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Metal Accumulation and Toxicity in the Submerged Aquatic Macrophyte *Vallisneria americana*

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Abstract Submerged aquatic vegetation (SAV) are ecologically important plant species, providing habitat and food for many aquatic organisms and enhancing water quality. Anthropogenic practices have increased the input of metal pollutants in aquatic systems. SAV are known to accumulate high concentrations of metal pollutants yet less is known about the toxic effects of metals on SAV. In this study, we examined metal accumulation, distribution, and photosynthetic toxicity in the commonly found SAV species *Vallisneria americana* after exposure to copper, cadmium, and lead in the laboratory. Metal content in the root and shoot system of *V. americana* was quantified and photosynthetic parameters were measured after exposure to three exposure concentrations (control, 10 and 50 µg/L) of copper, cadmium, and lead for 4 d. Significant metal accumulation in both leaf and root tissue was observed over time in *V. americana* and the distribution varied with exposure time, concentration, and metal. Photosynthetic impairment corresponded with increasing metal accumulation in *V. americana* with toxicity occurring in the 10 µg/L lead treatment. These findings could be used to help assess tolerance of *V. americana* in aquatic environments impacted by metals.

Keywords: SAV, metals, accumulation, toxicity, *Vallisneria americana*, photosynthesis.

Introduction

Submerged aquatic vegetation (SAV) are important plant species in both freshwater and brackish ecosystems (Pinto et al. 2019; Best 1981). SAV provide vital habitat for many aquatic organisms and food for waterfowl and marine mammals (Best 1981, White et al. 2002). SAV help to anchor sediments, reduce turbidity, and protect organisms from wave energy (Scheffer and Van Nes 2007, Poirrier et al. 2017). Further, SAV enhance water quality with light availability, aid in carbon and nutrient cycling, and absorb contaminants from the environment (Barnett and Schneider 1974, Laughlin 1982, Hauxwell et al. 2004, Li et al. 2007, Scheffer and Van Nes 2007, Camp et al. 2012, Poirrier et al. 2017). While SAV are crucial to the survival and health of many aquatic ecosystems, they have substantially declined over the past 50 years (Li et al. 2007, Orth et al. 2010, Pyati et al. 2012, Goldberg et al. 2018, Pinto et al. 2019, Goldberg and Trent 2020). Pollution, sedimentation, and deteriorating water quality are contributors to this phenomenon, which is excacerbated by the decline in SAV biomass (Alexander et al. 1993, Li et al. 2007, Goldberg and Trent 2020).

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Metals enter waterbodies naturally through the weathering of rock, and more substantially via anthropogenic sources like industrial effluents, domestic sewage, stormwater runoff from urban and agricultural lands, and as leachate from metal-based antifoulant paints (Bryan 1974, Guzmán and Jiménez 1992, Sundberg 1998, Jones 2010, Tchounwou 2012). Increased metal discharge into aquatic systems can lead to contamination of the water column and sediments, and cause toxicity to aquatic organisms when levels exceed acceptable limits (Nayar et al. 2004, Jaishankar et al., 2014, Jarvis and Bielmyer-Fraser 2015, Rani Das et al. 2017).

Many aquatic macrophytes have the capacity to accumulate significant concentrations of metals from water and sediment (Keskinkan et al. 2004, Peng et al. 2008, Xue et al. 2010, Harguinteguy et al. 2014, Jarvis and Bielmyer-Fraser 2015, Bai et al. 2018). The translocation of metals in plants can be monitored by comparing the root-to-shoot uptake of metals, assessing metal transport, and quantifying distribution of metals in the root and shoot system of aquatic plants (Page et al. 2006). Aquatic macrophytes have been used in remediation of metal-contaminated sites to remove metals and enhance water quality (Miretzky et al. 2006). High biomass of SAV can reduce resuspension of metals from sediment (Zhang et al. 2016). However, if metal concentrations in the aquatic environment exceed certain thresholds, SAV abundance will decline (Keskinkan et al. 2004). While metal accumulation in SAV has been well documented in field studies, fewer studies have focused on metal accumulation, distribution, and toxicity in SAV in a controlled laboratory environment. In field situations, measured toxic responses in organisms may be due to a variety of individual or a combination of environmental stressors. Elucidating metal-induced toxicity in the field can be challenging, which prompts the need for more laboratory experiments.

Responses of aquatic plants to metals include increased phytochelatin and chlorophyll production, antioxidant responses, decreased photosynthetic capabilities, and decreased growth (Baker and Walker 1989, Prasad and Strzałka 1999, Wang et al. 2008, Jarvis and Bielmyer-Fraser 2015). Chlorophyll fluorescence has been used to measure photosynthetic parameters in aquatic plants (Jarvis and Bielmyer-Fraser 2015, Siddiqui and Bielmyer-Fraser 2019). Fluorometry is a technique used to differentiate among photon energy captured by a chlorophyll-*a* pigment molecule used to drive photosynthesis, the energy emitted as fluorescence, and energy converted to heat (Schreiber and Bilger 1995, Rosenqvist and Van Kooten 2003). Measuring sub-lethal responses of plants to contaminants can be useful to assess plant health before they decline (Miller et al. 2017). Laboratory toxicity studies can effectively assess organism responses to specific contaminants and results can be applied to field situations.

The objective of this study was to measure metal accumulation, distribution, and toxicity in the perennial submerged aquatic macrophyte, *Vallisneria americana,* exposed to copper, cadmium, and lead in the laboratory. *V. americana* (commonly called wild celery, eelgrass and tape grass) is distributed throughout North American ranging from Canada southward to Texas and Florida in both non-tidal and tidal regions (Moore 2009, USDA NRCS 2020). *V. americana* can tolerate a range of salinity from freshwater to brackish ($<$10 ppt) and are commonly found

throughout river and estuarine systems (Lacoul and Freedman 2006, Dobberfuhl 2007, Sagan 2007, Goldberg et al. 2018). In several waterbodies, abundance of *V. americana* is indicative of ecosystem health and it has been used to monitor sediment-bound metal concentrations in the environment (St-Cyr and Campbell 2000).

Materials and Methods

Experimental organism. *V americana* was purchased from Buceplant and maintained at 25 °C in a large tank filled with synthetic hard freshwater and continuous aeration. The plants were acclimated to experimental conditions for 48 hours prior to use.

Experimental solutions. Synthetic hard freshwater was made by adding 120 mg/L $CaSO_4$, 8 mg/L KCl, 120 mg/L $MgSO_4$, and 192 mg/L $NaHCO_3$ to ultra-pure Milli-Q water following US. EPA guidelines (USEPA 2002). The water was mixed for at least 24 h prior to use (Bielmyer et al. 2012). *V. americana* have been found in aquatic systems with this level of water hardness (100-120 mg $CaCO_3$/L) (Bielmyer-Fraser et al. 2020). Using synthetic freshwater in controlled toxicity experiments reduces the influence of other variables like contaminants and dissolved organic carbon which could alter metal toxicity (Lauren and McDonald 1986; Playle et al. 1992; Bielmyer-Fraser et al. 2018). Certified stock solutions of 1000 µg/mL copper, as $Cu(NO_3)_2$, lead, as $Pb(NO_3)_2$ and cadmium as $Cd(NO_3)_2$) (Perkin Elmer, Norwalk, CT) were used to make 10 and 100 µg/L Cu, 10 and 100 µg/L Pb, and 10 and 100 µg/L Cd. The metal solutions were transferred into 2-L plastic beakers, each containing a plastic mesh, and equilibrated in their respective containers for 24 h.

Experimental design. There were seven treatments, including the control, each with three replicates and all experimental beakers were arranged in a randomized design Temperature was maintained at 24.2±0.56 °C using a water bath and recirculating chiller/heater (Model MC-1/4HP, AquaEuro Systems, Los Angeles, California) throughout the 4-day static-renewal experiment. A 16-h light: 8-h darkness cycle was maintained with cool white fluorescent lighting.

At the start of the experiment, one *V. americana* plant was added to each beaker and the plastic mesh was used to anchor the plant. Sediments were not used in this experiment to better characterize direct metal uptake by the plant. A 10 mL water sample was collected daily from each replicate, and the solution was filtered using a 0.45 µm filter, acidified with 50 µL trace metal grade HNO_3, and stored in a 15 mL polypropylene centrifuge tube. New solutions were made, equilibrated for 24 h, and used to renew the experimental solutions throughout the experiment. Three root and three leaf samples were collected from plants in the acclimation tank at the start of the experiment to be analyzed for background metal concentration. One leaf sample was then collected from each replicate at 2 and 4 d in the same manner. Additionally, at the end of the experiment, root samples were also collected for metal analysis. The plant tissue samples were measured for wet mass, dried in an oven at 60°C for 24 h, and then weighed to determine dry mass. Samples were then fully digested with addition of trace metal grade HNO_3 and heated in a 60°C hot water bath for 3 h.

Daily, temperature and dissolved oxygen (DO) were measured using a YSI meter (Model YSI-85; Pentair Aquatic Ecosystems, Apopka, Florida), *p*H was measured using a calibrated *p*H meter (WTW, Xylem Inc.), and light intensity was measured using a lux light meter (Extech, Nashua, NH). No algal growth was observed in any of the experimental beakers throughout the length of the experiment. At the end of the experiment, the mean ± standard deviation of DO, temperature, pH, and light intensity values were calculated (Table 1). Following American Public Health Association guidelines, hardness and alkalinity of the base water were measured using titrimetric methods (method 2340 and 2320, respectively; APHA 1985; Table 1).

Photosynthetic parameters. In each replicate, *V americana* were dark adapted for 30 min and then measured twice in the same location on the leaf for a variety of parameters using a modulated chlorophyll fluorometer (OS5p+; OptiScience, Hudson, NH) and a rapid light curve test (one minute illumination with saturating pulses at every 10 seconds; Genty et al. 1989, Ralph et al. 1999, Beer and Bjork 2000). At 0, 1, 2, and 4 d, the effective quantum yield of photosystem II (amount of energy used

Table 1. Water chemistry (mean ± standard deviation) in experimental solutions. Parameters were measured daily except for alkalinity and hardness which were measured at the start of the experiment in the base water.

Parameter	Measured Value
Dissolved Oxygen	7.78 ± 0.33 mg/L
Temperature	24.2 ± 0.56 °C
pH	8.60 ± 0.20
Light illumination	14501± 4628 lux
Alkalinity	128 mg $CaCO_3$/L
Hardness	118 mg $CaCO_3$/L

for photosynthesis; YPSII), the maximum electron transport rate (ETR_{max}), the minimum amount of light necessary to saturate the system (I_k), and the efficiency of the system (α) were measured in the leaves of *V. americana*.

Metal analysis. All the digested root and leaf samples were diluted using ultra-pure 18 mΩ Milli-Q water The diluted tissue and water samples were analyzed to determine cadmium, copper, and lead concentrations using an atomic absorption spectrophotometer with graphite furnace detection (AAnalyst 800, Perkin Elmer, Norwalk, CT). Certified metal standards dissolved in 2% HCl, ultra-pure Milli-Q water blanks, and QA/QC samples were analyzed throughout each analysis (detection limits: 0.1 µg/L cadmium, 0.7 µg/L copper, 0.7 µg/L lead). The plant tissue data are presented as µg metal/g dry weight.

Statistical analysis. At the end of the exposure, data from day 4 were analyzed using one-way analysis of variance (one-way ANOVA) to determine differences among treatments Normality and equality of variance of the data were tested using a Shaprio-Wilk test and Levene's test, respectively. Because the data were not normally distributed, a Kruskal-Wallis one-way ANOVA on ranks was used followed by a Tukey's test to determine pairwise comparisons ($p \leq 0.05$). The same procedures were used to analyze tissue metal concentrations and photosynthetic data over time.

Results

Experimental conditions. The water chemistry in the experimental solutions is presented in Table 1 The values remained stable and were within acceptable conditions. The measured metal concentrations in the experimental solutions were similar or just below nominal concentrations with one exception (50 µg/L cadmium treatment; Table 2). The highest Cd treatment was approximately double the nominal value, however, the concentration remained stable during the experiment.

Table 2. Measured metal concentrations (mean ± standard deviation) in experimental solutions.

Treatment	Measured Metal Concentration (µg/L)
Control - Cd	0.04 ± 0.04
Control - Cu	1.35 ± 0.98
Control - Pb	2.77 ± 1.51
10 µg/L Cd	9.43 ± 4.27
50 µg/L Cd	122 ± 25.6
10 µg/L Cu	7.20 ± 2.46
50 µg/L Cu	38.5 ± 8.23
10 µg/L Pb	6.91 ± 1.11
50 µg/L Pb	43.6 ± 9.39

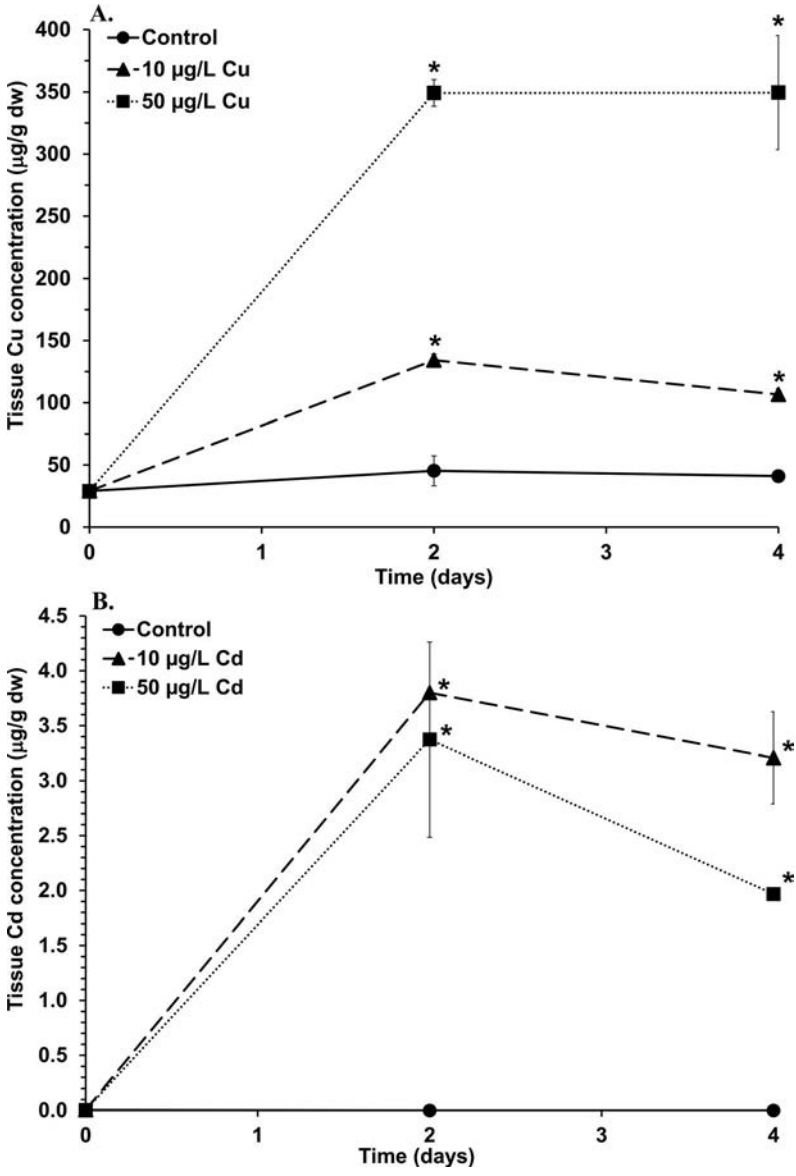

Figure 1. A. copper (Cu), B. cadmium (Cd), and C. lead (Pb) accumulation in the leaves of *V. americana* exposed to waterborne metal over 4 d. See Table 2 for measured metal concentrations. Asterisks denote significant difference from the control at a particular time point.

Metal accumulation. Metal significantly accumulated in the leaves of *V americana* over the duration of the experiment (P=0.005; Figure 1). For all metals, accumulation was highest after 2 d of exposure with significant differences observed in leaves of both treatments of cadmium and copper and the highest lead

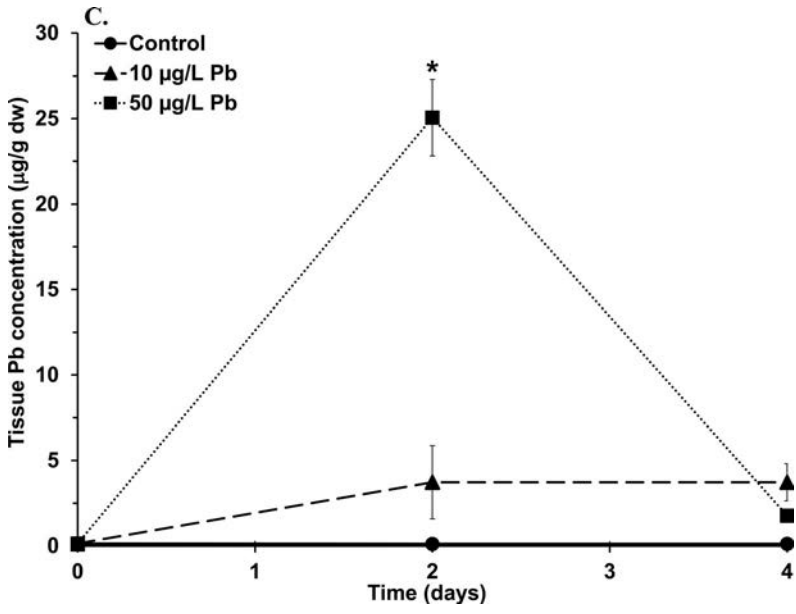

Figure 1. Continued.

treatment, as compared to control values (Figure 1). Metal accumulation in leaves of both cadmium treatments decreased slightly by 4 d, but cadmium and copper accumulation in both treatments remained significantly elevated, as compared to the control (Figure 1A, B). Alternatively, the lead concentration in the leaves of *V. americana* exposed to the highest lead treatment returned to control levels by 4 d (Figure 1C). The metal accumulation in leaves of the 10 µg/L lead treatment did not significantly differ from control values over the 4-d exposure.

The *V. americana* in the control treatments had no detectable cadmium or lead in the leaves or roots, however, the mean copper concentration in the leaves and roots of the control group were 29.1 and 24.1 µg/g dw, respectively (Figures 1, 2). At the end of the 4-d exposure, root copper concentration was significantly elevated in both copper treatments above control levels, similar to the pattern of copper accumulation in the leaves (Figure 2). No significant differences were detected in the root cadmium concentration in the 10 and 50 µg/L treatments as compared to controls. Alternatively, the root lead concentration was significantly elevated in the 10 µg/L treatment, as compared to control levels (Figure 2).

Photosynthetic parameters. The exposure of *V americana* to elevated metal concentrations showed notable changes in the photosynthetic parameters (Figure 3). The control values for all parameters did not significantly vary over the duration of the experiment or as compared to background levels (Figure 3). The YPSII and ETR_{max} in *V. americana* were significantly increased after 2 d of exposure to 10 µg/

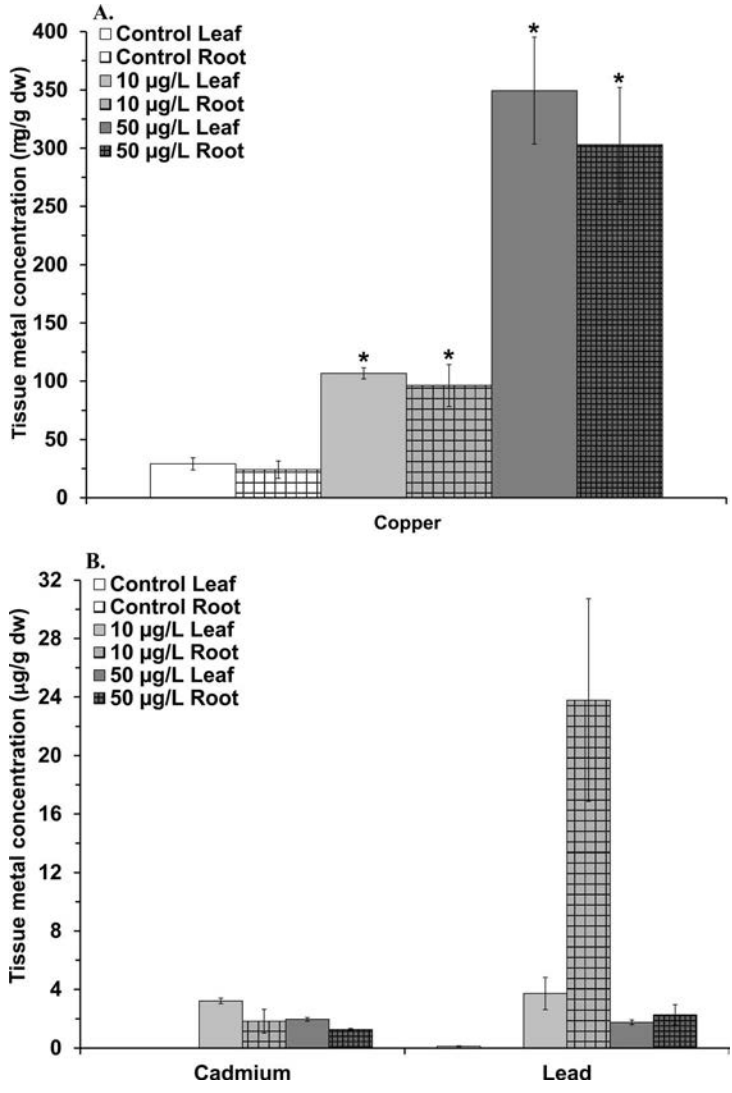

Figure 2. Cadmium, lead and copper concentrations in the tissues (leaves and roots) of *V. americana* exposed to control, 10, and 50 µg/L waterborne metal for 4 d. See Table 2 for measured metal concentrations. Asterisks indicate a significant difference from the control within a specified tissue type.

L copper (Figure 3A, B). Alternatively, a significant decrease in YPSII was observed in the 50 µg/L copper, 50 µg/L cadmium, and 10 µg/L lead treatments after 4 d of exposure (Figure 3). The Ik was significantly elevated in the 50 µg/L copper and 10 µg/L lead treatments and α was significantly decreased in the 10 µg/L lead treatment (Figure 3C, D).

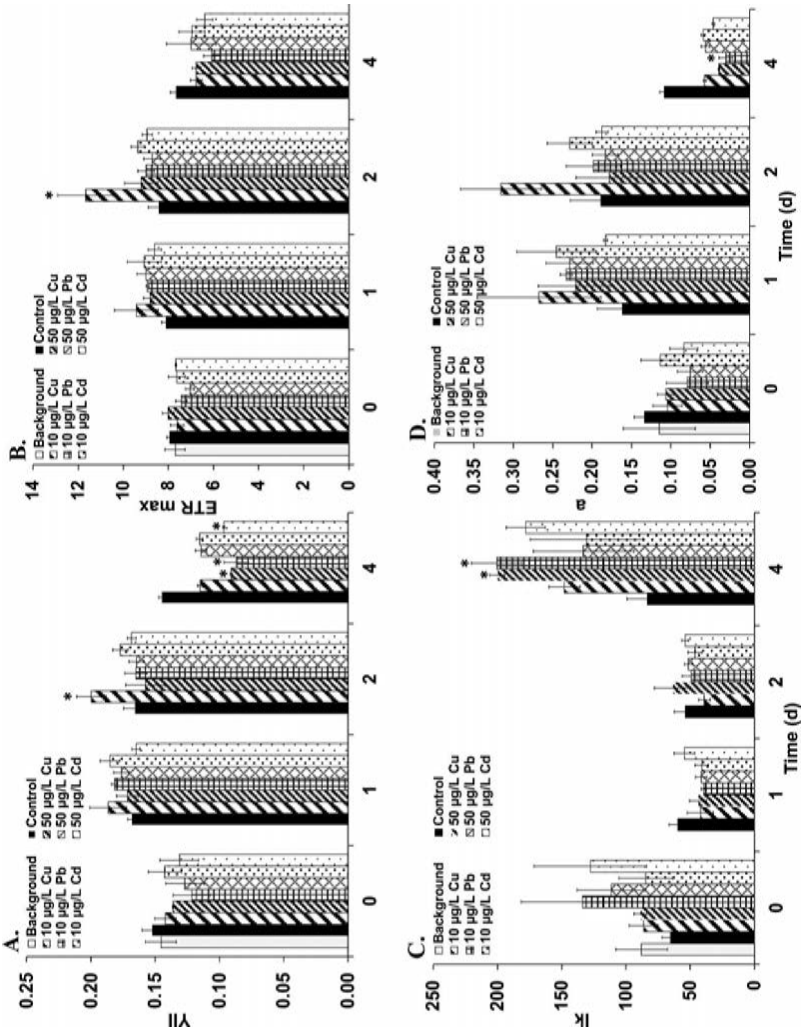

Fig. 3. A. effective quantum yield of photosystem II (YPSII), B. maximum electron transport rate (ETR_{max}), C. Ik (minimum amount of saturating light), and D. α (efficiency of photosystem II) in *V. americana* after exposure to control, 10 and 100 µg/L Cu, Pb, and Cd over 4 d. Asterisks denote significant difference from the control at a particular time point. "Background" denotes measurements taken at the start of the experiment prior to metal exposure.

Table 3. Shoot: root ratio for *V. americana* exposed to 10 and 100 μg/L cadmium, copper, or lead for 4 d.

Concentration	Lead	Copper	Cadmium
10 μg/L	0.24	1.11	1.74
100 μg/L	1.10	1.15	1.55

Discussion

Metal accumulation. The tissue metal concentrations in *V americana* in this study were similar to those reported in field monitoring studies in various rivers (St-Cyr and Campbell 1994, Hudon et al. 1998, St-Cyr and Campbell 2000). Mean concentrations of lead and copper in various aquatic plants were reported to be 11 and 48 μg/g dw, respectively (Hutchinson 1975). Bielmyer-Fraser et al. (2017) reported plant tissue concentrations of 53–798 μg/g dw copper, 3–95 μg/g dw cadmium, and 7–330 μg/g dw lead from a variety of macrophytes collected from two rivers with varying anthropogenic impact in southern Georgia. The lead concentration in leaf tissues of *Thlaspi praecox* collected from a heavily polluted site near a lead mine was 45 μg/g dw (Vogel-Mikus et al. 2008). Hudon (1980) reported 9.3–28.9 μg/g dw copper, 0.62–3.50 μg/g dw cadmium, and 0.85–2.68 μg/g dw lead in the above ground tissues of *V. americana* collected from St. Lawrence and Ottawa rivers in Canada. St-Cyr and Campbell (1994) reported concentrations of 9.1–39.6 μg/g dw copper, 0.35–5.12 μg/g dw cadmium, and 1.2–8.0 μg/g dw lead in the above-ground parts of *V. americana* collected from Lake St. Pierre, part of the St. Lawrence River. Further research by St-Cyr and Campbell (2000) showed concentrations of 6.2–35.4 and 4.4–39.4 μg/g dw copper, 0.28–4.3 and 0.07–2.35 μg/g dw cadmium, and 0.6–10.9 and 3.2–20.2 μg/g dw lead in the leaves and roots, respectively, of *V. americana*.

Wang et al. (2010) reported concentrations of 396 and 114 μg/g dw copper and 63.8 and 48.0 μg/g dw cadmium in the roots and shoots, respectively, of *V. spiralis* exposed to metal-contaminated sediments for 21 d. *V. spiralis* has been shown to rapidly accumulate copper, cadmium, and lead in the laboratory with copper accumulating evenly between the leaves and the roots; cadmium accumulating to a greater extent in the leaves, and lead accumulating to a higher degree in the roots of *V. spiralis* (Gupta and Chandra 1993, Sinha et al. 1994, Wang et al. 2008). After 4 d of metal exposure in the present study, using similar exposure conditions (e.g. without sediment), a similar pattern of accumulation was observed in *V. americana*. Likewise, Welsh and Denny (1980) reported a similar distribution pattern as compared to the present study for copper and lead in a variety of aquatic macrophytes in the field (English lakes) with shoot: root ratios ranging from 0.74–1.01 for copper and 0.17–0.25 for lead. In this study, shoot: root ratio ranged from 1.11–1.15 for copper, 0.24–1.10 for lead, and 1.55–1.74 for cadmium (Table 3). Metal distribution generally depends on the metal, the exposure concentration, and the plant species (Jarvis and Bielmyer-Fraser 2015, Bai et al. 2018).

Rooted submerged macrophytes can naturally absorb elements through their leaves from the surrounding water (Welsh and Denny 1980) and through their roots

from the sediment interstitial water (St-Cyr and Campbell 2000). Once absorbed, elements can be distributed to various tissues, dependent on biological requirements and/or detoxification (Ximenenz-Embun et al. 2002, Page et al. 2006, Horník et al. 2007). Copper is an essential element necessary for a variety of metabolic processes, including acting as a cofactor for many enzymes (Clemens et al. 2002). Cadmium and lead are nonessential and are therefore presumed in most cases to enter plant cells through essential transporters for other elements, such as zinc (Vogel-Mikus et al. 2008). Welsh and Denny (1980) reported two different pathways for copper and lead transfer from sediments to the shoot of various taxa of submerged aquatic macrophytes. Copper accumulation in these macrophytes was thought to occur primarily by absorption through the roots, translocation to the shoots, and re-translocation to the roots. Copper adsorption into the shoots from the water occurred to a lesser extent (Welsh and Denny 1980). Alternatively, lead accumulation in the shoots mainly occurred through adsorption from the surrounding water (Welsh and Denny 1980). Further, uptake of lead from the water occurred rapidly (Welsh and Denny 1980).

In this study, rapid lead accumulation in the leaves of *V. americana* was observed in the 50 μg/L treatment after 2 d of exposure. However, lead levels also rapidly declined in the leaves by 4 d of exposure. This observed accumulation may have been due to adsorption of lead from the surrounding water, similar to the observations of Welsh and Denny (1980). The significantly increased lead accumulation in the root tissue of *V. americana* exposed to 10 μg/L lead was likely due to uptake via the roots. It is important to note that the leaves and roots of *V. americana* exposed to 10 μg/L had higher lead concentration than those exposed to 50 μg/L lead after 4 d of exposure. Although not significant, the same pattern was observed with cadmium exposure. Jarvis and Bielmyer-Fraser (2015) observed a similar phenomenon in the seaweed, *Ulva lactuca,* after metal exposure. The decreased metal uptake with increasing exposure concentration was attributed to downregulation of specific metal transporters (Wang and Dei 1999, Jarvis and Bielmyer-Fraser 2015). Given that seaweed do not have root systems, further investigation is needed to assess this phenomenon in SAV, as this may be an important consideration if using *V. americana* for metal bioremediation.

For the St. Lawrence River, St-Cyr and Campbell (2000) concluded that roots of *V. americana* were better bioindicator organs than shoots for monitoring metal contamination in sediments. In addition to biota, sediments generally act as a sink for metals entering aquatic systems and therefore concentrations are generally higher in the sediments than the water column (Hall 1989, DiToro et al. 2001, Nayer et al. 2004, Pinto et al. 2019).

Martinez and Shu-Nyamboli (2011) also showed significant correlations between aquatic macrophytes and sediment bioavailable metal concentrations in the Gallinas River. Further, significantly higher metal concentrations were reported in the plant roots as compared to the shoots (Martinez and Shu-Nyamboli 2011). Sediment chemistry can influence the bioavailability of metals (Zhang et al. 2014). In the present study, sediment was eliminated from the metal exposure scenario, to assess metal uptake directly from the water into the plant tissue. It should be noted

that the presence of sediment and the anoxic interstitial water microenvironment could change the metal uptake kinetics in *V. americana* (Zhang et al. 2014). Additionally, developmental stage of the plant and type of root system have been shown to affect metal accumulation (Page et al. 2006). Several species of aquatic plants have mechanisms to protect against metal toxicity including rapid translocation and detoxification strategies (Geng et al. 2019); however, at elevated levels metals may exert toxic effects.

Metal toxicity. Excess copper, cadmium, and lead accumulation in plants can cause physiological effects and growth inhibition in a variety of species (Påhlsson 1989, Fernandes and Henriques 1991, Wang et al 2008, 2010, 2013, Jarvis and Bielmyer-Fraser 2015, Zhu et al. 2016). Reduction of chlorophyll in aquatic plants after copper, cadmium, and lead exposure has been shown in several studies (Gupta and Chandra 1993, Prasad et al. 2001, Moferran et al. 2009, Zhu et al 2016). For example, Wang et al. (2010) reported a decreased chlorophyll concentration in *V. spiralis* exposed to copper- and cadmium-contaminated sediments. Cadmium and lead exposure to the plant *Hydrilla verticillata* has been shown to inhibit chlorophyll synthesis enzymes (Singh et al. 2013). Zhu et al. (2016) reported reduced chlorophyll and reduced relative growth rate in *V. natans* exposed to copper-contaminated sediments. Further, the roots of *V. natans* were significantly damaged including stunted roots and poor growth initiation after exposure to increased copper in the sediments (Zhu et al. 2016).

In the current study, increased copper, cadmium and lead exposure to *V. americana* caused decreased YPSII. Decreased photosynthesis has been a reported consequence of metal exposure in other plant species as well (Jarvis and Bielmyer-Fraser 2015, Siddiqui and Bielmyer-Fraser 2019) and is likely a result of the reduction in chlorophyll, as previously mentioned. The decreased energy used for photosynthesis could have been used to upregulate production of phytochelatins (peptides that sequester metals) and antioxidant molecules (e.g. glutathione) which are used for metal detoxification (Wang et al. 2008, Main et al. 2010, Singh et al. 2013, Brock and Bielmyer 2013, Patel and Bielmyer-Fraser 2015). Wang et al. (2008) observed increased cadmium accumulation in leaves and roots, increased phytochelatin concentrations, increased glutathione concentrations, and decreased growth in *V. spiralis* exposed to 18–72 μg/L cadmium for 7 d as compared to controls. Similarly, decreased YPSII was observed in *V. americana* exposed to 50 μg/L cadmium for 4 d in the present study.

Tissue metal concentration correlated with toxicity in *V. americana*. Specifically, the treatments with the highest metal concentrations in the roots (50 μg/L copper and 10 μg/L lead) had the most severe impairment of photosynthetic parameters by 4 d. The 50 μg/L copper and cadmium treatments and 10 μg/L lead treatment had a lower YPSII which corresponded to decreased photosynthetic efficiency and an increase in the minimum amount of light necessary to saturate photosystems in *V. americana*. Decreased photosynthetic capability was likely a result of both metal accumulation in the leaf tissues (where photosynthesis occurs), in addition to metal accumulation in the root system and possibly impairment of water and nutrient uptake (Påhlsson 1989, Zhu et al. 2016).

Interestingly, a hormesis effect was observed after 2 d exposure to 10 μg/L copper, where YPSII and ETR_{max} were increased in the *V. americana*. Copper plays an essential role in electron transfer reactions in photosynthesis and may be beneficial in low concentrations over short-term exposure (O'Halloran and Culotta 2000, Clemens 2001, Clemens et al. 2002). However, in excess, the redox activity of copper may lead to the generation of damaging reactive oxygen species and declines in photosynthetic efficiency (Clemens et al. 2002).

This laboratory study demonstrated metal- and concentration-dependent differences in metal accumulation and distribution in *V. americana* as well as photosynthetic toxicity at environmentally realistic metal concentrations. SAV have been used to monitor metals and in bioremediation of metal-contaminated environments (Gupta and Chandra 1998). *V. americana* has the capacity to accumulate copper, cadmium, and lead from surrounding waters and these results highlight the utility of this species for both monitoring and remediation efforts. *V. americana* may be useful for bioremediation of copper- and cadmium-contaminated sites especially, given that many polluted areas have waterborne copper and cadmium concentrations below the 50 μg/L, which was the lowest concentration causing photosynthetic impairment in this study (Bielmyer-Fraser et al. 2020). Furthermore, this information could be used to help manage and protect SAV in aquatic systems impacted by metals.

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