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RESEARCH ARTICLE

Absorption and translocation of copper and arsenic in an aquatic macrophyte *Myriophyllum alterniflorum* DC. in oligotrophic and eutrophic conditions

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Abstract The aim of this study is to evaluate copper and arsenic accumulation and translocation at a concentration of 100 μg/L of a submersed macrophyte *Myriophyllum alterniflorum*. The trophic level (eutrophic and oligotrophic conditions) of the medium was also considered. To achieve this goal, plants were incubated for 21 days in the presence of 100 μg/L of Cu or AsV. The heavy metal transfers from the contaminated medium to plants and into plant tissues was discussed in terms of the bioconcentration factor (BCF) and the translocation factor (TF). Malondialdehyde (MDA) content in tissues was analyzed in order to study the toxicity of these two contaminants. Our results show that copper was more accumulated in shoots, than roots, whereas the opposite trend was observed for arsenic. In addition, the two contaminants were more accumulated in oligotrophic than eutrophic medium. The BCF of copper in shoots was 1356 in oligotrophic condition, while that of arsenic was higher in roots about 620 in the same condition. The TF was less than 1 for arsenic, and higher than 1 for copper, indicating that watermilfoil restrains the translocation of arsenic to shoots, while it has a low capacity to control the translocation of an essential micronutrient like copper. An increase in MDA content was observed under Cu and As stress. On the basis of this experiment, *M. alterniflorum* has a higher accumulation potential of copper and arsenic, and therefore, it can be a good candidate for the phytofiltration of these two contaminants from water.

Keywords *M. alterniflorum* · Arsenic · Copper · BCF · Phytofiltration · MDA

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Introduction

For several years, heavy metals have attracted attention due to their abundance and persistence in the environment and their toxicity to human health, animals, and plants (Xing et al. 2010). Some of these elements accumulate in the ecological food chain and become a serious threat to life (Nagajyoti et al. 2010). Two categories of heavy metals can be recognized: essential and non-essential elements. In aquatic environment, heavy metals have toxic effects on macrophytes. They lead to growth inhibition, photosynthesis reduction, and malondialdehyde (MDA) content increase. They can also induce an oxidative stress, leading to a cell membrane alteration due to lipoperoxidation (Tangahu et al. 2011).

Copper is an essential element for plants nutrition (Xing et al. 2010). It plays an important role in photosynthesis, and it is a cofactor of several enzymes like oxidase, superoxide dismutase, and ascorbate oxidase (Garcia et al. 2014). However, at excessive concentration, it can be also toxic and has deleterious effects on plants (Nagajyoti et al. 2010). Copper is mainly accumulated in the chlorophyllian parts of plants, and it is translocated from roots to shoots (Xue et al. 2010; Swain et al. 2014). Copper absorption in plants is predominantly controlled by the pH, the amount, and the oxidation

state of metal, the competition with other cations, the cation exchange capacity, and the organic carbon content. These interactions are very well explained in Biotic Ligand Model (BLM) (Ardestani et al. 2014; Smith et al. 2015).

Arsenic is considered as a non-essential element for plants and recognized as a carcinogenic (group 1) by CIRC since 1980. In drinking water, the limit for its concentration established by world health organization (WHO) is 10 μg/L. Arsenic is very toxic not only for human but also for plants (Singh et al. 2015). Arsenic is a metalloid and exists in three oxidation states −3, +3, and +5 (Sharma and Sohn 2009). Redox potential (Eh) and pH are the main factors controlling the speciation of arsenic (Mohan and Pittman 2007). In water surfaces, arsenic is therefore mainly found as arsenate form. In plants, As (V) is mainly accumulated in the root system and causes physiological alterations (Xue and Yan 2011; Chen et al. 2015). Therefore, plants restrain arsenate in the ground parts preventing its translocation to the chlorophyllian parts. It could be detoxified by phytochelatins through sequestration into root vacuoles (Zhao et al. 2009).

Eutrophication occurs in water due to nutrients enrichment especially phosphates and nitrogen (Xing et al. 2010). Nitrates and ammonium are the widespread inorganic forms of nitrogen found in waters and used by plants (Jampeetong and Brix 2009). An excessive concentration of nitrogen, and especially of ammonium, can have toxic effects on plants growth (Huang et al. 2013). In addition, eutrophication can affect the uptake of heavy metals in the environment. Indeed, organic matter, due to their complexing properties, affects the metal biodisponibility for plants (Tangahu et al. 2011). Arsenate chemical similarities with phosphates make it competes for plants phosphate transporters (Meharg and Macnair 1992; Zhao et al. 2009; Zangi and Filella 2012), and therefore, this eutrophic condition could reduce the arsenic uptake by plants.

Aquatic macrophytes and their biomarkers have been used in the bioindication of water pollution by Lagadic et al. (1998). Many authors have demonstrated their potential of accumulation of heavy metals and metalloids (Pollard et al. 2014). *Myriophyllum alterniflorum* is a submerged aquatic macrophyte, found in the oligotrophic rivers of France. Its capacity of accumulation of heavy metals such as copper and cadmium has been demonstrated in previous studies (Delmail et al. 2011). No study deals with copper and arsenic accumulation properties of *M. alterniflorum*, nor with its biochemical responses to Cu- and As-induced stress. In addition, few studies focused on the effect of nutrient enrichment on inorganic pollutant uptake (Wuana and Okieimen 2011) and physiology of submersed macrophytes (Zhu et al. 2014).

Thus, the aim of this study is to evaluate (i) the potential of watermilfoil plants to accumulate copper and arsenic under two trophic levels, oligotrophic and eutrophic, and (ii) the effect of this absorption on *M. alterniflorum* determined as MDA content.

Material and methods

Plant material, growth conditions, and acclimation

M. alterniflorum strain was previously established by Delmail et al. (2011). Watermilfoil was subcultured into culture boxes (Steri Vent High Model 80 × 100 × 100 mm, KALYS SA) containing 400 mL of sterile Murashige and Skoog's medium (1962) (Murashige and Skoog 1962) adjusted to pH 6.8 before autoclaving and supplemented with 30 % sucrose. The plants were maintained in a growth cabinet set at 26 ± 2 °C, with a photoperiod of 16 h and a light intensity of 13.07 ± 0.57 W/cm^2 (neon Supra'Lux Actizoo 30 W). After 30 days, plant clones were acclimatized during 21 days in a new oligotrophic medium having a chemical composition similar to the Vienne river ($NaHCO_3$ 0.18 mM; H_2SiO_3 0.15 mM; $MgSO_4{\cdot}7H_2O$ 0.045 mM; $CaCl_2$ 0.164 mM; $Ca(NO_3)_2{\cdot}4H_2O$ 0.013 mM; $KHCO_3$ 0.03 mM; $CoCl_2{\cdot}6H_2O$ 5.08E-06 mM; NH_4NO_3 0.00560 mM; H_3BO_3 0.0092 mM; $FeSO_4{\cdot}7H_2O$ 0.0037 mM; KH_2PO_4 0.0011 mM; $ZnSO_4{\cdot}7H_2O$ 4.58E-05; $MnSO_4$ H_2O 0.00043 mM; $NiCl_2{\cdot}6H_2O$ 5.092E-05 mM; and Na_2SeO_3 1.26E-05 mM). For eutrophic conditions, the concentrations of nitrates, ammonium, and phosphates were changed according to the system of surface water quality evaluation (MEDD et Agences de l'eau 2003). The final concentration of NO_3^-, NH_4^+, and PO_4^{3-} was respectively 2, 0.1, and 0.1 mg/L in oligotrophic medium and 25, 2, and 1 mg/L in eutrophic medium ($CaCl_2$ 0.058 mM; $Ca(NO_3)_2{\cdot}4H_2O$ 0.12 mM; $NaNO_3$ 0.053 mM; NH_4NO_3 0.11 mM; and KH_2PO_4 0.010 mM). These concentrations were chosen, on the one hand, to be close to the oligotrophic and eutrophic concentrations occurring in aquatic ecosystems, and, on the other hand, to exceed the limit of detection and quantification for medium analysis. All products used for the preparation of the synthetic medium were 99 %, Normapur, Prolabo.

Exposure to pollutants

After 21 days of acclimation, watermilfoil plants were transferred into culture boxes (Steri Vent High Model 80 × 100 × 100 mm, KALYS SA) containing 400 mL of the synthetic medium. $Na_2H_{1As}O_4{\cdot}7H_2O$ (Normapur, Prolabo 99.9 %) or $CuSO_4{\cdot}5H_2O$ (Normapur, Prolabo, 99.9 %) was added aseptically in order to reach a final concentration of 100 μg/L of Cu or As (in form of Arseniate), except for controls (nine replicates/condition). Plants were maintained in a growth cabinet set at 26 ± 2 °C, with a photoperiod of 16 h and

a light intensity of 13.07 ± 0.57 W/cm^2 (neon Supra'Lux Actizoo 30 W) for 21 days.

Cu and As analysis in watermilfoil plants

Watermilfoil plants were sampled in each box at t0 and tf (21 days) for Cu and As analysis. Five hundred milligrams of fresh watermilfoil samples were rinsed with distilled water, dried at 60 °C during 48 h, and then digested in a mixture of 2 mL 69 % HNO_3 (Xilab, for trace element analysis) and 2 mL of H_2O_2 (Prolabo, 30 %) at 150 °C for 2 h. The final volume was adjusted to 10 mL with MilliQ-Water and then filtered with 0.2-μm syringe filter (Whatman). Copper and arsenic concentrations were determined by ICP-MS (Agilent Technologies, ICP-MS 7700). They were measured separately in roots and shoots of watermilfoil plants. SLRS-5 (river water reference for trace metals, density = 1.0007 g/mL) was used as a internal reference for analysis. LQ (As) = 0.03 μg/L and LQ (Cu) = 0.05 μg/L.

Bioconcentration factor and Translocation Factor

To evaluate the bioaccumulation potential of watermilfoil, bioconcentration factor (BCF) and translocation factor (TF) were calculated (Liu et al. 2014). The TF is defined as the ratio of metal concentration in the shoots to those in the roots. While, the BCF is defined as the ratio of metal concentrations in the plants (shoots or roots) to those in water.

TF = [M]shoots/[M]roots where [M]shoots is the concentration of metal or metalloid in shoots in microgram per gram fresh weight (FW) and [M] roots is the concentration of metal or metalloid in roots in microgram per gram FW.

BCF = [M]plants/[M]water where [M] plants is defined as the concentration of metal or metalloid in plants (shoots or roots) in microgram per kilogram FW and [M] water is the concentration of metal or metalloid in water in microgram per liter metal or metalloid (M). All these calculations were performed on five replicates of plants.

MDA content

MDA quantification was based on the method adapted by (Delmail et al. 2011). Briefly, plants were grinded with 1 % trichloroacetic acid (TCA) with the aid of clean Fontainebleau sand (Prolabo, 150–200 μm). The mixture was then centrifuged at 5000 rpm for 10 min at 4 °C in a centrifuge (3–18 K, Sigma). To 1 mL of the supernatant, 1 mL of 0.5 % thiobarbituric acid (TBA) was added and heated for 30 min at 100 °C. After a second centrifugation (5000 rpm, 10 min, 4 °C), the absorbance was read at 532 nm. The amount of MDA was calculated from the extinction coefficient 155 mM/cm and expressed as mole per gram FW.

Statistical analysis

All results are mean of nine replicates except for BCF and TF factors calculated on five replicates. Normality of the data matrix was confirmed before others statistical analysis. Paired Student's *T* test was used to study the accumulation potential of Cu and As in the different parts of *M. alterniflorum* between t0 and tf (21 days). One-sample *T* test was used to study the significance of the translocation factor versus 1. While two-sample *T* test was used to study the difference in the accumulation of these two contaminants between eutrophic and oligotrophic conditions, the difference of BCF and TF between t0 and Tf (21 days) in the presence of copper and for the two trophic conditions and the evolution of MDA content in the presence of Cu and As were compared to control plants at tf (21 days). All the tests were performed at 5 % of significance (p value <0.05) with Systat 11 software package for Windows.

Results

Copper accumulation in watermilfoil

The potential of copper accumulation in watermilfoil shoots (including leaves) and roots was studied. Results are presented in Table 1 for both conditions: oligotrophic and eutrophic.

In oligotrophic conditions, Cu was more accumulated in shoots (196 ± 38 μg As/g dry weight (DW)) than in roots (99 ± 23 μg As/g DW), corresponding to an increase of 100 % μg Cu/g DW (p value <0.05). The same trend was observed in eutrophic condition with 145 ± 31 μg Cu/g DW in chlorophyllian parts against 56 ± 12 μg As/g DW (163 % μg Cu/g DW) in roots. In addition, our results showed that Cu was more accumulated in watermilfoil plants in oligotrophic than in eutrophic condition, this increase represented 33 % in shoots and 100 % in roots (p value <0.05).

Arsenic accumulation in watermilfoil

Arsenic accumulation in watermilfoil shoots (including leaves) and roots was also studied. Results are presented in Table 2 for both conditions: oligotrophic and eutrophic.

In oligotrophic conditions, As was more accumulated in roots (156 ± 22 μg As/g DW) than in shoots (104 ± 12 μg As/g DW), corresponding to an increase to 34 % μg As/g DW (p value <0.05). The same trend was observed in eutrophic condition with 107 ± 7 μg As/g DW in roots against 88 ± 9 μg As/g DW (18 % μg As/g DW) in the chlorophyllian parts. In addition, As was more accumulated in oligotrophic

Table 1 Copper accumulation (μg/g DW) in shoots and roots of watermilfoil plants controls and treated with 100 μg/L Cu^{2+} in eutrophic or oligotrophic conditions

	Trophic condition	Living parts	T0 controls	Tf (21 days)	
				Controls	Cu-treated plants
[Cu] μg/g DW	Oligotrophic condition	Shoots	8.94 ± 1.6^{a}	11.28 ± 1.80^{a}	196.06 ± 37.83^{c}
		Roots	4.22 ± 0.75^{b}	5.80 ± 0.90^{b}	98.67 ± 22.74^{d}
	Eutrophic condition	Shoots	9.15 ± 3.25^{a}	10.75 ± 3.61^{a}	145.44 ± 31.31^{e}
		Roots	5.32 ± 1.26^{b}	6.66 ± 1.75^{b}	55.57 ± 12.06^{f}

Values are means ± SD ($N = 9$). Different letters indicate significantly different values $p < 0.05$.

condition of about 19 % in shoots and 48 % in roots than in eutrophic ones (p value <0.05) as observed for Cu.

Translocation factor and bioconcentration factor of Cu and As in watermilfoil

The TF determined for *M. alterniflorum* tissues showed an opposite behavior for Cu and As. TF values obtained for the above and underground part of the plants were significantly >1.0 for copper and <1.0 for arsenic in both media (p value <0.05) (Table 3). For copper, the TF ratios at t0 in both media were not different (p value < 0.05) while at 21 days, it increased significantly from oligotrophic to eutrophic medium about 40 % (p value >0.05). For arsenic, the TF ratios at 21 days remained stable and did not differ between the two media.

In both conditions, for plants exposed to copper, the BCF in shoots (1356 ± 311 in OC and 592 ± 148 in EC) were higher than in roots (613 ± 44 in OC and 183 ± 42 in EC) (p value <0.05), and these BCFs are higher than those of copper-treated plants at t0. For arsenic, an opposite trend was observed for both conditions. The BCF was higher in roots (621 ± 102 in OC and 256 ± 73 in EC) than in shoots (475 ± 99 in OC and 228 ± 64 in EC) (p value <0.05).

At t0, copper-treated plants did not show any significant difference in BCF of shoots or roots if oligotrophic and eutrophic conditions are compared. While, at 21 days, in copper-treated plants in oligotrophic condition, BCF of shoots was 129 % higher (p value <0.05) than in eutrophic condition. In addition, the BCF of roots was higher in oligotrophic condition, corresponding to an increase of 234 % with respect to the eutrophic condition (p value <0.05).

The same trend was observed for arsenic, whereas at tf (21 days) the BCF of shoots in oligotrophic condition was more higher than that in the eutrophic condition corresponding to an increase of about 108 % (p value <0.05) compared to the plants exposed to arsenate in eutrophic medium. In addition, like shoots, BCF in roots of arsenate-treated plants was higher of about 143 % in oligotrophic condition than in eutrophic condition (p value <0.05).

MDA content

To study the effect of copper and arsenic on plants, the malondialdehyde content in watermilfoils was investigated. Results for both trophic conditions are presented in Table 4.

After 21 days, in oligotrophic medium, Cu at 100 μg/L did not change the MDA content in watermilfoil plants (p value >0.05) compared to the control plants. While in eutrophic medium, compared to the control, an increase (21 %; p value <0.05) of MDA in copper-treated plants at 21 days was observed.

In contrast for both conditions, arsenate was able to induce an increase in MDA content (from 6 ± 1 mol/g FW to 8 ± 1 mol/g FW in oligotrophic condition and from 12 ± 1 mol/g FW to 16 ± 3 mol/g FW) in eutrophic condition, corresponding to an increase of about 24 and 35 %, respectively, (p value <0.05) compared to the controls plants at 21 days of each condition.

In addition, it should be noted that the MDA content at 21 days in control plants (without copper and arsenate) showed a significant increase in eutrophic condition of about 88 % (p value <0.05), comparing to the control plants in oligotrophic conditions. While, in copper- and arsenic-treated

Table 2 Arsenic accumulation (μg/g DW) in shoots and roots of watermilfoil plants controls and treated with 100 μg/L As (V) in eutrophic or oligotrophic conditions

	Trophic condition	Living parts	T0 Controls	Tf (21 days)	
				Controls	As-treated plants
[As] μg/g DW	Oligotrophic condition	Shoots	$n.d^{a}$	$n.d^{a}$	103.85 ± 12.21^{b}
		Roots	$n.d^{a}$	$n.d^{a}$	156.53 ± 21.48^{c}
	Eutrophic condition	Shoots	$n.d^{a}$	$n.d^{a}$	87.48 ± 9.29^{d}
		Roots	$n.d^{a}$	$n.d^{a}$	106.65 ± 6.98^{e}

Values are means ± SD ($N = 9$). Different letters indicate significantly different values $p < 0.05$. *n.d* not detected

Table 3 Bioconcentration factor (BCF) and Translocation factor (TF) between shoots (including leaves) and roots of watermilfoil plants at t0 and after 21 days (Tf) of treatment with 100 μg/L of Cu or As individually in oligotrophic (OC) and eutrophic conditions (EC).

Pollutant level		Cu			As		
		TF	BCF Shoots	BCF roots	TF	BCF Shoots	BCF roots
OC EC	Copper-treated plants (T0)	$2.8 \pm 0.50^{a*}$	30.64 ± 5.26^{a}	10.97 ± 2.90^{b}			
	Copper-treated plants (Tf)	$2.20 \pm 0.44^{ab*}$	1355.70 ± 311.34^{c}	612.53 ± 43.55^{d}			
	Arsenic-treated plants (Tf)				$0.77 \pm 0.15^{a*}$	475.42 ± 99.42^{a}	620.67 ± 102.31^{b}
	Copper-treated plants (T0)	$2.47 \pm 0.90^{a*}$	24.13 ± 2.60^{a}	11.08 ± 4.73^{b}			
	Copper-treated plants (Tf)	$3.06 \pm 0.31^{ac*}$	592.43 ± 148.13^{g}	182.60 ± 42.00^{h}			
	Arsenic-treated plants (Tf)				$0.87 \pm 0.11^{a*}$	227.95 ± 63.97^{c}	255.02 ± 72.77^{d}

Values are means ($n = 5$) ± SD. Values are calculated on fresh weight basis. Different letters indicate significantly different values $p < 0.05$. *Asterisk* indicates that value is significantly different from 1.

plants, MDA showed an increase of 153 and 105 % (p value <0.05) after 21 days, respectively, comparing to those in oligotrophic conditions respectively.

Discussion

In this study, copper is more accumulated in shoots than in roots of *M. alterniflorum*, in both media, whereas it is more accumulated in oligotrophic medium than in eutrophic one (Table 1). Our results are similar to the observations of several terrestrial and aquatic macrophytes. Swain et al. (2014) showed that water hyacinth *(Eichhornia crassipes)* accumulated after 25 days more copper in stems (2314.2 μg/g DW) than in roots (464.9 2 μg/g DW) at a concentration of 1.05 mg/L in a bucket experiment. In addition, Xue et al. (2010) showed that after 4 days of treatments with Cu at a concentration of 4 mg/L, the Cu content in shoots of *Hydrilla verticillata* increased significantly and reached a maximum at 30,830 μg/g DW. It is probable that plants translocate the essential trace elements like Cu from the roots into the above-ground tissues for metabolic use. Also, these authors (Xue et al. 2010) found that the acropetal Cu translocation was higher than the basipetal translocation, and this translocation is done via the apoplast. This could explain the higher accumulation of copper in shoots. In addition, in oligotrophic medium, the concentration of phosphates and nitrates is low, and the concentrations of major cations especially Na^+ and K^+ are slightly lower (4.07 and 1.22 mg/L, respectively) compared to those in eutrophic medium (5.3 and 1.6 mg/L, respectively). This fact could decrease the competition of copper for plant receptors on the roots membranes and then explain the increase of its ad/absorption and bioaccumulation in watermilfoil plants (Ardestani et al. 2014). In contrast, in eutrophic medium, the concentration of phosphates and nitrates is higher, and the increase of organic carbon in the medium during 21 days (data not shown)\ could lead to a complexation of copper with organic matter, decreasing its bioavailability (Coquery and Welbourn 1995; Lin and Chen 1998; Liu et al. 2014). This behavior could explain the decrease of copper accumulation in eutrophic medium.

In contrast, arsenic is preferentially accumulated in roots than in shoots (Table 2). This fact is very well documented in literature (Robinson et al. 2006; Mazej and Germ 2009; Vromman et al. 2011; Yabanli et al. 2014). It is known that the root system acts as a barrier against toxic element translocation to the tops (Mazhoudi et al. 1997). Further, it has been hypothesized that the metal detoxification mechanism especially the sequestration by phytochelatins restricts the mobility of metals to shoot (Gupta et al. 2013) and could be responsible of the high As-content in roots. In addition, like copper, arsenic is more accumulated in oligotrophic medium than in

Table 4 Effect of copper or arsenate supply on the malondialdehyde (MDA) content in the shoots of watermilfoil controls and treated with 100 μg/L of Cu or As (V) in eutrophic or oligotrophic conditions

	Trophic condition	Tf (21 days)		
		Controls	Cu-treated plants	As-treated plants
MDA content mol/g FW	OC	6.47 ± 0.9^{a}	5.5 ± 1.2^{a}	8 ± 0.7^{b}
	EC	12.18 ± 0.93^{c}	13.93 ± 1.63^{d}	16.44 ± 2.74^{e}

Values are means ($N = 9$) ± SD expressed in mol/g FW. Different letters indicate significantly different values $p < 0.05$

eutrophic one (Table 2). In oligotrophic medium, the phosphates concentration in the medium is low, decreasing the competition of arsenate for plant transporters and then increasing its bioaccumulation in watermilfoil. In contrast, in eutrophic medium, the higher phosphate concentration increases their competition with arsenate, leading to a decrease of arsenic accumulation in plants (Zhao et al. 2009).

The BCF is a key index used to evaluate the accumulation potential of contaminants in plants. In our study, the BCF is higher in shoots for copper (600 in EC and 1400 in OC; on FW basis) and in roots for arsenic (250 in EC and 600 in OC; on FW basis) after 21 days. On DW basis, for copper, our BCF results in shoots are equivalent to 7000 in EC and 19,000 in OC, while for arsenic, these values in roots are equivalent to 4000 in EC and 10,000 in OC. So, our results indicate that *M. alterniflorum* has a higher bioaccumulation potential for Cu and As. Swain et al. (2014) showed that the maximum BCF values for Cu in shoots were 2918 (on DW basis), in the water hyacinth *E. Crassipes* exposed to 1.05 mg/L of Cu for 25 days. Xue et al. (2010) showed that the BCF of Cu in *H. verticillata* exposed to 128 μg/L of Cu was 901 (on DW basis) after 96 h of treatment in shoots. The same author showed that the BCF of *H. verticillata* exposed to 2 μM of As(V) for 4 days was equal to 4000 (on DW basis) in roots (Xue and Yan 2011). In a field experiment, Delmail et al. (2013) showed that Cu BCF in shoots of *M. alterniflorum* varied from 619 at 3 days to 226,024 after 28 days.

The translocation factor determined the ability of a pollutant to be restrained in the under parts or to be translocated to the upper parts of plants. In our study, copper is very well translocated to the chlorophyllian parts, while As is more accumulated in roots restraining its translocation to the upper parts. Our results are similar to those of Mazej and Germ (2009) who found in a field experiment that the TF of four aquatic macrophytes *Najas marina*, *Potamogeton lucens*, *Nuphar lutea*, and *Potamogeton nodosus* were less than 1 for As and higher than 1 for copper, indicating that the mobility to above-ground plant parts differs between these two elements. In addition, Liu et al. (2014) showed in a field experiment that a mangrove species *Kandelia obovata* in the presence of arsenic had a TF equal to 0.199. So, this plant accumulated more As in the roots and did not transfer it very well to shoots. Our results indicate that *M. alterniflorum* has a higher efficiency in restraining the translocation of a non-essential and very toxic element like As, but a lower capacity for controlling the translocation of an essential element like Cu.

It could be noted that *M. alterniflorum* grow normally when they are transferred into the new synthetic medium. Nevertheless, this growth is reduced in eutrophic conditions compared to the oligotrophic conditions due the nutrient loading in the medium (40 % decrease in the length of the chlorophyllian parts) (data not shown). And this effect was also evidenced by the measure of malondialdehyde content. Malondialdehyde content is a biomarker of lipoperoxidation that can be considered as an indicator of oxidative damage (Xing et al. 2010). In our study, controls plants in eutrophic medium showed an increase in MDA compared to the oligotrophic medium. This increase may be due to the high nutrient loading in eutrophic medium and especially to ammonium. This finding is similar to the observation in *Lemna minor* (Huang et al. 2013) and *Myriophyllum spicatum* (Zhang et al. 2013) under ammonium stress. The accumulation of copper and arsenic has a toxic effect on the metabolism of watermilfoil plants. Copper induced an increase of MDA content only in eutrophic medium and not in oligotrophic medium. The toxicity of copper appeared in eutrophic medium due the combined effect with ammonium. Our results are similar to several observations with *H. verticillata*, *Potamogeton pusillus*, and *Ceratophyllum demersum* (Mishra et al. 2006; Srivastava et al. 2006; Monferrán et al. 2009). In oligotrophic condition, the MDA content increase was not significant. Perhaps, the oxidative stress generated by copper was detoxified by the enzymatic and non-enzymatic defense system of watermilfoil, protecting the cell membranes from alteration and peroxidation by reactive oxygen species (Srivastava et al. 2006). While arsenic was able to induce an increase of MDA in both media, and it was more pronounced in eutrophic medium than oligotrophic medium. This increase in MDA content occurs with the increase in H_2O_2 content as shown recently by Krayem et al. (2016). This could be due to the combined effect of As and ammonium in eutrophic case. Many authors have shown an increase in MDA content in plants after an exposure to As, and our observations are similar to these studies (Singh et al. 2007; Duman et al. 2010; Chen et al. 2015).

Conclusion

Our results suggest that the uptake of copper and arsenic is affected by the trophic level. In the eutrophic medium, the absorption of these two contaminants is affected in one hand by the concentration of phosphate and nitrogen and by the organic carbon in other hand. In Addition, copper is more accumulated in shoots, than roots, whereas the opposite trend is observed for arsenic. *M. alterniflorum* has the capacity to restrain arsenic in its roots and prevents an excessive and toxic accumulation of this element in shoots. However, *M. alterniflorum* has a low capacity to prevent the

translocation of copper to shoots, because it is an essential micronutrient for plants. Cu and As have toxic effects on plants. This effect is demonstrated by the increase in MDA content. On the basis of this experiment, *M. alterniflorum* has a higher accumulation potential of copper and arsenic, and therefore, it can be a good candidate for the phytofiltration of these two contaminants in water.

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References

Ardestani MM, van Straalen NM, van Gestel CAM (2014) The relationship between metal toxicity and biotic ligand binding affinities in aquatic and soil organisms: a review. Environ Pollut 195:133–147. doi:10.1016/j.envpol.2014.08.020

Chen G, Liu X, Brookes PC, Xu J (2015) Opportunities for Phytoremediation and bioindication of arsenic contaminated water using a submerged aquatic plant: *Vallisneria natans* (lour.) Hara. Int J Phytoremediation 17:249–255. doi:10.1080/15226514.2014.883496

Coquery M, Welbourn PM (1995) The relationship between metal concentration and organic matter in sediments and metal concentration in the aquatic macrophyte *Eriocaulon septangulare*. Water Res 29:2094–2102. doi:10.1016/0043-1354(95)00015-D

Delmail D, Labrousse P, Hourdin P et al (2011) Differential responses of *Myriophyllum alterniflorum* DC (Haloragaceae) organs to copper: physiological and developmental approaches. Hydrobiologia 664:95–105. doi:10.1007/s10750-010-0589-9

Delmail D, Labrousse P, Hourdin P et al (2013) Micropropagation of *Myriophyllum Alterniflorum* (Haloragaceae) for stream rehabilitation: first *in vitro* culture and reintroduction assays of a heavy-metal hyperaccumulator immersed macrophyte. Int J Phytoremediation 15:647–662. doi:10.1080/15226514.2012.723068

Duman F, Ozturk F, Aydin Z (2010) Biological responses of duckweed (Lemna minor L.) exposed to the inorganic arsenic species As(III) and As(V): effects of concentration and duration of exposure. Ecotoxicology 19:983–993. doi:10.1007/s10646-010-0480-5

Garcia L, Welchen E, Gonzalez DH (2014) Mitochondria and copper homeostasis in plants. Mitochondrion 19(Part B):269–274. doi:10.1016/j.mito.2014.02.011

Gupta DK, Huang HG, Nicoloso FT et al (2013) Effect of Hg, As and Pb on biomass production, photosynthetic rate, nutrients uptake and phytochelatin induction in *Pfaffia glomerata*. Ecotoxicology 22:1403–1412. doi:10.1007/s10646-013-1126-1

Huang L, Lu Y, Gao X et al (2013) Ammonium-induced oxidative stress on plant growth and antioxidative response of duckweed (*Lemna minor* L.). Ecol Eng 58:355–362. doi:10.1016/j.ecoleng.2013.06.031

Jampeetong A, Brix H (2009) Effects of NH_4^+ concentration on growth, morphology and NH_4^+ uptake kinetics of *Salvinia natans*. Ecol Eng 35:695–702. doi:10.1016/j.ecoleng.2008.11.006

Krayem M, Deluchat V, Rabiet M et al (2016) Effect of arsenate on the biomarkers of M*yriophyllum alterniflorum* in oligotrophic and eutrophic conditions. Chemosphere 147:131–137

Lagadic L, Caquet TH, Amiard JC, Ramade F (1998) Utilisation de biomarqueurs pour la surveillance de la qualité de l'environnement., Tec & Doc Lavoisier

Lin J-G, Chen S-Y (1998) The relationship between adsorption of heavy metal and organic matter in river sediments. Environ Int 24:345–352. doi:10.1016/S0160-4120(98)00012-9

Liu C-W, Chen Y-Y, Kao Y-H, Maji S-K (2014) Bioaccumulation and translocation of arsenic in the ecosystem of the Guandu Wetland, Taiwan. Wetlands 34:129–140. doi:10.1007/s13157-013-0491-0

Mazej Z, Germ M (2009) Trace element accumulation and distribution in four aquatic macrophytes. Chemosphere 74:642–647. doi:10.1016/j.chemosphere.2008.10.019

Mazhoudi S, Chaoui A, Habib Ghorbal M, El Ferjani E (1997) Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum* Mill.). Plant Sci 127:129–137. doi:10.1016/S0168-9452(97)00116-7

MEDD et Agences de l'eau, 2003. Système d'évaluation de la qualité de l'eau des cours d'eau SEQ-Eau, Grilles d'évaluation version 2. 40 pages, 21 mars 2003 http://www.observatoire-eau-bretagne.fr/Ressources-et-documentation/Documents-deplanification/Systeme-d-evaluation-de-la-qualite-de-l-eau-des-cours-d-eau-SEQEau [archive] La grille d'évaluation SEQ-Eau (version 2)

Meharg AA, Macnair MR (1992) Suppression of the high affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L. J Exp Bot 43:519–524

Mishra S, Srivastava S, Tripathi RD et al (2006) Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65:1027–1039. doi:10.1016/j.chemosphere.2006.03.033

Mohan D, Pittman CU (2007) Arsenic removal from water/wastewater using adsorbents—a critical review. J Hazard Mater 142:1–53. doi:10.1016/j.jhazmat.2007.01.006

Monferrán MV, Sánchez Agudo JA, Pignata ML, Wunderlin DA (2009) Copper-induced response of physiological parameters and antioxidant enzymes in the aquatic macrophyte *Potamogeton pusillus*. Environ Pollut 157:2570–2576. doi:10.1016/j.envpol.2009.02.034

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497. doi:10.1111/j.1399-3054.1962.tb08052.x

Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8:199–216. doi:10.1007/s10311-010-0297-8

Pollard AJ, Reeves RD, Baker AJM (2014) Facultative hyperaccumulation of heavy metals and metalloids. Plant Sci 217–218:8–17. doi:10.1016/j.plantsci.2013.11.011

Robinson B, Kim N, Marchetti M et al (2006) Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand. Environ Exp Bot 58:206–215. doi:10.1016/j.envexpbot.2005.08.004

Sharma VK, Sohn M (2009) Aquatic arsenic: toxicity, speciation, transformations, and remediation. Environ Int 35:743–759. doi:10.1016/j.envint.2009.01.005

Singh HP, Batish DR, Kohli RK, Arora K (2007) Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. Plant Growth Regul 53:65–73. doi:10.1007/s10725-007-9205-z

Singh R, Singh S, Parihar P et al (2015) Arsenic contamination, consequences and remediation techniques: a review. Ecotoxicol Environ Saf 112:247–270. doi:10.1016/j.ecoenv.2014.10.009

Smith KS, Balistrieri LS, Todd AS (2015) Using biotic ligand models to predict metal toxicity in mineralized systems. Appl Geochem 57:55–72. doi:10.1016/j.apgeochem.2014.07.005

Srivastava S, Mishra S, Tripathi RD et al (2006) Copper-induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (L.f.) Royle. Aquat Toxicol 80:405–415. doi:10.1016/j.aquatox.2006.10.006

Swain G, Adhikari S, Mohanty P (2014) Phytoremediation of Copper and Cadmium from Water Using Water Hyacinth, *Eichhornia crassipes*. Int J Agric Sci Technol 2:1. doi: 10.14355/ijast.2014.0301.01

Tangahu BV, Sheikh Abdullah SR, Basri H et al (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 2011, e939161. doi:10.1155/2011/939161

Vromman D, Flores-Bavestrello A, Šlejkovec Z et al (2011) Arsenic accumulation and distribution in relation to young seedling growth in *Atriplex atacamensis* Phil. Sci Total Environ 412–413:286–295. doi:10.1016/j.scitotenv.2011.09.085

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. Int Sch Res Not 2011, e402647. doi:10.5402/2011/402647

Xing W, Huang W, Liu G (2010) Effect of excess iron and copper on physiology of aquatic plant *Spirodela polyrrhiza* (L.) Schleid. Environ Toxicol 25:103–112. doi:10.1002/tox.20480

Xue P, Li G, Liu W, Yan C (2010) Copper uptake and translocation in a submerged aquatic plant *Hydrilla verticillata* (L.f.) Royle. Chemosphere 81:1098–1103. doi:10.1016/j.chemosphere.2010.09.023

Xue P, Yan C (2011) Arsenic accumulation and translocation in the submerged macrophyte *Hydrilla verticillata* (L.f.) Royle. Chemosphere 85:1176–1181. doi:10.1016/j.chemosphere.2011.09.051

Yabanli M, Yozukmaz A, Sel F (2014) Heavy metal accumulation in the leaves, stem and root of the invasive submerged macrophyte *Myriophyllum spicatum* L. (Haloragaceae): an example of Kadın Creek (Mugla, Turkey). Braz Arch Biol Technol. doi: 10.1590/S1516-8913201401962

Zangi R, Filella M (2012) Transport routes of metalloids into and out of the cell: a review of the current knowledge. Chem Biol Interact 197:47–57. doi:10.1016/j.cbi.2012.02.001

Zhang L, Wang S, Jiao L et al (2013) Physiological response of a submerged plant (*Myriophyllum spicatum*) to different NH_4Cl concentrations in sediments. Ecol Eng 58:91–98. doi:10.1016/j.ecoleng.2013.06.006

Zhao FJ, Ma JF, Meharg AA, McGrath SP (2009) Arsenic uptake and metabolism in plants: Tansley review. New Phytol 181:777–794. doi:10.1111/j.1469-8137.2008.02716.x

Zhu G, Cao T, Zhang M et al (2014) Fertile sediment and ammonium enrichment decrease the growth and biomechanical strength of submersed macrophyte *Myriophyllum spicatum* in an experiment. Hydrobiologia 727:109–120. doi:10.1007/s10750-013-1792-2