Petroleum coke and soft tailings sediment in constructed wetlands may contribute to the uptake of trace metals by algae and aquatic invertebrates

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ABSTRACT

The fate of trace metals in pore water collected from wetland sediments and organisms exposed to petroleum coke were evaluated within *in situ* aquatic microcosms. Oil sands operators of Fort McMurray, Alberta, Canada produced 60 million tonnes of petroleum coke by 2008, containing elevated concentrations of sulphur and several trace metals commonly seen in oil sands materials. This material may be included in the construction of reclaimed wetlands. Microcosms were filled with a surface layer of petroleum coke over mine-waste sediments and embedded in a constructed wetland for three years to determine how these materials would affect the metal concentrations in the sediment pore water, colonizing wetland plants and benthic invertebrates. Petroleum coke treatments produced significantly elevated levels of Ni. We also found unexpectedly higher concentrations of metals in "consolidated tailings" waste materials, potentially due to the use of oil sands-produced gypsum, and higher background concentration of elements in the sediment used in the controls. A trend of higher concentrations of V, Ni, La, and Y was present in the tissues of the colonizing macrophytic alga *Chara* spp. Aeshnid dragonflies may also be accumulating V. These results indicate that the trace metals present in some oil sands waste materials could be taken up by aquatic macro-algae and some wetland invertebrates if these materials are included in reclaimed wetlands.

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1. Introduction

Constructed wetlands will be an important component in the final reclaimed landscape in northeastern Alberta's oil sands region (Harris, 2007). Some of these wetlands will be used to contain oil sands mine-tailings (clay-based fine tailings and contaminated water) produced during recovery of the oil sands bitumen (the raw oil product). The suitability of these materials in the successful colonization and establishment of wetland macrophytes and aquatic invertebrates has not been well established. The various types of fine tailings materials produced in the Alberta oil sands contain elevated concentrations of several contaminants, such as unrecovered bitumen, polycyclic aromatic

hydrocarbons, naphthenic acids and salts that could be detrimental to the establishment of invertebrate communities and macrophyte development (Nix and Martin, 1992). Additionally, these fine tailings have a physical consistency that may impede macrophyte establishment; having a very liquid consistency and a density of 25–40% solids by weight. Isolation of these products from direct contact with colonizing biota may accelerate reclamation success, and help to minimize environmental impacts.

Bitumen recovered from the oil sands is highly viscous and must be upgraded to lighter synthetic crude oil; this process produced approximately 60 million tonnes of stockpiled petroleum coke by 2008. Petroleum coke is more buoyant than the fine tailings. Covering the tailings in reclaimed wetlands with petroleum coke may provide a more stable colonization surface in such wetlands, and isolate colonizing organisms from the potential toxicity associated with the tailings. This in turn, could speed the rate at which these wetlands may establish functional invertebrate and macrophyte communities.

Petroleum coke produced during the upgrading of Athabasca oil sands bitumen has been observed to release trace metals in laboratory-conducted leaching studies (Puttaswamy et al., 2010; Squires, 2005). However, the concentration of the mixture of these leached elements was not great enough to cause mortality in the larval dipteran *Chironomus tentans* (Squires, 2005). The presence of petroleum coke in the environment can lead to higher concentrations of many trace elements in soil and water, but this may not preclude its

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use as a beneficial soil amendment (Chen et al., 2010). Several elements are expected to occur in elevated concentrations in petroleum coke produced by Syncrude Canada Ltd. (Chung et al., 1996). Higher concentrations of vanadium and nickel may be the best indicators of the presence of petroleum coke. These elements tend to be found in the asphaltenes associated with Syncrude bitumen in higher concentrations than are seen in more traditional crude oils (Zhao et al., 2002), and as a result, occur in fairly significant concentrations in petroleum coke produced by Syncrude Canada Ltd. (Zhao et al., 2001). Leachate waters collected from Syncrude petroleum coke stockpiles also have higher concentrations of both V and Ni than of any other element, but only the Ni was present in sufficient amounts to induce a toxic response in the cladoceran, *Ceriodaphnia dubia* (Puttaswamy et al., 2010).

Wetland sediments can act as a sink for metals (Weis and Weis, 2004). Although trace metals can exist in many compartments of aquatic sediments, freely dissolved ions in the aqueous phase are the most bioavailable to aquatic organisms, and have the greatest potential to cause adverse effects (Morgan, 1987; Peijnenburg et al., 1997). The effects of higher concentrations of trace metals in aquatic sediments on the structure of macrophyte and invertebrate communities may not be readily visible for several reasons, including organisms' tolerance and acclimation, evolution of genetically resistant populations, avoidance, and issues of contaminant bioavailability (Klerks and Levinton, 1993). The risk posed by higher metal concentrations can be assessed by directly measuring tissue metal concentrations in resident biota (Borgmann et al., 1993), particularly at low concentrations of contaminants, where community and individual-level stresses may not be apparent.

An *in situ* microcosm experimental study was designed to examine the potential for petroleum coke to limit the exposure of aquatic biota to the residual contaminants associated with fine tailings, two types of which are; mature fine tailings and composite tailings. The objectives of this investigation were to determine if petroleum coke used as a surface covering for the tailings sediments placed in constructed wetlands would affect trace metal concentrations in sediment pore water and if these in turn would be taken up by macrophytes and epibenthic invertebrates. Laboratory findings (Puttaswamy et al., 2010; Squires, 2005) suggest that petroleum coke amendments might increase the concentration of several trace metals in sediment pore waters, and potentially, this may result in elevated metal concentrations in the tissues of macrophytes and invertebrates that colonize coke-amended substrates relative to non-amended constructed wetland sediments.

2. Materials and methods

2.1. Study site description

Study wetlands were located on the Syncrude Canada Ltd. Mildred Lake Lease, approximately 50 km north of Fort McMurray, Alberta, Canada (Fig. 1). Sediment treatments were prepared in individual containers (microcosms) that were submerged within a constructed wetland, Shallow Wetland South Ditch (SWSD) in a reclaimed area, in summer 2002. This area had been excavated in 1993 and filled with water from a nearby stream draining from muskeg wetlands; recharge to this area is through surface runoff from precipitation. It had not received inputs of any oil sands waste materials prior to this experiment. Characteristics of this wetland were expected to be similar to those of wetlands of the same age located outside the oil sands reclamation area, being slightly brackish with a conductivity of 600 μS/cm and having a small concentration of naphthenic acids present naturally (6 μg/L). The long, narrow shape of SWSD (6 m wide × 120 m long) protected the wetland from wind and minimized the potential for sediment suspension by wave action.

For comparative purposes, two additional wetlands with water chemistry similar to SWSD (Fig. 1), but lacking experimental treatments were chosen as reference sites for pore water and invertebrate sampling. One was a beaver pond (BP) located near the south margin of the Syncrude lease area (known as SWSS Beaver Pond), which was larger, naturally formed by beaver activity, and older than the test site (age unknown, but estimated to be at least 30 years old at the time of sampling), and having conductivity of 450 μS/cm and naphthenic acid concentration of 6 μg/L. This section of the Syncrude lease had not been deforested for mining activities. It was sampled in 2005 to provide a comparative baseline for trace metal concentrations in natural wetlands in the Athabasca oil sands area. U-Shaped Cell (USC) is a constructed catchment area, built in 1995 on top of a large stockpile of tailings sand using a plastic liner to retain surface runoff from precipitation. This site was built adjacent to a coke storage area and received wind-blown, weathered coke and sand, so that its sediment consisted of ~20 cm depth of coke and coarse tailings sand. It had a conductivity of 900 μS/cm and naphthenic acid concentration of 3 μg/L.

2.2. Microcosm design

Experimental sediments were contained within 53-L Rubbermaid Roughneck Storage Totes® (dimensions $61 \times 40.6 \times 31.8$ cm deep, made of low-density polyethylene), to examine the effects of fluid fine tails and petroleum coke on epibenthic biota. A 3×2 factorial design of base and surface treatment sediments, respectively, (6 treatment combinations in total, Fig. 1.) were layered into the microcosms, which were then inserted into excavations in the natural wetland sediment, such that the top lip of the tote was nearly flush with the surrounding wetland bottom. The totes were initially placed in a water depth of approximately 50 cm, although seasonal variation in wetland water level sometimes resulted in water depths of over 1 m. Two replicates of each treatment were installed in a stratified random arrangement around SWSD in June 2002. Natural colonization of the microcosms was allowed for 3 years without experimental interference. After this period, the plant community of microcosms appeared to be indistinguishable from the surrounding wetland (personal observation).

Within each microcosm, the base layer consisted of either: native wetland (SWSD) sediment, consolidated tailings or mature fine tailings. The surface layer (10 cm thick) consisted of either petroleum coke (produced by Syncrude Canada Ltd.), or no coke (to these no-coke microcosms, an additional 10 cm layer of the base sediments was added, to maintain consistent volumes among treatments). Mature fine tailings are a form of fluid fine tailings consisting of an aqueous suspension of sand, silt, clay, and residual bitumen, at pH of ~8, conductivity of ~4000 μS/cm and naphthenic acid content of about 65 μg/L. In tailings ponds, fresh tailings settle, release some water, and condense to form a product termed mature fine tailings (MFT in figures). Compaction of this material can take several decades. To accelerate the rate of densification the fluid fine tailings can be treated with gypsum, which agglutinates the clays and speeds the dewatering process. The resulting materials are called composite or consolidated tailings (CT in figures). The gypsum is acquired from the residuals of the emission-desulfurization process used by another Athabasca oil sands operator, Suncor Energy Inc., in the burning of their own petroleum coke as boiler fuel. Suncor's flue gas desulfurization technique involves using calcium carbonate combined with crushed coke as a catalyst in this process, and this may contribute additional contaminants to the gypsum (Hall et al., 1982; Kappe and Ellison, 1986).

2.3. Pore water sample collection and extraction

Sediment cores were taken from each microcosm in SWSD in early September 2005, from an undisturbed quadrant of each microcosm. Sediment was also collected, in an identical manner, from five
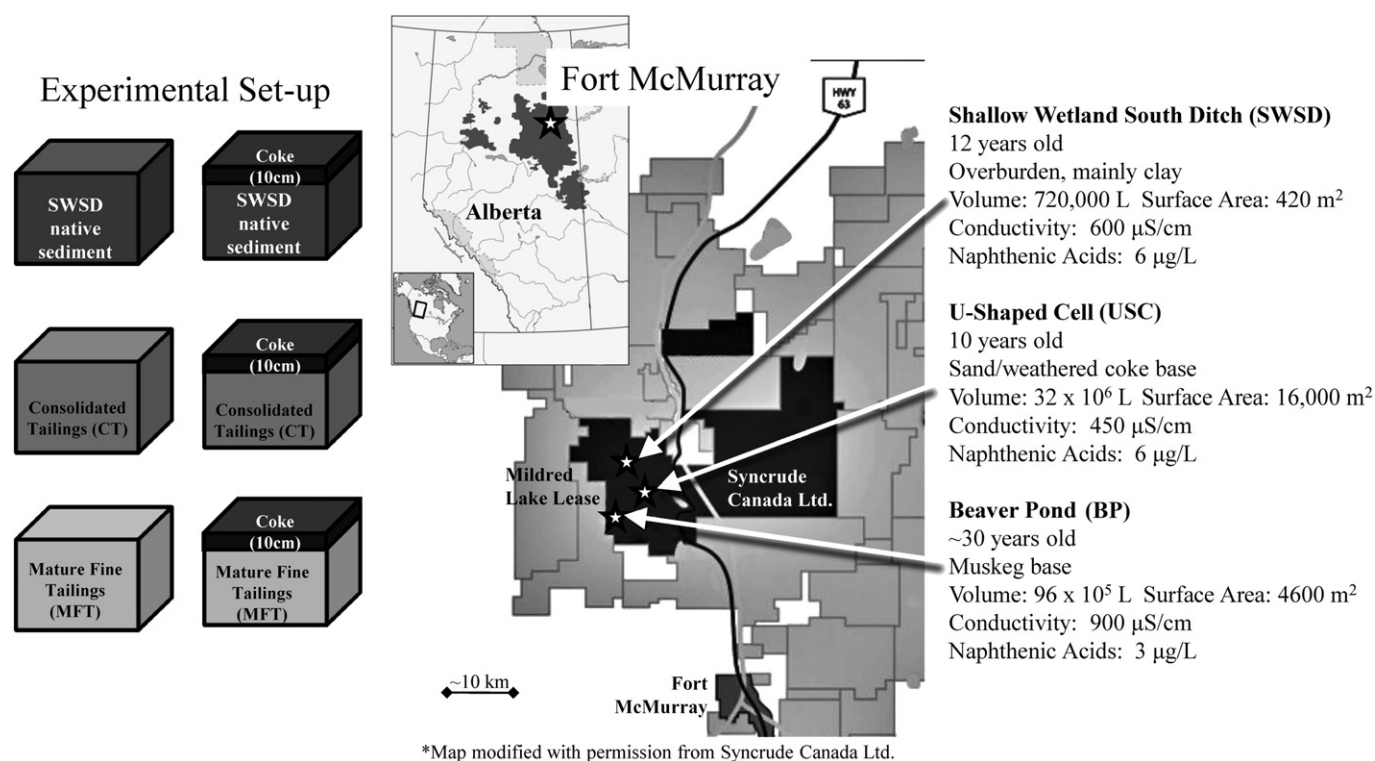
Fig. 1. Experimental design of microcosms, and study site location.

randomly selected locations of similar depth within both BP and USC in late July 2005. Coring tubes consisted of 40 cm long×5 cm inside diameter, acid-washed, high-density polycarbonate. Care was taken to limit exposure of the sediment to air, to prevent oxidation of metals in the pore water. Sediment cores were stored upright with a water cap over sediments, and frozen at −20 °C.

In the laboratory, sediment cores were thawed for 12 h in a refrigerator prior to analysis. All pore water extractions were conducted in an anoxic chamber to prevent oxidation of sediments, using aseptic techniques to prevent cross-contamination. Surface water was poured off and discarded, and the core was extruded from its tube. Surface treatment sediments (top 10 cm) were separated from the base sediment treatment and each was placed in sealed Nalgene® 50-mL centrifuge tubes (High-Speed polypropylene copolymer tube, or High-Speed Teflon® fluorinated ethylene propylene). Pore water was then extruded from the sediment by spinning down the solids at 4000 rpm (1073×g) in a refrigerated centrifuge, at 4 °C, for 60 min. The pH and electrical conductivity of the resulting supernatant water were measured using a Thermo Electron Corp. Orion 5 Star portable pH–conductivity–DO meter. The pore water was filtered through a disposable, Whatman 25-mm polypropylene, 0.45-μm syringe filter to remove suspended particles, acidified to 2% HNO_3, and stored in an airtight Nalgene container with nitrogen gas in the headspace. Only data from the metal concentrations of the pore waters collected from surface treatments are presented here, as this is the portion of sediment with which the algae and invertebrates would be associated. Surface pore water samples were collected from two replicates each of native sediments, consolidated tailings and mature fine tailings treatments, for a total of 6 microcosms without coke on the surface. An additional 6 microcosms had coke on the surface (refer to Fig. 1 for surface treatment sediments).

In this study, we compared our observed pore water metal concentrations against the Canadian water quality guidelines for protection of aquatic life created by the Canadian Council of the Ministers of the Environment (CCME, 1999a). Pore waters extracted without acids should contain only the metals that are freely dissolved in the water, and not those biologically unavailable metals bound to the sediment. We felt these were more appropriate guidelines than the interim Canadian sediment quality guidelines for the protection of aquatic life (CCME, 1999b) which are based on strong acid-extracted metal concentrations in the sediment, which would include both freely-dissolved metals and residually bound (biologically unavailable) metals. Metal toxicity can be greatly influenced by the presence of sulfides and oxyhydroxides in the pore waters (Ankley et al., 1991; Fortin et al., 1993). Equilibrium partitioning models based on acid-volatile sulfide normalizations are limited because they often result in an underestimation of the toxicity of metal-contaminated sediments (Simpson and Batley, 2007). The exposure route of benthic organisms to trace metals in the sediment is assumed to be principally through pore water. As well, the natural release of sediment contaminants to overlying waters is controlled by the concentration of metals in pore waters. Thus, water quality guideline values can be used to predict sediment metal bioavailability to aquatic organisms, and the potential risk sediment contamination poses to overlying waters (Giesy et al., 1990; Michaud et al., 2005).

2.4. Macrophyte and invertebrate sample collection

Individual samples of submerged vegetation (*Chara* spp.) were hand-pulled from each microcosm. All invertebrates and detritus were removed from plants by hand-picking. Invertebrates were rinsed with distilled water, blotted on paper towel and frozen at −20 °C, in August 2005. The surface sediment layer was sieved (mesh size 500 μm) and live invertebrates retained on the sieve were removed, identified, rinsed with distilled water and frozen at −20°C. Lymnaeid snails (shells removed), aeshnid dragonfly nymphs and larval chironomids (Tribe Chironomini) were present in sufficient quantities in microcosms for chemical analysis. Each invertebrate sample was a composite of many animals from one treatment, which were stored in acid-washed, 7-mL high-density polyethylene vials.

2.5. Analytical methods

Two to three grams of tissue were added to a 50-mL glass beaker along with 5 mL of equal parts ultra-trace grade sulfuric (12 M) and nitric acid (16 M) and allowed to digest for 10 min. The beaker was subsequently placed on a hot plate and the temperature increased to 120 °C over a 2-h period. After cooling, another 5 mL of 16 M HNO_3 was added and the sample was re-heated to 120 °C for 3 h after which the sample was cooled, amended with another 5 mL of 16 M HNO_3, covered with a watchglass, and heated to 120 °C for 12–16 h. After cooling to room temperature, 15 mL of 30% hydrogen peroxide was added to the beaker and the heat was gradually increased to 120 °C over several hours, care being taken to prevent sample boil over, until the digesta became clear and colorless. The digesta was subsequently transferred to a pre-weighed 125-mL low-density polyethylene bottle and made up to 100 g by weight with purified water. Pore-waters were acidified to 2% HNO_3 solution after collection from sediments and stored at 4 °C until analysis.

Samples were analyzed by a Thermo X Series II Inductively Coupled Plasma Mass Spectrometer (ICP-MS) at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory. Technique validation and laboratory performance were monitored by digesting two in-house tissue reference pools (Detroit River fish and caribou liver/kidney homogenate), certified biological reference materials (Dorm-2, Luts-1 and Dolt-2, (NIST, Gaithersburg, MD, USA)) and three method blanks for each batch of 25 samples analyzed. In addition, two randomly selected samples from each sample batch were digested in duplicate to provide an estimate of laboratory precision (recoveries were within ± 10%). Mass bias corrections were made using three in-house elemental standards (Be, In, and Tl).

2.6. Statistical analyses

Concentrations of elements that were lower than the method detection limits were assigned a value that was one-half of the detection limit (Clarke, 1998). All trace metal concentrations were $\log_{10(x+1)}$ transformed prior to analysis. Multivariate analyses were performed using CANOCO™ v4.56 software (Microcomputer Power, Ithaca, NY, USA). Each analysis was performed using only metals for which at least 50% of the sample values were above the analytical detection limits. A detrended canonical correspondence analysis (DCCA) was initially performed on each data set (pore water and algae) to determine whether unimodal or linear ordination methods were most appropriate. The longest gradient length for the pore water and algae data sets were 0.2 and 0.3, respectively, which are less than 4.0, indicating that the data fit a linear response model (Lepš and Šmilauer, 2003). Thus, a redundancy analysis (RDA) was used on each data set to determine how much of the variability in the trace metal concentrations was explained by the microcosm sediment treatments (the independent variables). RDA is a linear, direct gradient, multivariate analysis, producing canonical ordination axes that represent the variability in trace metal concentrations explained by the independent variables, and these independent variables can be correlated with the ordination axes (Lepš and Šmilauer, 2003). It is a multivariate analog of a regression analysis. Supplementary samples collected from BP and USC were not included in the model analysis; they were only plotted on the ordination diagrams for comparison purposes. Only the first two ordination axes were analyzed, as there can be significant distortion in successive ordination axes. Monte Carlo permutations (999 permutations) were used to test the likelihood-ratio of each of the ordination models against the null hypothesis that states that trace metal concentrations were not affected by the sediment treatments. This procedure produces an F-ratio (compared to the Wilks' lambda distribution) that is a multivariate counterpart of the traditional univariate F-ratio, at the critical p-value ≤ 0.05.

3. Results

3.1. Trace metal analysis of microcosm pore water

Generally, all surface sediment pore waters collected had low redox (Eh) potentials and neutral pH values (Table 1). When compared to Canadian water quality guidelines for the protection of aquatic life (CCME, 1999a) pore water concentrations of As, Cd, Cr, Cu, Mn, and Mo in all sediment treatments (including the native SWSD sediment) exceeded the guideline levels. Surface pore-waters collected from microcosms with a petroleum coke cap had higher concentrations of many metals including Ni, and lower concentrations of La and Y than all other microcosm sediments lacking coke amendments (Table 1). Vanadium was not detectable in these samples using this analytical method.

Redundancy analysis was performed on pore water concentrations of metals from all microcosm sediment samples taken from the four surface sediment treatments only (native n = 2, consolidated tailings n = 2, mature fine tailings n = 2 or petroleum coke n = 6). The sediment treatments explained 25% of the total variation in the trace metal concentrations in sediment pore water (the first and second axes constrained to the sediment treatment classes explained 22% and 3% of the total variation in the metal concentrations, respectively) (Fig. 2a).

The variation in the concentrations of Cd, Fe and Zn in pore water was not affected by the surface sediment treatments (i.e., trace metal species having very short vectors in the ordination space are not well explained by the sediment treatments). SWSD native wetland sediment, mature fine tailings sediment, and Syncrude petroleum coke had pronounced differences in the trace metals with which they were correlated (i.e., trace element vectors that point directly at, or directly away from a sediment treatment centroid, represent elements whose variability is controlled by the presence of that sediment treatment). Native sediments had the highest concentrations of Co, K and Mo, both types of fine tailings sediments had the highest concentrations of La, Y, and lowest concentrations of Cr, and petroleum coke had the lowest concentrations of Ba, Na, and Ga, and the highest concentration of Ni (Table 1). The two types of tailings sediments, mature fine tailings and consolidated tailings, were indistinguishable from each other in the ordination of the trace element concentrations in their pore water. The consolidated tailings treatment had high concentrations of Pb at 54.4 μg/L, greatly exceeding the Canadian Water Quality Guideline (CWQG) level of 1–7 μg/L (CCME, 1999a). All sediment treatment pore waters exceeded the CWQG for As, including SWSD native sediments and BP sediments.

Petroleum coke treatments had the largest influence on variability in the trace metal concentrations in pore water, as it had the highest negative loading on the first axis, with a correlation of −0.998. Native sediment was most highly correlated with axis 2 (+0.873, with the strongest correlation possible = 1.0) (Fig. 2a). However, we could not detect a statistically significant effect of any sediment treatment classes on the concentrations of trace metals in pore water extracted from those treatment sediments. Monte Carlo permutations of the first axis (which was most strongly correlated with coke treatment sediments) resulted in a p-value of 0.39, indicating that we could not detect an effect of petroleum coke treatments on pore water elemental concentrations. Permutations of all axes together had a p-value of 0.54.

Centroids (in gray, Fig. 2) of the samples collected from BP and USC native sediments were plotted as supplementary samples on in the RDA biplot, to illustrate the dissimilarity in metal concentrations to those in the microcosms, although these two reference wetlands did not contribute to the overall model. Both centroids were located on the positive side of the second RDA axis, indicating that the pore water metal concentrations were more similar to SWSD native sediment, than they were to petroleum coke; but concentrations of

Table 1
Trace metal concentrations (mg/L) measured in pore water extracted from the microcosm surface sediment treatments (CT = consolidated tailings, MFT = mature fine tailings, Native = SWSD native sediment), Beaver Pond (BP) and U-Shaped Cell (USC) sediment. CWQG = Canadian Water Quality Guidelines for the protection of aquatic life (CCME, 1999a).

Element	Det. Lim.	CWQG	Coke (±SE)	CT (±SE)	MFT (±SE)	Native (±SE)	BP (±SE)	USC (±SE)
As	0.0009	0.005	0.0134 (0.0047)	0.0311 (0.0233)	0.0071 (0.0004)	0.0119 (0.0034)	0.0071 (0.0016)	0.0866 (0.0587)
Ba	0.00004	n/a	*__0.05357 (0.00996)__*[a]	0.07653 (0.03255)	0.0793 (0.02946)	0.09798 (0.00855)	0.09116 (0.03997)	0.1006 (0.03698)
Ca	0.0288	n/a	37.02 (7.28)	25.13 (1.98)	26.31 (0.82)	35.5 (12.32)	113.43 (49.03)	35.68 (5.47)
Cd	0.00001	0.00002	0.00018 (0.00011)	0.00017 (0.00009)	0.00008 (0.00001)	0.00014 (0.00004)	0.0001 (0.0000)	0.0005 (0.0003)
Co	0.00005	n/a	0.00052 (0.00011)	0.00062 (0.00041)	0.00039 (0.00006)	**0.00108 (0.00032)**	0.00104 (0.00036)	0.00107 (0.00018)
Cr	0.0014	0.0089	0.0475 (0.0084)	*__0.0053 (0.0018)__*	*__0.0034 (0.0015)__*	0.0726 (0.058)	0.005 (0.0006)	0.0503 (0.0305)
Cu	0.0003	0.002–0.004	0.0026 (0.0011)	0.0081 (0.0071)	0.0013 (0.0004)	0.0024 (0.0000)	0.0022 (0.0005)	0.0226 (0.0117)
Fe	0.0449	0.3	0.413 (0.0738)	0.3978 (0.0599)	0.3504 (0.1057)	0.3514 (0.1007)	2.0423 (1.0816)	0.6181 (0.2339)
Ga	0.0002	n/a	*__0.0072 (0.0013)__*	0.0101 (0.0039)	0.0106 (0.0038)	0.0131 (0.0014)	0.0116 (0.0048)	0.0138 (0.005)
K	0.23	n/a	6.55 (1.11)	6.85 (0.45)	5.96 (0.76)	**9.72 (1.85)**	110.27 (95.26)	526.84 (468.92)
La	0.00002	n/a	0.00004 (0.00001)	**0.00066 (0.00009)**	**0.00014 (0.00013)**	0.00008 (0.00007)	0.0002 (0.0001)	0.0001 (0.00005)
Mg	0.01	n/a	18.68 (1.96)	14.91 (1.22)	17.21 (2.55)	21.2 (7.82)	46.31 (14.98)	7.71 (0.52)
Mn	0.0002	n/a	**0.3598 (0.1363)**[b]	0.1515 (0.0545)	0.2035 (0.0522)	0.2113 (0.1242)	0.0022 (0.0007)	0.0094 (0.0023)
Mo	0.00003	0.073	0.01097 (0.00352)	0.00632 (0.00057)	0.00463 (0.00142)	**0.03224 (0.02262)**	1.61365 (0.76167)	0.2093 (0.08954)
Na	0.48	n/a	*__20.49 (3.93)__*	62.05 (45.88)	48.86 (19.69)	45.5 (2.13)	89.7 (17.92)	38.92 (7.6)
Ni	0.0017	0.025–0.150	**0.009 (0.0031)**	0.0051 (0.0028)	0.0032 (0.0001)	0.0055 (0.0025)	0.0045 (0.0009)	0.0111 (0.0027)
Pb	0.0001	0.001–0.007	0.0013 (0.0004)	0.0544 (0.0535)	0.0007 (0.0004)	0.0016 (0.0001)	0.0017 (0.0012)	0.0083 (0.0048)
Rb	0.0001	n/a	0.0014 (0.0005)	0.003 (0.0017)	0.0019 (0.0015)	0.0037 (0.0026)	0.0063 (0.0042)	0.0213 (0.0162)
Sr	0.0006	n/a	0.2499 (0.0411)	0.2652 (0.0682)	0.2651 (0.0593)	0.3314 (0.0125)	0.6125 (0.2497)	0.3356 (0.0561)
Y	2.45×10^{-6}	n/a	6.23×10^{-5} (1.50×10^{-5})	$\mathbf{3.05 \times 10^{-4}}$ $\mathbf{(6.66 \times 10^{-5})}$	$\mathbf{9.75 \times 10^{-5}}$ $\mathbf{(6.18 \times 10^{-5})}$	7.99×10^{-5} (5.91×10^{-5})	1.60×10^{-4} (6.91×10^{-5})	7.88×10^{-5} (4.19×10^{-5})
Zn	0.0003	0.03	0.0337 (0.0103)	0.0466 (0.0188)	0.0094 (0.0005)	0.0292 (0.0135)	0.0156 (0.0038)	0.1489 (0.0936)
pH			7.54	7.87	7.47	7.71	7.09	7.68
Eh (mV)			−94.9	153.1	−88.4	−109.1	−36.7	−129

[a] Bold-faced and italicized metal values indicate a strong negative correlation (-0.70) with that treatment sediment.
[b] Bold-faced values indicate a strong (0.70) correlation with that treatment sediment.
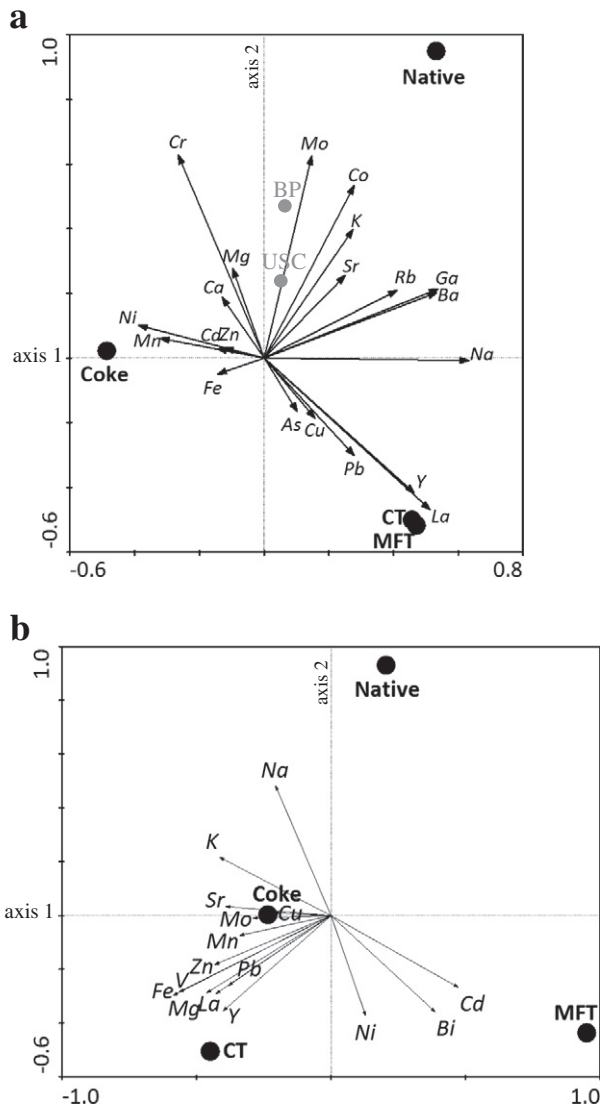
Fig. 2. RDA ordination diagrams of metal concentrations of a) sediment pore water and b) *Chara* spp. as described by the sediment treatment classes of the experimental microcosms.

trace metals from both supplementary wetlands were generally quite variable. These supplementary samples were also not included in the Monte Carlo permutation model and F-test. Of note is the difference in the concentrations of Y, Fe and Na between BP sediment pore water and all other sampled sediment types (Table 1), including USC. BP was the only site in this study that was not constructed by oil sands operators, and its pore waters had a concentration of Y that was 2–4 fold lower than any other sediment type. Additionally, both Fe and Na concentrations were significantly higher in BP pore waters than in the sediments from constructed wetlands and mining waste products.

A partial-RDA was used to test for the interactions between the base sediment treatments (native sediment, consolidated tailings or mature fine tailings) and surface sediment treatments (coke presence or absence), on the concentrations of trace metals from surface pore water (*i.e.*, did surface trace metal concentrations in surface sediments depend on the base sediment type?). The pore water metal concentrations in any type of surface sediment were not significantly influenced by the type of base material used underneath it. The first ordination axis (the only canonical axis) was a very good representation of the interaction term between base and surface treatments, where the interaction term was 100% correlated with this axis. However, this axis explained only 2% of the total variation in trace metal

concentrations in the surface sediments, and Monte Carlo permutations gave a p-value of 0.83 in the F-test. In other words, the P-RDA was able to create a good model of the interaction term of base and surface sediment metal concentrations, but the concentration of metals in surface sediments was not influenced by the type of base sediment in the treatment.

3.2. Concentration of trace metals in the alga Chara

The macrophytic green alga *Chara* spp. was the only plant species present in each microcosm in sufficient amounts to be used for chemical analyses. Tissues of *Chara* spp. collected from the microcosms containing consolidated tailings had higher concentrations of both La and Y (Fig. 3), than were seen in any other treatment. *Chara* spp. growing in petroleum coke and consolidated tailings treatments (which are partly created using residuals of the burning of coke) both had approximately three times higher V concentrations than algae in the other two sediment treatments. However, the difference in V concentrations among algal tissues from any of the four sediment treatments was not statistically significant. *Chara* spp. grown in petroleum coke, and both types of tailings had about 1.5 times the concentrations of Ni than was observed in the SWSD native sediment treatment.

Redundancy analysis was performed on trace metal concentrations measured in *Chara* spp. tissues (n = 4 for each surface treatment). Mature fine tailings treatments and SWSD native sediment treatments had the greatest impact on metal concentrations in *Chara* spp., although these effects were not strong (*i.e.*, mature fine tailings treatment showed the closest correlation with the first canonical axis (+0.587), and native sediment treatments were correlated with axis 2 (+0.670). The first and second ordination axes explained 17.7% and 8.4% of the variation in the trace metal concentrations) (Fig. 2b). Petroleum coke and consolidated tailings treatments had little effect on the total variation in metal concentrations observed in *Chara* spp. We could not detect a statistically significant difference in the trace metal concentrations in *Chara* spp. tissues that could be attributed to association with different microcosm sediment treatments (Monte Carlo permutations of the first axis resulted in a p-value of 0.52, all axes together had a p-value of 0.41).

The surface sediment treatments had the greatest influence on the concentrations of Bi, Cd, Cr, Ca, Fe, La, Mg, Na, V, and Y found in *Chara* spp. tissues (Fig. 2b). Algal tissues collected from mature fine tailings treatments were most closely correlated with increased concentrations of the metals Bi, Cd, Cr, and decreased concentrations of Na. Coke treatments were most strongly correlated with increased concentrations of Cu, Mo and Sr. Consolidated tailings were best described by increased concentrations of La and Y in *Chara* spp.

A partial-RDA was used to test for the interactions between the base sediment treatments (native sediment, consolidated tailings or mature fine tailings) and surface sediment treatments (coke presence or absence), on the concentrations of trace metals in *Chara* spp. tissues. The type of base material located beneath the surface treatment had no impact on the metal concentrations in *Chara* spp. tissues. The first axis generated by the p-RDA was moderately representative of the interaction between base and surface treatments, but did not significantly describe the variation in the concentrations of trace metals in *Chara*. The first ordination axis (the only canonical axis) represented 8.7% of the variation in the trace metal concentrations observed in *Chara* spp., and the interaction term was only 50.0% correlated with this axis. The Monte Carlo permutation test gave a p-value of 0.38 for the first axis.

3.3. Concentration of trace metals in macroinvertebrates

Trace metal concentrations were measured in macroinvertebrate tissues collected from the surface of the microcosms in SWSD.
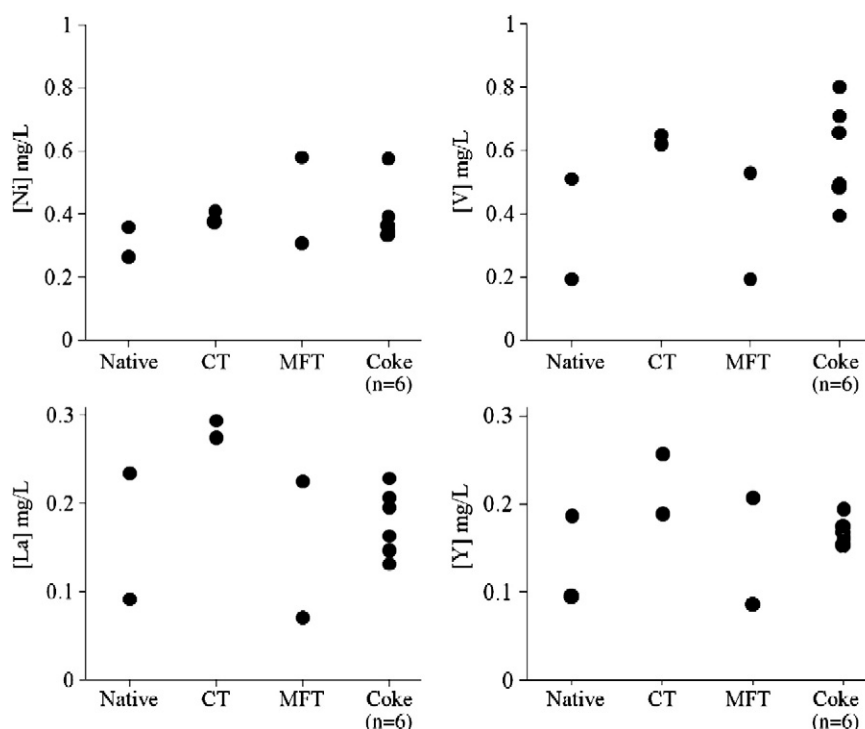
Fig. 3. Individual value plots of focal metal concentrations in *Chara* spp. tissues collected from each surface sediment treatment in the microcosm experiment. Sample sizes have been indicated where points overlap; please note the different scales on axes.

However, we lacked the replication necessary to conduct statistical analyses on the data. Three types of invertebrates were most frequently collected; snails (Gastropoda: Lymnaeidae), dragonflies (Odonata: Aeshnidae) and larval chironomids (Diptera: Chironomidae: Chironomini). In BP, only lymnaeid snails were collected in quantities sufficient for chemical analysis. No invertebrates were collected in USC. Each microcosm unit was relatively small, and this limited our ability to collect sufficient invertebrate tissue biomass from all treatments. Only a single, composite sample of each invertebrate type was available for each sediment treatment except petroleum coke treatments (n = 4).

Of the four focal metals, only V was found to be elevated in aeshnid dragonflies collected from microcosms containing petroleum coke (Fig. 4). La and Y concentrations were higher in the dragonfly larvae recovered from the petroleum coke and one replicate of the consolidated tailings sediment treatment than from the mature fine tailings and native sediment treatments. Chironomids generally had the highest mean concentrations of every metal measured compared to the other two taxa, including very high concentrations of V (913.1 ± 236.3 mg/L). However, this could be an analytical artifact related to the very small sample volumes available for analysis for this animal. The concentrations of metals in chironomids collected from the SWSD native sediment treatments were quite similar to those observed in chironomids from the consolidated tailings and petroleum coke treatments. The concentrations of metals in tissues of snails from SWSD native sediment treatments were consistently higher than those observed in snails collected from BP (Fig. 4).

4. Discussion

4.1. Trace elemental analysis of pore water

The pH of water can affect the valence state and speciation of metals. These will control the element's ionic mobility and bioavailability. Higher acidity increases the availability of free metal ions (Giesy, 1987). All extracted pore waters were generally neutral to somewhat basic (Table 1). Under these conditions, the availability of metals in these sediments should be lower than expected in an acidic environment. Water chemistry data confirmed that the porewaters extracted from both natural sediments and oil sands tailings materials located on the Syncrude lease contain concentrations of many trace metals that exceed Canadian water quality guideline critical concentrations, such as As, Cd, Cr, Cu, Mn, and Mo (Table 1).

Y and La were not found in higher concentrations in the pore waters of petroleum coke treatments than in other sediments, as was expected. Both of these elements were found in higher concentrations in mature fine tailings and in particular, consolidated tailings, than in other treatments. Rare earth elements such as these occur naturally in the earth's crust but tend to be associated with clay minerals (Laveuf and Cornu, 2009). This may explain their presence in both types of fine fluid tailings, which are composed of mainly fluidized clay. They likely occur in particularly higher concentrations in consolidated tailings because this product is formed by compacting (concentrating) mature fine tailings. Note that although the concentrations of La were higher in the pore water of the consolidated tailings, these levels were far below what has been shown to cause toxicity in lab toxicity tests on an aquatic species (Barry and Meehan, 2000). We postulate that the consolidated tailings may also contain elevated concentrations of Ni and V because the gypsum used to flocculate this sediment is acquired from end-products of desulfurization of gases produced by burning petroleum coke by Suncor Energy, Ltd. This process utilizes some crushed coke and calcium carbonate as a catalyst for producing gypsum, and can lead to minor contamination of this product with elements also associated with the coke itself (Kappe and Ellison, 1986).

The coke supplied for this project was taken from a weathered stockpile, and trace metal samples were collected three years after the initial installation of the experimental microcosms, so it is possible that these metals had been leached from the coke, or partitioned out by other mechanisms (precipitation, adsorption) prior to sample
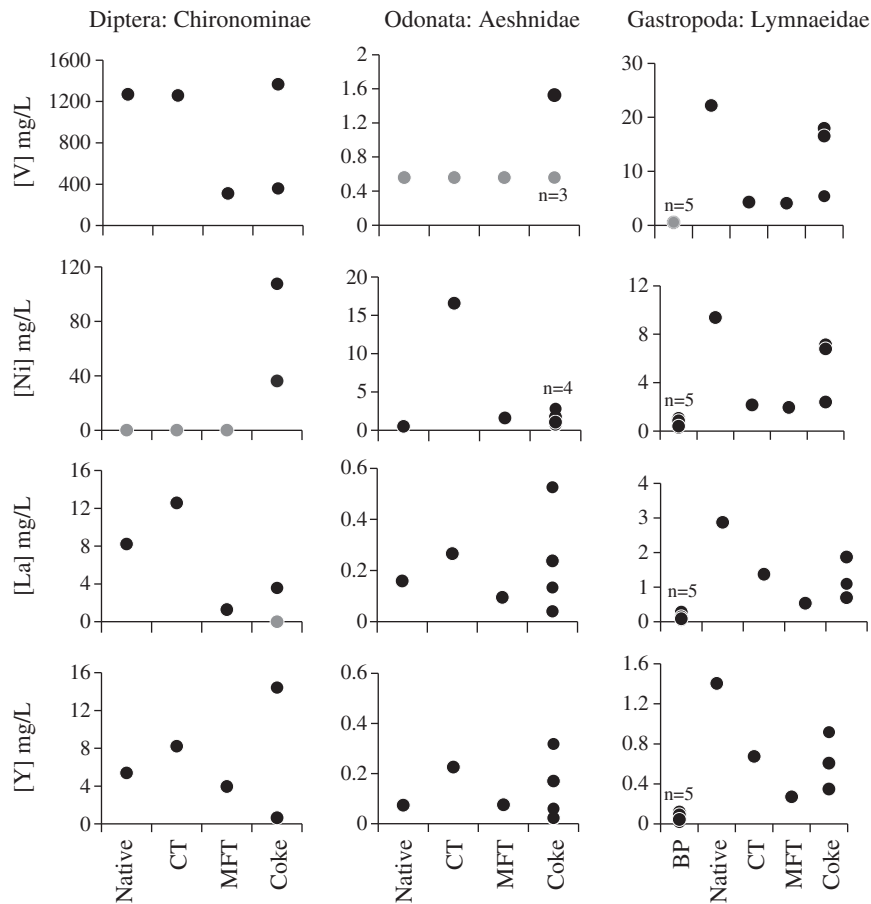
Fig. 4. Individual value plots of the concentrations of focal metals in benthic invertebrates (Chironominae, Aeshnidae, and Lymnaeidae) collected from the surface sediment treatments of the microcosms, and from Beaver Pond (BP). Where points overlap, the number of points is indicated. Gray points indicate samples for which metal concentrations were below detection limits. Note that axis scales are different for each graph.

collection. The grain size of the petroleum coke is similar to sand, and this may increase the rate of flushing of elements from this treatment, compared to the finer particle size and higher metal binding capacity of the other clay-based sediments. The highest concentration of Ni was observed in the coke pore waters (9.0 μg/L), almost double the concentration found any other type of sediment. Puttaswamy et al. (2010) found similar concentrations of Ni in pore water leachates from Syncrude's petroleum coke stockpiles as we did, and this concentration was acutely toxic to the cladoceran *Ceriodaphnia dubia* and contributed most of the toxicity associated with petroleum coke leachates. The elevated Ni concentrations in pore water of petroleum coke could pose a risk to aquatic invertebrate species.

In comparison to pore waters of natural freshwater wetlands in Alabama characterized by Donahoe and Liu (1998), we found higher concentrations of many elements in the pore waters of the microcosm sediment treatments (Ca, Mg, Mn, Na). Fe concentrations were much lower in all of the microcosm treatment sediments than in the natural Alabama wetland. Pore water concentrations of elements, except Na, are strongly controlled by adsorption to iron oxyhydroxides dissolved in the pore water (Fortin et al., 1993), and the lower concentration of Fe in the pore water could explain the higher concentration of other trace elements in these treatment sediments than was observed in BP wetland sediments. The most striking difference between the pore waters of any of the constructed wetlands sampled or oil sands waste products and those of BP was in the concentration of Y. In this sense, the higher concentration of Y in oil sands sediments could to be related to the mining activities.

Although the sediment treatments in microcosms did not significantly differ in the total of their trace metal concentrations, this

does not preclude closer evaluation of individual metals of interest. Concentrations of trace metals in pore water would only represent those metals that would be considered bioavailable. The sample sizes are likely too small to detect more subtle differences; sample sizes were small due to logistic constraints. We had expected to see larger differences in metals, because we had anticipated that the native SWSD sediments would be much lower in background element concentrations. The elevated concentrations of metals in native sediments meant that treatment sediments (consolidated tailings, mature fine tailings and coke) did not appear to be significantly different in terms of total metal concentration, but we note that many elements appear in fairly high concentrations, many exceeding Canadian water quality guideline levels. A retrospective power analysis of the detectable difference between the SWSD native sediment metals and the petroleum coke metals showed that a difference of ±158% or more would have to be observed to result rejection of the null hypothesis. Concentrations of Cd, Fe and Zn were the least affected by the sediment amendments.

4.2. Trace elemental analysis of algae

The variation in trace metal concentrations in *Chara* spp. tissues collected from surface of the experimental microcosms was not well correlated to the sediment treatments from which they were recovered. The concentrations of trace elements in *Chara* spp. samples may not be influenced by the sediment composition of the microcosms from which they were harvested. As a non-vascular, macrophytic alga, *Chara* spp. lacks a water transport system as well as true leaves, stems and roots (Forsberg, 1965). *Chara vulgaris* can

take up nutrients from its rhizoids and transfer them to other parts of the plant (Vouk, 1929). However, trace metals are less soluble than nutrients and may be less available for transport from surface sediments. Even though several trace metal concentrations in the pore water exceeded CCME water quality guidelines, this apparently did not influence the tissue metal concentrations of the alga. Although data were variable and differences were not statistically significant, *Chara* spp. tissues taken from petroleum coke and consolidated tailings treatments had V concentrations that averaged about 3 times higher than in the algae analysed from native or mature fine tailings treatments in the experimental microcosms. Despite lacking a true vascular system, some brown algae species have bioaccumulated V, after exposure to an oil spill (Villares et al., 2007). The concentration of Ni in *Chara* spp. tissues from the three mine waste materials was about 1.5 times greater than concentrations in native sediment treatments. Vanadium tends to be strongly associated with $CaSO_4$ (gypsum) in the petroleum coke (Jia et al., 2002). Gypsum added to the fresh mine tailings to create consolidated tailings was purchased from Suncor Energy, Inc. The elevated concentrations of V, Ni, La and Y, typically associated with the gypsum may have had its source in Suncor's coke, which is used as a catalyst during the production of gypsum (Hall et al., 1982). Elevated concentrations of Ni and V in *Chara* spp. tissues may be an indication of higher concentrations of these elements in all mine process materials used in this experiment.

4.3. Trace elemental analysis of macroinvertebrates

Aeshnidae dragonfly larvae are epibenthic, or plant-associated predators that feed on zooplankton, amphipods and chironomids (Folsom and Collins, 1984). Median concentrations of Ni were higher in Aeshindae than in snails. Where detectable concentrations of V were observed, they were associated only with the petroleum coke treatment. La and Y concentrations were higher in dragonfly larvae from coke sediment treatments than in larvae from non-coke treatments. Aeshnids are large, territorial, "sit-and-wait" predators (Corbet, 1957), and this tends to produce an even spatial distribution (Vanbuskirk, 1993), so these predators may be feeding mainly in and around the area where they were captured. They could have accumulated their elemental burdens from infauna associated with the experimental microcosms.

Chironomids generally had the highest mean concentrations of every metal measured compared to the other two invertebrates, including very high concentrations of V. Living directly in the sediments, chironomids are more likely to absorb metals through direct contact (Hare et al., 2003) than the other taxa. The larvae of Chironomini typically construct burrows within the surface sediments and consume mainly organic particles, detritus and algae, and feed on small worms (Loden, 1974).

Lymnaeid snails, which feed on plant tissues and epiphytic biofilms (Clarke, 1981) tend to be associated with aquatic plants. The concentrations of metals in tissues of snails from all Shallow Wetland South Ditch microcosms were consistently higher than those observed in snails collected from the natural Beaver Pond. Contrary to this finding, sediment from SWSD microcosm sediments had lower or similar concentrations of most metals than sediment from BP. However, BP also had the highest concentration of Fe in the pore water. Decomposition of the abundant organic matter in the sediment of BP (per. obs.) would consume free oxygen and lead to strongly reduced conditions, producing more dissolved Fe in the pore water (Donahoe and Liu, 1998). There is likely to be a greater concentration of iron oxyhydroxides present in the water, which are a strong complexing agent for most trace metals, making them less bioavailable (Donahoe and Liu, 1998). The surface water of BP was tea-coloured, indicative of the presence of dissolved organic acids (humic and fulvic acids associated with muskeg waters), while SWSD has little colour and low concentrations of organic acids (naphthenic acid

concentrations of <2 mg/L and dissolved organic carbon concentration of 20 mg/L). The detrital and dissolved organic matter in BP could act as natural ligands for metals, thus limiting their bioavailability. The metals found in BP are likely less bioavailable than those of SWSD. This suggests that reclaimed wetlands containing oil sands tailings or petroleum coke could also be amended with decomposing organic materials to produce anoxic conditions and higher concentrations of Fe oxyhydroxides as a protective measure for wetland biota, against higher concentrations of trace elements, but this hypothesis needs to be tested. The addition of peat and other organic materials to sediments is already recommended as a standard component of oil sands wetland reclamation (Harris, 2007).

5. Conclusions

The concentrations of several trace elements in pore water from all microcosm experimental sediments were found to exceed Canadian water quality guidelines for the protection of aquatic life in surface waters. Ni concentrations in the pore water of microcosms containing petroleum coke treatments were almost double those found in any other treatment sediment, at a concentration that is toxic to some invertebrate species. *Chara* spp. and benthic invertebrate taxa collected from microcosms containing petroleum coke or consolidated tailings sediment may have accumulated some nickel, vanadium, lanthanum and yttrium. These elements have been found in elevated concentrations in petroleum coke by other studies. La and Y were found in higher concentrations in both types of fluid tailings, probably due to their clay-based nature. They likely occur in even higher concentrations in consolidated tailings because this product is formed through the compaction of mature fine tailings. We postulate that the consolidated tailings may also contain elevated concentrations of metals because the gypsum used to agglutinate this sediment is acquired from the flue gas desulfurization of burned petroleum coke. Statistical comparisons of the concentrations of elements in pore waters of petroleum treatments to the native sediments of SWSD that were used as the baseline control sediments in the microcosms were confounded by unexpectedly higher in trace metal concentrations in SWSD sediments; in most cases exceeding available CWQG for the protection of aquatic life. This could potentially be the result of atmospheric deposition of particulate matter from nearby upgrading facilities on site. Aeshnid dragonfly larvae could be accumulating V from the petroleum coke treatments, but these levels were low. Chironomini larvae showed higher but variable metal concentrations in all treatments, likely not as a direct result of contact with the mine waste sediments. Tissues of lymnaied snails collected from the nearby natural wetland, Beaver Pond, harbored consistently lower trace elemental concentrations than those collected from Shallow Wetland South Ditch microcosms, and this is likely due to the intrinsic differences between these two wetlands. The use of petroleum coke as a surface sediment amendment in constructed wetlands in the Athabasca oil sands could pose some risk to wetland biota and warrants further evaluation on a larger scale.

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