

# WILDLIFE AS BIOSAMPLERS: CONTAMINANTS IN HAIR OF ELK HARVESTED NEAR THE ANACONDA SMELTER SITE

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## ABSTRACT

The purpose of this research was to test a new way of investigating biological uptake of smelting-related contaminants with a focus on harvested wildlife. Specific objectives were 1) to collect hair samples from elk (*Cervus elaphus*) harvested in the vicinity of the Anaconda Smelter National Priority List Site in Montana, 2) to analyze the samples using inductively coupled plasma – mass spectrometry (ICP-MS), and 3) to identify potential elements of concern from the data. Hair samples were collected from 56 elk, and concentration data were processed using a hazard quotient/index approach based on concepts commonly used in fields of ecological and human health risk analyses. Arsenic concentrations in the hair decreased as a function of increasing distance from the Anaconda smelter stack, and 57 % of the elk sampled were identified as animals of concern. For elk harvested within 25 km of the stack, elements of concern were aluminum, arsenic, barium, boron, lithium, manganese, molybdenum, strontium, and vanadium. For elk harvested within 76-101.5 km of the stack, elements of concern were aluminum, barium, boron, lithium, and manganese. Hazard indices for uranium, arsenic, cadmium, and lithium were larger by factors of ~17, 9, 7, and 6, respectively, for elk harvested within 25 km of the stack compared to hazard indices for elk harvested within 76-101.5 km.

**Key Words:** arsenic, pollution, biomonitoring, hair samples.

## INTRODUCTION

For more than a century, smelting activities in Anaconda caused wide-spread contamination in Montana. The United States Environmental Protection Agency (USEPA) listed the Anaconda Smelter Site on the Superfund National Priorities List (NPL) in 1983 (USEPA 1998). As a part of the Superfund activities, several contaminants have been characterized, risks to human health have been estimated, and some cleanup has taken place. Unfortunately, few data have addressed biological uptake of contaminants by human or wildlife populations.

During the past decade, we developed and tested a new way to study contaminant

uptake using domestic pets as bioindicators of environmental conditions in Butte and Anaconda (Peterson and Madden 2006). The technique involved sampling the hair of domestic dogs (*Canis lupus familiaris*) and cats (*Felis catus*), analysis by inductively coupled plasma-mass spectrometry (ICP-MS), and identification of elements of concern with a hazard index approach similar to methods employed in the field of risk analysis. More than 400 samples from the domestic pet population identified eight elements of concern (aluminum, arsenic, boron, lead, lithium, manganese, molybdenum, and selenium) in residential neighborhoods of Butte and Anaconda (Madden 2006, Barry 2006, Peterson and Barry 2006, and Robertson 2007).

Similar to our previous field campaigns, the overall goal of the research presented in this paper was to improve understanding of biological uptake of environmental contaminants. Instead of domestic pets, however, we addressed harvested wildlife. Specifically, we targeted the local elk (*Cervus elaphus*) population, and objectives were 1) to collect hair samples from elk harvested in the vicinity of the Anaconda Smelter NPL Site, 2) to analyze the samples with ICP-MS, and 3) to identify potential elements of concern from the data.

### **Uptake of Contaminants by Resident Wildlife**

As part of remedial investigation/feasibility studies of the Anaconda Smelter Site, numerous sampling campaigns were conducted to characterize risk and burden of pollutants on the surrounding environment (USEPA 1998). Few studies, however, were performed to characterize exposure and uptake of these contaminants for resident wildlife species, nor to monitor the efficacy of environmental cleanup. Initial assessment of ecological risk used a simple, predictive food chain model (USEPA 1998) without direct consideration of wildlife. Following the initial assessment, a handful of projects addressed contaminants in small mammals and avian species (Hopper et al. 2002). From recreational and wildlife management viewpoints, however, large mammal populations in the vicinity of the Anaconda Smelter Site were neither sampled nor monitored.

### **Hair Samples as Biosamplers of Environmental Exposure**

We were the first to propose domestic pet hair as a unique tool for studying residential exposure to mining-related contaminants (Peterson and Madden 2006). In human populations, however, hair and toenails have been used for many years in the field of forensics to determine possible cause of death by ingestion of toxic metals and/or medicines (Chatt and Katz 1988). Likewise, human hair samples have been used by law enforcement and by employers

as evidence of illegal drug and alcohol use (Pragst and Balikova 2006).

Elements in the bloodstream of mammals are transferred from the root cells into the hair shaft during growth stages (Beernaert et al. 2007). Hair consists of keratin with cysteine sulfhydryl groups capable of binding to metals and other elements (Mandal and Suzuki 2002). Siedel et al. (2001) and others presented uncertainties about external contamination, but Hinwood et al. (2003) concluded hair sampling to be a good “screening-level” technique for studying environmental exposure if care is taken to properly handle, rinse, and analyze the specimens.

In addition to our research in Butte and Anaconda, field campaigns elsewhere have been advancing the legitimacy of hair sampling as a research tool. Rashed and Soltan (2005), for example, analyzed hair of goats (*Capra hircus*), sheep (*Ovis aries*), and camels (*Camelus*) in Egypt, and concentrations of cadmium, cobalt, iron, lead, manganese, and nickel in the hair correlated to contaminants in vegetation consumed by the animals. D’Have et al. (2009) linked concentrations of lead and cadmium in hair of European Hedgehog (*Erinaceus europaeus*) to contaminant concentrations in the soil. Mercury concentrations were studied in hair of wild boars (*Sus scrofa*) by Sobanska (2005), in hair of deer mice (*Peromyscus maniculatus*) by Waring and Douglass (2007), and in hair of sled dogs by Dunlap et al. (2007). Beernaert et al. (2007) found linear relationships of lead and cadmium among hair, kidney, and liver samples in the Wood Mouse (*Apodemus sylvaticus*). McLean et al. (2009) also linked concentrations of lead and cadmium in soil with hair concentrations from small mammals residing near a decommissioned lead and zinc smelter in Australia. Finally, pollution in Nairobi, Kenya, was studied using hair samples from residential pets and wildlife (Mwaniki 2007). Prior to results summarized here, however, no data were available for wild game species residing on

or near contaminated Superfund sites in the United States.

## METHODS

As described in more detail by Gillespie (2011), we conducted field campaigns in the Anaconda, Montana area during two hunting seasons (October-November of 2009 and October-November of 2010). In 2009, we collected hair samples from wild game at the Montana Fish, Wildlife and Parks (FWP) hunting check station located along Mill Creek. In 2010, we obtained samples at the Mill Creek check station and at another FWP check station in Divide, Montana. Both stations were selected based on elk populations commonly harvested in the vicinity of the Anaconda Smelter NPL Site.

Regarding experimental protocol, we completed a questionnaire for each animal in our study. Specimens were assigned identification numbers. Hunters were also asked in which hunting districts and drainages the animals were harvested. Other information, such as sex and approximate age of the animal, was documented (Gillespie 2011).

Hair samples, ~ 150 milligrams (mg) in size, were removed from the harvested animals' coats with clean stainless steel scissors. When possible, the hair sample was collected from the region between the shoulder and neck of the animal. Samples were sealed in contaminant-free envelopes and stored until the end of each hunting season when they were sent to Trace Elements, Incorporated (Addison, Texas). Hair was examined with a microscope and rinsed repeatedly with de-ionized water to remove external soil particles prior to analysis by inductively coupled plasma-mass spectrometry. Trace Elements, Incorporated, is a licensed, certified clinical laboratory.

We analyzed the concentration data using the hazard index technique of Peterson and Madden (2006). The method is based on concepts commonly used in the fields of ecological and human health risk analyses.

A hazard quotient ( $HQ_{ij}$ ) of element  $i$  for animal  $j$  was calculated as:

$$HQ_{ij} = \frac{C_{ij}}{RfC_i} \quad (1)$$

where  $C_{ij}$  was concentration of element  $i$  in the hair sample of animal  $j$ ; and  $RfC_i$  values were the same reference concentrations used in our other research projects (TEI 2005, Peterson and Madden 2006, Madden 2006, Barry 2006, and Robertson 2007).

In addition to hazard quotients, two hazard indices were examined. A normalized animal hazard index ( $HI_j$ ) was calculated by summing the hazard quotients across the elements:

$$HI_j = \frac{\sum_{i=1}^{i=M} HQ_{ij}}{N} \quad (2)$$

where  $N$  was the number of elements. Likewise, a normalized element hazard index ( $HI_i$ ) was calculated by summing the hazard quotients across the number of samples:

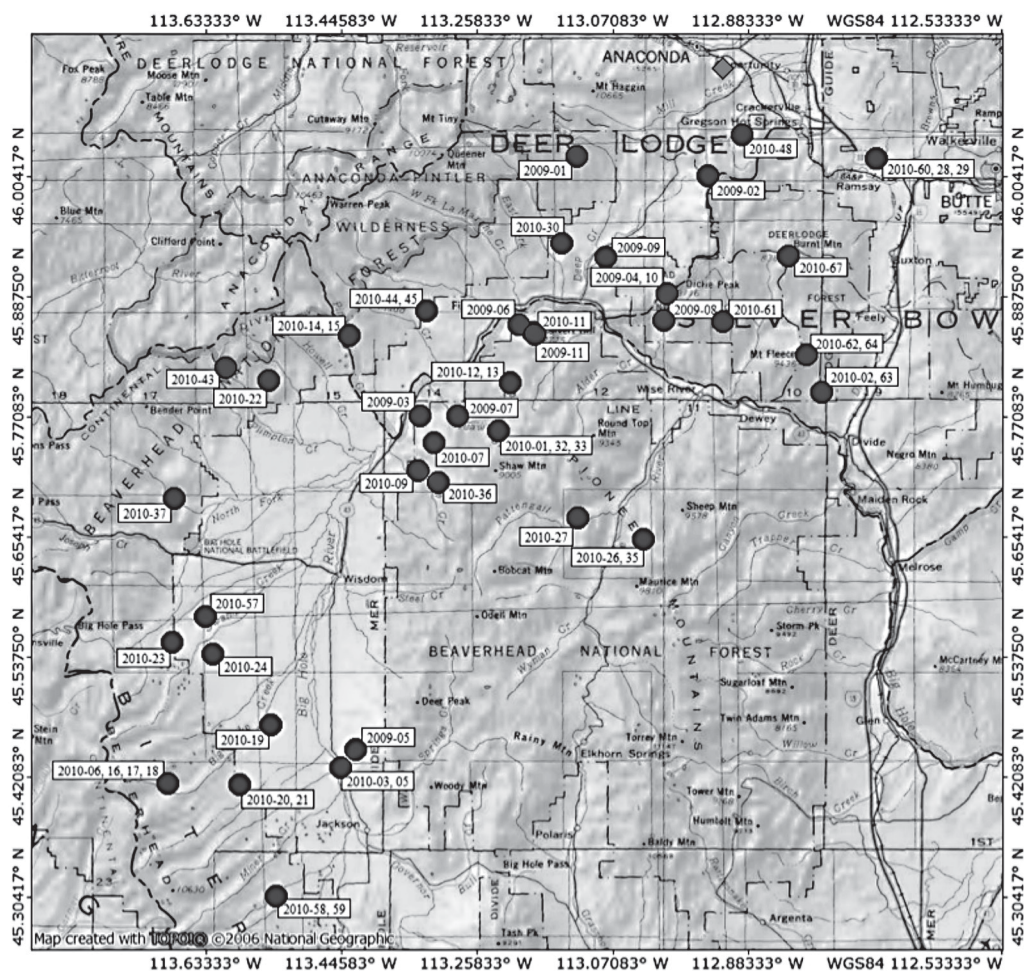
$$HI_i = \frac{\sum_{j=1}^{j=M} HQ_{ij}}{M} \quad (3)$$

where  $M$  was the total number of animals sampled. As per the method of Peterson and Madden (2006), the target value was 1.0 for both  $HI_j$  and  $HI_i$ . Animals with  $HI_j$  values  $\geq 1.0$  were defined as animals of concern, and elements with  $HI_i$  values  $\geq 1.0$  were defined as elements of concern.

## RESULTS

During field campaigns in 2009 and 2010, we collected hair samples from 56 elk harvested in the vicinity of the Anaconda Smelter NPL Site (Fig. 1). Harvest locations of the elk in the study corresponded to distances ranging 7.5-101.5 km from the Anaconda smelter stack, and our dataset consisted of hair samples from 31 adults, 25 sub-adults, 28 males, and 27 females (Table 1). Adult elk in this project were defined





**Figure 1.** Map of harvest locations (circles) for 56 elk sampled during field campaigns in 2009 and 2010. The Anaconda smelter stack is represented by a diamond, and harvest locations are labeled with sample identification numbers. As a scale of reference, the distance between the stack and harvest location is 19.1 km for Sample 2010-28, and the corresponding distance is 101.5 km for Sample 2010-59.

as 3 years of age and older, and sub-adults were younger than 3 years.

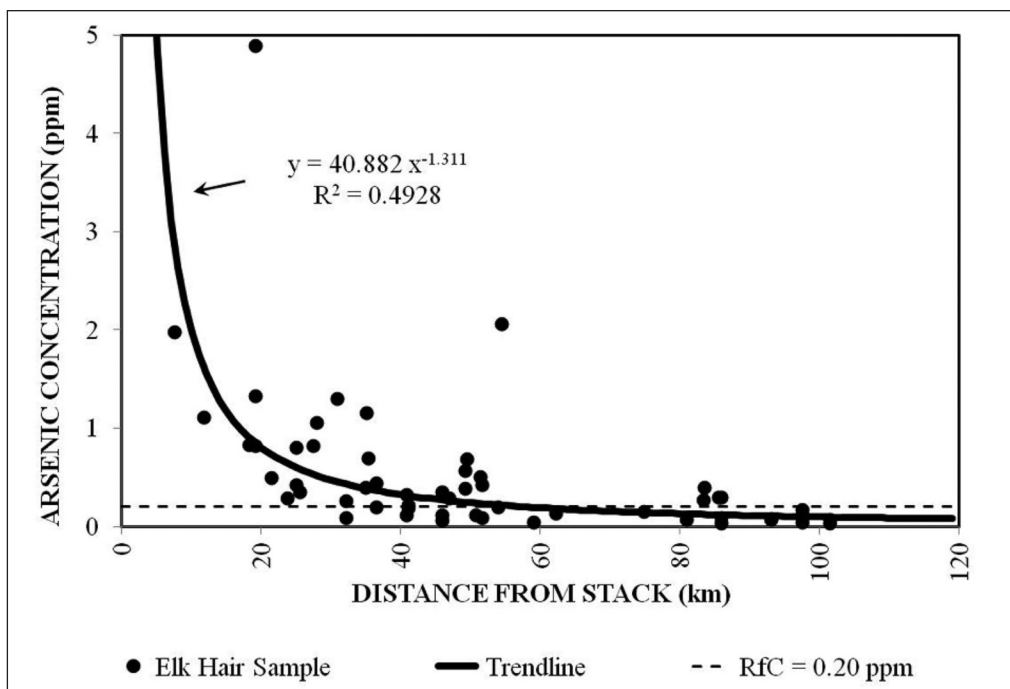
Concentrations of arsenic in the elk hair decreased as a function of increasing distance from the stack (Fig. 2). Thirty-six (~ 64 percent) of the samples contained arsenic concentrations greater than a reference concentration of 0.20 parts per million (ppm), and based on the best-fit equation in Figure 2, arsenic concentrations did not fall below 0.20 ppm until harvest distances were greater than ~ 58 km from the stack.

Using Equation (1) to calculate hazard quotients, Figure 3 depicts data for 14 elements in Samples 2010-28 and 2010-59. The elk for Sample 2010-28 was harvested ~19.1 km southeast of the smelter stack, and the elk for Sample 2010-59 was harvested ~101.5 km southwest of the stack. Thirteen elements in Sample 2010-28 exceeded a hazard quotient of 1.0, and elements with the highest  $HQ_i$  values were lithium (42.9), manganese (25.6), and arsenic (24.5). In contrast, the only elements in Sample 2010-59 with  $HQ_i$  values  $\geq 1.0$  were

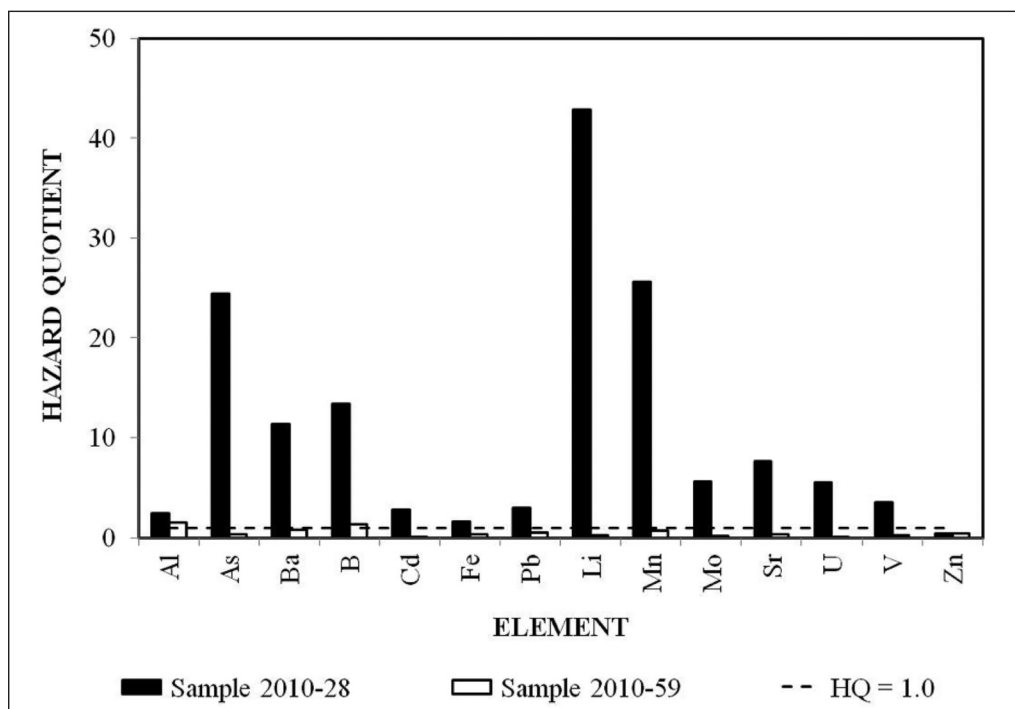
**Table 1.** Sample identification number, sex-age class, animal number (j), distance between harvest location and Anaconda smelter stack ( $X_j$ ), arsenic concentration ( $C_j$ ), arsenic hazard quotient (HQ<sub>j</sub>), and animal hazard index (HI<sub>j</sub>) for 56 elk harvested during field campaigns in 2009-2010.

Sample ID	Class*	j	$X_j$ (km)	Arsenic $C_j$ (ppm)	Arsenic HQ <sub>j</sub>	HI <sub>j</sub>	Sample ID	Class	j	$X_j$ (km)	Arsenic $C_j$ (ppm)	Arsenic HQ <sub>j</sub>	HI <sub>j</sub>
2009-01	MA	1	18.3	0.83	4.2	1.9	2010-20	FS	29	93.1	0.08	0.4	0.6
2009-02	MS	2	11.7	1.11	5.6	1.4	2010-21	FA	30	93.1	0.07	0.4	0.7
2009-03	MS	3	49.5	0.69	3.5	1.7	2010-22	MA	31	59.1	0.05	0.3	0.7
2009-04	MA	4	25.0	0.81	4.1	2.0	2010-23	FA	32	85.6	0.30	1.5	0.9
2009-05	MS	5	83.4	0.27	1.4	1.3	2010-24	MA	33	83.5	0.40	2.0	0.8
2009-06	FS	6	35.3	0.70	3.5	1.2	2010-26	MA	34	51.6	0.43	2.2	1.2
2009-07	FS	7	47.0	0.29	1.5	1.2	2010-27	MA	35	51.4	0.51	2.6	5.0
2009-08	FA	8	27.9	1.06	5.3	1.0	2010-28	MA	36	19.1	4.89	24.5	10.7
2009-09	FS	9	23.8	0.29	1.5	0.8	2010-29	FA	37	19.1	0.82	4.1	1.1
2009-10	FA	10	25.0	0.43	2.2	1.1	2010-30	MS	38	25.5	0.35	1.8	1.2
2009-11	FA	11	35.1	1.16	5.8	1.3	2010-32	MS	39	45.9	0.35	1.8	1.4
2010-01	FS	12	45.9	0.06	0.3	0.7	2010-33	MS	40	45.9	0.12	0.6	1.1
2010-02	MA	13	36.5	0.44	2.2	1.2	2010-35	MS41	51.6	0.09	0.5	0.6	
2010-03	MS	14	85.9	0.04	0.2	0.5	2010-36	FA	42	54.0	0.20	1.0	0.6
2010-05	US	15	85.9	0.09	0.5	1.8	2010-37	FA	43	74.8	0.15	0.8	1.7
2010-06	MS	16	97.5	0.11	0.6	0.8	2010-43	MA	44	62.2	0.14	0.7	2.0
2010-07	FA	17	50.8	0.12	0.6	0.7	2010-44	FA	45	41.1	0.22	1.1	0.7
2010-09	FA	18	54.4	2.06	10.3	4.1	2010-45	MS	46	41.1	0.19	1.0	0.7
2010-10	FA	19	30.9	1.30	6.5	1.8	2010-48	MA	47	7.5	1.98	9.9	1.7
2010-11	MS	20	34.9	0.40	2.0	0.7	2010-57	FS	48	81.0	0.07	0.4	0.6
2010-12	FS	21	40.8	0.12	0.6	0.7	2010-58	FA	49	101.5	0.04	0.2	0.5
2010-13	MS	22	40.8	0.33	1.7	1.4	2010-59	MA	50	101.5	0.07	0.4	0.5
2010-14	MA	23	49.2	0.39	2.0	1.6	2010-60	FS	51	19.1	1.33	6.7	1.1
2010-15	MA	24	49.2	0.57	2.9	1.4	2010-61	MS	52	27.4	0.82	4.1	1.8
2010-16	FS	25	97.5	0.17	0.9	0.8	2010-62	FA	53	32.1	0.26	1.3	0.6
2010-17	FA	26	97.5	0.05	0.3	0.4	2010-63	FS	54	36.5	0.20	1.0	0.7
2010-18	MS	27	97.5	0.12	0.6	1.1	2010-64	FA	55	32.1	0.09	0.5	0.5
2010-19	FA	28	85.9	0.30	1.5	2.2	2010-67	MA	56	21.4	0.50	2.5	1.1

\*Class of Animal: MA = Male Adult, MS = Male Sub-Adult, FA = Female Adult, FS = Female Sub-Adult, U = Unknown



**Figure 2.** Arsenic concentration in hair samples from elk harvested at distances ranging 7.5-101.5 km from the Anaconda smelter stack. A best-fit trendline of the data and an arsenic reference concentration of 0.20 ppm are also shown.



**Figure 3.** Hazard quotient values for Samples 2010-28 and 2010-59. These hair samples were collected from adult elk harvested 19.1 and 101.5 km from the Anaconda smelter stack, respectively. For 14 elements, the corresponding animal hazard indices ( $HI_i$ ) are 10.7 and 0.5.

aluminum (1.5) and boron (1.3). For these two samples, the animal hazard indices from Equation (2) were 10.7 and 0.5, respectively. Based on the  $HI_j$  value  $\geq 1.0$ , the elk corresponding to Sample 2010-28 was identified as an animal of concern, as were 32 (~ 57 percent) of the 56 elk sampled (Table 1).

As per concentration statistics of the data set, no samples contained concentrations exceeding the reference concentration for zinc (Table 2), and average and median concentrations were lower than reference concentrations for cadmium, iron, lead, molybdenum, strontium, uranium, vanadium, and zinc. However, because the main source of contamination was the smelter, we divided the samples into four zones according to distance (X) between harvest location and the Anaconda smelter stack: Zone 1 (X<25 km), Zone 2 (26-50 km), Zone 3 (51-75 km), and Zone 4 (76-101.5 km). Element hazard indices were calculated with Equation (3) for each of the four zones (Table 3), and elements of concern were identified.

While Zones 2 and 3 revealed more variability,  $HI_i$  values decreased from

Zone 1 to Zone 4 for most of the elements. In Zone 1,  $HI_i$  was  $\geq 1.0$  for aluminum, arsenic, barium, boron, lithium, manganese, strontium, and vanadium. Of these elements of concern, the largest hazard indices in Zone 1 were for arsenic (6.5), lithium (6.0), and manganese (5.3). For elk harvested in Zone 4, however, the only elements of concern were aluminum, barium, boron, lithium, and manganese, with manganese exhibiting the largest hazard index (2.8). Zinc showed no spatial variation among the zones, but the element hazard indices were dramatically larger in Zone 1 compared to Zone 4 by factors of 16.7 for uranium, 8.9 for arsenic, 6.7 for cadmium, and 5.6 for lithium.

## DISCUSSION

In this project, we proposed and tested a novel way to study contaminant uptake using elk as biosamplers of environmental conditions near the Anaconda Smelter NPL Site. Even though the Environmental Protection Agency has been directing cleanup activities in the area for many years, large mammal populations have not been addressed. Based on our data, elk in the

**Table 2.** Element (i), reference concentration ( $RfC_i$ ), minimum (Min), maximum (Max), average (Avg), median (Med), and standard deviation (Stdev) concentration ( $C_i$ ) measured in hair samples of 56 elk harvested during field campaigns in 2009-2010.

I	Element i	$RfC_i$ (ppm)	Min $C_i$ (ppm)	Max $C_i$ (ppm)	Avg $C_i$ (ppm)	Med $C_i$ (ppm)	Stdev $C_i$ (ppm)
1	Aluminum (Al)	32	5	648	73	49	103
2	Arsenic (As)	0.20	0.04	4.89	0.52	0.29	0.75
3	Barium (Ba)	4	1.8	45.6	6.3	4.6	6.3
4	Boron (B)	5.9	3.6	79.1	12.2	9.9	10.1
5	Cadmium (Cd)	0.20	0.01	0.56	0.07	0.03	0.10
6	Iron (Fe)	99	12	902	84	56	130
7	Lead (Pb)	2	1	8	1	1	1
8	Lithium (Li)	0.08	0.02	3.43	0.19	0.11	0.45
9	Manganese (Mn)	3.3	0.97	84.51	12.90	8.54	14.13
10	Molybdenum (Mo)	0.22	0.02	1.6	0.14	0.08	0.26
11	Strontium (Sr)	5.4	0.7	41.1	3.7	2.7	5.3
12	Uranium (U)	0.20	0.005	1.1	0.07	0.015	0.16
13	Vanadium (V)	0.60	0.02	2.47	0.35	0.14	0.52
14	Zinc (Zn)	200	70	140	100	100	10

**Table 3.** Element (i) and element hazard index (HI<sub>i</sub>) values for Zone 1 (X<sub>j</sub><25 km), Zone 2 (26-50 km), Zone 3 (51-75 km), and Zone 4 (76-101.5 km). Also shown is the ratio of the hazard indices for Zone 1:Zone 4.

i	Element i	Zone 1 HI <sub>i</sub>	Zone 2 HI <sub>i</sub>	Zone 3 HI <sub>i</sub>	Zone 4 HI <sub>i</sub>	Zone1:Zone4 Ratio
1	Aluminum (Al)	1.8	1.6	6.2	1.6	1.1
2	Arsenic (As)	6.5	2.2	2.3	0.7	8.9
3	Barium (Ba)	2.2	1.2	1.8	1.6	1.4
4	Boron (B)	3.2	2.0	1.8	1.6	2.0
5	Cadmium (Cd)	0.8	0.3	0.4	0.1	6.7
6	Iron (Fe)	0.7	0.5	2.1	0.8	0.9
7	Lead (Pb)	0.8	0.5	1.1	0.5	1.5
8	Lithium (Li)	6.0	1.5	2.5	1.1	5.6
9	Manganese (Mn)	5.3	3.3	5.9	2.8	1.9
10	Molybdenum (Mo)	1.0	0.4	1.2	0.4	2.6
11	Strontium (Sr)	1.3	0.6	0.6	0.6	2.3
12	Uranium (U)	0.8	0.2	0.6	0.1	16.7
13	Vanadium (V)	1.1	0.5	0.8	0.3	3.2
14	Zinc (Zn)	0.5	0.5	0.5	0.5	1.0

vicinity of the site are still being exposed to significant amounts of contamination. While results from this campaign are site-specific, our technique could be used at other sites where anthropogenic pollution is of concern, and where efficacy of remediation is in question.

Concentrations for many contaminants in our dataset increased for elk harvested closer to the Anaconda stack. Elk are migratory animals, however, and we know that contaminant concentrations in hair are not solely dependent on environmental conditions at the harvest locations. To advance our fundamental understanding of variability within and among the samples, we recommend future research to merge hair sampling with radio-collar tracking for a subset of elk during the growth period of the hair (i.e., for several months prior to hunting season). With subsequent environmental sampling along the migratory path, uptake of contaminants into hair could be correlated to pollution concentrations in the soil, vegetation, and water within a specific habitat. In addition, in-depth medical research should scrutinize health effects associated with uptake of these pollutants by the local elk population.

Specifically, studies should address the impact of contaminants on the health of the game animals; however, hunters and their families are also at risk of developing health problems if they routinely ingest wild meat contaminated with arsenic and other contaminants. This latter topic will be the focus of a follow-up paper by our research group.

## SUMMARY

We conducted the first known field campaign using hair samples to investigate uptake of environmental contaminants for harvested wildlife residing in a Superfund area. Based on 56 elk harvested in the vicinity of the Anaconda Smelter NPL Site during hunting seasons in 2009 and 2010, ~57% of the elk sampled were identified as animals of concern. Manganese, arsenic, and lithium were identified as elements of most concern, especially for elk harvested within 25 km of the smelter stack. In addition, hazard indices for uranium, arsenic, cadmium, and lithium were larger for elk harvested within 25 km of the stack by factors of ~17, 9, 7, and 6, respectively, compared to elk harvested within 76-101.5 km.



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