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Reviewer Anonymity

The identity of all reviewers shall remain anonymous to the authors, called a blind review process. All criticisms or comments by authors shall be directed to the EIC; they may be referred to the ME or the Editorial Board by the EIC for resolution.

Microhabitat Characteristics Relative to Lek Abandonment by Greater Sage Grouse in the Dakotas

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Abstract

We compared peripheral microhabitat characteristics to identify possible reasons for greater sage grouse (*Centrocercus urophasianus*) lek abandonment in North Dakota and South Dakota. Comparisons of active leks in the Dakotas were made with active leks in eastern Montana. We systematically selected 12 sample sites at equidistant points from each other within 1.5 km of the lek center. Only non-tilled areas were sampled, but tillage generally comprised <5 percent of sample sites and was evaluated in a separate landscape-level study. We detected no differences ($P > 0.10$) between sagebrush (*Artemisia spp.*) cover or density around active leks compared to the same attributes around historically active but now inactive leks in North and South Dakota. However, big sagebrush (*A. tridentata*) height, forb cover, and bare ground were greater ($P < 0.10$) around active leks compared to inactive leks in North Dakota. The area within 1.5 km of active leks in eastern Montana had much greater ($P < 0.10$) cover and density of sagebrush than active leks in either North or South Dakota. Sagebrush characteristics, i.e., coverage, density, and height, peripheral to active leks in the western Dakotas appeared desirable for sage grouse nesting sites compared to nesting habitat described in other areas of more classic habitat in Montana or Idaho. The substantial forb and grass cover association with marginal sagebrush coverage in the Dakotas apparently provides adequate nesting and brood rearing habitat.

Key words: *Artemisia spp.*, *Centrocercus urophasianus*, greater sage grouse, lek, Montana, North Dakota, sagebrush, South Dakota

Introduction

Total numbers of males among greater sage grouse (*Centrocercus urophasianus*) and males/lek have steadily declined in the past 50 years with many leks on the eastern edge of the species' range in the Dakotas becoming inactive (Smith et al. 2004). Our knowledge of sage grouse habitat in the Dakotas is limited, particularly that relative to habitat characteristics that may lead to lek abandonment by males or factors contributing to population declines. Researchers have attributed declining populations of sage grouse during the 1900s to the decrease of sagebrush (*Artemisia spp.*) due in part to conversion to tillage agriculture, overgrazing, fire, and/or drought (Patterson 1952, Rogers 1964, Gregg et al. 1994, Connelly and Braun 1997, Connelly et al. 2000). Distribution of sage grouse in the western Dakotas lies along an eastward extension of sagebrush steppe, including both big sagebrush (*A. tridentata*) and silver sagebrush (*A. cana*) (Schroeder et al. 1999). Laycock (1967) and Frischknecht and Harris (1973) reported that overgrazing by sheep negatively affected sagebrush, and in South Dakota, domestic sheep grazing

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reduced sagebrush production by 40 percent in 2 years (Bever 1951). Sage grouse rely on sagebrush for food, shelter, water, and escape cover (Swenson et al. 1987, Fischer et al. 1996, Paige and Ritter 1999, Schroeder et al. 1999). Connelly et al. (1991), Gregg et al. (1994), and Crawford (1997) have shown that along with sagebrush habitat herbaceous cover is important to sage grouse—a complex critical for nesting and early brood rearing (Connelly et al. 2000). Since male sage grouse also tend to establish display grounds (leks) in areas occupied by prenesting females (Gibson 1996), good habitat in proximity to leks likely improves chances of male sage grouse attracting females.

Our objectives were to 1) determine if differences occur in microhabitat characteristics, e.g., percent sagebrush canopy cover, big sagebrush height, silver sagebrush height, herbaceous cover of grass and forbs, etc., between active and inactive leks that may be related to abandonment, and 2) discern how microhabitat around leks on the eastern edge of the sage grouse range compares to microhabitat characteristics around leks more central to the sage grouse range in Montana. A separate landscape level study (Smith et al. 2005) evaluated macro-habitat influences that included influences of tilled ground.

Study Area

The study area (Fig. 1) was located in extreme western South Dakota in Fall River, Butte, and Harding counties, southwestern North Dakota in Bowman, Slope, and Golden Valley counties, and southeastern Montana in Garfield, Rosebud, Custer, and Powder River counties. Elevation in the South Dakota study area ranges from 525 to1050 m above sea level; unglaciated rolling prairie with occasional buttes and intermittent streams characterize topography of the area (Johnson 1976, Kalvels 1982, Johnson 1988). Annual precipitation ranges from 37.4 to 41.8 cm with ~ 80 percent falling from April to September (Johnson 1976, Kalvels 1982, Johnson 1988). Seasonal temperatures range from 14.3 to 31.1 °C during summer and from -14.6 to -1.4 °C during winter (Johnson 1976, Kalvels 1982, Johnson 1988).

Elevation in the North Dakota study area ranges from 660 to 1970 m above sea level; general topography resembles the South Dakota study area but with pinnacles, domes, canyons, gorges, ravines, and gullies associated with the Little Missouri Badlands (Opdahl et al. 1975, Thompson 1978, Aziz 1989). Annual precipitation ranges from 35.6 cm to 40.6 cm with ~ 80 percent falling from April to September (Opdahl et al. 1975, Thompson 1978, Aziz 1989). Summer temperatures range from 9.9 to 27.5° C and -15.6—0.2° C during winter (Opdahl et al. 1975, Thompson 1978, Aziz 1989).

Elevation in the Montana study area ranges from 575 to 2480 m above sea level; rolling to eroded sedimentary terraces dominate the landscape (Nunns 1943, Parker 1971, USDA Natural Resources Conservation Service 1996, Drummond 2003). Annual precipitation ranges from 25.5 cm to 35.6 cm with ~ 80 percent falling during April-September (Nunns 1943, Parker 1971, USDA Natural Resources Conservation Service 1996, Drummond 2003). Summer temperatures range from 13.8 to 22.6° C and from -9.3 to 0.7° C during winter (Nunns 1943, Parker 1971, USDA Natural Resources Conservation Service 1996, Drummond 2003).

Our study areas fall within the big sagebrush-wheatgrass plains vegetation type (Johnson and Larson 1999). Vegetation communities consist of a mixture of shrubs that include big sagebrush, silver sagebrush, and greasewood (*Sarcobatus vermiculatus*). We did not identify big sagebrush to subspecies at our study sites although Wyoming big sagebrush (*A .t. wyomingensis*) is ubiquitous across this region, and basin big sagebrush (*A. t. tridentata*) also overlapped this range. Perennial grasses include Kentucky bluegrass (*Poa pratensis*), western wheatgrass (*Agropyron smithii*), and Japanese brome (*Bromus japonicus*), and forbs include common dandelion

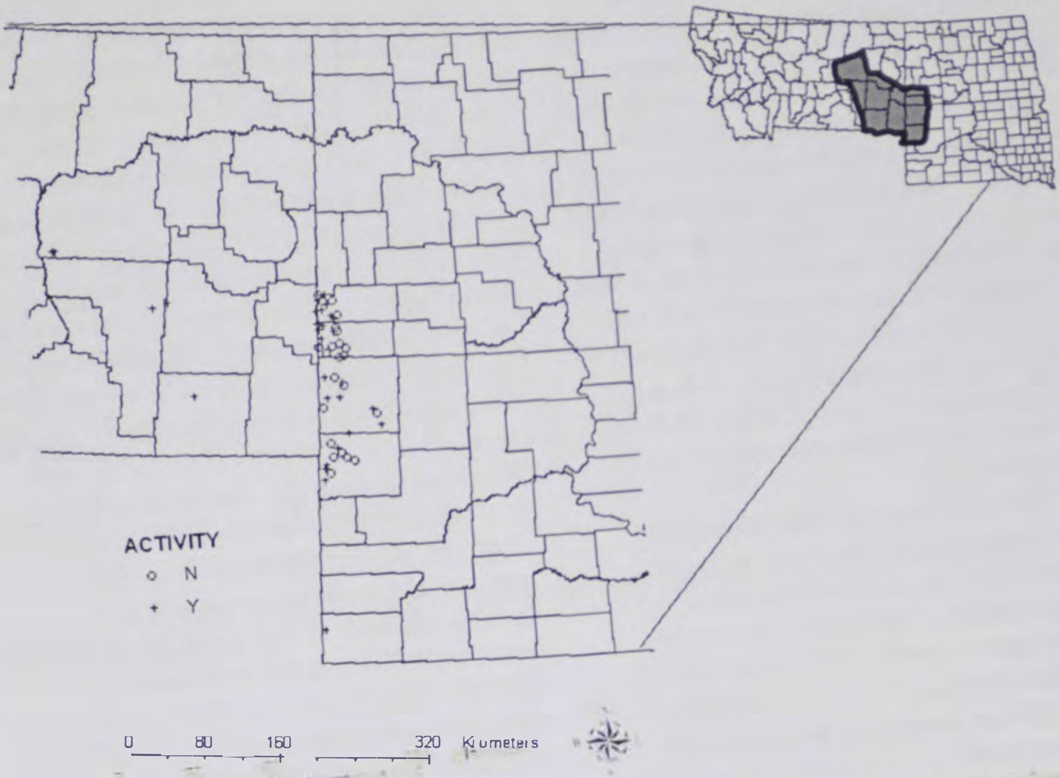

Figure 1. Approximate active and inactive lek locations used for microhabitat comparisons.

(*Taraxacum officinale*), common yarrow (*Achillea millefolium*) and cudweed sagewort (*Artemisia ludoviciana*). Agricultural lands included cash crops (corn, wheat, and alfalfa), tilled land, and open grassland.

Methods

Collecting microhabitat data

We sampled microhabitats during 14 May–27 June of 2001 and 2002 near sage grouse leks in North Dakota ($n = 27$), South Dakota ($n = 22$), and Montana ($n = 5$). Data were gathered from both active and inactive lek sites (Fig. 1). Connelly et al. (2000) defined an active lek as having >2 male sage grouse in >2 of the previous 5 years. We considered a lek inactive when it did not meet criteria for an active lek. An inactive lek may have at one time been active but was inactive at the time of the study (Smith et al. 2004). We received legal descriptions of lek locations, i.e., township, range, section, quarter-section, from North Dakota Game and Fish Department (NDGF), South Dakota Department of Game, Fish and Parks (SDGFP), Montana Fish, Wildlife and Parks, and USDA Forest Service (USFS). Visits to each lek were made in the spring of 2001 and 2002 to assist NDGF, SDGFP, and USFS in determining activity and to get Universal Transverse Mercator (UTM) coordinates of the lek centers. Centers of the quarter sections were used as the proximal inactive lek centers. We then entered coordinates into a computer, referenced, and overlaid onto a map of western South Dakota, southwestern North Dakota, eastern Montana using the geographic information system (GIS) ArcView 3.2a (Environmental Systems Research Institute [ESRI], Inc. 1999). We buffered the center each lek using an area with a radius of 1.5 km to use as an indicator of habitat quality for microhabitat analysis. Aldridge (2000) found an average lek-to-nest distance of 4.7 km in Alberta. Wakkinen et al. (1992) found that 92 percent of nests in Idaho occurred <3 km from leks where females bred. Breeding to nesting site movements generally range 1.1–6.2 km but can be >20.0 km (Connelly et al. 2000). We used a 1.5-km buffer to prevent overlap with buffers of other leks (active and inactive). These buffered areas were then overlaid with a 3 x 3-km regular grid (DeMars 2000) centered on the lek, with a cell size of 0.5 km^2. A total of 12 sample sites were placed systematically at equidistant points from each other around each lek. We assigned each sample site, located using state maps, county maps, and a hand-held 12-Channel GPS receiver, UTM coordinates within ArcView 3.2a.

From the center of each sample site, we established four 50-m line transects along each of the cardinal directions. Characteristics of the shrub community canopy cover, density, and height, were recorded along each transect (Ellis et al. 1989). Total live and dead shrub cover was measured using the line-intercept method (Canfield 1941, Connelly et al. 1991, Higgins et al. 1996). We determined densities of sagebrush shrubs by species by walking along all transects with a 1-m stick centered horizontally and perpendicular to the tape, counting the numbers of individuals that had >50 percent of their canopy or the entire area of the trunk within the area covered by the meter stick and then calculating density/ m^2 for each species (Higgins et al. 1996). Height (cm) of shrubs by species and maximum effective height of grass were measured at 5-m intervals along each transect to obtain mean values (Connelly et al. 1991, Musil et al. 1994, Nelle et al. 2000). We measured grass height at 5-m intervals and the height of the closest each shrub of each species within a 2-m radius of the tape to prevent bias of measuring the tallest or shortest of each species.

Herbaceous cover was measured within a 20- x 50-cm quadrat (Daubenmire 1959, Higgins et al. 1996) along each transect at 5-m intervals. We placed the 20- x 50-cm quadrat with the 50-cm side perpendicular to the edge of the right side of the tape relative to the starting point. If a shrub obstructed placement , we placed the quadrat along the tape between the two closest shrubs next to the 5-m interval point on the tape. At each 5-m interval we used a smaller 10 x 25-cm quadrat to measure understory herbaceous cover because we felt it would better represent herbaceous cover under the canopy. A standard quadrat would have included herbaceous cover that fell outside of the canopy a majority of the time and not really reflect understory cover. We placed the 10- x 25-cm quadrat beneath the closest sagebrush plant within a 2-m radius of the 5-m transect interval. The quadrat was laid at a randomly selected cardinal direction from the trunk of the plant. If no sagebrush was present, we used the closest other species of shrub. If first 10- x 25-cm quadrat placement failed to fall underneath the canopy of the shrub or if quadrat placement was obstructed, the next counterclockwise cardinal direction was used to place the quadrat. For each quadrat (20- x 50-cm and 10- x 25-cm) we assigned the following variables to one of six cover classes based on ocular estimates (Daubenmire 1959): total grass cover, total forb cover, total bare ground, and total litter (dead vegetation that was disconnected or not standing, dead insects, and animal feces) were recorded for both understory and herbaceous cover (Nelle et al. 2000, J.W. Connelly, IDGF, pers. comm.). Average cover estimates of the 20- x 50-cm quadrats and 10- x 25-cm quadrats for each sample site were used in the analysis. Percent visual obstruction was measured using the staff-ball method (Collins and Becker 2001) in each quadrant defined by the intersection of transects. We measured visual obstruction readings 10 m from the intersection point and at a 45° angle. Percent visual obstruction was measured at heights of 0.10 m, 0.25 m, and 0.50 m.

Microhabitat characteristics related to lek activity

A multiple t-test with a Bonferroni correction was performed to compare habitat variables (SAS Institute 1999). We used the

Table 1. Habitat variables ($x \pm SE$) variables used for comparison between active and inactive sage grouse leks [Active - North Dakota: leks = 15, sample sites (n) = 180; South Dakota: leks = 12, sample sites (n) = 144] [Inactive - North Dakota: leks = 12, sample sites (n) = 144; South Dakota: leks = 10, sample sites (n) = 120].

Variable[a]	North Dakota		South Dakota	
	Active	Inactive	Active	Inactive
Sagebrush canopy cover (%)	2.99 ± 0.26	2.24 ± 0.24	3.02 ± 0.28	3.53 ± 0.36
Sagebrush density (/m²)	0.41 ± 0.03	0.35 ± 0.03	0.62 ± 0.06	0.66 ± 0.07
Big sagebrush height (cm)	20.95 ± 1.16	15.50 ± 1.29	21.26 ± 0.90	18.10 ± 1.21
Silver sagebrush height (cm)	23.00 ± 1.41	22.26 ± 1.58	8.60 ± 1.29	10.83 ± 1.59
Grass height (cm)	10.21 ± 0.38	10.70 ± 0.57	12.22 ± 0.33	12.47 ± 0.34
Visual obstruction 0.25 m (%)	8.93 ± 1.02	12.12 ± 1.62	8.94 ± 1.12	8.46 ± 1.21
Visual obstruction 0.50 m (%)	1.72 ± 0.34	2.23 ± 0.52	0.42 ± 0.15	0.63 ± 0.28
Forb cover (%)	7.96 ± 0.42	5.44 ± 0.48	7.26 ± 0.41	7.54 ± 0.45
Grass cover (%)	41.10 ± 1.80	44.90 ± 2.06	46.48 ± 1.62	53.25 ± 1.78
Bare ground cover (%)	24.93 ± 1.70	16.53 ± 1.94	23.89 ± 1.67	20.74 ± 1.83
Litter cover (%)	26.07 ± 1.60	25.71 ± 1.82	31.41 ± 1.37	29.82 ± 1.50

[a] Average cover reading for 40 Daubenmire (1959) frames were used to categorize forb, grass, bare ground, and litter cover for each sample site.

Bonferroni correction to correct for type I error by using a P-value adjustment for all main effect means that takes into account the raw P-value and number of comparisons. North and South Dakota were analyzed separately due to differing landscape features around lek sites and may include different breeding populations because the closest active leks were >20 km apart (Connelly et al. 2000). Comparisons were also made between moderately large leks, i.e., >40 males, in Montana and active leks in North Dakota and South Dakota that were smaller. Variables compared were deemed to be important to sage grouse lek occurrence and activity. We set statistical significance at $\alpha = 0.10$.

Results

Microhabitat characteristics related to lek activity

Microhabitat comparisons in relation to lek activity in North Dakota were performed using data from 180 sample sites around 15 active and 144 sample sites around 12 inactive leks. We performed microhabitat comparisons relative to lek activity in South Dakota using data from 144 sample sites around 12 active and 120 sample sites around 10 inactive sage grouse leks. We calculated descriptive statistics for variables considered in lek activity analyses; sagebrush cover and sagebrush density were analyzed as a combination of both big and silver sagebrush (Table 1). We found greater big sagebrush height ($P < 0.05$) and greater forb cover and bare ground ($P < 0.01$) around active leks than around inactive leks in North Dakota (Table 2).

South Dakota comparisons showed that grass cover was higher ($P < 0.10$) around inactive leks (Table 2). None of the other variables differed ($P > 0.10$) between areas around active and inactive leks (Table 2).

Montana leks compared to leks in the Dakotas

We found greater sagebrush canopy cover and density, visual obstruction at 0.25-m height, and bare ground ($P < 0.0001$) around Montana active leks than around North Dakota active leks (Table 3). Height of silver sagebrush was considerably shorter ($P < 0.0001$) around Montana active leks than around North Dakota active leks (Table 3); however, forb cover and bare ground were greater ($P < 0.05$) around active leks in North Dakota. We found sagebrush greater canopy cover and density, visual obstruction at 0.25 m, and visual obstruction at 0.25 m and 0.50 m ($P < 0.10$) and greater height of

Table 2. Microhabitat comparisons of sample sites within 1.5 km of active versus inactive sage grouse leks. [North Dakota - active: leks = 15, sample sites (*n*) = 180; inactive: leks = 12, sample sites (*n*) = 144] [South Dakota - active: leks = 12, sample sites (*n*) = 144; inactive: leks = 10, sample sites (*n*) = 120].

	North Dakota				South Dakota			
Variables	Active	Inactive	Raw-P[a]	Bon[b]	Active	Inactive	Raw-P	Bon
Sagebrush canopy cover (%)	2.99	2.24	0.0398	0.4378	3.02	3.53	0.2574	1.0000
Sagebrush density (%)	0.41	0.35	0.1873	1.0000	0.62	0.66	0.6392	1.0000
Big sagebrush ht. (cm)	20.95	15.50	0.0019	0.0209	21.26	18.10	0.0248	0.2728
Silver sagebrush ht. (cm)	23.00	22.26	0.7247	1.0000	8.60	10.82	0.2734	1.0000
Grass ht. (cm)	10.21	10.70	0.4611	1.0000	12.22	12.47	0.5904	1.0000
Visual obstruction 0.25 m (%)	8.93	12.11	0.0856	0.9416	8.94	8.46	0.7704	1.0000
Visual obstruction 0.50 m (%)	1.72	2.23	0.3973	1.0000	0.42	0.63	0.4962	1.0000
Forb cover (%)	7.96	5.44	0.0001	0.0011	7.26	7.54	0.6476	1.0000
Grass cover (%)	41.10	44.09	0.1660	1.0000	46.48	53.25	0.0053	0.0583
Bare ground cover (%)	24.93	16.53	0.0013	0.0143	23.89	20.74	0.2050	1.0000
Litter cover (%)	26.07	25.71	0.8849	1.0000	31.41	29.82	0.4334	1.0000

[a] *P*-value not adjusted for number of comparisons.
[b] Bonferroni Correction *P*-value adjustment for all main effect means that takes into account the raw *P*-value and number of comparisons.

Table 3. Microhabitat comparisons of sample sites within 1.5 km of active sage grouse leks with >40 males in eastern Montana and active sage grouse leks in North Dakota. [Montana - leks = 5, sample sites (*n*) = 60; North Dakota - leks = 15, sample sites (*n*) = 180]

	Montana		North Dakota			
Variables	x	SE	x	SE	Raw-P[a]	Bon[b]
Sagebrush canopy cover (%)	9.25	1.10	2.99	0.26	<0.0001	<0.0001
Sagebrush density (%)	1.03	0.11	0.41	0.03	<0.0001	<0.0001
Big sagebrush ht. (cm)	25.93	1.17	20.95	1.16	0.0201	0.1324
Silver sagebrush ht. (cm)	7.13	1.82	23.00	1.41	<0.0001	<0.0001
Grass ht. (cm)	9.04	0.36	10.21	0.38	0.0934	0.4968
Visual obstruction 0.25 m (%)	24.04	3.14	8.93	1.02	<0.0001	<0.0001
Visual obstruction 0.50 m (%)	1.75	0.72	1.72	0.34	0.9693	1.0000
Forb cover (%)	5.48	0.34	7.96	0.44	0.0020	0.0220
Grass cover (%)	30.35	1.88	41.10	1.62	0.0003	0.0033
Bare ground cover (%)	59.12	2.45	24.93	1.76	<0.0001	<0.0001
Litter cover (%)	7.14	0.74	26.07	1.60	<0.0001	<0.0001

[a] *P*-value not adjusted for number of comparisons.
[b] Bonferroni Correction *P*-value adjustment for all main effect means that takes into account the raw *P*-value and number of comparisons.

big sagebrush ($P < 0.05$) around active leks in Montana than around active leks in South Dakota (Table 4). Grass height was less ($P < 0.0001$) around Montana active leks than around South Dakota active leks (Table 4). We found greater grass cover and litter cover ($P = <0.0001$) and less bare ground ($P = <0.0001$) around active leks in South Dakota around active leks in Montana.

Discussion

Leks provide key activity areas for sage grouse within spring and early summer habitat complexes. Other research has shown that sagebrush reduction adjacent to leks resulted in a decreased numbers of strutting males or caused complete abandonment of the lek (Enyeart 1956, Peterson 1970, Wallestad and Schladweiler 1974, Smith et al. 2005). Potential lek

Table 4. Microhabitat comparisons of sample sites within 1.5 km of active sage grouse leks with >40 males in eastern Montana and active sage grouse leks in South Dakota.
[Montana - leks = 5, sample sites (n) = 60; South Dakota - leks = 12, sample sites (n) = 144]

	Montana		South Dakota			
Variables	x	SE	x	SE	Raw-P[a]	Bon[b]
Sagebrush canopy cover (%)	9.25	1.10	3.02	0.28	<0.0001	<0.0001
Sagebrush density (%)	1.03	0.11	0.62	0.06	0.0009	0.0060
Big sagebrush ht. (cm)	25.93	1.17	21.26	0.90	0.0037	0.0259
Silver sagebrush ht. (cm)	7.13	1.82	8.60	1.29	0.5263	0.9946
Grass ht. (cm)	9.04	0.36	12.22	0.33	<0.0001	<0.0001
Visual obstruction 0.25 m (%)	24.04	3.14	8.94	1.12	<0.0001	<0.0001
Visual obstruction 0.50 m (%)	1.75	0.72	0.42	0.15	0.0111	0.0753
Forb cover (%)	5.48	0.34	7.26	0.42	0.0302	0.3322
Grass cover (%)	30.35	1.88	46.48	1.51	<0.0001	<0.0001
Bare ground cover (%)	59.12	2.45	23.89	1.77	<0.0001	<0.0001
Litter Cover (%)	7.14	0.74	31.41	1.40	<0.0001	<0.0001

[a] P-value not adjusted for number of comparisons.
[b] Bonferroni Correction P-value adjustment for all main effect means that takes into account the raw P-value and number of comparisons.

habitat may not be limiting (Schroeder et al. 1999), and vegetation on active sites may not likely change rapidly or dramatically, except with tillage of the area, so as to exclude sage grouse from actual strutting areas or other alternate sites nearby. Therefore, abandonment of leks by male sage grouse may be related to female migration from these areas to areas with more desirable nesting habitat or to an increase in human disturbance in the proximate area of the lek site, e.g., installation of oil and natural gas near leks in North Dakota, or to an inadequate food supply within 1.5 km of the lek (Tate et al. 1979, Call and Maser 1985, Braun 1998). Presence of good nesting habitat around leks is important in order to attract females to the display ground. Fidelity of male sage grouse to leks is related to the previous years' territory establishment and reproductive success (Dunn and Braun 1985, Gibson 1992). In our study, sagebrush canopy cover did not differ around active and inactive leks within our 1.5 km buffers in South or North Dakota, which suggested that factors other than sagebrush canopy led to lek abandonment. Big sagebrush was taller around active leks than around inactive leks in North Dakota, which might suggest that areas around active leks provided better quality nesting sites (Connelly et al. 1991, Wallestad and Pyrah 1974, Aldridge and Brigham 2002), hence attracting females to these areas. The larger active leks in Montana also had taller and denser sagebrush than the Dakotas, which may result in more females in the area, increased male breeding success, and thus, larger male numbers on the lek. Retention or attraction of females would help keep males in the area and maintain active leks (Gibson 1992). Connelly et al. (1991) found higher nest success (53%) for sage grouse nesting under sagebrush than those that nested under other species of shrubs (22%). Conversely, Sveum et al. (1998) found no difference between nest success under sagebrush versus nest success under other species of shrubs, indicating that if adequate overall cover is present the species comprising the cover may be of minor importance as suggested by Aldridge and Brigham (2002).

We found no relationship between sagebrush cover and density, big sagebrush height, silver sagebrush height, grass height, visual obstruction 0.25 and 0.50 m and abandonment of leks in South Dakota, which indicated that other factors may be involved. Comparisons of forb, grass, bare ground, and litter cover in North Dakota revealed that forb cover was greater around active leks and may thereby have provided greater food availability for females or broods. The presence of forbs may nutritionally enhance female reproductive output and also increase densities of insects, a food source for newly hatched chicks (Barnett and Crawford 1994, Drut et al. 1994). Bare ground was greater around active leks in North Dakota, and bare ground was greater around the large Montana lek than around leks in both North and South Dakota; this may be related to greater shrub cover in these areas since shrubs may shade out grasses or compete for water (Peterson 1995). Sage grouse nesting sites in Washington were associated with high percentages of bare ground cover (Sveum et al. 1998).

Sagebrush canopy cover, density, and visual obstruction (0.25 m) were greater in Montana and might be important to support large, active leks. Sage grouse in the Dakotas seemingly rely upon taller and denser grass or forb cover to supplement canopy of the shorter and smaller sagebrush (Aldridge and Brigham 2002), and other shrub species such as silver sagebrush might play more of a role in survival. We would expect greater forb cover in North Dakota and greater grass cover in both South and North Dakota because the area lies at the eastern edge of the sagebrush distribution and functions as an ecotone between sagebrush and prairie. Sagebrush communities in the Dakotas also offer more mesic habitat compared to Montana. Herbaceous cover located within the 1.5-km buffer around active leks in the Dakotas apparently provided adequate nesting sites (Connelly et al. 2000) and daily use areas for female sage grouse (Musil et al. 1994); mean forb coverage ranged from 5 to 25 percent in North Dakota and South Dakota and mean grass coverage ranged from 26-50 percent in North Dakota and South Dakota.

Sagebrush cover (>28%) and height (>40 cm) in proximity to the lek are important to male sage grouse lek fidelity in the Great Basin area of the Western U. S. (Ellis et al. 1989). Successful nest areas located within big sagebrush/bunchgrass in south-central Washington during 1992-1993 had sagebrush cover of 19-23 percent, sagebrush height of 23-27 cm, and bare ground of 35-44 percent (Sveum et al. 1998). Other studies (Connelly et al. 2000) across the country have shown that sage grouse nest sites have mean sagebrush heights of 29-80 cm, mean sagebrush canopy coverages of 15-38 percent, mean grass heights of 14-30 cm, and mean grass coverages ~ 3-51 percent. Our study showed that in the big sagebrush-wheatgrass plains of the western Dakotas (Johnson and Larson 1999), sagebrush cover and density likely limit sage grouse success. When compared to larger active leks in Montana the Dakotas have significantly less sagebrush cover, and the cover that was present was not as dense. Mean sagebrush canopy coverage peripheral to active lek sites was 3.0 percent in North and South Dakota; mean big sagebrush height was 21.0 cm in North Dakota and 21.3 cm in South Dakota; and mean silver sagebrush height was 23.0 cm in North Dakota and 8.6 cm in South Dakota. These values were lower than those observed at successful nesting sites (Connelly et al. 2000) and daily use areas (Musil et al. 1994) in more central portions of the sage grouse range. However, preferred habitat may exist outside of this buffered area, or the sage grouse in the Dakotas have adapted to marginal sagebrush habitats with less than preferred cover as nesting sites. The adequate forb and grass cover in the Dakotas may provide means for greater reproductive success (Crawford 1977) and allow for sage grouse populations to exist in these marginal habitats.

Average movements of female sage grouse from breeding to nesting sites range from 1.1 to 6.2 km, and patchy distribution of sagebrush habitat in the Dakotas means it is probably not distributed uniformly in relation to the leks. This might suggest managing areas ≤5 km from all active leks (Connelly et al. 2000). Given the extensive movements and not knowing whether the population is migratory, evaluation of nesting in radio-marked female sage grouse in the Dakotas could provide valuable information on their movements from breeding areas, selection of nest site and brood rearing areas, and nesting and brood rearing success in this marginal sagebrush habitat. Possible use of silver sagebrush or other shrubs by sage grouse would also be of interest in regard to potential nest site resources in the Dakotas.

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Use of Latitude-Adjusted Elevation in Broad-Scale Species Distribution Models

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Abstract

Using readily available spatial data and GIS, we developed a method to adjust elevation by latitude using an estimate of the elevation of alpine treeline across a latitudinal gradient. Latitude-adjusted elevation accounted for the influence of latitude on relationship between elevation and annual monthly maximum temperature, which demonstrates the ability of this method to apply elevation as a model predictor variable across latitudinal ranges. Elevation is particularly useful for predicting coarse-grained distribution patterns of many species because elevation integrates influences of climate, physiognomy, and vegetative cover into a single measurement. However, problems arise with use of elevation as a predictor across wide latitudinal gradients because climate and biotic distributions tend to respond to increases in elevation and latitude similarly. Latitude-adjusted elevation can be used to extrapolate species-distribution models beyond the latitudinal extent of data availability. Using this method we extrapolated a model predicting wolverine (*Gulo gulo*) habitat across a large region.

Key Words: alpine treeline, elevation, GIS, *Gulo gulo*, latitude, life zones, regional modeling, timberline, wolverine

Introduction

It has long been recognized that elevational and latitudinal gradients strongly influence distribution of biota at local, regional, and global scales. Merriam (1895) developed the concept of "life zones" and observed that changes in species associations with increasing elevations was the equivalent of moving to areas of increasing latitude. Since then, the interrelationship between elevation, latitude, habitats, and species distributions has become integral to the field of biogeography. The influence of latitude and elevation on species distributions is so fundamental that the life zone concepts developed by Merriam (1898) and later redefined by Holdridge (1947) are still used to map species occurrences. In some cases the relationship between species distributions and elevation-latitude relationships is extreme. For example, Lambert et al. (2005) found that the lower-elevation limit of Bicknell's sparrow distribution decreased by 81.63 m for every 1° increase in latitude. This relationship is nearly identical to the elevation-latitude relationship for treeline (-83 m/1° latitude) reported by Cogbill and White (1991) for the northern Appalachian Mountains. Therefore, models that attempt to predict distribution of elevation-dependent species over a range of latitudes must account for elevation-latitude interactions to determine suitable habitat. However, obtaining species occurrence data across the full extent of desired model application is not always practical or possible. Therefore, combined influences of elevation and latitude cannot be directly modeled. In these cases, latitude-adjusted elevation as a model input parameter should improve model performance over simple elevation.

Within appropriate areas, alpine treeline provides a convenient baseline for

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adjusting elevation due to latitude. Several estimates of elevation-latitude and alpine treeline relationships have been reported in the literature (Hermes 1955, Li and Chou 1984, Peet 1988, Cogbill and White 1991). However, treeline is predominantly controlled globally by growing season temperature (Jobbagy and Jackson 2000) and locally by topography (Cogbill and White 1991). Therefore, elevation of treeline can vary according to regional and local climate as well as fine-scale patterns of topography. Not surprisingly, previously reported estimates between elevation-latitude relationships and alpine treeline vary with location. Additionally, the relationship tends to be curvilinear over broad ranges of latitude with a shallower slope at lower latitudes (Peet 1988). The utility of using previously published estimates of elevation-latitude relationships to derive latitude-adjusted elevation parameters for model input relies on ability to find an estimate that matches the location and extent of the area being modeled. To address this problem, we developed a GIS-based method using readily available landcover data to estimate the elevation-latitude relationship of treeline and develop a latitude-adjusted elevation layer for input into spatially explicit models. In our case, the intended use was to predict wolverine habitat (*Gulo gulo*), a wide-ranging species typically found in boreal, taiga, and tundra ecotones, but with historic distribution including a peninsular extension south into the Rocky Mountains of the contiguous U.S. Wolverines are difficult and expensive to capture and locate, which places severe limitations on the extent of data available for developing habitat models. In addition, wolverines have been extirpated from much of their historic range in the Rocky Mountains within the U.S. Therefore, species occurrence data suitable for modeling habitat preferences in our study were only available from a 2° band of latitude (43 - 45° N) making it impossible to test the influence of latitude on habitat preference using regression analysis methods. Latitude-adjusted elevation solved this problem by allowing us to estimate the relationship between wolverine habitat preference and elevation across latitudinal gradients beyond the spatial extent of our location data.

METHODS

We developed a raster grid of latitude-adjusted elevation over a five-state region within the United States: Colorado, Idaho, Montana, Utah, and Wyoming (~ 49° N, -118° E to 37° N, -101°E). We conducted GIS analysis using ArcGIS 9.1 (ESRI, Inc., Redlands, CA), the National Landcover Dataset (NLCD) (Homer et al. 2001), and a Digital Elevation Model (DEM) from the National Elevation Dataset (NED) to estimate the elevation of treeline for each 1° band of latitude across the study extent (n = 13, 1° intervals). Both datasets were retained at their original 30-m resolution. We selected 50,000 forested pixels (either conifer or deciduous) at random and assigned each pixel with its corresponding elevation value from the DEM and the midpoint latitude value for the corresponding 1° latitude band (e.g. 44°-45° band = 44.5°). Midpoint latitudes provided an average approximation of the exact latitudes of samples used to estimate treeline within each 1° latitude interval. We estimated treeline elevation for each 1° latitude interval by calculating the maximum elevation that contained 95 percent of the sampled forested cells within the latitude band. That is, 95 percent of all forested cells occurred at or below our estimated treeline elevation for each 1° latitude interval. The smallest sample size for any of n latitude intervals was n = 875 with only three intervals containing < 4000 sample points. This resulted in a single estimate for treeline elevation in each of the 13 1° latitude intervals.

We calculated adjusted elevation using the following equation: Latitude-adjusted Elevation = $E - (\hat{Y} - T_{std})$, where E = Actual Elevation, $\hat{Y}$ = Predicted Treeline elevation at the latitude of E, and Tstd = Predicted Treeline elevation at 44.5 N Latitude. We chose 44.5 N Latitude as our standard treeline because it occurred at mid-latitude of our wolverine habitat model validation area. Thus, we adjusted elevation upward for locations north of the standard and adjusted downward for locations south of the standard.

We tested whether latitude-adjusted elevation can account for the influence of latitude in regional models using mean annual temperature within the Greater Yellowstone wolverine study area (45° N, -112° E to 43° N, -110° E) and within the elevation band (2743-3048 m) preferred as habitat by wolverine (Inman, unpublished data). The annual mean maximum temperature (AMMT) derived from the PRISM model within the GYA study area was 7.5° C. PRISM (Parameter-elevation Regressions on Independent Slopes Model) data are spatial datasets gridded from point climate data using a knowledge-based approach developed by Christopher Daly, Director, Spatial Climate Analysis Service, Oregon State University, Corvallis (Daly et al. 2001) (http://www.ocs.oregonstate.edu/prism). Temperature grids are available at 4-km resolution. We extracted all points with a AMMT of 7-8° C ($n = 2044$) and regressed latitude on elevation and then latitude on latitude-adjusted elevation using SAS (Cary, NC) statistical software. If latitude-adjusted elevation accounted for influence of latitude on AMMT, then the slope and correlation coefficient of the latitude on latitude-adjusted regression should approach zero and the predicted latitude-adjusted elevation of 7-8° AMMT would be the same at all latitudes.

Results

We observed a strong relationship between latitude and the estimated elevation where treeline occurs. A polynomial curve produced the best fit.

Treeline elevation (m) = (-10.727 * Latitude^2) + (792.47 * Latitude) - 11280
($n = 13$, $R^2 = 0.97$)

But a simple linear regression also provided a good fit:

Treeline elevation (m) = (-130.08 * Latitude) + 8427.5
($n = 13$, $R^2 = 0.91$)

We eliminated the effect of latitude vs. the elevation of AMMT by using latitude-adjusted elevation in place of elevation. Latitude is a reasonably good predictor of the elevation where AMMT = 7.5 ± 0.5 °C (Fig. 1):

AMMT Elevation (m) = (-155.93 * Latitude) + 9432.8
($n = 2043$, $R^2 = 0.73$)

Latitude was not a good predictor of the latitude-adjusted elevation where AMMT = 7.5° ± 0.5 °C occurred (Fig. 2):

AMMT Latitude-adjusted Elevation (m) = -22.963 * Latitude) + 3595.7
($n = 2043$, $R^2 = 0.05$)

Latitude-adjusted elevation reduced both the slope and correlation coefficient of the relationship. The predicted elevation, where AMMT = 7.5 ± 0.5 °C, was 2728 m ± 857 m, but the range of predicted latitude-adjusted elevation, where AMMT = 7.5 ± 0.5 °C occurs, was much smaller (2608 m ± 149 m).

Discussion

The life zone concept (Merriam 1895, Holdridge 1947) is important to consider when attempting to model species distributions and habitat across regional scales. This consideration becomes particularly important when modeling across mountainous terrain where the variable of concern may be associated with ≥1 elevational life zones. In such cases, elevation may be a useful predictor but the elevation-latitude relationship must be accounted for if the user intends to apply the model over a wide range of latitudes.

Using alpine treeline as a baseline for adjusting elevation provides an efficient method to predict elevational shifts across a latitude gradient for application in coarse-grained regional models. However, one must realize that treeline provides only a coarse approximation of the elevation-latitude relationships of other ecotones. For example, Cogbill and White (1991) reported that the upper and lower bounds of the spruce-fir (*Picea-Abies*) ecotone in the Appalachian Mountains resulted in a narrower zone at southern latitudes. However, they cautioned that both regional and local variation of the actual ecotone is large and within the magnitude of predicted convergence. Therefore, we recommend that methods described here be used with caution if applied to models intended to predict fine-grained patterns of species distributions or potential habitat. Likewise, our method for deriving latitude-adjusted elevation would likely prove unsatisfactory for modeling distributions of species strongly associated with a narrow range of habitat types unless it incorporates other regional and local predictors of habitat distribution.

Latitude-adjusted elevation did not completely eliminate the relationship between latitude and temperature. Although latitude-adjusted elevation greatly reduced the slope of the relationship (Figs. 1 and 2), the slope was still significantly different from zero (α = 0.05). Errors in the GIS input data, such as misclassification of landcover types, generate noise but do not explain the residual negative relationship of the results. However, this residual relationship was not unexpected since annual temperatures are determined by factors other than elevation and latitude, such as regional weather patterns or prevailing aspects.

Our technique provides a useful method for using latitude-adjusted elevation for modeling wildlife habitat and other variables over large extents. This method relies only on readily available digital landcover and elevation data that eliminates a need for extensive collection of field data to measure the elevation-latitude relationship of a parameter. Using these methods, one may

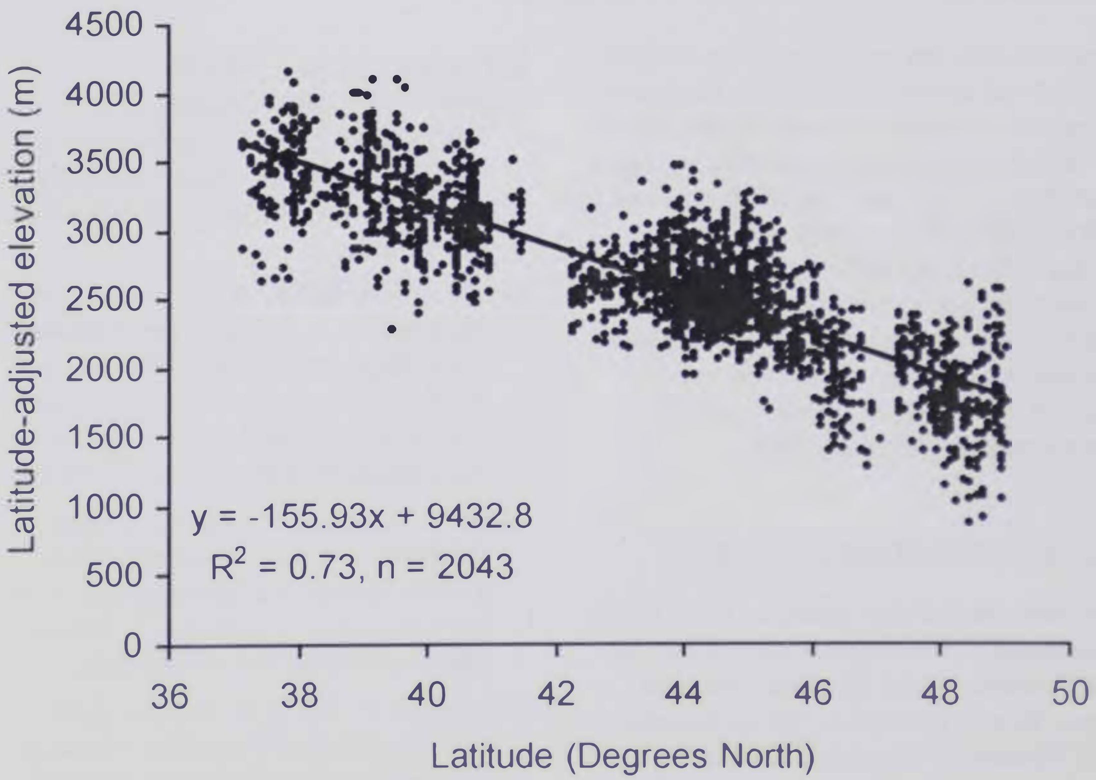

Fig. 1. Latitude vs. Elevation of Equal AMMT (7.5 ± 0.5°C). Linear regression between latitude and elevation of points with equal annual mean maximum temperatures across a five-state region.

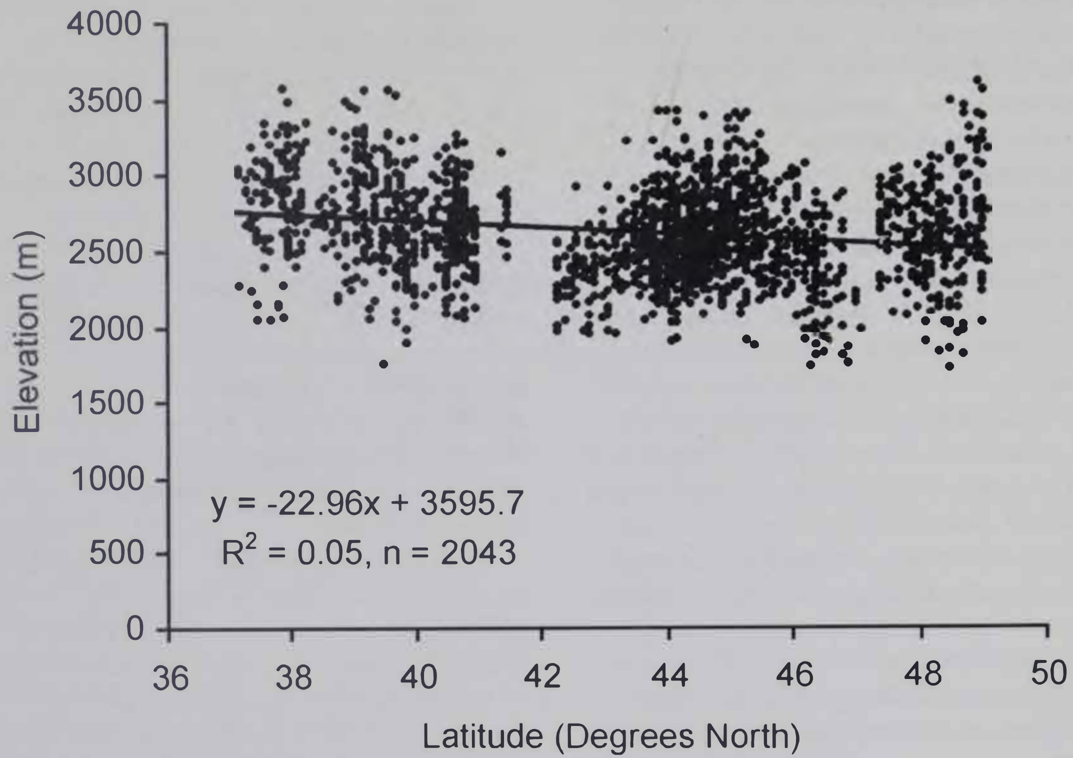

Fig. 2. Latitude vs. Latitude-Adjusted Elevation of Equal AMMT (7.5 ± 0.5 °C). Linear regression between latitude and latitude adjusted elevation of points with equal annual mean maximum temperatures across a five-state region.

improve accuracy by confining the analysis to the focal extent of a particular study and eliminating errors introduced by differences in regional and local climate and topography outside the study area. This method shares the same limitations as any estimator of broad-scale trends and should not be relied upon to predict fine-scale patterns. Given these limitations, this technique is useful for modeling species occurrences or habitats that are influenced by elevation and that span broad latitudinal gradients.

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Mercury Levels in Vegetation Growing on Contaminated Soils in Southwestern Montana

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Abstract

Contamination at former gold mine sites is common because mercury was used to extract gold by amalgamation of which some was lost to soils. Mobilization from soil to plants at these sites could result in high levels of mercury in plant tissues causing concern over spread of mercury through natural pathways such as grazing. We determined whether vegetation growing on three sites in southwestern Montana mobilized mercury from soil into roots or leaves. Two sites were known or suspected to have mercury contamination from past mining activity. The third site was an engineered repository for mining wastes. Soil mercury levels were highly elevated at the sites with past mining activity. Two grass species growing on the most contaminated site did not accumulate substantial amounts of mercury in either roots or leaves. Ponderosa pine (*Pinus ponderosa*) showed similar results (no accumulation) but we found Douglas-fir (*Pseudotsuga menziesii*) roots to have an accumulation of mercury. Repository soil had levels of mercury double the amount reported for U.S. topsoils, but levels in grass roots and leaves were not substantially elevated above soil levels. Hence, this repository is currently preventing movement of mercury from covered tailings into the vegetation on its surface. Species that were not accumulating mercury (thus not mobilizing mercury) could be utilized for vegetation rehabilitation on other mercury-contaminated sites.

Key Words: mercury, vegetation, soils, mining reclamation

Introduction

Mercury (Hg) has been used to amalgamate precious metals throughout history and was used extensively in Western U.S. gold fields including those in Montana. During 1850-1900, gold mining in the U.S. consumed an estimated 63 million kg of Hg (de Lacerda 1998). Efficiency of mercury utilization/recovery in gold processing was low and most estimates show a mass loss of mercury at least equivalent to the mass of gold recovered (de Lacerda 1998). Today, mercury is creating environmental concerns because there is potential for movement into food chains.

Many historical mining districts now have residual mercury in their environs – either from traditional milling and processing, or from placer mining operations. Placer mining operations often used mercury as an amalgam to remove gold from sand concentrates recovered during dredging operations (Lyden 1987). Mineral processing operations typically placed milled tailings into nearby drainages and floodplains. Some such tailings remain in place today. Flood events washed others down drainages and redeposited them. Some have been relocated into repositories.

Movement of mercury from soil into plants is a plausible scenario with variable pathways once in the plant. Mercury can be sequestered within plant tissues. In a broad study of a mine tailing field in Nova Scotia, Wong et al. (1999) found that several herbaceous plants accumulated mercury in addition to other heavy metals and suggested their use in phytoremediation. Alternatively, movement could occur through multiple pathways leading to wider mercury movement in the environment. Such pathways include consumption of vegetation by herbivorous or decomposer organisms that utilize dead plant components.

Patra and Sharma (2000) have discussed the wide variation in mercury uptake and movement within different plant species. Variation among plants followed by the uncertain pathways of mercury from plants into the environment provides a basis for research into these questions on a site-specific level. Few studies have documented plant mercury uptake. Without research specific to a site and vegetation present, it is difficult for land managers to provide restoration plans that utilize plants efficiently without causing further spread of mercury through the environment.

The objectives of this study were to determine whether different species (1) accumulated mercury when growing on contaminated soils, (2) varied in accumulation between roots and leaves, and (3) would be appropriate for revegetating contaminated sites.

Considerable research on the environmental movement and behavior of mercury has been published and summarized (U.S. Environmental Protection Agency 1997a, Nriagu 1994). Extensive research in aquatic environments has shown bioaccumulation of mercury in fish a widely recognized pollution problem (U.S. Environmental Protection Agency 1997b). Less research has been done regarding the movement of mercury in terrestrial ecosystems or uptake by plants. The bulk of the literature regarding mercury uptake by plants revolves around species of cultural or economic importance, such as garden vegetables or forest trees. Patra and Sharma (2000) reported that plant uptake through roots is correlated with the mercury level in the soil, i.e., plants exhibit higher mercury uptake at higher soil mercury levels. Comparatively, uptake for trees, especially in needles of conifers, seems as much related to atmospheric deposition as to soil concentrations (Patra and Sharma 2000).

Materials and Methods

Study Areas

We selected three field sites (Fig. 1): two sites with historical mining operations and a mine waste repository. The Silver Creek site, located approximately 24 km north of Helena, Montana, was extensively placer mined using hand and hydraulic methods from the mid 1860's until 1904 (Lyden 1987). Later in 1939-1940, a dragline dredge operation recovered gold from an estimated 1.1 million cubic meters of gravel in about a 3-km stretch of this stream bottom (Lyden 1987). The study site was located in this dredged area. Upstream about 6.5 km, mineral deposits were discovered near Marysville, Montana in 1875. The last mining activity in the drainage occurred from the mid 1970s thru 1986 in operations that reprocessed previously deposited tailings from the Marysville area. This site is contaminated with mercury. The U.S. Geological Survey reported in a survey paper that the mercury content of U.S. soils averages about 0.1 μg/g (U.S. Geological Survey 1970). It further reports that anomalies around the world's mercury deposits fall in a range of 10 to 100 μg/g. Studies by Sambathkumar (2002) and Nagulapaty (2001) found mercury concentrations from 0.06 to 30 μg/g in the Silver Creek Drainage.

The Ranch site was located ~1.6 km northwest of Silver Creek along Trinity Creek, an intermittent stream that had been placer mined as evidenced by gravel piles parallel to its path. This mining likely took place sometime from 1875 to 1921—the "period of greatest prosperity for the area" (Lyden 1987). We found no information to indicate whether mercury was used in the mining process along Trinity Creek.

The Comet site was a mine waste repository. The wastes came from the abandoned Comet Mine and mill near Basin, Montana. The last of its tailings and waste rock were moved in 2001 from the drainage below the mine into an engineered repository located near a ridge top about 1.6 km southeast of the mine.

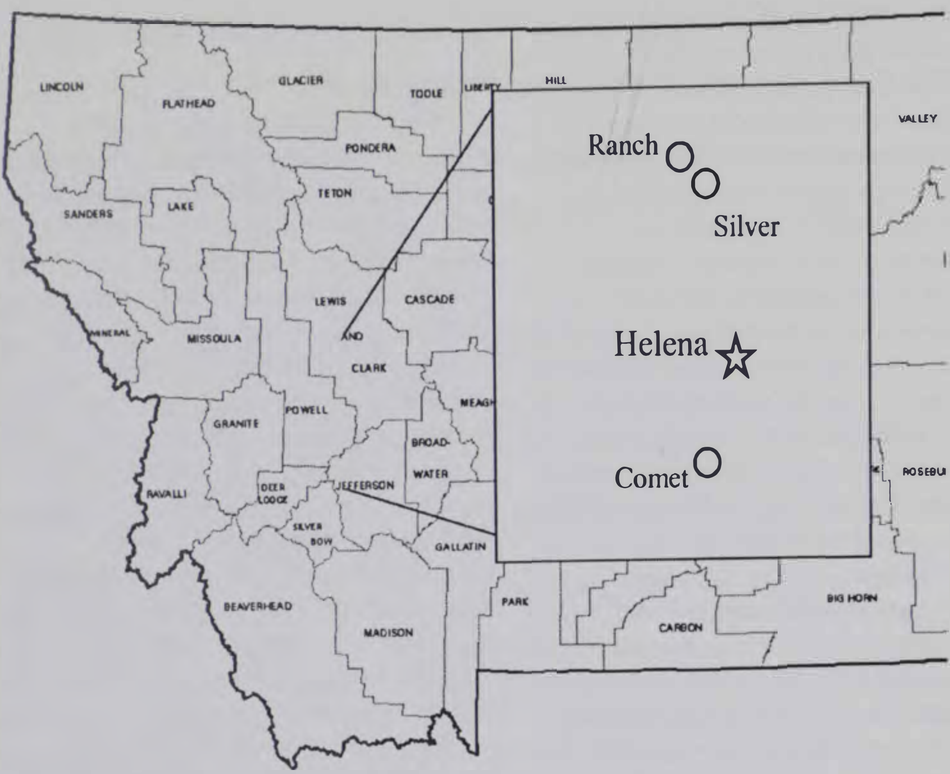

Fig 1. Location of three mercury contaminated mine sites in southwestern Montana. Helena is the state capital.

After relocation, the waste materials were covered sequentially with a geotextile cushion, a geosynthetic clay liner, a Geonoet filter fabric geocomposite, an organically amended cover soil and then with about 15-25 cm of topsoil salvaged earlier from the site (M. Browne, personal communication, April 2003). The cover soil was planted with a grass and forb seed mixture in fall 2001, and the area was fenced to prevent grazing. We chose this site to determine whether the repository was working as intended to prevent movement of mercury into the cover vegetation.

The climate of southwestern Montana is a cool and dry continental type. Wide seasonal and daily variations characterize temperatures. Daytime temperatures in winter average from 4 to 10 °C but may be as low as -20 °C. Daytime temperatures in summer typically range from 15 to 25 °C. The frost-free period averages ~ 70 days—mid-June to late August. Precipitation varies with location and altitude. There is a distinct spring/summer rainy season in May/June with an average of 89 mm of precipitation/month. Approximately half of annual precipitation falls as snow (U.S. Department of Commerce, NOAA.1988). The closest weather stations to the study sites were 6.4 km from Boulder, Montana (~ 240 m lower than the repository site) and at Austin, Montana (~ 6 km south of the Silver Creek site). Highest average monthly temperatures occur in July at both sites with temperatures of 17 °C at Austin and 18°C near Boulder. Lowest monthly temperatures occur in January with average temperatures of -7°C at Austin and -6°C at Boulder (U.S. Department of Commerce, NOAA. 1997). Precipitation isopleths indicate that the Silver and Ranch sites lie in the 41- to 46-cm precipitation zone, whereas the Comet repository site is in the 46- to 51-cm precipitation zone (U.S. Department of Agriculture 1977).

Field Procedures

Soil samples were obtained at 10- to 20-m intervals along transects crossing the approximately 20-ha sample areas. Each sample was collected at a depth of 10 to 20 cm using a spade to first lift the soil and then obtaining the sample with a plastic spoon. To avoid cross-contamination among samples, care was taken to only sample soil that had not contacted the shovel surface.

For each soil sample, we obtained a composite by combining eight sub-samples, which were collected by sampling at 1 and 2 m away from the center point in each cardinal direction. After obtaining all the sub-samples, we discarded the spoon and placed the sample on ice in the cooler for transport to the laboratory where it was frozen for later analysis. Samples were cooled and frozen to preserve mercury concentrations as collected.

We obtained vegetation samples at the same locations as the soil samples. Samples of leaves/needles were clipped directly into plastic sampling bags taking care to clip only leaves and avoid stems. New growth and one-year needles were separately sampled from ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*) trees. Scientific names of all plant species sampled appear in Table 2. We distinguished new growth needles from older needles by their lighter color. For root samples, the soil around selected plants was lifted as in the soil sampling above. We grasped roots with forceps and clipped with scissors into sampling bags. To avoid cross contamination among samples, the forceps and scissors were rinsed with acidified water and then with deionized water between each vegetative sample. Samples were placed on ice for transport to the laboratory and freezing.

All vegetative samples were composites taken from three to five plants of each species at each sample location. The number of plants composited into each sample varied because each sample location included variable numbers of plants and species. Sometimes there were no plants of some species. In such cases, we added sampling locations in which selected species occurred within the study area. Soils were also obtained at these added locations.

Laboratory Procedures

We analyzed samples for total mercury according to U.S. Environmental Protection Agency method 245.5 (U.S. Environmental Protection Agency 1991) using Cold Vapor Atomic Absorption Spectroscopy (CVAAS). The matrix for the CVAAS calibration standard was the same as used for sample preparation. We removed soil samples from the sampling bag, mixed, and placed in beakers to air dry. Upon reaching constant air-dry weight, the sample was mixed again. We placed samples of 0.2 g into a digestion bottle with 5.0 ml Aqua regia (3:1 conc. HCl:conc. HNO3) and heated for 2 min in a water bath of 95 °C. After cooling, 50 ml of de-ionized water and 15 ml of 5-percent w/v potassium permanganate solution were added, and the sample was mixed and placed into a 95 °C water bath for 30 min. After cooling, 6.0 ml of 35-percent w/v sodium chloride-hydroxylamine-hydrochloride solution and 55 ml of de-ionized water was added before filtering and measuring the final sample volume. The sample was then analyzed for total Hg using CVAAS.

Leaves and roots were rinsed with de-ionized water and dried. Upon reaching constant air-dry weight, the sample was fully mixed. Samples of 0.2 g were weighed and placed into a digestion bottle with 6 ml 35-percent hydrogen peroxide in a 95 °C water bath for 5 min. We then added 1.0 ml of 1:1 v/v concentrated nitric acid and the bottle returned to the water bath for ten min. After cooling, 50 ml of de-ionized water, 15 ml 5-percent w/v of potassium permanganate,

Table 1. Soil mercury concentrations at each study site.

Site	Hg (µg/g) Mean	Std. Dev	Range
Silver Creek	22.357	28.56	0.366-79.949
Comet Mine			
Soil cap	0.192	0.029	0.167-0.231
Tailings	0.924	0.084	0.820-1.02
Ranch	1.155	1.546	0.073-5.809

Table 2. Mercury concentrations by species and site, southwestern Montana.

Species	Site		Root Hg (µg/g)	Leaf Hg (µg/g)	Root/shoot ratio
Slender wheatgrass *(Elymus trachycaulus)*	Comet	N	5	5	
		Mean	0.01	0.013	0.8
		Std. Dev.	0.007	0.005	
	Ranch	N	5	5	
		Mean	0.003	0.005	0.6
		Std. Dev.	0.002	0.002	
	Silver	N	5	5	
		Mean	0.008	0.004	2.0
		Std. Dev.	0.011	0.003	
Needleandthread grass *(Stipa comata var. comata)*	Silver	N	5	5	
		Mean	0.027	0.006	4.5
		Std. Dev.	0.042	0.001	
Canada bluegrass *(Poa compressa)*	Comet	N	5	5	
		Mean	0.005	0.006	0.8
		Std. Dev.	0.005	0.003	
Big sagebrush *(Artemisia tridentata)*	Ranch	N	5	3	
		Mean	0.017	0.002	8.5
		Std. Dev.	0.021	0.002	
	Silver[1]	N	5	2	
		Mean	0.229	0.008	28.6
		Std. Dev.	0.375	0.011	
Skunkbrush *(Rhus trilobata)*	Ranch	N	4	5	
		Mean	0.003	0.001	3.0
		Std. Dev.	0.001	0.001	
	Silver[2]	N	4	4	
		Mean	0.104	0.001	104.0
		Std. Dev.	0.201	0.001	
Spotted knapweed *(Centaurea biebersteinii)*	Ranch	N	4	6	
		Mean	0.007	0.001	7.0
		Std. Dev.	0.007	0.001	
	Silver	N	5	4	
		Mean	0.012	0.075	0.2
		Std. Dev.	0.024	0.145	
Common yarrow *(Achillea millefolium)*	Comet	N	5	5	
		Mean	0.005	0.005	1.0
		Std. Dev.	0.003	0.004	
Ponderosa pine *(Pinus ponderosa)*	Ranch	N	5	10	
		Mean	0.003	0.001	3.0
		Std. Dev.	0.001	0.001	
	Silver	N	5	10	
		Mean	0.003	0.003	1.0
		Std. Dev.	0.002	0.002	
Douglas-fir *(Pseudotsuga menzeisii)*	Ranch	N	4	9	
		Mean	0.002	0.002	1.0
		Std. Dev.	0.001	0.001	
	Silver	N	9	10	
		Mean	0.061	0.002	30.5
		Std. Dev.	0.062	0.002	

[1].One outlier removed from root sample = 4.078 µg/g

[2].One outlier removed from root sample = 2.108 µg/g

5 ml of 0.25 M sulfuric acid, and 8 ml of 5-percent w/v potassium persulfate were added, the solution was then mixed and placed in the water bath at 95 °C for two hours. After cooling 50 ml of de-ionized water was added before filtering, volume measurement, and analysis for total Hg using CVAAS.

Data were compiled by species and site and graphically compared. Small and variable sample sizes precluded use of direct statistical comparisons between the three sites; however, correlations between mercury levels among plant components and soil were done across sites. We removed two extreme outliers from the data set prior to analysis. Both samples were from roots and likely contaminated by soil particles not removed during the lab cleansing procedure.

Results

Mercury in field soils

We found highly elevated concentrations of mercury at the Silver Creek site, lower but still elevated levels at the Ranch Site, and the lowest levels in the cover soils at the Comet repository site (Table 1). Estimated worldwide concentrations of surface crustal mercury average 0.07 µg/g (Lindberg et al 1979) and 0.1 µg/g for US topsoil (U.S Geological Survey 1970). The average levels found at the three sites were 22.36 µg/g at Silver Creek, 1.16 µg/g at the Ranch Site and 0.19 µg/g at the Comet Repository. These respective levels were approximately 225, 12, and 2 times greater than the average U.S. topsoil levels (Fig. 2). Tailings beneath the Comet Repository soil cap and liner averaged 0.85 µg/g or approximately eight times greater than the U.S. topsoil level.

Mercury in vegetation

The vegetative species growing at the Silver Creek and nearby Ranch site were similar (Table 2); hence, it was possible to sample two in-common tree species and one in-common grass species at each site. The Comet repository had no trees and one in-common grass species, i.e., slender wheatgrass, with the other two sites.

The concentrations of mercury found in the leaves of the species sampled were highest at the Comet site and similar at the Silver and Ranch sites (Fig. 2). Root mercury concentrations were similar at the Comet and Ranch sites but much higher with more variation at the Silver site (Fig. 2). The only species common to the three areas was slender wheatgrass (*Elymus trachycaulus).* The higher soil concentrations at the Silver Creek and Ranch sites did not result in increased mercury concentrations in leaves or roots of slender wheatgrass (Table 2). Similarly, needleandthread (*Stipa comata*) at the ranch site and Canada bluegrass (*Poa compressa)* at the comet site did not have elevated mercury concentrations even though growing in soils with elevated mercury content.

The two species of trees studied were ponderosa pine and Douglas-fir. Like the grasses, ponderosa pine did not show elevated mercury levels in either roots or leaves (needles) even though the trees grew in the Silver Creek soils with mercury levels averaging > 22 µg/g (Table 2, Fig. 2). However, Douglas-fir trees had slightly elevated root and needle mercury levels. Ponderosa pine growing in the heavily contaminated Silver Creek site soils accumulated very low levels of mercury in their roots and even less in first and second year needles. Comparatively, Douglas-fir trees accumulated some mercury in their roots and far lesser amounts in their needles. Our results indicated that ponderosa pine could likely grow on mercury-contaminated soils without causing mercury accumulation in roots or shoots. However, Douglas-fir trees had a high (25 to 1) root to needle ratio of mercury showing that this species has the potential to mobilize mercury into roots.

One objective was to determine the root to shoot mercury levels of plants growing on these sites. Some research has found root/shoot ratios of mercury of approximately 10:1 (Lindberg et. al. 1979). At the Silver Creek site, we found high root/shoot ratios for the shrub species and Douglas-fir but

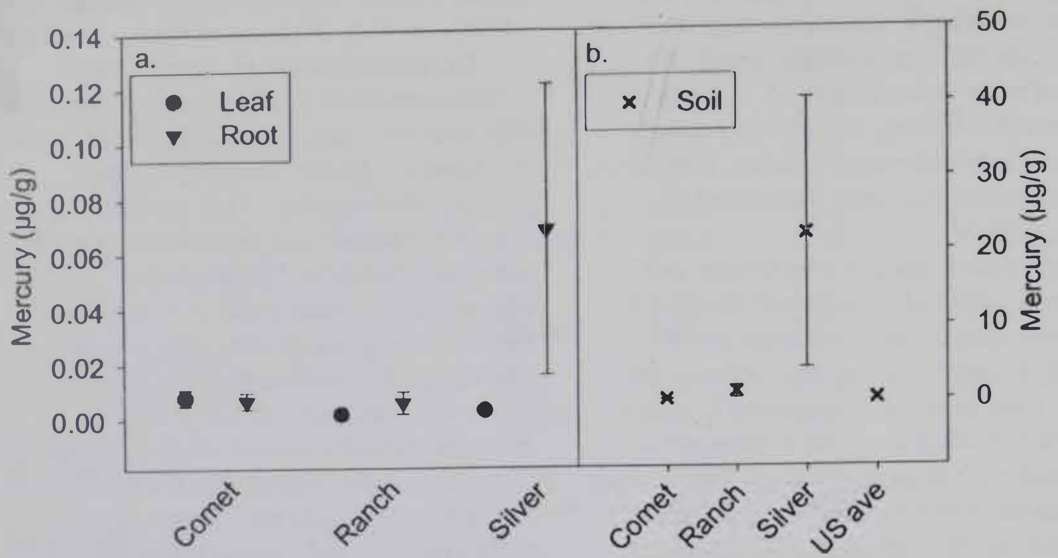

Figure 2. Mean mercury concentrations in roots and leaves at three contaminated sites in southwestern Montana; and b. comparative soil mercury concentrations at the same three sites and U.S. average topsoil (USGS, 1970). Points represent means; lines represent 95% confidence intervals.

not for the other species (Table 2). These results indicated that the three species with high root/shoot mercury ratios take up mercury into their roots but have a barrier that prevents transport of mercury from root to shoot. Correlations between root and soil mercury levels were significant (Pearson's = 0.408, P = 0.002) whereas the correlation between leaf and soil levels was only mildly so (Pearson's = 0.227, P = 0.069). The correlation between root and leaf mercury levels was quite low and not significant (Pearson's = 0.049, P = 0.685).

Discussion

Slender wheatgrass and needleandthread grass from the contaminated Silver Creek site did not accumulate large amounts of mercury in either roots or shoots. Likewise slender wheatgrass at the Comet site had low levels of mercury. Our findings indicated that these species could be used in revegetation of mercury-contaminated soils without danger of causing increased mercury mobilization.

We recommend that slender wheatgrass, needleandthread grass, and Canada bluegrass all be considered as acceptable grass species to be used in revegetating sites that might be contaminated with mercury. None of these species selectively accumulated mercury even though grown or growing on soils with elevated mercury concentrations. Similarly, ponderosa pine did not selectively accumulate mercury and could potentially be used to revegetate wastes containing mercury without the likelihood of mercury mobilization via roots or needles.

Vegetation type may impact movement of mercury throughout the plant. For example, Ellis and Eslick (1997) found correlations between root and leaf levels of mercury in shrubs and herbaceous species to soil mercury difficult to predict and highly variable on an abandoned mine site in Idaho. Barghigiani and Bauleo (1992) found levels of mercury in older leaves of silver fir (*Abies alba*) correlated with soil mercury. Overall correlations between root and soil and leaf and soil mercury were low in our study; however, we did not conduct species-specific correlations.

The techniques for reclaiming mining wastes used at the Comet Mine Repository appear to currently be preventing the movement of mercury from the covered wastes into vegetation. The long-term success of repositories in preventing the environmental movement of mercury or other metals could be demonstrated using the periodic assessment of on-site biomonitors like vegetation.

Rehabilitation of mine sites may include phytoremediation using plants known to accumulate toxic metals. The potential continued movement of accumulated metals through herbivory or decomposition may hinder such efforts. In non-woody plants, the root apparently provides a barrier to the movement of mercury throughout the plant since root levels of mercury are often on the order of 10 to 20 times levels in the leaves or shoot (Lindberg et al 1979). The root/shoot ratio of mercury could be important in the selection of species used in reclaiming mine wastes or revegetation of repositories as mercury that remains in the root is far less susceptible to movement through grazing, wind or water. Further study of the levels of mercury in vegetation growing on mercury contaminated soils is warranted to find other species that do not accumulate the metal and that could be used as restoration species on contaminated sites.

Conclusions

1. Slender wheatgrass, needle-and-thread, and Canada bluegrass should be considered for revegetation on mercury contaminated soils because they did not accumulate mercury when growing on sites with elevated mercury concentrations.
2. Ponderosa pine did not accumulate mercury when growing on a contaminated site and could also be used to revegetate wastes containing mercury without the likelihood of mercury mobilization via roots or needles.
3. The techniques for reclaiming mining wastes used at the Comet Mine Repository are currently preventing movement of mercury from covered wastes into vegetation.
4. Further study of mercury concentrations in vegetation growing on mercury contaminated soils is warranted to find other species that do not accumulate the metal and which could be used as restoration species on contaminated sites.

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Development of a New Biomonitoring Technique Using Domestic Pets as Sentinel Species

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Abstract

The goal of this research project was to develop a new way of investigating residential exposure to environmental contaminants. Specific objectives were to 1) develop a new method of monitoring biological exposure, 2) test the method in the field, and 3) develop a technique for analyzing the data. Domestic pets were chosen as the sentinel species, and the protocol involved collection of hair samples with subsequent analysis using inductively coupled plasma–mass spectrometry (ICP-MS). The method was tested using ~ 100 pets residing in the Butte area, and a new technique was devised that defines hazard quotients and hazard indices commonly employed in the field of risk assessment to identify pets of concern (POCs) and elements of concern (EOCs). In the field campaign, 76 percent of hair samples had arsenic concentrations ≥ the reference concentration of 0.02 mg percent. Twenty-five pets were identified as POCs based on pet hazard indices (HI_j) ≥ 1.0, and only 10 of the 25 POCs resided within the Butte Priority Soils Operable Unit (BPSOU), a boundary set by the EPA to represent the bulk of residential contamination in Butte. We also identified the following elements as EOCs based on element hazard indices (HI_i) ≥ 1.0: sodium, copper, manganese, selenium, boron, molybdenum, arsenic, lead, aluminum, lithium, and zirconium. The new biomonitoring technique was designed as a screening-level tool for studying residential exposure to environmental contaminants, but pets are companion animals and results may have implications for human health risk assessment.

Key Words: biomonitoring, domestic pets, hair sampling, residential contamination

Introduction

Nearly a century of mining and smelting activities in the Butte/Anaconda area of Montana resulted in widespread contamination. Arsenic, cadmium, copper, lead, and zinc are pollutants commonly identified in tailings and mine waste. Some remediation has taken place over the past two decades as a direct outcome of the National Priorities List (NPL) designation of sites in the area. However, much contamination remains in the residential neighborhoods, and little is known about long-term health impact on populations exposed on a daily basis to residual contaminants.

We describe a new way of investigating residential exposure to environmental pollutants. The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as Superfund, employs simple methods for addressing human health risk based on concentrations of contaminants measured in the soil, sediments, water, and air at a site (U.S. Environmental Protection Agency 1989); general assumptions are made about intake to estimate potential cancer risks and health hazards for "hypothetical" receptors, such as residents, workers, adults, and children. These methods were not designed for communities such as Butte where the Superfund site is in the backyard, receptors are not hypothetical, and contaminated waste is in the yards, gardens, water supply, and house dust.

To improve our understanding of actual exposure to contamination in a residential area such as Butte, we propose using domestic pets as our sentinel species. Although levels of contact with contaminated soil and water might be much higher for pets than humans, they might act as good surrogate monitors for humans in contaminant exposure assessments.

This paper describes the first in a series of research campaigns for which the main objectives of this project were to 1) design a simple biomonitoring method, 2) test the method in the field, and 3) develop a technique for analyzing the data. Regarding objective 1, our novel protocol involved collection of hair samples with subsequent analysis using inductively coupled plasma mass spectrometry (ICP-MS) as an easy, non-invasive way to obtain biological data regarding toxin/contaminant environmental exposure. For objective 2, we tested the method in winter of 2004 by sampling ~ 100 pets, including a variety of breeds, ages, and residence locations. Finally, because a technique was not available for interpreting the data from samples of pet hair, a simple method was devised that utilized some of the concepts currently used in the field of risk analysis (objective 3).

Although use of animals as sentinel species is not a new idea, this project is the first of its kind to draw on domestic pets as biosamplers with subsequent hair sampling and analysis to study chronic exposure of environmental contaminants in a residential Superfund area. Although results from our campaign were site specific, the method developed and tested here has potential for applications elsewhere.

Background

Mining History in the Butte Area

Gold mining began in Butte in 1864 (U.S. Environmental Protection Agency 2005), and by 1884, more than 300 mines, nine stamp mills, and as many as seven copper smelters were present (MacMillan 2000). Heap roasting was a common form of smelting in the late 1800s, and the heaps in Butte consisted of large masses of sulfide ore intermixed with layers of logs harvested from the local forests. According to MacMillan (2000), the heaps were "*as large as city blocks, as wide as city streets, and as high as six feet.*" Heaps commonly burned for weeks at a time, releasing thick smoke containing undiluted oxides of sulfur, arsenic, particulates, and fluorides. Furthermore, smoke lingered in the valley for days in the wintertime during inversions. By-products of these localized mining and smelting activities included accumulation of waste piles, dumps, and tailings throughout the community, along Silver Bow Creek, and within the Clark Fork watershed (U.S. Environmental Protection Agency 2005).

Cancer Statistics in Silver Bow County

Butte is located in Silver Bow County, the only county in Montana that was assigned a "priority 1" index by the National Cancer Institute in 2004 (U.S.

Table 1. Cancer Rates from National Cancer Institute for 1997-2001.

Area	Annual Death Rate from Cancer per 100,000 people	Higher or lower than National Rate	Annual Percent Change in Death Rate from Cancer (95 % CI)	Rising or Falling Trend
Silver Bow County	**238.6** (218.3, 260.6)	**Higher**	**+3.2** (0.4, 6.1)	**Rising**
Montana	195.0 (191.0, 199.0)	Lower	-0.6 (-1.0,-0.2)	Declining
United States	199.8 (199.6, 200.0)	-	-1.1 (-1.2, -1.0)	Declining

Reference: National Cancer Institutes of Health 2004

National Institutes of Health 2004). Priority 1 indicates an area where the annual death rate from cancer is above the U.S. rate, and an area that also exhibits a rising trend of deaths from cancer (Table 1). The annual death rate from cancer for Silver Bow County between the years of 1997 and 2001 was 238.6/100,000 people compared to rates for Montana and the U.S. of 195.0/ and 199.8/100,000 people, respectively. In addition, trends showed that the annual rate of cancer death was rising in Silver Bow County but declining in the rest of Montana and the rest of the U.S.

In addition to data from the National Cancer Institute, a request from the Montana Department of Public Health and Human Services (MDPHHS) in 2001 prompted the Agency of Toxic Substances and Disease Registry (ATSDR) to conduct a health consultation, and the report was released in December of 2003 (Dearwent and Gonzalez 2002). Cancer incidences in Silver Bow County for the years 1979 to 1999 were compared to data from the entire state of Montana, and to the United States as a whole. Six types of cancer (urinary bladder, kidney, liver, lung, prostate, and skin) were chosen as those most often linked to arsenic exposure. Based on the average standardized incidence ratios (SIRs), Silver Bow County had higher cancer rates than the rest of Montana, and higher rates than the rest of the U.S., in at least one age group for nearly all six types of cancer. The only exception was for prostate cancer for which Silver Bow County dropped below the national rate, but even in that case the incidence of prostate cancer for Silver Bow County was dramatically higher than the rest of Montana.

Cancer data described above were averaged over the whole county. However, a variety of mining and smelting operations over the years resulted in levels of soil contamination with extreme spatial variations throughout the area. Spatial variations in residential exposure and health effects throughout Silver Bow County are likely. However to date, incidence of cancer or of other health problems has not been investigated on neighborhood scales with the purpose of relating environmental exposure to health effects.

Superfund Operable Units

Within the Clark Fork Basin Superfund complex, four units are listed on the NPL: Silver Bow Creek/Butte Area Site, Montana Pole Site, Anaconda Smelter Site, and Milltown Reservoir Sediment Site. Operable units (OUs) are separate components within an NPL site, and the Butte Priority Soils Operable Unit (BPSOU), a major focus of this study, is one of seven operable units within the Silver Bow Creek/Butte Area Site. Other operable units within the Silver Bow Creek/ Butte Area include Butte Mine Flooding, West Side Soils, Active Mining Area, Streamside Tailings, Rocker Timber Treating and Framing Plant, and Warm Spring Ponds OUs (U.S. Environmental Protection Agency 2005).

The remedial investigation/feasibility study (RI/FS) of the BBPSOU focused on contaminants in soils, mine waste, surface water, and alluvial groundwater in the urban area (U.S. Environmental Protection Agency 2005). One important feature of the BPSOU is the inclusion of inhabited residential areas of Butte and Walkerville. Although some remediation has been performed since 1988 (U.S. Environmental Protection Agency, 2005), residents in this area have been directly exposed to the mining-related contaminants in the air, soil, water, and dust for more than a century.

Mining-Related Contaminants of Concern and Health Effects

According to the EPA's proposed remedy for the BPSOU, the contaminants of concern (COCs) in the soils are arsenic, lead, and mercury (U.S. Environmental Protection Agency 2005). Adverse health effects in humans have been documented for these three elements (Table 2). Exposure to arsenic, lead, and mercury generally can cause minor problems, e.g., a sore throat, and major problems, e.g., nervous system disorders, cardiovascular diseases, and cancer.

Table 2. Table of Health Effects for Arsenic, Lead, and Mercury.

Contaminant	Health Effects	References
Arsenic	Sore throat, irritated lungs, nausea, vomiting, decreased red/white blood cell counts, abnormal heart rhythm, damage blood vessels, pins and needles, darkening of skin, wart/corns on palms/soles/torso, redness/swelling of skin, and lung/skin/bladder/liver/kidney/prostate cancers.	ATSDR 2005, IRIS 2005a, Klaassen 2001
Lead	Decreased reaction time, weakness in fingers/wrists/ankles, memory and intelligence effects, anemia, blood disorders, organs/nervous system effects, and damaged kidneys/ reproductive systems.	ATSDR 2005, IRIS 2005b, Klaassen 2001
Mercury	Death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects, respiratory, cardiovascular, and gastrointestinal effects.	ATSDR 2005, IRIS 2005c, Klaassen 2001

Historically, metals have been recognized primarily for their acute effects to humans exposed in the workplace, but more recently, deeper investigation into the subacute and chronic effects has evolved. Assigning responsibility for long-term toxic impact of metals has been extremely difficult because cause-and-effect relationships are hard to establish when the endpoint lacks specific symptoms. Such symptoms relate to metal exposure (Klaassen 2001) and additive effects from exposure over time to multiple contaminants at once. Research in Butte, however, has potential to improve our understanding of chronic exposure to a handful of mining-related contaminants and associated health risks in humans and in other species.

Because of the health hazards associated with arsenic and lead, residential preliminary remediation goals (PRGs) were developed for the BPSOU. The initial PRG for lead in the BPSOU soil was 1200 ppm based on a risk assessment published in 1994, but the value increased to 1575 ppm in 2003. The PRG for arsenic in soil and house dust was set at 250 ppm, a threshold that equates to a cancer risk of 1 in 10, 000 (U.S. Environmental Protection Agency 2005). These PRGs are less conservative than PRGs set for other Superfund sites; in fact, EPA Region 9 lists a PRG of 0.39 ppm for arsenic in residential soils (U.S. Environmental Protection Agency Region 9 2004), and the Montana Department of Environmental Quality (DEQ) recently published their action level in surface soil as 40 ppm based on 209 native soil samples collected in unimpacted areas throughout Montana (Department of Environmental Quality 2005). Unfortunately, no reliable research has been performed to date to document whether the cleanup actions based on these elevated PRGs in Butte have been effective at reducing residential exposure.

Sentinel Species for Studying Exposure

According to O'Brien et al. (1993), *"Sentinels are organisms in which changes in known characteristics can be measured to assess the extent of environmental contamination and its implications for human health and to provide early warning of those implications."* Over the years, many animals have been used as sentinel species to advance the field of toxicology (O'Brien et al. 1993). Heyder and Takenaka (1996) noted that small mammals have been used as primary sentinel species in most laboratory studies, but they also concluded that dogs (*Canis familiaris)* were preferred for evaluating pulmonary responses to air

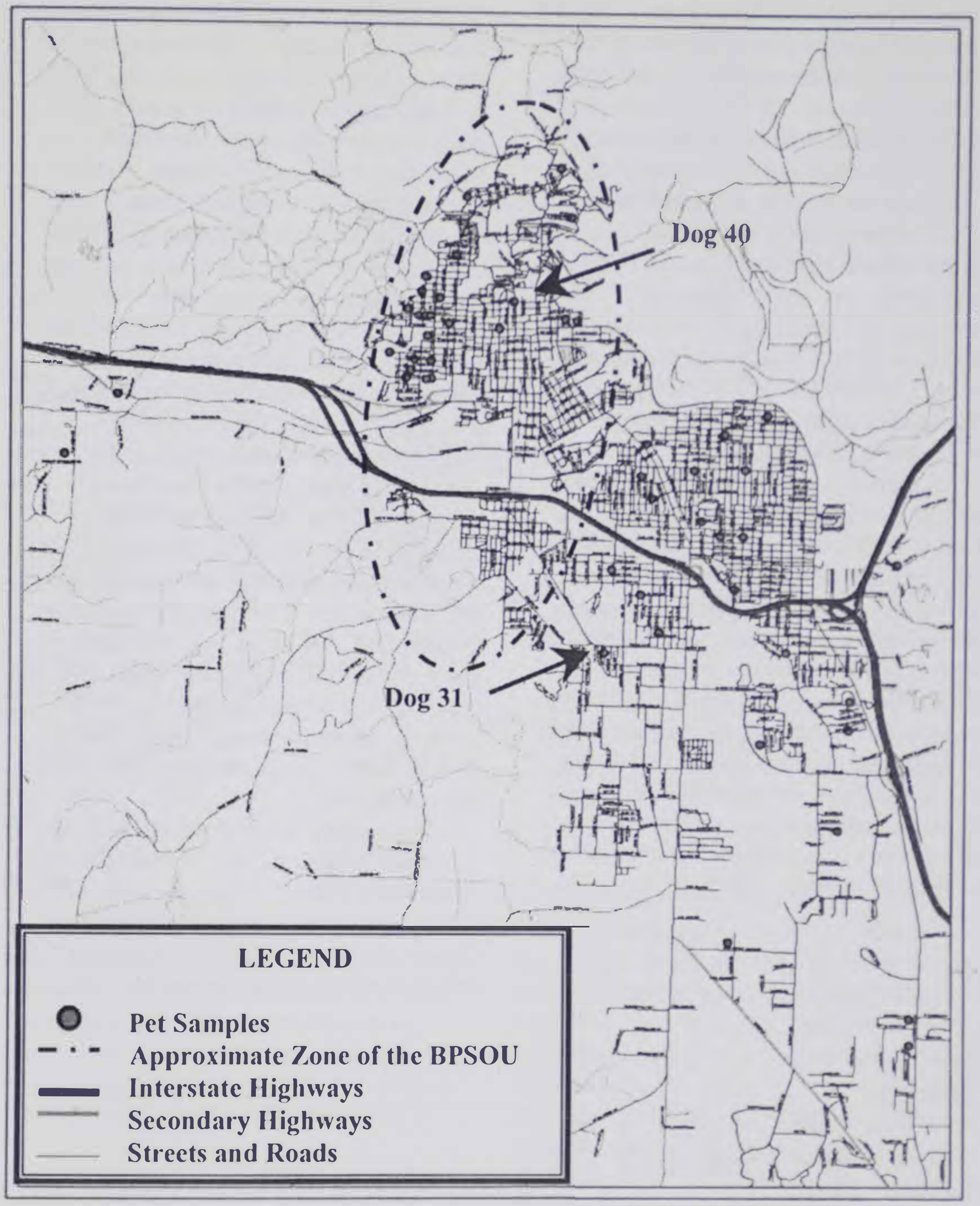

Figure 1. Map of Butte showing residence locations for the Butte pets sampled during the 2004 Hair Sampling Campaign. (Dogs 31 and 40 are discussed in the text.)

pollutants in controlled laboratory settings. Calderon-Garciduenas et al. (2001a, 2001b) expanded on this idea and examined dogs residing in Mexico to study effects of air pollution on respiratory, cardiac, and brain pathology. Thomas (1976) and Berny et al. (1995) found correlations between blood lead levels in dogs and children residing in the same households, and Hayes et al. (1981) related incidence of bladder cancer in dogs to bladder cancer in humans. O'Brien et al. (1993) also found house cats (*Felis domesicus*) to exhibit similar responses as humans to methyl mercury poisoning, and Berny et al. (1995) observed no significant difference in blood lead levels between house cats and humans.

Compared to other species, dogs and cats live parallel to humans and are most likely, if not more, exposed to the same toxins in soils, water, and house dust in a residential setting. In addition, dogs and cats are known to develop clinical signs more rapidly than humans after exposure, thus providing an "early warning" of threat to human health for reasons such as shorter life spans and decreased latency (O'Brien et al. 1993). Prior to our work, however, no one addressed exposures to mining-related contaminants or incidence of disease among domestic pets living in the Butte area.

Hair Analysis for Studying Chronic Exposure

The body has physiological mechanisms that remove contaminants after exposure such as excretion through the hair, which immobilizes the toxin in the keratin. Hair analysis, according to Lauwerys and Hoet (2001), is a good indicator of long-term absorption of inorganic arsenic, and concentrations in the hair shaft represents exposure periods of 1-10 months and longer (United States Department of Public Health and Human Services 2000). Controversial views exist about how much of the hair analysis might be contamination externally adsorbed to the hair shaft (Siedel et al. 2001), but the method is considered a good "screening" level indicator of chronic exposure to environmental toxicants (Hinwood et al. 2003), and the Agency for Toxic Substances and Disease Registry (ATSDR) has used the method with human subjects in recent health consultations elsewhere (Orloff and Mistry 2001). We found no data in the literature, however, for levels of contaminants in hair samples from human or pet populations living on Superfund sites.

In summary, many residents of Butte live inside a contaminated zone known as the Butte Priority Soils Operable Unit. Although cancer statistics for Silver Bow County are elevated when compared to rest of Montana and the rest of the U.S.,

Table 3. Summary of Information for Pets in the 2004 Hair Sampling Campaign.

Description	Number
Range of pet numbers	1-101
Total pets with complete lab reports	99
Total cat hair samples	7
Total dog hair samples	93
Pets sampled that resided in Butte	90
Pets sampled that resided elsewhere	10
Range of ages of pets (yr)	0.2-16
Average age of pets (yr)	6
Range of weight of pets (kg)	0.01-0.73
Average weight of pets (kg)	0.27
Number of male pets	57
Number of female pets	43
Pets residing within the Butte Priority Soils Operable Unit	48
Pets residing outside of the Butte Priority Soils Operable Unit	51

Table 4. Concentration Statistics of Elements for Hair Samples Collected in 2004.

Element	RfC (mg %)	Range (mg %)	Average (mg %)	Standard Deviation (mg %)	≥RfC (%)
Calcium (Ca)	129	13-272	93	55	26
Magnesium (Mg)	27	1.6-50.2	18.1	11.7	22
Sodium (Na)	205	9.6-1014	281	186	58
Potassium (K)	62	2-211	44	41	20
Copper (Cu)	1.7	0.9-10.3	1.7	1.3	23
Zinc (Zn)	20	14-41	18	3	11
Phosphorus (P)	35	16-46	31	6	20
Iron (Fe)	9.9	1-144.4	9.7	17.3	22
Manganese (Mn)	0.33	0.018-6.426	0.569	0.864	42
Chromium (Cr)	0.12	0.05-0.69	0.11	0.08	15
Selenium (Se)	0.16	0.09-0.49	0.22	0.08	72
Boron (B)	0.59	0.01-16.8	0.93	1.97	34
Cobalt (Co)	0.026	0.001-0.067	0.008	0.012	8
Molybdenum (Mo)	0.022	0.003-0.272	0.026	0.035	33
Sulfur (S)	4885	3726-5433	4466	326	9
Uranium (U)	0.02	0.000-0.0296	0.002	0.004	2
Arsenic (As)	0.02	0.01-1.397	0.065	0.149	76
Beryllium (Be)	0.002	0.001-0.003	0.001	0.0004	3
Mercury (Hg)	0.04	0.01-0.04	0.01	0.01	0
Cadmium (Cd)	0.02	0.001-0.318	0.014	0.045	8
Lead (Pb)	0.2	0.1-4.1	0.3	0.5	26
Aluminum (Al)	3.2	0.6-75.1	6.0	9.4	49
Germanium (Ge)	0.04	0.005-0.022	0.008	0.002	0
Barium (Ba)	0.4	0.02-1.93	0.24	0.29	16
Lithium (Li)	0.008	0.001-0.286	0.018	0.034	53
Nickel (Ni)	0.25	0.01-2.07	0.18	0.31	20
Platinum (Pt)	0.02	0.001-0.006	0.001	0.0005	0
Vanadium (V)	0.06	0.008-0.154	0.029	0.028	8
Strontium (Sr)	0.54	0.02-1.3	0.18	0.18	10
Tin (Sn)	0.04	0.001-0.09	0.020	0.02	8
Tungsten (W)	0.04	0.0003-0.073	0.005	0.011	2
Zirconium (Zr)	0.06	0.003-0.36	0.068	0.065	36

residential exposure and health problems have not been investigated on neighborhood scales. Our research introduces a new type of screening tool using domestic pets as sentinel species with hair sampling and analysis to study incidental, chronic contact with contaminants in the environment.

Experimental Methods

Our sampling campaign was conducted during February and March of 2004. We sampled ~ 100 pets, and the protocol consisted of the following components: 1) documentation, 2) hair sample collection, 3) hair sample analysis, 4) data entry, and 5) data analysis. Regarding documentation, pet owners were asked a series of standard questions about their dog or cat at the time of sampling, i.e., name, sex, breed, age, weight, home address, length of time residing in Butte, hours/day spent indoors, hours/day spent outdoors, brand of pet food, source of drinking water, etc. We collected each hair specimen between the shoulder and neck of the pet using stainless steel scissors, and care was taken to avoid cross contamination by disinfecting the scissors between collections. Specimen size was at least 150 mg, and immediately following collection, each sample was cataloged and placed in a sealed envelope specifically provided by the testing laboratory, Trace Elements, Incorporated (4501 Sunbelt Drive, Addison, TX 75001). Hair specimens were mailed to the laboratory within several days of collection.

Trace Elements, Incorporated is a

licensed, certified clinical laboratory. They specialize in analysis of human hair, mainly for nutritional purposes and are regularly inspected by the Clinical Laboratory Division of the Department of Health and Human Services. The laboratory personnel at Trace Elements, Incorporated use a state-of-the-art Sciex Elan 6100 for the inductively coupled plasma-mass spectrometry (ICP-MS) measurements. They factor extensive quality assurance and quality control (QA/QC) checks into all analytical procedures (Trace Elements, Incorporated 2000), and we submitted split samples with each batch of specimens as an external check.

Lab reports from Trace Elements contained concentrations for 32 elements, and they assigned a concentration equal to the instrument detection limit in the event that a concentration was below detection. Concentration units on each report were mg percent (mg of the element/100 g hair), and 1mg percent (mg %) = 10 ppm. Upon receipt of the lab reports, we entered concentrations for each pet into a spreadsheet, and at least one person checked data entry for accuracy.

The laboratory reports also listed reference concentrations (RfCs) for the elements. Reference ranges were established from a study of healthy dogs including all common breeds (Trace Elements, Incorporated 2005). A single reference level was given for toxic elements, e.g., uranium, arsenic, beryllium, etc., although nutritional elements, e.g., calcium, magnesium, etc. had a lower reference limit and an upper reference limit that described a zone of preferred concentrations. For example, the RfC for arsenic was 0.02 mg percent, but an acceptable RfC range for calcium was 41-129 mg percent. Concentrations above these RfCs are not necessarily toxic, but the values can be considered "guidelines for comparison with reported test values" (Trace Elements, Incorporated 2005).

Results and Discussion

General Information

Background data gathered during the sampling campaign included a suite of information for the study (Table 3). Pet numbers ranged from 1 to 101, but one young puppy (#34) did not have enough hair to add up to a 150-mg sample, and one number (#55) was not used as a pet number,

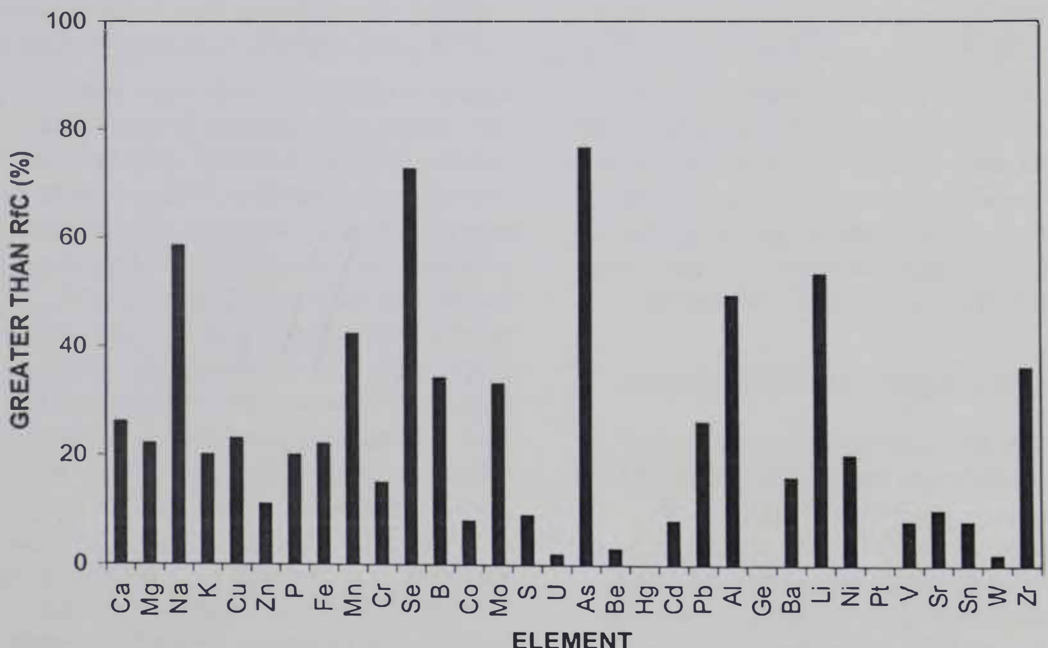

Figure 2. Percent of samples in the 2004 campaign where the concentration exceeded the reference level for each element.

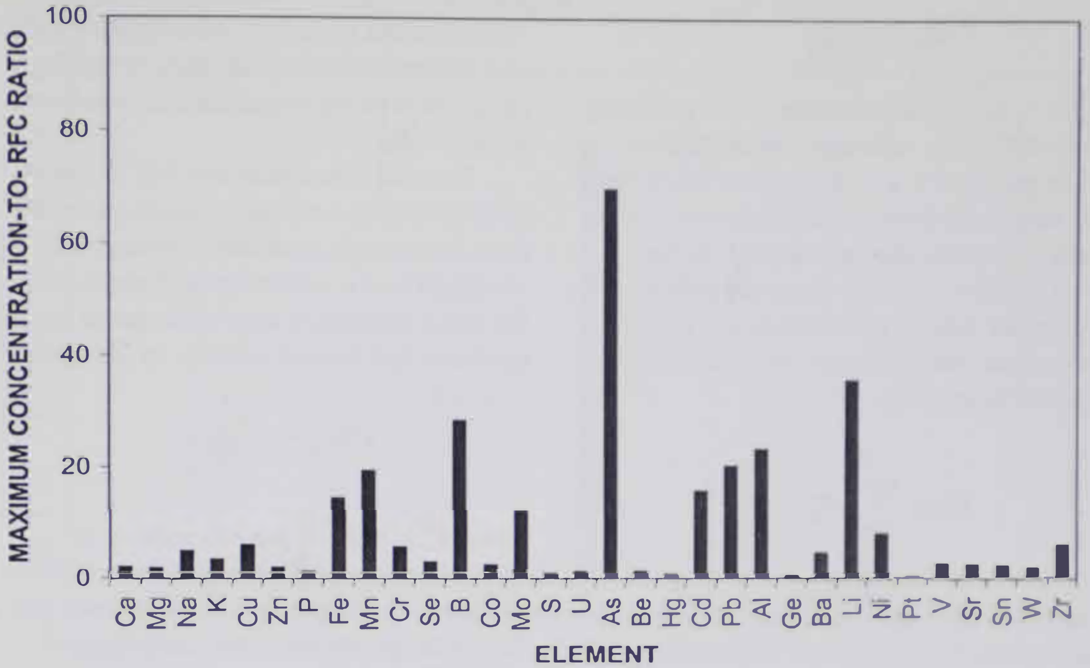

Figure 3. Ratio of the maximum concentration to the reference concentration for each element.

so 99 complete lab reports were available. Ten of the animals were cats, and 90 were dogs. Pet age in the study ranged between 0.2 and 16 years with an average of 6 years, and weight of the pet ranged 0.01-0.73 kg with an average of 0.27 kg. Of the dogs, 57 were males and 43 were females. In addition, 48 of the dogs resided within the envelope of the Butte Priority Soils Operable Unit (BPSOU) while 51 lived outside of the BPSOU boundary. Figure 1 shows the residence locations for the pets living in Butte.

General Statistics

We observed a wide range of concentrations in the dataset (Table 4), but mercury, germanium, and platinum were the only elements that did not show levels above reference concentrations. Arsenic concentrations in 76 percent of the samples exceeded the RfC value of 0.02 mg percent, and sodium, manganese, selenium, aluminum, and lithium exceeded the RfCs in 58, 42, 72, 49, and 53 percent of the samples, respectively (Table 4 and Fig. 2). In addition, lead concentrations in 26 percent of the samples were higher than the RfC.

As a worst-case scenario, the maximum arsenic concentration was 69.9 times higher than the reference level (Fig. 3). Several other elements also appeared to have dramatically-elevated maximum concentrations, such as iron, manganese, boron, molybdenum, cadmium, lead, aluminum, and lithium. Some of these elements may have nutritional sources, and others may be environmental. At this stage of the research, we did not try to distinguish between the two sources, but the mining-related contaminants, e.g., such as arsenic, aluminum, lead, etc., were elevated in many of the samples. We will examine these and additional data to identify nutritional versus environmental sources in future analyses.

Risk Analysis

In the field of risk assessment for air toxics (U.S. Environmental Protection Agency 2004), a non-cancer hazard quotient (HQ) is calculated for each air pollutant (i) as follows:

$$HQ_i = \frac{C_i}{RfC_i}$$

where C_i is the concentration of air pollutant i, and RfC_i is the reference concentration for air pollutant i. Concentrations below the RfC threshold should result in no adverse health effects (excluding cancer), so the target HQ is < 1.0 for a single air pollutant. Also in the field of risk assessment for air toxics, multiple pollutants are addressed via a hazard index (HI):

$$HI = \sum_{i=1}^{i=N} HQ_i$$

where N is the total number of air pollutants. This approach assumes that health effects are additive from exposure to multiple contaminants at once. Again, the target HI is < 1.0, so it is possible to have individual HQ values < 1.0 but still exceed the target HI.

Similar calculations of hazard values are utilized for human health risk assessments on Superfund sites (U.S. Environmental Protection Agency 1989). Instead of air concentration units, however, intake values and reference doses (with units of mg/[kg day]) are used for contaminants in soil and water media.

No established protocol was available for performing a risk assessment on results from hair sample analyses; consequently, we developed a simple method employing the same commonly used concepts of hazard quotients and hazard indices. In our case, however:

$$HQ_{ij} = \frac{C_{ij}}{RfC_i}$$

where HQ_{ij} was the hazard quotient of element i for pet j; C_{ij} was the concentration of element i in the hair sample of pet j; and RfC_i was the reference concentration for element i.

We also defined two hazard indices. A pet hazard index (HI_j) was calculated for each pet by summing the hazard quotients across the elements:

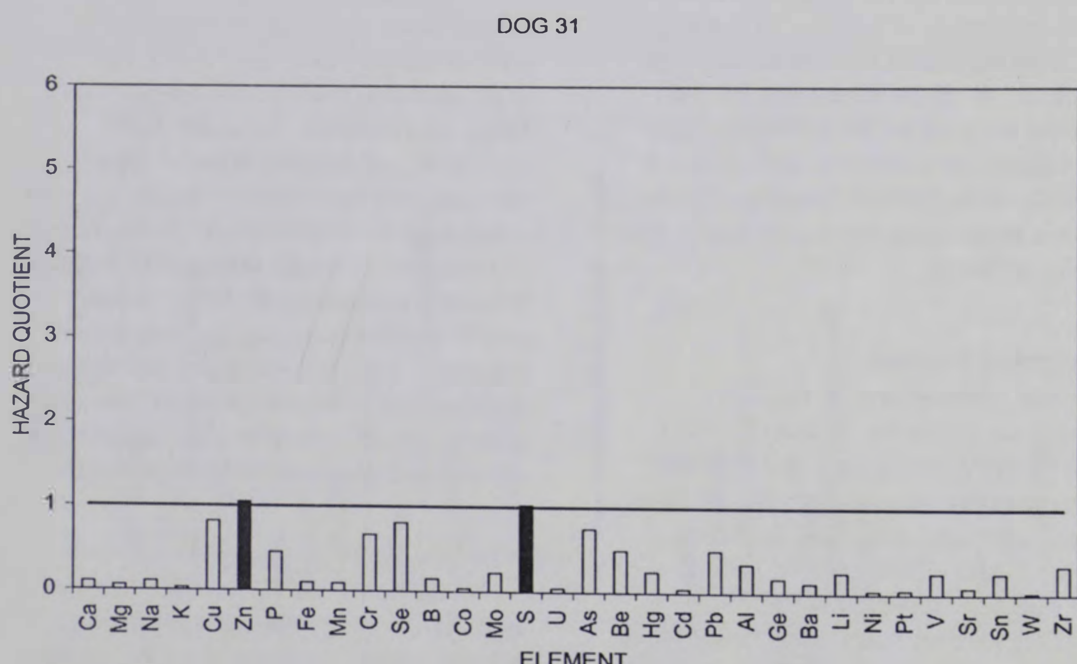

Figure 4. Hazard quotient (HQ) graph for Dog 31. White shading indicates HQ values < 1.0, and black shading corresponds to HQ values ≥ 1.0.

DOG 40

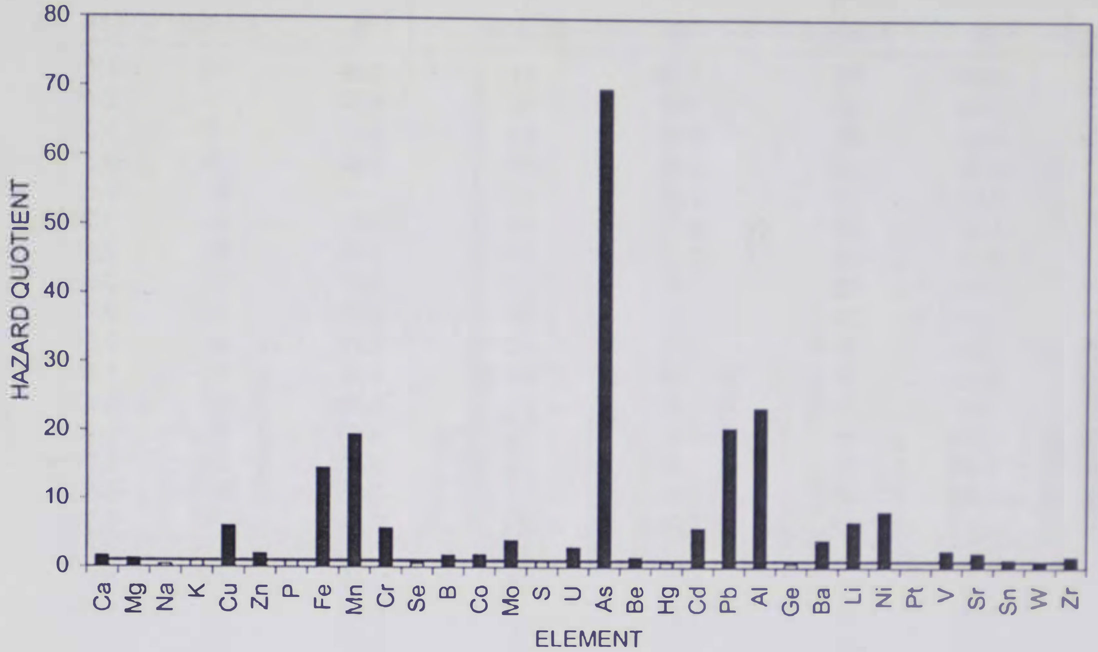

Figure 5. Hazard quotient (HQ) graph for Dog 40. White shading indicates HQ values < 1.0, and black shading corresponds to HQ values ≥ 1.0.

$$HI'_j = \sum_{i=1}^{i=N} HQ_{ij}$$

where N was 32 elements, and an element hazard index (HI'_i) was calculated for each element by summing the hazard quotients across the pets:

$$HI'_i = \sum_{i=1}^{i=M} HQ_{ij}$$

where M was the total number of pets. The hazard indices were then normalized by the number of elements and pets as follows:

$$HI_j = \frac{\sum_{i=1}^{i=N} HQ_{ij}}{N}$$

and

$$HI_i = \frac{\sum_{j=1}^{i=M} HQ_{ij}}{M}$$

where the target value was 1.0 for both HI_j and HI_i. Pets with HI_j values ≥ 1.0 were defined as pets of concern (POCs), and elements with HI_i values ≥ 1.0 were defined as elements of concern (EOCs).

Table 6. Element Hazard Index (HI_i) Data for the 2004 Campaign.

Element	HI_i	Element	HI_i	Element	HI_i	Element	HI_i
Calcium	0.72	Manganese	1.72	Arsenic	3.25	Lithium	2.30
Magnesium	0.67	Chromium	0.88	Beryllium	0.54	Nickel	0.73
Sodium	1.37	Selenium	1.40	Mercury	0.30	Platinum	0.05
Potassium	0.72	Boron	1.57	Cadmium	0.71	Vanadium	0.48
Copper	1.00	Cobalt	0.30	Lead	1.58	Strontium	0.51
Zinc	0.89	Molybdenum	1.20	Aluminum	1.86	Tin	0.50
Phosphorus	0.87	Sulfur	0.91	Germanium	0.19	Tungsten	0.12
Iron	0.98	Uranium	0.20	Barium	0.59	Zirconium	1.13

Table 5. Pet Hazard Index (HI_j) Data for Pets in the 2004 Campaign.

Pet Number	HI_j	Pet Number	HI_j	Pet Number	HI_j	Pet Number	HI_j
1	0.62	26	1.79	51	1.20	76	1.02
2	0.48	27	1.50	52	0.77	77	0.66
3	0.58	28	0.44	53	0.65	78	0.76
4	0.89	29	0.48	54	0.44	79	0.57
5	0.55	30	0.38	55	-	80	0.72
6	0.44	31	0.31	56	1.37	81	1.28
7	0.44	32	0.51	57	1.12	82	0.51
8	0.44	33	0.46	58	0.57	83	0.56
9	0.90	34	-	59	0.56	84	0.79
10	1.31	35	0.57	60	0.74	85	0.54
11	0.44	36	0.82	61	0.88	86	1.35
12	0.44	37	1.38	62	0.78	87	0.52
13	1.21	38	0.64	63	0.73	88	0.46
14	0.80	39	1.44	64	0.83	89	0.94
15	0.48	40	6.74	65	2.27	90	0.52
16	0.83	41	2.34	66	0.88	91	0.54
17	0.85	42	0.50	67	0.41	92	0.53
18	0.66	43	1.55	68	0.64	93	0.61
19	2.09	44	1.08	69	0.60	94	0.58
20	0.68	45	1.88	70	0.65	95	1.53
21	0.96	46	0.94	71	0.99	96	2.06
22	0.49	47	0.74	72	0.74	97	1.69
23	0.73	48	0.71	73	3.07	98	0.38
24	0.63	49	0.37	74	0.59	99	0.52
25	0.95	50	0.64	75	0.79	100	3.03
						101	1.46

To illustrate hazard quotient method in this paper, we selected the two extreme cases from the 2004 field campaign. Dog 31 was a 13-year old male Lhasa apso that weighed 0.5 kg and lived at 3518 Oregon Avenue located outside of the southern boundary of the BPSOU (Fig. 1). Dog 40 was a 12-year old male border collie that weighed 0.25 kg and lived at 125 West Copper in the uptown area of Butte, inside the BPSOU (Fig. 1). Hazard quotients for Dog 31 were < 1.0 for all but two of the elements, and most were much less than 1.0 (Fig. 4). The HQs for zinc and sulfur were the highest but still barely above the level of concern at values of 1.05 and 1.03, respectively. When averaged over all of the elements, the HI_j for Pet 31 was 0.31, the smallest pet hazard index for the whole campaign.

Dog 40's hair sample revealed much different results from Dog 31's. Hazard quotients for Dog 40 were < 1.0 for sodium, potassium, phosphorous, selenium, sulfur, mercury, germanium, and platinum, but all of the rest of the elements exceeded the level of concern (Fig. 5). The top five HQs were 69.9, 23.5, 20.5, 19.5, and 14.6 for arsenic, aluminum, lead, manganese, and iron, respectively. When averaged over the elements, this dog had highest pet hazard index for the whole campaign, a HI_j value of 6.74.

Identification of Pets of Concern and Elements of Concern

While Dogs 31 and 40 represented the two extremes of the campaign, 25 pets were identified as pets of concern based on pet hazard indices (HI_j) ≥ 1.0 (Table 5 and Fig. 6). Ten of the POCs resided inside the BPSOU and 15 resided outside the BPSOU. This suggested that the envelope of the BPSOU might not describe a clear division of contaminated soils inside and non-contaminated soils outside the boundary.

The following eleven elements were

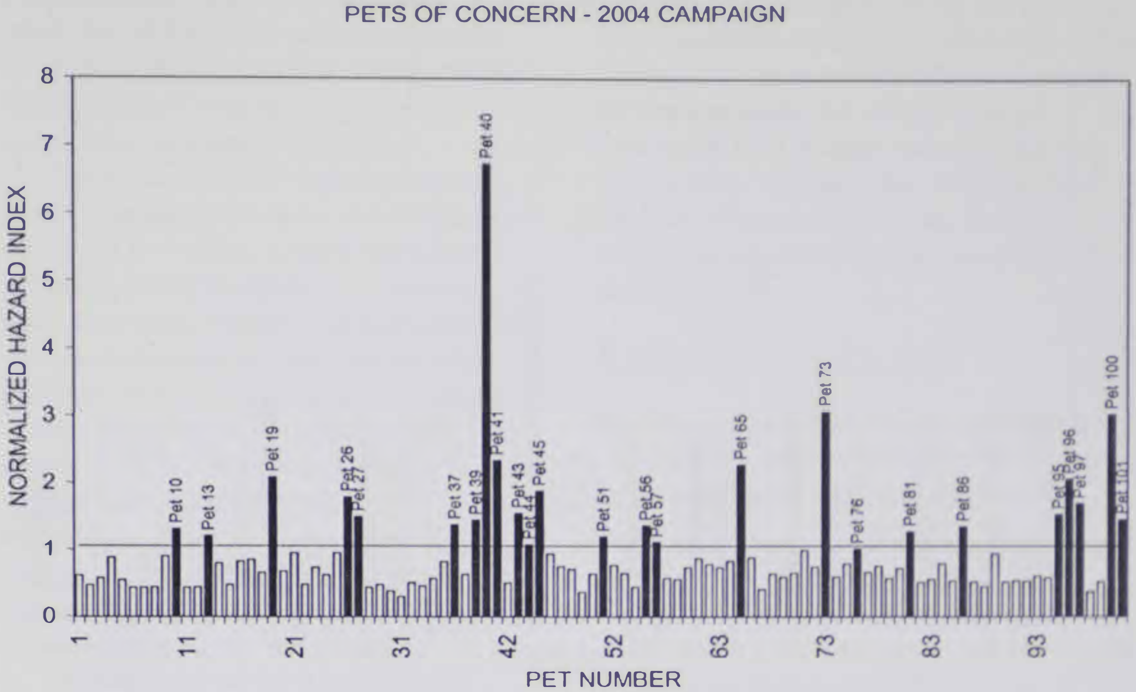

Figure 6. Hazard index (HI_j) graph for all pets in the 2004 campaign. While white shading indicates HI_i values < 1.0, black shading corresponds to HI values ≥ 1.0. Pets with HI_j values ≥ 1.0 are defined as pets of concern.

identified as elements of concern for our campaign based on element hazard indices (HI_i) ≥ 1.0: sodium, copper, manganese, selenium, boron, molybdenum, arsenic, lead, aluminum, lithium, and zirconium (Table 6 and Fig. 7). Of these elements, several may be mining-related, e.g., copper, iron, manganese, molybdenum, arsenic, lead, aluminum, etc., whereas others may be diet-related, e.g., such as sodium, selenium, boron, etc.

Based on our study, contaminant levels continue to be elevated in Butte's domestic pet population after nearly 20 years of cleanup activities. Of the mining-related contaminants, arsenic and lead stand out as major elements of concern because of possible links to health effects previously discussed. Manganese and aluminum, however, are also important because they have not been addressed in the local Superfund activities, and both are believed to play roles in serious health problems such as Parkinson's or Alzheimer's disease (Klaassen 2001).

Although these results are site specific, they illustrate the potential for developing this method into a simple screening-level tool for identifying elements in a residential setting that should be further investigated. In addition, citizens are normally hesitant about providing blood or urine samples from family members, especially from their children; however, residents in Butte were fascinated and enthusiastic about participating in a study that only required a small amount of hair from their pet.

Variability, Limitations, and Applications

As with all biological or ecological indices, our data contained variability in accumulation of the elements of concern. In this paper, we focused on introducing the method, presenting results from our field campaign, and devising a way to examine the data. Sources of variability (such as breed type, sex, age of the pet, etc.) were not discussed here but will be addressed in a future publication regarding a larger dataset.

In terms of limitations, exposure to environmental toxins is likely extreme for pets compared to exposure for humans

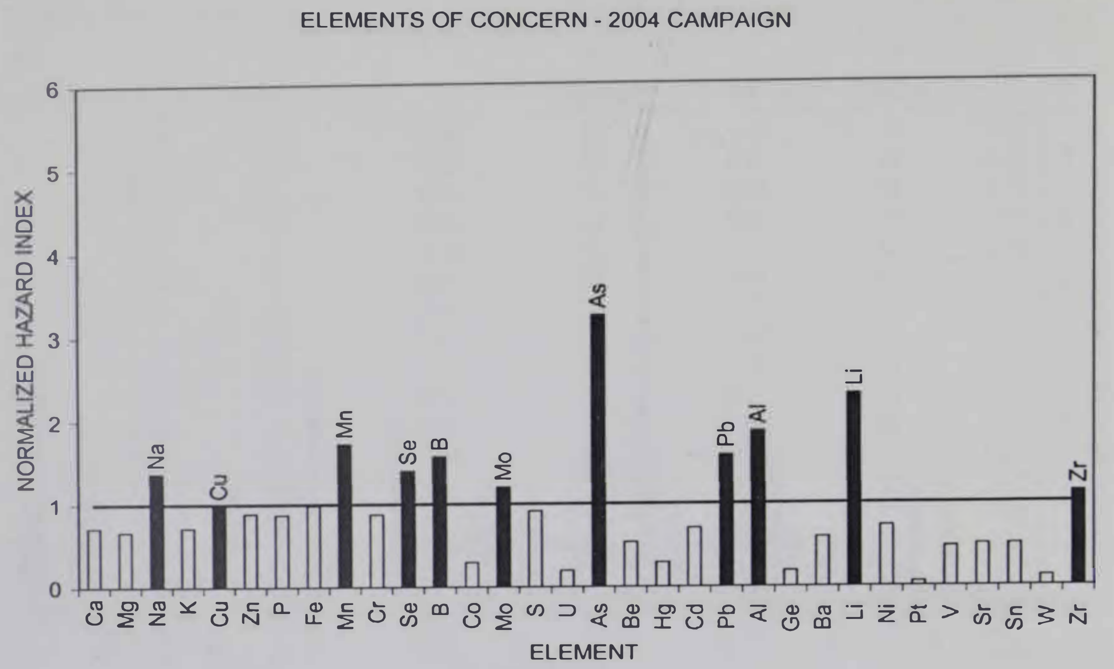

Figure 7. Hazard index (HI_i) graph for all elements in the 2004 campaign. While white shading indicates HI_i values < 1.0, black shading corresponds to $HI_i \geq 1.0$. Elements with HI_i values ≥ 1.0 are defined as elements of concern.

because of self-grooming habits and because pets have more direct contact with soil, house dust, and mud puddles; thus, we cannot assume that the method might be directly extrapolated to humans. Furthermore, our method of assigning hazard quotients and hazard indices is based on concentrations relative to reference concentrations. This is a simple, initial step toward evaluating incidental exposure to environmental contaminants, but relative toxicity of individual elements is not incorporated. Refinement of the method could include relative toxicity factors derived from data employed in the field of human health risk assessment.

Overall, our new biomonitoring technique was designed as a screening-level tool to identify contaminants that may be of concern in a community. For a residential area like Butte, the method may be useful for discovering homes or neighborhoods that might need of further investigation and/or remediation. Justification of the project involved use of dogs and cats as sentinels to protect the health of adults and children residing in a contaminated area, but protection of the health and well being of local pets is also important.

Conclusions

The purpose of this project was to develop a new way of investigating residential exposure to environmental pollutants by utilizing domestic pets as a sentinel species. We met our objectives by developing a biomonitoring technique involving pet hair samples with subsequent analysis for an array of elements, including metals. We tested the technique in the field during early 2004 using pets residing primarily in the Butte area. Results from the sampling campaign were entered into a database, and to examine the data we developed a hazard quotient technique similar to the methods used in the field of risk analysis.

We calculated a pet hazard index for each pet by summing HQs across the elements and by normalizing by the number of elements, and calculated an element hazard index for each element by summing HQs across the pets and by normalizing by the number of pets. We identified 25 pets as pets of concern based on $HI_j \geq 1.0$ of which only 10 resided inside the BPSOU. This suggested that the envelope of the BPSOU does not necessarily define a distinct zone of environmental contaminant exposure in Butte.

Likewise, the following 11 elements were identified as elements of concern based on $HI_i \geq 1.0$: sodium, copper, manganese, selenium, boron, molybdenum, arsenic, lead, aluminum, lithium, and zirconium. Of these eleven elements, only two (arsenic and lead) of the five contaminants (arsenic, cadmium, copper, lead, and zinc) commonly investigated in the Butte and Anaconda mine wastes were included. These results confirm the EPA's designation of arsenic and lead as contaminants of concern, but our study also indicated that other elements of concern probably also should be addressed.

In summary, we have introduced a new way of studying incidental exposure to environmental contaminants using pets as biosamplers. Because pets are companion animals, results from the method have implications for human health risk assessment. While this paper focused on describing the method and summarizing results from samples collected during winter of 2004, additional research is ongoing, and a subsequent paper will address data from approximately 250 more pets sampled during the past two years. Future effort will statistically compare exposure levels for pets residing inside and outside the boundary of the BPSOU and demonstrate the potential of using domestic pet hair as a tool for quantifying efficacy of remediation. A longer-term goal of the research is to identify links between concentration data of elements of concern on neighborhood scales from the biomonitoring technique with 1) concentrations of contaminants in the soils, water, and house dust, and 2) incidence of disease, including cancer, in pets and humans. Butte is the ideal community for conducting this type of research.

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