PHYTOREMEDIATION OF SELENIUM-IMPACTED WATER BY AQUATIC MACROPHYTES[1]

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Abstract. Stormwater runoff raises concern over potential downstream impacts of selenium (Se) on aquatic ecosystems. Constructed wetland phytoremediation is a sustainable, inexpensive, eco-friendly technology with potential to remove Se from stormwater. The objectives of this study were to: 1) evaluate the bioavailability of Se chemical form and concentration on plant uptake and 2) determine the potential of aquatic macrophytes to improve water quality in a constructed wetland[4]. The experiment was arranged as a 2 X 2 factorial nested within a split-split plot design replicated three times. Cattail (CT; *Typha angustifolia* L.), duckweed (DWD; *Lemna minor* L.), fanwort (CAB; *Cabomba caroliniana* A. Gray), soft rush (SR; *Juncus effuses* L.), muskgrass (MG; *Chara* spp.), and unplanted controls (UNP) were acclimatized 14 d in 115-L microcosms containing 0.035 m^3 of Catalpa silty clay loam with 26 L of water supplemented with 0.1 N Hoagland's solution. Selenium treatments were applied as a 4-L solution of either sodium selenite (SeO_3^{2-}) or sodium selenate (SeO_4^{2-}) to a total volume of 30 L at 0, 500, or 1000 μg Se L^{-1}. Water samples were collected daily for six days. Plant and soil samples were collected prior to Se application and at three-day intervals post Se application. Water, plant, and soil samples were analyzed for total [Se] by inductively coupled plasma-mass spectrometry. Data were analyzed with PROC GLM at $\alpha=0.05$. After six days, CT and MG-planted microcosms significantly decreased aqueous [Se] by 75 and 74%, respectively, compared to 61% for UNP. The aqueous fraction of microcosms planted to CAB, DWD, and SR were similar to UNP controls. Plant tissue Se content in CT was significantly less than CAB, DWD, or MG, suggesting CT has the potential to volatilize Se. Given its abundance and efficacy, CT is likely a suitable species for Se removal in constructed wetlands supplied with either selenite or selenate-impacted waters.

Additional Key Words: cattail, duckweed, fanwort, muskgrass, phytoremediation, selenate, selenite, selenium, softrush

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Introduction

Degradation of environmental quality in both aquatic and terrestrial ecosystems from wastewaters and stormwater runoff associated with anthropogenic activities is a growing concern. Flue gas desulfurization (FGD) is a clean coal technology that adsorbs sulfur/nitrogen oxides (SNOX) and trace elements to a calcareous material during the coal combustion process. Coal fly ash is a byproduct of FGD that is primarily used as a roadbed for heavy mining equipment. Extreme weather events produce large volumes of rapid runoff that can accumulate and transport trace elements, such as Se. In Mississippi, the majority of the annual rainfall (140 cm) is received during the winter and spring. Stormwater runoff is typically retained by impoundments or in sedimentation ponds until Se concentrations meet water quality standards before discharge into local watersheds. Water quality standards for Se set forth by the National Pollutant Discharge Elimination System (NPDES) are daily discharge cannot exceed 11.8 μg Se L^{-1} (ppb) and mean monthly discharge not exceeding 4.6 ppb Se. Long-term retention of wastewaters or runoff imposes watershed management constraints and potentially compromises structural integrity possibly resulting in Se contamination events. Under certain circumstances, rapid treatment of Se-impacted waters may be crucial to meet low level NPDES limitations.

Selenium is a metalloid essential to humans and livestock, but can be toxic to fish and waterfowl. Being chemically similar to sulfur (S), nonspecific substitution in protein structures can lead to deficiencies or toxicities. Selenium bioaccumulation in lower trophic level organisms can result in biomagnification up to 35,000 times greater than aqueous Se concentrations (Lemly, 2014). Teratogenic and reproductive deformities in fish and waterfowl have been documented as a result of Se toxicity (Ohlendorf et al., 1986; Lemly, 2014). Aqueous Se thresholds vary by ecosystem based on species, food-web models, hydrology, and other watershed characteristics. Soil Se concentrations typically range from 0.01 to 2 ppm (Fordyce, 2005). Inorganic Se exists in four oxidation states (-II, 0, IV, VI). Selenium solubility is influenced by microbial decomposition of organic matter that alters reduction-oxidation potential (Eh). Selenide (Se^{2-}) and elemental Se (Se^0) are insoluble at Eh below 0 mV, but can become bioavailable under oxidized conditions. Selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) are bioavailable oxyanions and the primary chemical forms of concern. When aqueous conditions are moderately reduced (200 to 0 mV), selenite is the predominant oxyanion, whereas, selenate dominates oxidized (Eh > 300 mV) aqueous environments (Masscheleyn et al., 1991). Active treatment technologies rely on these characteristics to remove Se from wastewaters. However, active treatment technologies require capital, energy, and maintenance inputs that can offset the overall profitability of energy derived from coal combustion. Constructed wetlands (CWs) are an inexpensive, sustainable, and eco-friendly option for treating FGD wastewaters and Se-impacted runoff at remote locations.

Constructed wetland phytoremediation is a passive treatment technology that gained notoriety during the Kesterson Reservoir (CA) restoration in the early 1990's. Phytoremediation is simply plants removing pollutants via three primary elimination pathways: Rhizofiltration, phytoaccumulation, and phytovolatilization. Rhizofiltration is the process by which Se is concentrated in the rhizosphere where plants can phytoaccumulate Se through the sulfur assimilatory pathway (Dushenkov et al., 1995; Pilon-Smits and Quinn, 2010). Select terrestrial and aquatic plants can transform inorganic Se into organic dimethylselenide (DMSE) or dimetheyldiselenide (DMDSE) that volatilize into the atmosphere (Terry et al. 2000). These organic forms are 600 times less toxic compared to inorganic Se compounds (Wilber, 1980). Phytovolatilization is an attractive elimination pathway in that Se is completely removed from the aqueous system.

Aquatic macrophytes were selected for biomass production and growth habit (Table 2). Preference was given to native species or those not considered invasive to the Southeast. Fanwort (*Cabomba caroliniana* L.; CAB; Fig. 1a) exhibits tolerance to anaerobic, alkaline conditions most likely occurring in constructed wetlands receiving runoff from coal fly ash surfaces. Cattail (*Typha angustifolia* L.; CT; Fig. 1b) is ubiquitous in wetland environments and several studies indicate effective Se removal of 89 and 46% of applied Se as selenite and selenate, respectively (Hansen et al., 1989; Shardendu et al., 2003). Duckweed (*Lemna minor* L.; DWD; Fig. 1c) is a floating macrophyte selected for its rapid vegetative reproduction and inherent ability to bioaccumulate Se. Literature indicates DWD has the ability to remove 55 to 99% of applied Se (Zayed et al., 1998; Carvalho and Martin, 2001; Miranda et al., 2014). Muskgrass (*Chara sp.* Desv. & Lois.; MG; Fig. 1d) is a freshwater macroalga known to accumulate S and prefers alkaline environments. Literature indicates MG removed 70 to 75% of applied Se from aqueous solutions (Lin et al., 2002). Soft rush (*Juncus effusus* L.; SR; Fig. 1e) was chosen for its abundance in Southeast wetlands and is less aggressive than CT.

Figure 1. Aquatic plant species evaluated for phytoremediation of aqueous selenium. a) Carolina fanwort (CAB), b) cattail (CT), c) duckweed (DWD), d) muskgrass (MG), and e) soft rush (SR).

The objectives of this study were to: 1) evaluate the influence of Se chemical form and concentration on plant uptake and 2) determine the potential of aquatic macrophytes to improve water quality of selenium-impacted storm water runoff.

Materials and Methods

A pilot-scale microcosm experiment was conducted during the fall of 2017 at the R.R. Foil Research Facility at Mississippi State, MS. The experiment was designed as 2 X 2 factorial arrangement of treatments nested within a split-split plot design replicated over time. Aquatic plants were collected locally, rinsed of debris, and transplanted into 115-L microcosms containing 0.035 m^3 of a Catalpa silty clay loam (fine, smectitic, thermic Fluvaquentic Hapludolls). Table 1 shows the soil chemical properties and extractable nutrient concentrations. Table 2 shows the aquatic plant species and population densities. Shoots of emergent species were trimmed to 45.7 cm (18 in.) above the plant crown (Fig. 2). Planted and unplanted (UNP) microcosms were filled with 26 L of water augmented with a 0.1 N Hoagland's solution for 14 d. Selenium treatments were applied to microcosms as a 4-L solution to bring microcosms to a volume of 30 L at 500 or 1000 ppb Se as the Na salt of selenite (Na_2SeO_3) or selenate (Na_2SeO_4). A zero Se block served as a control. Plant and soil samples were collected prior to Se application and at three-day intervals post Se application. Water samples were collected daily for six days. Water (EPA Method 200.8), plant, and soil samples (EPA Method 6020A) were analyzed for total Se concentrations by inductively coupled plasma-mass spectrometry (ICP-MS) (Waypoint Analytical, Inc., Memphis, TN). Main and interaction effects were determined with ANOVA using PROC GLM (SAS, v. 9.4, SAS Inst., Cary, NC) and mean separation at α = 0.05.

Table 1. Catalpa silty clay loam soil chemical properties and Mehlich-III extractable nutrient concentrations (ppm) in microcosms.

pH	CEC	OM	P	K	Ca	Mg	S	Na	Fe	Mn
	$cmol_c\ kg^{-1}$	%	- ppm -							
6.1	15.9	3.1	38.5	55.3	2480.4	124.2	14.5	46.1	22.2	255.9

Figure 2. Constructed wetland microcosms made from 115-L plastic containers (left). Microcosms contained 0.035 m^3 of a Catalpa silty clay loam planted to five aquatic macrophytes, or unplanted, and flooded with 30 L of water at 0, 500, or 1000 ppb Se as selenite or selenate (right).

Results and Discussion

Statistical Analysis

Only main effects were significant for aqueous selenium removal and plant Se accumulation (Table 3). Significant differences among monthly replications (Sep, Oct, and Nov) were expected ($P < 0.0001$). These differences can be attributed to changes in photoperiod and temperature that drive plant metabolic processes. For aqueous Se removal and plant Se accumulation, only main effects were significant.

Table 3. Main and interaction effects ANOVA (α=0.05) for aqueous selenium removal and plant Se accumulation in the simulated constructed wetland microcosm study.

Source	Aqueous selenium removal	Plant selenium accumulation
Rep (R)	<0.001	<0.0001
Oxidation (O)	0.0003	<0.001
Concentration (C)	0.001	0.03
Plant (P)	0.0169	<0.001

Aqueous Selenium Removal

Selenite

Microcosms planted with CT and MG removed the greatest amount of applied SeO_3^{2-}-Se (Table 4). On average, CT and MG-planted microcosms removed 89 and 96% of applied SeO_3^{2-}-Se, respectively, compared to 78% for UNP microcosms. Soft rush, CAB, and DWD-planted microcosms were not different from the UNP control. When supplied with 1000 ppb, Se removal was 8 to 11% greater in planted microcosms compared to the UNP microcosm, with the exception of DWD. These results indicate at this level of Se, CT and MG have the potential to effectively phytoremediate storm water containing SeO_3^{2-}-Se.

Selenate

Selenium removal was significantly less for microcosms supplied with SeO_4^{2-} -spiked water compared to SeO_3^{2-} (Table 4). Overall, planted microcosms removed 7 to 19% more of the applied Se in six days compared to the UNP microcosm. Muskgrass-planted microcosms removed 55% of the applied Se. Fanwort and CT-planted microcosms removed 49% of the applied Se. Microcosms planted to SR or DWD demonstrated similar Se removal efficiencies. After six days at 500 ppb SeO_4^{2-} -Se, MG, CAB, and DWD removal efficiencies were greater than 50% of applied aqueous Se. At 1000 ppb SeO_4^{2-} -Se, MG and CT-planted microcosms removed 52% of applied SeO_4^{2-} -Se compared to 44% in microcosms planted to CAB and SR. Duckweed-planted microcosms were similar to UNP microcosms. These results suggest the potential of aquatic plants to phytoremediate water impacted by SeO_4^{2-} -Se; however, greater than six days are necessary to achieve the removal efficiencies equivalent to microcosms supplied with SeO_3^{2-}-Se.

Table 4. Mean aqueous Se removal, as a percentage of applied aqueous selenium, for planted and unplanted microcosms when supplied with selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}). Means with same letter are not statistically different at α=0.05.

Aquatic plant species	SeO_3^{2-}	SeO_4^{2-}	Aquatic plant mean
	- - - - - - - - Aqueous selenium removal (% of applied aqueous Se) - - - - - - - -		
CAB	80	51	65B
CT	87	64	75A
DWD	82	41	62B
MG	91	50	70AB
SR	85	46	66AB
UNP	80	41	61B
Selenium oxidation mean	84A	49B	66

Plant Selenium

Selenite

All aquatic plant species evaluated show potential to remove Se from water containing either SeO_3^{2-} or SeO_4^{2-}. Overall, plant tissue Se content (mg kg^{-1} DW) was greater when plants were supplied with SeO_3^{2-}-Se compared to SeO_4^{2-}-Se (Fig. 3). As expected, plant tissue Se content was generally greater on day six compared to day three. In MG-planted microcosms supplied with SeO_3^{2-}-Se, greater tissue Se content at three days after application compared to six days after application suggests MG has the potential to phytovolatilize Se. Duckweed accumulated the greatest amount of Se, followed by CAB. Both species also demonstrated similar aqueous Se removal. Selenium content in CT and SR tissue was less than DWD and CAB, yet decreases in aqueous Se concentrations were greater in microcosms planted with CT or SR. These data suggest CT, MG, and SR can putatively augment and phytovolatilize Se from water containing SeO_3^{2-}-Se. Based on these findings, DWD and CAB can be used in constructed wetlands for phytoremediation of water impacted by SeO_3^{2-}-Se. Cattail, MG, and SR should also be considered for use in constructed wetlands because suspected phytovolatilization removes Se from the aqueous fraction. However, Se contained in decomposing plant tissue is likely to be released back into the system.

Selenate

Plant tissue total Se content was less when supplied with SeO_4^{2-} compared to SeO_3^{2-}, but followed a similar trend. Results indicate total Se plant tissue content was greatest for DWD followed by CAB. Selenium concentrations in CT, MG, and SR tissue were less than DWD or CAB, yet greater aqueous Se removal was observed. These results suggest CT, MG, and SR also show potential to phytovolatilize Se supplied as SeO_4^{2-}.

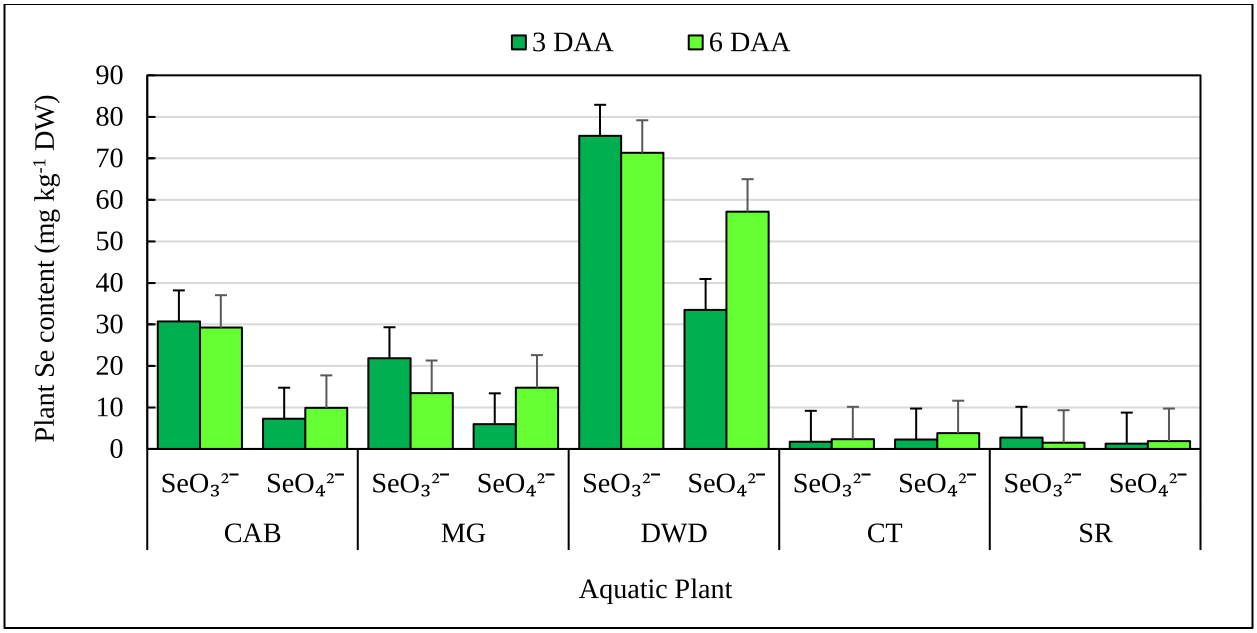

Figure 3. Mean plant tissue total selenium concentration (mg kg^{-1} DW) for aquatic plant species supplied with selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}) at three and six days after application (DAA).

Selenium Mass Balance

Aqueous Se removal occurs primarily through rhizofiltration, phytoaccumulation, or phytovolatilization. Soil Se was not significantly different among planted and unplanted microcosms. Therefore, phytoaccumulation or phytovolatilization are the suspected Se elimination pathways in this study. A mass balance of aqueous and plant Se was determined for all microcosms (Table 5). Results indicate DWD primarily phytoaccumulates Se in plant tissue with a small portion possibly lost through phytovolatilization. These results agree with previous studies evaluating Se accumulation in DWD (Zayed et al., 1998; Carvalho and Martin, 2001; Miranda et al., 2014). Cattail and MG Se volatilization is well documented under both laboratory and field conditions (Hansen et al., 1998; Pilon-Smits et al., 1999; Lin et al., 2002). Literature evaluating CAB and SR for Se phytoremediation is lacking. Results from this study show CAB removes aqueous Se primarily through phytoaccumulation and SR is suspected to phytovolatilize Se.

Table 5. Selenium mass balance (mg) in planted and unplanted microcosms.

Selenium species	Plant ID	Aqueous selenium removal	Plant selenium content	Missing selenium content
		- - - - - - - - - - - - mg	- - - - - - - - - - - -	
Selenite	CAB	21.0	3.4	17.6
	CAT	17.9	0.6	17.3
	DWD	15.9	6.4	9.5
	MG	25.8	3.1	22.7
	SR	20.6	0.2	20.4
	UNP	18.1	0.0	18.1
Selenate	CAB	14.1	1.3	12.8
	CAT	15.7	1.1	14.6
	DWD	9.1	4.6	4.5
	MG	13.6	4.3	9.3
	SR	11.9	0.2	11.7
	UNP	10.8	0.0	10.8

Conclusions

Aquatic plant species evaluated in this study do show potential to improve water quality in constructed wetlands receiving Se-impacted wastewater or runoff. Greater aqueous Se removal and plant Se accumulation was observed when microcosms were supplied with SeO_3^{2-}-impacted water. Cattail and MG demonstrated the greatest aqueous Se removal efficiencies and show potential to phytovolatilize Se when supplied as SeO_3^{2-}. Duckweed removes aqueous Se through phytoaccumulation in plant tissue. Overall, aquatic species in this study are best suited for constructed wetlands receiving water impacted by SeO_3^{2-}, but can enhance water quality when supplied with SeO_4^{2-}-impacted waters. Implementing CWs can provide mining operations an inexpensive water treatment option to meet federal and state water quality criteria for Se in storm water runoff. Future research is planned to determine an overall Se mass balance.

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Literature Cited

Carvalho, K.M. and D.F. Martin. 2001. Removal of aqueous selenium by four aquatic plants. J. Aquat. Plant Manage. 39:33-36.

Dushenkov, V., P.B.A. Nanda Kumar, H. Motto, and I. Raskin. 1995. Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. Environ. Sci. Technol. 29:1239-1245. https://doi.org/10.1021/es00005a015

Fordyce, F., 2007. Selenium geochemistry and health. Ambio 36:94-97. https://doi.org/10.1579/0044-7447(2007)36[94:SGAH]2.0.CO;2

Hansen, D., P.J. Duda, A. Zayed, and N. Terry. 1998. Selenium removal by constructed wetlands: Role of biological volatilization in the environment. Environ. Sci. Technol. 32:591–597. https://doi.org/10.1021/es970502l

Lemly, A.D., 2014. Teratogenic effects and monetary cost of selenium poisoning of fish in Lake Sutton, North Carolina. Ecotoxicol. Environ. Saf. 104:160–167. https://doi.org/10.1016/j.ecoenv.2014.02.022

Lin, Z.Q., M. de Souza, I.J. Pickering, and N. Terry. 2002. Evaluation of the macroalga, muskgrass, for the phytoremediation of selenium-contaminated agricultural drainage water by microcosms. J. Environ. Qual. 31:2104-2110. https://doi.org/10.2134/jeq2002.2104

Masscheleyn, P.H., R.D. Delaune, and W.H. Patrick. 1991. Arsenic and selenium chemistry as affected by sediment redox potential and pH. J. Environ. Qual. 20:522-527. https://doi.org/10.2134/jeq1991.00472425002000030004x

Miranda, A. F., N. Muradov, A. Gujar, T. Stevenson, D. Nugegoda, A. S. Ball, and A. Mouradov. 2014. Application of aquatic plants for the treatment of selenium-rich mining wastewater and production of renewable fuels and petrochemicals. J. Sust. Bio. Sys. 4:97-112. https://doi.org/10.4236/jsbs.2014.41010

Ohlendorf, H.M., D.J. Hoffman, M.K. Saiki, and T.W. Aldrich. 1986. Embryonic mortality and abnormalities of aquatic birds – apparent impact of selenium from irrigation drainwater. Sci Total Environ 52: 49–63. https://doi.org/10.1016/0048-9697(86)90104-X

Pilon-Smits, E. A. H, M.P. deSouza, G. Hong, A. Amini, R.C. Bravo, S.T. Payabyab, and N. Terry. 1999. Selenium volatilization of twenty aquatic plant species. J. Environ Qual. 28:1011-1018. https://doi.org/10.2134/jeq1999.00472425002800030035x

Pilon-Smits, E. A. H. and C. F. Quinn. 2010. Selenium metabolism in plants. *In*: Hell R., Mendel RR. (eds) Cell biology of metals and nutrients. Plant Cell Monographs, vol 17. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-10613-2_10

Shardendu, S., N. Salhani, S.F. Boulyga, and E. Stengel. 2003. Phytoremediation of selenium by two helophyte species in subsurface flow constructed wetland. Chemosphere 50:967-973. https://doi.org/10.1016/S0045-6535(02)00607-0

Terry, N., A.M. Zayed, M.P. de Souza, and A.S. Turan. 2000. Selenium in higher plants. Annual Rev. of Plant Phys. And plant Mol. Bio. 51:401-432. https://doi.org/10.1146/annurev.arplant.51.1.401

Wilber, C.G. 1980. Toxicology of selenium: A review. Clin. Toxicol. 17:171-230. https://doi.org/10.3109/15563658008985076

Zayed, A., S. Gowthaman, and N. Terry. 1998. Phytoaccumulation of trace elements by wetland plants: I. Duckweed. J. Environ. Qual. 27:715:721. https://doi.org/10.2134/jeq1998.00472425002700030032x