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Copper, Zinc, and Cadmium Concentrations in *Peromyscus maniculatus* Sampled Near an Abandoned Copper Mine

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Abstract. Concentrations of zinc, copper, and cadmium were determined in soil and liver, kidney, bone and stomach contents of deer mice (*Peromyscus maniculatus)* from two sites near an abandoned mine and one control site, on Vancouver Island, British Columbia, Canada. Soil concentrations of copper were significantly elevated at the mine and off site vs the reference site. In contrast, there was no difference in soil cadmium and zinc concentrations between the mine and reference site. Concentrations of copper, cadmium and zinc in livers of mice from the mine site were significantly elevated relative to the reference and off site locations. Cadmium kidney concentrations tended to be greater in mice from the mine versus the off site and reference site. No differences in bone cadmium, copper and zinc and, kidney copper and zinc concentrations were noted among mice from the three locations. Diet of mice from mine and off sites contained significantly greater copper concentrations than the reference population; no differences in cadmium or zinc diet concentrations in mice from the three sites were noted. Comparison of ratios of metal concentrations in diet:soil and concentrations in liver:soil suggest that for zinc and copper, soil and diet are of equal importance as a source of metal contamination to these mice. In contrast, cadmium diet:soil and cadmium liver:soil ratios were much greater than one indicative of bioconcentration of cadmium from soil to diet and from soil to liver. For assessing routes of metal exposure, in this case for deer mice inhabiting an abandoned mine site, for copper and zinc, soil will most likely be indicative of exposure conditions. In contrast, concentrations of cadmium in diet will be more representative of amounts that the animal is potentially ingesting. Of further importance is that relative to reference sites, mice inhabiting an abandoned copper mine site have significantly elevated tissue levels of copper. This is turn will provide a route of metal exposure to carnivorous birds such as owls and hawks. The toxicological significance of this exposure to birds of prey has yet to be assessed adequately.

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Ore extraction of metals results in the generation of large amounts of mine tailings waste. For many mines operated from the turn of the century to the early 1960s, close-out procedures were not in place or required by law. Consequently, many mines were abandoned once the mine was exhausted. Little or no effort was expended to correct or minimize potential environmental impacts. There are numerous such abandoned mine sites located throughout British Columbia, Canada, with at least five of these generating acid mine drainage (Environment Canada 1993). Although these abandoned mines pose several environmental threats (ground water contamination, acid mine generation, exposed mine tailings, etc.), the potential threat of introducing metal contamination into the food chain is unknown.

Small ground mammals such as the common deer mouse (*Peromyscus maniculatus*) are key intermediates in the transfer of contaminants from soils to higher trophic levels such as raptures. Metal concentrations in these small mammals provide information about potential bioaccumulation, mobility within the ecosystem and relative food chain transport (Johnson *et al.* 1978). Mice are highly suited for studies related to metal exposure because they are found in a variety of habitats and established adults remain localized in the same general area (within one km) throughout most of their lives (Stickel 1968).

Mice are exposed to elevated metal levels directly through their diet (Ma and Faber 1991), or through soil ingestion (Anthony and Kozlowski 1982). Therefore small mammals living in and around abandoned mine sites could accumulate metal burdens to the point that may be of concern to higher trophic levels. The objectives of this study were to determine whether measured metal concentrations in mice sampled from an abandoned copper mine site were elevated relative to reference sites and to assess whether mouse tissue metal levels could be a potential concern to carnivorous birds such as owls and hawks.

Methods

Study Area

The study site was located on the abandoned Blue Grouse Mountain Copper Mine, near Honeymoon Bay, on Vancouver Island, B.C. (Figure

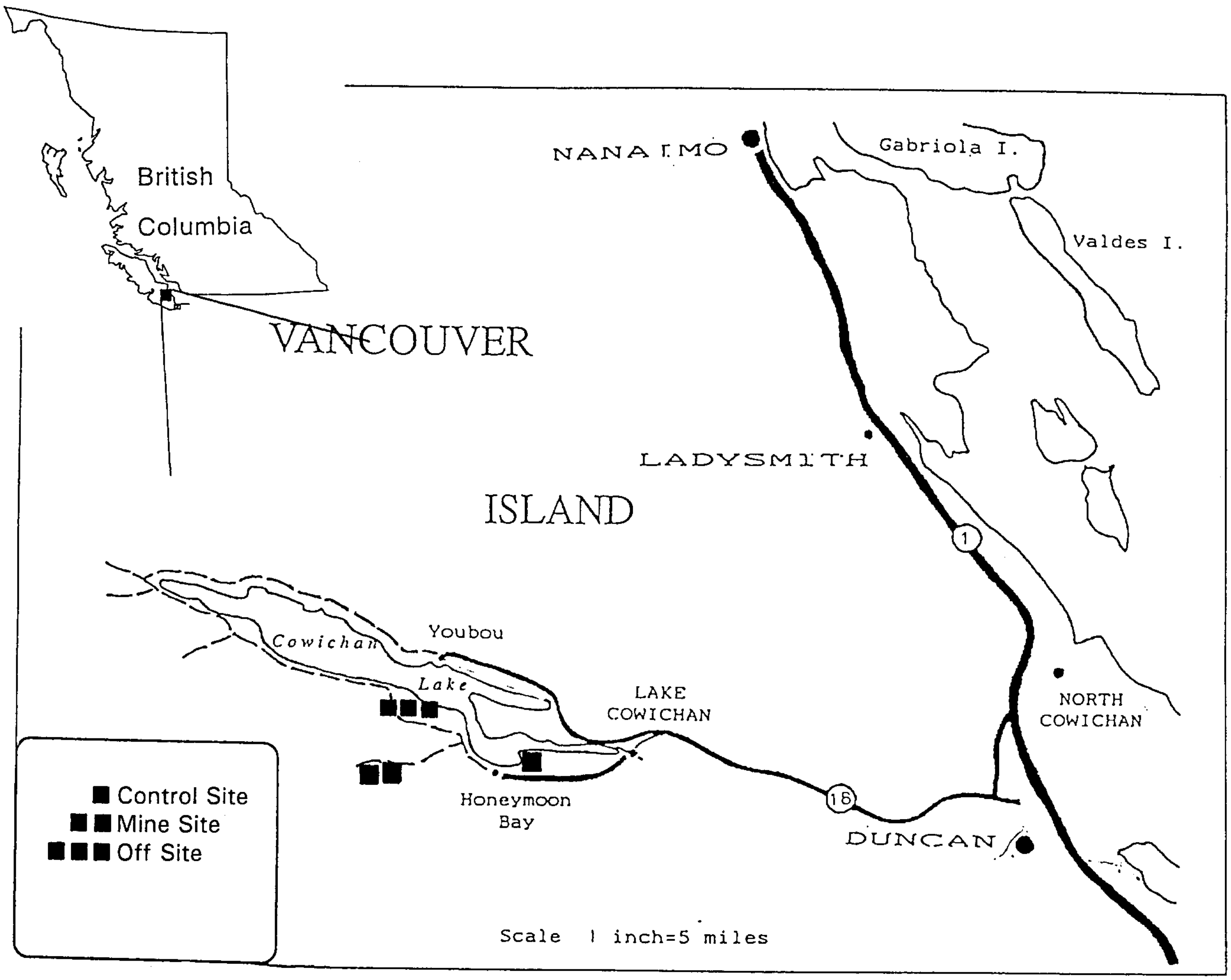

Fig. 1. Location of mine site, off-mine site, and reference site on Vancouver Island, BC, Canada

1). This area is underlain by basalt and andesite flows of the Franklin Creek volcanic formations. The high-grade copper ore (averaging 3% copper and 0.3 oz silver/tonne) was mined from 1915 to 1962, during which time approximately 300,000 tonnes were processed (Phendler 1980). The mine processed three million dollars worth of ore (Bates 1993). Following cessation of operations the mine shaft was dynamited shut. However, throughout the mined area, large amounts of exposed mine tailings and rock were left behind (Bates 1993). These exposed materials contain high amounts of chalcopyrite ($CuFeS_2$), pyrite (FeS_2), pyrrhotite ($Fe_{11}S_{12}$), and sphalerite (ZnS), (Phendler 1980). Two locations within the impacted area were sampled, one adjacent to the mine shaft and the other located approximately one km from the centre of the mining shaft. The reference site was in a grassy field with no exposed rock, located five km from the mine site.

Geochemical Analysis

Soils were collected with plastic spoons and scoops from ten locations at each of three study sites. The uppermost surface layers (2 cm) of soil was carefully removed from the ground for metal analysis. Soils were placed into plastic bags for transport. Two extraction procedures, acid extractable, and total soil metal content, were employed: a 6-h digest at 70°C in 6 M HCl (acid extractable fraction); aqua regia, a 3:1 v/v concentrations HCl/HNO_3 at 70 °C for 24 h. Supernatants from the digests (filtered through a 0.45 μm cellulose acetate filter), were analyzed for copper, zinc, and cadmium by atomic absorption spectrophotometry (AAS), within the Department of Chemistry, Simon Fraser University. Absorbances were converted to concentrations via standard curves. Standard soil samples and blanks were routinely run to ensure the quality of analysis.

Mice Collection and Metal Analysis

Deer mice were collected by baited (peanut butter and oatmeal balls) snap-traps from May 21/94–June 1/94. Trapped mice were identified to species (Whitaker 1980) and frozen until metal analysis. Mice were sexed, weighed frozen (to the nearest 0.1 g) and total-body and hind-foot length measured (to the nearest 0.1 mm). Whole liver, kidney, stomach and one femur bone were dissected from the mice and placed into acid-washed polyethylene vials and stored until metal analysis. Stomach contents were viewed under compound and dissecting microscopes to identify diet composition. All tissues were dried at 70° C to a constant weight, prior to acid digestion. Tissue digests were performed in a CEM MDS-2000 model Microwave Sample Preparation System. Preweighed samples were placed into acid washed CEM Teflon digestion vessels with 1.5–3-mL concentrated analytical grade nitric acid. To ensure quality of the analysis, blanks were included with every third running of the microwave and a National Bureau of Standards

Table 1. Cadmium, zinc, and copper concentrations (mg kg^{-1} ± S.D.) in soil samples sampled from the mine site, 1 km from the mine site, and the reference sites. Results of a one-way ANOVA for differences in metal concentrations among the three sites are given within the table. Means with the same letter superscript are not significantly different (Bonferroni multiple-range tests). Bold values are at least twofold above the CCME (1991), Interim Canadian Environmental Quality Criteria for Contaminated Soils for residential/parkland area (remediation criteria that are used as benchmarks to evaluate the need for further investigation or remediation with respect to specified land use, in this case for residential/parkland use)

Metal	Site	n	6M HCl	Aqua regia (AR)	HCl/AR
Cadmium	Reference	9	0.031[a] (0.04)	5.62[a] (11.04)	0.005
	Mine site	7	0.021[a] (0.006)	5.02[a] (2.88)	0.004
	Off site	7	0.009[b] (0.002)	0.68[b] (0.71)	0.013
	ANOVA	F	3.85	8.73	
		P	0.042	0.002	
Copper	Reference	10	53.7[b] (55.0)	63.4[b] (41.9)	0.84
	Mine site	7	**315.0**[a] (3.15)	**293.0**[a] (2.7)	1.07
	Off site	7	**306.0**[a] (20.14)	**252.9**[a] (25.3)	1.05
	ANOVA	F	135.0	156.5	
		P	0.0001	0.0001	
Zinc	Reference	10	87.8[a] (6.4)	89.0[a] (4.8)	0.98
	Mine site	9	80.6[a] (10.4)	84.0[a] (7.7)	0.95
	Off site	9	58.7[b] (2.6)	59.0[b] (15.5)	0.98
		F	40.9	12.61	
		P	0.0001	0.001	

(NBS) bovine liver standard was digested with each running. Liver tissues were digested at 70% power for 5 min and then 100% power for an additional 5 min to obtain a complete digestion. Kidney and bone samples required 5 min at 70% and 6 min at a 100% for complete digestion. Stomach contents required 5 min at 70% and an additional 6–10 min at 100% power depending on the composition of the sample. Digests were diluted to 5 mL with NANO pure distilled deionized water and analyzed for cadmium, copper and zinc via Inductively Coupled Plasma, AtomComp Series 1100 at the University of British Columbia. Percent recovery of the analysis as indicated by the NBS standard was 104, 112, and 93% for cadmium, copper and zinc, respectively. Limits of detection of the analysis was 0.001, 0.002, and 0.001 $\mu g\ mL^{-1}$ for cadmium, copper and zinc, respectively.

Data Analysis

All data analyses was performed by SAS (Statistical Analysis Systems) for the personal computer made available by the Simon Fraser University Computing services (SAS Institute Inc. 1988). Concentrations of easily extractable and aqua regia metal as well as mice tissue metal concentrations were converted to $\mu g g^{-1}$ dry weight for data analysis. To improve the normality of the data and to meet the assumptions of the ANOVA, all metal concentrations were log transformed prior to statistical analysis (Sokal and Rohlf 1990). Significance was accepted at 0.02 (Bonferonni corrected value for multiple comparisons).

Results

Acid Extractable Versus "Total" Metal: The total amount of copper and zinc recovered from the soil fraction was the same for both extraction procedures (Table 1). In contrast, only 0.5% of cadmium was recovered by the 6 M HCl versus the aqua regia acid extract (Table 1).

Differences in soil metal content among study sites: As expected, both acid extractable and total copper soil concentrations were significantly elevated at the two sites located closest to the mine as compared to the reference site (Table 1). In contrast, soil concentrations of zinc and cadmium tended to be higher in the reference and one of the two mine study sites (the site located most closely to the original mine shaft) compared to the off site (Table 1).

Table 2. Morphological characteristics (mean ± S.D.) and sex ratios of mice captured from the three sampling locations

Feature	Reference site	Mine site	1 km off site
Sex Ratio (male/female)	5/4	4/4	3/3
Body weight (g)	18.44 (3.3)	23.0 (4.5)	22.3 (1.36)
Body length (mm)[a]	77.9 (8.9)	80.0 (9.07)	76.4 (3.58)
Hind foot length (mm)	18.8 (0.93)	18.7 (0.79)	18.9 (0.44)
Femur length (mm)	15.1 (1.07)	15.7 (0.87)	15.49 (0.39)
Liver dry wt (g)	0.34 (0.122)	0.35 (0.088)	0.389 (0.047)
Kidney dry wt (g)	0.06 (0.01)	0.058 (0.018)	0.065 (0.005)
Femur bone dry wt (g)	0.029 (0.011)	0.028 (0.004)	0.029 (0.002)

[a] Excluding tail

Differences in Mice Body and Organ Size: As morphological characteristics such as organ weight can be indicative of exposure to stress (Linzey and Grant 1994), in addition to weight and length of mice, comparisons among whole organ weights (liver and kidney) were performed. A one-way ANOVA on the various measures of body size, plus a comparison of dry weights of whole liver and kidney, indicated that there was little difference in these measures among the reference, mine and off sites (Table 2).

Differences in Mouse Tissue and Stomach Contents Metal Concentrations among Study Sites: Cadmium concentrations were significantly greater in liver and tended to be greater in kidney

Table 3. Mean metal concentrations ($\mu g\ g^{-1}$ dry weight) and standard deviations in liver, kidney, bone, and diet of mice from the reference, mine, and off site locations. Values for the one-way ANOVA are for differences in mice metal concentrations among the three sample locations are given within the table. Means with the same letter superscript are not significantly different (Bonferroni multiple-range tests)

Site	Tissue/n	Cadmium	Copper	Zinc
Reference	Liver/9	0.35^{b} (0.30)	17.4^{b} (2.2)	79.5^{b} (9.8)
Mine	Liver/8	0.74^{a} (0.52)	52.9^{a} (41.1)	100.0^{a} (15.5)
Off site	Liver/6	0.18^{b} (0.09)	29.4^{b} (11.8)	90.3^{ab} (12.7)
	ANOVA:F/P	**4.37/0.02**	**10.86/0.0006**	**6.32/0.007**
Reference	Kidney/9	0.20 (0.11)	2.54 (2.35)	15.6 (3.69)
Mine	Kidney/8	0.38 (0.23)	3.96 (2.35)	15.9 (5.55)
Off site	Kidney/6	0.15 (0.11)	2.67 (0.57)	14.8 (3.9)
	ANOVA:F/P	**3.7/0.04**	**0.82/0.46**	**0.07/0.93**
Reference	Bone/9	0.57 (0.37)	3.2 (8.9)	180.0 (50)
Mine	Bone/8	0.63 (0.56)	1.2 (2.6)	199.0 (34)
Off site	Bone/6	0.33 (0.28)	2.67 (0.57)	205.0 (19)
	ANOVA:F/P	**3.02/0.07**	**1.31/0.29**	**0.95/0.44**
Reference	Diet/9	0.75 (0.82)	32.0 (36.0)b^{b}	68.0 (50)
Mine	Diet/8	2.70 (6.9)	413.7 $(362)^{a}$	150.0 (137)
Off site	Diet/6	1.05 (1.1)	296.0 $(51)^{a}$	130.0 (147)
	ANOVA:F/P	**0.18/0.83**	**12.37/0.0004**	**0.95/0.44**

Table 4. Ratio of metal concentrations in diet:soil (acid extractable), liver:soil (acid extractable), and liver:diet

Metal/Site	Diet:Soil (AE)[a]	Liver:Soil (AE)	Liver:Diet
Cd/Reference	24.2	11.5	0.47
Cd/Mine site	128.0	35.3	0.27
Cd/Off site	116.0	20.1	0.17
Cu/Reference	0.59	0.32	0.53
Cu/Mine site	1.31	0.17	0.12
Cu/Off site	1.0	0.1	0.1
Zn/Reference	0.77	0.91	1.16
Zn/Mine site	1.87	1.25	0.66
Zn/Off site	2.24	1.55	0.69

[a] Acid extractable soil metal

of mice sampled from the mine versus the off and reference sites (Table 3). No differences in bone cadmium were noted among mice sampled from the three sites. Copper was significantly higher in liver of mice collected from the mine site versus those from the off and reference sites. In contrast, no difference in copper kidney or copper bone concentrations were noted among mice sampled from the three locations. Zinc concentrations in liver from mice collected from the mine site were significantly greater and tended to be greater in those mice collected from the off site as compared to concentrations of zinc in liver of reference mice. Zinc kidney and bone concentrations did not differ among mice sampled from the three locations. Visual inspection of stomach contents indicated that in general, stomachs contained 20% insects, with the remaining 80% comprised of plant material. Diet concentrations of copper were significantly higher and cadmium tended to be higher in mice from the mine site compared to the other two sites. No difference in diet zinc concentrations were noted among the three sampling locations.

Bioconcentration Factors: To assess whether mice were concentrating metals from soil or diet, bioconcentration factors that is, the ratio of metal in diet:acid extractable soil, metal in liver:acid extractable soil and metal in liver:diet, were calculated (Table 4). Only liver metal concentrations showed any significant difference in metal content among the three sampling sites, hence only these tissue values were used in the calculations. Each metal showed a different trend; for diet:soil comparisons, ratios were approximately 1, 2, and 100 for copper, zinc and cadmium, respectively. Liver:soil ratios were less than 1, equal to 1 and approximately 20 for copper, zinc and cadmium, respectively. For liver:diet ratios, values were less than 1, approximately 1, and less than 1 for copper, zinc and cadmium, respectively.

Discussion

Metal Concentrations in Soils: Recovery of metals from the two extraction procedures illustrate the different forms of each metal within the surface soils of the three sampling locations. Only a fraction of cadmium was recovered by the weaker acid extract compared to the more complete aqua regia digest. It is possible that soil cadmium occurs largely in mineralized form, i.e. within the lattice of silicates as compared with zinc and copper which could be present in a more readily extractable form. These latter forms could include sulphide species such as chalcopyrite ($CuFeS_2$), pyrite (FeS_2), pyrrhotite ($Fe_{11}S_{12}$), and sphalerite (ZnS), bound to oxides of Fe and Mn as well as being bound by organic matter (Brummer 1986). That concentrations of copper and zinc recovered by the aqua regia extract were the same as that recovered by the less severe acid extract, suggests that total soil concentrations of these two metals are more readily available to biota from soil versus that of cadmium.

Contrasting Metal Concentrations in Liver, Kidney, Bone and Diet Items of Mice from the Three Sampling Locations: Liver tissue showed significant differences in metal concentrations among mice from the three sampling sites. Copper, zinc, and cadmium were significantly greater in mice from the mine site compared to the reference site. This is in contrast to Hunter *et al.* (1987) who noted that whole body copper concentrations for *Microtus agrestis, Apodemus sylvaticus,* and *Sorex araneus* from a copper-contaminated grassland were independent of the degree of habitat contamination. These authors attributed the absence of copper accumulation to the ability of the mammals to homeostatically regulate tissue levels of copper. Similarly, Pascoe *et al.* (1994) noted that the bioavailability of metals, which included copper, cadmium, and zinc, to the herbivores *Peromyscus maniculatus* and *Microtus pennsylvanicus* was quite small, that is, the herbivores did not appear to be accumulating significant amounts of these metals relative to background exposures. However, Folkeson *et al.* (1990), in their study on the influence of acidity and other soil properties on metal concentrations in forest plants and animals, noted that of the 22 elements analyzed in the two biotic compartments, concentrations of copper in the liver of *Apodemus flavicollis* were significantly correlated with those in *Fagus sylvatica* nuts, the dominating food item. In our study, that mice from the mine site contained elevated liver copper concentrations relative to the reference site suggests that tissue burdens are elevated as a consequence of elevated exposure.

Of further interest are that concentrations of both cadmium and zinc are significantly greater in liver of mice from the mine site versus the reference site. This is despite the similarity in soil concentrations of cadmium and zinc among the sites as well as similar diet concentrations of these two elements. Metal–metal interactions i.e., enhanced uptake of one metal following exposure to elevated concentrations of another chemically similar metal are well known (Bremner and Marshall 1974; Wesenberg 1983; Elsenhans *et al.* 1987; Shukla and Chandra 1987). For example, Shukla and Chandra (1987) reported the enhanced accumulation of lead and cadmium in liver of growing rats following concurrent exposure to a mixture of lead, manganese and cadmium. Elsenhands *et al.* (1987) noted that increasing dietary concentrations of nickel to the rat increased the retention of lead in the rat kidney and cadmium in the small intestine. Wesenberg (1983) noted that the administration of small amounts of cadmium to suckling rats was followed by an increase of zinc content in liver and kidney.

In this study, high levels of copper may have stimulated the induction of metallothionein, a metal-binding protein in mammalian liver and kidney which sequesters and detoxifies some metals such as cadmium (Cherian and Goyer 1978). An increase in metallothionein could account for the enhanced accumulation of cadmium and zinc in liver of mice from the most copper contaminated site. An important implication of possible metal–metal interactions is that metal tissue concentrations, such as those determined in mice tissues in the present study, will not simply be related to environmental levels of the metal, i.e., as a consequence of metal–metal interactions, in this case the enhanced uptake of zinc and cadmium by copper exposed mice, tissue metal levels will be higher than that predicted based on exposure levels to a single metal alone.

A Comparison of Diet:Soil, Liver:Diet, and Liver:Soil Metal Concentrations

Comparison of ratios of metal concentrations in liver:soil and concentrations in liver:diet suggest that for zinc and copper, soil and diet are of equal importance as a source of metal contamination to these mice. Venugopal and Luckey (1978) have reported that rats can only tolerate 200 ppm of copper in their diet. A comparison of diet to liver concentrations of copper suggest that sampled mice are only assimilating 50% of that copper present in the diet. Hence, although dietary values are high, only a fraction of the potentially available metal appears to be incorporated into the liver tissue. In contrast to zinc and copper, cadmium diet:soil and liver:soil ratios suggest that diet items concentrate cadmium from the environment, i.e., concentrations of cadmium in diet items are much greater than acid extractable soil concentrations of cadmium. Hence, for this element, cadmium soil levels will not be representative of exposure conditions, rather, amounts that have accumulated within the diet items (plants and invertebrates) will be more indicative of amounts that the animal is potentially exposed to.

Implications in Regards to the Trophic Transfer of Metals via Deer Mice

Livers from mice sampled from an abandoned copper mine contained elevated concentrations of copper, cadmium and zinc. Diet copper concentrations in mice from the mine and off site were also significantly greater as compared to mice from the reference site. Based on the mass of copper in liver, kidney, bone, and diet of mice from the mine site, daily exposure to a hawk that feeds on 10 mice/day (D. Lank, personal communication) would be approximately 5 mg. Although there have been a number of studies which address the question as to whether exposure to elevated metal levels in the environment correlate to elevated levels of metal in associated bird populations (e.g., Scheuhammer, 1987 for review), there are few studies which address the toxicological significance of elevated dietary metal concentrations, on the overall fitness of birds of prey such as hawks and raptures. Studies of Rangachar and Hedge (1973) cited in Jackson (1977) suggest that amounts of copper that carnivorous birds feeding on mice from the mine site would be exposed to on a daily basis are within the range of amounts that have shown to have a physiological effect on birds. These authors noted that 10 mg of copper per day administered to 14-week-old White Leghorn pullets resulted in higher packed cell volume and higher haemoglobin levels. Indeed, the toxicological significance to birds of prey of long-term exposure to elevated levels of metals from areas such as abandoned mine sites warrants further investigation.

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