A NOVEL APPROACH TO ASSESSING THE CAUSE OF IMPACTS ON BENTHIC MACROINVERTEBRATE COMMUNITIES, AND DISTINGUISHING BETWEEN SEDIMENT VS WATERBORNE EFFECTS DOWNSTREAM OF A METAL MINING DISCHARGE

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Hudbay has owned and operated a base metal mine and metallurgical complex in the Flin Flon area of northwestern Manitoba for over 90 years. Zinc and copper ores are processed from local mines and concentrates from external sources. The Flin Flon tailings impoundment system (FFTIS) receives input from several processes, such as mill effluent waste, dewatering from underground workings, surface runoff and storm water drainage, and runoff from the town of Creighton. FFTIS effluent is discharged into Flin Flon Creek, where it comprises approximately 70% of the creek volume. The effluent concentration decreases as it flows into the Northwest Arm of Schist Lake, where it is approximately 30%.

Benthic invertebrate community surveys downstream of the FFTIS have shown that benthic invertebrate communities in Flin Flon Creek are severely impacted and dominated by a single pollution-tolerant species: the midge larva *Chironomus* (Stantec 2005, 2007, 2009, 2011). In the NW Arm of Schist Lake, benthic communities are also impacted but not to the same extent.

An integrated assessment of multiple lines of evidence including toxicity testing and modeling approaches was carried out to assess metal bioavailability and causes of toxicity on benthic invertebrate communities. Assessment of Flin Flon Creek and the NW Arm of Schist included extensive water and sediment chemistry, assessment of simultaneously extracted metals/acid volatile sulfides (SEM/AVS), and benthic community composition. A novel approach to toxicity testing was conducted in the field and laboratory using *Hyalella azteca* (*Hyalella*), and bioaccumulation of metals in all surviving test organisms was measured. In addition, wild chronically exposed *Hyalella* were collected for metals analysis from both areas. Finally, the Metal Effects Addition Model (MEAM) and Biotic Ligand Models (BLM) were applied to assess metal speciation and predict toxicity to daphnids and fish.

In the Flin Flon Creek exposure area, aluminum exceeded the CCME WQG 60% of the time and cadmium, copper, iron, selenium and zinc exceeded the CCME WQGs 100%. In the NW Arm of Schist Lake, cadmium, copper, iron and zinc were also above guideline values 26% to 59% of the time, and selenium 100%. Relative to background, selenium concentrations were highest followed by copper and cadmium. For reference, Table 1 shows the water quality guidelines and results.

The biotic ligand model was used to predict the toxicity (LC50) of copper, zinc, and cadmium for fish and daphnids under site-specific conditions in Flin Flon Creek and the NW Arm of Schist Lake. Overall, metal toxicity to fish and daphnids decreased in the order Cu>Zn>Cd. Comparison of predicted LC50 values to site concentrations, along with long term pH values in Flin Flon Creek, indicated that daphnids could be affected by copper, especially at lower pH levels in Flin Flon Creek. Metal speciation confirmed that the observed cadmium and zinc concentrations are unlikely to be the cause of acute toxicity in Flin Flon Creek, whereas it appears that copper could at times be bioavailable, particularly at low pHs. Farther downstream in Schist Lake, the model did not predict Cu, Zn or Cd toxicity to fish or daphnids.

Metals in sediment followed a similar trend to that observed in water. Most of the guideline exceedances occurred in Flin Flon Creek, where CCME Interim Sediment Quality Guideline Probable Effect Levels (ISQG PEL) for As, Cd, Cr, Cu, Pb, Hg, and Zn were exceeded. In the NW Arm of Schist Lake, 90% of the samples exceeded the ISQG PEL for cadmium, and 100% of the samples exceeded the ISQG PEL for zinc. Copper, arsenic, and mercury concentrations were also higher than the ISQG, but not above the PEL, with exceedences occuring 81%, 29%, and 29% of the time, respectively. All metals in the sediment from the reference area were below the ISQG PEL. For reference, Table 2 shows the water quality guidelines and results.

Sediment core samples were collected from exposure and reference areas to determine the SEM/AVS ratios and further assess whether specific free metals (Ag, Cd, Cu, Ni, Pb, and Zn) are bioavailable in pore water at sufficient levels to cause toxicity or be linked to metal bioaccumulation in biota. The SEM/AVS molar ratios can be used to estimate sediment porewater concentrations of divalent metals and provide a better indication of sediment toxicity than total metals analyses on bulk sediment (DeWitt et al. 1996; Hansen et al. 1996a, 1996b).

The $\text{SEM}^*_{x,OC}$ values for cadmium, copper, nickel, zinc, and lead was calculated according to Di Toro et al. (2005), and were normalized for organic carbon, which was very high in Flin Flon Creek. A threshold value of >100 µmol/g was used to predict whether metal-toxicity might be expected. The range of $\text{SEM}^*_{x,OC}$ in Flin Flon Creek was 430 to 988 µmol/g C-org, and Schist Lake ranged from 13 to 219 µmol/g C-org with three of five samples being greater than the threshold value of 100 µmol/g. Based on SEM/AVS results, zinc was identified as the predominant metal contributing to sediment toxicity in both areas. Redox values in Flin Flon Creek ranged from -153 to -278 mV and from -205 to -236 mV in Schist Lake indicating that the sediment in both areas is anoxic and highly reduced.

To distinguish between sediment and waterborne toxicity, a novel experimental design using slightly modified standard sediment toxicity test methods for *Hyalella azteca* was carried out in the laboratory under controlled conditions and in the field, thereby integrating the complexity of receiving environment. In the laboratory, water only tests were also conducted (in addition to sediment toxicity tests) with receiving water to further help separate sediment vs waterborne toxicity.

Exceedances of water and sediment quality guidelines provided an initial screening tool for evaluating toxicity in the receiving environment, as do models, but they do not conclusively demonstrate that a metal is causing adverse effects in aquatic biota. Guidelines are based on laboratory data, with safety factors applied to the most protective toxicity test results. In contrast, field conditions that affect metal bioavailability and toxicity are far more complex. In the natural environment, metals also exist within a mixture. Table 3 provides and overview of the factorial experimental design that was implemented, showing each treatment (i.e. how different types of overlying water were combined with the various sediment samples collected from the same location). At the end of the toxicity tests, surviving organisms from each treatment (and wild *Hyallela*) were analyzed for metals and used as inputs to the metal effects addition model (MEAM).

Sediment from Flin Flon Creek resulted in complete (100%) mortality of *Hyallela* in lab toxicity test exposures under controlled conditions. *Hyallela* did survive test exposures with reference or lab sediment and overlying Flin Flon Creek water, and actual survival (76 to 94%) was better than predicted (16% to 23%). However, had exposures been prolonged, mortality would have likely increased given that growth was significantly reduced, and both copper and selenium exceeded their LBC25s (Table 3). The *in situ* caged *Hyalella* toxicity tests deployed in Flin Flon Creek resulted in 100% mortality, with or without Flin Flon Creek sediment.

Survival of *Hyalella* in aqueous-only toxicity tests with Flin Flon Creek water was 70%; however, growth was significantly lower than the lab controls, confirming sublethal effects from Flin Flon Creek water. The water-only tests were conducted in conjunction with sediment toxicity tests to further distinguish between historical contamination associated with sediment vs current site water/effluent effects (Environment Canada 2013). Based on results of the biotic ligand model and toxicity tests, it appears that Flin Flon Creek sediment and receiving water can cause toxicity, however; effects are associated moreso with sediment (i.e. historical contamination and site sediment condition).

Sediment from Schist Lake exhibited survival rates of that ranged from 64% to 96% in lab tests, and from 63 to 85% in field toxicity tests. When *Hyalella* was exposed to Schist Lake sediment or surface water alone, metals did not accumulate to levels that exceeded the LBC25s. However, when they were exposed to Schist Lake sediment and water together, in the lab or field, selenium and zinc bioaccumulation was higher than the LBC25s. Wild *Hyalella* accumulated selenium to the LBC25. Actual survival was always better than predicted, indicating that other factors, possibly due to acclimation of the organisms, genetic divergence among *Hyalella azteca* clades and other factors that modify metal bioavailability, uptake and toxicity.

The ability to model the mixture of metals under various scenarios provides insight into metals that may be causing observed benthic community effects. The MEAM (Norwood et al. 2013) was used with one modification, to account for 14 day rather than 28 day exposures. *Hyalella* tissue metal concentrations from the lab and field toxicity test treatments were used as inputs to the model, as well as bioaccumulation data from wild *Hyalella* collections carried out in 2011 and 2014. Results showed that potentially copper or selenium is causing toxicity in Flin Flon Creek. In Schist Lake, metals potentially causing toxicity are selenium and zinc.

Summary

Flin Flon Creek is devoid of resident fish populations and benthic invertebrate communities are severely impacted. Sediment in the creek is anaerobic and conducive to anaerobic microbiological activity as demonstrated by very low redox values. In general, the highly reduced conditions in Flin Flon Creek sediment may contribute to accelerated migration of metals, which supports the SEM/AVS results showing that metals in the sediment of Flin Flon Creek could cause toxicity. Toxicity in Flin Flon Creek to *Hyalella* and potentially other aquatic biota appears to be associated with both sediment and water, But moreso the historical contamination associated with the sediment. The receiving environment is complex but it appears that under certain conditions (especially low pH, high temperature, and low oxygen levels), metals become mobilized and available for uptake. The water column and sediment are in a constant state of flux, a situation that is not fully understood and is being further investigated. However, the weight of evidence to date points to copper, selenium and zinc associated with the sediment and water as being the metals preventing *Hyalella* (or other aquatic biota) from becoming established in Flin Flon Creek.

Benthic invertebrate communities in the NW Arm of Schist Lake are moderately impaired. Results from lab and field based sediment toxicity tests conducted with Schist Lake sediment revealed that survival was > 65% and growth was actually significantly higher compared to the lab controls. Schist Lake water or sediment alone does not appear to cause toxicity. MEAM modeling with bioaccumulation data from lab and field toxicity tests revealed that selenium and zinc accumulated to LBC25 values. However, actual survival was typically better than predicted. Wild Hyalella azteca also accumulated selenium and zinc to LBC25s, indicating that wild populations have acclimated to elevated metal levels, or different local species of *Hyalella* may exhibit different responses.

Table 1: Summary of water quality guideline exceedances for metals in exposure area waterbodies (2004-2014)

Parameter	Fraction	Unit	Guidelines		Total Number of Samples	Sample Values Not Meeting Guidelines				Descriptive Statistics			
						CCME WQG		MB PSOG[1]		Concentrations			
			CCME WQG[a]	MB PSOG[b]		Number Exceeding	Percent	Number Exceeding	Percent	Min.	Max.	Mean	Std. Dev.
Flin Flon Creek (upstream of former Trout Lake Mine discharge)													
Aluminum	T	µg/L	5	5	5	3	60%	3	60%	49	101	71.4	21.1
Arsenic	T	µg/L	5	150	5	1	20%	0	0%	3.1	6	4.6	1.1
Cadmium	T	µg/L	0.37	1.1	5	5	100%	2	40%	0.61	1.88	1.16	0.46
Copper	T	µg/L	4	60	5	5	100%	2	40%	15.9	89	48	34.5
Iron	T	µg/L	300	300	5	5	100%	5	100%	454	640	547	89.7
Selenium	T	µg/L	1	1	5	5	100%	5	100%	6.5	46	30	16
Zinc	T	µg/L	30	750	5	5	100%	0	0%	86	390	231	127
Northwest Arm of Schist Lake													
Cadmium	T	µg/L	0.37	0.83	17	5	29%	0	0%	0.053	0.60	0.22	0.18
Copper	T	µg/L	4	34	17	10	59%	0	0%	2.0	13	5.9	3.4
Iron	T	µg/L	300	300	19	5	26%	5	26%	10	400	120	150
Selenium	T	µg/L	1.0	1.0	19	19	100%	19	100%	2.63	10	4.4	2.0
Zinc	T	µg/L	30	420	19	11	58%	0	0%	27	110	57	29

Table 2: Summary of sediment quality guideline exceedances for metals in exposure area waterbodies (2004-2014)

Parameter	Unit	CCME Guideline		Total Number of Samples	Number of Samples Not Meeting Guidelines				Summary Statistics					
					ISQG		PEL		Concentration					
		ISQG[a]	PEL[b]		Number Exceeding	Percent	Number Exceeding	Percent	Min.	Max.	Mean	Med.	Std. Dev.	Geo. Mean
Flin Flon Creek (upstream of former Trout Lake Mine discharge)														
Arsenic	mg/kg	5.9	17	16	16	100%	14	88%	11	1260	297.5	150	376	133.5
Cadmium	mg/kg	0.6	3.5	16	16	100%	16	100%	5	510	115.4	70.5	148.7	54.9
Chromium	mg/kg	37.3	90	16	10	63%	3	19%	17	180	57.9	43	43.3	46.9
Copper	mg/kg	35.7	197	16	16	100%	16	100%	214	14000	4098.8	2455	4293.2	2192.7
Lead	mg/kg	35	91.3	16	13	81%	11	69%	19	739	309.4	221	277.7	166.6
Mercury	mg/kg	0.17	0.486	16	13	81%	11	69%	0.09	6.9	1.8	0.97	1.91	0.96
Selenium	mg/kg	none	none	16	n/a	n/a	n/a	n/a	19	1050	290	-	338	-
Zinc	mg/kg	123	315	16	16	100%	16	100%	472	33500	9428.3	5490	9721.8	5479.8
Northwest Arm of Schist Lake														
Arsenic	mg/kg	5.9	17	21	6	29%	0	0%	3	12	5.74	4.85	2.81	5.21
Cadmium	mg/kg	0.6	3.5	21	21	100%	19	90%	1.8	25.4	7.81	6.25	5.31	6.53
Copper	mg/kg	35.7	197	21	17	81%	0	0%	29	190	78.7	58.5	47.2	67.1
Mercury	mg/kg	0.17	0.486	21	6	29%	0	0%	0.025	0.27	0.105	0.051	0.083	0.074
Selenium	mg/kg	None	None	21	n/a	n/a	n/a	n/a	2.6	24.4	7.8	-	6.2	-
Zinc	mg/kg	123	315	21	21	100%	21	100%	430	3790	1511.5	1200	986.9	1282.9

Table 3: **Results of field and lab based sediment toxicity tests, bioaccumulation and metal effects addition modeling (MEAM) for Flin Flon Creek and Schist lake exposure scenarios.**

		Overlying Water	**Background-Corrected Concentration (nmol/g)**							
			As	**Cd**	**Cu**	**Pb**	**Se**	**Zn**	**Hyalella Survival (%)**	
Test	**Sediment**	**LBC25x24hr**	**83**	**585**	**1850**	**650**	**72.4**	**938**	**Predicted**	**Actual**
Lab 2011	Flin Flon Creek	Lab	No Survival						n/a	0
		Cran-REF	No Survival						n/a	0
		Flin Flon Creek	No Survival						n/a	0
	Cranberry-REF[a]	Flin Flon Creek	26	0.8	**1992**	5.8	**178**	150	22.5	94.0
	Lab	Flin Flon Creek	4.7	0	**1685**	0.1	**220**	0	16.3	76.0
In situ Caged 2011	Flin Flon Creek	Cran-REF	16.1	0	0	23	20	111	63.4	32.0
		Flin Flon Creek	No Survival						n/a	0
	Cranberry-REF	Flin Flon Creek	No Survival						n/a	0
Wild 2011	Flin Flon Creek		No *Hyalella* in Flin Flon Creek						n/a	0
Lab 2011	Schist Lake	Lab	6.8	0	0	5.5	34.6	337	70.7	86.0
		Cran-REF	8.9	0.2	663	1.5	41.4	45.7	69.5	64.0
		Schist Lake	6.2	7.7	428	1.7	**90.4**	398	53.0	97.8
	Cranberry-REF	Schist Lake	5.3	5.9	0	1.6	35.1	320	72.4	93.3
	Lab	Schist Lake	0	0	0	0	33.8	0	72.7	77.8
In situ Caged 2011	Schist Lake	Cran-REF	14.4	0	140	8.8	6.8	0	80.4	63.0
		Schist Lake	14.3	0	855	16.5	54.7	**1017**	23.6	85.0
	Cranberry-REF	Schist Lake	15	0	180	6.1	29	368	74.1	60.0
Wild	Schist Lake 2011 (n=3)		24.5	8.7	0	16.8	**116**	**556**	34.0	na[b]
	Schist Lake 2014 (n=8)		4.5	0	0	4.8	**63.0**	**249**	54.9	na
	Schist Bay (n=3)		20.9	0	0	5.2	**79.1**	206	58.6	na
	Mirond Lake 2011 (n=1)		4.0	1.9	0	4.5	6.2	104	76.5	na
	Mirond Lake 2014 (n=8)		0	0	0	0.8	2.4	0	77.0	na

Note: All data corrected by Norwood et al (2013) background concentrations

[a] REF – Reference Area

[b] na – not applicable since wild *Hyalella* collected

Bold – indicates mean values exceed the LBC25

Bold – indicates one or more replicates exceeds the LBC25

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