly higher (estimate: −0.15, S.E.: 0.07, *t*: −2.16, *P*: 0.049) concentrations in leaves than stems (Table S3).

Differences in As Species Accumulation within *P. amphibia*. Inorganic As in roots was substantially higher (86.9 ± 75.8 mg kg^{-1} d.w.) than mean concentrations of any other identified As species measured in *P. amphibia*, which were ≤0.12 mg kg^{-1} d.w. (s.d. 0.07; Figure 1, Table S1). All analyzed organic species of As were measured in each tissue of *P. amphibia*, while unknown As species were only detected in plant roots (mean 0.17 mg kg^{-1} d.w. ± 0.22).

The below-sediment tissues of *P. amphibia* contained significantly higher concentrations of inorganic As, DMA, MMA and total organic As and identified As species than the above-sediment tissues (Table 3). There were no significant differences between concentrations of arsenobetaine and Tetra between tissues above and below the sediment surface.

DISCUSSION

Macrophytes in Kinghorn Loch Accumulated Potentially Toxic Concentrations of As. The present study showed that lake macrophytes contained relatively high concentrations of As, 30 years following the diversion of red mud leachate from Kinghorn Loch. The As concentrations in shoots reported here were higher than those reported in two other lakes (i.e., 1.4 to 5.3 mg kg^{-1} in Lake Dianchi in China[35] and <1 mg kg^{-1} in Lake Velenjsko, Slovenia[36]) characterized by elevated concentrations of As in surface sediment. In addition, the As concentration in *E. nuttallii* leaves in Kinghorn Loch was in excess of the maximum plant shoot content of As reported for wetlands affected by the Aznalcóllar mine spill in Spain (0.01−28.5 mg As kg^{-1}).[37] However, the upper concentration range in plant roots documented by Taggart et al.[37] (1.39−1089 mg kg^{-1}) was greater than that measured in root samples collected from Kinghorn Loch (40.5−218 mg kg^{-1}).

There are no widely accepted toxicity threshold concentrations for As in plant tissues.[17] Most of the mean concentrations measured in macrophyte tissues in Kinghorn Loch, however, were higher than the ranges 0.01−1.00 mg kg^{-1} and 3.00−10.0 mg kg^{-1} that were suggested by Chaney[38] as "normal" and potentially phytotoxic ranges of inorganic As concentrations in dry foliage, respectively. These results indicate that, although the total As concentrations measured

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in water in Kinghorn Loch 30 years after the pollution ceased entering the lake meet Standards for Protection of Aquatic Life, the high content of As in lake bed sediments remains a toxic hazard to macrophytes.

Concentrations of Inorganic As in Macrophytes Were Significantly Higher Compared to Organic As. Inorganic As was the dominant species in the studied macrophytes in Kinghorn Loch. Although As speciation in plants in the aquatic environment has not been extensively studied, these field observations are in agreement with the results from controlled laboratory experiments that have shown much higher uptake of arsenate and arsenite, compared to DMA and MMA, by *Spirodela polyrhiza* L.,[9] and by *Azolla caroliniana* Willd. and *Azolla filiculoides* Lam.,[18] and higher arsenate uptake in comparison to DMA by *Salvinia natans* L.[20] The present study also confirms the results of the survey investigating As species accumulation in a range of aquatic plants sampled in a wetland affected by As from mining activities in Yellowknife, Canada.[19] These observations made on aquatic plants are generally consistent with the results of wider research, which have investigated As speciation in several plants used for human consumption, e.g., rice *Oryza sativa*,[21,39] as well as other terrestrial plants.[14]

It is not known if the presence of various organic As compounds in tissues is a result of uptake or transformation of As by plants.[14] The present study identified four organic As species within macrophyte tissues, which except for arsenobetaine in *Chara* spp., were present at concentrations not indicated as potentially phytotoxic.[38] DMA, MMA, and Tetra, but not arsenobetaine, were previously identified in aquatic plants from Yellowknife, Canada.[19] A review of As speciation in terrestrial plants showed that methylated As forms were also reported (as a minor fraction of the total accumulated As) in a wide number of plant species.[14] The presence of arsenobetaine has been previously reported in terrestrial plants, however, in a smaller number of species compared to methylated As compounds.[14]

In addition to the As compounds discussed above, the results of the present study indicate that *Chara* spp., *P. pectinatus* and *M. spicatum* all accumulated a small fraction of As species that were not identified by the analysis procedure employed. A few aquatic plant species from the Yellowknife study, such as *Myriophyllum* sp., contained arseno-sugars.[19] Koch et al.[19] suggested that the presence of arseno-sugars in submerged plant tissues could be explained by contamination of samples with organisms containing this form of As, e.g., epiphytic algae growing in physical contact with some macrophytes. Thus, it is possible that arseno-sugars accounted for some of the unidentified As species present in macrophytes at Kinghorn Loch, despite the washing procedure, although the effect of this procedure was not quantified for this study.

Accumulation of As Species Varied between Above-Sediment Tissues of Macrophytes and among Macrophyte Species. Most studies have examined variation in As accumulation only for total As. The extent of metal(loid) accumulation investigated at the total concentration level has been shown to differ between macrophyte species and tissues[40−42] and thus, it was hypothesized that the concentrations of different As chemical forms within plants in Kinghorn Loch would also vary depending on plant tissue and species. Variation in total identified As, and consequently variation of inorganic As, which had the highest contribution to the total As concentrations in this study, confirmed that hypothesis. With the exception of DMA, organic As species concentrations were also reported to vary significantly with plant species and tissue type. Across-species As variation observed in the present study is in agreement with the differences in the uptake of inorganic and organic As forms by different macrophyte species previously reported.[18,19] Variation in metal(loid) uptake may be related to differences in plant growth rate and absorption efficiency for metals.[15] In addition, some macrophytes can accumulate greater concentrations of As than others, but might show fewer signs of damage due to more effective mitigation mechanisms.[43] In the present study, the highest shoot concentrations of inorganic As were observed in *E. nuttallii*. Previous estimates of metal (Hg and Cd) uptake by *E. nutallii* shoots indicated high and rapid bioaccumulation of metals, with shoots having a high tolerance due to the protection of cellular machinery by highly effective tolerance mechanisms.[44] This might explain the capability of this species to accumulate high concentrations of As in Kinghorn Loch. Lower content of inorganic As in above-sediment tissues of other taxa in the present study might indicate different plant defense mechanisms in comparison to *E. nuttallii*, such as limiting As absorption. In order to decrease As uptake and enhance their survival in the presence of arsenate, plants can suppress phosphate uptake pathways used for arsenate absorption,[14] which may be the defense process occurring in some of the macrophyte species in Kinghorn Loch. Across-tissue variation observed in As species may be a result of differences in translocation of As species between tissues by plants or reflect differences in tissue-specific metabolism, although knowledge of the ability of plants to transform inorganic As into methylated species is limited.[14]

Concentrations of all detected As species in macrophytes in Kinghorn Loch were higher (apart from DMA in *Chara* spp.) in leaves compared to stems of the four macrophyte taxa sampled. The only exception was *P. amphibia*, in which total As concentrations were significantly higher in stems than leaves. The significantly higher concentrations of inorganic and organic As in leaves compared to stems in *M. spicatum* in the present study were in agreement with total As distribution in shoots of this species in Kadin Creek, Turkey.[45]

Roots of *P. amphibia* Accumulated Significantly Higher Concentrations of Most Identified As Species Compared to the Above-Sediment Tissues. Most previous studies have shown that a major fraction of total As is accumulated in the roots of aquatic plants.[15−17,42,46] To date, few plants have been identified with the ability to translocate high amounts of As from roots to above-sediment tissues.[46] This was also demonstrated for *P. amphibia*, in the present study. Chou et al.[47] suggested that minor translocation of As from roots to shoots in rice plants was indicative of adaptive survival behavior in As contaminated sediments. The results for inorganic As from Kinghorn Loch indicate that this might also be the strategy of *P. amphibia*. Favas et al.[42] suggested that As speciation appears to play an important role in both the uptake mechanism and further translocation of As between plant tissues. Arsenate is an analogue of phosphate[14] and uptake, accumulation and translocation of inorganic As is thought to be affected by its relationship with phosphate transporters[42] and the presence of Fe and sulfur (S).[25] Fe plaque on plant roots can act either as a sink or a source of As to plants and S can help detoxify As through complexation and restricting its translocation to shoots.[25] However, the role of the Fe plaque in the translocation of As into the macrophytes in Kinghorn Loch

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cannot be verified as the As content of the Fe plaque observed was not determined. In contrast, Rahman et al.[21] reported that uptake of the organic As species, DMA, through roots and shoots of rice plants was not affected by Fe. Moreover DMA and arsenate are suggested to be taken up by different pathways.[9] Despite these differences, concentrations of DMA in the present study were, similarly to inorganic As species, significantly higher in the plant roots than in shoots. Knowledge of the mechanisms of translocation and accumulation of organic As species in plants is, however, limited.

Toxicological Implications of Measured As Species Concentrations in Macrophytes in Kinghorn Loch. Information about the relative concentrations of As species present in macrophytes can provide important toxicological context. Organic As species, recorded in the present study at concentrations comparable to background levels of total As, are generally less toxic to organisms, including aquatic plants, than inorganic As compounds.[48] Moreover, arsenobetaine, the organic As species present in the highest concentration of those analyzed in the present study is reported to have nontoxic properties.[49]

The dominance of inorganic As forms, which are present in elevated concentrations in all macrophyte taxa in Kinghorn Loch indicates that, even following 30 years of recovery, red mud pollution still poses a risk to the macrophytes of this lake, and potentially to higher organisms in the food web. Inorganic As species are highly toxic to plants.[14] It is possible that the macrophyte community that has developed in Kinghorn Loch is tolerant to elevated As concentrations. For example, two of the dominant macrophytes species included in the study, *M. spicatum* and *P. pectinatus*, have been reported to accumulate high metal concentrations, persisting under conditions of concentrations in tissues higher than those regarded as toxic for plants.[50] In addition, *P. pectinatus* can quickly colonize polluted waters and environments unsuitable for other species[50] and *Elodea* sp. is known to be able to adapt to a wide range of environmental conditions.[51] These traits have led researchers to consider the use of *M. spicatum*, *P. pectinatus*, and *E. nuttallii* for phytoremediation.[44,52,53] The presence of species that have been shown to be persistent in heavily polluted waters suggests that the elevated As concentrations in the sediment may still influence the macrophyte community structure in Kinghorn Loch.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b00942.

Macrophyte sampling sites, a detailed description of the method for determination of As speciation, As species concentrations in macrophytes, ranking of mixed models, and coefficient estimates of the optimal mixed models explaining variation in arsenic species concentrations in macrophytes (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Power, G.; Gräfe, M.; Klauber, C. Bauxite residue issues: I. Current management, disposal and storage practices. *Hydrometallurgy* **2011**, *108*, 33−45.

(2) Klebercz, O.; Mayes, W. M.; Anton, Á.D.; Feigl, V.; Jarvis, A. P.; Gruiz, K. Ecotoxicity of fluvial sediments downstreams of the Ajka red mud spill, Hungary. *J. Environ. Monit.* **2012**, *14*, 2063−2071.

(3) Dubey, K.; Dubey, K. P. A study of the effect of red mud amendments on the growth of cyanobacterial species. *Biorem. J.* **2011**, *15*, 133−139.

(4) Edwards, R. A. *Impact of a long-term caustic discharge from red mud disposal on a freshwater lake*. Ph.D. Dissertation, University of Edinburgh, 1985.

(5) Gelencsér, A.; Kováts, N.; Turóczi, B.; Rostási, A.; Hoffer, A.; Imre, K.; Nyiró-Kósa, I.; Csákberényi-Malasics, D.; Tóth, A.; Czitrovszky, A.; Nagy, A.; Nagy, S.; Ács, A.; Kovács, A.; Ferincz, A.; Hartyáni, Z.; Pósfai, M. The red mud accident in Ajka (Hungary): characterization and potential health effects of fugitive dust. *Environ. Sci. Technol.* **2011**, *45*, 1608−1615.

(6) Mayes, W. M.; Jarvis, A. P.; Burke, I. T.; Walton, M.; Feigl, V.; Klebercz, O.; Gruiz, K. Dispersal and attenuation of trace contaminants downstream of the Ajka bauxite residue (red mud) depository failure, Hungary. *Environ. Sci. Technol.* **2011**, *45*, 5147−5155.

(7) Spears, B. M.; Carvalho, L.; Perkins, R.; Kirika, A.; Paterson, D. M. Long-term variation and regulation of internal phosphorus loading in Loch Leven. *Hydrobiologia* **2012**, *681*, 23−33.

(8) Sharpley, A.; Jarvie, H. P.; Buda, A.; May, L.; Spears, B.; Kleinman, P. Phosphorus legacy: overcoming the effects of past management practices to mitigate future water qualities impairement. *J. Environ. Qual.* **2013**, *42*, 1308−1326.

(9) Rahman, M. A.; Hasegawa, H.; Ueda, K.; Maki, T.; Rahman, M. M. Arsenic uptake by aquatic macrophyte *Spirodela polyrhiza* L.: Interactions with phosphate and iron. *J. Hazard. Mater.* **2008**, *160*, 356−361.

(10) Shah, A. Q.; Kazi, T. G.; Arain, M. B.; Jamali, M. K.; Afridi, H. L.; Jalbani, N.; Baig, J. A.; Kandhro, G. A. Accumulation of arsenic in different fresh water fish species − potential contribution to high arsenic intakes. *Food Chem.* **2009**, *112*, 520−524.

(11) Hirata, S. H.; Hayase, D.; Eguchi, A.; Itai, T.; Nomiyama, K.; Isobe, T.; Agusa, T.; Ishikawa, T.; Kumagai, M.; Tanabe, S. Arsenic and Mn levels in Isaza (Gymnogobius isaza) during the mass mortality event in Lake Biwa, Japan. *Environ. Pollut.* **2011**, *159*, 2789−2796.

(12) Chen, C. Y.; Pickhardt, P. C.; Xu, M. Q.; Folt, C. L. Mercury and arsenic bioaccumulation and eutrophication in Baiyangdian Lake, China. *Water, Air, Soil Pollut.* **2008**, *190*, 115−127.

(13) Rowe, C. L.; Hopkins, W. A.; Congdon, J. D. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: a review. *Environ. Monit. Assess.* **2002**, *80*, 207−276.

(14) Meharg, A. A.; Hartley-Whitaker, J. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytol.* **2002**, *154*, 29−43.

(15) Mishra, V. K.; Upadhyay, A. R.; Pathak, V.; Tripathi, B. D. Phytoremediation of mercury and arsenic from tropical opencast coalmine effluent through naturally occuring aquatic macrophytes. *Water, Air, Soil Pollut.* **2008**, *192*, 303−314.

DOI: 10.1021/acs.est.6b00942
Environ. Sci. Technol. 2016, 50, 9044−9052

(16) Mazej, Z.; Germ, M. Trace element accumulation and distribution in four aquatic macrophytes. *Chemosphere* **2009**, *74*, 642−647.
(17) Taggart, M. A.; Carlisle, M.; Pain, D. J.; Williams, R.; Green, D.; Osborn, D.; Meharg, A. A. Arsenic levels in the soils and macrophytes of the 'Entremuros' after the Aznalcóllar mine spill. *Environ. Pollut.* **2005**, *133*, 129−138.
(18) Zhang, X.; Lin, A.-J.; Zhao, F.-J.; Xu, G.-Z.; Duan, G.-L.; Zhu, Y.-G. Arsenic accumulation by the aquatic fern *Azolla*: comparison of arsenate uptake, speciation and efflux by *A. caroliniana* and *A. filiculoides*. *Environ. Pollut.* **2008**, *156*, 1149−1155.
(19) Koch, I.; Wang, L.; Ollson, C. A.; Cullen, W. R.; Reimer, K. J. The predominance of inorganic arsenic species in plants from Yellowknife, Northwest Territories, Canada. *Environ. Sci. Technol.* **2000**, *34*, 22−26.
(20) Rahman, M. A.; Hasegawa, H.; Ueda, K.; Maki, T.; Rahman, M. M. Influence of EDTA and chemical species on arsenic accumulation in Spirodela polyrhiza L. (duckweed). *Ecotoxicol. Environ. Saf.* **2008**, *70*, 311−318.
(21) Rahman, M. A.; Rahman, M. M.; Kadohashi, K.; Maki, T.; Hasegawa, H. Effect of external iron and arsenic species on chelant-enhanced iron bioavailability and arsenic uptake in rice (*Oryza sativa* L.). *Chemosphere* **2011**, *84*, 439−445.
(22) Abedin, M. J.; Cotter-Howells, J.; Meharg, A. A. Arsenic uptake and accumulation in rice (*Oryza sativa* L.) irrigated with contaminated water. *Plant Soil* **2002**, *240*, 311−319.
(23) Alam, M. G. M.; Tanaka, A.; Allinson, G.; Laurenson, L. J. B.; Stagnitti, F.; Snow, E. T. A comparison of trace element concentrations in cultured and wild carp (*Cyprinus carpio*) of Lake Kasumigaura, Japan. *Ecotoxicol. Environ. Saf.* **2002**, *53*, 348−354.
(24) Gómez, R.; Baos, R.; Gómara, B.; Jiménez, B.; Benito, V.; Montoro, R.; Hiraldo, F.; González, M. J. Influence of mine tailing accident near Doñana National Park (Spain) on heavy metals and arsenic accumulation in 14 species of waterfowl (1998 to 2000). *Arch. Environ. Contam. Toxicol.* **2004**, *47*, 521−529.
(25) Zhao, F. J.; McGrath, S. P.; Meharg, A. A. Arsenic as a Food Chain Contaminant: Mechanisms of Plant Uptake and Metabolism and Mitigation Strategies. *Annu. Rev. Plant Biol.* **2010**, *61*, 535−559.
(26) Rahman, M. A.; Hasegawa, H.; Ueda, K.; Maki, T.; Okumura, C.; Rahman, M. M. Arsenic accumulation in duckweed (*Spirodela polyrhiza* L.): a good option for phytoremediation. *Chemosphere* **2007**, *69*, 493−499.
(27) Olszewska, J. *Long term chemical and ecological recovery of Kinghorn Loch (Scotland, U.K.) following red mud pollution*. Ph.D. Dissertation, University of Edinburgh, 2016.
(28) EEC.. Council Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community (Dangerous Substances Directive)—List II substances. *Official Journal* **1976**, *L129*, 23−29.
(29) Canadian Council of Ministers of the Environment. Canadian sediment quality guidelines for the protection of aquatic life: Arsenic. In *Canadian Environmental Quality Guidelines*, 1999; Canadian Council of Ministers of the Environment: Winnipeg, 1999.
(30) Gunn, I. D. M.; O'Hare, M.; Carvalho, L.; Roy, D. B.; Rothery, P.; Darwell, A. M. Assessing the condition of lake habitats: a test of methods for surveying aquatic macrophyte communities. *Hydrobiologia* **2010**, *656*, 87−97.
(31) Hempel, M.; Blume, M.; Blindow, I.; Gross, E. M. Epiphytic bacterial community composition on two common submerged macrophytes in brackish water and freshwater. *BMC Microbiol.* **2008**, *8*, 58.
(32) Zuur, A. F.; Ieno, E. N.; Walker, N. J.; Saveliev, A. A.; Smith, G. M. *Mixed Effects Models and Extensions in Ecology with R*; Springer: New York, 2009.
(33) R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013; URL http://www.R-project.org/.
(34) Pinheiro, J.; Bates, D.; DebRoy, S.; Sarkar, D. and the R Core Team. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1−117. 2014; http://CRAN.R-project.org/package=nlme.
(35) Zhang, N.; Wei, C.; Yang, L. Occurance of arsenic in two large shallow freshwater lakes in China and a comparison to other lakes around the word. *Microchem. J.* **2013**, *110*, 169−177.
(36) Jackson, L. J.; Rassmusen, J. B.; Peters, R. H.; Kalff, J. Empirical relationships between the element composition of aquatic macrophytes and their underlying sediments. *Biogeochemistry* **1991**, *12*, 71−86.
(37) Taggart, M. A.; Mateo, R.; Charnock, J. M.; Bahrami, F.; Green, A. J.; Meharg, A. A. Arsenic rich iron plaque on macrophyte roots − an ecotoxological risk? *Environ. Pollut.* **2009**, *157*, 946−954.
(38) Chaney, R. L. Toxic element accumulation in soils and crops: protecting soil fertility and agricultural food-chains. In *Inorganic Contaminants in the Vadose Zone*; Bar-Yosef, B.; Barrow, N. J.; Goldshmid, J., Eds.; Springer-Verlag: Berlin, 1989; pp 140−158.
(39) Rahman, M. A.; Hasegawa, H.; Rahman, M. M.; Miah, M. A. M.; Tasmin, A. Arsenic accumulation in rice (*Oryza sativa* L.): human exposure through food chain. *Ecotoxicol. Environ. Saf.* **2008**, *69*, 317−324.
(40) Cardwell, A. J.; Hawker, D. W.; Greenway, M. Metal accumulation in aquatic macrophytes from southeast Queensland, Australia. *Chemosphere* **2002**, *48*, 653−663.
(41) Grudnik, Z. M.; Germ, M. *Myriophyllum spicatum* and *Najas marina* as bioindicators of trace element contamination in lakes. *J. Freshwater Ecol.* **2010**, *25*, 421−427.
(42) Favas, P. J. C.; Pratas, J.; Prasad, M. N. V. Accumulation of arsenic by aquatic plants in large-scale field conditions: opportunities for phytoremediation and bioindication. *Sci. Total Environ.* **2012**, *433*, 390−397.
(43) Leão, G. A.; Oliveira, J. A.; Farnese, F. S.; Gusman, G. S.; Felipe, R. T. A. Sulfur metabolism: different tolerances of two aquatic macrophytes exposed to arsenic. *Ecotoxicol. Environ. Saf.* **2014**, *105*, 36−42.
(44) Larras, F.; Regier, N.; Planchon, S.; Poté, J. Physiological and proteomic changes suggest an important role of cell walls in the high tolerance to metals of *Elodea nuttallii*. *J. Hazard. Mater.* **2013**, *263*, 575−583.
(45) Yabanli, M.; Yozukmaz, A.; Sel, F. Heavy metal accumulation in the leaves, stem and root of the invasive submerged macrophyte *Myriophyllum spicatum* L. (Haloragaceae): an example of Kadin Creek (Mugla, Turkey). *Braz. Arch. Biol. Techn.* **2014**, *57*, 434−440.
(46) Rahman, M. A.; Hasegawa, H. Aquatic arsenic: Phytoremediation using floating macrophytes. *Chemosphere* **2011**, *83*, 633−646.
(47) Chou, M. L.; Jean, J. S.; Sun, G. X.; Hseu, Z. Y.; Yang, C. M.; Das, S.; Teng, J. H. Distribution and accumulation of arsenic in rice plants grown in arsenic-rich agricultural soil. *Agron. J.* **2014**, *106*, 945−951.
(48) Tamaki, S.; Frankenberger, W. T. Environmental biochemistry of arsenic. *Rev. Environ. Contam. Toxicol.* **1992**, *124*, 79−110.
(49) Caumette, G.; Koch, I.; Reimer, K. J. Arsenobetaine formation in plankton: a review of studies at the base of the aquatic food chain. *J. Environ. Monit.* **2012**, *14*, 2841−2853.
(50) Samecka-Cymerman, A.; Kempers, A. J. Toxic metals in aquatic plants surviving in surface water polluted by copper mining industry. *Ecotoxicol. Environ. Saf.* **2004**, *59*, 64−69.
(51) Regier, N.; Baerlocher, L.; Münsterkötter, M.; Farinelli, L.; Cosio, C. Analysis of the *Elodea nuttallii* transcriptome in response to mercury and cadmium pollution: development of sensitive tools for rapid ecotoxicological testing. *Environ. Sci. Technol.* **2013**, *47*, 8825−8834.
(52) Lesage, E.; Mundia, C.; Rousseau, D. P. L.; de Moortel, A.; du Laing, G.; Tack, F. M. G.; de Pauw, N.; Verloo, M. G. Removal of heavy metals from industrial effluents by the submerged aquatic plant *Myriophyllum spicatum*. L. In *Wastewater Treatment, Plant Dynamics and Management in Constructed and Natural Wetlands*; Wymazal, J., Ed.; Springer: New York, 2008; pp 211−221.

DOI: 10.1021/acs.est.6b00942
Environ. Sci. Technol. 2016, 50, 9044−9052

(53) Peng, K.; Luo, C.; Lou, L.; Li, X.; Shen, Z. Bioaccumulation of heavy metals by the aquatic plants *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. and their potential use for contamination indicators and in wastewater treatment. *Sci. Total Environ.* **2008**, *392*, 22−29.

DOI: 10.1021/acs.est.6b00942
Environ. Sci. Technol. 2016, 50, 9044−9052