

Hair Analysis as an Environmental Health Bioindicator: A Case-Study using Pelage of the California Ground Squirrel (*Spermophilus beecheyi*)

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Abstract

*This study investigated hair elemental concentrations as an indicator of environmental health with the California Ground Squirrel (*S. beecheyi*). 181 hair specimens were analyzed for 35 elements. Soil and hair analysis results showed that for some elements, concentrations were much higher in the environment than in the hair of *S. beecheyi*. Conversely, some elements were much lower in the environment but have a long biological half-life such that while undetectable in soils they are often slowly excreted in the hair follicle and therefore bioaccumulate (e.g. Copper, Lead, Zinc). On average *S. beecheyi* did not exhibit toxicological symptoms, though several results were well above the published minimal risk level (MRL). The information presented here may help establish an interval above which undue or excessive exposure may be indicated. These data represent the first large scale investigation of hair analysis of a burrowing animal globally and provide a reservoir of baseline data and information for future studies.*

Keywords: Environmental health, hair analysis, wildlife, California, *S. beecheyi*

1.0 Introduction

Pesticides significantly contribute to the productivity of the multibillion-dollar agricultural industry in the San Joaquin Valley of California, USA. Although pesticides are regulated and monitored to protect human health, many questions remain regarding the long-term effects of pesticides on wildlife, from which we may also draw inference to human health. For example, studies of heavy metal concentrations are needed to lend insight into the potential environmental problems caused by inorganic and heavy metal pesticides. Pesticides of specific concern often include inorganic and heavy metal pesticides, including various arsenic compounds, e.g., Paris Green ($(\text{CH}_2\text{COO})_2 \text{Cu} \cdot 3\text{Cu}(\text{AsO}_2)_2$) and cryolite (Na_3AlFe), which is still used extensively. These compounds, plus others, have a long history of use in the San Joaquin Valley (Matsumura 1975). Other sources of potentially toxic metal exposure include lead from gasoline and industrial emissions.

There is a continual influx of many other pollutants into the biosphere from both natural and anthropogenic sources. A complex variety of abiotic and biotic processes affect their speciation (physical-chemical forms) and distribution, including adsorption onto and desorption from mineral surfaces, incorporation in precipitates or coprecipitates, release through the dissolution of minerals, and interactions with plants and microbes. Some of these processes can effectively isolate heavy metals from the biosphere, whereas others cause their release or transformation to different species that may be more (or less) bioavailable and/or toxic to organisms (Brown et al. 1999).

Naturally occurring minerals in the soil have been shown to lead to various toxicological responses. Although heavy metals are naturally present in the environment, human activities can increase their levels. When the pH of soil decreases as an effect of acid rain, the solubility and mobility of many metals (e.g., cadmium) will increase. Their uptake by plants may increase and herbivorous animals feeding on such plants may accumulate the metals in their organs (Medvedev 1999). Tataruch (1984) showed that tissues of animals inhabiting acid soils contained cadmium levels that were higher than those in animals inhabiting alkaline soils. This is an important consideration since trace element nutrition and inorganic composition of the body play a very important role in the physiopathologic status and reproductive efficiency of animals.

Long-term metabolic changes of various elements, as well as past and present nutritional events in an individual can be sequestered in many tissues, but may be best reflected in hair (Dey et al. 1999). The prospect of being able to study the general health and well being of any animal without sacrificing the animal has become increasingly appealing to contemporary bio-ecologists. Since trace elements have been measured in hair, hair has proven to be a practical dose meter for heavy metal environmental pollutants (Hammer et al. 1971), and can thus serve as an indicator of environmental health.

One approach to assessing the status of environmental health is to compare the level of trace element concentration of hair in populations living in areas with varying degrees of pollution (Obrusnik 1986). Corridan (1973) suggested that hair and nail samples provide reliable indications of exposure to many toxic metals over periods of a year or more and can thus be used in epidemiological studies related to environmental pollution.

The use of hair as an indicator is not new. Over 200 years ago, hair was analyzed to measure arsenic levels in the body (Gouille and Kintz 1996). Hair was proposed as a useful biopsy specimen over 50 years ago as an indicator of body stores of trace elements based on its function as a minor excretory organ (Katz and Chatt 1988). With rapid advances in laboratory technology and modern analytical techniques, hair has emerged as a valuable medium for providing important clinical information about long-term nutritional status, toxic element exposure, drug and substance abuse, and other important bio-physiological parameters (Ho et al. 2001). Further, hair is painlessly removed, normally discarded, easily collected, and may better reflect the total body pool of some elements than either blood or urine (Hammer et al. 1971). Unlike short-term indicators (e.g., body fluids) hair retains an incorporated substance for long periods, since there is no active metabolism/excretion to remove the substance once deposited (Tsatsakis et al. 1998).

The elemental composition of human hair has been studied in depth (Bos et al. 1985). However, despite the extent of previous studies, interpretation of the results has been difficult. Thus, fundamental, basic research is warranted that seeks to better document contaminant levels in both hair and the environment. Extensions of hair elemental analysis should be combined with monitoring mineral metabolism to make hair analysis a potentially useful technique worthy of continued use for wildlife species (Franzmann et al. 1975).

California ground squirrels (*Spermophilus beecheyi*) are ideal mammals for such studies. They are common and feed on a wide array of vegetation. Secondly, *S. beecheyi* may be particularly susceptible to chemicals and heavy metals in the soil because of their close living arrangements within the soil environment. It is possible that ground squirrels are subjected to higher doses of heavy metals by virtue of being fossorial, and consequently may accumulate more heavy metals in their system than non-burrowing mammals. Conceivably, ground squirrels may be an excellent bio-indicator for heavy metal toxicity.

The following study compares concentrations of selected heavy metals in both soil and the hair of *S. beecheyi*. Data were gathered from three different geographic locations over the course of one year to provide an average metric for heterogeneous environmental conditions (assuming environmental conditions change spatially and temporally). Soil samples were collected and tested for select heavy metals and minerals, and an attempt was made to identify potential toxicities that may be important for land-use and wildlife management considerations.

2.0 Methods

2.1 Study Sites

Study sites were located in Fresno and Madera counties in the central region of the San Joaquin Valley California (Figure 1). Specimens were collected strictly from the valley floor so as not to introduce other ecological and/or environmental variables. Either of which may have produced confounding results in metal and mineral content of hair or color due to diet and other habitat differences found in foothill or mountain environments.

Figure 1. Study areas used for the current project from spring 2001 through winter 2002. Highlights 1, 2, 3 are study sites, KMJ (permanent preserve), 5S Ranches (permanent pasture), and Almond Growers (permanent agriculture) respectively.

Study site one (KMJ Radio tower site) was located in Kerman, CA, USA. The site was approximately 32 hectares in size with Latitude: 36°41'37"N, Longitude: 120°03'17"W. Elevation of the site is approximately 65 m. At the time of this study, vegetation of this study site was classified as "California annual grassland series", including species of *Bromus*, *Hordeum*, *Vulpia*, and *Erodium* (Sawyer and Keeler-Wolf 1995).

Annual precipitation ranges from 20-89 cm, and average annual temperatures range from 12°-17° C. Average frost-free season ranges from 225-250 days (historical record). Soils at site one are classified as Hesperia sandy loam “saline-alkali” consisting of well-drained, moderately coarse textured soils. These soils have accumulated a slight to moderate amount of lime below the surface layer and in places are saline-alkali affected. The natural vegetation is presumed to have been comprised mainly of annual grasses and forbs, together with saline-alkali tolerant plants in some locations. The profile of 0-13 cm (including the profile analyzed in this study) is represented by light brownish-gray fine sandy loam. PH is neutral (7.0). Soil depth exceeds 1.5 m in most soils. Surface texture ranges from coarse sandy loam to clay loam. Available water holding capacity ranges from 15-23 cm, depending on soil depth (Huntington 1971). Study site two (5S Ranches) was located in Madera, CA, USA at Latitude: 36°57'56"N, Longitude: 119°49'52"W. Elevation of this site is approximately 112 m. At the time of this study, vegetation at the site was classified as “California annual grassland series” with dominant vegetation including genera such as *Bromus*, *Hordeum*, *Vulpia*, and *Erodium* (Sawyer and Keeler-Wolf 1995).

Figure 2. Study sites used for the current research from spring 2001 through winter 2002. Top) KMJ radio transmitter study site (permanent preserve), middle) 5S Ranches Inc. study site (permanent pasture), and bottom) Central California Almond Growers study site (permanent agriculture). Photos by author.

Soils at site two are classified as Atwater loamy sand. The profile of 0-61 cm (including the profile analyzed in this study) is represented by pale-brown and soft loamy sand that can be slightly acidic, very weak and very fine and granular in structure when moist. This soil type is very low in organic matter. Runoff is slow, and internal drainage is moderately rapid. The rooting zone is very deep, and the available water holding capacity and the natural fertility is considered moderate. The soil is free of excess salts and alkali. The soils in this unit were derived from wind-reworked granitic alluvium. Typically these soils occur on the leeward side of present or abandoned stream courses on low terraces (Ulrich and Stromberg 1962). Study site three (the Central California Almond Growers Association) was located in Sanger, CA, USA at Latitude: 36°46'02"N, Longitude: 119°35'36"W. Elevation is approximately 114 m. Vegetative community was considered Ruderal (Sawyer and Keeler-Wolf 1995), and was relatively low in vegetation and diversity due to frequent use of herbicides. Dominant vegetation generally located along fencerows included: puncture vine (*Tribulus terrestris*), *Echinochloa sp.*, *Salsola tragus*, *Conyza bonariensis*, and *Amaranthus sp.* Soils at site three were generally similar to those at site two.

2.2 Sample Collection Techniques

Dey et al. (1999) were able to show significant differences (95% CI) in the concentrations of heavy metals in the hair of leopard cats (*Felis bengalensis*), civet cats (*Viverra zibitha*), flying squirrels (*Petaurista magnificus*) and leopards (*Panthera pardus*) with 10-15 specimens of each species. Therefore, the attempted minimum number of hair samples collected per sampling period per study site was 15. Hair samples were collected from preserved specimens of *S. beecheyi* collected during the course of previous research (see Anderson 2000, Hubbart 2002, Hubbart et al. 2011). At least 0.25 g of hair was shaved with a single edged razor blade from the back of the base of the neck and between the shoulder blades of each specimen (Hubbart et al. 2011, Jachowski and Hubbart 2012). The selection of this area for specimen collection is based on the criterion that “new growth” hair be collected, assuming that the most recent hair growth is more representative of the body’s elemental status during previous months, and that external contamination tends to increase with hair length (DiPietro et al. 1989). Tsatsakis et al. (1998) tested these assumptions by analyzing the hair of rabbits and showed statistically reliable results at 95% CI that length of hair was directly correlated to an increased concentration of a given element. Hair was weighed on a mechanical scale to the nearest 0.01g and then promptly sealed in a new 473 ml Ziploc[®] bag. The new Ziploc[®] bag was then labeled with the specimen catalog number and placed in an appropriate box to await shipment with the remaining hair samples from the given season.

2.3 Hair Analysis

Hair samples were analyzed for the metals: Aluminum (Al), Antimony (Sb), Barium (Ba), Beryllium (Be), Boron (Bo), Cadmium (Cd), Calcium (Ca), Cesium (Cs), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Magnesium (Mg), Manganese (Mn), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Potassium (K), Rubidium (Rb), Sodium (Na), Strontium (Sr), Thallium (Tl), Titanium (Ti), Uranium (U), Vanadium (V) and Zinc (Zn), and minerals: Arsenic (As), Phosphorus (P), Selenium (Se) and the complexes, Ca/Mg, Ca/P, Mg/K, Na/K and Zn/Cu.

Accuracy of laboratory tests is best ensured through the use of standards of known value to calibrate tests. Internationally recognized groups, such as the National Institute of Science and Technology, provide standard materials of known value, and other organizations, such as the United States Environmental Protection Agency, provide specifications for those standards (Steindel and Howanitz 2001). This methodology requires that laboratory control samples be analyzed for each analytical method. Therefore, a method blank was analyzed with each batch of samples, thereby maintaining instrumental calibration. Instrument standardization was verified by calibration every 10 samples using a calibration blank and a check standard as recommended by U.S. EPA Method 6010 (1983).

2.4 Soil Sampling and Analysis

The elements that were measured in this study are relatively immobile in the soil. Therefore, soil samples were collected from each study site and analysis conducted on a composite sample at the end of the study. A single core of soil 2.54 cm × 15.24 cm in depth from each site was collected during each sampling period (seasonally). The depth of the soil sample was designed to include the root zone of the majority of the vegetation that the squirrels eat (Korschgen 1970). The soil corer used was made with 15 cm of Poly Vinyl Chloride (PVC) schedule 40 standard plumbing grade pipe and capped with a 2.54 cm PVC cap (Figure 8). This material was used to avoid possible soil sample contamination by the use of conventional steel soil samplers. Each sample was promptly placed in a sterile Ziploc[®] bag, sealed and stored. At the end of the study, composite samples were prepared. The three composite soil samples were delivered for analysis to Twining Laboratories, Fresno, California, USA. Soil samples were analyzed for the metals: Aluminum (Al), Beryllium (Be), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Magnesium (Mg), Mercury (Hg), and Zinc (Zn), and minerals Arsenic (As), and Selenium (Se). Soil samples were analyzed using Sequential Inductively Coupled Plasma (ICP) Absorption technology. Except for elemental mercury (analyzed with EPA method 7471, cold-vapor method), Twining labs used the same protocols for digestion and ICP (methods 3050 and 6010 respectively) as those supplied by the Environmental Protection Agency and used by NHA to analyze the hair samples (mg/kg).

2.5 Elemental Concentration and Environmental Risk Assessment

Toxicological research and toxicity testing conducted and interpreted by toxicologists are critical components of the scientific core of elemental hair analysis, sometimes referred to as “risk assessment” (Faustman and Omenn 2001). An effort was made to consider the elements surveyed in this study in terms of toxicological effects, normal route of ingestion and the minimal risk level (MRL) or reference dose (RFD: the minimum level of a given element that can be ingested without resulting toxicosis in humans). Also considered was lethal dose killing 50% (LD/50) of experimental animals (often times laboratory rats) and the reference range (RR) which is the detectable range of the given element using ICP/MS. Data were derived from standard texts and monographs, government documents, technical reports, material safety data sheets (MSDS) and primary journal literature (the reader is referred to Literature Cited and the Appendix for more information).

4.0 Results and Discussion

One hundred eighty-one hair samples were analyzed between April 2001 and January 2002. Means and standard deviations of the elements and elemental complexes analyzed in this study (Al, Sb, As, Ba, Be, B, Cd, Ca, Ca/Mg, Ca/P, Cs, Cr, Co, Cu, Fe, Pb, Mg, Mg/k, Mn, Hg, Mo, Ni, P, K, Rb, Se, Na, Na/k, Sr, Tl, Ti, U, V, Zn, Zn/Cu) are provided in Table 1 and illustrated in Figure 3.

Aluminum (489.01ppm), Calcium (1665.67ppm), Iron (588.96ppm), Magnesium (539.38ppm), Phosphorus (511.31ppm), Potassium (404.95ppm), Sodium (270.49ppm), and Zink (150.00ppm) had the highest average concentration from all 181 hair samples during the year of sample collections. Lead had an average concentration of 9.05ppm, but also had a standard deviation of 19.26 indicating a great deal of variability in results. As shown in Table 1 (standard deviations), one of the greatest findings in this work is in fact the variability in concentrations between individuals.

Many factors can influence the observed results of this work including (but not limited to) total concentration and speciation (physical-chemical forms) of metals, mineralogy, pH, redox potential, temperature, total organic content (both particulate and dissolved fractions) and suspended particulate content, as well as volume of water, water velocity and duration of water availability, particularly in arid and semi-arid environments. In addition, wind transport and removal from the atmosphere by rainfall must be considered.

Many of these factors vary seasonally and temporally and most factors are interrelated. Consequently, changing one variable may affect several others (Luoma 1989).

Table 1. Means and standard deviations of hair elemental analysis results from hair samples collected from California ground squirrels from spring 2001 through winter 2002 from three geographic locations in the San Joaquin Valley, California. T = Trace (< 0.01 of the given element).

Figure 3. Illustration of hair elemental analysis results from hair samples collected from California ground squirrels from spring 2001 through winter 2002 from three geographic locations in the San Joaquin Valley, California. Chart shows percent of total of higher concentration elements (Table 1).

Climate also strongly influences soil types, which in turn largely controls element (metals and metalloids) mobility and availability (Chaney 1988). Metal uptake rates are also controlled by plant species and relative abundance and availability of necessary elements. Abundant bioavailable amounts of essential nutrients, including phosphorous and calcium, can decrease plant uptake of non-essential but chemically similar elements, including arsenic and cadmium, respectively. More complex interactions have also been observed: bioavailability may be related to multi-element amounts or ratios. For example, copper toxicity is related to low abundances of zinc, iron, molybdenum and (or) sulfate (Chaney 1988).

Retention is one of the key processes affecting the fate of organic chemicals in the soil environment. Retention refers to the ability of the soil to hold a pesticide or other organic molecule and to prevent the molecule from moving either within or outside of the soil matrix. Retention strongly influences chemical transport to the atmosphere, groundwater, and surface waters (Koskinen and Harper 1990). Not surprisingly, retention is a primary factor influencing the efficacy of soil-applied pesticides (e.g., Bailey et al. 1964, Hamaker et al. 1972, Green 1974, Weed and Weber 1974, Calvet 1980).

Soil pH plays a large role in the bioavailability of elements in the soil environment. Nearly all California soils have a pH ranging from 5.0 to 8.5. Notably, a low pH (e.g., an acidic soil with a pH of 5.5) suggests that soil nutrient concentrations and microbial activity are low (Tucker et al. 1987). In strongly acidic soils, most heavy metals and some nutrients are soluble and aluminum and manganese may be present in toxic concentrations. A high pH (e.g., an alkaline soil with a pH of 8.0) suggests that concentrations of some soil nutrients (particularly calcium and magnesium) are high, which can limit plant growth and affect the type of crops that can be grown on a site (Donahue et al. 1983). Soil pH also greatly affects the solubility of minerals and many heavy metals, and therefore affects their availability for plant growth and uptake in biomass and their potential to be leached from the soil.

4.1 Pesticide and Herbicide Use

The use of synthetic chemicals to control pests, principally insects, weeds, and fungi, became an integral part of agriculture and disease control after World War II. These chemicals provide an inexpensive means of increasing crop production, preventing spoilage of stored foods, and saving many millions of human lives by the prevention of insect-borne diseases (Moyle 1997). The history of DDT use in the U.S. illustrates the gradual development of awareness to the ecological consequences of pesticide application. DDT is classified as an "organochlorine" chemical, a descriptive label that reflects its chemical structure, consisting of a combination of carbon (organic molecules are defined as those comprised of at least some amount of carbon) and chlorine atoms. Other organochlorines that are important environmental pollutants include polychlorinated biphenyls (PCBs) and dioxins. Polychlorinated biphenyls were used as insulators in the electrical industry until the environmental threat posed by their toxicity was realized in the mid-1970s. Dioxins are the most potent chemical carcinogens known, and are present in the environment largely as a byproduct of various industrial activities (e.g. bleaching of paper) (Moyle 1997).

The properties that make DDT and other organochlorine pesticides toxic to insect pests also make them hazardous to wildlife. Organochlorine pesticides are extremely persistent. For example, some of the DDT applied in the USA in the early 1970s is still present in the environment today. An additional important characteristic of organochlorines is their tendency to be accumulated by living organisms (bioaccumulation). Organochlorines are strongly attracted to fats present in cells and tissues of living organisms. Since organochlorines resist degradation, these compounds can gradually accumulate to high concentrations in tissues of vertebrates (Moyle 1997).

Other types of synthetic insecticides have been developed that pose lesser environmental threats than the organochlorines. Organophosphates, with a chemical structure consisting of carbon and phosphorus atoms, are widely used. These compounds degrade much more readily than the organochlorines, and therefore have less of an impact on nontarget species. Malathion, large quantities of which are used in attempts to control the Mediterranean fruit fly in California, is a well-known organophosphate insecticide. Other synthetic pesticides are used to control weeds, fungi, and other pests. Although the more recent generations of chemicals pose a lesser ecological threat than the organochlorines, they still have been shown to produce adverse effects in wildlife populations (Moyle 1997).

In urban areas industries and automobiles release large amounts of toxic metals into the air that eventually settle onto the ground. During rainstorms, water picks up these pollutants as it flows over the ground surface. This "storm water runoff" ultimately flows into either a local sewer system or a local water body (i.e., creek, stream, river, lake, estuary, or ocean). Storm water runoff from urban areas is one of the most significant sources of toxic water pollution (Moyle 1997). Combustion of leaded gasoline in the 1900's resulted in widespread contamination of terrestrial and aquatic ecosystems, particularly near roads with dense traffic. Wildlife living near roads have been shown to accumulate elevated concentrations of lead (Clark 1979).

4.2 Soil Analyses

Of the 12 elements analyzed in the soil analysis conducted in this study, Ca, Hg, and Se were not detected (Table 2, Figure 5). It is possible that these elements were present in amounts smaller than the lower detection limits of the ICP/MS, which was 0.1, 0.02 and 2.1 mg/kg for each element, respectively. This could explain why these elements were detected at relatively low levels in the hair analysis of *S. beecheyi*. Rozman and Iatropoulos (1989) explained that as a chemical is biotransformed or excreted from the body, more is released from the storage site. The storage site may have been hemoglobin, lungs, bone or soft tissues. As a result, the biological half-life of stored compounds can be very long. Therefore, although some metals may be undetectable (or at very low levels) in soils they can and often are slowly excreted in the hair follicle such that they become amplified.

Relationships between soil and hair elemental analysis are clearly complex. Soil analysis concentrations for Cu, Pb, and Zn were well below the concentration levels found in the hair analysis (Table 1, Table 2). For these three elements, as discussed above, it is possible that given their respective slow excretion rates, they may become amplified in the hair follicle. However, soil analysis concentrations of Al, As, Be, Cr, Fe, and Mg were all well above the detected concentrations of the hair. This may be attributable to metabolic release of these elements to urinary and / or fecal excretion, which for many minerals is regulated to approximately equal the intake so that a balance is maintained (Beers and Berkow 1999). A spatial and temporal investigation of soil species may better elucidate the causes of many of the elemental concentration differences seen here.

Table 2. Soil elemental analysis results (ppm) from soil samples from three geographic locations in the San Joaquin Valley of California, USA.

In this study, the elemental concentrations found from hair analysis for the complexes, Ca/P, Zn/Cu, Ca/Mg and Na/K are assumed to be dependent upon metabolic processes of *S. beecheyi*, of which there is currently no known documentation. Therefore, a discussion of these complexes and *S. beecheyi* would be purely speculative and will therefore not be discussed here.

Hammer et al. (1971) tested hair trace metal levels and environmental exposure of fourth grade boys in cities representing exposure dose gradients for As, Cd, Cu, Pb, and Zn. City exposure ratings were determined from aerometric, geologic, and industrial data. The mean concentrations of As, Cd, Cu, and Pb did not follow the estimated exposure gradient. Conversely, hair Zn distributions, unlike those for As, Cd, Cu, and Pb were quite similar to the estimated city means.

Figure 4. Illustration of soil elemental analysis results (ppm) from three geographic locations in the San Joaquin Valley of California, USA, where Iron, Aluminum, and Cadmium were greatest contributors to total soil concentration (for elements tested).

Quarles et al. (1974) conducted a study of Pb and Cd in small mammals at varying distances from a highway (increased Pb concentrations have been found in air, soil, and plants within this zone).

They discovered that while Pb concentrations increased dramatically with decreasing distance from a highway, Cd concentrations varied significantly, revealing no obvious pattern. Causative factors for the Cd irregularities were not discovered. It is possible that the results in the current work result from spatial and temporal variables that can be clarified in future work. Brazil and Ferguson (1989) found that Cd concentrations in livers of moose varied in six different regions from eastern North America. They were unable to determine what caused the region related differences since results were widely dispersed and various. The results for Cd in this study seem to agree with the irregularities found in the work of Quarles et al. (1974) and Brazil and Ferguson (1989). Zachwieja et al. (1995) evaluated Zn content in children's hair living in various rural and industrial settings. Although the most industrial setting did have the highest concentrations of Zn, it was not the most remote (rural) settings that had the least. There were no statistically significant differences among the four different settings. They were able to confirm however that the place of residence did affect the Zn content in children's hair depending on the degree of environmental pollution in the given area. With regard to the current study, whether the observed concentrations have to do with poisons applied, industrial activities, or naturally occurring deposits remains unknown but supplies direction for future investigation.

4.3 Toxicology

A discussion of elements of toxicological concern found to exist in this study is carried out here based largely upon the data found in the literature, and materials safety data sheets (MSDS). For more information pertaining to each of the elements studied, the reader is referred to Literature Cited and the Appendix. Notably, while the results presented here are based upon varying published sources, they should be considered inconclusive. This research provides a wealth of baseline data. However, whether results are indicative of toxicities or environmental health must be supported by additional investigation.

For Ca the mean hair concentration ($1665.67\mu\text{g/g}$) exceeded the documented Minimal Risk Level (MRL) of $2.0\mu\text{g/m}^3$ (based on inhalation). However, all samples were much less than the lethal dose killing 50% of specimens (LD/50) for rats of 6450 mg/kg/day , which is based on ingestion. It may be reasonable to assume that the mean concentration (based on the results of this study), which is well below the LD/50 for rats toxic upper limit, is within healthy parameters for this species. This is further reasonable since calcium sequestered in the hair follicle of these animals is probably ingested through food consumption and not inhaled. There is no evidence that indicates that Ca is available for inhalation within the soil environment of burrowing animals. For Co the mean observed hair concentration was $0.33\mu\text{g/g}$, which exceeded the documented MRL of $.03\mu\text{g/m}^3$ (based on inhalation), but was much less than the LD/50 for rats of 6171 mg/kg , which is based on ingestion. It is likely that the observed mean level of $0.33\mu\text{g/g}$ observed in this work is within healthy parameters for this species since *S. beecheyi* does not live in association with cobalt powder, cobalt oxide and cobalt sulfides, which are capable of inducing local sarcomas (malignant tumors)(Goyer and Mehlman 1977).

For Mn the mean hair concentration of all specimens was $22.49\mu\text{g/g}$. The documented MRL was $0.04\mu\text{g/m}^3$ (based on inhalation). However, this figure is much less than the LD/50 for rats of 9.0 g/kg , which is based on ingestion. Here again, it is likely that the observed mean concentration, which is well below the LD/50 for rats toxic upper limit, is within healthy parameters for this species. There are few reported cases of manganese toxicity in humans from oral ingestion (Goyer and Clarkson 2001). The most common form of manganese toxicity is the result of chronic inhalation of airborne manganese in mines, steel mills, and some chemical industries (ATSDR 1997), none of which necessarily apply to the natural history of *S. beecheyi*.

For Hg the mean hair concentration was $0.2\mu\text{g/g}$. This figure is equal to the documented MRL of $0.2\mu\text{g/m}^3$ (based on inhalation). This concentration level is also the lower detection limit for this element using ICP/MS. It therefore may be that actual concentration levels of this element were somewhere below $0.2\mu\text{g/g}$. The major source of mercury (as mercury vapor) in the atmosphere is the natural degassing of the earth's crust. Mercury is also found as a residual from seed treatments in agricultural practices. It is doubtful that the Hg detected in this study is due to agricultural practices since no seed sowing of any kind had taken place in any of the study sites for at least the previous 10 years (though wind transport may be a factor). It is difficult to assess what quantities of mercury come from human activities, but these are believed to be similar in magnitude to natural sources (Goyer and Clarkson 2001). For Ni the mean hair concentration was $0.77\mu\text{g/g}$. This concentration exceeds the documented MRL of $0.2\mu\text{g/m}^3$ (based on inhalation), which is also the lower detection limit for ICP/MS. It has not been shown that this element is available for inhalation within the soil environment and is therefore unavailable for inhalation by *S. beecheyi*.

Presumably, any detected Ni in the hair analysis should have been acquired by ingestion. The LD/50 for nickel in pure form is not known for oral ingestion. Nickel (inhalation) is known to cause lung cancer, nasal cancer and other respiratory tract related cancers. Studies with rats have shown similar respiratory effects as well as malignant sarcomas at sites of injection. Other serious consequences of long-term exposure to nickel are not apparent, but severe acute and sometimes fatal toxicity may follow exposure to nickel carbonyl. Further, allergic contact dermatitis is common among the general population (Goyer and Clarkson 2001). None of these symptoms were observed with specimens collected for this study. It is unknown what metabolic differences exist between humans and *S. beecheyi* with regard to nickel metabolism.

For Na the mean hair concentration was 270.49 µg/g. This concentration exceeds the documented MRL of 2 µg/g/day (based on oral ingestion), but was much less than the LD/50 for rats of 3,000 mg/kg, which is based on ingestion. It may be that living in the soil environment predisposes *S. beecheyi* to increased levels of Na. It may also be that *S. beecheyi* has developed a higher tolerance for this element. For Ti the mean hair concentration was 37.73 µg/g. This concentration exceeds the documented MRL of 0.1 µg/m³ (based on inhalation), but all were much less than the LD/50 for rats of 400mg/m³, which is also based on inhalation. It is known that approximately 3% of an oral dose of titanium is absorbed in humans. The majority of that absorbed is excreted in the urine (Goyer and Clarkson 2001). It is unknown whether or not titanium is inhaled in the *S. beecheyi* environment. It is also unknown whether titanium metabolism is similar between *S. beecheyi* and humans.

Although on average *S. beecheyi* did not exhibit any overt toxicological symptoms, there were a number of specimens (individuals) whose hair analysis results were well above the published MRL. This is not surprising given that individuals within populations may exhibit an ability to tolerate much higher doses of certain toxic elements, without developing symptomatic toxicosis. Conversely, it has been just as well documented that certain individuals within a population will exhibit a high degree of intolerance to certain toxic elements and will exhibit extreme symptoms of toxicosis well before any other members of that population (Bascietto et al. 1990). Due to factors such as age, genetic variations and the presence of disease, toxic doses among individuals may vary. Whether this is something that is being seen in this here will only be supported with further investigation. These findings represent some of the first in a fossorial wildlife population globally, and therefore provide a rich data set from which to draw inference for management and future investigations.

5.0 Conclusions

This study investigated the value of hair as an indicator of environmental health in a case-study of the California Ground Squirrel (*S. beecheyi*). As far as is known, these data represent the first large scale investigation of hair analysis of a burrowing animal globally and provide a reservoir of baseline data and information for future studies. The purpose of this work was to quantify concentrations of the following metals and minerals (Al, Sb, As, Ba, Be, B, Cd, Ca, Ca/Mg, Ca/P, Cs, Cr, Co, Cu, Fe, Pb, Mg, Mg/K, Mn, Hg, Mo, Ni, P, K, Rb, Se, Na, Na/K, Sr, Tl, Ti, U, V, Zn, Zn/Cu) from hair samples from 181 *S. beecheyi* specimens collected during previous work. Soil and hair analysis results showed that for some elements, concentrations are much higher in the environment than in the hair of *S. beecheyi*. Conversely some elements were much lower in the environment but have a long biological half-life such that while undetectable in soils they are often slowly excreted in the hair follicle and therefore bioaccumulate (such as what may have been the case here with Cu, Pb, and Zn).

Not unlike other studies that have been performed on hair elemental analysis, elemental concentrations were widely dispersed. Information concerning presence and bioavailability of the elements surveyed from different (replicated) study sites could make understanding the differences between a given element and its respective habitat much easier, although still inconclusive since available vegetation, dietary preference and other factors need to be considered. These complexities further illustrate the difficulty of identifying conclusive reasons for the elemental concentration variability observed in this study. Perhaps the most generally accepted use of hair analysis is documentation of exposure to potential toxic elements from the external environment. In this regard, the data presented here may help establish an interval above which undue or excessive exposure may be indicated, even without the defining of the mechanism of deposition (external "air pollution" or internal excretion). More controversial is the use of hair elemental data to evaluate nutritional status. With seemingly rare exceptions, the pattern of hair trace metals is not well correlated with the nutritional or disease status of the individual. It is presumed that the populations of *S. beecheyi* in the current study were "normal" with respect to disease and nutritional status. The elucidation of the relationship of hair element concentrations and adverse health outcomes cannot therefore be predicted from these data, but provide ample impetus for future research.

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Appendix: Toxicology

Appendix for elements studied in the current work. Where ATSDR is the Agency For Toxic Substances And Disease Registry, EPA is the Environmental Protection Agency, OSHA is the Occupational Safety And Health Agency, ESPI is Electronic Space Products International, USGS is United States Geological Survey, NHA is Nutri World Hair Analysis, Route is the “route of ingestion”, Oral or Inhalation (Inh), MRL is Minimal Risk Level, ATSDR RFD is Reference Dose, EPA MRL and RFD are estimates of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse health effects, published by the Agency For Toxic Substances And Disease Registry (ATSDR) and the Environmental Protection Agency (EPA) respectively. LD/50 is Lethal Dose killing 50% of specimens, RR is Reference Range. Represents average concentration of nutrients found in hair samples of the general public (Nutri World Hair Analysis 2001). Superscripts correspond to citation source(s).

Element	Route	MRL/RFD	LD/50 (Rat)	RR (µg/g, ppm)	Source(s)
Aluminum	Oral	² 2.0 mg/kg/day	na	³ 2 - 13.1	¹ (OSHA 2001a) ² (ATSDR 2001a) ³ (NHA 2001)
Antimony	Oral	² .0004 mg/kg/day	³ 7 gm/kg	⁴ .06 - .84	¹ (ATSDR 1995a) ² (EPA 2001a) ³ (Sigma 2000a) ⁴ (NHA 2001)
Arsenic	Oral	² .0003 mg/kg/day	¹ 8 mg/kg	³ .03 – 1.1	¹ (Sigma 2000b) ² (ATSDR 2001b) ³ (NHA 2001)
Barium	Inh	¹ 5-15 mg/m ³	^{1,2} 50 mg/kg	³ .05 – 5.97	¹ (ATSDR 1995b) ² (Sigma 2000c) ³ (NHA 2001)
Beryllium	Oral	¹ .002 mg/m ³	na	² .06	¹ (ESPI 1992) ² (NHA 2001)
Boron	Oral	² .01 mg/kg/day	¹ 650 mg/kg	³ .04 – 4.17	¹ (Sigma 2000d) ² (ATSDR 2001c) ³ (NHA 2001)
Cadmium	Oral	² .0002 mg/kg/day	¹ 2330 mg/kg	³ .02 - .79	¹ (Aldrich 1999a) ² (ATSDR 2001d) ³ (NHA 2001)
Calcium	Inh	² .002 mg/m ³	¹ Oral 6450 mg/kg	³ 194 – 2700	¹ (Sigma 2000e) ² (USGS 2001) ³ (NHA 2001)
Calcium/ Magnesium	na	na	na	¹ 5 – 15	¹ (NHA 2001)
Calcium/ Phosphorus	na	na	na	¹ 2.5 – 6.5	¹ (NHA 2001)
Cesium	na	na	¹ 1400 mg/kg	² .01 – 5	¹ (Sigma 2000f) ² (NHA 2001)
Chromium	Inh	² .001 mg/m ³	¹ Oral 3360 mg/kg	³ 0 - .52	¹ (Sigma 2000g) ² (ATSDR 2001e) ³ (NHA 2001)
Cobalt	Inh	² .00003 mg/m ³	¹ Oral 6171 mg/kg	³ 0 - .18	¹ (Aldrich 1999b) ² (ATSDR 2001f) ³ (NHA 2001)
Copper	Inh	² .1 mg/m ³	¹ Oral 300 mg/kg	³ 9 – 40	¹ (Aldrich 2001a) ² (ATSDR 1999)

Iron	Inh	¹ 7 mg/m ³	² Oral 1520 mg/kg	³ 5.2 – 20.5	³ (NHA 2001) ¹ (Aldrich 2000a) ² (Aldrich 2001b) ³ (NHA 2001)
Lead	na	na	¹ Oral 220 mg/kg	² .04 – 10.8	¹ (Sigma 2001a) ² (NHA 2001)
Magnesium/ Potassium	na	na	na	na	¹ (NHA 2001)
Manganese	Inh	² .00004 mg/m ³	¹ Oral 9 gm/kg	³ 0 – 1.07	¹ (Sigma 2001b) ² (ATSDR 2001g) ³ (NHA 2001)
Mercury	Inh	² .0002 mg/m ³	na	³ .2 – 5.52	¹ (Fluka 2000a) ² (ATSDR 2001h) ³ (NHA 2001)
Molybdenum	Oral	² .005 mg/kg/day	³ 318 mg/kg	⁴ 0 - .12	¹ (Aldrich 2000b) ² (EPA 2001b) ³ (Aldrich 2000c) ⁴ (NHA 2001)
Nickel	Inh	² .0002 mg/m ³	na	³ .2 – 1.5	¹ (Aldrich 2000d) ² (ATSDR 2001i) ³ (NHA 2001)
Phosphorous	Oral	² .0002 mg/kg/day	na	³ 118 – 181	¹ (Sigma 2000h) ² (ATSDR 2001j) ³ (NHA 2001)
Potassium	Oral	na	¹ 750 kg/mg	² 8 – 38	¹ (ESPI 2001) ² (NHA 2001)
Rubidium	Oral	na	¹ 2625 mg/kg	² .01 – 5	¹ (Fluka 2000b) ² (NHA 2001)
Selenium	Oral	² .005 mg/kg/day	na	³ 0 – 8.08	¹ (Aldrich 2000e) ² (ATSDR 2001k) ³ (NHA 2001)
Sodium	Oral	¹ .002 mg/kg/day	² 3000 mg/kg	³ 11.2 – 219	¹ (EPA 2001c) ² (Mallinckrodt 1999) ³ (NHA 2001)
Sodium/ Potassium	na	na	na	na	na
Strontium	Oral	² .06 mg/kg/day	¹ 7500 mg/kg	³ .02 – 32.1	¹ (Hummel 2001) ² (EPA 2001d) ³ (NHA 2001)
Thallium	Oral	² .0008 mg/kg/day	¹ 30 mg/kg	³ .01 – 3.15	¹ (Radian 1991) ² (EPA 2001e) ³ (NHA 2001)
Titanium	Inh	² .0001 mg/m ³	³ 400 mg/m ³	⁴ .05 – 1.45	¹ (ATSDR 1997) ² (ATSDR 2001l) ³ (EPA 2001f) ⁴ (NHA 2001)
Uranium	Oral	² .002 mg/kg/day	na	³ .006 - .2	¹ (OSHA 2001b) ² (ATSDR 2001m) ³ (NHA 2001)
Vanadium	Oral	² .003 mg/kg/day	³ 10 mg/kg	⁴ 0 - .15	¹ (Fluka 2000c) ² (ATSDR 2001n) ³ (ESPI 1994) ⁴ (NHA 2001)
Zinc	Oral	² .3 mg/kg/day	na	³ 113 – 207	¹ (EPA 2001g) ² (ATSDR 2001o) ³ (NHA 2001)
Zinc/ Copper	na	na	na	¹ 5 – 11	¹ (NHA 2001)

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Table 1. Means and standard deviations of hair elemental analysis results from hair samples collected from California ground squirrels from spring 2001 through winter 2002 from three geographic locations in the San Joaquin Valley, California. T = Trace (< 0.01 of the given element).

Element	Concentration Average (PPM)	Std. Dev (±) (n=181)	Percent of Total
Aluminum (Al)	489.01	385.96	10.3%
Antimony (Sb)	0.24	0.42	0.0%
Arsenic (As)	0.15	0.13	0.0%
Barium (Ba)	9.52	6.91	0.2%
Beryllium (Be)	0.06	T	0.0%
Boron (B)	1.35	1.73	0.0%
Cadmium (Cd)	0.05	0.08	0.0%
Calcium (Ca)	1665.67	1020.27	35.1%
Cal/Mag (Ca/Mg)	3.20	0.66	0.1%
Cal/Phos (Ca/P)	3.34	1.95	0.1%
Cesium (Cs)	0.10	0.08	0.0%
Chromium (Cr)	0.45	0.57	0.0%
Cobalt (Co)	0.33	0.27	0.0%
Copper (Cu)	13.14	3.33	0.3%
Iron (Fe)	588.96	449.10	12.4%
Lead (Pb)	9.05	19.26	0.2%
Magnesium (Mg)	539.38	336.23	11.4%
Mag/Pot (Mg/K)	1.96	1.63	0.0%
Manganese (Mn)	22.49	18.52	0.5%
Mercury (Hg)	0.20	T	0.0%
Molybdenum (Mo)	0.17	0.14	0.0%
Nickel (Ni)	0.77	0.68	0.0%
Phosphorous (P)	511.31	98.21	10.8%
Potassium (K)	404.95	308.39	8.5%
Rubidium (Rb)	1.67	1.27	0.0%
Selenium (Se)	0.59	0.49	0.0%
Sodium (Na)	270.49	303.36	5.7%
Sod/Pot (Na/K)	0.57	0.39	0.0%
Strontium (Sr)	9.63	8.33	0.2%
Thallium (Tl)	0.01	T	0.0%
Titanium (Ti)	37.73	29.07	0.8%
Uranium (U)	0.14	0.17	0.0%
Vanadium (V)	1.54	1.26	0.0%
Zinc (Zn)	150.00	23.24	3.2%
Zinc/Cop (Zn/Cu)	11.95	2.89	0.3%

PPM = Parts Per Million = $\mu\text{g/g}$

Std. Dev = Standard Deviation

T = Trace (<0.01)

Table 2. Soil elemental analysis results (ppm) from soil samples from three geographic locations in the San Joaquin Valley of California, USA.

Element	Wet Weight Concentration (PPM)	Dry Weight Concentration (PPM)	Percent Difference Wet vs. Dry (%)
Aluminum (Al)	6000.0	6200.0	3.2%
Arsenic (As)	1.6	1.7	3.0%
Beryllium (Be)	0.2	0.2	3.7%
Cadmium (Cd)	ND	ND	ND
Chromium (Cr)	10.8	11.3	4.4%
Copper (Cu)	6.4	6.7	3.5%
Iron (Fe)	8333.3	8500.0	2.0%
Lead (Pb)	4.5	4.7	4.3%
Magnesium (Mg)	1269.8	1303.3	2.6%
Mercury (Hg)	ND	ND	ND
Selenium (Se)	ND	ND	ND
Zinc (Zn)	23.7	24.7	4.1%

ND = None

Detected

% moisture wet

(3.43%)

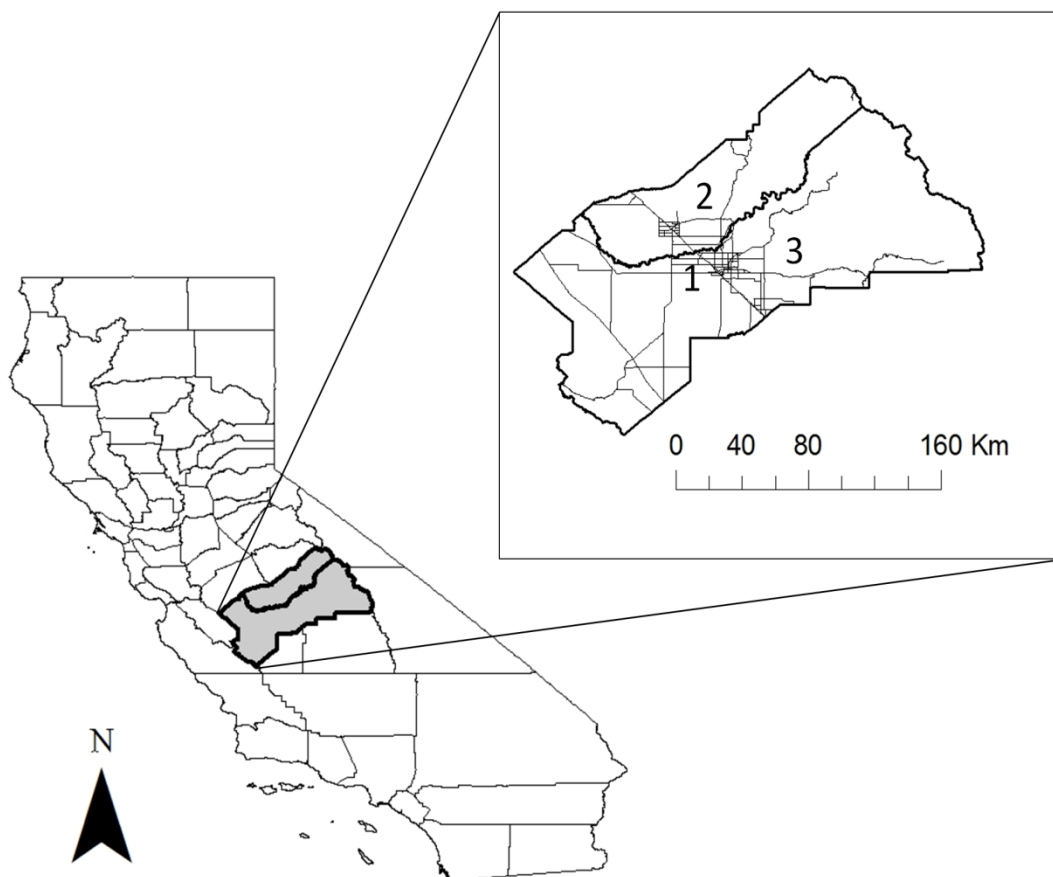
PPM = $\mu\text{g/g}$ **Figure 1.** Study areas used for the current project from spring 2001 through winter 2002. Highlights 1, 2, 3 are study sites, KMJ (permanent preserve), 5S Ranches (permanent pasture), and Almond Growers (permanent agriculture) respectively.



Figure 2. Study sites used for the current research from spring 2001 through winter 2002. Top) KMJ radio transmitter study site (permanent preserve), middle) 5S Ranches Inc. study site (permanent pasture), and bottom) Central California Almond Growers study site (permanent agriculture). Photos by author.

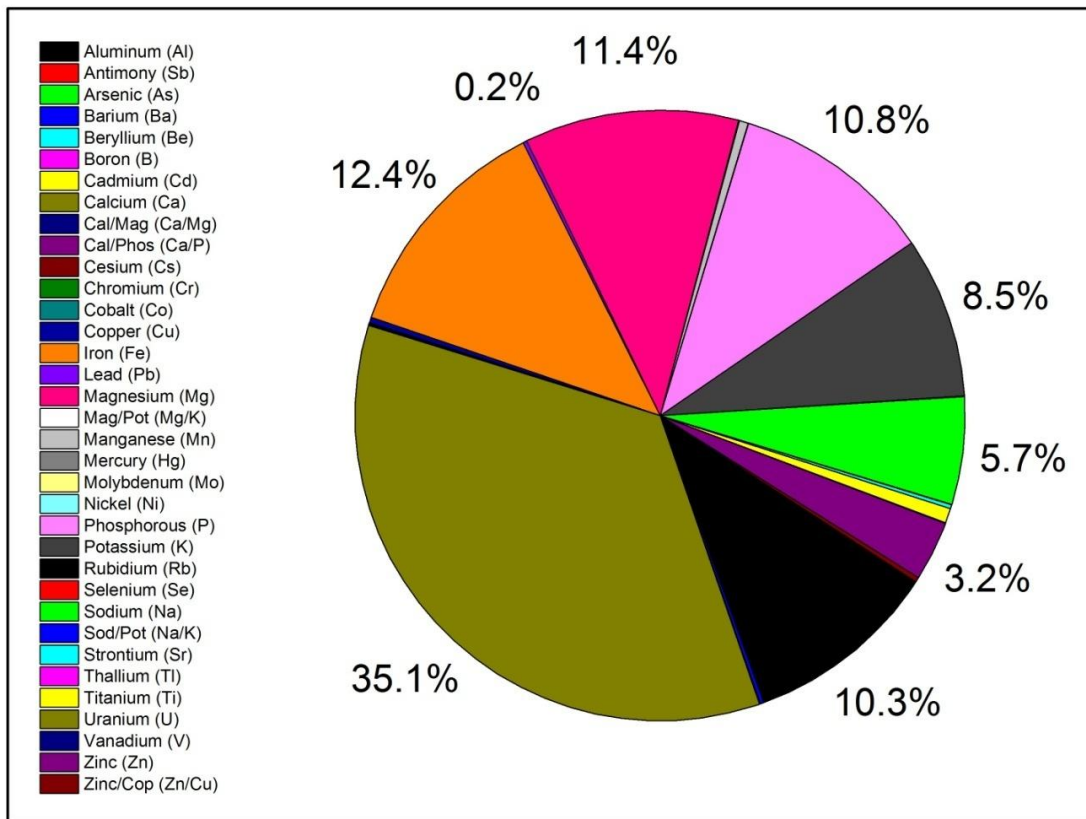


Figure 3. Illustration of hair elemental analysis results from hair samples collected from California ground squirrels from spring 2001 through winter 2002 from three geographic locations in the San Joaquin Valley, California. Chart shows percent of total of higher concentration elements (Table 1).

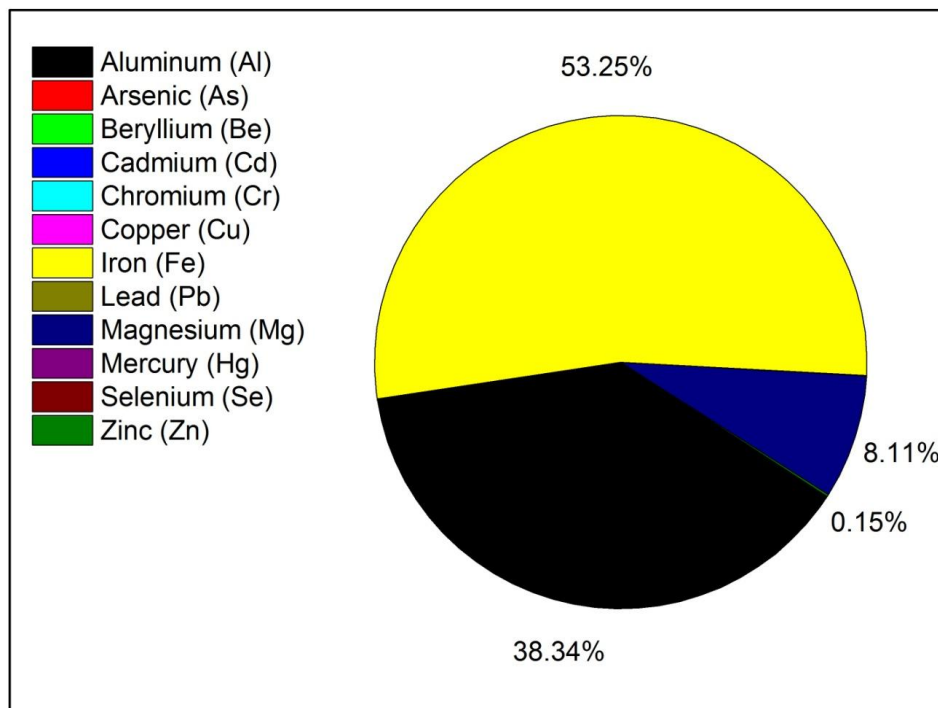


Figure 4. Illustration of soil elemental analysis results (ppm) from three geographic locations in the San Joaquin Valley of California, USA, where Iron, Aluminum, and Cadmium were greatest contributors to total soil concentration (for elements tested).