Metal uptake in northern laboratory-scale wetlands treating synthetic mine drainage

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Abstract

Constructed wetlands (CWs) have been employed as passive treatment systems for metal contaminated mine drainage in Canada. However, relatively few CWs have been documented in northern environments and further studies are needed to understand the metal removal mechanisms in wetlands operating under cold climates, with short growing seasons. The goal of this study was to evaluate the performance of laboratory-scale CWs for the removal of Cd, Cu, Se and Zn, as well as to evaluate Cu uptake in two northern plant species (*Carex aquatilis* and *Juncus balticus*). Eight laboratory-scale wetlands were constructed using local materials, including locally harvested plant species and microorganisms, and operated under northern summer conditions for ten weeks. The CWs had a three-day retention time and were fed continuously with synthetic influent containing Cd, Cu, Fe, Se, and Zn, at concentrations predicted at mine closure. Average removal efficiencies of 96%, 99%, 79%, and 97% were observed for Cd, Cu, Se, and Zn respectively. There were no significant differences in plant growth between the CW treatments, or any evidence of increasing Cu uptake with increasing availability in either northern plant species. Our study suggests that CWs could operate as successful passive treatment solutions in a northern environment, at least during the summer months. However, further studies are required to examine potential contaminant uptake in a suite of northern plant species and examine the efficacy of CWs under winter conditions.

Introduction

Constructed wetlands (CWs) are biogeochemical systems where an effluent flows through a plant-soil matrix, and natural processes reduce pollutant levels to a given discharge limit (Bathia and Goyal, 2014). CWs have been applied for treatment of municipal, agricultural, and industrial effluents with complex physical, chemical, and/or biological mechanisms decreasing contaminant levels (Kalder and Knight, 1996;

Kaldec and Wallace, 2008). Once established, CWs can become self-sustaining ecosystems with the plants providing yearly renewal of carbon to fuel microbial activity (Contango Strategies, 2014). CWs have been proposed as a sustainable and long-term solution for water treatment at mine closure in Canada due to their low maintenance and operational cost requirements and high removal capacity (Eger and Kairies Beatty, 2013; Sheoran and Sheoran, 2006). However, relatively few wetlands have been used in northern environments, and further studies are needed to design systems that will best fit the remediation objectives and environmental constraints (Kaldec and Reddy, 2001).

The processes involved in metals removal from mine-impacted water include, but are not limited to, reduction/oxidation, precipitation, bio-sorption, bioaccumulation, and volatilization (Sobolewski, 1999; Guittonny-Philippe et al., 2014). Microbial sulfate reduction processes and metal precipitation as sulfide salts in the anaerobic zone of the substrate is considered a major mechanism for metal sequestration in CWs (Arroyo et al., 2013). While wetland sediments are known to act as a sink for heavy metals (Sheoran and Sheoran, 2006; Baldwin and Hodaly, 2003; August et al., 2002), bioaccumulation in plants is also considered to some extent a metal removal pathway. Metal uptake by plants growing in wetlands treating mine-impacted waters has been sparsely studied with records of metal uptake by *Carex aquatilis* and *C. rostrata* (August et al., 2006; Stoltz and Greger, 2002; Nyquist and Greger, 2009), *Juncus maritimus* and *J. effuses* (Conesa et al., 2011; Rahman et al., 2011), *Typha latifolia* and *T. domingensis* (Mitsh and Wise, 1998; Taylor and Crowder, 1983; Maine et al., 2006), *Phragmites australis* (Batty and Younger, 2004; Stoltz and Greger, 2002; Nyquist and Greger, 2009) *Eichhornia crassipes* (Maine et al., 2006) and *Salix Sp.* (Stoltz and Greger, 2002). In most of these cases heavy metals were reported to be largely found in plant roots with minimal or no uptake into shoots. Metal uptake potential in aboveground shoots should be well characterized in CWs that are used for mine closure, as it could pose a risk by exposing foraging wildlife to contaminants. Uptake by wetlands plants can be strongly affected by the water chemistry, the plant species (Deng et al., 2004; Sheoran, 2006), as well as the redox conditions and geochemistry in the wetland substrate (Sobolewski, 2010).

Implementation of two large CWs has been proposed as part of the billion-ton Copper-Gold Casino deposit project, located in the Yukon, 300 km northwest of Whitehorse. CWs have been proposed as a passive option for remediation to mitigate the risk of metals discharge into the downstream environment. One 10 ha CW has been proposed to treat discharge from the 3.14 km^2 open pit, which then flows into the proposed 1,120 ha Tailings Management Facilities (TMF). A second 6 ha CW has been proposed down-gradient of the TMF for final water treatment before release into the Casino Creek watershed (Casino Mining Corp., 2014). This plan was submitted earlier this year for revision under the Yukon Socio-Economic and Environmental Assessment Act, one of the regulations framing environmental permitting in Yukon Territory. However, very few data on northern wetlands with northern plants are available in the

literature, and a deeper understanding of northern wetlands systems and plant uptake capacity is required for assessment and development of passive water treatment in the North.

The three objectives of this study were to: 1) Assess the short term efficiency of laboratory-scale CWs for mine effluents containing Cd, Cu, Fe, Se and Zn; 2) assess the potential uptake of Cu by two northern wetlands plants; and 3) examine the influence of a methanol-amendment on metals removal by the laboratory-scale CWs.

Material and methods

Lab-scale wetland setup

Eight laboratory-scale CWs were established in late June 2015 in the Yukon Research Centre greenhouse (Figure 1). Each wetland consisted of a 47 L tote filled with 35 L soil substrate (13 cm height) made up of a homogeneous mixture of 5% (v/v) peat (Premier®, PremierTech Horticulture, Rivière-du-Loup, Quebec), 55% (v/v) washed sand (GE Cement plant, Whitehorse) and 40% (v/v) washed pea gravel (GE Cement plant, Whitehorse). *Carex aquatilis* and *Juncus balticus*, two plant species common in northern natural wetlands with different root oxygen exchange rates, were collected from a natural wetland located on McIntyre Creek in Whitehorse, YT (60°44'48.6"N 135°06'17.5"W). Eight plugs of either species containing rhizomes, roots, and approximately 250 ml of natural wetland substrate were transplanted from the natural wetland into each laboratory-scale CW. Each wetland contained only one species; therefore, we had four wetlands with *C. aquatilis* and four with *J. balticus*. Two days after transplant, fertilizer was added (Alaska® Fish Fertilizer 5-1-1, Lilly Miller, dosage of 20 ml/m^2). The CWs were then saturated with tap water up to the level of the substrate surface and left undisturbed (no flow) for two weeks to allow the rhizomes to establish and the microorganisms within the transplanted substrate to incubate. Tap water was then circulated for another two weeks through the CWs. After four weeks of incubation with tap water, the aboveground biomass was removed at a height of 2 cm, leaving no shoots. Synthetic influents containing metals were then circulated through the CWs for ten weeks and new shoot growth was monitored.
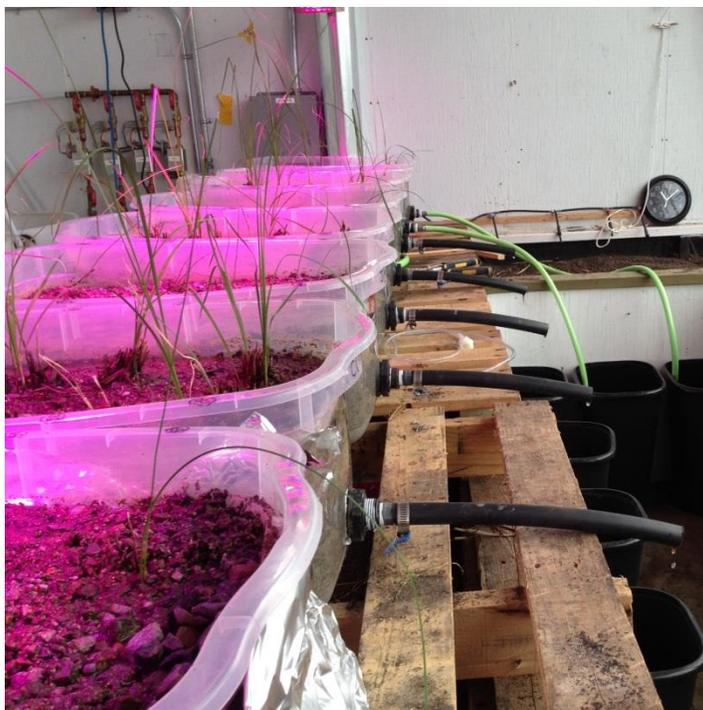
Figure 1: Laboratory-scale CWs

Synthetic influent preparation

Synthetic influents were prepared weekly by dissolving $CdSO_4*8/3H_2O$ (Acros Organics, ACS Reagent), $CuSO_4*5H_2O$ (Fisher Scientific, Fisher Bioreagents), $FeSO_4*7H_2O$ (Fisher Scientific, Reagent Grade), SeO_2 (anhydrous; Acros Organics, 99.8%) $ZnSO_4*7H_2O$ (Acros Organics, ACS Reagent) and Na_2SO_4 (anhydrous; Fisher scientific; Lab Grade) in DI water. We had 4 CWs with different water treatments (4 water treatments $\times$ 2 species for a total of 8 CWs): i) TMF with metal concentrations that reflected the concentrations predicted at closure in the Tailings Management Facility, ii) Pit with metal concentrations predicted at closure in the open pit, iii) Pit(MeOH) with Pit metal concentrations and the addition of 1% MeOH (Fisher Scientific) added weekly and iv) City of Whitehorse tap water that was considered a Control treatment. pH was similar in all influents while Cd, Cu and Se concentrations were about twice in the Pit treatment as in the TMF treatment (Table 1). Cadmium, Se and Zn concentrations in the tap water Control were below quantification limits or quantified under 5% of the measured concentrations in TMF, Pit and Pit(MeOH) (Table 1). We did detect Cu concentrations in tap water (35.3 ± 14.4 ug/L) that were approximately 30% of the concentrations found in the TMF influent.

Table 1: Average and standard deviation for pH, SO₄, Cd, Cu, Se and Zn concentrations in the influents between Week 2 and 10[1]

	Control	TMF	Pit	Pit(MeOH)
pH	7.9 ± 0.3	7.8 ± 0.1	7.9 ± 0.1	7.7 ± 0.1
Cd (ug/L)	0.07 ± 0.05	1.6 ± 0.9	6.1 ± 0.44	5.5 ± 0.74
Cu (ug/L)	35 ± 14	121 ± 33	644 ± 181	607 ± 192
Se (ug/L)	0.7 ± 0.0	2.8 ± 1.2	4.4 ± 1.7	4.5 ± 2.3
Zn (ug/L)	24 ± 3.7	525 ± 63	576 ± 53	540 ± 84
SO4 (mg/L)	37 ± 3.3	600 ± 243	610 ± 237	496 ± 215

[1](Values below quantification limit are assumed to be equal to the quantification limit of 0.05 ug Cd/L, 0.6 ug Cu/L, 0.7 ug Se/L and 0.4 ug Zn/L)

Laboratory-scale constructed wetlands operation and monitoring

The 8 CWs were operated and monitored over 10 weeks (excluding 4 weeks of incubation) in a greenhouse under northern summer conditions. Temperature was 11°C with no light from 23:00-5:00 and 16°C with 175 μmol/m²/s of light from 5:00-23:00. Synthetic influents or tap water were pumped at the bottom of the substrate using a multi-channel peristaltic pump (Masterflex pump, head and C-flex tubing) at 5 ml/min with sub-surface vertical flow. The hydraulic residence time was approximately 5 days. Effluents from the CWs were discharged into outlet collection containers. The volume of the effluent accumulated in the collection containers over a week were recorded while pH measurements and samples were collected weekly. Samples for total metal analysis were preserved with 5% HNO3 (trace metal grade) and stored at 4°C and samples for sulfate analysis were stored frozen.

At the end of the experiment period, Oxygen Reduction Potential (ORP) and pH were measured in the center of the CW at a depth of 5 cm and 10cm from the outlet. CWs were drained and interstitial water that remained within the substrate was sampled and preserved with 5% HNO_3 (trace metal grade) for total Cu and Se analysis. All plant materials were extracted from the wetland and above- and belowground biomass were carefully washed with DI water, brushing the materials to remove any remaining soil particles. Root and shoot length were recorded and dry biomass was determined following drying at 105°C for 72 hrs. Three replicates of live shoots and roots were further analyzed for metal content. Wetland substrates from each CW were thoroughly mixed and also analyzed for metal contents.

Analytical techniques

Total Cd, Cu, Se and Zn metal concentrations in effluents were measured using Perkin Elmer PinAAcle Atomic Absorption (AA) analyzer (Perkin Elmer, Waltham, MA). Cadmium, Cu and Se were analyzed by Graphite Furnace (GFAA) and Zn by Flame (FAA). The Quantification Limits (QL) used in this work have been defined for each element as 10-σ and are 0.05 ug Cd/L, 0.6 ug Cu/L, 0.7 ug Se/L and 0.4 ug Zn/L.

Calibrations were completed on a daily basis using single element standards (SCP Science, Baie D'Urfé, QC), and blanks and mixed verification standards (Perkin Elmer, Waltham, MA) were analyzed every 15 samples. pH was measured using Oakton PCD650 meter (Vernon Hills, IL) with a double junction pH electrode. Sulfate was analyzed by spectrophotometry using a SmartChem 170® Automated Discrete Analyzer (Westco, Guelph, ON) according to the STM Method D516-90, 02. Biomass samples were digested with nitric acid (trace metal grade) according to the method described by Zarcinas et al. (1987) while substrate samples were digested with aqua regia according to USEPA reference method 3050B.

Results and discussion

More than 96%, 99%, 79% and 97% average removal efficiencies were observed for Cd, Cu, Se and Zn respectively in the four CWs treating Pit and TMF synthetic influents (Figure 2). Although, our Pit treatment had higher metal concentrations in the influent (Table 1), we observed similar removal efficiencies between the Pit and TMF treatments. No changes in pH were observed between the CWs. Although, our CWs were only at a small laboratory-scale, the Cd, Cu and Zn removal efficiencies we observed were similar to the efficiencies observed in other pilot or full-scale wetlands. Removal efficiencies of 94-99% for Cd (Gammons et al., 2000; Yang et al., 2006), 89-97% for Cu (Banks et al., 1997; Contango Strategies, 2014; Gammons et al., 2000; Lesage et al., 2007) and 87-98% for Zn (Banks et al., 1997; Lesage et al., 2007; Sobolewski, 1996; Yang et al., 2006) have been reported. Selenium treatment of mine-impacted water by CWs seems less common than transition metals treatment. Efficiencies above 83% were observed in this study, but this diverges from an efficiency of 26% reported for a northern wetlands planted with the same *Carex* species (Contango Strategies, 2014). This low efficiency was attributed to the elevated concentrations of nitrate, which competes over Se as an electron-acceptor.
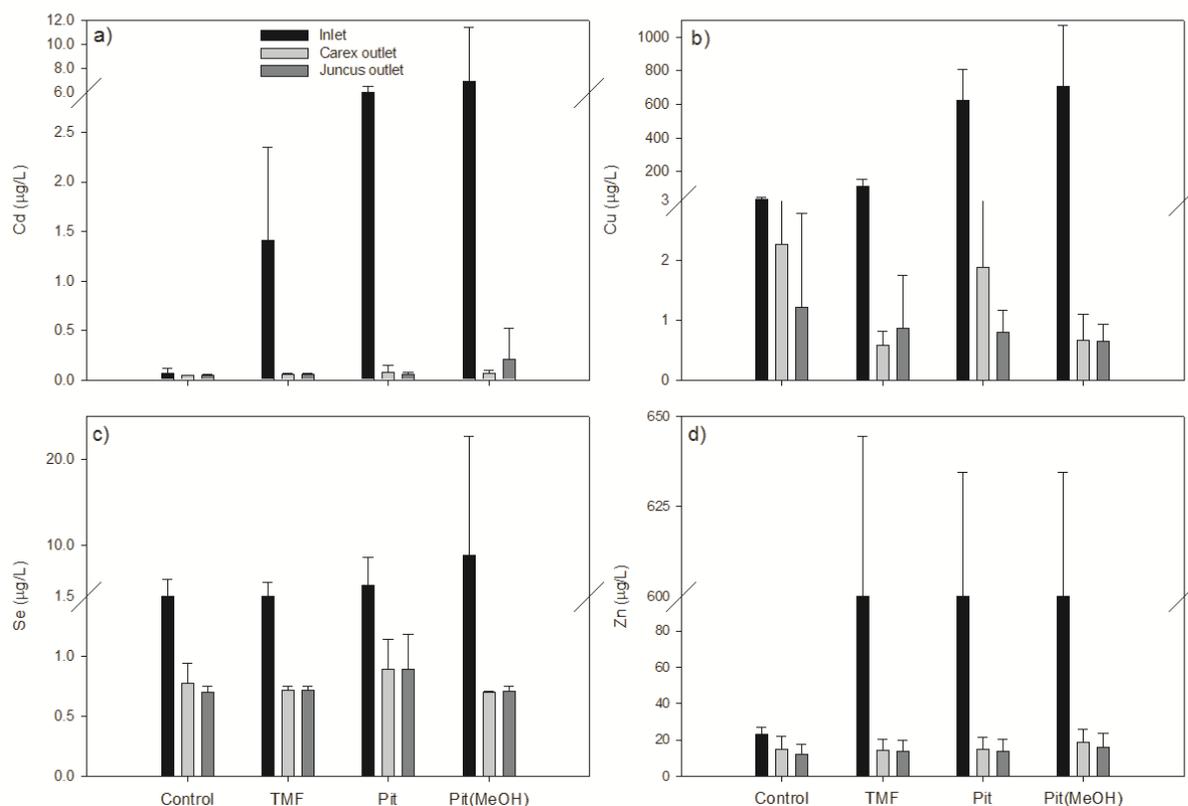
Figure 2: Cd (a), Cu (b), Se (c) and Zn (d) concentrations in CWs with *C. aquatilis* (Carex) and *J. balticus* (Juncus) fed with Control, TMF, Pit or Pit(MeOH) waters (Note the broken Y-axis which accommodate inlet concentrations)

Wetland plant growth

We found no significant difference in the above and belowground length or in the above and belowground biomass across our different CW treatments for either of our northern plant species (Table 2; ANOVA with TukeyHSD post hoc, p>0.05 for all comparisons). Although, we did not observe any treatment effects on establishment, *C. aquatalis* had a 53% survival rate and *J. balticus* only a 38% survival rate following transplanting. If using locally transplanted materials high numbers of replicate plants need to be used to account for transplant related mortality. However, for those transplants that did survive we observed strong growth within 10 weeks with stems as long as 74 cm and 50 cm for *C. aquatalis* and *J. balticus* respectively.

Table 2: Average and standard deviation of root and shoot length and biomass (dry weight) of *Carex aquatilis* (Carex) and *Juncus balticus* (Juncus) after ten weeks of growth in CWs treated with either control, pit, TMF and pit(MeOH) waters

	Root length	Shoot length	Root dry weight	Shoot dry weight
	(cm)	(cm)	(g)	(g)
Control-Carex	20 ± 8.9	35 ± 21	0.38 ± 0.23	0.76 ± 0.47
Control-Juncus	11± 10	25 ± 24	0.29 ± 0.31	0.30 ± 0.19
TMF-Carex	24 ± 21	35 ± 22	0.50 ± 0.23	9.2 ± 23
TMF-Juncus	11 ± 7.1	15 ± 12	0.27 ± 0.21	0.20 ± 0.14
Pit-Carex	25 ± 20	24 ± 23	0.42 ± 0.27	1.4 ± 1.2
Pit-Juncus	10 ± 6.1	21± 15	0.25 ± 0.15	0.21± 0.07
Pit(MeOH) Carex	21 ± 9.1	28 ± 18	0.49 ± 0.23	0.91 ± 0.31
Pit(MeOH) Juncus	7.2 ± 2.8	16 ± 8.8	0.30 ± 0.21	0.23 ± 0.09

Cu uptake by wetlands plant

Copper content in three replicates of roots and shoots was analyzed from plants collected in the natural environment, as well as, in the laboratory-scale CWs which were treated with tap water (Control), TMF, Pit or Pit(MeOH) synthetic waters (Figure 3). Copper content in shoots from *C. aquatilis* and *J. balticus* from the natural wetlands were 7.07 ± 2.79 and 7.14 ± 2.71 mg/kg respectively. Contango Strategies (2014) reported similar Cu contents (4 to 20 mg Cu/kg) in *C. aquatilis* growing in a natural wetland area where Cu-loaded seepages are known to occur within the Minto Cu-Au mine area in Yukon Territory.

There was no significant difference in below or aboveground Cu content in either plant species across the different CWs treatments or in comparison to the natural control (ANOVA, p=0.80 for belowground biomass and p=0.15 for aboveground biomass). Hence, no significant uptake of Cu into below or aboveground biomass by *C. aquatilis* or *J. balticus* was observed in this study. Even through small-scale experiments have been shown to overestimate metal plant uptake capability (Conesa et al., 2007), both the results from this study and those observed from the natural wetland located at Minto mine (Contango Strategies 2014) suggest a low tendency for Cu uptake in these wetland plants. Despite the differences in the root oxygen exchange between these two species (*C. aquatilis* 6.7 μmol O_2 loss/g dry root/day vs *J. balticus* 9.9 μmol O_2 loss/g dry root/day [Taylor, 2009]) we did not see any differences in their uptake of Cu within the CWs.
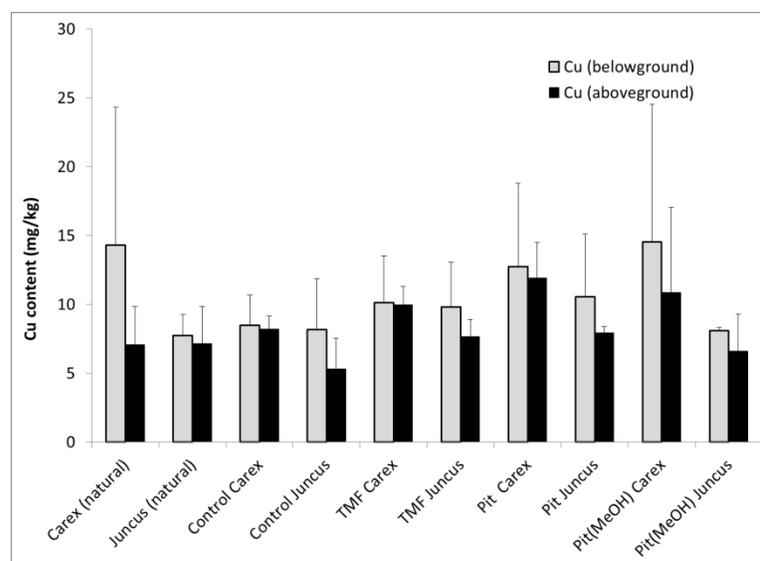
Figure 3: Copper content in *C. aquatilis* and *J. balticus* in the natural environment and in constructed laboratory-scale wetlands after 10 weeks of operation with tap water (Control), TMF, Pit or Pit(MeOH)synthetic waters

High removal efficiencies were observed in the CWs for all the metals monitored and especially for Cu (>99% removal efficiencies). The % Cu uptake, defined as the Cu that was taken up by the plants versus Cu that was retained within the wetland substrate, was calculated according to Equation 1. $Cu_{plant\ content}$ was calculated by taking the average uptake in roots and shoots and multiplying this by the total below and aboveground biomass in each CW. $Cu_{retained}$ was calculated as the amount of Cu in wetland influent minus the amount of Cu discharged in the wetland effluent for each CW. Less than 0.06 % of Cu retained in the CWs was actually taken up by the plants, except for the Cu uptake in the CW with *C. aquatilis* fed with TMF (0.21 % Cu uptake), suggesting that metal uptake by *Carex aquatilis* or by *Juncus balticus* is clearly not a major Cu removal mechanism in the CWs studied (Table 3). Similar observations were made for Fe uptake by Mitsh and Wise (1998) who observed less than 0.07 % uptake in a constructed wetland in Ohio (USA) and by August et al. (2002) who measured less than 0.5 % uptake in a natural wetland in Colorado (USA). Our study supports the findings of others that suggest metal removal mechanisms are most likely driven by chemical and microbial reactions occurring within the substrates (Sobolewski, 1999; Sheoran and Sheoran, 2006).

$$Eq.\ 1 \qquad \%\ Uptake = \frac{Cu_{plant\ content}}{Cu_{retained}}$$

Table 3: Percent uptake in *C. aquatilis* or *J. balticus* in CWs fed with tap water (Control), TMF, Pit and Pit(MeOH) Synthetic Waters

	C. aquatilis	J. balticus
	% Uptake	% Uptake
Control	N/A	N/A
TMF	0.209%	0.057%
Pit	0.055%	0.011%
Pit(MeOH)	0.036%	0.008%
Average	0.10%	0.03%

Effect of liquid carbon feed

Metal uptake by plants is thought to depend on the geochemistry in the substrate and is affected by the speciation and availability of the metals. In order to assess the impact of a change in the substrate conditions on metal uptake, one of the CWs was fed with liquid methanol at 1% (v/v). Organic matter decomposition typically decreases the ambient redox potential. Organic carbon acts as an electron donor to microorganisms, such as sulfate-reducing bacteria, which reduce sulfate (SO_4) releasing sulfide (S^2) (Sobolewski 2010). Sulfide is highly reactive and forms insoluble metal salts, such as CdS, CuS and ZnS. In our study when MeOH was added at the influent (i.e. Pit(MeOH) treatment) we observed an increase in reducing conditions in the CW substrate, with an average ORP of -341.9 mV , compared to an average of -109.4 mV when no MeOH was added. In addition, a significant reduction of the sulfate concentration was observed in the Pit(MeOH) CWs compared with the Pit CWs, which were operated in the same conditions but without MeOH (Figure 4). Low ORP, high SO_4 and high metal removal efficiencies (Figure 2) detected in this study are strong indications that sulfate-reduction mechanisms and sulfide precipitation are occurring. We also observed pitch black deposit characteristic of sulfide precipitates and odiferous evidence of H_2S from the Pit(MeOH) CWs, however at the end of the 10 weeks of treatment shoots of both *C. aquatilis* and *J. balticus* were yellowed and dead. Sulfide is known as a strong phytotoxcin to plants by causing basic disturbance to cell metabolism and energy transfer, which can hamper plant nutrient uptake (Lamers et al., 2013). This possibly indicates that the concentration of organic carbon used was too high. Further studies are needed to examine the influence of sources and concentrations of carbon in northern CWs and how these may influence metal sequestration in both wetland substrates and plants.
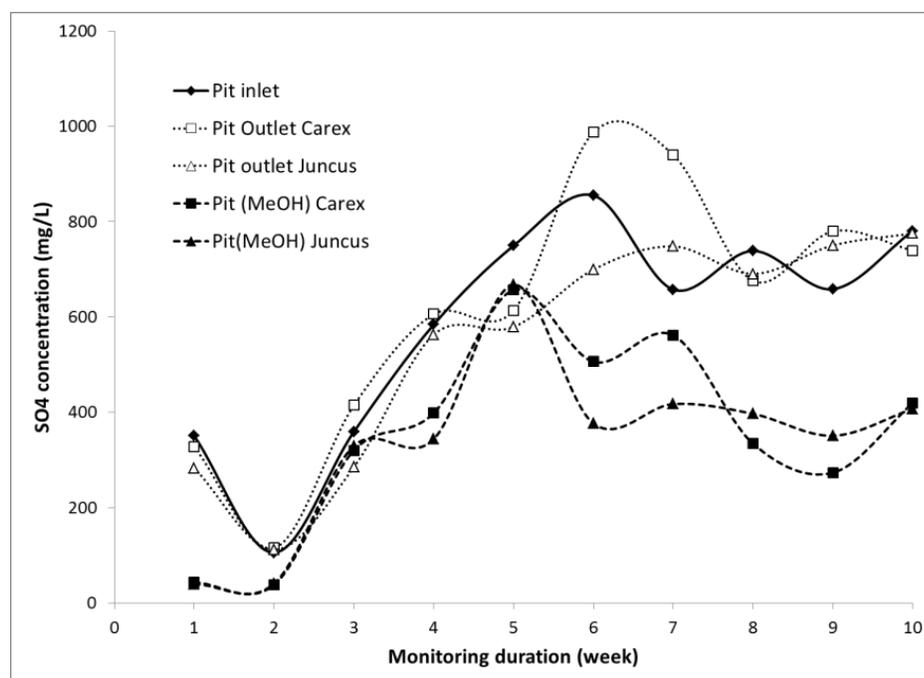
Figure 4: Sulfate concentrations in the influent and effluent from the Pit and Pit(MeOH) CWs planted with either *C. aquatilis* (Carex) or *J. balticus* (Juncus)

Conclusion

Our laboratory-scale CWs demonstrated a strong ability to remove contaminants from synthetically contaminated waters with average removal efficiencies of 96%, 99%, 79% and 97% for Cd, Cu, Se, and Zn respectively. Even with increased contaminant concentrations in the influent waters (i.e., Pit treatment compared to TMF treatment) we did not see a decline in removal efficiencies. While transplant related mortality did reduce the overall biomass accumulation in our CWs, we did not see any treatment effect on the growth of our two northern plant species *C. aquatilis* and *J. balticus*. Furthermore, there was no evidence of Cu uptake in either species with increasing Cu contaminant availability. Overall our study suggests that CWs could operate as successful passive treatment solutions in a northern environment, at least during the summer months. Further studies are required to examine potential contaminant uptake in a suite of northern plant species and examine the efficacy of CWs under winter conditions.

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