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Cadmium, Copper, Iron, and Zinc Concentrations in Kidneys of Grey Wolves, *Canis lupus*, from Alaska, Idaho, Montana (USA) and the Northwest Territories (Canada)

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Abstract Cadmium, copper, iron, and zinc levels were measured in the kidneys of 115 grey wolves (*Canis lupus*) from Idaho, Montana and Alaska (United States), and from the Northwest Territories (Canada). No significant differences in the levels of iron or copper were observed between locations, but wolf kidneys from more northern locations had significantly higher cadmium levels (Alaska > Northwest Territories > Montana ≈ Idaho), and wolves from Alaska showed significantly higher zinc than other locations. Additionally, female wolves in Alaska had higher iron levels than males, and adult wolves in Montana had higher copper levels than subadults.

Keywords Grey wolf · *Canis lupus* · Heavy metals

Elevated heavy metal concentrations have been documented in a wide range of vertebrates, including caribou (*Rangifer tarandus*) (Elkin and Bethke 1995), polar bear (*Ursus maritimus*) and arctic fox (*Alopex lagopus*) (Hoekstra et al. 2003), and bald eagles (*Haliaeetus leucocephalus*) (Bechard et al. 2009). Within tissues, high levels of essential heavy metals such as copper (Cu), iron (Fe) and zinc (Zn) and the presence of nonessential heavy metals such as cadmium (Cd) are associated with numerous physiological impairments and may be lethal (Jumba et al. 2007; Wren 1986). Heavy metals occur naturally in the environment, although some are released in high amounts as a result of human activities such as ore mining, mineral refining, and coal burning (Hoekstra et al. 2003). Such

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metals have been documented to bioaccumulate and to biomagnify within wildlife food chains (Smith and Rongstad 1981, Hoekstra et al. 2003).

Grey wolves *(Canis lupus*) are found in the northern regions of North America, ranging from Minnesota to the western United States, and in Canada and Alaska. Gamberg and Braune (1999) documented the levels of several metals in grey wolves from the Yukon Territory, Canada. However, few studies have reported the presence of heavy metals in wolves from other parts of North America. The purpose of this investigation is to document the presence and quantify levels of Cd, Cu, Fe and Zn in kidneys of wolves from four geographic areas of North America, including areas far from the Arctic that may experience greater direct anthropogenic impact.

Materials and Methods

The distribution and abundance of the heavy metals Cd, Cu, Fe and Zn were measured in tissue samples of wolf kidneys. The kidneys analyzed in this study were obtained from individuals trapped, found dead, or lethally controlled and collected from 2005 to 2008 by federal and state wildlife officials in Alaska (n = 13), Idaho (n = 28), Montana (n = 32), United States, and by hunter harvest collections in the Northwest Territories, Canada (n = 42). One kidney was excised from each wolf and placed in a high-density polyethylene jar that met US EPA analyte specifications for metals. The jars were then placed in insulated coolers with ice packs for transport to the lab. Kidneys were shipped frozen on dry ice to Illinois Wesleyan University where they were stored at −80°C until they were analyzed.

Eight hours prior to digestion, kidneys were thawed to room temperature. Approximately 200–250 mg of mediodorsal cortical kidney sample (weighed wet to ±0.1 mg) were delivered directly into acid-cleaned 30-mL Teflon screw-cap vials. Within each digestion set, approximately 25% of the kidneys were sampled, weighed, and digested in triplicate, as a measure of precision and metal level homogeneity within the kidney. Additionally, in each digestion set, one previously analyzed sample was re-thawed, sampled, and analyzed. To each vial, exactly 4.00 mL of ultrapure HNO_3 (JT Baker Ultrex), followed by 1.00 mL of ultrapure H_2O_2 (JT Baker Ultrex) were added. The vials were capped loosely and the samples were allowed to dissolve at room temperature for 12–16 h. Following cool digestion, the caps were tightened and the vial was placed in a 60°C water bath for 4 h.

All samples completely dissolved following the hot digestion step. The samples were diluted by a factor of about 12 (precisely measured) with ultrapure water and transferred directly into acid-cleaned autosampler tubes. These solutions (acid content ∼8%) were then analyzed directly by ICP-optical emission spectroscopy, using a six-point external calibration for each metal. Samples remained in autosampler tubes for less than 2 weeks prior to analysis. To ensure quality control, approximately 10% of vials in each digestion set were 'digestion blanks' (no sample added, but otherwise treated identically) and an additional 10% of vials analyzed a standard reference material (DOLT-3, National Research Council Canada). To further monitor contamination in autosampler tubes and check for instrument replicability, analysis was performed in triplicate on 10% of the digestion vials. Each set also included autosampler tubes with only 8% Ultrex HNO_3, to check for potential contamination in transport to the ICP lab.

The analytical limit of detection, calculated as three times blank standard deviation and converted to kidney mass units using standard volume and mass targets, was less than 0.1 ppm (wet weight) for all metals on all analysis dates. Digestion replicate relative error was typically less than 10%, though sometimes higher for low-level Cd samples. Metal levels in reagent, digestion, vial, transport, and analysis blanks were small and consistent, and subtracted from all analyses. Average analysis of the standard reference material was within 10% of the certified value for all metals.

The data were analyzed statistically using SPSS version 17.0 (SPSS 2009). Wolves were categorized by collection location (Alaska, Idaho, Montana, Northwest Territories), sex and age class ("adults" >1 year and "subadults" <1 year). Data were tested for normality for each metal via a Kolmogorov–Smirnov normality test (Sokal and Rohlf 1995). Cd, Cu, and Fe data had non-normal distributions and were log transformed. Within each location a two-way analysis of variance (ANOVA) was performed for each metal to examine the effects of sex and age class. However, because the wolves sampled from the Northwest Territories were all adults, a one-way ANOVA was performed for each metal to examine the effects of sex. Data with non-significant differences between sexes and age classes were pooled within each location and analyzed with one-way ANOVA followed by a Hochberg's GT2 post-hoc test to compare differences among locations. Metals that differed significantly by sex and age class were analyzed separately among locations. Data reported are untransformed arithmetic means with upper and lower 95% confidence limits (Sokal and Rohlf 1995), and untransformed geometric means.

Results and Discussion

Each of the four metals was detected and quantified in all but two individuals from Montana, which did not show detectable levels of Cd (Table 1). For the entire sample of

Table 1 Concentrations, in ppm wet weight, of Cd, Cu, Fe and Zn in kidneys of grey wolves: arithmetic mean (geometric mean in parentheses), and lower and upper 95% confidence intervals are listed

Category	Cadmium		Copper		Iron			Zinc	
	Mean	95% confidence interval	Mean	95% confidence interval	Mean	n[a]	95% confidence interval	Mean	95% confidence interval
Alaska									
Male (n = 5)	1.83 (1.54)	0.26–3.39	8.76 (6.79)	0.26–17.25	**105.2 (95.6)***	(n = 3)	23.1–187.2	35.5 (33.8)	20.4–50.9
Female (n = 8)	3.14 (1.55)	0.13–6.15	7.20 (6.66)	4.85–9.54	**210.8 (203.9)***	(n = 7)	155.0–266.6	41.3 (40.1)	33.1–53.9
Adult (n = 10)	3.04 (1.71)	0.73–5.35	8.29 (7.16)	4.86–11.73	177.3 (160.9)		111.3–243.2	38.2 (36.4)	29.4–46.2
Subadult (n = 3)	1.28 (1.12)	−0.79–3.35	6.15 (5.41)	−3.76–16.06	159.4 (139.8)		−48.6–367.5	42.3 (41.6)	24.0–55.8
Overall (n = 13)	2.63 (1.55)	0.87–4.40	7.80 (6.71)	5.04–10.55	172.4 (154.8)		121.1–223.6	39.1 (37.6)	32.6–45.6
Idaho									
Male (n = 8)	0.31 (0.25)	0.12–0.51	6.31 (6.14)	4.98–7.64	105.0 (96.1)		65.4–144.6	26.0 (25.7)	22.2–29.9
Female (n = 9)	0.43 (0.30)	0.16–0.70	6.26 (5.85)	4.83–7.69	114.0 (96.0)	(n = 8)	48.7–179.3	25.0 (24.6)	21.0–29.0
Adult (n = 12)	0.41 (0.30)	0.20–0.62	6.14 (5.78)	4.98–7.30	116.6 (101.1)		69.7–163.5	25.3 (24.9)	22.2–28.3
Subadult (n = 5)	0.28 (0.23)	0.01–0.55	6.64 (6.51)	4.89–8.39	93.9 (85.9)		37.9–149.8	26.0 (25.6)	19.3–32.8
Overall (n = 28)	0.37 (0.28)	0.26–0.51	6.29 (5.98)	5.37–6.70	102.3 (96.1)		80.4–124.1	25.8 (25.4)	23.9–27.7
Montana									
Male (n = 11)	0.38 (0.39)	0.28–0.49	4.57 (4.83)	3.19–5.96	97.0 (86.2)	(n = 10)	53.9–140.1	27.4 (29.2)	24.3–30.5
Female (n = 7)	0.46 (0.00)	−0.01–0.92	7.34 (6.57)	2.60–12.08	124.4 (112.3)		74.3–174.5	27.5 (27.5)	23.7–31.2
Adult (n = 11)	0.43 (0.30)	0.15–0.70	**7.36 (6.72)***	4.77–9.95	125.9 (116.3)		89.6–162.2	28.8 (28.5)	26.1–31.4
Subadult (n = 11)	0.38 (0.37)	0.27–0.50	**2.96 (2.92)***	2.48–3.45	76.0 (64.9)	(n = 10)	19.6–132.4	25.3 (25.0)	21.3–29.3
Overall (n = 32)	0.46 (0.00)	0.34–0.58	6.27 (5.55)	5.02–7.52	111.3 (101.1)		93.7–129.0	28.7 (28.0)	25.9–31.6
NWT									
Male (n = 17)	0.84 (0.63)	0.55–1.14	7.57 (6.83)	5.47–9.67	92.0 (87.2)	(n = 14)	72.4–111.7	29.1 (28.7)	24.4–33.8
Female (n = 19)	0.91 (0.69)	0.38–1.45	7.23 (7.18)	5.37–9.10	139.5 (117.1)	(n = 13)	81.4–197.6	25.4 (26.9)	23.1–27.6
Overall (n = 42)	0.97 (0.70)	0.70–1.19	7.57 (7.01)	6.62–8.52	117.4 (100.5)		92.6–142.3	28.0 (27.8)	26.2–29.8

* Two values given in bold differ significantly ($p < 0.05$). No other comparisons of male-to-female and adult-to-subadult for each metal and location showed a statistically significant difference

[a] When listed, sample size is different from overall sample sizes listed; iron concentrations were not available for all individuals due to failed analyses

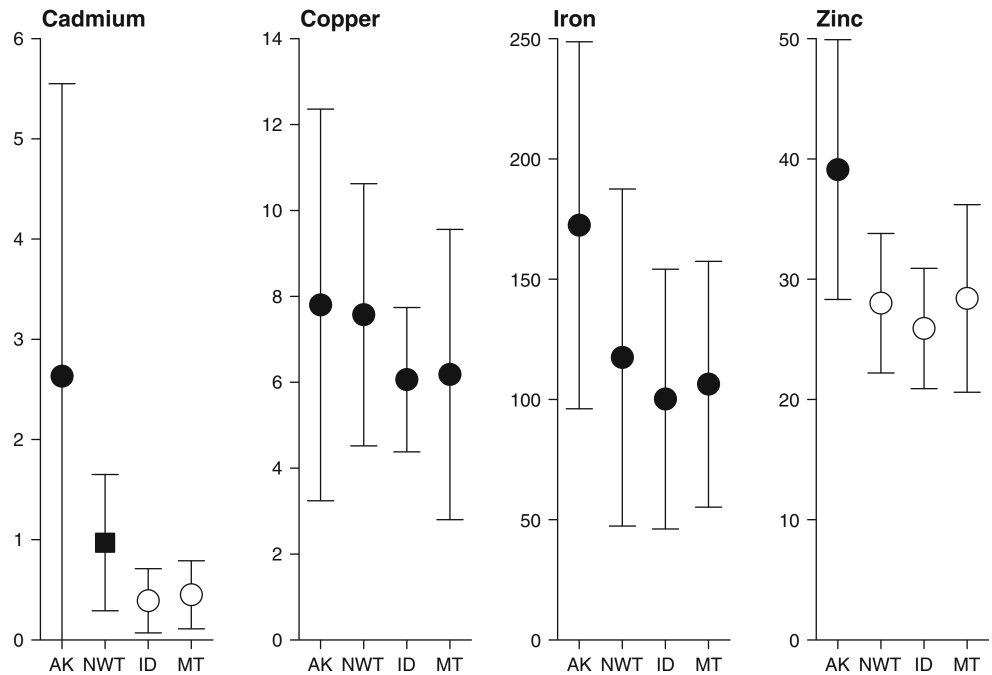

Fig. 1 Mean concentration and standard deviation of metal levels (ppm wet weight) in wolf kidneys from four locations (*AK* Alaska, *NWT* Northwest Territories, *ID* Idaho, *MT* Montana). If symbols are different between two locations, then a statistically significant ($p < 0.05$) difference was observed between the means

115 wolves, metal level ranges (10th–90th percentile) were 0.13–1.92 ppm for Cd, 4.01–10.66 ppm for Cu, 58.4–188.7 ppm for Fe, and 21.9–36.5 ppm for Zn. Female wolves from Alaska had significantly higher Fe levels than males ($F_{1,7} = 10.91$, $p = 0.013$). In addition, adult wolves from Montana had significantly higher Cu levels than subadults ($F_{1,14} = 21.17$, $p < 0.001$). No other comparisons of male-to-female or adult-to-subadult for any metal in any location showed a significant difference. The pooled data (Fig. 1) indicated that wolves from Alaska had significantly higher levels of Cd and Zn than wolves from Idaho, Montana, or the Northwest Territories. Wolves from the Northwest Territories had significantly higher levels of Cd than wolves from Idaho and Montana, while wolves from Montana had significantly higher levels of Fe than wolves from Idaho. No other significant differences in metal concentrations between the locations were observed.

An analysis of the 13 Alaska wolves showed a bimodal distribution of metal concentrations: six of the 13 wolves had relatively high metal levels for all four metals, while levels in seven wolves were much lower. The ratio of average metal levels in the high set to low set was 5.1 for Cd, 2.7 for Cu, 1.6 for Zn, and 1.5 for Fe. For Zn, the low Alaska set was not significantly different from any other site. However, for Cd, even the low Alaska set was still significantly higher than Idaho and Montana, showing a consistently higher observation of Cd levels for all Arctic or sub-Arctic wolves relative to wolves from lower latitudes. Within the Alaska set, there was no observable pattern in location, sex, or age correlating with the assignment of individuals into the high set or low set. If contamination of the high Alaska set can be traced to local conditions, the relatively large range of Arctic wolves (Walton et al. 2001) could indicate that individual wolves with high metal levels may have dispersed from areas with elevated environmental metal levels.

Heavy metals in the food chain may originate either through natural local mineral deposits or through long-distance atmospheric transport. Contamination from local mineral sources are often exacerbated by mining and subsequent riverine transport; the Alaska wolves were primarily collected in areas with significant mineral deposits (Eppinger et al. 2007) and a high level of recent and historic gold placer mining (LaPerriere et al. 1985).

The levels of Cd in wolves from Alaska were higher than those from wolves from the Yukon Territory, Canada (Gamberg and Braune (1999), and from other wildlife including arctic foxes and wolverines (*Gulo gulo*) (Hoekstra et al. 2003). Wolves in this study from the Northwest Territories had Cd levels comparable to those found in arctic foxes from the Arviat region in Canada (Hoekstra et al. 2003), while Cu and Zn concentrations from wolves collected in all regions were higher than those from wolves from the Canadian Arctic (Gamberg and Braune 1999).

The spatial variability observed of metal levels in wolf kidneys is consistent with variability observed in other studies on metal levels in Arctic and sub-Arctic wolf prey, including moose (*Alces alces*) (Venalainen et al. 2005; Danielsson and Frank 2009) and caribou (Aastrup et al. 2000; Larter and Nagy 2000; Elkin and Bethke 1995); Aastrup et al. (2000) further noted that Zn, Cu, and Cd in caribou livers were higher in late winter after a season of feeding on long-lived (and therefore long-term bioaccumulating) lichens.

The significantly higher concentration of Fe in females than in males in Alaska is surprising and inconsistent with other studies. Addison et al. (1972) found that iron levels are generally lower in females than in males, which is due to differences in iron storage and hemoglobin amounts (Gochfeld 1997). However, Gochfeld (1997) found that some heavy metals are often found in higher concentrations in females than males in a number of species, which may be attributed to different behaviors of heavy metals in the two sexes. In addition, one sex may show higher levels of a particular metal in the liver, while the other may show higher levels in the kidney (Gochfeld 1997). Examination of heavy metal levels in wolf liver tissues would be necessary to determine if this factor contributed to the higher level of Fe in females than males.

Acute toxicity data for heavy metals in wolves is lacking, but comparison to the veterinary literature for kidneys of domestic dogs (*Canis lupus familiaris*) suggests that the levels observed are significantly below acute toxicity levels (Puls 1994). Cadmium in the kidneys in more than 80% of the individual wolves in this study were above the "normal" range for dogs (0.12–0.18 ppm wet wt.), but none approached "toxic" levels (>200 ppm wet wt.). Similarly, no individual wolves approached the "toxic" level for dogs for Fe, Cu, or Zn.

The relatively small sample sizes in some subsamples may have affected the results of this study. In addition, only adult wolves were collected from the Northwest Territories, making it impossible to determine the effect of age class on metals within this region. Heavy metal concentrations determined within this study should be considered as baseline values for comparison in future investigations, such as those that may determine if biomagnification of heavy metals has occurred in wolf food chains.

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