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Selection of macrophytes with Cu-enriched root biomass intended for ecocatalyst production

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Abstract

Four macrophytes commonly used to clean up Cu contaminated effluents, i.e. *Arundo donax* L., *Cyperus eragrostis* Lam., *Iris pseudacorus* L. and *Phalaris arundinacea* L., were assessed to produce Cu–rich plant biomass intended for ecofriendly catalyst preparation. 7-month-old plants were exposed to a Cu gradient (0.08, 2, 10, 20 and 40 µM Cu) in batch conditions during 2 months. Copper exposure affected the root DW yield of *C. eragrostis* from 2 µM Cu, whereas *I. pseudacorus* and *A. donax* developed well. Maximum Cu concentration in the biomass of *C. eragrostis* and *P. arundinacea* (i.e. 255 and 838 mg Cu kg^{-1} DW respectively) did not reach the 1000 mg Cu kg^{-1} DW threshold value needed to produce Cu-ecocatalysts. Copper concentrations in the roots of *I. pseudacorus* and *A. donax* exceeded this threshold value at 40 µM and over 10 µM Cu, i.e. 1099 and 1809 mg Cu kg^{-1} DW, respectively, making them relevant candidates for producing Cu-ecocatalysts.

Keywords: Copper, Ecocatalysis, Biosourced chemistry, Phytoremediation, Rhizofiltration

1. Introduction

In Aquitaine, France, the Bordeaux mixture (BM, $Ca(OH)_2$ + $CuSO_4$) is generally used as Cu-based fungicide in a concentration range of 10–20 g L^{-1} (Oustriere et al., 2017). Its long-lasting application in the Bordeaux vineyards contributes to locally increase total soil Cu (e.g. >1000 mg Cu kg^{-1}) above the common values in French topsoil (Réseau de Mesures de la Qualité des Sols: 40.2 mg Cu kg^{-1} DW) (El Hadri et al., 2012). Diffuse migration and soil erosion result in Cu concentrations in surface waters in the Gironde estuary (i.e. <0.2 – 2.5 µg L^{-1}) (IFREMER, 2014) possibly above the mean value in French running freshwater (0.9 µg L^{-1} Cu) (Salpeteur and Angel, 2010). This may compromises biodiversity of flora and fauna, e.g. marine organisms such as mollusks and arthropods (Baker et al., 2014), as well as bacterial, fungal (Taylor and Walker, 2016), and algal communities (Rocha et al., 2016). Copper bioaccumulation in plant and animal communities, with adverse consequences in the food web, can also be of great concern (Garrouj et al., 2017).

Rinsing the tanks of BM crop sprayers may generate high effluent amounts, estimated at 2.500.000 L $year^{-1}$ in Aquitaine, France (Oustriere et al., 2017). Their spreading on field borders is authorized by the French legislation (Article L. 253-1 of the rural Code, 2014). The management of BM effluents in constructed wetlands (CW) is a green, efficient and cost-effective alternative to avoid such spreading and prevent ecosystem exposure to Cu excess (Oustriere et al., 2017). Such CW using macrophytes successfully allowed purification of Cu-contaminated effluents derived from paper industry (Arivoli et al., 2015), swine farms (Cortes-Esquivel et al., 2012), or BM use in vineyards (Oustriere et al., 2017). Floating CW based on aquatic vegetation, which forms buoyant filters by their dense interwoven roots and rhizomes sometimes supported by rafts or other floating materials, also provides Cu removal from effluents (Headley and Tanner, 2006). In both planted and floating CW, Cu is immobilized in the rhizosphere, stored in the belowground biomass (Marchand et al., 2010) and/or trapped in the biofilm (Oustriere et al., 2017). Consequently, root and shoot Cu concentrations of CW macrophytes may exceed common Cu values in plant parts (Tremel-Schaub and Feix, 2005). One concern is the handling and disposal of these metal-enriched plant biomasses (Jiang et al., 2015).

An expanding body of work tries to combine soil remediation or water clean-up with biomass processing technologies to valorize harvested biomass and fully develop the financial viability of such techniques (Jiang et al., 2015). Among them, ecocatalysis is an emerging technology exploring the use of metal species originating from plant biomass with high metal(loid) concentrations (e.g. unusual oxidation levels, new associated chemical species, and effects of synergy) (Hechelski et al., 2018). Such biomass produces metal-ligand complexes, used as "Lewis acids" to catalyze fine organic chemical reactions for the synthesis of molecules with high added value: pharmaceuticals (e.g. anticancer and antiviral agents), cosmetics, agrochemicals (e.g. green pesticides) and textiles. New ecocatalysts are needed to increase the number of potential reactions, especially Cu-based

ecocatalysts, so-called Eco-Cu® (Clavé et al., 2016). High Cu concentrations (i.e. ≥1000 mg kg^{-1} DW) in plant biomass are needed to meet the requirement for ecocatalysis. Such concentrations are unusual in plants, except for aerial parts of Cu-hyperaccumulators, i.e. >300 mg kg^{-1} shoot DW (Van der Ent et al., 2013), and belowground biomass of some Cu excluder plants. Only a few studies have reported Cu concentration ≥1000 mg kg^{-1} DW in the roots of macrophyte species exposed to Cu excess (Table 1). Moreover, aboveground biomass produced by macrophytes can be substantial, e.g. 1 to 4 kg dry matter m^{-2} yr^{-1} or more, with similar amounts produced belowground (Craft, 2013), making them suitable candidates for producing high amounts of metal-enriched root biomasses.

The novelty of this study was to bring new insights into identifying dual-use biomass for cleaning-up Cu-contaminated effluents followed by a valorization in bio-sourced chemistry sector. A thorough selection of macrophyte species is required prior the implementation of CW for managing Cu contaminated effluents (Oustriere et al., 2017). This study aimed at identifying local macrophytes from the Aquitaine region that can be used for cleaning up Cu contaminated effluents and that provide a Cu-rich belowground biomass with the potential to be used as Cu-ecocatalyst. Biomass production of *Arundo donax* L., *Cyperus eragrostis* Lam., *I. pseudacorus* L., and *Phalaris arundinacea* L. was assessed along a Cu concentration gradient [0.08-40 µM Cu] in controlled batch conditions for two months. Copper and nutrient concentrations in roots and shoots were determined, as well as root and shoot dry weight (DW) yields and chlorophyll fluorescence as a biomarker of Cu-derived phytotoxicity.

2. Material and Methods

2.1. Plant sampling and growing

Sampling of *A. donax, C. eragrostis, I. pseudacorus,* and *P. arundinacea* was performed in October 2014. These species were selected based on their high root Cu accumulation potential and/or their ability to treat Cu contaminated effluent (Table 1). As Marchand et al. (2014) reported an intra-specific variability in the root DW yield of some macrophytes exposed to Cu excess, the four macrophyte species tested here were collected at sites known to host some of the most tolerant populations. *Iris pseudacorus* was sampled in a riverbank sandy soil with acidic pH soil of the Sanguinet Lake (3.3 mg Cu kg^{-1}, 44°30’20’’N; 1°08’01’’E, France). *Phalaris arundinacea* was collected nearby a drainage ditch with neutral soil pH, located in the vineyards of Saint-Emilion (27 mg Cu kg^{-1}, 44°54’54’’N; 0°08’23’’W, France). *Cyperus eragrostis* was sampled at the Jalle d’Eysines riverbank, located 1 km downstream from a wastewater treatment plant, with neutral soil pH (33 mg Cu kg^{-1}, 44°53’36’’N; 00°40’40’’W, France). *Arundo donax* was sampled at a drainage ditch (43°51'21.4"N 7°51'21.5"E, Italy). For each plant species, 30–40 samples of rhizomes (*I. pseudacorus,* 7 cm-length), young plants (*C. eragrostis,* <10 cm high), and bud-bearing stems (*P. arundinacea* and

A. donax, 10–20 cm length) were individualized, then cultivated and rooted in individual pots (9×8×9 cm^3), on perlite imbibed with a quarter-strength Hoagland Nutrient Solution (1/4HNS) (Marchand et al., 2014) for 6 months in a greenhouse at INRA-Bordeaux, Villenave d'Ornon, France. Culture medium was renewed every month to avoid anoxia and/or nutrient depletion. In March 2015, 25 standardized plants (with similar stem and root size or volume, and rhizome for *I. pseudacorus*) of either *I. pseudacorus, P. arundinacea, C. eragrostis* and *A. donax* were isolated, transplanted individually in a plastic bottle (1.5L), filled with 1L of 1/4HNS and grown for 1 month until the experiment was performed.

2.2. Plant exposure to Cu

In April 2015, just before Cu exposure, all macrophyte roots were blackened with activated plant coal (concentration: 1.5%, Marchand et al., 2014). This staining method allowed a rapid and highly accurate measurement of root growth during the exposure (i.e. length/biomass of new white root parts). It proved to be non-invasive in pilot experiments. In parallel, 100 plastic bottles filled with 1L of 1/4HNS were spiked with Cu ($CuSO_4 \cdot 5H_2O$) to achieve five Cu concentrations: 0.08, 2, 10, 20 and 40 µM Cu (four series of five replicates $concentration^{-1}$, one for each plant species).

For each of the four series, 25 standardized individuals (5 replicates for each of the five Cu concentrations) were placed in 1L bottles containing the Cu-spiked solutions. All plants were then randomly placed in the greenhouse and cultivated for 2 months from April to May 2015 (15/9 h light/darkness; 65 ± 5% relative humidity; 25 ± 5°C). Culture medium of each replicate was changed every six days to maintain targeted Cu concentrations and avoid nutrient depletion and/or anoxia.

2.3. Growing solution and plant analyses

The pH, redox potential (Eh) (Hanna instruments, pH 210, combined electrode Ag/AgCl – 34) and Cu^{2+} concentration (Cupric ion electrode, Fischer Bioblock, USA) were monitored during the test. Each of the five Cu concentrations made a total of 20 samples (4 plant species and 5 replicates per concentration). For all treatments, solutions were weekly, randomly selected and analyzed, just before (T_6) and after (T_0) changing growing solutions at the end of a 6-day exposure (Table 2). Only 2 out of the 20 solutions for each concentration were analyzed to limit the number of measures. In parallel, Cu^{2+} concentration in solutions at T_0 was computed using the MINEQL+4.6 software (Table 2). After a 2-month Cu exposure, chlorophyll fluorescence parameters, i.e. maximum efficiency of Photosystem II (PSII) (Fv/Fm ratio), real efficiency of PSII [Y(II)] and non photochemical quenching (qN), were measured for all plants using a portable modulated fluorometer (Pam-2500 Waltz, Germany). Then, roots and shoots were harvested. The black-stained and white parts of roots produced before and after Cu exposure, respectively, were separated. Root and shoot samples were washed twice with deionized

water, blotted with filter paper, placed in paper bags and oven dried at 60°C to constant weight for 72h and then weighed for determining the shoot and root DW yields. For all plants, dried white roots and shoots were then ground (< 1 mm particle size, Retsch MM200) and weighed aliquots (0.5 g DW) were wet-digested using microwaves (CEM Marsxpress 1200 W) with 5 mL supra-pure 14M HNO_3, 2 mL 30% (v/v) H_2O_2 not stabilized by phosphates and 1 mL MilliQ water. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Mineral composition (Al, B, Ca, Cu, Fe, Mg, P, K and Na) in digested samples was determined by ICP-MS (Thermo X series 200, INRA USRAVE laboratory, Villenave d'Ornon, France). All elements were recovered (>95%) according to the standard values and the standard deviation for replicates was <5%. All concentrations in plant parts are reported on DW basis. Copper removal (or mineral mass) was calculated as follows: Cu (mg $plant^{-1}$) = DW yield (kg $plant^{-1}$) × Cu concentration (mg kg^{-1} DW).

2.4. Statistical analyses

One-way analysis of variance (ANOVA) was performed to assess how pH, Eh and Cu^{2+} concentrations in the growing medium vary according to Cu concentrations at T_0 and T_6. ANOVA was also carried out to test the influence of Cu exposure and plant species on (1) root and shoot Cu concentrations, (2) root and shoot DW yields and (3) Cu removal, for the four macrophytes, after a 2-month exposure. Dead plants were removed from the statistical analysis. Normality and homoscedasticity of residuals were met for all data sets (Shapiro and Levene's test). When significant differences were identified between treatments, multiple comparisons of mean values were conducted using post-hoc Tukey HSD tests. Differences were considered statistically significant at $p<0.05$. When assumptions were not met, Wilcoxon pairwise tests adjusted with a Bonferroni correction were used. All statistical analyses were made using R software (version 3.0.3, Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

It is worth exlploring the potential sustainable harvest of Cu-rich root mats to produce Cu-ecocatalysts for green chemistry, especially when taking advantage of the Cu-excluder phenotype of many macrophytes (Marchand et al., 2010). The ability of macrophyte species to accumulate and tolerate Cu is mainly a function of genotype and a cascade of underlying molecular mechanisms, notably Cu uptake, homeostasis, detoxification and translocation between plant parts, in line with plant physiology and phenology, anatomy, and biomass production (Marchand et al., 2014; Printz et al., 2016).

3.1. Physico-chemical parameters of the culture medium

At T_0 and T_6, pH in the culture medium was neutral, without significant changes across the Cu gradient. However, pH values significantly decreased between T_0 and T_6 for the 0.08-10 µM Cu range (Table 2). For both T_0 and T_6, Eh value was slightly oxidative and did not significantly change across the Cu gradient. At T_0, soluble Cu^{2+} concentration significantly increased along the Cu gradient. This mirrored the increasing total Cu concentration in the spiked solutions and was consistent with the modeled Cu^{2+} concentrations. Soluble Cu^{2+} concentration was lower at T_6 than at T_0 for all Cu levels, except at 40 µM Cu. Such soluble Cu^{2+} decrease across time may be due to Cu precipitation, sorption on both the bottle surface and root mats, Cu uptake by roots and microbes, and complex formation with ligands of the rhizodeposition (Marchand et al., 2014).

3.2. Anatomical and functional traits

Copper is pivotal in redox control and electron transport in the plant cells, but Cu excess can lead to uncontrolled production of reactive oxygen species (ROS) with many deleterious effects, notably for the chlorophyll fluorescence parameters (Printz et al., 2016). Here, despite high chronic Cu exposure, none of the three chlorophyll fluorescence parameters used as stress biomonitors varied along with the 0.08 – 40 µM Cu exposure range, for the four studied macrophytes (Table 3). The Fv/Fm values even remained close to the optimal value (around 0.8), which is typical for an intact photosystem II in higher plants (Björkmann and Demmig, 1987). In parallel, biomass production in response to the increasing Cu exposure ranged from no effect to severe inhibition (Fig. 1-4 A) and in a wide range of root and shoot Cu concentrations (Fig. 1-4 B). Despite some ecological similarities, differences in root anatomical and functional traits among the four studied macrophytes should be kept in mind to explain their response to Cu excess: e.g. *A. donax* rhizomes are tough and fibrous and form knotty, spreading mats and deep roots; *I. pseudacorus* has fleshy roots (10-30 cm long) and thick, pink tuberous rhizomes (2-3 cm diameter); *P. arundinacea* displays highly branched, scaly rhizomes with densely fibrous roots and numerous adventitious roots at the rhizome nodes; *C. eragrostis* has coarse fibrous roots. Thus, these four macrophyte species may have adopted differential tolerance strategies to cope with Cu excess.

3.2.1. *Cyperus eragrostis* and *P. arundinacea* - Cu dilution in the whole plant biomass

For *C. eragrostis,* the shoot DW yield (g $plant^{-1}$) remained steady on the Cu gradient, after the 2 month-Cu exposure (Fig. 1A), ranging from 8 ± 4.6 at 40 µM Cu to 16 ± 4.7 at 0.08 µM Cu. Conversely, its root DW yield (g $plant^{-1}$) was 5 fold lower when exposed to 2 µM Cu as compared to 0.08 µM Cu and roots did not grew at 40 µM Cu. Similarly, *C. alternifolius* exposed for 15 days to poly-contaminated wastewater from electroplating (0.7 µM Cu) showed a marked decrease in root length even though it produced a high leaf biomass (Sun et al., 2013). For *P. arundinacea,* the shoot and root DW yields (g $plant^{-1}$) significantly decreased when plants were exposed to ≥ 20 µM Cu (from

25 ± 3 to 6 ± 3 in shoots and from 2 ± 0.6 to 0.6 ± 0.2 in roots) being, respectively, 2.2-fold and 2.7-fold lower at 40 µM Cu than at 0.08 µM Cu, although not significantly for the roots exposed to 40 µM Cu (Fig. 2A). To detoxify and sequester high metal amounts, plants need to spend energy, leaving less resources for growth, reproduction, and other processes (Maestri et al., 2010). This decrease in biomass production was correlated with increasing root Cu, which may indicate an increase in plant maintenance cost. For *P. arundinacea,* decrease in shoot and root DW yields was correlated with increasing root Cu concentration (linear relationship, R^2: 0.57; $y = -0.002x + 2.3$ and R^2: 0.75; $y = -0.03x + 33$, respectively). Both shoot and root Cu concentrations (mg Cu kg^{-1}) significantly increased at 10 µM Cu and shoot Cu concentration peaked at 40 µM Cu (i.e. 838 ± 71) (Fig. 2B). These results are in line with Marchand et al. (2014) who found that root biomass production of *P. arundinacea* was correlated with Cu exposure. Consequently, Cu removal (mg Cu $plant^{-1}$) increased from 0.075 ± 0.022 at 0.08 µM Cu to 5.286 ± 1.210 at 40 µM Cu for the shoots and from 0.011 ± 0.002 at 0.08 µM Cu to 0.723 ± 0.300 at 40 µM Cu for the roots (Fig. 2C). In parallel, Cu concentrations (mg Cu kg^{-1}) in *C. eragrostis* significantly increased with the Cu gradient, ranging from 3.4 ± 0.8 at 0.08 µM Cu to 246 ± 111 at 40 µM Cu in shoots and from 9 ± 0.7 at 0.08 µM Cu to 256 ± 58 at 10 µM Cu in roots (Fig. 1B). *Cyperus eragrostis* and *P. arundinacea* may have adopted internal detoxification mechanisms to cope such high shoot Cu concentrations. This early shoot Cu accumulation must result from Cu dilution in the whole plant biomass as vacuole storage capacities in roots are exceeded. This response is interpreted by some authors as an opportunity to dispose of these temporary organs, which are periodically lost and regenerated every years (Bonanno et al., 2017).

As the biomass production of *C. eragrostis* (especially the roots) was affected by Cu excess, its shoot and root concentrations did not meet the requirement for Cu-ecocatalysts (> 1000 mg Cu kg^{-1}) (Clavé et al., 2016). Higher Cu concentrations in both roots and shoots were reported for other *Cyperus* sp. exposed at lower concentrations (e.g. *C. alternifolius*: 1310 mg kg^{-1}) (Sun et al., 2013) (Table 1). Maximum capacity for Cu sorption into the cell walls and Cu accumulation in vacuoles of roots may be reached more rapidly for some plant species, notably for *C. eragrostis*, which displays a high formation of additional aerenchyma in the root cortex and large variations in the internal structure of roots under flooded conditions and hydroponics (Sharma et al., 2016). The use of *C. alternifolius* to produce Cu-rich biomass for Cu-ecocatalysts production may be an alternative option (Table 1). Even though root and shoot Cu concentrations of *P. arundinacea* were relatively high, they were insufficient for their potential use as Cu-ecocatalysts. Plant inoculation with endophytic bacteria may be an option to promote root Cu concentration of *P. arundinacea* and better fit Cu-ecocatalyst requirements. Enhanced root Cu accumulation, Cu translocation, biomass production, nutrient availability, and plant Cu tolerance were reported in plants inoculated with endophytic bacteria (Ma et al., 2011).

3.2.2. *Iris pseudacorus* and *A. donax* - Cu accumulation in roots and rhizomes

Root and shoot biomass of both of *I. pseudacorus* and *A. donax* showed moderate and non-significant differences across the Cu gradient (Fig. 3A and 4A). The low impact of Cu excess on root DW yields of *A. donax* and *I. pseudacorus* may indicate that both species tend to maximize their belowground biomass as Cu exposure increased and have efficient mechanisms to maintain cellular Cu homeostasis. The shoot DW yield of *A. donax* was significantly higher at 2 µM Cu than at 0.08 µM Cu, highlighting a hormesis effect, as defined by Calabrese and Blain (2009), but did not significantly differ in the 10–40 µM Cu range. The root DW yield of *A. donax* was only significantly lower at 40 µM Cu as compared to 0.08 µM Cu (Fig. 4A). Shoot and root biomass of *A. donax* also slightly decreased only at 42.5 µM Cu in a spiked-nutrient solution (Elhawat et al., 2014). For *I. pseudacorus*, the shoot and root DW yields (g plant^{-1}) remained similar across the Cu gradient, varying from 15 ± 8 at 20 µM Cu to 26 ± 9 at 0.08 µM Cu and from 0.9 ± 0.2 at 40 µM Cu to 1.9 ± 1.2 at 0.08 µM Cu, respectively (Fig. 3A). Preferential allocation of trace elements in macrophyte aboveground storage organs, as survival strategy to overcome abiotic stresses, was previously reported (Bonanno et al., 2013). Weak changes in both root and shoot DW yields of *I. pseudacorus* and *A. donax* (Fig. 3 and 4A) matched with their Cu tolerance on this Cu range and the unavoidable presence of a rhizome for *I. pseudacorus* (Marchand et al., 2014). Shoot Cu concentration of *A. donax* significantly raised at 10 µM Cu and culminated at 20 µM Cu (i.e. 175 ± 103 mg Cu kg^{-1}) while its root Cu concentration increased linearly (R^2: 0.75; y = 83x + 563) with Cu exposure, although not significantly in the 10-40 µM Cu range (i.e. 1809 ± 386 and 3512 ± 1372 mg Cu kg^{-1}) (Fig. 4.B). As a consequence, its shoot Cu removal (mg Cu plant^{-1}) started significantly to rise at 20 µM Cu (Fig. 4C) and its root Cu removal plateaued at 2 µM Cu. For *I. pseudacorus*, shoot Cu concentration increased at 20 µM Cu and peaked at 40 µM Cu, whereas its root Cu concentration linearly increased (R^2: 0.82; y = 27x - 41) with the Cu gradient, ranging (mg Cu kg^{-1}) from 9 ± 0.7 at 0.08 µM Cu to 1099 ± 434 at 40 µM Cu (Fig. 3B). Its root Cu removal (mg Cu plant^{-1}) increased with Cu exposure and peaked up to 0.846 ± 0.298 at 40 µM Cu. The shoot Cu concentrations of *I. pseudacorus* and *A. donax* were relatively low on the 0.08 – 40 µM Cu range as compared to their high root Cu concentrations (Fig. 2 & 4 B). Such pattern is widely accepted for *I. pseudacorus* due to the large storage capacity of its rhizomes (Sun et al., 2013), but the ability of *A. donax* to accumulate such root Cu concentration is less reported (Table 1). Accumulation of metal(loid)s in excess in the roots and rhizomes is a common sequestration strategy of macrophytes to quench their potential phytotoxic effect (Marchand et al., 2010). To maintain cellular homeostasis and limit Cu phytotoxicity, *A. donax* and *I. pseudacorus* may have set physiological processes to bind Cu by ligands, e.g. nicotianamine, phytochelatins and metallothioneins, and compartmentalize Cu in the roots (Printz et al., 2016; Sharma et al., 2016). Plants adopting this strategy (excluders) have efficient root cellular mechanisms to exclude Cu from the shoots, e.g. improved efflux Cu pumping at the plasma membrane, vacuolar Cu compartmentalization (Sharma et al., 2016), in addition to root efficient detoxification mechanisms (Printz et al., 2016).

At 40 µM Cu, *I. pseudacorus* produced high root and shoot biomasses with low shoot Cu concentration but high root Cu concentration (i.e. 1099 mg Cu kg^{-1}), just achieveing the minimum required concentration for Cu-ecocatalysts. As its shoot Cu concentration was within the common Cu values in aerial plant parts (3 – 20 mg Cu kg^{-1}) (Tremel-Schaub and Feix, 2005), this shoot cellulosic biomass can be merged with other biomass for multiple potential uses: (1) energy sector (i.e. biofuel, bioethanol) (2) derived bioproducts, and (3) cellulose and paper industry (Vigil et al., 2015). In the 10-40 µM Cu range, root Cu concentration for *A. donax* also met the required concentration for Cu-ecocatalysts. Such Cu-rich biomass may be used to catalyze fine organic chemical reactions to synthesize molecules with high added value: pharmaceuticals (e.g. anticancer and antiviral agents), cosmetics, agrochemicals (e.g. green pesticides) and textiles (Clavé et al., 2016). Conversely, Cu concentration in *A. donax* shoots was insufficient for their use as Cu-ecocatalysts, while it may be too high to be combined with other plant biomass, even though they may be used to fertilize Cu-deficient soils and substrates. One option may be to limit Cu root-to-shoot transfer by adding silicon in the culture medium. Silicon deposition and cell wall thickening at the rhizodermis and induced-suberization of the endodermal tissue of roots of some plant species may at least partially block the apoplast bypass flow across the roots and restrain the apoplastic and symplastic transport of Cu, thus limiting Cu root-to-shoot transfer (Li et al., 2008). The resulting shoot biomass of *A. donax* with low Cu concentration could integrate local biomass processing chains (e.g. energy sector: bioethanol, biofuels, combustion; potential fertilizers: compost, biochar, litter; bioproducts: construction eco-materials and plant fiber/plastic composites) (Vigil et al., 2015).

3.3. *Experimental limit*

Plant resistance to transplantation is an important factor, in addition to Cu tolerance or Cu accumulation, to be consider when selecting a plant species. At the beginning of the experiment seven macrophyte species were sampled and three of them, *Spartina anglica, Phragmites australis* and *Typha latifolia* were not included in the experiment because they did not resist the stress of transplantation, standardization and growth in culture medium. Moreover, our four studied macrophytes were 7-month-old, showing relatively low shoot and root biomasses during our experiment, as compared to mature plants. According to Brisson and Chazarenc (2009), extrapolating results from young plants may mislead the prediction of treatment benefits of a plant species in a mature CW system; however due to management constraints, many studies still use young plants. In a 1-month experiment with a mature pilot-scale CW, root and shoot Cu concentrations of *A. donax* plants exposed to a 69 µM Cu-contaminated Bordeaux mixture effluent (i.e. 623 ± 140 and 8 ± 2.5 mg Cu kg^{-1}, respectively) were lower than the values measured in this study (Fig. 4B) (Oustriere et al., 2017), demonstrating the dilution effect of Cu in a higher biomass. Accounting for a potential dilution

effect in biomass, the root and shoot Cu concentrations obtained here for the 7-month-old plants may lead to overestimation when transposed to mature CW.

5. Conclusions

The four macrophytes achieved different biomass yields, Cu concentrations and Cu removals on the 0.08-40 µM Cu gradient. *Iris pseudacorus* and *A. donax,* which accumulated Cu in their roots and likely in rhizomes, can deliver root mats potentially usable as Cu-ecocatalyst when treating effluents with 40 µM Cu and 10 µM Cu, respectively. For *P. arundinacea* and *C. eragrostis,* Cu concentration in the whole plant was more diluted, notably at high Cu exposure, with roots not reaching the 1000 mg Cu kg^{-1} DW required to be used as Cu-ecocatalyst.

E-supplementary data of this work can be found in online version of the paper

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Figure captions

Fig. 1. *Cyperus eragrostis*: (A) shoot and root DW yields (g DW $plant^{-1}$), (B) shoot and root Cu concentrations (mg kg^{-1}) and (C) Cu removal (mg $plant^{-1}$) by the shoots and roots after a 2 month-exposure to the Cu gradient. Mean values per treatment (n=3). Values with different letters differ significantly (one way ANOVA, p-value<0.05). # These plants did not produce either new roots or enough biomass to be wet-digested.

Fig. 2. *Phalaris arundinacea*: (A) shoot and root DW yields (g DW $plant^{-1}$), (B) shoot and root Cu concentrations (mg kg^{-1}) and (C) Cu removal (mg $plant^{-1}$) by the shoots and roots after a 2 month-exposure to the Cu gradient. Mean values per treatment (n=4). Values with different letters differ significantly (one way ANOVA, p-value<0.05).

Fig. 3. *Iris pseudacorus*: (A) shoot and root DW yields (g DW $plant^{-1}$), (B) shoot and root Cu concentrations (mg kg^{-1}) and (C) Cu removal (mg $plant^{-1}$) by the shoots and roots after a 2 month-exposure to the Cu gradient. Mean values per treatment (n=4). Values with different letters differ significantly (one way ANOVA, p-value<0.05).

Fig. 4. *Arundo donax:* (A) shoot and root DW yields (g DW $plant^{-1}$), (B) shoot and root Cu concentrations (mg kg^{-1}) and (C) Cu removal (mg $plant^{-1}$) by the shoots and roots after a 2 month-exposure to the Cu gradient. Mean values per treatment (n=5). Values with different letters differ significantly (one way ANOVA, p-value<0.05).

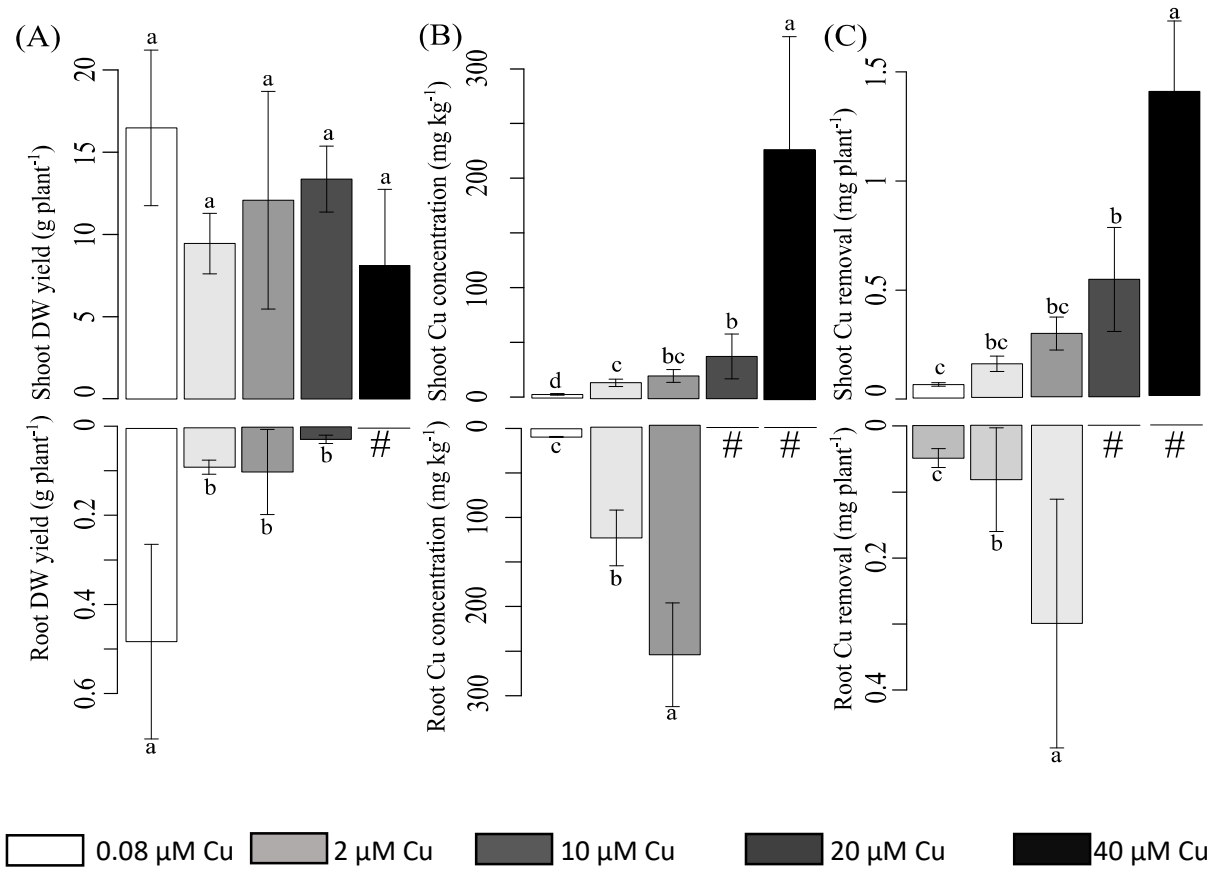
(A)
(B)
(C)
Shoot DW yield (g plant-1)
Root DW yield (g plant-1)
Shoot Cu concentration (mg kg-1)
Root Cu concentration (mg kg-1)
Shoot Cu removal (mg plant-1)
Root Cu removal (mg plant-1)
0.08 µM Cu
2 µM Cu
10 µM Cu
20 µM Cu
40 µM Cu

Fig. 1.

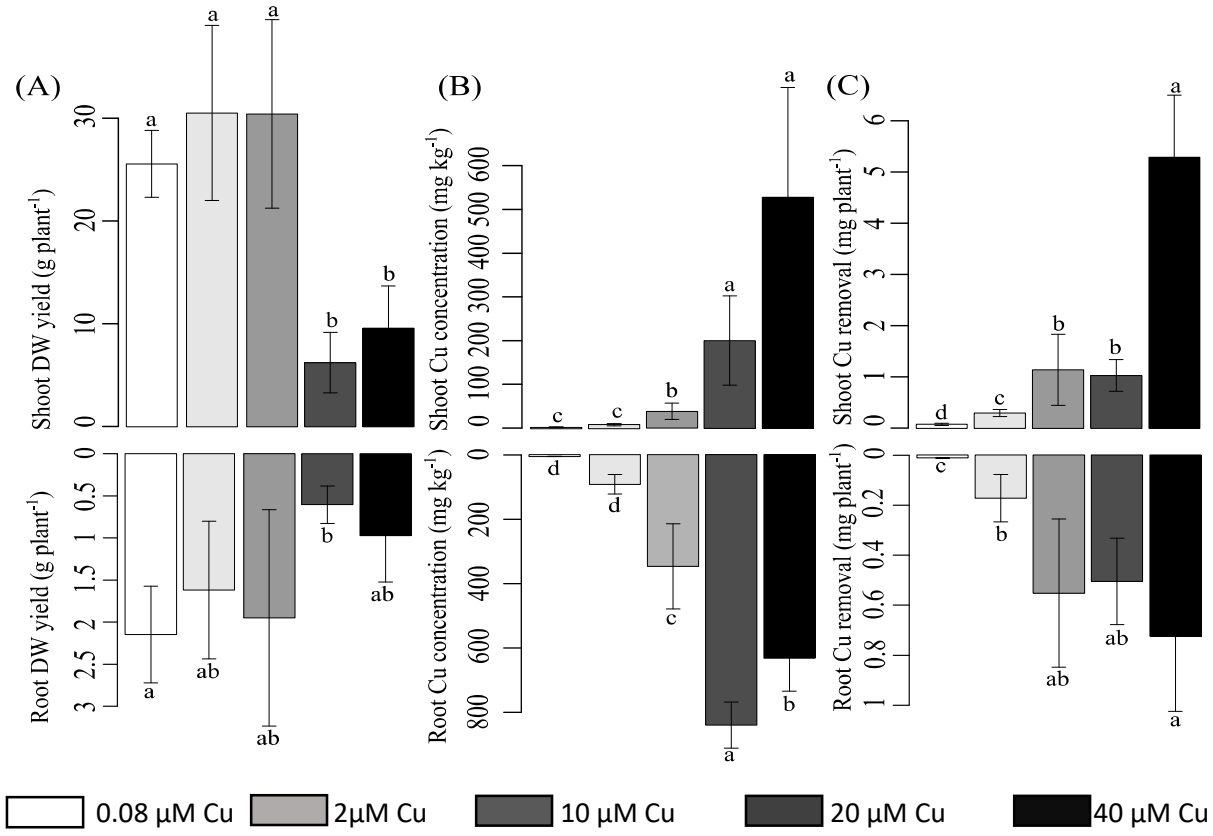
(A)
(B)
(C)
Shoot DW yield (g plant-1)
Root DW yield (g plant-1)
Shoot Cu concentration (mg kg-1)
Root Cu concentration (mg kg-1)
Shoot Cu removal (mg plant-1)
Root Cu removal (mg plant-1)
0.08 µM Cu
2µM Cu
10 µM Cu
20 µM Cu
40 µM Cu

Fig. 2.

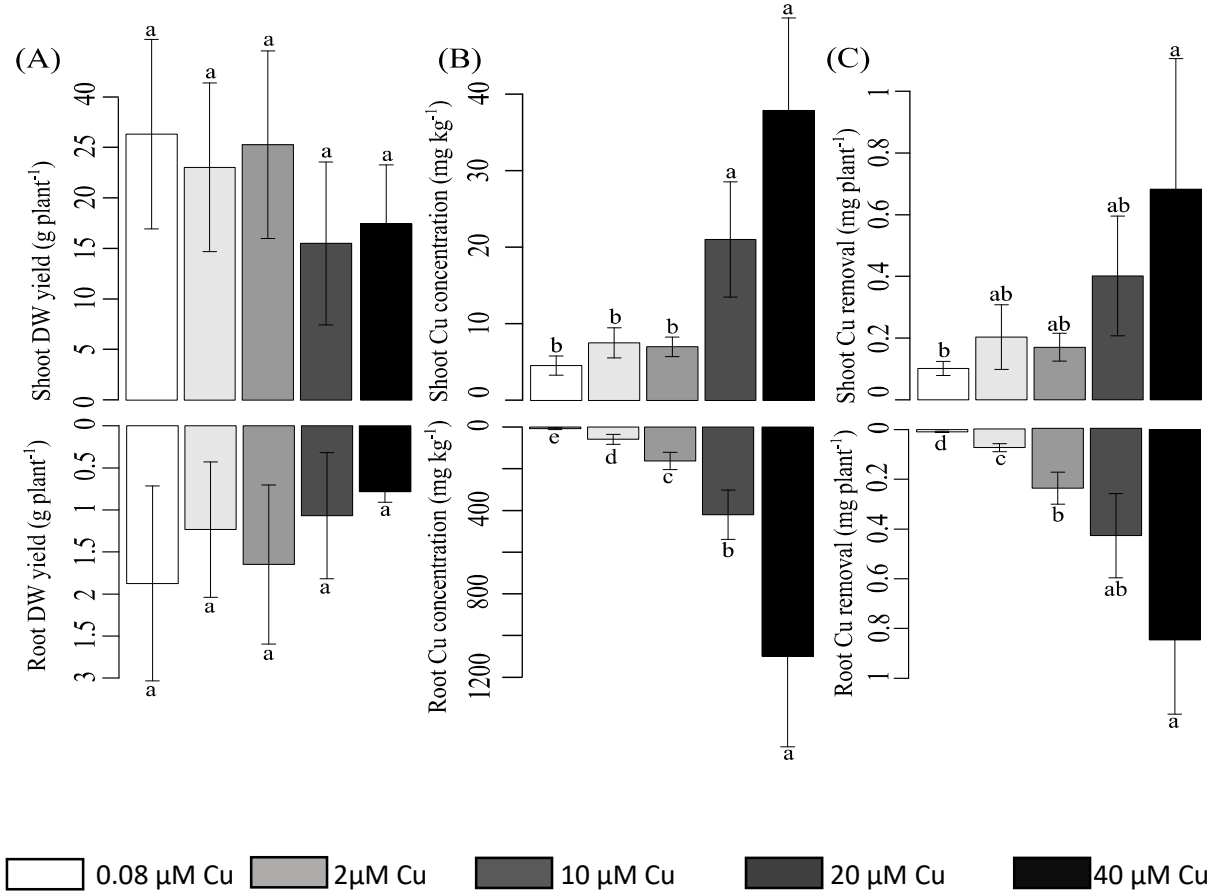
(A)
(B)
(C)
Shoot DW yield (g plant-1)
Root DW yield (g plant-1)
Shoot Cu concentration (mg kg-1)
Root Cu concentration (mg kg-1)
Shoot Cu removal (mg plant-1)
Root Cu removal (mg plant-1)
0.08 µM Cu
2µM Cu
10 µM Cu
20 µM Cu
40 µM Cu

Fig. 3.

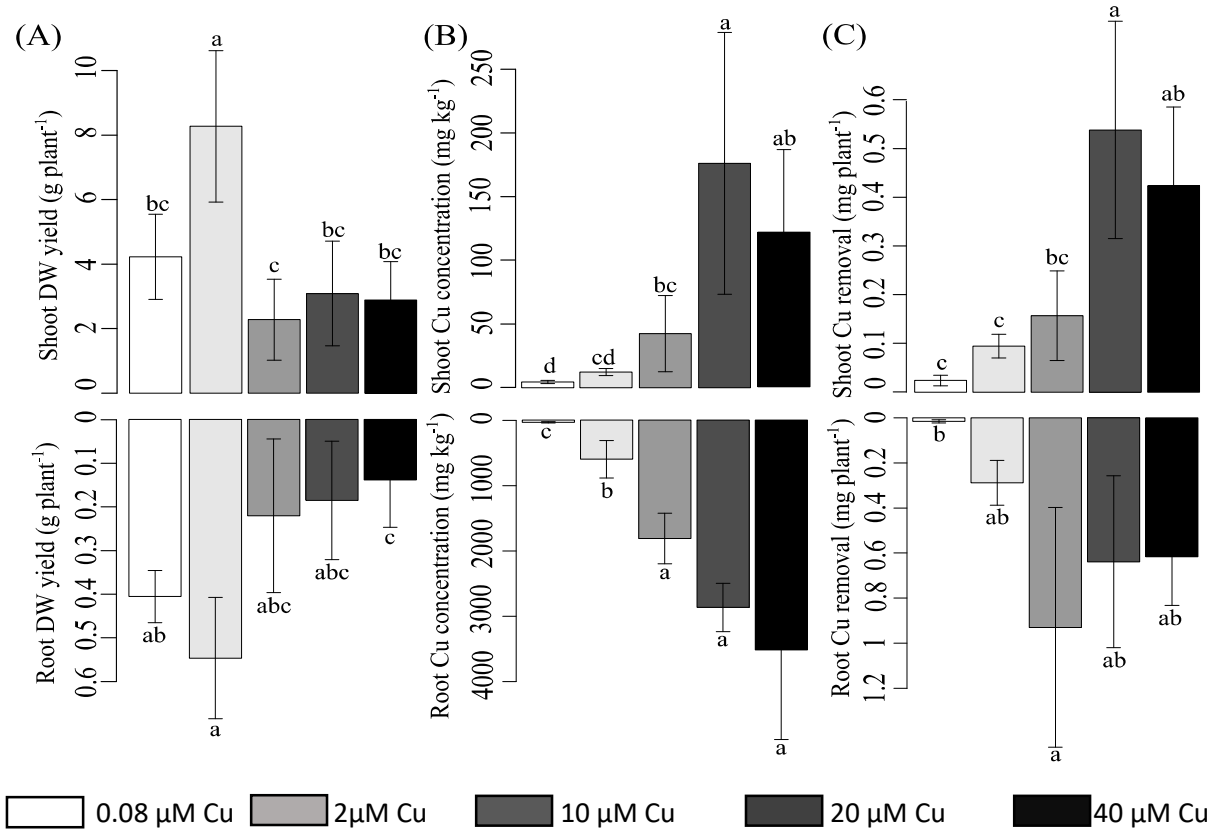
(A)
(B)
(C)
Shoot DW yield (g plant-1)
Root DW yield (g plant-1)
Shoot Cu concentration (mg kg-1)
Root Cu concentration (mg kg-1)
Shoot Cu removal (mg plant-1)
Root Cu removal (mg plant-1)
0.08 µM Cu
2µM Cu
10 µM Cu
20 µM Cu
40 µM Cu

Fig. 4.

Table. 1. Frequent Cu concentrations in plant parts of emerged, rooted macrophytes potentially usable for Cu rhizofiltration

Organ	Concentration	Conditions	Exposure Concentration	Exposure Time	References
Cyperus alternifolius					
Leaves	7 ± 0.3	Constructed wetland	16.5 µM Cu	5 months	Cheng et al., 2002
Shoots	8 ± 0.1				
Rhizomes	309 ± 16				
Roots	2610 ± 380				
Lateral roots	15600 ± 238				
Shoots	670 ± 70	Hydroponic experiment	0.7 µM Cu	15 days	Sun et al., 2013
Roots	1310 ± 10				
Shoots	1000	Constructed wetland	2.14 µM Cu	3 months	Soda et al., 2012
Roots	386	Constructed wetland	0.16 µM Cu	1 month	Yadav et al., 2012
Stem	70				
Leaves	79				
Cyperus rotundus					
Roots	689	Plant sampling	1756 mg kg^{-1} (Soil)	-	Ashraf et al., 2011
Shoots	78				
Leaves	134				
Flowers	85				
Cyperus involucratus					
Leaves	112 ± 14	Plant sampling	9902 ± 1 089 mg kg^{-1} (Soil)	-	Kříbek et al., 2011
Shoots	112 ± 23		1114 ± 342 mg kg^{-1} (Soil)	-	
Iris pseudacorus					
Shoots	5.8 ± 0.5	Plant sampling	7 ± 0 .2 µg L^{-1} (Water) 6 ± 0.5 mg kg^{-1} (Sed)	-	Samecka-Cymerman et al., 2001
Shoots	430 ± 30	Hydroponic experiment	0.7 µM Cu	15 days	Sun et al., 2013
Roots	1430 ± 170				
Iris ensata					
Shoots	257 ± 5	Plant sampling	27–120 mg kg^{-1} (Soil)	-	Usman et al., 2012
Roots	263 ± 16		0.4 ± 0.01 mg kg^{-1} (Sed)		
Phalaris arundinacea					
Shoots	7 ± 0.6	Plant sampling	198 ± 12 µg L-1 (Water) 0.4 ± 0.01 mg kg-1 (Sed)	-	Samecka-Cymerman et al., 2001
Shoots	1.6 - 9.7	Plant sampling	7.4 - 31 µg L^{-1} (Water)	2-4 years	Vymazal et al., 2007
Stem	5.9 - 6.3	Field experiment	-	2-8 years	Pahkala and Pihala, 2000
Leaf sheath	4.1 - 7.3				
Leaf blade	6.0 - 8.2				
Leaves	6 - 10	Plant sampling	2.6 - 15 mg kg^{-1} (Sed)	-	Parzych et al., 2015
Rhizomes	10 - 20				
Leaves	0.6 - 12	Plant sampling	1.93 - 8.09 µg L^{-1} (Water) 1.35 - 247 mg kg^{-1} (Sed)	-	Łojko et al., 2015
Roots and Rhizomes	3.8 - 24	Plant sampling	0.54 - 6.50 µg L^{-1} (Water)	-	Polechońska and Klink, 2014
Stems	0.9 - 5.6		2.71 - 42.5 mg kg^{-1} (Sed)		
Leaves	3.6 - 8.7				
Arundo donax					
Shoots	600	Hydroponic experiment	0.7 µM Cu	15 days	Sun et al., 2013
Roots	630				
Roots	7.4 ± 0.9	Plant sampling	22.7 ± 3.45 µg L^{-1} (Water)	-	Bonanno, 2013
Stems	1.9 ± 0.2		115 ± 17.6 mg kg^{-1} (Sed)		
Leaves	1.1 ± 0.1				

Sed: Sediment

Table. 2. Physico-chemical parameters of the culture medium along the Cu gradient at T_0 (n=14, day 0, before solution replacement) and T_6 (n=8, day 6 after solution replacement)

Total Cu added			(mV)	Cu^{2+} (µg L^{-1})	
(µM)	**(mg L^{-1})**	**pH**	**Eh**	**Measured Cu**	**Modeled Cu**
T_0					
0.08	0.005	7.6 ± 0.3 a	259 ± 43 a	1.1 ± 1.2 d	-
2	0.13	7.4 ± 0.2 ab	248 ± 29 a	1.5 ± 1.7 cd	3.5
10	0.64	7.4 ± 0.1 ab	262 ± 26 a	9 ± 8 abc	14
20	1.3	7.3 ± 0.1 abc	282 ± 38 a	16 ± 9 a	17
40	2.5	7.3 ± 0.2 abc	285 ± 37 a	19 ± 10 a	19
T_6					
0.08	0.005	7.0 ± 0.3 d	272 ± 16 a	1.2 ± 1.6 d	
2	0.13	7.0 ± 0.2 d	254 ± 24 a	0.6 ± 0.7 d	
10	0.64	6.9 ± 0.2 d	258 ± 24 a	1.2 ± 2.2 d	
20	1.3	7.1 ± 0.2 cd	265 ± 25 a	2.0 ± 1.7 bcd	
40	2.5	7.2± 0.1 bcd	268 ± 19 a	9 ± 7 ab	

Mean value ± SD for each treatment. Values with different letters in a column differ significantly (one way ANOVA, p-value <0.05).

Table. 3. Response surface for the maximum efficiency of PSII (Fv/Fm), real efficiency of PSII [Y(II)], and non-photochemical quenching (qN), in the macrophyte leaves after the 2-month-exposure to the Cu gradient. Mean values per treatment (n= 3 for *C. eragrostis,* n= 4 for *P. arundinacea* and *I. pseudacorus* and n= 5 for *A. donax*).

Cu exposure (µM Cu)	Fv/Fm	Y(II)	qN
A. donax			
0.08	0.85 ± 0.009 a	0.24 ± 0.1 a	0.39 ± 0.1 a
10	0.78 ± 0.03 a	0.37 ± 0.1 a	0.58 ± 0.2 a
40	0.51 ± 0.3 a	0.29 ± 0.2 a	0.64 ± 0.2 a
C. eragrostis			
0.08	0.82 ± 0.03 a*	0.37 ± 0.1 a	0.46 ± 0.1 a
10	0.8 ± 0.05 a*	0.52 ± 0.02 a	0.53 ± 0.1 a
40	0.77 ± 0.03 a*	0.51 ± 0.05 a	0.54 ± 0.1 a
I. pseudacorus			
0.08	0.83 ± 0.04 a*	0.18 ± 0.04 a	0.32 ± 0.3 a
10	0.8 ± 0.05 a*	0.35 ± 0.2 a	0.43 ± 0.1 a
40	0.82 ± 0.02 a*	0.38 ± 0.2 a	0.5 ± 0.1 a
P. arundinacea			
0.08	0.8 ± 0.004 a	0.22 ± 0.05 a	0.72 ± 0.2 a
10	0.77 ± 0.07 a	0.33 ± 0.1 a	0.62 ± 0.1 a
40	0.67 ± 0.1 a	0.37 ± 0.09 a	0.6 ± 0.1 a

Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value <0.05). * Wilcoxon pairwise tests.

Graphical Abstract