U.S. Fish and Wildlife Service
Region 2
Contaminants Program

TRACE ELEMENTS IN BATS ROOSTING IN MINES AT KOFA NATIONAL WILDLIFE REFUGE, ARIZONA, 2001 - 2002

by

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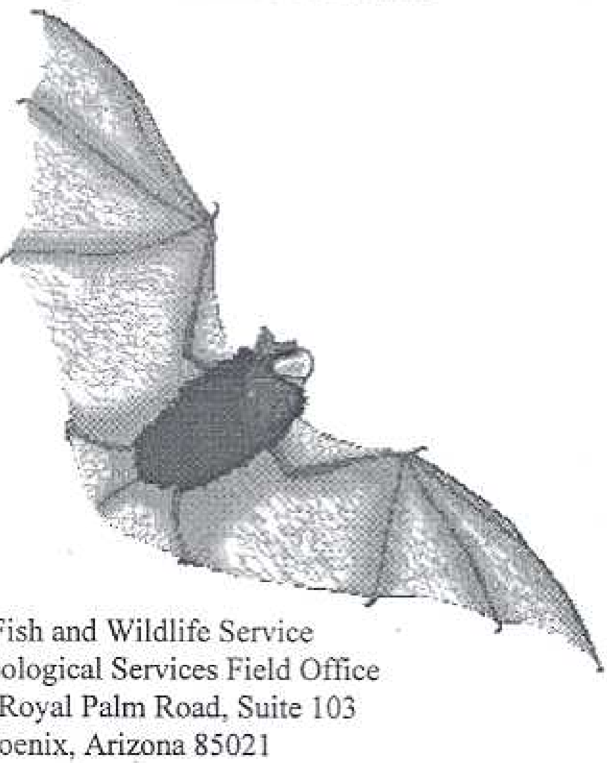

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July 2003

ABSTRACT

Californiamyotis*(Myotis californicus)* and California leaf-nosed bats *(Macrotus californicus)* were collected from five mines used as roost sites to assess concentrations and potential adverse effects of trace elements. Geometric mean carcass lead concentrations in bats collected from two lead mines (62 and 102 µg/g dry weight) were significantly (P< 0.0001) higher than those in individuals from nearby gold and silver mines (0.96 - 2.59 µg/g dry weight). Carcass concentrations were within the range reported in bats collected from known lead-contaminated sites. A geometric mean of 16.6 µg/g dry weight lead in leaf-nosed bat liver samples was among the highest recorded in apparently healthy individuals of any species. Arsenic, cadmium, mercury, and selenium in bat carcass and liver tissues were below biologically significant thresholds.

Bats roosting in lead mines may be accumulating a significant portion of their total body burden at the roost site by ingesting lead particles while grooming or through inhalation of lead contaminated dust. Soil from two lead mine roost sites contained from 56,900 to 102,000 µg/g lead. These concentrations were up to three orders of magnitude higher than those in soil from reference mines (37 - 250 µg/g). Water used to wash bats before dissection contained 18 trace elements; whereas, only three were detected in reference water samples. Lead concentrations in the different media tested (soil, wash water, carcass, and liver tissues) were consistently higher in samples from lead mines than in samples from gold and silver mines. The detection of trace elements in wash/rinse water indicates that these elements were present as particles on the bats fur and were available to be ingested during grooming.

Project No. 22410-1261-2N41
DEC ID No. 200120001.2

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INTRODUCTION

An alarming 56% of the 43 bat species found in the United States and Canada are endangered or are under consideration for listing (Anonymous 1995). Bats now have the highest percentage of endangered and candidate species among all land mammals in the United States and Canada. More than 20% of Arizona's bat populations are declining (Noel and Johnson 1993). Nineteen of 28 species found in Arizona use mines to some extent as day, night, transitory, maternity, or hibernating roosts (Castner et al. 1995). A 1997 survey of 173 mines on Kofa NWR revealed that 105 contained potential roosting habitat (Snow 1998). Evidence of bat use was documented in 58 of the 105 (55%) suitable sites which was slightly higher than other Arizona areas (Castner et al. 1994, Snow 1998). All refuge mines were surveyed except those that appeared unsafe to enter or areas where land ownership was in question.

A previous study of bats roosting in Sheep Tank mine on Kofa NWR documented high levels of several trace elements in mine soil and in bat carcass tissues (King et al. 2001). For example, barium, manganese, and zinc were detected in soil at concentrations at least 10-times higher than previously reported in Arizona. Big brown bats *(Eptesicus fuscus)* from the same mine also contained significantly higher concentrations of these elements than big brown bats collected from three reference sites. King et al. (2001) concluded that trace elements acquired at the roost site could be a major component of total accumulation.

This report documents trace element bioaccumulation in bats roosting in five mines on Kofa NWR and discusses the potential for contaminant-induced adverse effects. We further tested the hypothesis that body burdens of certain elements may, in part, reflect ingestion of elements at the roost site through grooming.

STUDY AREA

Five mines selected for study included the Hub, Hull, Keystone, Northstar No. One (Northstar), and Puzzler. Specific study sites were selected based on a history of bat use as described by Snow (1998). Mine names and their latitude and longitude coordinates are presented in Table 1. Relative locations are shown in Figure 1. The maximum distance between mines was approximately 31 km (~19 miles).

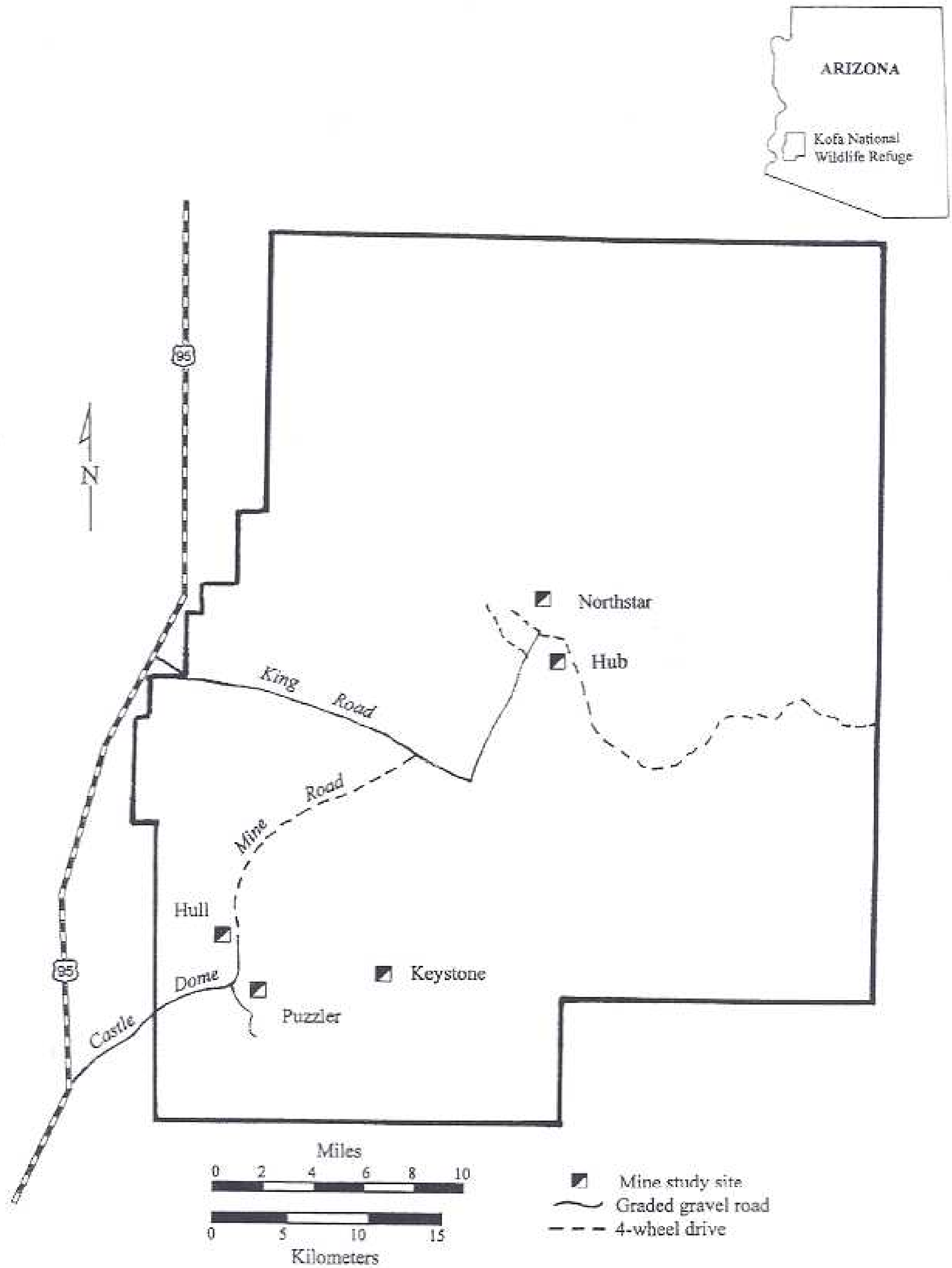

Figure 1. Kofa National Wildlife Refuge study sites, 2000 - 2001

Table 1. Bat and mine soil samples collected at Kofa National Wildlife Refuge Arizona, 2001 - 2002

Study sites	Latitude - longitude	Date coll.	Bats		Soil	
			N	Common name *(Scientific name)*	N	(g)
Hub Mine	N 33°16'15" W 113°57'43"	08/09/01	9 4	California myotis *(Myotis californicus)* California leaf- nosed bat *(Macrotus californicus)*	1 1	256 245
Hull Mine	N 33°03'56" W 114°10'53"	11/16/01	8	California leaf-nosed bat *(Macrotus californicus)*	1	445
Keystone Mine	N 33°03'02" W 114°01'13"	11/14/01	4	California leaf-nosed bat *(Macrotus californicus)*	1	263
Northstar Mine	N 33°17'48" W 113°58'26"	11/15/01	8	California leaf- nosed bat *(Macrotus californicus)*	1	360
Puzzler Mine	N 33°01'55" W 114°09'22"	02/01/02	6	California leaf- nosed bat *(Macrotus californicus)*	1 1	108 86

METHODS

Sample collections: Field collections were completed in August and November 2001 and in February 2002. We selected two bat species for study, the California myotis *(Myotis californicus)* (Family Vespertilionidae) and the California leaf-nosed bat *(Macrotus californicus)*, (Family Phyllostomidae) based on abundance. Most bat species in northern and mid-latitudes of the U.S. hibernate during winter months (Tuttle 1991). However, the California myotis remains 'fairly active' throughout the winter (Noel and Johnson 1993). California myotis roost in any convenient shelter and do not regularly return to the same place every day (Noel and Johnson 1993). In contrast, California leaf-nosed bats neither hibernate nor migrate. Also, they are relatively roost site specific (Noel and Johnson 1993) although they may switch roost sites within a given area during the course of a year (K. Hinman pers. comm., T. Snow pers. comm.). Thus, the California leaf-nosed bat is a better biomonitor of contamination of the local environment than California myotis. Bats were usually collected during exit flights using mist nets placed across the mine entrance. At the Northstar mine, bats were collected by sweep nets during daylight hours. Individuals were euthanized by cervical dislocation and individually placed in labeled plastic bags. The bags were then stored on wet ice until they could be transferred to a commercial freezer.

We also collected one or two soil samples from each mine. Soil samples were taken from the mine floor near areas where the bats roosted. Each soil sample consisted of five sub-samples collected from the same general area. Soil samples were individually stored in plastic bags. We compared trace element concentrations in mine soil with Arizona background levels (Boerngen and Shacklette 1981, Earth Technology 1991) and with human health guidelines (ADEQ 1998). Arizona Department of Mines and Mineral Resources (ADMMR) records were examined to determine major minerals extracted from the mine study sites (ADMMR 1996).

Sample preparation: Individual bats were weighed, measured, and sexed. The bats were then "washed" before dissection. One hundred milliliters (ml) of distilled water was poured into a pre-cleaned and labeled sample jar. From that jar, about 25 - 30 ml of water was then poured into a plastic spray bottle that had been triple-rinsed with distilled water. The bat was removed from the sample bag and set aside. The bag was rinsed with distilled water from the larger sample jar to remove any dust or dirt particles that may have been dislodged from the bat. The water in the bag was then poured back into the sample jar. An individual bat was then placed into the jar with water and the jar gently agitated for two minutes. The bat was then held over the jar with forceps while water from the spray bottle was squirted over the bat for a final rinse. Usually from 2 to 4 ml of water was "lost" through adhering to sample bag and to bat fur. The sample jar was brought to 100 ml by adding clean distilled water. We also analyzed three 100 ml reference samples of clean water. Each reference water sample was poured into an empty plastic bag identical those in which the bats had been stored. After the bags were rinsed, the water was poured back into the sample jar and increased, if needed, to 100 ml.

After washing, the bats were dissected and gastrointestinal tracts were removed and discarded. We had hoped to individually analyze livers from each bat, but California myotis livers weighed less than 0.1 g and were too small for individual analyses. Myotis livers were replaced in the carcass and analyzed along with the individual carcass remainder. Livers of all California leaf-nosed bats were dissected out and analyzed individually. Carcass remainders also were analyzed for trace elements.

Chemical analyses: Soil, water, carcass, and liver samples were analyzed for aluminum, arsenic, barium, beryllium, boron, cadmium, chromium, copper, iron, lead, mercury, magnesium, manganese, molybdenum, nickel, selenium, strontium, vanadium, and zinc at Laboratory and Environmental Testing, Columbia, Missouri. Arsenic and selenium concentrations were determined by graphite furnace atomic absorption spectrophotometry (USEPA 1984). Mercury was quantified by cold vapor atomic absorption (USEPA 1984). All other elements were analyzed by inductively coupled plasma atomic emission spectroscopy (Dahlquist and Knoll 1978, USEPA 1987). Blanks, duplicates, and spiked samples were used to maintain laboratory quality assurance and quality control (QA/QC). QA/QC was monitored by the Services' Patuxent Analytical Control Facility. Analytical methodology and reports met or exceeded the Service's standards. Trace element concentrations are expressed in µg/g (ppm) dry weight. The lower limits of analytical quantification varied by element and by sample and are listed in each appendix. Percent moisture is also presented to permit wet weight to dry weight conversions. Dry weight values can be converted to wet weight equivalents by subtracting the percent moisture (as a decimal) from 1.0 and multiplying the resulting number by the dry weight value as illustrated in the following equation:

$$\mu g/g \text{ wet weight} = (1 - \% \text{ moisture}) \times \mu g/g \text{ dry weight}$$

Statistical analysis: Data for most elements were skewed and arithmetic means of skewed data do not accurately reflect central tendency; therefore, we used geometric means to more accurately estimate central tendency. The geometric mean was calculated when a element was detected in ≥50 % of the samples. For those samples for which no residues were detected, a value of one-half the lower limit of detection was substituted for the not-detected-value to facilitate the calculation of means. Residue data were normalized by log-transformation before mean comparisons. Geometric means were first compared using a 2-way ANOVA (species, location). Results of a 2-way ANOVA indicated that there were significant differences in geometric means of several elements between species and among mines; therefore, we further analyzed the data using a series of one-way ANOVAs. The Bonferroni multiple comparison method (Neter and Wasserman 1974) was used to test for mean separation when ANOVA showed significant differences.

RESULTS

The Keystone mine was primarily a silver mine and both gold and silver were taken from the Northstar mine (ADMMR 1996). The Hull and Puzzler were primarily lead mines. The Hub was not listed in the ADMMR (1996). Apparently, no minerals were extracted from the Hub mine as it served as an exploratory shaft only (P.A. "Doc" Burdick, pers. comm.). California myotis were collected only from the Hub mine and California leaf-nosed bats were collected from all mines. All females were post-lactation.

Soil– Two soil samples were collected from the Hub and Puzzler mines and one each from the others (Table 2). Background data were available for 14 of 19 elements studied. Soil from the Puzzler mine was the most contaminated; concentrations of 9 of 14 trace elements (64%) exceeded either human health guidelines for non-residential soil, or the maximum Arizona background level, or both. At other mines, five or fewer elements (≤36%) in any single soil sample exceeded either the human health or Arizona background level. Lead in particular showed striking differences among mines. Lead was detected in Hull mine soil at 102,000 µg/g which was more than 1,000-times higher than the maximum recorded Arizona background level and more than 51-times higher than the non-residential human health guideline. Two soil samples collected from the Puzzler mine contained 56,900 and 95,200 µg/g lead, also far in excess of background levels and human health guidelines. The next highest lead concentration was 250 µg/g recorded in the Keystone mine soil (Table 2).

Table 2. Trace elements in soil samples from mines on Kofa National Wildlife Refuge (µg/g dry weight) compared with Arizona background levels and human health guidelines[1]

					Mine						
	Arizona Background[2]		Human Health Guidelines[3]		Hub		Hull	Key-stone	North-star	Puzzler	
Element	Mean	Maximum	Residential	Non-resident	Interior	Mouth	Interior	Interior	Interior	Interior	Interior
Aluminum	55,213	100,000	77,000	1,000,000	9,650	13,500	11,500	20,100	22,000	21,000	32,700
Arsenic[4]	9.8	97	10.0	10.0	***87.0***	***75.0***	***32.0***	***52.0***	***38.0***	***1,320***	***550***
Barium	565	1,500	5,300	110,000	123.	1,310	414	242	3,120	***3,560***	***2,660***
Beryllium	0.52	5.0	1.4	11.0	***5.40***	***5.90***	0.99	1.60	2.20	***20.0***	4.10
Cadmium	NA	NA	38	850	2.90	<0.20	3.00	0.99	0.50	7.00	18.0
Chromium	61.3	300.	2,100	4,500	26.0	29.0	3.50	23.0	6.10	39.0	22.0
Copper	30.	200.	2,800	63,000	81.0	12.0	94.0	27.0	43.0	***233***	173
Lead	23.4	100.	400	2,000	37.0	40.0	***102,000***	***250***	***100***	***95,200***	***56,900***
Mercury	0.10	0.57	6.7	180	***6.10***	***2.90***	***4.20***	0.32	***7.10***	***5.00***	***5.10***
Molybd.	3.0	3.3	380	8,500	<5.00	<5.00	***33.0***	<5.00	<5.00	***686***	***2,350***
Nickel	27.5	150.	1,500	34,000	18.0	10.0	<5.00	16.0	10.0	19.0	9.00
Selenium	0.30	1.6	380	8,500	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Vanadium	71.3	300.	540	12,000	28.0	31.0	14.0	37.0	16.0	***10,100***	***1,360***
Zinc	62.1	150.	23,000	510,000	130.0	53.0	***5,320***	***635***	130	***1,990***	***3,830***

[1]There are no background data available for boron, iron, magnesium, manganese, and strontium.

[2]Data from Earth Technology (1991) and Boerngen and Shacklette (1981).

[3]ADEQ (1998).

[4]Elements in bold italics are of special concern because concentrations in mine soil exceed the Arizona background maximum and/or concentrations exceed non-residential human health guidelines.

Wash/rinse water– Traces of all elements except molybdenum were detected in bat wash water (Table 3, Appendix 1). Aluminum, magnesium, and strontium were the only elements recovered in all three reference water samples. Geometric mean concentrations of aluminum and magnesium were significantly greater ($P = 0.0001$) in wash water than in reference samples. Strontium concentrations in reference water samples were similar ($P > 0.05$) to those in all bat wash water samples except those of leaf-nosed bats from the Hub mine ($P = 0.0436$).

Geometric mean copper concentrations were significantly ($P<0.05$) lower in the myotis water samples (0.005 µg/g) than in leaf-nosed bat samples (0.011 - 0.026 µg/g) (Table 3, Appendix 1). Mercury and selenium were not detected in myotis wash water samples, but mercury and selenium were present in 4 of 5 leaf-nosed bat water samples. Lead concentrations were significantly ($P< 0.0001$) higher in Hull (0.706 µg/g) and Puzzler (1.356 µg/g) wash water samples than in all other samples (0.006 - 0.068 µg/g).

Table 3. Comparison of trace elements (µg/g wet weight) in water used to “wash” California myotis (CAMY) and California leaf-nosed bats (CLNB) collected from mines on Kofa National Wildlife Refuge, Arizona 2001 - 2002

			Geometric mean concentration (number of samples with detectible concentrations) / range[1]								
Species	Mine	N	aluminum	arsenic	barium	boron	beryllium	cadmium	chromium	copper	iron
NA	Reference	3	0.04 (2) A[2] ND[3] - .085	---- (0)	---- (0)	---- (0)	---- (0)	---- (0)	---- (0)	---- (0)	---- (0)
CAMY	Hub	8	5.32 (8) B 2.86 - 11.0	0.003 (8) A .002 - .005	0.053 (8) A .032 - .095	---- (0)	---- (0)	---- (0)	0.006 (8) AB .003 - .013	0.005 (8) A .003 - .009	3.47 (8) A .180 - 6.45
CLNB	Hub	4	17.8 (4) C 13.0 - 22.8	0.010 (4) B .005 - .015	0.165 (4) B .124 - .219	0.071 (4) .06 - .09	0.0010 (4) .0008 - .0010	---- (0)	0.019 (4) C .013 - .026	0.026 (4) B .019 - .041	13.1 (4) B 8.73 - 19.5
CLNB	Hull	8	2.40 (8) B 1.30 - 4.39	0.004 (8) A .002 - .009	0.172 (8) B .073 - .373	---- (1) ND - 0.04	---- (0)	0.0005 (4) A ND - .001	0.005 (8) A .003 - .009	0.018 (8) BC .006 - .034	2.28 (8) AC 1.20 - 4.30
CLNB	Keystone	4	5.84 (4) BC 4.12 - 9.30	0.011 (4) B .007 - .016	0.086 (4) A .062 - .139	---- (0)	---- (1) ND - 0.0060	0.0005 (2) A ND - .0009	0.011 (4) BC .010 - .013	0.017 (4) BC .012 - .030	3.98 (4) A .260 - 6.38
CLNB	Northstar	8	2.66 (8) B 1.40 - 6.89	0.004 (8) A .002 - .012	0.359 (8) B .109 - .917	---- (0)	---- (0)	0.0005 (4) A ND - .0026	0.004 (8) A .003 - .008	0.011 (8) C .005 - .014	1.62 (8) C 0.96 - 4.40
CLNB	Puzzler	6	5.37 (6) B 4.45 - 6.24	0.019 (6) B .014 - .029	0.153 (6) B .127 - .180	---- (0)	---- (0)	0.0010 (6) A .0008 - .002	0.015 (6) C .012 - .018	0.018 (6) BC .015 - .021	3.95 (6) A 3.50 - 4.40

			Geometric mean concentration (number of samples with detectible concentrations) / range[1]								
Species	Mine	N	lead	magnesium	manganese	mercury	nickel	selenium	strontium	vanadium	zinc
NA	Reference	3	---- (0)	0.09 (3) A .075 - .110	---- (0)	---- (0)	---- (0)	---- (0)	0.017 (3) A .012 - .022	---- (0)	---- (1) ND - 0.16
CAMY	Hub	8	0.006 (7) A ND - 0.01	1.80 (8) B 0.91 - 3.77	0.086 (8) A 0.04 - 0.16	---- (0)	0.006 (5) AB ND - 0.10	---- (0)	0.025 (8) A 0.02 - 0.04	0.007 (8) A .003 - .014	0.054 (8) A 0.03 - 0.09
CLNB	Hub	4	0.019 (4) AB .010 - .031	6.92 (4) B 4.60 - 10.2	0.299 (4) B .180 - .450	0.0006 (3) A ND - .0010	0.014 (4) A .010 - .020	0.0004 (3) A ND - .0006	0.071 (4) B .054 - .091	0.027 (4) BC .019 - .040	0.216 (4) B .130 - .350
CLNB	Hull	8	0.706 (8) C .190 - 6.36	0.55 (8) C 0.63 - 1.75	0.126 (8) AB .039 - .309	---- (0)	---- (1) ND - 0.006	0.0004 (5) A ND - .0006	0.029 (8) AB .019 - .049	0.004 (8) A .002 - .009	0.332 (8) B .084 - 1.22
CLNB	Keystone	4	0.068 (4) B .036 - .110	2.43 (4) B 1.73 - 3.72	0.401 (4) BC .272 - .661	0.0004 (2) A ND - 0.0008	0.004 (2) B ND - .008	0.0004 (3) A ND - .0006	0.038 (4) AB .028 - .053	0.008 (4) A .005 - .013	0.229 (4) B .130 - .350
CLNB	Northstar	8	0.018 (8) AB .009 - .089	0.88 (8) C 0.60 - 2.46	0.091 (8) A .047 - .443	0.0017 (8) C .0009 - .0042	---- (1) ND - 0.006	---- (2) ND - .0006	0.029 (8) AB .018 - .038	0.003 (8) A .001 - .009	0.045 (8) A .023 - .430
CLNB	Puzzler	6	1.356 (6) C .998 - 2.04	2.30 (6) B 1.98 - 2.68	0.408 (6) C .292 - .568	0.0052 (6) B .0036-.0087	0.007 (6) AB .005 - .010	0.0005 (6) A .0002-.0009	0.052 (6) AB .044 -.064	0.073 (6) C .047 - .121	0.288 (6) B .220 - .410

[1]Molybdenum was not detected in any samples.
[2]Means sharing a common letter (A,B,C) are not significantly different (P> 0.05).
[3]ND = Not detected.

Carcasses– California myotis carcasses included liver tissues and therefore are not directly comparable to California leaf-nosed bat carcasses from which livers had been dissected for separate analysis. The most striking among mine differences in leaf-nosed bat carcass concentrations were those of lead (Table 4, Appendix 2). Geometric mean lead concentrations were more than 20-times higher ($P < 0.0001$) in carcasses from the Puzzler (102 µg/g dry weight) and Hull (62 µg/g) mines than in those from the Hub, Keystone, and Northstar mines (0.96 - 2.59 µg/g).

Beryllium and molybdenum were not detected in leaf-nosed bat carcass samples (Table 4, Appendix 2). Nickel was recovered only in two of four leaf-nosed bats from the Hub mine. Geometric means of all elements except cadmium, chromium, and selenium were significantly different ($P \leq 0.05$) among mines. Concentrations of all elements except beryllium and molybdenum were detected in myotis carcasses (Table 5, Appendix 3)

Liver– Copper, lead, manganese, and mercury concentrations were significantly ($P \leq 0.05$) higher in leaf-nosed bat livers from the Puzzler mine than from other mines (Table 6, Appendix 4). Arsenic, barium, and boron were detected in fewer than one-half of the samples from all mines. Aluminum was present in fewer than one-half of the samples except those from the Northstar mine (geometric mean = 27.3 µg/g dry weight). Beryllium occurred in fewer than one-half of the samples from all mines except Puzzler (geometric mean = 0.14 µg/g). Lead was recovered in all samples from the Hull and Puzzler mines (geometric means = 3.77 and 16.6 µg/g, respectively) and in one sample from the Northstar mine. Strontium and vanadium were detected only in samples from the Hub and Puzzler mines (Table 6). Concentrations of cadmium, molybdenum, selenium, strontium, and vanadium were similar ($P > 0.05$) among all mines.

Table 4. Comparison of trace elements in the carcass of California leaf-nosed bats (µg/g dry weight) collected from five mines on Kofa National Wildlife Refuge, Arizona 2001 - 2002

		Geometric mean concentration (number of samples with detectible concentrations) / range[1]							
Mine	N	aluminum	arsenic	barium	boron	cadmium	chromium	copper	iron
Hub	4	180. (4) A 92.0 - 379.	0.28 (4) A 0.19 - 0.44	5.65 (4) AB 4.64 - 7.07	1.67 (4) A 0.38 - 3.84	0.28 (4) A 0.21 - 0.42	1.67 (4) A 0.73 - 6.07	7.85 (4) A 6.51 - 9.83	461. (4) A 345. - 655.
Hull	8	90.1 (8) ABC 47.3 - 433.	0.24 (8) A 0.17 - 0.40	6.55 (8) A 4.43 - 11.8	6.98 (8) B 5.26 - 10.5	---- (3) ND - 0.14	0.97 (8) A 0.41 - 3.76	5.10 (8) B 3.80 - 6.72	245. (8) B 120. - 329.
Keystone	4	54.9 (4) BC 46.9 - 67.0	0.31 (4) A 0.25 - 0.37	2.91 (4) B 2.70 - 3.20	6.61 (4) B 5.81 - 7.78	---- (0)	0.61 (4) A 0.41 - 0.85	4.84 (4) B 3.97 - 5.88	248. (4) B 214. - 279.
Northstar	8	40.1 (8) C 22.2 - 65.2	0.23 (8) A 0.13 - 0.78	9.29 (8) A 3.62 - 18.6	7.48 (8) B 6.48 - 9.01	---- (1) ND - 0.25	0.69 (8) A 0.35 - 1.25	5.21 (8) B 3.73 - 6.36	256. (8) B 155. - 313.
Puzzler	6	91.5 (6) ABC 14.7 - 130.	1.02 (6) B 0.40 - 1.54	7.00 (6) A 4.02 - 9.63	0.74 (3) A ND - 1.20	0.22 (6) A 0.11 - 0.42	1.46 (6) A 0.77 - 2.34	7.16 (6) A 5.57 - 8.95	334. (6) AB 242. - 449.

		Geometric mean concentration (number of samples with detectible concentrations) / range[1]							
Mine	N	lead	magnesium	manganese	mercury	selenium	strontium	vanadium	zinc
Hub	4	0.96 (4) A 0.62 - 1.74	1261 (4) A 1154 - 1397	6.14 (4) AB 3.76 - 10.3	0.46 (4) AB 0.29 - 0.67	0.89 (4) A 0.76 - 1.11	15.3 (4) AB 11.8 - 17.4	1.22 (4) A 0.91 - 1.66	103. (4) A 95.0 - 113.
Hull	8	62.0 (8) B 21.5 - 138	862 (8) B 641 - 1103	3.34 (8) B 2.14 - 8.17	0.25 (8) B 0.13 - 0.50	0.80 (8) A 0.67 - 0.96	11.7 (8) BC 10.3 - 13.4	---- (0)	75.8 (8) AB 62.0 - 95.1
Keystone	4	2.59 (4) A 1.41 - 7.33	865 (4) B 718 - 1226	5.94 (4) AB 5.03 - 6.61	0.25 (4) B 0.21 - 0.37	0.84 (4) A 0.59 - 1.60	11.9 (4) BC 10.8 - 13.5	---- (0)	64.5 (4) BC 60.3 - 70.2
Northstar	8	1.28 (8) A 0.59 - 2.72	959 (8) AB 778 - 1213	2.82 (8) B 1.61 - 8.12	0.36 (8) B 0.22 - 0.52	0.95 (8) A 0.79 - 1.34	10.3 (8) C 7.10 - 13.7	---- (0)	73.4 (8) C 60.7 - 82.8
Puzzler	6	102. (6) B 54 - 174	1136 (6) AB 897 - 1355	13.2 (6) A 5.12 - 29.0	0.88 (6) A 0.33 - 1.62	1.14 (6) A 0.90 - 1.26	19.7 (6) A 14.7 - 27.3	3.87 (6) B 1.45 - 6.29	94.0 (6) ABC 78.0 - 103.

[1]Beryllium and molybdenum were not detected in any samples.
[2]Nickel was present in two samples from the Hub mine at 0.52 and 0.59 µg/g.
[3]Means sharing a common letter (A,B,C) are not significantly different (P> 0.05).

Table 5. Trace elements in carcasses California myotis and California leaf-nosed bats (µg/g dry weight) collected from the Hub mine, Kofa National Wildlife Refuge, Arizona, 2001 - 2002[1]

		Geometric mean concentration (number of samples with detectible concentrations) / range[2]							
Species	N	aluminum	arsenic	barium	boron	cadmium	chromium	copper	iron
California myotis	9	178 (9) 112 - 370	0.48 (9) 0.33 - 0.73	7.28 (9) 4.73 - 16.1	1.27 (9) 0.81 - 2.76	0.23 (9) 0.14 - 0.38	1.03 (9) 0.37 - 2.20	8.40 (9) 7.19 - 10.3	431 (9) 367 - 601
California leaf-nosed bat[3]	4	180 (4) 92 - 379	0.28 (4) 0.19 - 0.44	5.65 (4) 4.64 - 7.07	1.67 (4) 0.38 - 3.84	0.28 (4) 0.21 - 0.42	1.67 (4) 0.73 - 6.07	7.85 (4) 6.51 - 9.83	461 (4) 345 - 655

		Geometric mean concentration (number of samples with detectible concentrations) / range							
Species	N	lead	magnesium	manganese	mercury	selenium	strontium	vanadium	zinc
California myotis	9	0.29 (9) 0.22 - 0.46	1185 (9) 859 - 1351	7.24 (9) 5.71 - 10.6	1.33 (9) 1.10 - 1.84	1.32 (9) 1.12 - 1.55	16.98 (9) 12.9 - 21.5	0.24 (6) <0.50 - 0.39	78 (9) 65.3 - 96.0
California leaf-nosed bat[2]	4	0.96 (4) 0.62 - 1.74	1261 (4) 1154 - 1397	6.14 (4) 3.76 - 10.3	0.46 (4) 0.29 - 0.67	0.89 (4) 0.76 - 1.11	15.28 (4) 11.8 - 17.4	1.22 (4) 0.91 - 1.66	103 (4) 95 - 113

[1]Because California myotis carcass samples included liver tissues, and livers were removed from all leaf-nosed bats, carcasses body burdens are not directly comparable.

[2]Beryllium and molybdenum were not detected in any samples.

[3]Nickel was present in two California leaf-nosed bat samples at 0.52 and 0.59 µg/g, but nickel was not detected in California myotis.

Table 6. Comparison of trace elements in liver tissues of California leaf-nosed bats (µg/g dry weight) collected from five mines on Kofa National Wildlife Refuge, Arizona 2001 - 2002

		Geometric mean (number of samples with detectible concentrations) / range[1]					
Mine	N	cadmium	copper	iron	lead	magnesium	manganese
Hub	4	1.92 (4) A[2] 0.79 - 3.10	17.2 (4) A 15.0 - 19.0	1833 (4) AB 1520 - 2200	---- (0)	793 (4) A 652 - 858	12.45 (4) A 11.0 - 14.0
Hull	8	1.15 (7) A ND - 3.10	14.0 (8) A 11.0 - 17.0	1233 (8)B 519 - 1850	3.77 (8) A 1.80 - 14.0	661 (8) B 583 - 694	7.28 (8) B 3.40 - 8.50
Keystone	4	1.01 (4) A 0.72 - 1.20	19.7 (4) A 13.0 - 43.0	1949 (4) AB 1720 - 2480	---- (0)	745 (4) AB 705 - 804	11.03 (4) A 8.80 - 14.0
Northstar	8	0.61 (8) A 0.20 - 1.70	15.9 (8) A 13.0 - 20.0	1689 (8) AB 1390 - 2030	---- (1) ND - 1.00	735 (8) AB 681 - 803	10.27 (8) A 8.00 - 12.0
Puzzler	6	2.34 (6) A 1.50 - 3.40	31.1 (6) B 26.0 - 37.0	2166 (6) A 1530 - 2900	16.6 (6) B 11.0 - 36.0	763 (6) A 697 - 863	22.16 (6) C 17.0 - 25.0

		Geometric mean (number of samples with detectible concentrations) / range[1]					
Mine	N	mercury	molybdenum	selenium	strontium	vanadium	zinc
Hub	4	0.42 (3) A ND - 0.50	4.00 (4) A 4.0 - 4.0	2.45 (3) A <2.00-4.00	0.22 (2) A ND - 0.75	1.94 (4) A 1.70 - 2.10	107 (4) A 102 - 110
Hull	8	0.62 (8) A 0.40 - 1.00	2.67 (7) A ND - 4.0	3.05 (8) A 2.00 - 4.00	---- (0)	---- (0)	75 (8) B 67.4 - 83.4
Keystone	4	0.57 (4) A 0.40 - 0.90	3.22 (4) A 3.0 - 4.0	3.35 (4) A 3.00 - 4.00	---- (0)	---- (0)	97 (4) AC 83.2 - 123
Northstar	8	0.36 (4) A ND - 1.00	3.59 (8) A 3.0 - 4.0	3.33 (8) A 2.00 - 4.00	---- (0)	---- (1) <0.50 - 0.20	82 (8) BC 76.5 - 90.2
Puzzler	6	2.27 (6) B 1.30 - 3.90	3.15 (6) A 3.0 - 4.0	3.43 (6) A 2.00 - 7.20	0.42 (6) A 0.20 - 0.80	2.23 (6) A 1.50 - 2.90	116 (6) A 103 - 143

[1]Arsenic, barium, and boron were detected in fewer than one-half of the samples from all mines. Aluminum was present in fewer than one-half of the samples from all mines except Northstar (geometric mean = 27.3 µg/g). Beryllium also occurred in fewer than one-half of the samples from all mines except Puzzler (geometric mean = 0.14 µg/g).

[2]Means sharing a common letter (A,B,C) are not significantly different ($P > 0.05$).

DISCUSSION

Trace elements in mine soil– Of the 14 elements for which background data were available, concentrations of five fell within established Arizona background parameters and/or human health guidelines. Only arsenic, beryllium, and lead exceeded both the maximum Arizona background level and human health guidelines for non-residential soil from one or more mines. Arsenic in all soil samples exceeded human health guidelines. Arsenic was present in one sample from the Puzzler mine at more than 130-times the human health threshold. Beryllium was detected at 20 µg/g, almost twice the human health guideline of 11.0 µg/g, in one soil sample from the Puzzler mine. Lead concentrations in soil from the Hull and Puzzler mines were up to 1,000-times higher than the maximum Arizona background level and from 28- to 51-times higher than the human health threshold.

Because only one or two soil samples were collected at each mine, means cannot be calculated, and differences among mines can not be assessed statistically. The element with the greatest among-mine difference was lead. Lead concentrations in Hull (102,000 µg/g) and Puzzler (56,900 and 95,200 µg/g) soil samples were at least 200-times higher than the next highest concentration (250 µg/g). We emphasize this difference in soil lead levels among the different mines because similar relationships were evident for lead in wash water and lead in bat tissues. Lead concentrations were consistently higher in all samples from the Hull and Puzzler mines than in samples from the other mines.

Trace elements in wash water– Traces of all elements except molybdenum were detected in bat wash water (Table 3). Lead was present at significantly ($P < 0.0001$) higher levels in wash water from the Puzzler and Hull mine bats than in all other water samples. The presence of these elements in the wash/rinse water indicates that these elements were present as particles on the bat's fur and available for ingestion by the bats during grooming.

Trace element concentrations in tissues– Comparing "whole body" or "carcass" concentrations among studies is difficult because dissection methods often differ among studies. What one author refers to as whole body or carcass may not meet the definition of other authors. Even in our investigation, carcass results are not directly comparable between species because California myotis carcasses contained liver tissue, but the livers were removed from the leaf-nosed bat samples. Whole body and carcass concentrations provide an estimation of total body burden and the safest comparisons are within individual studies. Liver tissue data provide more reliable among-study comparisons.

When comparing results of this study with those of others, we limited comparisons of our data to bioaccumulation of trace elements in bats or other insectivorous mammal species such as shrews (*Sorex* spp.). This study documented concentrations of 19 elements in bat tissues; however, only arsenic, cadmium, lead, mercury and selenium are likely to bioaccumulate to toxic levels (Eisler 1985a, 1985b, 1987, 1988a, 1988b, Hoffman et al. 1990, Cooke and Johnson 1996, Ma 1996) and we will limit our discussion to these five elements.

Arsenic acts as a cumulative poison (Jenkins 1981) and is listed by the USEPA as one of 129 priority pollutants (Keith and Telliard 1979). Arsenic may be absorbed by ingestion, inhalation, or through permeation of the skin or mucous membranes (Gearheart and Waller 1994). Most arsenic is excreted in the urine during the first few days after exposure (Gearheart and Waller 1994). Background arsenic concentrations are usually less than 1 µg/g wet weight (about 4 µg/g dry weight) in most terrestrial fauna (Eisler 1988). Elevated levels of arsenic are normal in mineralized zones containing gold, silver, and sulfides of lead and zinc (Eisler 1988a). Only background concentrations were present in carcass samples, and arsenic was not detected in liver tissues; thus, there appears to be little potential for arsenic related problems in bats at the Kofa NWR mines we sampled.

Cadmium has no known biological function (Eisler 1985a). Its toxicity may originate through exposure via respiration or ingestion (Cooke and Johnson 1996). In most mammals, absorption from the diet is low; usually less than 5% of the ingested cadmium is absorbed. In a recent review of data from 13 studies conducted between 1974 and 1987, the average carcass cadmium concentration in shrews (an insectivorous mammal) from uncontaminated sites ranged from 1.2 to 4.0 µg/g dry weight (Talmage and Walton 1991). In our study, cadmium was detected in carcass samples from only two of five mines, and the maximum individual concentration was 0.42 µg/g, well within the background range. The kidney is the critical organ for cadmium toxicity in mammals as it is the first organ in which damage is observed or adverse functional changes start to occur (Cooke and Johnson 1996). The proposed toxic threshold for cadmium is 350 µg/g dry weight in the kidney of most mammals (Cooke and Johnson 1996). No corresponding threshold has been proposed for the liver. Cadmium concentrations in livers of 3 bat species collected alive (n = 37) ranged from 2.45 to 4.64 µg/g dry weight (converted from wet weight) as summarized by Clark and Shore (2001). Lower mean concentrations of cadmium, 0.84 µg/g dry weight, were reported in the livers of 10 individuals of 5 species found dead from 1986 - 1989 in Germany (Streit and Nagal 1993a). However, in insectivorous mammals such as shrews, liver concentrations are usually higher than those in the kidney suggesting the toxic threshold also should be higher. The maximum cadmium concentration in bat liver tissue was 3.40 µg/g dry weight; therefore, cadmium does not appear to have bioaccumulated to potentially hazardous concentrations.

Lead is neither essential nor beneficial to living organisms; all metabolic effects are negative (Eisler 1988b, Pain 1995). Ingestion and inhalation are the most important exposure routes of lead to terrestrial mammals. Only two studies were located that assessed lead levels in bat carcasses (Clark 1979, Martin 1992). Although not directly comparable, lead concentrations in carcasses of California myotis from the Hub mine, 0.22 - 0.46 µg/g dry weight, were considerably lower than those in big brown bats, 31.49 - 46.56 µg/g wet weight (~126 - 186 µg/g dry weight), and little brown bats *(Myotis lucifugus),* 16.97 µg/g wet weight (~68 µg/g dry weight), collected from lead-contaminated highway areas in Maryland (Clark 1979). Lead concentrations in California leaf-nosed bats collected from the Hub, Keystone, and Northstar mines (maximum concentration = 7.33 µg/g) also were below levels in bats from known contaminated sites. In contrast, lead in carcasses of leaf-nosed bats from the Hull mine (geometric mean = 62.0 µg/g dry weight, range = 21.5 - 138) approached levels that suggest a significant degree of contamination. California leaf-nosed bats from the Puzzler mine (carcass geometric mean = 102 µg/g dry weight, range = 54 - 174) contained lead concentrations that were well within the range of those in bats collected from lead-contaminated areas.

In an extensive review of levels and effects of contaminants in bats, Clark and Shore (2001) summarized the findings of five studies that addressed lead concentrations in live and dead bats. There was considerable overlap in liver lead concentrations in bats found dead and those collected alive. Lead in livers of three species of fruit-eating bats that died of lead poisoning near Brisbane, Australia ranged from 1.69 to 13.1 µg/g dry weight (Sutton and Hariono 1987, Hariono et al. 1993). In Germany, livers of five insectivorous bat species found dead contained an average of 4.4 µg/g dry weight lead (Streit and Nagel 1993a). Average lead concentrations of 12.2 µg/g dry weight were recorded in livers of three live *Pipistrellus pipistrellus* in Germany (Streit and Nagel 1993b). Much higher levels of 4.35 and 5.37 µg/g wet weight (~ 17 - 21 µg/g dry weight) were reported in the livers of the insectivorous *Tadarida brasiliensis* collected alive from caves in New Mexico and Oklahoma (Thies and Gregory 1994). The geometric mean of 16.6 µg/g dry weight lead in the livers of leaf-nosed bats from the Puzzler mine is among the highest yet recorded in apparently healthy individuals.

Lead mines used as maternity roosts may present additional potential problems for bats. Lead crosses the placenta and is passed in milk producing early intoxication of the fetus during pregnancy and the newborn during lactation (Eisler 1988b, Streit and Nagel 1993b, Ma 1996). Younger development stages are the most sensitive, and the effects are exacerbated by elevated temperatures, a particular concern for Arizona bat populations. It is not known for certain what concentrations of lead in bat carcasses and liver tissues are associated with sublethal effects, or what effects current concentrations would have on the learning ability and behavior of young bats.

Mercury concentrations are of special concern because mercury can bioconcentrate in organisms and biomagnify through the food chain (Eisler 1987). Mercury has no known biological function and its presence in cells of living organisms is undesirable and potentially hazardous. It is generally accepted that the organic form of mercury (methylmercury) is more toxic to wildlife than the inorganic form (Ma 1996). Therefore, if bats are accumulating mercury at the mine roost, it would be difficult to selectively assess the accumulation and potential effects of each mercury form.

Elevated mercury concentrations were detected in all soil samples except the Keystone sample. Mercury was detected in ≥50% of the wash/rinse water samples from the Hub, Keystone, Northstar, and Puzzler mines. It was recovered in all carcass samples and in 25 of 30 liver samples. The maximum concentration in an individual carcass sample was 1.62 µg/g dry weight. Liver mercury concentrations in bats collected in our study (<0.05 - 3.90

µg/g dry weight) were generally lower than levels (4.60 - 11.08 µg/g dry weight) (converted from wet weight) in livers of bats collected alive in Sweden, Japan, and western Virginia, USA as summarized by Clark and Shore (2001). Background concentrations of mercury in mammals from uncontaminated sites usually are <1.0 µg/g wet weight (Eisler 1987) (approximately <4.0 µg/g dry weight). No data were located that assess lowest adverse effect levels such as low concentrations that may adversely affect learning and behavior. Data summarized by Thompson (1996) for several field and laboratory studies indicate that diagnostic lethal levels of mercury in the liver usually exceed 30 µg/g wet weight (approximately 120 µg/g dry weight). The maximum concentration of mercury in liver tissues of bats in our study was 3.90 µg/g dry weight. Therefore, there seems to be little potential for adverse affects of mercury alone on adult bats. Mercury, however, when ingested in combination with other compounds and elements such as parathion, cadmium, and copper can have additive or synergistic toxic effects (Eisler 1987, Hoffman et al. 1990, Calabrese and Baldwin 1993).

Selenium is an essential trace element in animal diets, but it is toxic at concentrations only lightly above required dietary levels. Mammals are much less susceptible to selenium toxicosis than birds (Eisler 1985b), but little is known about the toxicity of selenium to bats. Individual selenium concentrations in bat carcasses collected during the present study (2001 - 2002) ranged from 0.59 to 1.60 µg/g dry weight and were similar (P> 0.05) to those (0.80 - 1.14 µg/g dry weight) in bats collected from Kofa NWR in 1998 - 1999 (King et al 2001). No comparable data for selenium in bat carcasses was located. Selenium in ornate shrews *(Sorex ornatus)* collected from a selenium contaminated area of Merced County, California, averaged 47.9 µg/g dry weight (Clark 1987). Selenium in bats from Kofa NWR was far lower than concentrations in shrews collected from selenium contaminated areas.

The interpretation of selenium concentrations in liver tissues is complicated due to a lack of comparative mammalian data. The lower toxic threshold of selenium in bird liver tissue is about 10 µg/g dry weight (Ohlendorf 1993). Since mammals are more resistant to selenium poisoning than birds, the toxic threshold in mammals also should be higher than that in birds, i.e. higher than 10 µg/g. Based on relatively low carcass (0.59 - 1.60 µg/g) and liver (<2.0 - 7.20 µg/g) concentrations, selenium does not appear to be a contaminant of concern for California myotis and California leaf-nosed bats roosting at Kofa NWR.

Soil, wash water, and tissue relationships: Are body burdens solely a reflection of bioaccumulation of trace elements through the food chain, or could contaminant levels be influenced by ingestion or inhalation of element-laden particles at the mine roost site? Although the California leaf-nosed bat is relatively roost-site-specific, individuals may use one or more roosts within an area during the course of a season. In general, this species does not migrate which reduces the variable of contaminants accumulated from distant areas. To fully address this question, one must focus on elements with high accumulation potential and a high degree of variability in soil concentrations among the mines. Two elements assessed in this study, zinc and lead, fit the parameters of an elevated accumulation potential and a high degree of geographic variability.

There was more than a 100-fold difference in zinc soil concentrations among the mines. While zinc accumulates in body tissues, tissue residues are not reliable indicators of zinc contamination (Eisler 1993, Opresko 1992, USDI 1998). Lead, on the other hand, bioaccumulates throughout the lifetime of an individual and body burdens are highly correlated with exposure (Eisler 1988b, Ma 1996, USDI 1998). Also, there was more than a 2,500-fold difference in soil lead concentrations among the mines.

Lead concentrations in the different media tested (soil, wash water, carcass, and liver tissues) were consistently higher in samples from the Hull and Puzzler mines than in samples from other areas. Lead in soil from the Hull and Puzzler mines was at least two orders of magnitude higher than concentrations in soil from the other mines sampled. Lead in wash water from the Hull and Puzzler mine bats also was more than 10-times greater than concentrations in the next highest water sample. Geometric mean lead concentrations were significantly ($P = 0.0001$) higher in carcass samples from the Hull and Puzzler mines than in those from all other locations. Similarly, lead was detected in all liver tissue samples from the Hull and Puzzler mines, but lead was present in only 1 of 16 samples from the other mines. These data suggest a strong association between lead in soil, lead in wash water, and lead in bat tissues. Bats roosting in lead mines may be accumulating a significant portion of their total body burden at the roost site by ingesting lead particles while grooming or through inhalation of lead contaminated dust.

RECOMMENDATIONS

Bats collected from Kofa NWR showed a distinctive roost site specific pattern of lead accumulation. Further field and laboratory research is needed to better understand the dynamics of lead and other trace element bioaccumulation and the potential adverse effects of these elements on bat populations. Potential adverse effects of lead should be assessed through determinations of delta-aminolevulinic acid dehydratase (ALAD) levels in the blood of bats from contaminated areas compared with those from reference sites. Histopathological differences also should be noted.

Field and laboratory studies should assess lead tissue levels associated with reduced reproduction, impaired motor skills, adversely affected hearing, and possible aberrant behavior. If further research indicates that current levels are adversely affecting bats, then the mines may be modified or closed.

Although more than 20% of Arizona's bat populations are declining (Noel and Johnson 1993), little is known about population trends of bats roosting on Kofa NWR. Long-term population trends need to be assessed. Also, population dynamics at specific lead-contaminated mine roosts should be documented.

ACKNOWLEDGMENTS

We thank Mike Rabe and Lin Piest of the Arizona Game and Fish Department, Susanna Henry, and Jena Moon of the USFWS, and volunteer Jason Knight for assistance with field work. Appreciation is expressed to Ray Varney, Manager, Kofa NWR for providing logistical support necessary to complete field work. Special acknowledgment is extended to John Moore of the Service's Patuxent Analytical Control Facility for his determination to maintain high QA/QC analytical standards. We also thank P.A. “Doc” Burdick for providing otherwise undocumented historical information on area mines. This manuscript was reviewed by Denise Baker, Don Clark, Katy Hinman, Laila Lienesch, Steve Robertson, Tim Snow, and Stan Wiemeyer who made numerous helpful comments and suggestions.

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Appendix 1. Trace elements in water used to wash California leaf-nosed bats *(Macrotus californicus)*. Bats collected from five mines on Kofa National Wildlife Refuge, Arizona

		Concentration, µg/g wet weight[1]																	
Sample Number	Area	Al	As	B	Ba	Be	Cd	Cr	Cu	Fe	Hg	Mg	Mn	Ni	Pb	Se	Sr	V	Zn
Control1	NA	0.060	<.0005	<0.04	<.001	<.0005	<.0005	<0.002	<0.002	<0.05	<.0005	0.110	<.002	<.005	<.005	<.0002	0.022	<.001	<.005
Control2	NA	0.085	0.001	<0.04	<.001	<.0005	<.0005	<0.002	<0.002	<0.05	<.0005	0.100	<.002	<.005	<.005	<.0002	0.019	<.001	.016
Control3	NA	<0.02	<.0005	<0.04	<.001	<.0005	<.0005	<0.002	<0.002	<0.05	<.0005	0.075	<.002	<.005	<.005	<.0002	0.012	<.001	<.005
Wash1	Hub	22.8	0.013	0.08	0.184	0.001	<.0005	0.023	0.029	16.9	0.0006	9.10	0.385	0.019	0.023	0.0006	0.082	0.035	0.250
Wash2	Hub	22.6	0.015	0.09	0.219	0.001	0.0007	0.026	0.041	19.5	0.0010	10.2	0.450	0.020	0.031	0.0006	0.091	0.040	0.350
Wash3	Hub	13.0	0.005	0.06	0.124	0.0006	<.0005	0.013	0.019	8.73	<.0005	4.60	0.180	0.010	0.010	<.0005	0.054	0.019	0.190
Wash4	Hub	15.1	0.009	0.06	0.149	0.0008	<.0005	0.016	0.020	10.2	0.0010	5.36	0.255	0.010	0.017	0.0004	0.063	0.021	0.130
Wash 1	Hull	1.80	0.003	<0.04	0.126	<0.005	<0.0005	0.005	0.017	1.60	<0.0005	0.63	0.070	<0.005	0.671	<0.0005	0.022	0.003	0.320
Wash 2	Hull	3.38	0.005	<0.04	0.373	<0.005	0.0009	0.005	0.020	3.20	<0.0005	1.21	0.130	<0.005	1.32	0.0005	0.036	0.005	0.559
Wash 3	Hull	1.90	0.003	0.04	0.092	<0.005	<0.005	0.003	0.011	1.50	<0.0005	0.85	0.046	<0.005	0.420	0.0005	0.022	0.003	0.220
Wash 4	Hull	3.65	0.009	<0.04	0.266	<0.005	0.001	0.008	0.034	4.30	<0.0005	1.23	0.110	<0.005	6.36	0.0005	0.049	0.007	1.220
Wash 5	Hull	4.39	0.004	<0.04	0.321	<0.005	0.001	0.009	0.014	3.60	<0.0005	1.75	0.140	<0.005	0.490	<0.0005	0.043	0.008	0.380
Wash 6	Hull	1.50	0.002	<0.04	0.073	<0.005	<0.005	0.004	0.006	1.20	<0.0005	0.68	0.039	<0.005	0.190	<0.0004	0.019	0.003	0.084
Wash 7	Hull	3.01	0.004	<0.04	0.322	<0.005	0.0007	0.007	0.011	2.70	<0.0005	1.25	0.309	0.006	0.280	0.0005	0.033	0.009	0.290
Wash 8	Hull	1.30	0.004	<0.04	0.090	<0.005	<0.0005	0.004	0.010	1.90	<0.0005	0.66	0.084	<0.005	0.996	0.0006	0.020	0.002	0.330
Wash 1	Keystone	7.18	0.016	<0.04	0.098	<0.005	<0.0005	0.011	0.030	5.06	0.0006	3.09	0.496	0.006	0.110	0.0005	0.044	0.010	0.320
Wash 2	Keystone	9.30	0.014	<0.04	0.139	0.006	0.0008	0.013	0.019	6.38	0.0008	3.72	0.661	0.008	0.094	0.0006	0.053	0.013	0.350
Wash 3	Keystone	4.23	0.011	<0.04	0.062	<0.005	0.0009	0.010	0.012	3.00	<0.0005	1.75	0.291	<0.005	0.059	0.0004	0.028	0.005	0.190
Wash 4	Keystone	4.12	0.007	<0.04	0.066	<0.005	<0.0005	0.010	0.012	2.60	<0.0005	1.73	0.272	<0.005	0.036	<0.0004	0.031	0.005	0.130

Appendix 1 (Cont.). Trace elements in water used to wash California leaf-nosed bats *(Macrotus californicus)*. Bats collected from five mines on Kofa National Wildlife Refuge, Arizona

		Concentration, µg/g wet weight[1]																	
Sample Number	Area	Al	As	B	Ba	Be	Cd	Cr	Cu	Fe	Hg	Mg	Mn	Ni	Pb	Se	Sr	V	Zn
Wash 1	Northstar	6.89	0.012	<0.04	0.109	<0.005	<0.0005	0.008	0.014	4.40	0.0009	2.46	0.443	0.006	0.089	0.0006	0.038	0.009	0.430
Wash 2	Northstar	1.50	0.002	<0.04	0.315	<0.005	0.0008	0.005	0.014	0.96	0.0016	0.64	0.050	<0.005	0.010	<0.0004	0.023	0.002	0.032
Wash 3	Northstar	1.80	0.002	<0.04	0.159	<0.005	<0.0005	0.004	0.005	1.10	0.0009	0.63	0.063	<0.005	0.010	<0.0005	0.018	0.002	0.024
Wash 4	Northstar	3.22	0.004	<0.04	0.593	<0.005	0.0006	0.003	0.014	1.90	0.0042	1.14	0.080	<0.005	0.018	0.0005	0.032	0.003	0.041
Wash 5	Northstar	1.40	0.003	<0.04	0.185	<0.005	<0.0005	0.003	0.008	0.98	0.0010	0.60	0.047	<0.005	0.009	<0.0004	0.023	0.001	0.023
Wash 6	Northstar	3.52	0.005	<0.04	0.917	<0.005	<0.0005	0.003	0.013	2.00	0.0020	0.72	0.099	<0.005	0.021	<0.0004	0.037	0.003	0.027
Wash 7	Northstar	1.90	0.003	<0.04	0.753	<0.005	0.0026	0.007	0.009	1.20	0.0017	0.72	0.080	<0.005	0.010	<0.0004	0.032	0.003	0.037
Wash 8	Northstar	4.45	0.005	<0.04	0.674	<0.005	0.0006	0.003	0.012	2.30	0.0036	1.03	0.100	<0.005	0.045	<0.0005	0.036	0.004	0.059
Wash5	Puzzler	6.24	0.014	<0.04	0.151	<.0005	0.0009	0.018	0.016	4.40	0.0047	2.46	0.292	0.007	1.260	0.0005	0.064	0.047	0.340
Wash6	Puzzler	5.17	0.017	<0.04	0.127	<.0005	0.0008	0.016	0.015	3.50	0.0036	2.12	0.346	0.006	1.040	0.0004	0.049	0.055	0.220
Wash7	Puzzler	4.45	0.021	<0.04	0.150	<.0005	0.001	0.017	0.023	3.70	0.0060	1.98	0.453	0.006	1.470	0.0009	0.044	0.085	0.280
Wash8	Puzzler	5.52	0.029	<0.04	0.180	<.0005	0.0017	0.013	0.021	4.20	0.0087	2.35	0.568	0.007	2.040	0.0004	0.051	0.121	0.410
Wash9	Puzzler	4.98	0.023	<0.04	0.180	<.0005	0.0010	0.012	0.019	3.80	0.0064	2.68	0.486	0.005	1.580	0.0006	0.061	0.089	0.290
Wash10	Puzzler	6.05	0.016	<0.04	0.135	<.0005	.00008	0.013	0.015	4.20	0.0036	2.28	0.365	0.010	0.998	0.0002	0.046	0.063	0.230

[1]Molybdenum was not detected in water samples.

Appendix 2. Trace elements in carcasses of California leaf-nosed bats *(Macrotus californicus)* collected from five mines on Kofa National Wildlife Refuge, Arizona

		Concentration, µg/g dry weight[1]																	
Mine	Sex	Al	As	B	Ba	Cd	Cr	Cu	Fe	Hg	Mg	Mn	Ni	Pb	Se	Sr	V	Zn	Moist (%)
Hub	M	379.	0.44	3.46	7.07	0.21	1.69	9.83	655	0.29	1397.	10.3	0.59	1.74	1.11	16.6	1.66	113.	70.1
Hub	M	288.	0.38	3.84	5.22	0.42	6.07	8.71	520	0.67	1175.	8.82	0.52	0.97	0.78	11.8	1.41	105.	67.9
Hub	M	104.	0.19	1.55	5.93	0.21	1.05	6.83	385	0.50	1333.	3.76	<0.50	0.80	0.95	17.4	0.91	101.	68.3
Hub	M	92.0	0.19	0.38	4.64	0.31	0.73	6.51	345	0.46	1154.	4.15	<0.50	0.62	0.76	16.0	1.04	95.0	67.6
Hull	F	50.1	0.28	5.26	5.75	<0.10	0.96	4.86	219	0.13	761.	2.71	<0.50	110.	0.70	12.6	<0.50	63.7	51.5
Hull	F	92.1	0.20	6.34	11.8	0.10	0.41	6.72	291	0.50	956.	3.39	<0.50	138.	0.91	13.4	<0.50	95.1	58.5
Hull	F	129.	0.33	6.44	9.87	<0.10	0.63	3.80	293	0.40	1103.	3.90	<0.50	63.9	0.80	11.7	<0.50	87.9	58.3
Hull	F	433.	0.17	6.43	4.43	<0.10	3.76	4.64	253	0.16	692.	2.14	<0.50	130.	0.67	10.3	<0.50	62.0	53.6
Hull	M	65.0	0.40	6.98	5.61	<0.10	0.88	5.96	329	0.20	641.	3.55	<0.50	21.5	0.95	10.3	<0.50	70.8	61.7
Hull	F	71.2	0.21	10.5	5.43	0.14	0.83	5.09	120	0.22	883.	2.26	<0.50	24.0	0.68	11.5	<0.50	76.7	55.2
Hull	F	78.2	0.24	7.52	8.46	0.11	0.90	5.49	246	0.16	923.	8.17	<0.50	44.8	0.76	13.1	<0.50	73.2	57.0
Hull	M	47.3	0.19	7.39	4.44	<0.10	1.25	5.84	286	0.45	1055.	3.06	<0.50	74.6	0.96	11.1	<0.50	83.0	60.5
Keystone	F	48.9	0.37	7.78	2.96	<0.10	0.54	5.88	279	0.37	1226.	5.84	<0.50	7.33	0.79	10.8	<0.50	70.2	57.9
Keystone	F	58.6	0.25	6.64	2.70	<0.10	0.75	4.40	214	0.22	718.	6.40	<0.50	1.41	0.59	12.5	<0.50	61.7	51.1
Keystone	F	67.0	0.36	5.81	3.20	<0.10	0.41	5.36	279	0.21	863.	5.03	<0.50	2.74	0.67	13.5	<0.50	66.2	53.6
Keystone	F	46.9	0.29	6.35	2.81	<0.10	0.85	3.97	226	0.22	737.	6.61	<0.50	1.59	1.60	11.0	<0.50	60.3	52.7

Appendix 2 (Cont.). Trace elements in carcasses of California leaf-nosed bats *(Macrotus californicus)* collected from five mines on Kofa National Wildlife Refuge, Arizona

		Concentration, µg/g dry weight[1]																
Area	Sex	Al	As	B	Ba	Cd	Cr	Cu	Fe	Hg	Mg	Mn	Ni	Pb	Se	Sr	V	Zn	Moist (%)
Northstar	F	65.2	0.78	8.37	3.62	<0.10	0.39	5.79	313	0.26	927.	8.12	<0.50	2.72	0.90	11.1	<0.50	82.8	56.6
Northstar	F	39.4	0.19	7.62	11.8	<0.10	0.47	5.22	249	0.46	953.	2.64	<0.50	1.06	0.79	11.3	<0.50	73.1	57.8
Northstar	M	50.3	0.31	9.01	9.41	<0.10	1.15	3.73	292	0.52	1213.	3.87	<0.50	0.98	1.34	13.7	<0.50	80.8	58.9
Northstar	M	31.8	0.20	6.48	10.2	<0.10	1.25	5.73	268	0.29	895.	2.63	<0.50	0.82	0.88	7.10	<0.50	60.7	56.0
Northstar	F	34.6	0.17	7.05	7.68	<0.10	0.76	5.45	291	0.46	1023.	2.67	<0.50	0.89	0.94	11.3	<0.50	83.2	59.2
Northstar	F	39.9	0.15	6.52	15.2	<010	0.75	4.74	264	0.22	862.	2.25	<0.50	2.54	0.93	8.47	<0.50	66.7	55.2
Northstar	F	22.2	0.13	7.16	6.25	0.25	0.96	5.10	257	0.35	778.	1.90	<0.50	0.59	0.82	8.07	<0.50	68.9	57.6
Northstar	M	53.3	0.27	8.02	18.6	<0.10	0.35	6.36	155	0.46	1085.	1.61	<0.50	2.35	1.09	13.5	<0.50	74.3	61.4
Puzzler	F	56.2	0.40	<2.00	4.02	0.36	1.05	5.57	296	0.33	897.	6.36	<0.50	54.	1.01	16.8	1.45	78	60.7
Puzzler	F	14.7	0.81	1.20	5.70	0.11	2.34	6.70	412	0.78	1151.	14.1	<0.50	72.	1.04	14.7	3.14	94	65.2
Puzzler	M	118.	1.46	1.11	9.19	0.12	1.24	8.95	449	1.62	1229.	29.0	<0.50	104.	0.96	23.0	6.29	94	66.0
Puzzler	F	88.5	1.08	<2.00	7.15	0.42	1.75	7.68	258	0.98	1105.	21.0	<0.50	122.	0.90	19.8	4.95	98	63.8
Puzzler	F	76.0	1.47	<2.00	9.63	0.39	0.77	7.37	242	0.95	1146.	18.6	<0.50	131.	1.90	19.3	4.04	103	66.6
Puzzler	M	130.	1.54	0.75	8.11	0.15	2.34	7.11	407	1.20	1355.	5.12	<0.50	174.	1.26	27.3	5.87	100	66.9

[1]Beryllium and molybdenum were not detected in any samples.

Appendix 3. Trace elements in California myotis *(Myotis californicus)* carcass tissues, µg/g dry weight, collected at the Hub mine, Kofa NWR, August, 2001

		Concentration, µg/g dry weight[1]																	
Sample number	Sex	Al	As	B	Ba	Cd	Cr	Cu	Fe	Hg	Mg	Mn	Ni	Pb	Se	Sr	V	Zn	Moist (%)
MYCA1	F	205	0.44	1.35	7.84	0.30	1.56	10.3	454	1.84	1128	7.68	0.85	0.46	1.34	19.9	0.23	79.2	66.3
MYCA2	M	156	0.33	2.76	4.73	0.38	0.64	9.65	422	1.59	1324	6.41	0.48	0.23	1.26	21.5	<0.50	85.8	68.7
MYCA3	M	184	0.37	1.11	5.41	0.34	0.37	7.81	397	1.10	859	6.72	0.39	0.22	1.37	15.1	0.26	65.3	68.0
MYCA4	F	131	0.39	1.23	6.49	0.26	1.38	7.30	392	1.13	1214	5.71	0.46	0.33	1.12	16.5	<0.50	76.5	67.9
MYCA5	F	370	0.73	1.17	7.16	0.14	0.99	8.89	601	1.21	1351	10.6	0.52	0.27	1.24	14.7	0.39	72.4	70.1
MYCA6	F	182	0.52	1.26	7.09	0.20	1.71	8.10	383	1.19	1270	7.58	0.53	0.42	1.55	17.2	0.24	78.7	67.5
MYCA7	M	126	0.51	0.81	6.56	0.14	0.70	7.19	367	1.13	1174	5.72	0.40	0.24	1.24	12.9	0.10	96.0	68.0
MYCA8	F	240	0.57	1.12	16.1	0.20	2.20	8.62	535	1.68	1257	9.33	0.77	0.26	1.54	17.3	0.30	77.0	69.3
MYCA9	F	112	0.60	1.30	8.18	0.20	1.02	8.18	379	1.30	1165	6.79	0.67	0.26	1.24	19.5	<0.50	72.0	68

[1]Beryllium and molybdenum were not detected in any carcass tissues.

Appendix 4. Trace elements in liver tissues of California leaf-nosed bats *(Macrotus californicus)* collected from five mines on Kofa National Wildlife Refuge

		Concentration, µg/g dry weight																
Mine	Sex	Al	As	B	Be	Cd	Cu	Fe	Hg	Mg	Mn	Mo	Pb	Se	Sr	V	Zn	Moist (%)
Hub	M	30.0	<0.90	<9.00	<0.10	0.79	17.0	2180	0.50	825.	14.0	4.00	<0.90	4.00	0.75	1.70	110.	65.1
Hub	M	<10.0	<1.00	<10.0	0.10	2.50	19.0	1520	0.50	858.	12.0	4.00	<1.00	3.00	<0.20	2.10	102.	68.7
Hub	M	<9.00	<0.90	<9.00	<0.10	2.20	18.0	2200	<0.50	652.	13.0	4.00	<0.90	3.00	0.30	2.00	106.	68.2
Hub	F	<8.00	<0.80	<8.00	<0.10	3.10	15.0	1550	0.50	857.	11.0	4.00	<0.80	<2.00	<0.20	2.00	110.	68.8
Hull	F	<5.00	1.80	<2.00	<0.10	1.90	14.0	1300	0.50	669.	8.10	4.00	2.70	3.10	<0.20	<0.50	71.8	66.2
Hull	F	6.00	<0.50	2.00	<0.10	1.80	15.0	1010	0.88	694.	6.50	2.00	6.60	3.00	<0.20	<0.50	83.4	68.7
Hull	F	<9.00	1.00	2.00	<0.10	2.30	11.0	519	0.80	630.	3.40	<2.00	2.00	3.00	<0.20	<0.50	67.4	69.2
Hull	F	<6.00	<0.60	<2.00	<0.10	1.50	17.0	2180	0.60	693.	11.0	4.00	3.20	3.00	<0.20	<0.50	82.9	67.9
Hull	M	<5.00	0.90	<2.00	<0.10	<0.10	12.0	1470	0.50	698.	7.10	3.00	5.30	2.00	<0.20	<0.50	70.0	69.7
Hull	F	<6.00	<0.60	4.00	<0.10	3.10	14.0	1280	0.40	654.	8.10	3.00	2.70	3.70	<0.20	<0.50	80.2	66.7
Hull	F	<6.00	<0.60	<2.00	<0.10	2.50	16.0	1030	0.50	583.	8.20	3.00	1.80	4.00	<0.20	<0.50	73.2	66.7
Hull	M	<7.00	<0.70	<2.00	<0.10	0.69	14.0	1850	1.00	678.	8.50	3.00	14.0	3.00	<0.20	<0.50	73.5	66.7
Keystone	F	<7.00	<0.70	<2.00	<0.10	1.20	15.0	1840	0.90	709.	12.0	3.00	<0.70	3.00	<0.20	<0.50	85.6	68.5
Keystone	F	8.00	<0.60	<2.00	<0.10	1.00	18.0	1840	0.40	766.	10.0	3.00	<0.60	4.00	<0.20	<0.50	99.6	68.0
Keystone	F	<9.00	1.00	<2.00	<0.10	1.20	43.0	2480	0.60	804.	14.0	4.00	<0.90	3.00	<0.20	<0.50	123.	68.6
Keystone	F	<6.00	<0.60	<2.00	<0.10	0.72	13.0	1720	0.50	705.	8.80	3.00	<0.60	3.50	<0.20	<0.50	83.2	70.4
Northstar	F	<8.00	2.00	3.00	<0.10	1.70	17.0	2030	0.40	723.	9.80	4.00	<0.80	4.00	<0.20	<0.50	87.1	69.6
Northstar	F	<10.0	<1.00	<2.00	<0.10	0.70	13.0	1500	<0.60	750.	8.00	4.00	<1.00	2.00	<0.20	<0.50	76.5	66.9
Northstar[1]	M	98.0	<0.80	<2.00	<0.10	0.20	15.0	1450	1.00	803.	12.0	3.00	<0.80	3.00	<0.20	<0.50	79.5	69.3
Northstar	M	42.0	<0.40	<2.00	<0.10	0.20	14.0	1390	<0.20	735.	9.90	3.00	<0.40	3.00	<0.20	0.20	77.4	68.6
Northstar	F	130.	<0.90	<2.00	<0.10	1.50	16.0	1960	0.80	717.	11.0	4.00	<0.90	4.00	<0.20	<0.50	90.2	67.7
Northstar[2]	F	98.0	<0.80	<2.00	0.44	1.70	20.0	1830	<0.40	681.	11.0	4.00	<0.80	4.00	<0.20	<0.50	84.1	67.6
Northstar	F	98.0	<0.70	3.00	<0.10	0.81	18.0	1850	<0.40	704.	11.0	4.00	<0.70	3.40	<0.20	<0.50	79.2	67.7
Northstar[3]	M	<6.00	0.70	<2.00	<0.10	0.20	15.0	1630	0.80	777.	10.0	3.00	1.00	3.90	<0.20	<0.50	84.5	68.7
Puzzler[4]	F	<7.00	<0.70	<7.00	0.10	3.40	36.3	2090	1.30	863.	22.0	3.00	36.0	4.20	0.20	1.90	143.	64.4
Puzzler	M	<8.00	<0.80	10.0	0.10	1.60	28.0	2420	2.80	783.	24.0	4.00	12.0	2.00	0.30	1.50	122.	66.3
Puzzler	F	<10.0	1.00	<10.0	0.20	1.50	37.0	2200	3.90	740.	24.0	3.00	17.0	3.00	0.60	2.20	120.	65.8
Puzzler	F	<10.0	<1.00	<10.0	0.20	3.40	28.0	2090	2.30	764.	22.0	3.00	20.0	3.00	0.80	2.60	103.	67.6
Puzzler[5]	M	<10.0	1.00	<10.0	0.20	2.80	33.0	2900	1.50	743.	25.0	3.00	13.0	7.20	0.50	2.60	109.	66.1
Puzzler	M	<9.00	<0.90	<9.00	0.10	2.10	26.0	1530	2.80	697.	17.0	3.00	11.0	3.00	0.40	2.90	106.	66.9

[1]This sample also contained 0.70 µg/g nickel.

[2]This sample also contained 1.0 µg/g barium and 0.80 µg/g nickel.

[3]This sample also contained 1.0 µg/g barium.

[4]This sample also contained 0.60 µg/g chromium.

[5]This sample also contained 0.50 µg/g chromium.