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RESEARCH ARTICLE

Copper uptake by *Eichhornia crassipes* exposed at high level concentrations

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Abstract The objective of this study was to assess the growth of water hyacinth (*Eichhornia crassipes*) and its ability to accumulate Cu from polluted water with high Cu concentrations and a mixture of other contaminants under short-term exposure, in order to use this species for the remediation of highly contaminated sites. Two hydroponic experiments were performed under greenhouse conditions for 7 days. One of them consisted of growing water hyacinth in Hoagland solution supplemented with 15 or 25 mg Cu/L and a control. The other one contained water hyacinth growing in polluted river water supplemented with 15 mg Cu/L and a control. Cu was accumulated principally in roots. The maximum Cu concentration was 23,387.2 mg/kg dw in the treatment of 25 mg Cu/L in Hoagland solution. Cu translocation from roots to leaves was low. The mixture of 15 mg Cu/L with polluted water did not appear to have toxic effects on the water hyacinth. This plant showed a remarkable uptake capacity under elevated Cu concentrations in a mixture of pollutants similar to pure industrial effluents in a short time of exposure. This result has not been reported before, to our knowledge. This species is suitable for phytoremediation of waters subject to discharge of mixed industrial effluents containing elevated Cu concentrations (≥15 mg Cu/L), as well as nutrient-rich domestic wastewaters.

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Introduction

Environmental pollution by trace metals is, in many cases, accompanied by pollution with foreign organic compounds and vice versa. Not only phytoremediation but also phytostabilization, in areas affected by multiple pollution sources, is complicated, and only a few plant species have been shown to survive under such adverse conditions. Among heavy metals, copper (Cu) is an essential element for plants. Toxic concentrations of metals, even the essentials, can exert primary and secondary effects on plants at different levels of integration (molecular, cellular, and tissue) depending on the metal(s) in question (Ernst et al. 1992).

The Matanza-Riachuelo (MR) river (Argentina, South America) and its banks contain an important load of urban and industrial waste composed of nutrients, organic matter, and a variety of chemical elements, such as heavy metals (Ronco et al. 2008). Metal contaminant discharges during the past century have made the MR river sediments into a large repository of metal pollutant. In the anoxic sediments, sulfide species are important binding forms for many trace metals promoting their incorporation into sediments (Rendina and Fabrizio de Iorio 2012).

River restoration projects aim to recover ecosystem goods and services while protecting downstream and coastal ecosystems (Palmer et al. 2005). Frequently, remedial procedures of disturbed aquatic environments involve dredging and construction activities that can strongly modify the distribution of metals between the dissolved and particulate phases and their speciation, transport phases, and bioavailability (Hamzeh et al. 2014). In addition, restoration involves revegetation with tolerant native plants. The Matanza-Riachuelo restoration plan

(ACUMAR (Autoridad de Cuenca Matanza-Riachuelo) 2007) includes remediation and revegetation of the river banks. In these environments, the levels of toxic compounds in the water frequently exceed those recommended in national and international water quality guidelines for the protection of aquatic life [2.00–4.00 μg Cu/L, Law No. 24051 (1991); 1.45–2.33 μg Cu/L, USEPA (United States Environmental Protection Agency) (2007); 1.00–28.00 μg Cu/L, UKTAG (United Kingdom Technical Advisory Group on the Water Framework Directive) (2008); 1.00–2.50 μg Cu/L, ANZECC & ARMCANZ (Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand) (2000); 0.30–1.40 μg Cu/L, DWAF (Department of Water Affairs and Forestry) (1996)]. However, these levels allow the presence of tolerant organisms, and tolerance implies adaptation to multiple stress conditions. At these concentrations, heavy metals, as well as organic xenobiotics, have been found to be beneficial for plants because they stimulate reactive oxygen species (ROS) and induce resistance against other stressors (Verkleij et al. 2009). The presence of other stressors, such as oxygen deficit, latent pathogen infection, and moderate concentrations of toxic ions typical of a polluted river, could contribute to stress alleviation and stimulate macrophyte growth through cross-protection (Poschenrieder et al. 2013). Interactions between metals and other compounds occurring inside, on the surface, or outside plants may disrupt the expected toxic responses. The metabolic ability of exposed macrophytes may weaken the effects of metal pollutants due to the interactions occurring in the macrophyte and in the surrounding water. These interactions and the metabolic ability of certain aquatic plants may offer an approach for the establishment of a bioremediation plan for restoring the water quality (Ali et al. 2013).

Water hyacinth (*Eichhornia crassipes*) is one of the free floating macrophytes most studied for phytoremediation purposes. Previous experiments indicate that it is capable of accumulating metal ions from water, including As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, and Zn (Azizur Rahman and Hasegawa 2011; Cordes et al. 2000; Gupta et al. 2012) and also acting as a biosorbent (Mahamadi 2011). It is also capable of removing organic matter from wastewater and domestic effluents (Gupta et al. 2012; Ndimele et al. 2011) as well as other organic xenobiotic compounds (Ndimele 2010). *E. crassipes* is native to South America, and it is abundant in highly polluted rivers such as the MR river.

According to Saygideger et al. (2005), the bioavailability of metals to aquatic plants is dependent on many factors including environmental metal concentration, pH of water, concentration of ligands, competition with other metals for binding sites, and mode of exposure. In polluted water bodies, all these factors are combined with a diverse mixture of toxic compounds. This situation results in an intricate set of conditions that floating macrophytes and other riparian plants are exposed to, but they do not always survive successfully. The Cu removal efficiency by water hyacinth from contaminated water bodies has been studied in long-term experiments (Ajayi and Ogunbayo 2012; Buta et al. 2011). There are also several studies that followed the Cu uptake after the first few days of exposure (Yapoga et al. 2013; Mishra et al. 2008; Upadhyay et al. 2007). However, in all cases, the Cu concentrations present in, or added to, those polluted water bodies are very low, and the performance of water hyacinth under high Cu concentrations (>10 mg/L) in highly polluted waters in short-term experiments remains poorly known (Soltan and Rashed 2003; Sutton and Blackburn 1971). Thus, the objective of this study was to assess the growth of water hyacinth and its ability to accumulate Cu from polluted water bodies with high Cu concentrations and a mixture of other contaminants under short-term exposure, in order to use this species for the remediation of highly contaminated sites.

Materials and methods

E. crassipes plants and water were collected from the lower MR river (34°38′12″ S, 58°21′05″ W), Buenos Aires, Argentina. The lower part of the basin was considered as one of the ten most polluted sites in the world by Green Cross Switzerland in 2013 (Blacksmith Institute & Green Cross 2013; ECyT-AR (La Enciclopedia de Ciencias y Tecnologías en Argentina) 2011).

Plants were washed several times with tap water and placed in two plastic tanks filled with 30 and 40 L of diluted Hoagland solution during 60 days for greenhouse acclimatization.

After acclimatization, individuals of similar size and weight grown in the greenhouse were selected. Homogeneous plant biomass of water hyacinth was placed in 6-L plastic reactors containing Hoagland solution (experiment H) (So et al. 2003) or MR polluted river water (experiment MR). The composition of each media is detailed in Table 1. Three extra plants were separated for initial determinations for each experiment. Experiment H consisted of two treatments and a control: Hoagland solution supplemented with 15 mg Cu/L (T_H15), or 25 mg Cu/L (T_H25), and the control containing only Hoagland solution (T_H0). Experiment MR was prepared with MR river water supplemented with 15 mg Cu/L ($T_{MR}15$) and a control containing only MR river water ($T_{MR}0$). Three replicate reactors containing 200 g of fresh biomass (one or two plants) were prepared for each treatment and the control. In both experiments, the Cu for treatments was added as $CuSO_4 \cdot 5\ H_2O$. Analytical-grade reagents were used to prepare media and treatments.

Table 1 Initial physicochemical characteristics of Hoagland solution (H) and Matanza-Riachuelo River water (MR water)

	H	MR water
Macronutrients		
Ca (mg/L)	80.23	55.95
K (mg/L)	118.26	13.33
Mg (mg/L)	12.15	26.78
N–NH_4^+ (mg/L)	18.01	14.03
NO_2^- (mg/L)	n/m	0.014
NO_3^{-2} (mg/L)	186.01	0.18
SRP (as orthophosphate) (mg/L)	94.99	1.70
SO_4^{-2} (mg/L)	48.22	72.30
Micronutrients		
Cl (mg/L)	0.86	255.85
Cu (mg/L)	0.025	0.033
Fe (mg/L)	n/m	0.62
Mn (mg/L)	0.033	n/m
Na (mg/L)	n/m	150.00
Zn (mg/L)	0.068	0.073
Non-essential heavy metals		
Cd (mg/L)	n/m	0.070
Cr (mg/L)	n/m	0.073
Ni (mg/L)	n/m	0.17
Pb (mg/L)	n/m	0.073
Other physicochemical parameters		
Alkalinity (as CO_3^{-2}) (mg/L)	n/m	543.76
TOC (total organic carbon) (mg/L)	n/m	15.22
DOC (dissolved organic carbon) (mg/L)	n/m	9.52

n/m not measured

The reactors were placed in a greenhouse with a daylight period of 12 h and temperature of 20±2 °C for 7 days. Distilled water was added daily to compensate for evaporation or transpiration losses, keeping the initial volume constant.

Sample collection and analysis

Water samples were collected from each reactor at the beginning and at the end of the experiments. Conductivity and pH were measured during the experiments. Initial and final physicochemical characteristics of the culture media (alkalinity as CO_3^{-2}, Ca, Cd, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, N–NH_4^+, Ni, NO_2^-, NO_3^{-2}, Pb, SO_4^{-2}, SRP as orthophosphate, Zn, temperature, and pH) were determined following Standard Methods test protocols (APHA (American Public Health Association) 2000). The N–NH_3^+ speciation from NH_4^+ in MR river water was estimated (Körner et al. 2001), and Cu speciation was estimated using MINEQL+ v4.6 speciation software (Environmental Research Software).

After the exposure period in the experimental reactors, the plants were harvested, washed with deionized water, and separated into roots and leaves. The initial and final plant material was dried at 70 °C until constant dry weight. The initial dry weight of plants collected for experiment H was 2.01±0.19 and 5.01±0.27 g (mean±standard error) for roots and leaves, respectively. For experiment MR, the initial dry weight of plants was 1.38±0.12 and 1.82±0.21 g (mean± standard error) for roots and leaves, respectively. The dry mass of plant samples (0.25–1 g) was ground and placed in 100-mL Teflon beakers for digestion (HNO_3/$HClO_4$/HCl) at 140 °C until the minimum volume was reached. The suspension obtained was diluted to 50 mL with double deionized water and stored in polyethylene bottles for metal determination in flame-AAS (Perkin Elmer 1100B, Perkin Elmer, Inc. Waltham, MA, USA). Calibration standards and a blank reagent were analyzed every 10 samples. The detection limit of flame-AAS was 0.077 mg Cu/L. Initial plant samples selected for experiment H contained 19.26±3.33 and 16.64±1.01 mg Cu/kg in roots and leaves, respectively, while the plant samples selected for experiment MR contained 3.64±0.15 and 4.70±0.76 mg Cu/kg in roots and leaves, respectively. The initial range of Cu concentrations measured in tissues of *E. crassipes* was below the toxic limit (20 mg/kg, Burkhead et al. 2009).

Growth estimation and Cu accumulation

To estimate the growth of *E. crassipes*, the relative growth rate (RGR) and the growth inhibition percentage (GI) were calculated. The RGR (day^{-1}) was calculated as follows (Rizzo et al. 2012):

$$\mathrm{RGR} = \frac{\ln(\mathrm{DW}_f) - \ln(\mathrm{DW}_i)}{t}$$

where DW_f=final dry weight (treatment or control), DW_i= initial dry weight, and t=duration (days) of the experiment. The GI (%) was calculated as follows (Park et al. 2011):

$$\mathrm{GI} = \left(1 - \frac{\mathrm{RGR}_{\mathrm{treatment}}}{\mathrm{RGR}_{\mathrm{control}}}\right) \times 100$$

where $RGR_{treatment}$=relative growth rate (day^{-1}) for treatment x, and $RGR_{control}$=relative growth rate (day^{-1}) for respective control.

A plant's ability to accumulate metals from the substrate can be estimated using the bioconcentration factor (BCF), which is defined as the ratio of metal concentration in the roots to that in the substrate. The ability to translocate metals from the roots to the shoots is measured using the translocation factor (TF), which is defined as the ratio of metal concentration in the shoots to the roots. The BCF was calculated as follows (Ali et al. 2013):

$$\mathrm{BCF} = \frac{C_\mathrm{r}}{C_\mathrm{w}}$$

where C_r=final Cu concentration (mg/kg dw) in roots, and C_w=initial Cu concentration (mg/L) in the medium. The TF was calculated as follows (Ali et al. 2013):

$$\mathrm{TF} = \frac{C_\mathrm{a}}{C_\mathrm{r}}$$

where C_a=final Cu concentration (mg/kg dw) in leaves.

Statistical analysis

An analysis of variance (ANOVA) test was performed for each experiment to identify any statistically significant differences in biomass, growth, and Cu accumulation in tissues depending on water Cu concentrations in each experiment.

Another ANOVA test was performed to identify statistically significant differences in growth and Cu accumulation in tissues comparing treatments supplemented with 15 mg Cu/L from both experiments.

Data were tested for normal distribution using Shapiro–Wilks' test and for homogeneity of variance applying Levene's test. Data were natural-log transformed when these assumptions were not satisfied. Tukey's test was applied to differentiate means where appropriate. All tests were compared at a level of $p<0.05$.

Results

Physicochemical characteristics of aquatic media

The initial pH and temperature range were 5.55–7.14 °C and 19.7–21.0 °C, respectively. The initial Cu concentration was determined after metal addition to each treatment, and it was similar to the nominal concentration in all cases. However, according to the calculations of MINEQL+ speciation software, more than 90 % of the Cu precipitated in the treatments where metal was added (Table 2).

Plant biomass and growth estimation

Table 3 shows the final dry weights of root and leaf biomass, RGR, and GI in both experimental sets.

Table 2 Initial Cu concentration in experiment H and experiment MR and its estimated speciation according to MINEQL+ software

	Cu (mg/L)	Dissolved complexes (%)	Precipitated solids
Experiment H (Hoagland solution)			
T_H0 (control)	0.025	100	0 %
T_H15	15.56	0.7	99.3 % (as $Cu_3(PO_4)_2$)
T_H25	23.20	5.2	94.8 % (as $Cu_3(PO_4)_2$)
Experiment MR (MR water)			
$T_{MR}0$ (control)	0.033	100	0 %
$T_{MR}15$	14.75	0.6	99.4 %

T_H0 control, T_H15 supplemented with 15 mg Cu/L, T_H25 supplemented with 25 mg Cu/L, $T_{MR}0$ control, $T_{MR}15$ supplemented with 15 mg Cu/L

In both experiments, the leaf dry weights in the controls increased in comparison to the initial values. In experiment H (Table 3), root biomass in T_H15 increased by 43 %, whereas leaf biomass decreased by 19 % in respect to the control. Thus, the RGR in T_H15 was significantly lower than the control, showing the highest growth inhibition (63 %). On the other hand, plant biomass and RGR in T_H25 did not differ from the control.

However, in experiment MR (Table 3), the root biomass in $T_{MR}15$ was significantly lower than the control, by almost half, whereas the leaf biomass did not differ from the control. However, the decrease in RGR in respect to the control was not statistically significant.

To evaluate the effects of Cu addition and the mixture of contaminants (in experiment MR) on the growth of *E. crassipes*, treatments T_H15 and $T_{MR}15$ were compared. The RGR was much higher for plants grown in MR water ($F=160.21$, $p<0.0002$), and consequently, they had a smaller GI ($F=31.54$, $p<0.0049$).

Table 3 Dry weights of *E. crassipes* roots and leaves, relative growth rate (RGR), and growth inhibition percentage (GI) of plants after 7 days of metal exposure

Treatments	Roots (g)	Leaves (g)	RGR (day^{-1})	GI (%)
Experiment H (Hoagland solution)				
T_H0 (control)	1.84 ± 0.07^a	7.01 ± 0.21^a	0.048 ± 0.004^a	–
T_H15	2.64 ± 0.07^b	5.66 ± 0.07^b	0.017 ± 0.002^b	63.4 ± 3.9^a
T_H25	1.47 ± 0.07^a	6.80 ± 0.11^a	0.043 ± 0.002^a	8.7 ± 5.0^b
Experiment MR (MR water)				
$T_{MR}0$ (control)	3.34 ± 0.19^a	3.50 ± 0.17^a	0.092 ± 0.009^a	–
$T_{MR}15$	1.76 ± 0.12^b	3.02 ± 0.03^a	0.073 ± 0.002^a	21.2±1.9

Values expressed as mean±SD error. Different letters under the same column indicate significant differences ($p<0.05$) among treatments

T_H0 control, T_H15 supplemented with 15 mg Cu/L, T_H25 supplemented with 25 mg Cu/L, $T_{MR}0$ control, $T_{MR}15$ supplemented with 15 mg Cu/L

Copper accumulation in plants

Table 4 shows the Cu concentration in roots and leaves, the BCF, and the TF of *E. crassipes*. In both experiments, Cu accumulated principally in roots. These concentrations were much greater than those of leaves, except for $T_{MR}0$, which had similar Cu concentrations in both parts. The maximum concentration (23,387.2 mg/kg dw) was observed in roots grown in T_H25 (Table 4).

In experiment H, Cu concentrations in roots and leaves tended to increase with increasing metal concentration in water, although Cu in leaves appeared to stabilize at 25 mg Cu/L. Cu in roots from T_H15 was almost 250 times higher than the control, and over 450 times higher in plants from T_H25. Cu in leaves was almost 5-fold higher than the control (T_H0) for both treatments (T_H15 and T_H25). BCFs were high, while translocation factors were lower than unity (TF<1) (Table 4). Plants in T_H15 had a lower BCF than the control (T_H0). The BCF of plants in T_H25 presented an intermediate value that could not be statistically differentiated from the other treatments.

In experiment MR, Cu concentration in roots from $T_{MR}15$ exceeded over 2000 times that of the control. For leaves, Cu concentration was over 45 times higher than the control. Plants in $T_{MR}15$ had a higher BCF than the control but a lower TF (Table 4).

To evaluate the effects of Cu addition and the mixture of contaminants on Cu concentrations in plants, treatments T_H15 and $T_{MR}15$ were compared. The Cu concentration in roots grown in Hoagland solution (T_H15) was over 1.5-fold higher ($F=26.02$, $p<0.007$) than those grown in MR water ($T_{MR}15$). The BCF was also higher in a similar proportion ($F=9.35$, $p<0.0378$). The opposite occurred in the leaves: Cu concentration in plants grown in MR water was almost 3-fold higher ($F=10.17$, $p<0.0332$) than the ones grown in Hoagland solution, and therefore showed a higher TF ($F=12.57$, $p<0.0239$). Plants from this experiment could translocate more Cu than plants from Hoagland treatments.

Discussion

Physicochemical characteristics of aquatic media: Cu speciation

According to metal speciation calculations, almost all the Cu added to the treatments in both experiments was not dissolved in the aquatic media.

In MR water, the presence of dissolved organic carbon (DOC) provided mainly by domestic discharge was probably involved in Cu speciation, affecting its dissolution and bioavailability in the medium. DOC is often the dominant factor in determining the speciation and partitioning behavior of trace metals in surface waters (Linnik 2003; Wen et al. 1999). Trace metal binding by DOC derived from various sources, or by broadly isolated fractions of DOC (e.g., humic or fulvic acid fractions), has been investigated and general orders of binding affinities have been documented (Kerndorff and Schnitzer 1980; Langmuir 1997).

In the Hoagland solution, other factors could be involved in Cu speciation. According to MINEQL+ calculations, the precipitated Cu was associated with phosphate in both treatments, which is one of the main macronutrients in this aquatic medium. In a previous study by Kamal et al. (2004), they performed a mass balance of Cu in a hydroponic experiment with three aquatic plants (*Myriophylhum aquaticum*, *Ludwigina palustris*, and *Mentha aquatica*). They observed 94.02–98.18 % precipitation of Cu in the controls (1.06 mg Cu/L) and 38.96–60.75 % precipitation of Cu in the treatment (5.60 mg Cu/L), both with an initial P concentration of 28.5 mg/L from a nutrient-rich solution. They suggested that this Cu was not absorbed by plants because it may have been removed from water through the formation and precipitation of $Cu_3(PO_4)_2$. Reichman (2002) also mentioned that non-renewed solution cultures, like the ones used in this work, require high basal nutrient concentrations, which may result in the precipitation of the metals in high metal treatments. In particular, this occurs with metal-phosphates (Asher and

Table 4 Cu concentration in roots and leaves, bioconcentration factor (BCF), and translocation factor (TF) in *E. crassipes* after 7 days of metal exposure

Treatments	Cu in roots (mg/kg dw)	Cu in leaves (mg/kg dw)	BCF	TF
Experiment H (Hoagland solution)				
T_H0 (control)	51.2 ± 3.63^a	12.4 ± 0.6^a	1184.0 ± 80.1^a	0.26 ± 0.03^a
T_H15	$12{,}588.8 \pm 196.1^b$	60.3 ± 3.4^b	744.3 ± 30.6^b	0.0048 ± 0.0004^b
T_H25	$23{,}387.2 \pm 1070.6^c$	59.5 ± 4.4^b	823.2 ± 8.2^{ab}	0.0026 ± 0.0002^b
Experiment MR (MR water)				
$T_{MR}0$ (control)	3.71 ± 0.02^a	3.81 ± 0.01^a	113.4 ± 7.6^a	1.03 ± 0.01^a
$T_{MR}15$	7622.5 ± 645.1^b	179.6 ± 31.8^b	515.0 ± 37.7^b	0.025 ± 0.006^b

Values expressed as mean±SD error. Different letters under the same column indicate significant differences ($p<0.05$) among treatments

T_H0 control, *T_H15* supplemented with 15 mg Cu/L, *T_H25* supplemented with 25 mg Cu/L, *$T_{MR}0$* control, *$T_{MR}15$* supplemented with 15 mg Cu/L

Blarney 1987). Reichman (2002) presented examples of studies that used high Cu and P concentrations (80–725 μM Cu= 5.08–46.07 mg Cu/L and 0.2–1.0 mM P=6.19–30.97 mg P/L), together with a percentage of the precipitated Cu as phosphates forms. In all cases, Cu precipitation ranged from 42.8 to 94.8 %, according to GEOCHEM speciation software, also suggesting that this Cu-phosphate precipitate was not bioavailable for plant uptake. The evidence reported by Kamal et al. (2004) and Reichman (2002) suggests that there is a high probability that Cu, in the concentrations used in experiment H, could precipitate as copper phosphates considering the condition of high P concentration in the Hoagland solution.

However, in the present study, Cu precipitation did not prevent its incorporation by plants. When the plant material was digested, not only was Cu found associated to the roots (it could have been absorbed, but also adsorbed or physically retained by them), but also it was found in the leaves, which can only have reached them through translocation, i.e., internal metabolism. Therefore, at least a fraction of the organic or inorganic complexes of Cu present in the culture medium must have been absorbed by the plants, indicating that there is a part of the Cu appearing as precipitated according to MINEQL estimations but that remained bioavailable for the water hyacinth (Casares 2012).

Plant biomass and growth estimation

The RGR and the GI had varying results with the increase in Cu exposure. Cu concentration in roots and leaves reached much higher values than 20 mg/kg, which is the tolerance limit for Cu toxicity in shoots or leaves (Burkhead et al. 2009). This means that the accumulated Cu could be toxic to most plants. In experiment H, Cu could have had a toxic effect on the water hyacinth in T_H15, reflected in a significant decrease in RGR and increase in the GI. RGR and GI did not change significantly in T_H25, but the plants in this treatment could have also suffered Cu toxicity, since they exhibited leaf and petiole chlorosis during the experiment as well as signs of dehydration (results not shown) that might be due to the toxic effect of exposure to high Cu concentration (Hammad 2011; Akpor and Muchie 2010; Hu et al. 2007). Mahmood et al. (2005) also reported that *E. crassipes* growth was not affected, but they found some yellowish necrotic spots on the leaves.

When both media were compared, the effect of Cu in combination with other pollutants in $T_{MR}15$ (experiment MR) on the RGR and GI was not as great as the effect of Cu alone in T_H15 (experiment H). Other studies also indicate that *E. crassipes* growth was not affected by the presence of Cu mixed with other contaminants from different types of industrial effluents (textile effluent, Mahmood et al. 2005; coal mining effluent, Mishra et al. 2008) or wastewaters (secondary treated municipal wastewater, Upadhyay et al. 2007; wastewater, Buta et al. 2011). Since MR water is nutrient-enriched with domestic discharge, it possibly provides an appropriate environment in which plants can thrive (Gupta et al. 2012; Rai 2012; Upadhyay et al. 2007). Furthermore, protective benefits of pollutant mixture at low concentrations, such as the presence of other heavy metals found in MR water, could have been achieved by the plants growing in this medium. Metal ions can act as elicitors of defense responses that in turn can stimulate the growth of plants, particularly under stress conditions (Poschenrieder et al. 2013). There are several detoxification mechanisms, e.g., amino acids play a significant role in Cu chelation, by which heavy metal detoxification and tolerance in plants take place (Xiong et al. 2006). It is possible that these mechanisms could be triggered above a certain concentration threshold for the toxicity in question, protecting growth metabolism.

Copper accumulation in plants

Cu accumulation in *E. crassipes* was considerably high, particularly in roots. Cu concentrations in treated roots as well as bioconcentration factors in this study far exceed the reported values for this species, both in synthetic growth media and in different types of effluents or polluted water (Table 5). There were only two exceptions: Johnson and Sheehan (1977), who reported a higher value, or Sutton and Blackburn (1971), who reported a comparable value. The main differences between this study and the ones reported in the literature are the duration and the addition of Cu to the treatments. Even when the experiments in this study lasted for only 7 days (half or a third of the time reported for most studies), Cu concentrations in roots were between 4 and 23 times the ones reported for synthetic growth media, or 3–165 times the ones reported for the polluted water or effluent culture solutions. A striking result is that *E. crassipes* is capable of great uptake under a short period of exposure to high Cu concentration. The metal uptake dynamics are divided into periods: fast metal uptake during the first day and then slower uptake afterwards (Smolyakov 2012). The quick decrease in the water's metal results from biosorption on the roots. For heavy metals speciating in the aquatic environments as cations (Van Leeuwen et al. 2005), apoplastic immobilization by negatively charged cell wall polymers has been reported as the mechanism accounting for most of the removal capacity exhibited by aquatic macrophytes (Dalla Vecchia et al. 2005). Plants can also increase the uptake of Ca, K, Mg, and P as a detoxifying strategy, since a change in the vacuole and apoplast pools, where the majority of absorbed toxic elements are deposited, could induce the uptake of these nutrients to form aggregates with the toxic elements and then attach the aggregates to cell walls. Other possible defensive mechanisms include regulation of ion influx (stimulation of transporter activity at low intracellular ion supply, and inhibition at high concentrations),

Table 5 Comparison of Cu concentrations in roots and leaves in *E. crassipes* in different laboratory studies, Cu treatment, culture solution, and time of exposure

Cu treatment (mg/L)	Culture solution	Time of exposure (days)	Cu in roots (mg/kg dw)	Cu in leaves (mg/kg dw)	Reference
Synthetic growth media					
15	Hoagland	7	12,588.8	60.3	This study
25	Hoagland	7	23,387.2	59.5	This study
6	Water	18	1190–1265	138–147[b]	Majid and Siddique 2013
5.5	Water	5	997.5	115.5[b]	Mokhtar et al. 2011
10[a]	Water	7	2900	700	Soltan and Rashed 2003
10	Hoagland	49	52,600	29,000	Johnson and Sheehan 1977
16	Hoagland	7	14,801	132	Sutton and Blackburn 1971
Polluted water and effluents (mixture of contaminants)					
15	MR water	7	7622.5	179.6	This study
0.096	Titanium sponge effluent	15	46	69	Sukumaran 2013
0.15	Coal mining effluent	21	470	440	Mishra et al. 2008
0.11	Secondary-treated municipal wastewater	20	570	340	Upadhyay et al. 2007
0.01	Nile water	10	141	53	Soltan and Rashed 2003
0.03	Wastewater	4	142	68	Soltan and Rashed 2003
2.5–4.9	Textile dye wastewater	12	2269	286	Sharma et al. 2000

[a] Mixture of heavy metals

[b] Cu accumulated in shoots

extrusion of intracellular ions back into the external solution (Haque et al. 2009), chemical species of toxic ions, chelator—metal ion interactions in nutrient solutions, phosphate, and chelator—metal ion interactions in nutrient solutions and in planta interactions (Verkleij et al. 2009).

Cu was accumulated much less in leaves than in roots, but Cu concentrations in leaves were still considerably high. This means that a portion of the metal absorbed, or adsorbed onto roots, has had time to transfer to leaves. Smolyakov (2012) demonstrated that these two processes are complete at 4 or 8 days. Therefore, one could assert that during the experiment both processes could have occurred. All previous studies show higher values of Cu accumulation in leaves in the synthetic media (Table 5). When comparing polluted water and effluents, our value exceeded some of the existing data (Sukumaran 2013; Soltan and Rashed 2003, Table 5), but it was still below most of the others. Translocation factors in our experiments were also much lower than in the literature (Mishra et al. 2008; Upadhyay et al. 2007). In particular, Cu produces ROS causing the peroxidative destruction of biomembranes and cellular damage (Freedman et al. 1989). Hence, most of the metals are excluded from the shoot via root sequestration, probably to protect the photosynthetic system in leaf cells, which is extremely sensitive to heavy metals (Küpper et al. 2000) due to the lack of hypertolerance mechanisms in the shoots (Chardonnens et al. 1999).

As a conclusion, most of the Cu added to the treatments was considered as precipitated according to chemical speciation by software calculations. However, this fact did not affect the Cu uptake in the experiments. We suggest that the plants in the experiments absorbed Cu from fractions with a low potential Cu bioavailability. *E. crassipes* showed signs of Cu toxicity in the Hoagland experiment in both treatments (15 and 25 mg Cu/L), but it did not appear to be affected in the MR experiment (15 mg Cu/L added to polluted river water). This is an interesting result since it is possible that the presence of organic matter enriched the culture medium improving the water hyacinth growth and/or the protective detoxification mechanisms in the plant could have been triggered above a certain level of contamination. In both situations (with or without signs of toxicity), the water hyacinth was able to accumulate important amounts of Cu in its tissues far beyond the toxic limit for other species. Cu was mainly phytofiltered by the roots. In particular, this study showed the remarkable uptake capacity of water hyacinth under elevated Cu concentrations in a mixture of pollutants, such as those present in pure industrial effluents, in a short time of exposure. This result has not been reported before, to our knowledge, and makes this plant suitable for phytoremediation of waters subject to discharge of mixed industrial effluents containing elevated Cu concentrations (≥15 mg Cu/L), as well as nutrient-rich domestic wastewaters.

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