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Heavy and Trace Metals in Wild Mink (*Mustela vison*) and River Otter (*Lontra canadensis*) Captured on Rivers Receiving Metals Discharges

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The Fraser and Columbia Rivers are large, complex systems whose combined watersheds account for most of the land base in British Columbia (Canada) and Washington (U.S.) as well as that of the northern edge of Oregon (U.S.). Both rivers are dammed for hydro-electric power extraction and both carry effluents discharged from multiple municipalities and pulp mills. In Trail, B.C., along the Columbia River, a copper and gold smelter built in the early 1900s is currently focusing production on zinc and lead (Aquametrix Research 1994). Improvements in effluent treatment over the past decade have significantly reduced the release of metals to the river; however, measurable concentrations of lead, zinc, arsenic, cadmium, mercury and copper may still be found in the riverine receiving environment (Aquametrix Research 1994). Although the Fraser River does not receive discharges from metal smelters, it does receive pollutants from many other sources that likely contain traces of metals.

Mustelids were chosen as mammalian complements to traditionally monitored avian predators (Elliott et al. 1989; Wilson et al. 1996), because they do not migrate great distances and their habits and prey species are predominantly aquatic (Toweill 1974; Gilbert and Nancekivell 1982; Novak et al. 1987). Also, ranch mink have shown an extreme sensitivity to chlorinated organics such as PCBs in laboratory studies (Aulerich and Ringer 1970; Tillitt et al. 1996). We have reported elsewhere on chlorinated hydrocarbon contamination in animals trapped along the two rivers (Elliott et al. 1999; Harding et al. 1999) and we focus here on concentrations of metals in the same individuals. We hypothesized that mink and otter resident in streams receiving metals discharges should have higher tissue concentrations of elements such as lead, cadmium and mercury, compared to animals resident in more pristine reference areas.

MATERIALS AND METHODS

Skinned mink and river otter carcasses were collected from commercial trappers during the winters of 1990/91 (Oct.), 1991/92 (Dec.-Jan.), 1994/95 (Nov.-March) and 1995/96 (Nov.-Jan.). Traplines were located on the Fraser River near Prince George (upper river locales) and Chilliwack (lower locales), B.C. Most traplines on the Columbia River extended between Revelstoke and Trail, B.C., but two individual mink and one pooled sample each of mink and otter were collected proximate to Portland, OR (Fig. 1). In addition, individuals were collected from two tributaries of the Columbia in B.C., the Kootenay and Slocan Rivers (Fig. 1).

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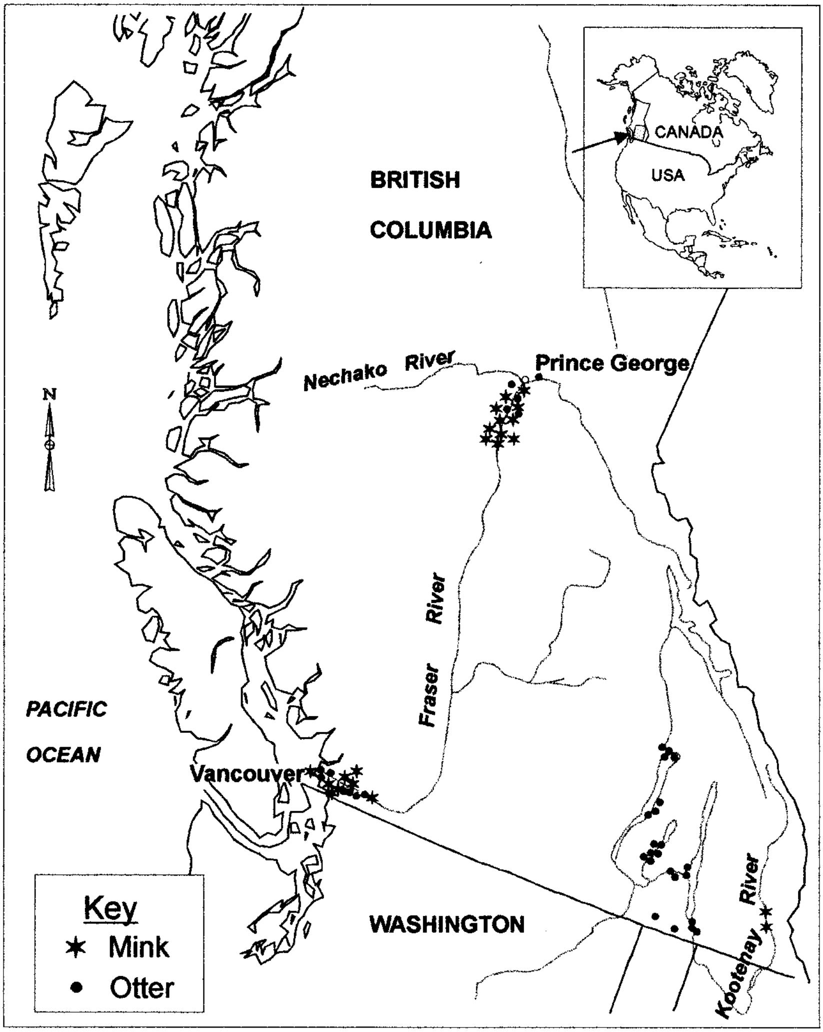

Figure 1 Location of collection sites for mink and otter.

Trappers were provided with collection kits to ensure consistent and adequate quality of tissue storage. Skinned carcasses were frozen before shipment by air to the laboratory. Necropsies were completed on carcasses collected in the last two seasons prior to tissue collection for contaminants analysis. The methods and results of biological condition assessments are reported elsewhere in relation to chlorinated hydrocarbon contamination (Harding et al. 1999). Associations between age, sex, organ somatic indices (liver, kidney, spleen) and metals concentrations were conducted here using Pearson correlation matrices and one-way analyses of variance for sex differences on SYSTAT 5.0 (Wilkinson 1990).

Kidneys from mink and livers from mink and river otter were excised with hexane-washed utensils, weighed, and stored frozen in hexane-washed, heat-treated jars until metals analyses were conducted. Six mink had both kidney and liver removed for a comparison of concentrations between the two organs. Tissues were freeze-dried and digested in acid (in three stages, 65% HNO_3, 97% H_2SO_4, 37% HCl) before analysis. Metals and selenium were assessed in most samples at the Environment Canada Pacific Environment Science Centre using inductively coupled argon-plasma emission spectroscopy (ICP), graphite furnace spectrometry (cadmium and lead) and atomic absorption spectroscopy (mercury). Detection limits were 0.02, 0.2 and 0.01 µg/g dry weight for cadmium, lead and mercury, respectively. Detection limits for ICP data ranged between 0.08 and 4 µg/g dry weight and are reported in the results. Four liver samples collected during the first two seasons were analyzed for mercury, lead cadmium, zinc and copper only at the Environment Canada National Wildlife Research Centre. Mercury was assessed using cold vapor atomic absorption spectroscopy (AAS), while lead was assessed using graphite furnace spectrometry. Cadmium, copper and zinc were assessed using flame AAS with a high sensitivity nebulizer. The detection limits for mercury, lead, cadmium, copper and zinc were 0.002, 0.001, 0.01, 0.1, and 0.01 µg/g dry weight, respectively. Quality assurance procedures included the analysis of replicate samples, reagent blanks and appropriate Standard Reference Materials (lobster and dogfish tissues).

In order to statistically compare specimens from different collection areas, those collected during the last two seasons were divided into regions as follows: upper Fraser River (near Prince George), lower Fraser River (near Chilliwack), upper Columbia River (above Arrow Lakes), lower Columbia River (below Hugh Keenleyside Dam), and Kootenay River. Where differences with age or sex existed, analyses of covariance (ANCOVAs) were conducted to assess site differences with age or sex as the covariate. In all other cases, ANOVAs were applied to check for site differences. Where mink and otter livers were collected from the same regions, ANOVAs were conducted to assess differences in metal and trace organic concentrations among species. Paired t-tests were completed to determine differences in element concentrations between the two organs in six mink. Also, associations among the many heavy metals and trace organics were evaluated using Pearson correlation matrices, independently for each species. All statistical evaluations were conducted on dry weight values using SYSTAT 5.0 (Wilkinson 1990). Non-detections were expressed as half of the detection limit for the preceding analyses.

RESULTS AND DISCUSSION

Metals concentrations in tissues of mink and river otter were generally low (Tables 1-3) and within the range of values reported for ranch and wild populations (Anderson-Bledsoe and Scanlon 1983; Ogle et al. 1985; Blus et al. 1987; Wren et al. 1988; Stejskal et al. 1989; Halbrook et al. 1996; Henny et al. 1996). A small number of differences among collection areas, sexes and species were detected, and the river otter collected below the metal smelter at Trail. B.C. showed elevated lead concentrations.

The female river otter collected near the Cominco smelter on the lower Columbia River showed a liver lead concentration several orders of magnitude higher than individuals from other areas ($p<0.001$; Table 2). The other otter sample collected downstream of Cominco (CO-5b = a pool of 3 animals trapped near Portland, Oregon) also showed an elevated lead concentration (Table 3); however, given the distance in river miles separating the two otter groups, it seems unlikely that the higher values are directly related. The two lead values of 7.2 and 5.66 µg/g dry weight were higher than values reported in Ontario (Wren et al. 1988), Illinois (Halbrook et al. 1996), Virginia (Anderson-Bledsoe and Scanlon 1983) and Oregon (Henny et al. 1996) river otter. However, the otter were far less contaminated than mink (Blus et al. 1987) trapped downstream of the heavily mined Kellogg area on the Coeur d'Alene River in Idaho (up to 22.0 µg/g wet weight versus 2.17 and 1.87 µg/g wet weight in our study). Although lower than a suggested toxic threshold of 10 µg/g wet weight (Buck et al. 1976), these lead burdens might be toxicologically relevant when considered in combination with the other contaminants - both chlorinated organics and metals - detected in the samples (Elliott et al. 1999; Harding et al. 1999). River otter also exhibited site-specific variability in copper concentrations. Fraser River populations had higher copper levels (p=0.011, Table 2), but the differences were not extreme.

Table 1. Concentrations of metals and selenium in kidneys of mink.

Element	Kootenay River	L. Fraser River
Sample Size (n)	4	8
Aluminum	3.25±0.75	7.18±1.75
Barium	0.29±0.10	0.15±0.05
Cadmium	3.61±1.16	0.73±0.21
Calcium	494±145	381±31
Cobalt	<0.4	0.25±0.05
Chromium	0.98±0.34	1.68±0.33
Copper	12.13±1.65	12.4±0.66
Iron	715±56	456±33
Lead	1.13±0.73	0.59±0.21
Magnesium	433±34	533±26
Manganese	2.4±0.25	3.56±0.22
Mercury	3.37±1.16	3.13±0.64
Molybdenum	<0.8	0.48±0.08
Potassium	7010±1010	8680±447
Selenium	4.0±1.23	<4
Sodium	4360±383	4710±274
Strontium	0.52±0.24	0.45±0.06
Tin	6.25±0.25	5.5±0.60
Titanium	0.13±0.03	0.41±0.25
Zinc	67±8.8	65±2.6

Values are means ± standard error in µg/g dry weight. Antimony (14 µg/g d.w.), beryllium (<0..08 µg/g d.w.), nickel (<2 µg/g d.w.), and vanadium (<0.8 µg/g d.w.) were not detected in any samples.

Kidney cadmium concentrations in mink collected from the Kootenay River were above those described for other mink populations in Ontario (Wren et al. 1988), Virginia (Ogle et al. 1985) and Illinois (Halbrook et al. 1996). They were also significantly higher than cadmium in mink collected from the lower Fraser River during this study (p=0.012; Table 1). However, the Kootenay mink, with an average cadmium concentration of 3.6 µg/g dry weight, were not likely to be experiencing any toxic effects from this level of contamination. Although cadmium toxicity is not well documented in mustelids, studies with other mammals suggest that renal dysfunction occurs at kidney concentrations around 40 to 200 µg/g wet weight (Friberg et al. 1974).

Table 2. Concentrations of metals and selenium in livers of mink and river otter.

	Mink		Otter				
	LFR	UFR	KOT	LCO	UCO	LFR	UFR
(n)	8	12	12	1	4	3	6
Al	3.63±0.91	3.5±0.47	<4	<4	5.0±1.08	<4	3.33±0.99
Ba	0.06±0.02	0.08±0.02	0.05±0.01	<0.08	0.46±0.42	<0.08	0.05±0.01
Cd	0.26±0.07	0.10±0.05	0.68±0.29	0.66	0.30±0.10	0.12±0.01	0.07±0.03
Ca	380±49	551±66	226±28	180	253±23	213±6.7	195±9.9
Co	0.23±0.03	0.33±0.06	0.29±0.06	<0.4	<0.4	0.30±0.10	<0.4
Cr	1.16±0.40	1.98±0.28	1.44±0.30	<0.4	0.53±0.33	1.40±0.12	1.42±0.22
Cu	23±3.4	24±3.3	21±1.6	20.7	23±3.2	32±3.1	31±2.7
Fe	1050±76	982±74	1100±57	1310	921±115	993±204	1330±108
Pb	0.45±0.14	0.21±0.06	0.79±0.17	7.2	0.23±0.03	0.43±0.13	0.17±0.02
Mg	634±18	580±15	587±25	600	613±31	643±15	607±39
Mn	8.98±0.55	8.53±0.55	9.99±1.02	9.43	13.1±1.3	11.5±1.24	10.7±1.20
Hg	4.62±1.08	4.05±1.42	2.08±0.53	3.3	3.31±1.19	3.50±1.72	2.93±0.64
Mo	1.5±0.19	0.98±0.11	1.83±0.17	1.0	1.75±0.25	<4	2.33±0.42
K	7470±339	8110±148	7460±340	7500	8250±717	7980±354	7800±456
Se	2.06±0.06	2.42±0.29	7.41±0.97	5.0	6.25±2.66	5.30±2.00	6.67±0.42
Na	5050±257	4490±128	5260±156	5100	5170±233	5670±888	5270±278
Sr	0.45±0.14	0.32±0.04	0.16±0.03	<0.08	0.44±0.23	0.27±0.07	0.17±0.03
Sn	5.53±0.64	5.17±0.17	<4	<4	<4	2.67±0.67	<4
Ti	<0.2	<0.2	0.23±0.04	<0.2	0.15±0.05	0.27±0.09	0.38±0.05
Zn	95±3.1	95±4.8	77±6.2	77.2	87±10	86±3.0	78±7.2

Values are means ± standard error in µg/g dry weight. Antimony (<4 µg/g d.w.), beryllium (<0.08 µg/g d.w.), nickel (<2 µg/g d.w.), and vanadium (<0.8 µg/g d.w.) were not detected in any samples. (n) = sample size. LFR = lower Fraser River, UFR = upper Fraser River, KOT = Kootenay River, LCO = lower Columbia River, UCO = upper Columbia River.

Kidney mercury concentrations were also high in Kootenay mink compared to Illinois mink (Halbrook et al. 1996) or those collected here from the lower Fraser River. However, values were intermediate between animals collected from control and mercury-contaminated basins in Manitoba (Kucera 1983) and Ontario (Wren et al. 1986). The maximum value detected in mink from this study was 6.68 µg/g dry weight (2.27 µg/g wet weight), whereas individuals from mercury-contaminated areas had up to 14.83 µg/g dry weight or 5.54 µg/g wet weight mercury in kidney tissue. Although no tissue concentrations are available for wild mercury-poisoned mink, Wren (1985) found 58 µg/g wet weight in the kidney tissue of a mercury-poisoned wild river otter.

Table 3. Metal concentrations (µg/g dry weight) in livers of mink and river otter collected upstream and downstream on the Columbia River.[a]

Species	Sample	Cadmium	Copper	Lead	Mercury	Zinc
Mink	CM-4	<0.01	13.08	0.36	2.39	9.2
	CM-5	1.35	27.39	0.43	1.52	10.1
	CM-6	<0.01	38.67	0.18	10.85	14.2
River otter	OTUCO-009	0.29	31.1	0.3	1.44	89.3
	OTUCO-023	0.54	18.6	0.2	6.67	113.9
	OTUCO-025	0.07	24.3	0.2	1.84	78.9
	OTUCO-026	0.31	17.0	0.2	3.28	64.6
	OTLCO-024	0.66	20.7	7.2	3.30	77.2
	CO-5b	0.24	18.62	5.66	2.26	9.51

[a] - river otters OTUCO-009 to 026 were collected upstream of the metal smelters on the Columbia, while all other individuals were collected downstream.

In addition to cadmium and mercury regional differences, mink from the Kootenay River had higher levels of iron ($p=0.002$), and lower levels of magnesium ($p=0.045$) and manganese ($p=0.01$) than mink from the lower Fraser River. Kidney concentrations of cadmium, iron, magnesium and manganese, and liver concentrations of cadmium all ranged higher than those detected in ranch mink during a dietary background study (Stejskal et al. 1989), but values are still likely not sufficient to produce toxic effects in exposed mink.

In six mink collected from the lower Fraser River, approximately half of the compounds tested were preferentially partitioned to one or the other of the two organs assessed. Cadmium levels were greater in the kidney ($p<0.001$, Fig. 2b), while copper ($p=0.046$), iron ($p=0.001$), manganese ($p<0.001$), magnesium ($p=0.002$) and zinc ($p=0.002$) levels were greater in the liver. Tissue concentrations of calcium, chromium, lead, mercury and sodium did not differ. These results reflect those of Stejskal et al. (1989) for ranch mink, except for calcium (which they found to be higher in livers) and sodium (which they found to be higher in kidneys).

When liver concentrations of metals in mink and river otter were compared on the Fraser River system, only calcium ($p<0.001$), copper ($p=0.039$), manganese ($p=0.009$), sodium ($p=0.032$) and zinc ($p=0.017$) concentrations were species-specific. Copper and manganese were higher in otter, while calcium, sodium and zinc were higher in mink. Although statistically significant, differences in these metals were not extreme and values were within the ranges described in the literature. No species differences in liver concentrations of the heavy metals, cadmium, lead and mercury, were detected.

When correlations were tested between metals and biological parameters, a significant association was found between mink age, and liver ($r=0.636$, $p=0.003$) and kidney ($r=0.841$, $p=0.036$) cadmium concentrations. Average adult liver cadmium concentration was 0.55 µg/g dry weight, compared to 0.09 and 0.13 µg/g dry weight for yearlings (1 yr) and juveniles (<1 yr), respectively. However, the relationship must be considered with caution given the small sample size involved in the evaluation (two adults, three yearlings and fourteen juveniles). If the association is real, it suggests that cadmium may accumulate with age in this species. Unfortunately, those mink with the highest cadmium concentrations, the Kootenay River group, were not aged. There were no other significant correlations between metals and liver, kidney or spleen somatic indices or age or sex in either species ($r<0.6$, $p>0.05$).

The data presented here on heavy and trace metals in mustelids from the Fraser and Columbia River systems suggests that these populations are not at risk from discharges of metals. Further sampling should be undertaken along the reaches from which otter female OTLCO-024 was collected in order to obtain a better indication of lead exposure of mustelid populations in the region surrounding the Cominco smelter. Otherwise, values documented here may be considered as a contribution to a base of reference (control) concentrations of metals in wild mink and river otter.

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