ANALYSIS OF TRACE METAL CONTAMINATION OF COAL STRIPMINES AND BIOACCUMULATION OF TRACE METALS BY *PEROMYSCUS LEUCOPUS*

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Chapter I

INTRODUCTION

Problem and objectives

Trace metals may contaminate terrestrial environments by several pathways. Sewage sludge, fertilizers, pesticides, industrial emissions, and mining operations are well known point sources of trace metal contamination. Coal mining is an important industry and possible point source for heavy metal contamination in eastern Oklahoma [1-4]. Removal of coal by underground mining and strip mining techniques may result in terrestrial and aquatic contamination through disposal of mine tailings, waste water run-off, and other activities associated with mining [5]. Strip mining methods disturb large areas of land and expose trace elements which are found within the coal as well as in various metal ores sometimes associated with coal deposits [6].

Present strip mining laws include Oklahoma's Open Cut Land Reclamation Act, Mining Lands Reclamation Act, and the federal Surface Mining Control and Reclamation Act that were enacted in 1968,1971, and 1977, respectively. These laws force mining companies to reclaim mined areas, which may reduce the bioavailability of various metal contaminants. Although reclamation seems to be a reasonable treatment for recently abandoned stripmines [7,8], Johnson et al. [9] stated that reclamation of old stripmines which have naturally revegetated will destroy valuable wildlife habitat. Unless wildlife species are susceptible to heavy metal contaminants in the soil, reclamation of these habitats may not be advantageous to wildlife species. Because many stripmines were abandoned prior to 1968, many old, orphaned stripmines will not be reclaimed. In 1977, 36,000 acres of abandoned coal stripmines in Oklahoma remained unreclaimed [1]. These sites and other unreclaimed mines across Oklahoma and the United States may remain point sources of heavy metal contamination for indigenous biota.

The objective of this thesis was to analyze animal tissues and soils from orphaned stripmines in eastern Oklahoma for the presence of select trace metals. Two essential and two non-essential trace metals were chosen for analysis due to their close relationship with coal deposits. These were copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd). Soil samples were analyzed to provide information on the present status of these metals in the various abandoned stripmines. Bone, kidney, and liver tissues were analyzed to determine if indigenous populations of *Peromyscus leucopus* (white-footed mice) are bioaccumulating trace metals from the stripmine sites.

It is hypothesized that concentrations of trace metals in soils from abandoned stripmines are significantly higher than from reference sites. Due to feeding and grooming habits of *P. leucopus*, individuals inhabiting abandoned coal stripmines are hypothesized to accumulate significantly higher levels of trace metals than individuals from reference sites.

Study sites

Selection of mine sites was based on an earlier study which documented elevated Zn concentrations in soils of stripmines throughout eastern Oklahoma [9, Oklahoma Biological Survey, unpublished data]. These sites have been abandoned for up to 70 years and were allowed to naturally revegetate. However, soil characteristics and vegetation types suggested the sites had not completely recovered from mining disturbance [10]. Sites selected in Craig Co. included two abandoned colliery stripmines and a matched reference site of similar habitat, identified as Wayland and Moss mines and Wayland reference. Okmulgee Co. sites also included two colliery stripmines, named Marler and Hamilton mines. A portion of the Eufala Wildlife Refuge, near Coal Creek, was selected as the reference site within Okmulgee Co. These reference sites were similar to their respective stripmine sites in geographic area and vegetation type so that variation due to factors other than trace metal concentrations were minimized between stripmine and reference sites. Because of the increase in Zn and other trace metals in the soil of this region [9], undisturbed soil from reference sites within this region also may contain elevated levels of trace metal contamination. Wildlife inhabiting undisturbed habitats on trace metal-enriched soils have been found to bioaccumulate trace metals [12]. Therefore, soils also were collected from a remote reference site near Lake Carl Blackwell within Payne Co. to determine baseline trace metal concentrations in soil and small mammals outside the coal belt of eastern Oklahoma.

Justification

The objectives of this project are to quantify the levels of Cd, Cu, Pb, and Zn in colliery spoil piles and determine if the metals present are bioaccumulating in indigenous populations of *P. leucopus.* Most studies involving abandoned colliery stripmines focus on decreased soil and water pH, increased sedimentation and erosion, and various forms of pollution other than heavy metals [4,6,13-16]. Most stripmine studies that quantify heavy metal contamination involve metalliferous stripmines, which undoubtedly would have greater concentrations of heavy metals [17-20]. Trace metal contamination due to colliery strip mining has received limited attention.

Data reported in this thesis will be combined with data from collaborative studies at Oklahoma State University and Texas Tech University. These collaborative studies will implement a suite of genetic and morphometric biomarker assays to determine if populations of small mammals show evidence of environmental stress while occupying abandoned coal stripmines. Animals analyzed for heavy metal contamination also will be analyzed for genetic and morphometric abnormalities. Correlations between soil and tissue metal concentrations may indicate that *P. leucopus* bioaccumulates trace metals through contact with colliery spoil. Also, correlations between metal content of tissues and the presence of genetic damage or morphometric abnormalities may provide information on the toxicological effects of heavy metals on *P. leucopus, in situ.*

The following two chapters are manuscripts prepared for submission to the journal Environmental Toxicology and Chemistry. References, tables, figures, and abbreviations are formatted to satisfy the manuscript requirements of this journal.

LITERATURE REVIEW

Presence of Trace Metals in the Environment

Cadmium, Cu, Pb, and Zn are fairly common in industrial effluents and many commercial products. These trace metals also constitute some of the raw materials used by industry. Copper and Zn are essential trace metals for plants and animals. For this reason, mineral-poor soils may receive fertilizers containing these metals. Due to the use of these metals by industry and agriculture, pollution of ecosystems by these trace metals is inevitable.

Cadmium is an element of global concern due to its widespread industrial use, ability to bioaccumulate, and toxic properties. Cadmium is a naturally rare element with background concentrations in United States soils ranging from 0.5 μg/kg to 2.4 mg/kg with mean concentrations of 0.31 mg/kg [21,22]. Cadmium contamination is widespread due to its role in the production of many consumer goods including corrosion-resistant metal, pigments, plastics, batteries, and electronics [21]. Approximately 50 percent of the total Cd produced eventually is released to the environment [23]. Cadmium normally does not occur as a pure element in nature. It has been observed in complexes with sulfur, chlorine, and various chelators such as metallothionein [24]. Most pure Cd is produced by removing Cd impurities from Zn, Zn-Pb, and Zn-Pb-Cu ores [21]. For this reason, mining and smelting of Zn and Pb ores are a point source of Cd contamination. Naturally high Cd levels can be found in areas with black shale deposits or volcanic activity [23]. Concentrations of Cd in contaminated soils have been documented in many studies [8, 17, 19, 20, 25-29]. Clevenger [20] found spoil piles from metalliferous mines closed in 1972 to contain 4 to 29 ppm Cd. Johnson et al. [26] found Cd concentrations of two Pb - Zn mine sites in England to range from 268 to 435 μg Cd/g .

Lead is also an important trace metal due to industrial uses and toxicity to plants and animals. Concentrations of Pb in uncontaminated soils vary widely. Point sources of Pb contamination include mining and smelting industries; industries which produce solder, batteries, paints; and the disposal of these products. Ma [30] reported automobile exhaust and spent Pb shot to be the first and second most important sources of Pb in the Netherlands, respectively. Prior to legislation which reduced the emission of Pb from automobiles in the U.S., Raymond and Forbes [31] also found aerial deposition of Pb from auto exhaust to be significant. Uncontaminated soils in Wales and England ranged from 15 to 106 μg Pb/g with a mean concentration of 42 μg Pb/g [32]. Heavily contaminated areas may have Pb concentrations as high as 42,400 μg/g [26].

As well as being an important industrial element, Zn is an essential trace element in biological systems. Thus, it is reasonable to assume that small concentrations of Zn occur naturally in soils supporting flora and fauna. Although this element is essential, excessive concentrations are toxic. Main point sources of Zn contamination include mining, application of sewage sludge/manure, and application of artificial fertilizers [8,33]. Elinder [34] reported Zn concentrations in unpolluted soils to range from 10 to 300 mg Zn/kg. Polluted soils may have Zn concentrations as high as 24,000 mg/kg [35].

Copper, like Zn, is an essential trace element for biological systems and is toxic at high concentrations. It is also an important raw material for many industrial processes and manufactured products. Baker [36] reported Cu pollution to result from mining and smelting, sewage sludge/ manure, and fertilizers. Background concentrations of Cu in the soil may range from 2 to 100 mg Cu/ kg.

Presence of Trace Metals in Coal Deposits

Most research on the presence of trace elements in stripmine spoil piles involves abandoned Pb and Zn stripmines and their respective smelters. This work has focused on problems such as acidification of soil and surrounding water, increased erosion and sedimentation, contamination and leaching of iron, and nutrient deficiencies [13-16]. Down [37], Kimber et al. [38], and Johnson et al. [9] analyzed colliery spoil for trace elements. Down [37] found Zn to persist in colliery spoil at low concentrations for longer than 100 years. Kimber [38] indicated that elevated levels of Cu were present in colliery spoil. Zn and Pb also were found at levels that may be phytotoxic. Johnson et al. [9] found elevated and, in some cases, phytotoxic levels of Zn in colliery spoil from eastern Oklahoma.

Trace metal contamination of colliery spoil piles may originate from metals found in the overburden or within remaining low grade coal. Overburden is composed of the soil horizons and parent rock above the coalbed. Trace metal concentration of the overburden will vary depending on the mineralogy of the soil. Due to the close proximity of Oklahoma coalbeds to the tri-state mining district, it is reasonable to assume Zn- and Pb-rich deposits are associated with Oklahoma coal. The tri-state mining district of Kansas, Missouri, and Oklahoma is characterized by extremely rich deposits of Zn and Pb ores which were mined until the 1960's [39]. One mine complex of the tri-state district, Picher mine field of Ottawa county, is a point source of Zn and Pb contamination as well as other pollutants [40]. This mine field is within approximately 20 miles of several stripmine sites sampled in this study. Because Cd are often found within Pb and Zn deposits [5,41,42], soils rich in Pb and Zn ores also may have elevated levels of Cd and Cu.

Trace metals may be released from the extraction and processing of coal as well as from remaining low grade coal exposed to weathering. According to Ausmus [4], inorganic portions of coal are partially composed of galena (Pb sulfide) and sphalerite (Zn sulfide). Thus, wastes created by the removal of overburden and coal extraction may increase the levels of Cd, Cu, Pb, and Zn in abandoned stripmine soils.

Movement of Trace Metals in Soils and Plants

Availability of environmental trace metals to wildlife species depends on the speciation and location of the metals in soils and plants. Soil properties that affect the presence and speciation of metals in soils include soil pH, organic matter, clay minerals, cation exchange capacity (CEC), and redox potential [43]. The presence of other metal ions also affect the activity of these metals. Highest concentrations of Cd, Cu, Pb, and Zn are found in the surface soil horizons because of vegetation cycling, adsorption by soil constituents, and aerial deposition. In stripmine spoils, vegetative cycling and adsorption of metals may be most important in allowing metals to remain in upper layers of soil.

Cadmium and Zn are chemically similar and have similar soil properties. Both are slightly mobile in soils, and when taken up by plants, are quickly translocated from the roots to other portions of the plant [5,22,33,44]. However, food portions of vegetation, seeds, fruits, and mast have been shown to contain lower concentrations of trace metals than other portions of the plant. Beyer et al. [35] found plants growing 2 km downwind from a Zn smelter to have accumulated only a small proportion of the metals present at the site in the foliage and even less in acorns and berries. Adriano [5] reported soil pH to be the most important soil characteristic regulating plant uptake of Cd and Zn. Depending on the soil environment, Cd and Zn may be found as a free ion, organic complex, oxide, or bound to other soil constituents [5,33]. Clevenger [20] found highest concentrations of Cd and Zn in the residual fraction of soil and stripmine spoil. Significant quantities of Zn were also found in oxide and carbonate forms. Residual fractions of trace metals in soil are not phytoavailable. Under some situations, oxides and carbonates may become available to plants.

Soil pH, CEC, and organic matter were reported to be the most important soil properties in Pb retention [32]. Copper is also fairly immobile in soils due to its strong affinity to organic matter, clay minerals, and iron and manganese oxides. Absorption of Pb and Cu by vegetation results in accumulation of Pb in the roots. Rarely will Pb translocate to higher portions of plants [5,45]. Because Cu is essential for normal plant growth, some Cu moves to other portions of the plant. Under contaminated conditions, most Cu absorbed by plants will accumulate in the roots [5]. In metalliferous stripmines, most Pb and Cu seem to be bound in the residual fraction. Copper is also found in the form of oxides, carbonates, and organic complexes [20].

Toxicological properties of Trace Metals

Trace metals, which may be essential or non-essential elements to biota, are normally found in small quantities in soil, flora, and fauna. The definition of an essential trace metal is one which an organism cannot live without or cannot be replaced by another element [43]. When concentrations of essential and non-essential trace metals exceed normal limits, biota may or may not experience toxic effects. Contamination levels that result in a toxic response vary according to the metal contaminant, presence of other metals or minerals, biochemical factors, and the organism exposed. Metallothioneins (MT), cysteine rich metal-binding proteins found in floral and faunal tissues, are important proteins involved with regulating metal distribution and toxicity through metal chelation [46-48]. Production of MT is enhanced by several types of stress, including heavy metal exposure, cold stress, and food deprivation [49-53]. Although much information on MT function exists, normal metabolic functions of MT still are not well understood

[48]. Following inactivation of MT-I and MT-II genes in embryonic stem cells, laboratory mice have exhibited normal reproduction and development [54]. This indicated that MT is not directly involved with these processes.

Zinc and Cu are environmental trace elements which are essential for plants and animals. Due to homeostatic mechanisms, Zn compounds are relatively nontoxic at low to moderate doses [17,55]. However, toxic responses due to excessive exposure to Zn compounds include depressed growth rate and appetite as well as arthritis, lameness, and gastrointestinal tract inflammation in pigs, other domestic species and laboratory animals [34,55]. In laboratory rats, ingestion of 0.5 - 1.0 % Zn in the diet resulted in various toxic effects depending on the compound [55]. On the metabolic level, Zn toxicity results in reduced iron, Cu, ferritin, cytochrome, and catalase activities in the liver, blood, and other tissues. Metallothioneins have been shown to reduce the toxic effect of excessive Zn concentrations [46]. Main target organs of Zn toxicity include bone, kidney, and liver [8,17,56]. Ogle et al. [56] reported the average Zn concentration in kidneys of *P. leucopus* was <25 μg/g. Talmage and Walton [57] reported Zn concentrations in kidney and liver tissues of reference *P. leucopus* averaged 140 and 160 μg/g (dry weight), respectively. No variation in Zn concentration of the kidney and liver was observed between sexes or among ages. Zn concentration in bone varied with sex but not age. Calcium, phosphorus, iron, and vitamin-D in the diet will effect Zn accumulation and function in tissues [17]. High Zn concentrations may reduce Cd toxicity and Cu absorption [17,34]. Johnson et al. [17] recognized the pancreas as a long-term storage unit and excretory organ for Zn.

As with Zn, homeostatic mechanisms cause Cu compounds to be relatively nontoxic at low to moderate doses [55]. Even so, extreme concentrations will result in a toxic response [34,58]. Toxic responses of inhaled Cu compounds in laboratory rats are reported to involve micronodular lesions and macrophage invasion of the lungs, specifically the alveolar space [58]. Injections of toxic concentrations of Cu compounds in lab rats resulted in reductions in hemoglobin content, red cell count, and hematocrit. Pigs receiving 700 mg Cu/kg body weight experienced iron-deficiency anemia, gastric ulcers, and hepatic centric lobular necrosis. Excess concentrations of Cu have caused mutagenesis by inducing infidelity during DNA synthesis [59]. Mink dosed with 200 μg Cu/g body weight experienced no toxic response [56]. Copper toxicity is ameliorated by excess levels of Zn, selenium (Se), molybdenum (Mo), and nickel (Ni) by either limiting adsorption or enhancing excretion of Cu [55]. Metallothionein has been found to regulate Cu concentrations in various organs and production of this protein also ameliorates Cu toxicity [52]. Copper concentrations in wild mink also varied with age, sex, and home range [56].

Most organisms can tolerate low concentrations of Cd, but it has no known function in normal metabolism. On the average, reference *P. leucopus* have Cd concentrations of 0.4 and 0.6 μg/g (dry weight) in liver and kidney respectively [57]. For human exposure, Mhatre [60] reported 0.1 mg Cd/L and <0.5 ppm Cd to be the maximum allowable concentrations in water and food respectively. Laboratory rats with 40 μg Cd/g in renal tissue experienced hypertension [42]. Chronic Cd toxicity results in anemia, renal dysfunction, testicular damage, cardiovascular toxicity, reduced appetite, growth rate, and bone mineralization [53,55,61,62]. *In vitro* and *in vivo* studies have shown Cd to interfere with nucleic acid base pairing [59,63]. The genome also may be altered through interference of spindle fiber formation, chromosomal, and chromatid aberrations resulting from Cd exposure. The distribution of Cd in tissues has been found to vary according to species, exposure, the presence of viral infection, and other variables. Alberici et al. [8] found highest concentrations of Cd in bone tissue of meadow voles (*Microtus pennsylvanicus*) exposed to mine land reclaimed with sewage sludge. In wild and laboratory populations of rats, Andrews et al. [19] found kidney and liver to be the primary and secondary target organs, respectively. *Peromyscus leucopus* living in forested areas irrigated with waste water preferentially accumulated Cd over Zn in the kidney [64]. Johnson et al. [17] reported 75% of ingested Cd to be accumulated by the liver and kidney in laboratory rats. In a field study, Johnson et al. [17] found kidney and liver tissue from small mammal species accumulated 13 to 70% of the total Cd body burden depending on species. Laboratory rats also accumulated Cd in mammary tissue [55]. When compared with controls, virus-infected mice accumulated highest levels of Cd in the spleen and kidneys [65]. Calcium, phosphorous, fiber, and protein content in diet may affect the regulation of Cd [19]. Accumulation and toxicity of Cd is effected by Zn, Cu, iron (Fe), selenium (Se) and Pb [66,67]. The presence of excess MT has been shown to reduce Cd toxicity [53,67].

Lead may be present in biota in small concentrations even though it has no normal metabolic function. Talmage and Walton [57] reported kidney and liver tissues of *P. leucopus* from reference sites contained 13 and 3 μg Pb/g (dry weight), respectively. Sanderson and Bellrose [68] reported 20.0 ppm (dry weight) in mallard (*Anas platyrhynchos*) liver indicated Pb toxicosis. Chronic sublethal exposure of animals to Pb resulted in anemia, neurological damage, renal dysfunction, reproductive and hematopoietic impairment [55,69,70]. Lead also affects the gastrointestinal, cardiovascular, hepatic, and endocrine organs and systems. Low Pb concentrations have been weakly correlated with elevated lesion counts, aberrant cell counts, and variation in mean nuclear DNA content [71]. Main target organs of Pb include bone, kidney, and liver. However, Pb levels in the soft tissues such as brain, kidney, and liver have greater toxicological importance [55]. Alberici et al. [8] and Johnson et al. [17] reported the primary storage organ to be bone; although, kidney tissue is most sensitive to Pb toxicity. Growing rats will retain Pb in their skeleton when diet concentrations are as low as 2 ppm [72]. Absorption and toxicity of Pb is reduced when mallards are fed diets high in calcium, phosphorous, and protein [17,68]. Aside from diet, sex, metabolism, territory, and behavior also affect Pb accumulation and toxicity [18,73].

Exposure of Peromyscus leucopus *to trace metals*

In terrestrial ecosystems, routes of exposure to environmental contaminants include passive diffusion through the skin and absorption through the gastrointestinal tract and alveoli. In the terrestrial environment, most trace metals, including Cd, Cu, Pb, and Zn, are not available to *P. leucopus* or other wildlife species through passive diffusion. Trace metal speciation in the environment, as determined through sequential extraction procedures, results in compounds that are either hydrophilic or strongly bound to soil particles [N. Basta, personal communication, 20]. Unless an active transport pathway can be utilized, hydrophilic compounds and macromolecules do not readily cross biological membranes, including the epidermis and dermis [74].

Many field studies have shown wildlife species, including *P. leucopus*, to be susceptible to inhalation of trace metals [75]. Several laboratory studies also have demonstrated the effect of inhaled trace metals on the reproductive and pulmonary systems in small mammal and human subjects [76-78]. However, trace metal emissions from Zn smelters and other sources were estimated to have a finite range and inhalation exposure occurred only within this range [79]. Trace metal contamination at the stripmine sites in this study resulted from physical disturbance of the top soil and parent material of the area. This disturbance probably resulted in little past or present exposure to trace metals via inhalation.

In the case of *P. leucopus* inhabiting the present study sites, the most critical exposure pathway may be ingestion of contaminated soils. Baker [80] reported the diet of *Peromyscus* consisted of grains, seeds, fruits, and insects. Collins [44] and Koeppe [45] reported that grains, seeds, and fruits are less likely to contain excessive quantities of trace metals than other portions of the plant. Also, the ability of soil to bind heavy metals may reduce the uptake of metals by plants [81]. Clevenger [20] found significant portions of Cd, Cu, Pb, and Zn in soil and spoil to be unavailable to plants. Beyer et al. [82] found slightly elevated levels of metals in *Mus musculus* although vegetation did not contain significantly elevated metal concentrations. If plants have not accumulated excessive concentrations of trace metals, elevated trace metals in herbivorous animals must have been absorbed directly from the soil through ingestion.

Peromyscus leucopus occupying stripmine sites may be exposed to heavy metals by ingestion of soils, through burrowing and nest building activities, as well as grooming [19,83,84]. Garten [83] found 12-14 g of insoluble ash, partially composed of ingested soil, in the gastrointestinal tract of *P. leucopus*. Beyer et al. [84] found the diet of *P. leucopus* to contain an average of less than two percent ingested soil. However, soil may account for as much as 16% of the diet of *P. leucopus* [W.N. Beyer, personal communication].

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Chapter II

ANALYSIS OF TRACE METAL CONTAMINANTS IN SOILS FROM COAL STRIPMINES AND REFERENCE SITES IN OKLAHOMA

Abstract - Although coal stripmines probably have less trace metal contaminants than metalliferous stripmines, low to moderate levels of some trace metal contaminants present at coal stripmines may cause adverse ecological effects. To quantify levels of trace metal contamination due to coal stripmining, soils from a series of abandoned coal stripmines and unmined sites in Oklahoma were analyzed for cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). Background levels of Cd, Cu, Pb, and Zn within Oklahoma's coal belt were elevated compared to other regions of Oklahoma. Coal stripmining significantly increased soil concentrations of Cu, Pb, and Zn at two of three stripmines surveyed. Although, levels of Cd, Cu, and Pb at these sites did not exceed concentrations which are toxic to earthworms and plant seeds in toxicity assays, low soil pH and relatively high concentrations of Zn may have increased significantly the bioavailability of these trace metals.

Keywords - trace metals, pH, soil, coal strip-mining, Oklahoma

INTRODUCTION

One source of heavy metal pollution is metalliferous and coal mining and processing [1-5]. In most cases, mining results in acidification of surrounding soil and water, increased erosion and sedimentation, nutrient deficiencies for terrestrial and aquatic plants, and metal contamination [6-10]. Coal stripmining also may expose trace elements found within coal deposits or overburden [11-14]. Trace metal concentrations of the overburden, which includes the soil horizons and parent material above coalbeds, will vary depending on the mineralogy of the soil. Stripmines within the Oklahoma coal belt are located near the tri-state mining district of Kansas, Missouri, and Oklahoma. This region is characterized by rich deposits of zinc (Zn) and lead (Pb) ores which were mined until ~1960 [17]. Because cadmium (Cd) and copper (Cu) are often found in association with Pb and Zn deposits [6, 15, 16], elevated concentrations of these metals also may be present in this region. Trace metal contamination may result from erosion of remaining low grade coal. According to Ausmus [4], inorganic portions of coal are partially composed of galena (lead sulfide) and sphalerite (zinc sulfide). Therefore, it is reasonable to assume that soils of stripmines within Oklahoma's coal belt have elevated levels of Cd, Cu, Pb, and Zn compared to areas outside the coal belt.

Many coal stripmines of Oklahoma's coal belt are not reclaimed [1] because present strip-mining laws, Oklahoma's Open Cut Land Reclamation Act, Mining Lands Reclamation Act, and the federal Surface Mining Control and Reclamation Act, were not enacted until 1968, 1971, and 1977, respectively. Stripmines surveyed in this study are not reclaimed and are hypothesized to expose plants and wildlife to excess levels of trace metals.

To determine if coal stripmine soils in Oklahoma contain elevated concentrations of essential and non-essential heavy metals, soil samples were collected from stripmine and reference sites in Oklahoma and analyzed for Cd, Cu, Pb, and Zn. Trace metal concentrations in coal stripmine spoil were hypothesized to be significantly elevated over reference site soils.

METHODS

Study sites

Abandoned coal stripmines sampled in this project were located in Craig and Okmulgee counties, OK. The stripmine sites have been abandoned for up to 70 years and were allowed to naturally revegetate. All mines were reported to have low soil pH due to the presence of sulfur ores [13]. The Craig Co. mine site was identified as Wayland mine. Sites in Henryetta Co. were identified as Marler mine and Hamilton mine. Exact locations of Moss and Wayland mines are sections 7 and 8, T28N, R20E and section 15, T29N, R20E, Craig Co., respectively. Marler and Hamilton mines are located in sections 8 and 17, T11N, R13E, Okmulgee Co.

Wayland mine has been abandoned for ~60 years and has naturally revegetated. However, habitat disturbance caused by stripmining was still prevalent due to the presence of steep spoil piles and a large pond in the last cut of the mine. The shale-like nature of the surface soils at this stripmine indicated that true soil horizons had not yet developed. Although partial revegetation has occurred, exposed surface soils, more appropriately termed mine spoil, were still prevalent. Large trees with a diameter at breast height (DBH) ranging from 35 to 50 cm were present on the site; however, dominate vegetative types were shrubs and trees of seedling to sapling size. Shrubs and small trees that dominated the area include dogwood (*Cornus* sp.), willow (*Salix* sp.), sumac (*Rhus* sp.), and elm (*Ulmus* sp.). Representative large tree species included cottonwood (*Populus deltoides*) and oak (*Quercus* sp.). Poison ivy (*Toxicodendron radicans*) also was prevalent at this and other mine sites. This suggested that stripmine sites have not completely recovered from mining disturbance because poison ivy commonly colonizes disturbed or formerly disturbed habitats [18]. Heaviest vegetation occurred on the tops of ridges and in bottoms of valleys, and was sparse on steep slopes. For these reasons, Wayland mine and the other mine sites were considered to be disturbed habitats.

Marler and Hamilton mines differed considerably in age. Mining on Marler mine ceased in 1917 and on Hamilton mine in 1930. This difference in age was reflected in the steepness of the spoil piles. Spoil piles were visible at Marler mine, but their size was reduced due to the slumping of loose spoil. Hamilton mine spoil piles were very steep. Vegetative types were similar between Craig Co. and Okmulgee Co. stripmine sites. Mine ponds were still present at Marler and Hamilton mines.

Reference sites surveyed in this study included sites in Craig, Okmulgee, and Payne counties. The Craig Co. reference site, Wayland reference, included three small woodlots within a kilometer of Wayland mine. Vegetation was similar to the oak-hickory savannah described by Bruner [19]. The Okmulgee Co. reference site was located on the Eufala Wildlife Management Area, Deep Fork Section and served as the intracounty reference site for Marler and Hamilton stripmines. Eufala reference site vegetation also resembled the oak-hickory savannah including a field dominated by *Andropogon* sp. The Payne Co. reference site was located in the Lake Carl Blackwell area. Historically dominated by the *Stipa-Koeleria* association [19], the area was characterized by a mix of grasslands dominated by the genera *Stipa*, *Koeleria*, and *Andropogon,* and oak-hickory woodlands.

Smith and Rongstad [20] indicated that undisturbed soils which cover mineral deposits may contain low levels of the minerals found in deeper deposits. Therefore, soils were collected from a remote reference site near Lake Carl Blackwell, Payne Co. to determine if trace metal concentrations in soil of the coal belt in eastern Oklahoma was naturally higher than other regions of Oklahoma. Soil from the Lake Carl Blackwell area were assumed to have little heavy metal contamination due to the lack of mining and other industrial activities within a 6 km radius of the site.

Selection of mine sites initially was based on an earlier study which documented Zn concentrations in soils of stripmines throughout eastern Oklahoma [Oklahoma Biological Survey, unpublished data, 13]. These studies found stripmines from Okmulgee and Craig counties to contain 130 to 163 ppm and 40 to 46 ppm Zn, respectively, within the top 20 cm of soil. Accessibility and presence of indigenous small mammal populations also were factors in stripmine and reference site selection.

Collection and analysis of soils

Soil samples were collected randomly from stripmine and reference sites along transects. Along each transect, sample points were selected as a random distance from the edge of the site. Each soil sample consisted of ~1 kg of the top 20 cm of soil, excluding litter. Samples were collected by metal shovel and stored in clean, water-tight, polyetheylene bags. Sampling depth was selected due to the tendency of trace metals to remain in the topsoil [21] and the probability of direct contact with this portion of the soil by small mammals [22, 23]. Within one week of collection, soils were air dried for 48 h and stored in polyetheylene bags. Before digestion and metal analysis, soils were homogenized with ceramic mortar and pestle and passed through a 2 mm sieve. All materials collected by the sieve were discarded as non-soil. Soil pH was measured in $CaCl_2$ by the standard procedure reported by McRae [24]. For each sample, two 10 g aliquotes of soil were placed in separate beakers and combined with 20 ml of 0.01 M $CaCl_2$. Soil suspensions were stirred for 15 minutes and allowed to settle for 15 minutes or until the supernatant cleared. pH of the clear supernatant was determined with a pH meter.

Stripmine and reference site soils were digested by EPA Method 3050 [25]. From the homogenized soil sample, ~2 g of soil were placed in a 250 ml beaker and digested with nitric acid by refluxing for 45 to 60 minutes. The resulting suspension was evaporated to ~5 ml and combined with Type II reagent-grade water and 30% hydrogen peroxide. After the solution ceased effervescing, additional hydrogen peroxide was added in 1 ml increments until the solution was unchanged. Samples were cooled and centrifuged at 10,000 rpm to clear the supernatant which was diluted to 50 ml and transferred to polyethylene or glass storage bottles. Digested samples were analyzed for total adsorbed Cd with a graphite furnace Perkin-Elmer Atomic Absorption Spectrophotometer, Model 5000 (GF-AAS). Samples were analyzed for total adsorbed Cu, Pb, and Zn by flame atomization with the same instrument (flame-AAS).

To reduce field and laboratory contamination of soil and tissue samples, trace metal analysis was conducted according to the Quality Assurance/Quality Control Plan of the Water Quality Research Laboratory at Oklahoma State University. This plan was developed according to EPA's quality control protocol [26] and included the analysis of reagent and soil blanks to determine if processing and digestion procedures resulted in contamination. Reagent blanks consisted of digestion reagents and Type II reagent-grade water. Soil blanks contained certified inorganics blank soil exposed to the same sampling and processing techniques applied to stripmine and reference samples. Percent recovery of Cd, Cu, Pb, and Zn was determined through analysis of reagent and soil spikes. Periodically during sample analysis, EPA Certification Standards were analyzed to ensure proper calibration of the instrument. Absorbance values of reagent blanks from a given group were subtracted from all samples in that group to account for contamination of samples via digestion reagents.

Statistical analysis

Bartlett's test for homogeneity of variance and the skewness and kurtosis of data distributions indicated that trace metal concentrations were not normally distributed. Because these data were not consistent with the assumptions of parametric statistical tests, data were rank transformed before analysis by ANOVA and LSD multiple comparisons. This technique has been shown to be the equivalent of analyzing data by non-parametric statistical tests [27]. If sites were found to differ significantly ($p \leq 0.05$) in soil trace metal concentration or pH with ANOVA, each stripmine site was compared with intracounty and remote reference sites with pairwise LSD tests. Although comparisons among sites were calculated with transformed data, untransformed data were reported for comparison among sites.

RESULTS

Analysis of quality assurance-quality control data

Collection and processing of soil samples did not cause Cd, Cu, Pb, or Zn contamination of soil blanks. Average concentrations of soil blanks were 0.12 mg Cd/kg, 7.98 mg Cu/kg, 7.44 mg Pb/kg, and 3.18 mg Zn/kg. Certified values for the inorganics blank soil were < 1.0 mg Cd/kg, < 20.0 mg Cu/kg, <30.0 mg Pb/kg, and < 50.0 mg Zn/kg. Average percent recoveries of Cd, Cu, Pb, and Zn from reagent spikes were 95, 98, 93, and 86 percent, respectively. Average percent recoveries of Cd, Cu, Pb, and Zn from soil spikes were 90, 107, 96, and 93 percent, respectively. Data were not corrected for the observed percent recoveries.

Trace metal analysis of soils

Trace metal concentrations and soil pH differed significantly among stripmine and reference sites (Table 1). Soils from Hamilton and Marler mines were found to have the lowest pH values and had significantly greater concentrations of Cd and Zn than Blackwell reference site soils. However, soil Cd concentrations at Eufala and Wayland reference sites were not significantly different from these mine sites. Soil Zn concentrations from Eufala and Wayland reference sites were significantly lower than Hamilton and Marler stripmines but were significantly elevated when compared with Blackwell reference site soil. Soil Cu and Pb concentrations at Hamilton, Marler, or Wayland mine sites were not significantly greater than levels of these trace metals in Wayland reference site soils (Table 1). However, Eufala and Blackwell reference site soils contained significantly lower levels of Cu and Pb than all other sites.

DISCUSSION

Mean concentrations of Cd, Cu, Pb, and Zn in soils of stripmine and coal belt reference sites were higher than average concentrations of these metals at Blackwell reference site and throughout Oklahoma soils [28, 29]. These data suggested that trace metal concentrations of undisturbed soils within the coal belt of eastern Oklahoma were naturally higher than concentrations of these metals in soils outside of this region. This agreed with research comparing trace metal content of soils from different regions of Oklahoma [29]. Thus, Blackwell reference site was not an appropriate reference to determine if stripmining activities resulted in trace metal contamination of surface soils. Also for this reason, it was more appropriate to compare trace metal concentrations of stripmine and coal belt reference sites with regional, rather than state-wide, background trace metal concentrations.

Copper and Pb concentrations at stripmine and coal belt reference sites were similar to average background concentrations reported for eastern Oklahoma [29]. This suggested that variation in Cu and Pb concentrations observed among stripmine and reference sites may occur naturally rather than result from coal stripmining. Also, Cu and Pb concentrations in stripmine soils were less than EC_{50} and LC_{50} values for standard lettuce and radish seedling emergence and earthworm survival toxicity assays [30, 31]. Cadmium and Zn levels at stripmine and coal-belt reference sites were greater than the average background concentrations reported for eastern Oklahoma and also exceeded the maximum concentrations of these metals in undisturbed Oklahoma soils [29]. Scott [29] reported maximum Cd and Zn concentrations in the upper soil horizons to be <1 ug/g and 136.5 ug/g, respectively. Although Cd values exceeded background concentrations, observed levels of Cd in stripmine and coal-belt reference sites were lower than the NOEC, EC_{50}, or LC_{50} levels of Cd in soil toxicity tests with plant or invertebrate test organisms [30 - 33]. Only Zn concentrations in soils from Oklmulgee stripmines were above background concentrations for this region and significantly higher than Zn levels in soils from the intracounty reference site. This suggested that elevated Zn levels observed at Marler and Hamilton stripmine sites were the result of coal stripmining activities. Also, levels of Zn in stripmine soils were greater than EC_{50}

values reported for lettuce and radish seedling emergence and root elongation toxicity assays; but were less than LC_{50} concentrations reported for earthworm survival assays [30]. Within Craig Co., concentrations of Cd, Cu, Pb, and Zn were not found to increase significantly with stripmine disturbance.

Relatively low soil pH at Marler and Hamilton stripmines may have increased the bioavailability of Cd and Zn at these sites. These metals were reported to be relatively mobile in soils due to their association with the exchangeable soil fraction [34, 35]. Acidic soil pH has been shown to increase the mobility of Cd and Zn within soils and between soils and plants [36, 37]. Relative toxicity of trace metals has been shown to increase as their mobility increases. Harrison [38] reported that pH-dependent metal speciation greatly affected mobility and gastro-intestinal absorption of ingested trace metals. Due to the low concentrations and relative mobility of Cu and Pb in stripmine soils, bioavailability of these metals was expected to be relatively low. These metals were reported to be relatively immobile in soils due to their association with organic matter [21, 35, 38, 39, 40] and residual fractions of the soil profile [41]. Davis et al. [42] found Pb from contaminated spoil to be relatively unavailable when ingested due to the oxidation of galena, which forms a coating of anglesite and K-jarosite precipitates.

Trace metal concentrations of coal stripmines varied among geographical regions. In Craig Co., coal-stripmining did not significantly increase the level of trace metals in the surface soils; but, a significant increase in Zn concentration was observed at Okmulgee stripmine sites as compared with Eufala reference site. As compared to sites impacted by metalliferous mining and smelting operations [43-45], trace metal concentrations at stripmine sites was relatively low. This suggested the risk of bioaccumulating trace metals to be relatively low for plants and animals occupying abandoned coal stripmines. However, Cd and Zn may have been more bioavailable due to low soil pH, thus increasing the risk of trace metal bioaccumulation by plants and animals.

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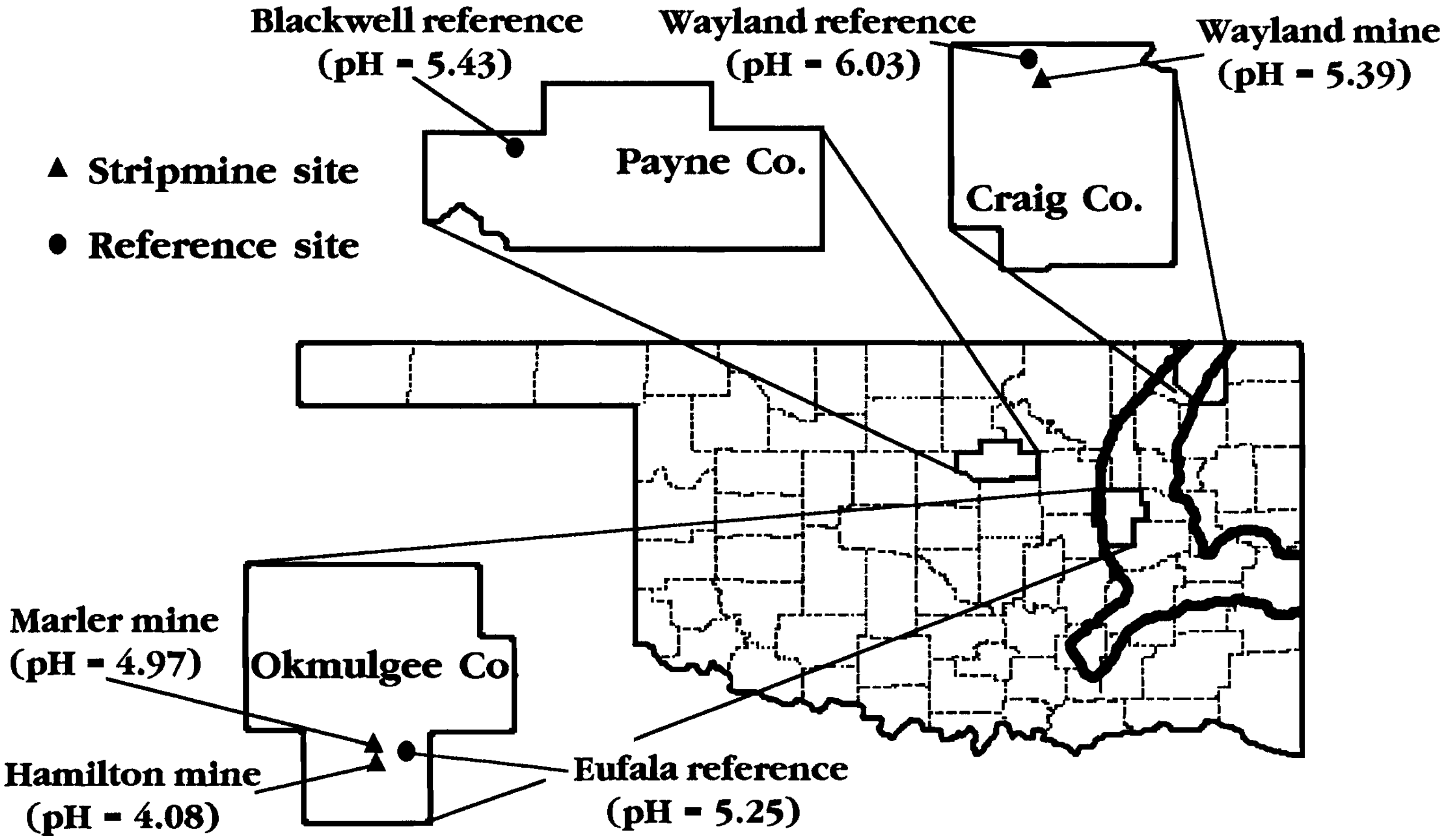
Table 1. Mean trace metal concentrations in ug/g soil, dry weight, and pH of colliery stripmine and reference soils from Oklahoma. Values in parentheses are standard error.

	Soil parameters *					
Site (*n*)	pH (CaCl2)	Cd	Cu	Pb	Zn	% Cd:Zn
Okmulgee Co.						
Marler mine (30)	4.97(0.10)a	1.99(0.37)a	16.86(0.91)a	32.80(3.31)a	544.36(64.38)a	0.29(0.03)ab
Hamilton mine (30)	4.08(0.10)b	1.23(0.24)b	22.09(0.97)b	27.06(2.62)a	373.98(35.91)a	0.26(0.04)bc
Eufala ref. (30)	5.25(0.06)c	1.25(0.32)ab	10.43(1.24)c	27.12(12.24)bc	231.18(40.23)b	0.48(0.11)e
Craig Co.						
Wayland mine (25)	5.39(0.11)c	0.32(0.05)c	22.40(1.39)b	18.92(3.81)b	253.60(28.89)b	0.13(0.01)d
Wayland ref. (29)	6.03(0.09)d	0.88(0.12)ab	20.34(1.02)b	24.65(1.11)a	256.98(23.88)b	0.31(0.02)ae
Payne Co.						
Blackwell ref. (30)	5.43(0.09)c	0.10(0.01)d	8.79(1.94)d	10.27(1.08)c	56.51(3.22)c	0.18(0.02)cd

* means in the same column with the same letter do not differ significantly ($p > 0.05$) as determined by LSD multiple comparisons

Fig. 1. Locations of the coal belt (outlined area) and study sites within Oklahoma, including mean soil pH of each study site.


Blackwell reference
(pH = 5.43)
Wayland reference
(pH = 6.03)
Wayland mine
(pH = 5.39)
▲ Stripmine site
● Reference site
Payne Co.
Craig Co.
Marler mine
(pH = 4.97)
Okmulgee Co
Hamilton mine
(pH = 4.08)
Eufala reference
(pH = 5.25)

Chapter III

TRACE METAL RESIDUES IN *PEROMYSCUS LEUCOPUS* INHABITING ABANDONED COAL STRIPMINES

Abstract - Some coal stripmine soils have been typified by elevated levels of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). The objective of this study was to determine if *Peromyscus leucopus* indigenous to abandoned coal stripmines bioaccumulated these trace metals. Although hepatic Pb concentrations were correlated positively with age, mice from stripmine sites did not bioaccumulate large amounts of Pb in renal or hepatic tissue. However, small amounts of Pb bioaccumulated in the bone of stripmine mice. Renal and hepatic Cd concentrations and Cd:Zn ratios of mice from some stripmines were elevated compared with reference mice and correlated significantly with age and relative kidney weight. Elevated Cu and Zn concentrations in liver and kidney tissues of stripmine mice were observed in some trapping periods. However, levels of these metals did not exceed normal ranges for small mammals. Skeletal concentrations of Cd, Cu, and Zn were detectable, but not consistently associated with stripmine disturbance. Soil pH and Cd, Pb, and Zn concentrations in the soil were correlated significantly with elevated renal and hepatic Cd concentrations. This indicated direct exposure to soil was possibly an important exposure pathway.

Keywords - Trace metals, Tissue residues, *Peromyscus leucopus*, Coal stripmines

INTRODUCTION

Coal mining is an important industry and possible point source of trace metal contamination [1-4]. Strip-mining techniques disturb large areas of land and expose trace elements through disposal of mine tailings, waste water runoff, and other mining activities [5, 6]. Trace metals may originate from within coal and various metal ores sometimes associated with coal deposits [6]. Abandoned colliery stripmines of eastern Oklahoma may contain elevated concentrations of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) due to their close proximity to the tri-state mining district of Kansas, Missouri, and Oklahoma which contains rich deposits of Zn and Pb ores [4, 5, 7]. Because laws regulating the mining and reclamation of stripmines were promulgated after 1968 [8], reclamation is not required for stripmines abandoned prior to 1968. After 1977, 36,000 acres of abandoned coal stripmines in Oklahoma, including the sites investigated in this study, remain unreclaimed [1]. Although many of these sites are naturally revegetating, they may continue to be possible point sources of trace metal contamination.

Trace metal contamination of abandoned stripmines, habitat alteration due to strip-mining, and subsequent revegetation or reclamation may result in trace metal bioaccumulation by small mammals [9-11], changes in small mammal distribution [12, 13], and population diversity [14, 15]. Exposure of small mammals to excess trace metals may result in bioaccumulation and toxicity [9-11, 16, 17] as well as transference of metals to higher trophic levels [9]. However, the effects of chronic exposure to low levels of trace metals in small mammals have not been thoroughly investigated.

Exposure of small mammals to environmental contaminants in terrestrial ecosystems may occur by passive diffusion through the skin and absorption through the gastrointestinal tract and lung alveoli. Diffusion of Cd, Cu, Pb, and Zn through mammalian skin is unlikely because trace metal compounds in the environment are either hydrophilic or strongly bound to soil particles [N. Basta, personal communication, 18, 19]. Although small mammals are susceptible to inhalation of trace metals [20], this pathway probably is not a significant route of trace metal exposure for these animals at abandoned stripmines because most metal contamination at these sites probably was not in aerosol form.

Peromyscus leucopus and other small mammals inhabiting abandoned stripmines may have been exposed to trace metals through ingestion of contaminated soils while feeding, burrowing, nest building, and grooming [11, 21, 22]. Research has indicated that the probability of trace metal assimilation from ingested vegetation is low. Baker [23] and Lackey et al. [24] reported that *Peromyscus* are opportunistic consumers with main food items including grains, seeds, and fruits which are less likely to contain excessive quantities of trace metals than other portions of the plant [25, 26]. Also, plant uptake of trace metals is reduced by the ability of soil to bind these elements [18, 27]. Beyer et al. [28] found slightly elevated levels of metals in *Mus musculus* although vegetation did not contain significantly elevated metal concentrations. Carnivorous small mammals have accumulated high levels of trace metals through consumption of soil invertebrates [16, 17, 29, 30]; however, invertebrates such as insects were not reported to be a large dietary component of the diet of *P. leucopus* [23, 24].

Animals chronically exposed to Cd, Cu, Pb, and Zn experience a variety of systemic and localized effects which directly or indirectly affect body or organ size. Because of their roles in normal metabolism, Cu and Zn are relatively non-toxic trace metals [31]. However, excessive uptake of Zn may result in depressed growth rate, reduced appetite, and gastrointestinal tract inflammation [31, 32]. In large concentrations, Cu also may result in gastric ulcers and hepatic necrosis [33]. Renal dysfunction, reduced growth rate, and reproductive impairment result from chronic Pb exposure [31,34]. Chronic Cd exposure results in renal dysfunction, testicular atrophy, and reduced growth rate [31,35, 36]. Due to physical and chemical similarities, Cd and Zn compete for binding sites on proteins and influence the severity of Cd induced renal damage [37]. Cadmium induced renal damage is proportional to the ratio of Cd to Zn concentrations. Thus, chronic exposure to trace metals may cause changes in body and organ weight or dimension.

To determine if small mammals inhabiting orphaned stripmines were bioaccumulating trace metals, kidney, liver, and bone tissues were collected from *Peromyscus leucopus* (white-footed mice) inhabiting stripmine and reference sites in eastern Oklahoma and analyzed for Cd, Cu, Pb, and Zn. Percent ratios of Cd:Zn in kidney, liver, and bone tissue were also determined and compared due to the competitive interaction of these elements in biological tissues. *Peromyscus leucopus* collected from abandoned colliery stripmines were hypothesized to accumulate significantly higher levels of trace metals than mice from reference sites. Trace metal concentrations in tissues were correlated with biological factors including age, total body weight, relative tissue weights, and testicular volume to determine if variation in selected biological traits corresponded with tissue levels of trace metals.

To investigate the movement of trace metals from soil to exposed mice, trace metal concentrations in the soil were correlated with these concentrations in tissues from mice. Soil analyses of these stripmine and reference sites were reported in a seperate manuscript (Hausbeck and McBee, *in litt*). Elevated trace metal concentrations in the soil were hypothesized to be correlated with elevated trace metal concentrations in the tissues.

METHODS

Study sites

Sites studied in Craig Co. included two abandoned colliery stripmines and an intracounty reference site, identified as Wayland and Moss mines and Wayland reference (Fig. 1). Okmulgee Co. sites also included two colliery stripmines and were referred to as Marler and Hamilton mines. A portion of the Eufala Wildlife Refuge, near Coal Creek, was selected as the reference site within Okmulgee Co.. Because of the assumed increase in metal content of the parent material in this region, mice inhabiting reference sites within this region also may be exposed to trace metal contaminants [38]. Within Payne Co., a remote reference site was selected near Lake Carl Blackwell. Mice collected from this site provided data on a population of *P. leucopus* living outside the coal belt of eastern Oklahoma.

Mines selected for this study have been abandoned for ~ 65 years and have been documented to contain elevated Zn concentrations in soils (Oklahoma Biological Survey, unpublished data) [8]. Stripmines from Okmulgee Co. were found to contain 130 to 163 ppm Zn and Craig Co. sites had 40 to 46 ppm Zn within the first 20 cm of topsoil. Undisturbed areas with similar vegetative types to that of the stripmines were selected as intracounty and remote reference sites. Accessibility and presence of indigenous small mammal populations were also factors in stripmine and reference site selection.

Tissue collection and analysis

Peromyscus leucopus were collected from stripmine and reference sites with aluminum Sherman live-traps during five trapping periods: spring (March), summer (July), and fall (October) 1992; and spring (March) and summer (May) 1993. Mice were not collected from Moss mine during the summer 1993 trapping period because the land tenants denied access to the property after March 1993. Animals were sacrificed by carbon dioxide asphyxiation and measured for total body, tail, hind-foot, and ear lengths as well as total body weight. Relative age of individuals was recorded as an index with five age groups. Lopez-Gonzales [39] collected these data according to molar tooth wear by Hoffmeister's [40] method. Liver and kidney tissues were excised from each animal and stored frozen at -70°C prior to further analysis. Collection of bone samples was limited to the recovery of femurs after bone marrow was flushed with a 0.075 M KCl solution for related genetic studies. Femurs were stored in plastic tubes on ice prior to further analysis.

Prior to digestion, frozen tissues were weighed to the nearest 0.01 g. Because all available kidney tissue was analyzed for trace metals, these tissues were not homogenized prior to digestion. Liver tissue collected during spring and summer 1992 trapping periods was homogenized prior to removing a 0.05 to 0.20 g subsample for related genetic studies. Tissues were homogenized with mortar and pestle while frozen. Because liver weights were recorded after homogenization, total liver weights of these samples were unknown. In order to decrease the possibility of tissue contamination and to increase the amount of tissue available for analysis, liver tissue from the remaining seasons were not homogenized prior to removing a subsample.

Liver and kidney tissues were digested by a method adapted from E.P.A.

Method 3050 [41]. Wet liver and kidney tissues were digested for an additional 12 h in nitric acid for increased oxidation of organic compounds. After samples were clear, they were diluted to 100 ml with Type II reagent-grade water [42]. Due to the small size of femurs, samples from two to three individual mice were composited for each season-site combination to obtain a sample size of ~0.1 g. Femur samples were dissolved in 2 to 3 ml concentrated nitric acid heated at 50°C in a water bath for 24 h. After this period, 0.3 to 0.6 ml of 30% Type II reagent-grade water and hydrogen peroxide were added and each sample was returned to the water bath for an additional 24 to 48 h. When samples were clear, they were diluted to 25 ml with Type II reagent-grade water. All digested samples were analyzed for Cd, Cu, and Pb with a graphite furnace Perkin-Elmer Atomic Absorption Spectrophotometer, Model 5000 (GF-AAS). Samples were analyzed for Zn by flame atomization with the above instrument (flame-AAS).

Quality control protocol

To reduce field and laboratory contamination of soil and tissue samples, trace metal analysis was conducted according to the Quality Assurance/Quality Control Plan of the Water Quality Research Laboratory at Oklahoma State University. This plan was developed according to EPA's quality control protocol [43] and included the analysis of reagent and tissue blanks to determine if processing and digestion procedures resulted in contamination. Reagent blanks consisted of digestion reagents and Type II reagent-grade water. Tissue blanks contained liver, kidney, or bone tissue from laboratory reared cotton rats (*Sigmodon hispidus*). Tissue blanks were designed to simulate collection and preparation of tissue samples to determine if these steps contaminated tissue samples. Reagent and tissue spikes, of known metal concentration, were analyzed to determine percent recovery of Cd, Cu, Pb, and Zn. Average percent recovery of Cd, Cu, Pb, and Zn from reagent spikes was 105%, 97%, 106%, 96%, respectively. Percent recovery of Cd, Cu, Pb, and Zn from liver, kidney, and bone tissue spikes was 107%, 99%, 98%, and 98%, respectively. Periodically during sample analysis, EPA Certification Standards were analyzed to insure proper calibration of the instrument. To account for contamination from glassware or digestion reagents, absorbencies of reagent blanks were subtracted from sample and spike absorbance readings before calculating concentrations.

Statistical analysis

Bartlett's test for homogeneity of variance and the skewness and kurtosis of metal concentration distributions indicated that these data were not normally distributed. Data were rank transformed before performing analysis of variance (ANOVA) procedures and least significant difference (LSD) multiple comparisons. This technique has been shown to be the equivalent of analyzing data by non-parametric statistical tests [44]. Significance was obtained at $p \leq 0.05$ level. Two-way ANOVA tests were performed with trapping period, site, and their interaction as factors. Because significant interaction was observed between trapping period and site, further statistical analyses among sites were calculated for each of the five trapping periods, separately. Sex was not found to significantly affect tissue metal concentrations within sites after two-way ANOVA tests with site and sex as factors. For this reason, sex was not considered to be a factor in further statistical analyses. If sites were found to differ significantly in tissue trace metal concentration, each stripmine site was compared with intracounty and remote reference sites with pairwise LSD tests. Although comparisons among sites were calculated with transformed data, original data were reported in this paper for comparison among sites.

Pearson correlation coefficients were calculated on ranked data to determine if relationships existed between trace metal concentrations of soil and tissue samples. Correlation analysis was performed between mean trace metal concentrations in soil and tissues because these media within a site were not directly related. Trace metal concentrations in soil were assumed to not vary significantly within the period of one or two years. Therefore within each site, mean soil metal concentrations were compared with tissue metal concentrations from all trapping periods.

Pearson correlation coefficients also were calculated on ranked data to determine if relationships existed between biological factors and tissue trace metal concentrations. In this study, biological factors investigated included total body weight, relative organ weight (organ to body weight ratio), and testicular volume. Testicular volume (V) was calculated by:

$$V = 4(\underline{a}^2 * \underline{b}\pi)/ 3$$

where $\underline{a}$ was one half the width and $\underline{b}$ was one half the length of the testicle. These factors have been reported to vary seasonally. For this reason, these correlation coefficients were calculated for each trapping period, separately. Testicular volumes were not calculated for male mice from the spring 1992 trapping period because complete testicular measurements were not recorded. Comparisons were not calculated between these factors and skeletal metal concentrations because the later were composites of at least two individuals.

RESULTS

Tissue metal concentrations

In the first trapping period, renal and hepatic Cd concentrations in mice from Okmulgee Co. mines were significantly greater than mice from Wayland mine, Eufala, and Wayland reference sites (Fig. 2 and 3). Renal Cd concentrations in mice from Moss mine also were significantly greater than concentrations in mice from Wayland mine and reference sites (FIg. 3). In the remaining trapping periods, mice from both Okmulgee Co. mine sites did not differ significantly from mice caught at the Eufala reference site in renal or hepatic Cd concentration (Fig. 2 and 3). However, mice from all Okmulgee Co. sites and Moss mine continued to have significantly greater Cd concentrations in renal and hepatic tissue than mice from Wayland and Blackwell reference sites.

Most kidney and liver samples did not contain detectable levels of Pb. Detection limit for Pb analysis on the GF-AAS was 0.01 ug/g for kidney and liver tissue. Mice collected during spring 1992 from Marler and Hamilton mine sites had significantly higher hepatic Pb levels than mice from Eufala reference site (Fig. 2). Significant elevations in Pb were not observed in renal or hepatic tissues from mice collected during summer and fall 1992 trapping periods. Because Pb concentrations were undetectable in most mice, Pb analysis was not perfomed on tissue collected during spring and summer 1993.

During the summer 1992 trapping period, hepatic and renal Cu concentrations were significantly greater in mice collected from Marler and Hamilton stripmines than mice from Blackwell and Eufala reference sites, respectively (Fig. 2 and 3). Mice collected from Moss mine during the spring 1993 collecting period had significantly greater hepatic Cu concentrations than mice from

Wayland and Blackwell reference sites (Fig. 2). Also, renal Cu concentrations were significantly greater in mice collected from Hamilton and Moss mines than mice from Eufala and Blackwell reference sites during the spring 1993 collecting period (Fig. 3). During the summer 1993 collecting period, mice collected from Wayland reference site differed significantly from mice from all other sites in hepatic and renal Cu concentration; however, the remaining sites did not differ significantly from each other (Fig. 2 and 3).

Similar to Cu, renal and hepatic Zn concentrations did not follow a consistent trend throughout the five trapping periods. In spring 1992, Zn levels in the kidneys were significantly higher in mice from Hamilton mine compared to mice from Eufala reference site (Fig. 3). Mice collected from Marler and Hamilton mines during summer 1992 had significantly greater concentrations of Zn in the liver and kidneys than mice from either Wayland or Blackwell reference sites (Fig. 2 and 3). Renal Zn levels in mice collected from Moss mine during summer 1992 also were significantly greater than Blackwell reference mice (Fig. 3). Mice collected from Wayland and Moss mines during fall 1992 had significantly higher hepatic Zn concentrations than mice from Wayland and Blackwell reference sites (Fig. 2). However, mice collected from Eufala reference and Moss mine sites in the fall 1992 trapping period had greater renal Zn concentrations than Blackwell reference mice (Fig. 3). Hepatic Zn levels in mice collected during spring 1993 did not vary significantly (Fig. 2). However, mice collected from Moss, Marler, and Hamilton mines during spring 1993 had significantly elevated renal Zn levels compared to mice from Wayland and Blackwell reference sites (Fig. 3). Mice collected from Hamilton and Moss mine sites during summer 1993 had significantly greater Zn concentrations in the liver and kidney than mice from Blackwell or

Wayland reference sites, respectively (Fig. 2 and 3).

Cadmium concentrations and Cd:Zn ratios in kidney and liver tissues followed the same trends in all trapping periods (Fig. 4). The largest Cd:Zn ratios occurred in mice from Marler, Hamilton, and Eufala mine or reference sites. These ratios were significantly greater than ratios in mice from Wayland reference and Blackwell reference sites.

Lead was detectable in bone tissue of *P. leucopus* collected from stripmine and reference sites during fall 1992, spring, and summer 1993 trapping periods (Fig. 5). In all of these periods, Marler and Hamilton mine mice had significantly higher Pb concentrations in skeletal tissue than mice from Eufala reference; however, only mice from Marler mine collected in the spring 1993 trapping period had significantly greater skeletal Pb concentrations than Blackwell reference mice.

Analysis of skeletal Cd, Cu, and Zn concentrations did not indicate mice accumulated these metals in bone. Low Cd concentrations were observed in bone tissues of mice collected during the fall 1992, spring 1993, and summer 1993 trapping periods (Fig. 5). Only mice collected from Okmulgee Co. mines during spring 1993 had significantly greater skeletal Cd concentrations than mice collected from Blackwell reference site. Mice collected from most stripmine sites did not have significantly greater Cu levels in the bone than Blackwell reference mice (Fig. 5). Bone tissue contained greater concentrations of Zn than either liver or kidney tissue (Fig. 5); however, it seemed to be less indicative of contaminant stress because Blackwell reference mice were significantly greater than or not significantly different from stripmine mice in all trapping periods. Skeletal %Cd:Zn ratios also did not seem to be affected by stripmine disturbance (Fig. 4).

Correlation of soil and tissue trace metals

Significant simple correlations were observed between soil parameters and trace metal concentrations in the tissues of *P. leucopus* (Table 1). Soil pH was negatively correlated with trace metal concentrations in the tissues except for hepatic Cu and Zn, renal Pb, and skeletal Cu and Zn concentrations. Hepatic Cd and Pb concentrations, and Cd:Zn ratios were correlated positively with soil Cd and Pb concentrations. Soil Cd concentrations were correlated positively with renal Cd, Cu, and Zn concentrations, and % Cd:Zn ratios. Soil Zn concentrations were correlated positively with hepatic and skeletal Pb concentrations.

Partial correlations of Cd and Zn levels between soil and tissue samples were calculated because the relationship of these metals in the soil was expected to influence the simple correlation of each other between these media. For all values of Cd in the soil, Zn in the soil was negatively correlated with hepatic and renal Cd and %Cd:Zn ($r < -0.95$, $p < 0.05$). Cadmium in the soil was positively correlated with hepatic and renal Cd and %Cd:Zn ($r > 0.975$, $p < 0.02$) at all values of Zn in the soil. Partial correlation analysis did not find Cd and Zn in the soil to be correlated with any other trace metals in the tissues.

Variation in body and tissue weights

Total body, relative kidney, and relative liver weights varied significantly among sites in all trapping periods. Relative kidney and liver weights varied significantly between male and female mice collected during the spring 1992, summer 1992, and summer 1993 collecting periods. Significant differences among sites in total body weight and relative liver weights were not consistent among trapping periods, and they did not seem to be associated with stripmine disturbance. However within each season, lower relative kidney weights were associated with mice collected from Okmulgee Co. sites which had the highest renal Cd concentrations.

Correlation of metal levels with biological measurements

In all trapping periods, age was positively correlated with renal and hepatic Cd concentrations and Cd:Zn ratios (Table 2). Positive correlations also were observed between age and Cu, Pb, and Zn concentrations in the kidney and liver for some trapping periods. This indicated that these metals, especially Cd, were bioaccumulated over time. Total body weight was correlated significantly with Cd, Cu, Zn concentrations, and Cd:Zn ratios in the kidney and liver; however, these correlations were not consistent in magnitude or direction, suggesting that other environmental or biological factors may affect this variable. During fall 1992 through summer 1993 trapping periods, liver weights were correlated negatively with hepatic Cu and Zn concentrations. Relative kidney weights were correlated negatively with Cd concentrations and Cd:Zn ratios for all trapping periods except spring 1992 (Table 2). Relative liver weights, as with total liver weights, were correlated negatively with hepatic Cu and Zn concentrations in the fall 1992 through summer 1993 trapping periods. Testicular volumes were significantly correlated with renal and hepatic Cd, Zn concentrations and Cd:Zn ratios in mice from many of the trapping periods. However, seasonal shifts in direction and significance of these correlations indicated variation in testicular volume was more likely due to variation in breeding condition than trace metal concentrations.

DISCUSSION

Peromyscus leucopus did not consistently bioaccumulate renal and hepatic concentrations of Cu from abandoned colliery stripmines in eastern Oklahoma. In summer 1992 collections, mice from stripmines contained elevated renal and hepatic Cu concentrations; however, this was not consistent among other trapping periods. Seasonal fluctuations in renal Cu concentration were illustrated in Figure 3. Similar fluctuations were observed for hepatic Cu concentrations. Due to this inconsistency, elevated renal and hepatic Cu concentrations may have been the result of biological variation between populations rather than environmental contamination. In all mice collected, neither renal or hepatic Cu levels exceeded normal ranges of monogastric mammals [45]. Also, these levels did not exceed those observed in laboratory reared cotton rats (Fig. 3). Skeletal Cu concentrations in mice from fall 1992 through summer 1993 collections also did not support the hypothesis of Cu bioaccumulation. Other studies involving granivorous species did not find mice from contaminated sites to have significantly elevated levels of essential elements compared with reference sites [10, 46, 47]. In mice collected for this study, the lack of consistently elevated renal and hepatic Cu may indicate the dietary levels of these metals were not sufficiently elevated to overload the homeostatic mechanisms which regulate levels of essential elements. Other studies have provided evidence in support of this hypothesis [10, 47-50].

Although many mice had non-detectable levels of Pb in the kidney and liver, correlative analysis between age and hepatic Pb concentrations suggested that older mice bioaccumulated higher levels of Pb in the liver. Low tissue Pb concentrations in *P. leucopus* may have been due to their relatively short lifespan. Wildlife with longer lifespans may be at greater risk for bioaccumulation of Pb.

Liver and kidney tissues have been indicative of exposure to Pb [10, 16, 46, 47, 51-53]; however, the main storage organ of Pb is skeletal tissue [10, 16, 45, 53-55]. Analysis of bone samples from fall 1992 through summer 1993 revealed detectable concentrations of Pb. Lead levels in bone of mice from stripmines were extremely low compared with mice from other sites contaminated from metalliferous mining and smelting industries [9, 53]. Renal, hepatic, and skeletal Pb concentrations did not exceed normal levels determined by the Subcommittee on Mineral Toxicity in Animals [45]. Although significant differences did exist among stripmine and reference sites, the low magnitude of Pb concentrations in the bone indicated the ecological significance of Pb in the bone was relatively low.

Analysis of hepatic and renal tissue for Zn provided some evidence that mice from stripmine sites bioaccumulated this element. However, Zn residue levels in tissues of mice from stripmines were not consistently elevated among the trapping periods. Figure 3 illustrated seasonal trends observed in renal Zn concentration. Similar trends were observed in hepatic Zn concentration. As with tissue Cu concentrations, renal and hepatic Zn concentrations were similar to that of laboratory reared cotton rats (Fig. 2 and 3). Biological or ecological factors may have caused Zn concentrations to vary among seasons. Studies have shown *P. leucopus* and other small mammals to experience seasonal variation in diet according to availability [24, 29]. Metal concentrations in the diet of small mammals was found to vary considerably depending on season and diet composition [9, 29]. Ecological stressors such as extreme temperatures and food and water deprivation may cause induction or enhancement of metallothionein (MT) production [56-58]. Excess MT could result in altered metal concentrations, including Zn, in kidney and liver tissues.

Conversely, mice from stripmines consistently showed elevated levels of Cd in kidney and liver but not bone tissue (Fig. 2 and 3). These data were consistent with other studies which found Cd bioaccumulated in herbivorous, omnivorous, and carnivorous species of small mammals [9-11, 17, 29, 48, 49, 59, 60]. Acute renal damage was reported to occur in laboratory reared rabbits when Cd concentration in the renal cortex reached 200 ppm [61]; renal Cd levels of *P. leucopus* in this study were well below this value. However, elevated Cd levels in kidney and liver of stripmine mice may still result in sub-acute toxic effects. Bousquet [62] reported sub-lethal renal damage to occur when 3 to 24 ug Cd/g accumulated in renal tissue. Snow [63] reported Cd to be carcinogenic through direct interaction with nucleic acids and inhibition of DNA repair mechanisms. Evidence also has shown Cd to interfere with biochemical reactions and protein formation involving essential elements such as calcium, copper, manganese, and zinc. Hypertension and degeneration of intestinal villi were also reported as sublethal effects in domestic animals [45].

Renal Cd:Zn ratios as high as 52.5% in mice from Marler, Hamilton mines and Eufala reference site indicated these animals may have experienced renal damage. Dosage ratios of 33% Cd:Zn and higher were found to cause a significant increase in renal damage as measured by the excretion of glucose, aspartate aminotransferase, and protein [37]. Cd:Zn ratios in this study and that of Kojima et al. [37] were not directly comparable because the former were metal residue ratios acquired through in situ chronic exposures and the later were from acute exposure. Because the concentration of a dose reaching the target organ is lower than the administered dose, it should be expected that a given Cd:Zn ratio in tissues would cause an equal or greater toxic effect than the same ratio in an administered dose.

Although mice from stripmines may have accumulated some Cd through foods in the diet, partial correlations between tissue and soil Cd concentrations suggested that direct exposure to soil was a factor in the bioaccumulation of Cd. Also, negative partial correlations between Zn levels in the soil and Cd levels in the kidney and liver suggested that exposure to Zn in the soil regulated the bioaccumulation of Cd. Significant correlations were not observed between tissue and soil Zn concentrations. This indicated that either Zn was accumulated by a pathway other than direct exposure to contaminated soil; or, homeostatic regulation of Zn in the liver, kidney, and bone reduced the bioaccumulation of Zn in these organs and reduced the usefulness of this correlation.

Stripmine and reference sites did not seem to contain large amounts of Cu and Pb in the soil. Due to the low concentrations and relative mobility of these metals in soil, Cu and Pb were not expected to accumulate in small mammals at the mine sites. These metals were reported to be relatively immobile in soils due to their association with organic matter [29, 64-66] and residual fractions of the soil profile [18]. Davis et al. [67] found Pb from contaminated spoil to be relatively unavailable when ingested due to the oxidation of galena, which forms a coating of anglesite and K-jarosite precipitates. Correlations supported this suggestion with respect to Cu; however, soil Pb concentrations and hepatic Pb concentrations were significantly related. Although most Pb in stripmine soils may be unavailable through the food chain, studies have found soil Pb continued to be hazardous when inhaled or ingested directly [18, 65]. This evidence supported the hypothesis that the main exposure pathway for Pb was via ingestion of contaminated soil. These data suggested that hazards of trace metal contamination were determined by soil parameters, such as pH and trace metal concentrations.

Correlation between biological factors and trace metal concentrations in the liver and kidneys suggested that levels of renal and hepatic Cd, Cd:Zn ratios, and renal Zn did not consistently affect total body weights. However, a consistent relationship between these metals and relative kidney weights was demonstrated. These results were consistent with other studies which found Cd and Zn to cause growth inhibition at sublethal concentrations in domestic and wild mammals [45, 68]. Reduced Cd:Zn ratios were related significantly to higher relative kidney weights. This result also was supported by other studies which have shown Zn to reduce the toxicity of Cd through competitive interaction [36]. Testicular atrophy has been shown to result from exposure to excess Cd [31]. However in this study, correlations between trace metals and testicular volumes indicated biological and ecological factors, rather than trace metal concentrations, regulated testicular volumes. *Peromyscus leucopus* collected in this study were subject to seasonal breeding periods [24]. Thus, changes in testicular volume among seasons were probably due to changes in reproductive activity. Because correlations between trace metals and testicular volume were inconsistent in magnitude and direction, it was assumed that reproductive activity had a greater effect on testicular size than trace metal concentrations in the tissues.

Because Cu concentrations in tissue of *P. leucopus* inhabiting abandoned coal stripmines were within normal ranges for monogastric mammals, this metal was not expected to cause an adverse effect. Extremely low levels of Pb in renal, hepatic and skeletal tissues of *P. leucopus* indicated little environmental exposure to this metal. This result was consistent with analysis of trace metals in the soil. Cadmium was found to bioaccumulate in the kidneys and livers of mice from Marler and Hamilton mines. Renal Cd concentrations in these mice were greater than levels considered to be sub-lethally toxic [62]. Threshold levels of Cd in the diet that have produced toxic effects were reported to range from 0.10 to 1.0 ug Cd/g [45, 68]. This indicated that predators feeding on mice from Hamilton and Marler mines had an increased risk of Cd accumulation and toxic effects. Although these threshold values were conservative, Cd levels in kidney and liver from mice inhabiting Okmulgee Co. stripmines exceeded these levels by up to 10-fold. Also, correlations between renal and hepatic Cd concentrations and relative kidney weights indicated possible sublethal toxic effects. These results suggested that small mammals are bioaccumulating Cd and may have experienced toxic effects due to the elevated levels of Cd in liver and kidneys.

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Table 1. Correlation coefficients (*n*) between mean trace metal concentrations in P. leucopus tissues and mean trace metal concentrations and pH of colliery stripmine and reference soils from Oklahoma.

	Soil parameters					
	pH	Cd	Cu	Pb	Zn	%Cd:Zn
Liver						
Cd	-0.785(29)*	0.631(29)*	-0.181(29)	0.631(29)*	0.262(29)	0.364(29)*
Cu	-0.127(29)	-0.020(29)	0.404(29)*	-0.020(29)	0.168(29)	-0.277(29)
Pb	-0.518(17)*	0.512(17)*	-0.051(17)	0.512(17)*	0.497(17)*	0.140(17)
Zn	-0.127(29)	0.118(29)	-0.012(29)	0.118(29)	0.047(29)	-0.019(29)
%Cd:Zn	-0.789(29)*	0.646(29)*	-0.165(29)	0.646(29)*	0.293(29)	0.358(29)
Kidney						
Cd	-0.802(28)*	0.667(28)*	-0.220(28)	0.667(28)*	0.301(28)	0.333(28)
Cu	-0.378(29)*	0.412(29)*	0.000(29)	0.412(29)*	0.340(29)	0.173(29)
Pb	-0.151(16)	0.176(16)	0.094(16)	0.176(16)	0.315(16)	-0.038(16)
Zn	-0.575(28)*	0.427(28)*	-0.032(28)	0.427(28)*	0.267(28)	0.170(28)
%Cd:Zn	-0.791(28)*	0.679(28)*	-0.233(28)	0.679(28)*	0.320(28)	0.350(28)
Bone						
Cd	-0.624(18)*	0.314(18)	-0.354(18)	0.314(18)	0.107(18)	0.060(18)
Cu	-0.292(18)	0.0040(18)	-0.060(18)	0.0040(18)	0.110(18)	-0.191(18)
Pb	-0.536(18)*	0.385(18)	-0.147(18)	0.385(18)	0.511(18)*	-0.016(18)
Zn	0.166(18)	-0.266(18)	-0.329(18)	-0.266(18)	-0.292(18)	-0.235(18)
%Cd:Zn	-0.646(18)*	0.351(18)	-0.273(18)	0.351(18)	0.169(18)	0.056(18)

* significant correlation coefficient ($p < 0.05$)

Table 2. Correlation analysis of age, body and tissue weights, and testicular volume of *P. leucopus* with recorded levels of trace metals in tissues (n>100).

Variables	Hepatic residues					Renal residues				
	Cd	Cu	Pb	Zn	%Cd:Zn	Cd	Cu	Pb	Zn	%Cd:Zn
					Spring 1992					
Age[a]	0.301*	0.065	0.202*	0.132	0.282*	0.383*	-0.008	0.077	0.156	0.401*
Total body weight	-0.233*	0.248*	-0.089	0.145	-0.256*	-0.163	-0.121	-0.053	-0.179	-0.134
Relative kidney weight	-0.091	0.042	-0.082	0.030	-0.095	-0.128	-0.193*	0.099	-0.093	-0.138
Relative liver weight	———	———	———	———	———	———	———	———	———	———
Testicular volume	———	———	———	———	———	———	———	———	———	———
					Summer 1992					
Age[a]	0.374*	0.118	0.225*	0.189*	0.357*	0.373*	0.107	0.122	0.089	0.385*
Total body weight (g)	0.100	-0.098	0.142	-0.057	0.103	0.073	-0.127	-0.088	-0.115	0.093
Relative kidney weight	-0.324*	-0.054	-0.157	-0.102	-0.319*	-0.288*	-0.144	0.092	-0.260*	-0.279*
Relative liver weight	———	———	———	———	———	———	———	———	———	———
Testicular volume (mm^3)	-0.436*	-0.225	0.030	-0.036	-0.429*	-0.423*	-0.203	-0.025	-0.222	-0.412*
					Fall 1992					
Age[a]	0.464*	-0.065	0.059	-0.095	0.480*	0.518*	-0.008	0.017	0.219	0.521*
Total body weight (g)	0.345*	-0.242*	-0.034	-0.008	0.358*	0.425*	0.080	0.150	0.377*	0.403*

Table 2. Continued.

Relative kidney weight	-0.497*	0.176	-0.093	-0.014	-0.507*	-0.520*	-0.067	-0.206	-0.334*	-0.499*
Relative liver weight	0.122	-0.295*	0.002	-0.351*	0.164	-0.092	-0.130	-0.212	-0.408*	-0.054
Testicular volume (mm^3)	-0.024	-0.123	0.003	-0.082	-0.004	-0.118	-0.129	-0.126	-0.026	-0.126
Spring 1993										
Age[a]	0.263*	0.167*	——	0.278*	0.230*	0.329*	0.148	——	0.230*	0.318*
Total body weight (g)	0.108	-0.018	——	0.088	0.097	0.180*	0.164*	——	0.195*	0.150
Relative kidney weight	-0.397*	0.022	——	-0.172*	-0.383*	-0.493*	-0.248*	——	-0.233*	-0.476*
Relative liver weight	-0.200*	-0.413*	——	-0.501*	-0.124	-0.140	-0.241*	——	-0.210*	-0.120
Testicular volume (mm^3)	0.254	-0.060	——	0.198	0.232	0.343*	0.404*	——	0.261*	0.316*
Summer 1993										
Age[a]	0.334*	0.142	——	0.139	0.340*	0.436*	0.235*	——	0.153	0.451*
Total body weight (g)	-0.139	-0.266*	——	-0.276*	-0.107	-0.030	-0.021	——	-0.194*	-0.002
Relative kidney weight	-0.464*	0.135	——	-0.071	-0.485*	-0.500*	0.020	——	-0.291*	-0.498*
Relative liver weight	-0.556*	-0.432*	——	-0.599*	-0.524*	-0.525*	-0.125	——	-0.513*	-0.500*
Testicular volume (mm^3)	-0.273*	0.132	——	-0.136	-0.269*	-0.186	0.105	——	-0.206	-0.159

[a] age based on a relative index of tooth wear

* correlation coefficient significant at ($p < 0.05$)

Fig. 1. Locations of the coal belt (outlined area) and study sites within Oklahoma.

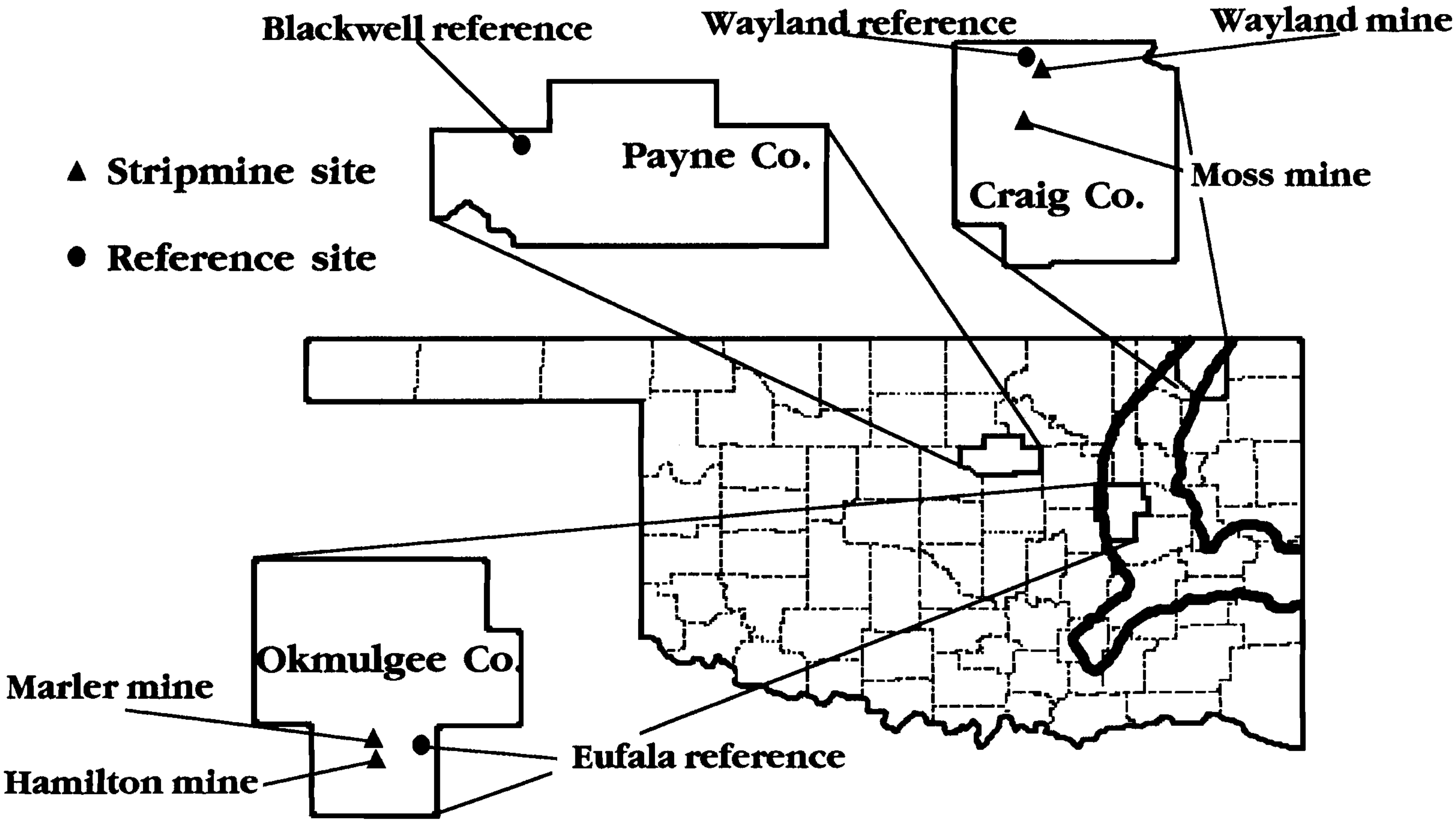
Fig. 2. Mean Cd (A), Cu (B), Pb (C), and Zn (D) concentrations in ug/g liver, wet weight, from white-footed mice (*P. leucopus*) trapped on colliery stripmine and reference sites during five trapping periods. Vertical bars denote standard error of the mean. Number at the base of each bar and asterisks above SE bars denote sample size and statistical significance of the mean, respectively. *, **, and *** denote means which are significantly greater than the intracounty reference, Blackwell reference, and both references, respectively. The dashed line represents the average concentration of trace metal observed in laboratory raised cotton rats (*Sigmodon hispidus*).

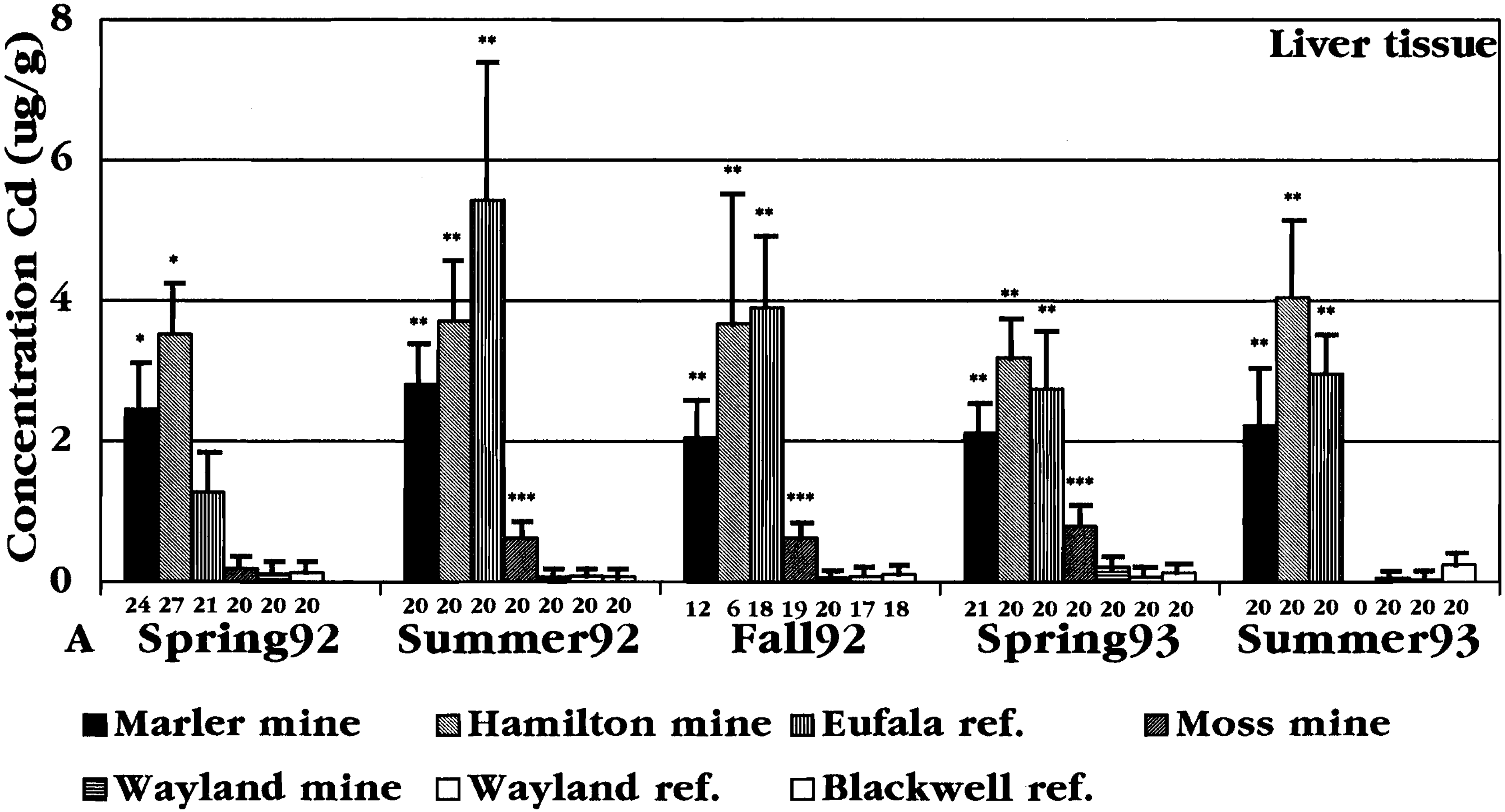
Fig. 3. Mean Cd (A), Cu (B), Pb (C), and Zn (D) concentrations in ug/g kidney, wet weight, from white-footed mice (*P. leucopus*) trapped on colliery stripmine and reference sites during five trapping periods. Vertical bars denote standard error of the mean. Number at the base of each bar and asterisks above SE bars denote sample size and statistical significance of the mean, respectively. *, **, and *** denote means which are significantly greater than the intracounty reference, Blackwell reference, and both references, respectively. The dashed line represents the average concentration of trace metal observed in laboratory raised cotton rats (*Sigmodon hispidus*).

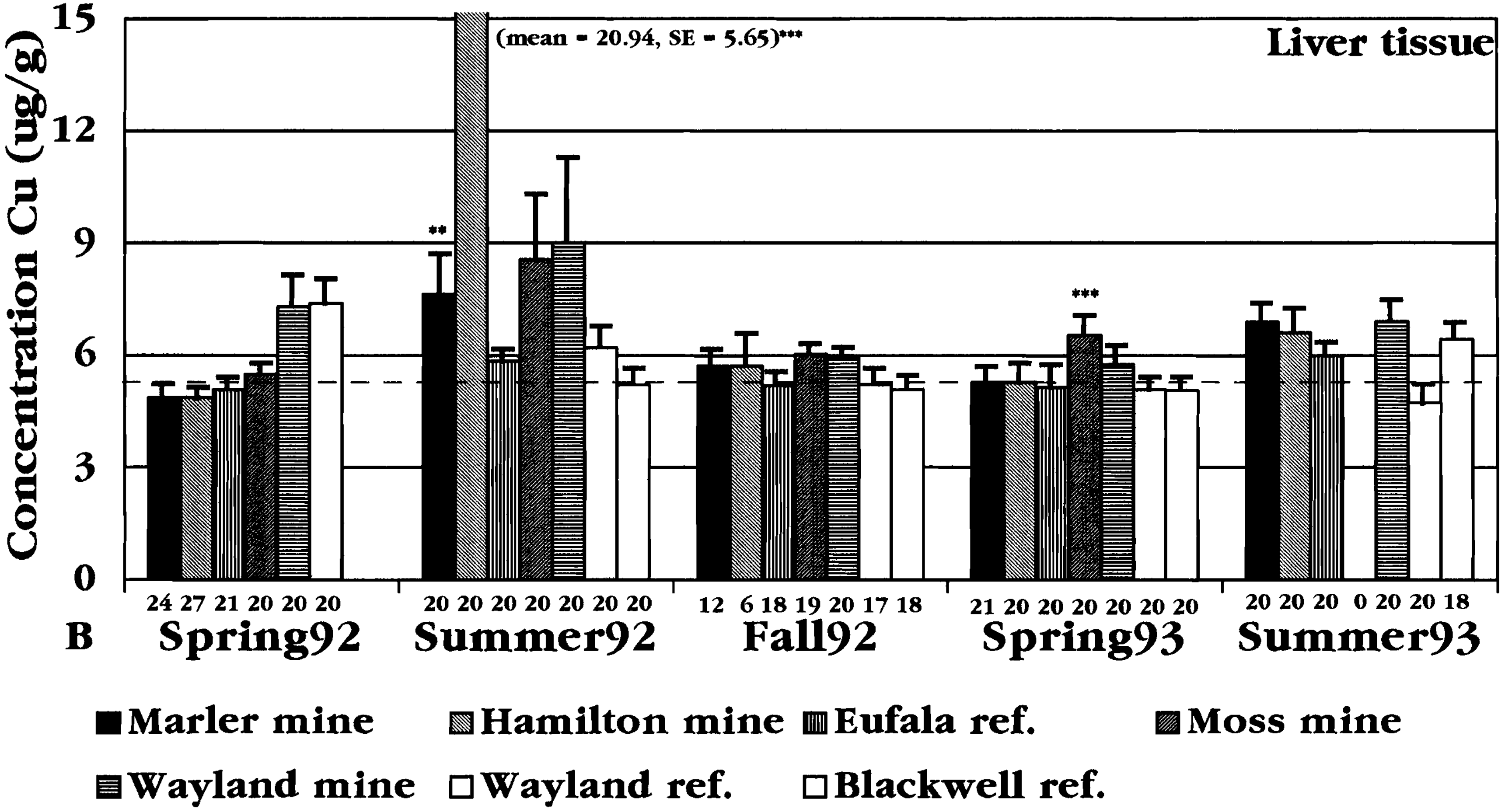
Fig. 4. Mean % Cd:Zn ratios in liver (A), kidney (B), and bone (C) from white-footed mice (*P. leucopus*) trapped on colliery stripmine and reference sites during five trapping periods. Vertical bars denote standard error of the mean. Number at

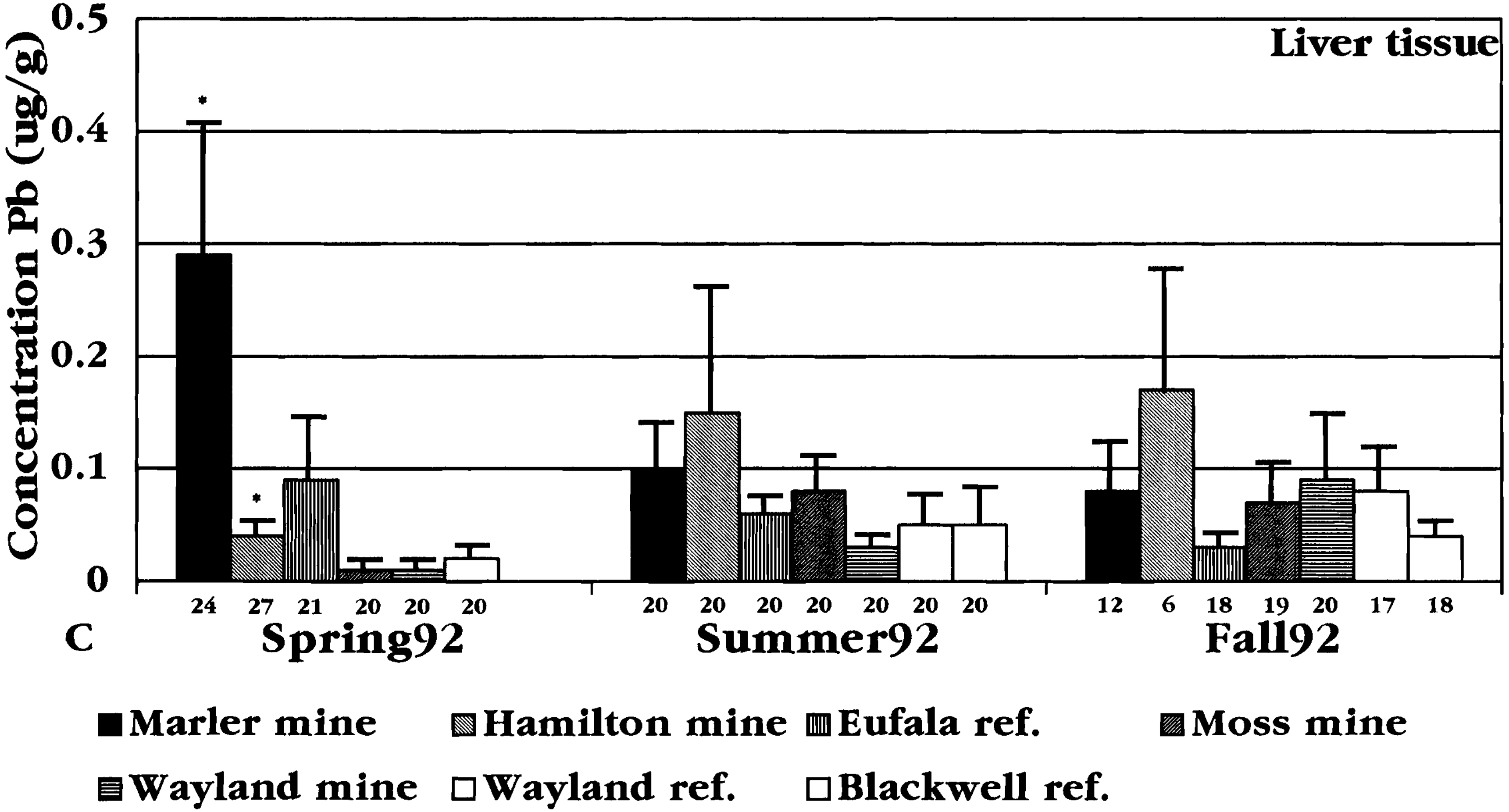
the base of each bar and asterisks above SE bars denote sample size and statistical significance of the mean, respectively. *, **, and *** denote means which are significantly greater than the intracounty reference, Blackwell reference, and both references, respectively. The dashed line represents the % Cd:Zn ratio found to increase renal damage in laboratory rats [37].

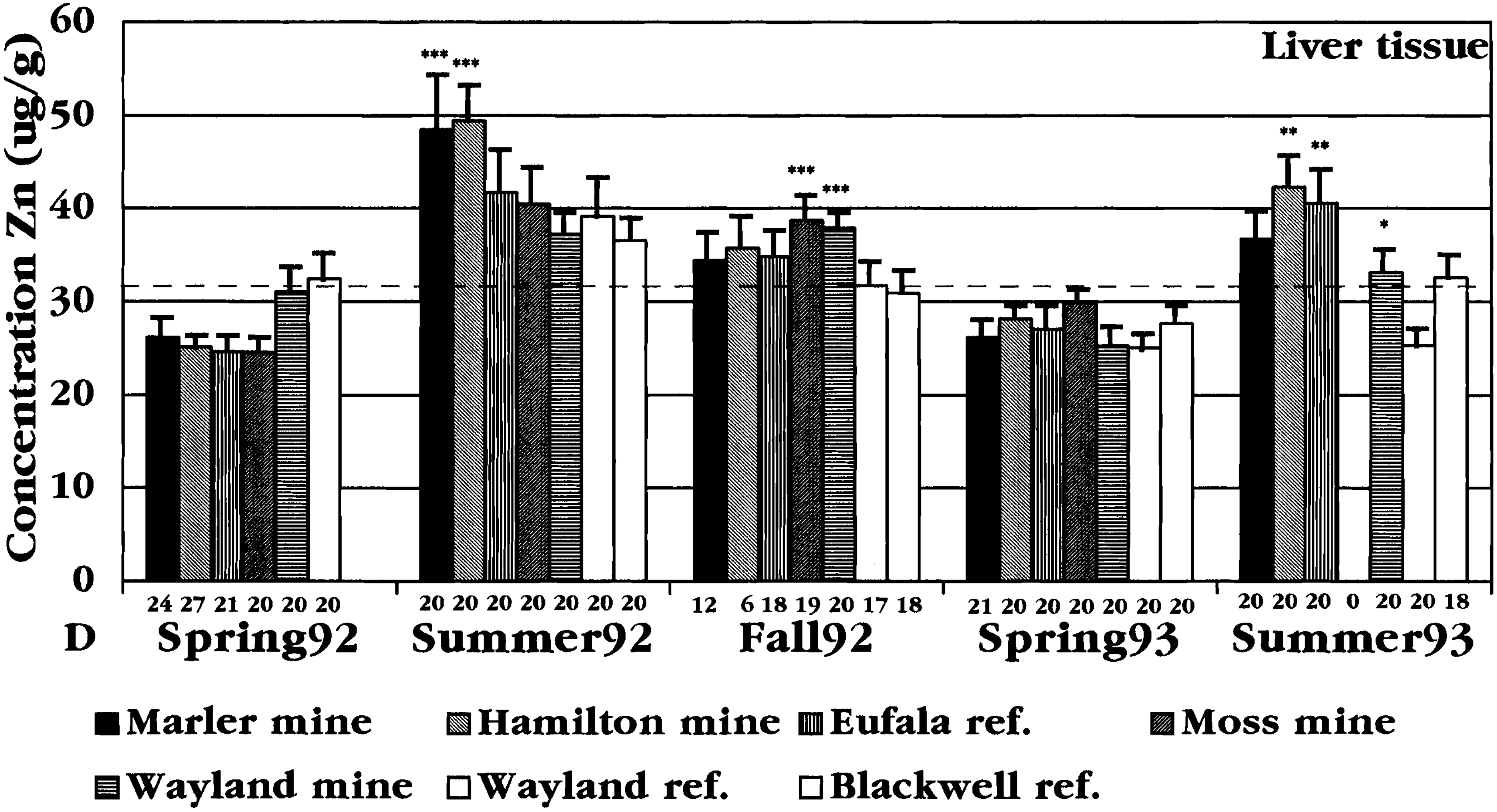
Fig. 5. Mean Cd (A), Cu (B), Pb (C), and Zn (D) concentrations in ug/g bone, wet weight, from white-footed mice (*P. leucopus*) trapped on colliery stripmine and reference sites during three trapping periods. Vertical bars denote standard error of the mean. Number at the base of each bar and asterisks above SE bars denote sample size and statistical significance of the mean, respectively. *, **, and *** denote means which are significantly greater than the intracounty reference, Blackwell reference, and both references, respectively.

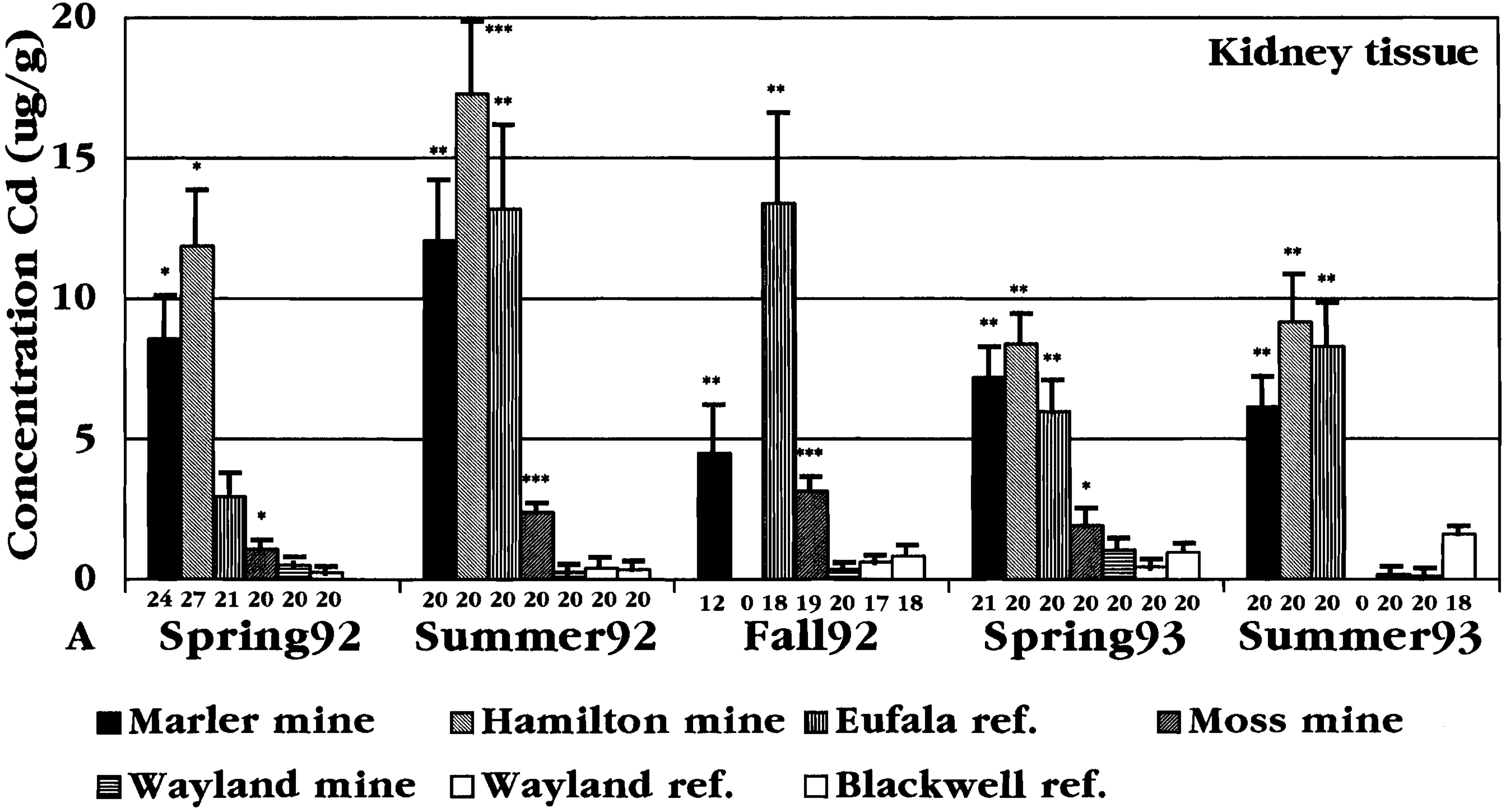

▲ Stripmine site
● Reference site
Blackwell reference
Payne Co.
Wayland reference
Wayland mine
Craig Co.
Moss mine
Okmulgee Co.
Marler mine
Hamilton mine
Eufala reference

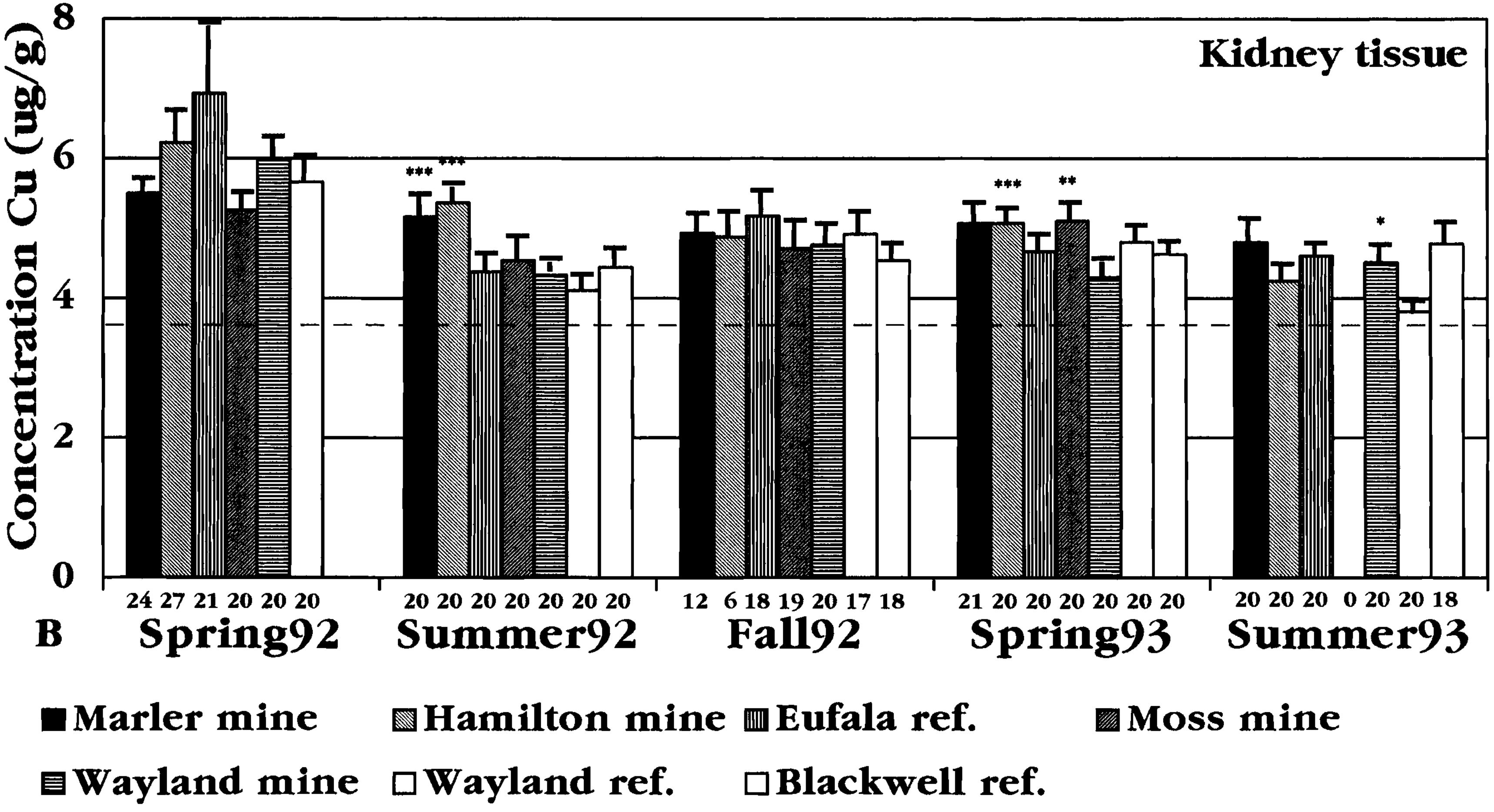

Liver tissue
Concentration Cd (ug/g)
0
2
4
6
8
*
**
***
24 27 21 20 20 20
20 20 20 20 20 20 20
12 6 18 19 20 17 18
21 20 20 20 20 20 20
20 20 20 0 20 20 20
A
Spring92
Summer92
Fall92
Spring93
Summer93
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▨ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.

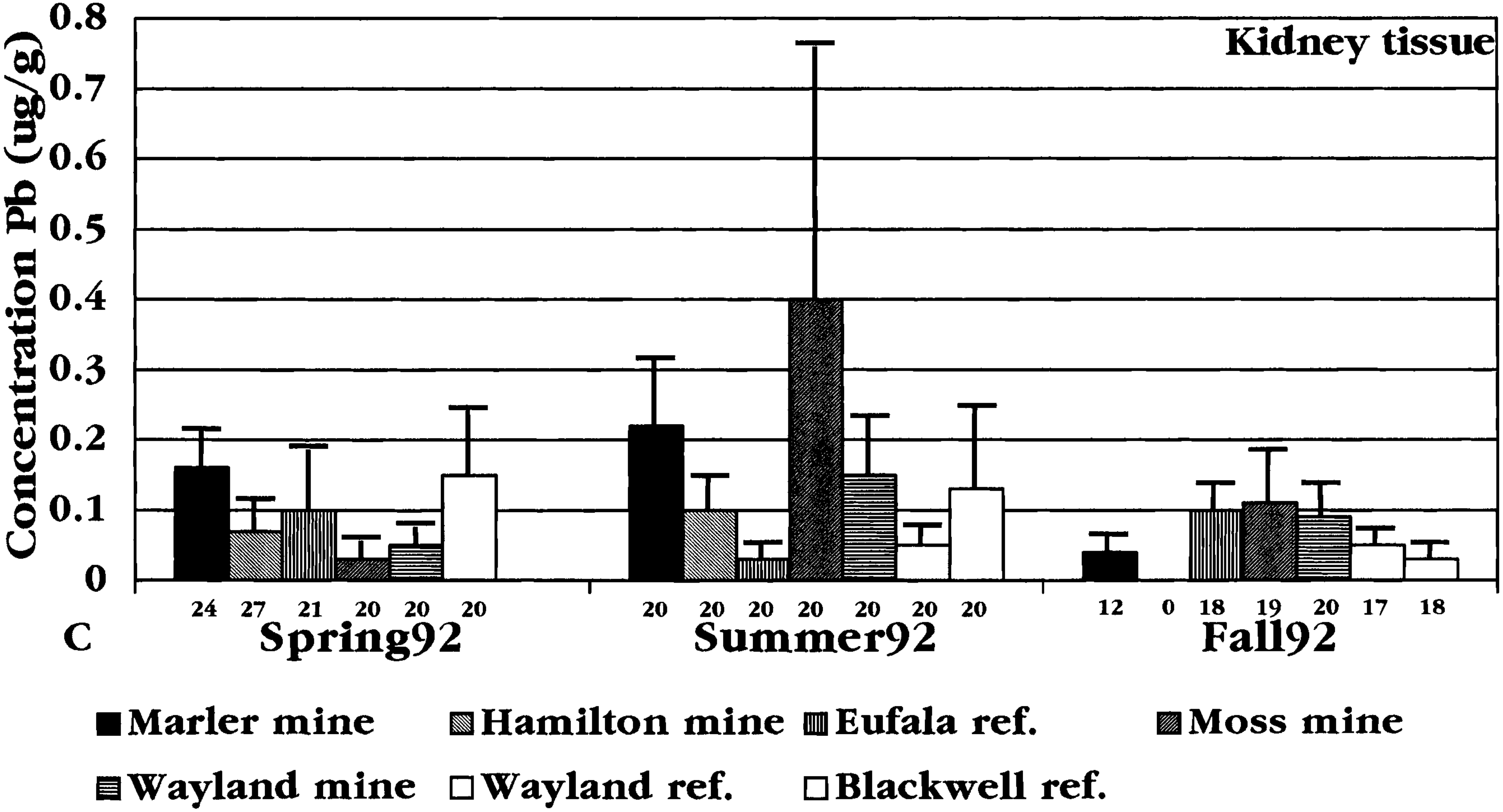

B
Concentration Cu (ug/g)
15
12
9
6
3
0
(mean = 20.94, SE = 5.65)***
Liver tissue
**
***
24 27 21 20 20 20
20 20 20 20 20 20 20
12 6 18 19 20 17 18
21 20 20 20 20 20 20
20 20 20 0 20 20 18
Spring92
Summer92
Fall92
Spring93
Summer93
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▨ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.

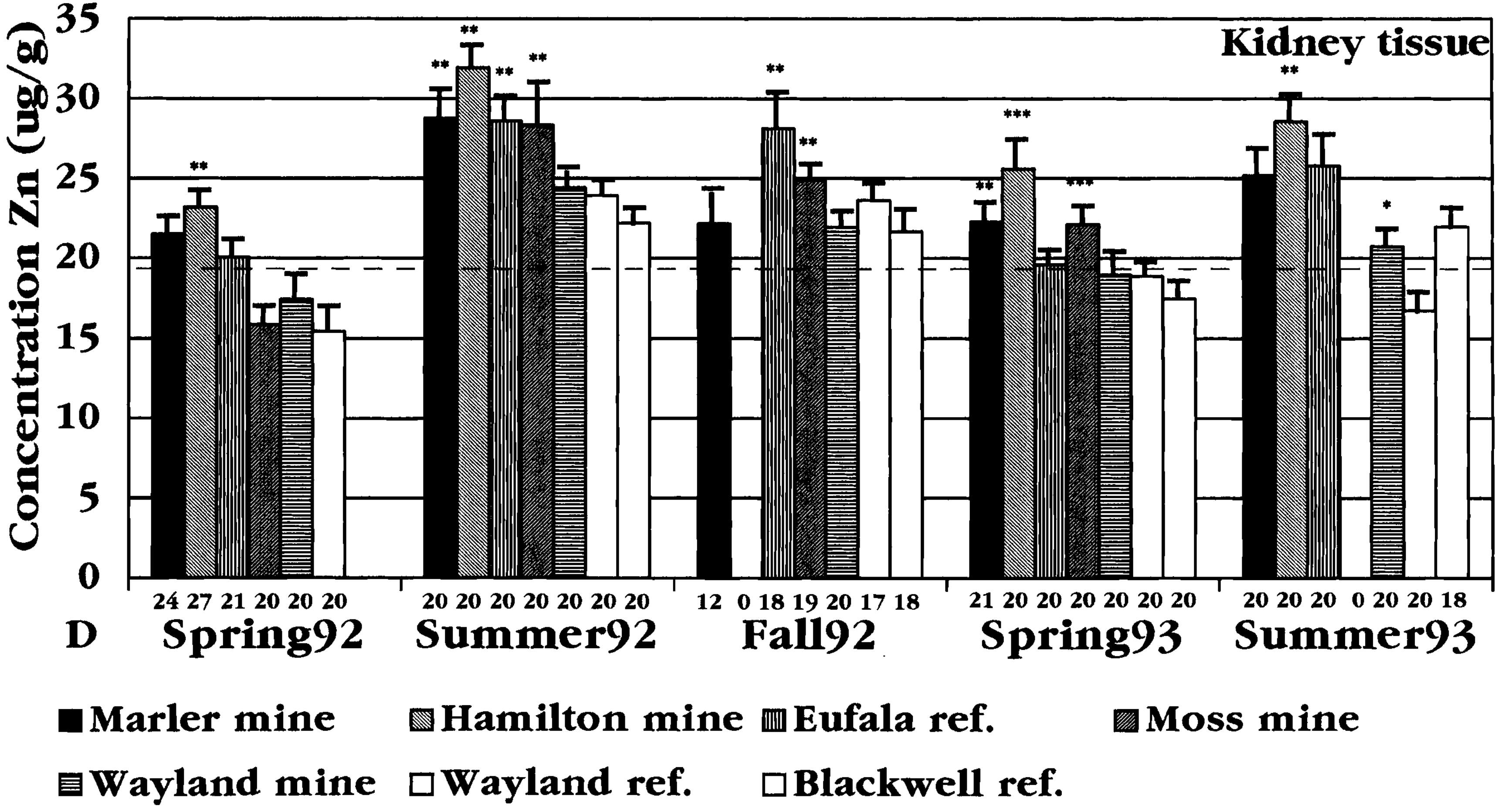

Liver tissue
Concentration Pb (ug/g)
0.5
0.4
0.3
0.2
0.1
0
*
*
24 27 21 20 20 20
20 20 20 20 20 20 20
12 6 18 19 20 17 18
C
Spring92
Summer92
Fall92
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▩ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.


Liver tissue
Concentration Zn (ug/g)
0
10
20
30
40
50
60
*** ***
*** ***
** **
*
24 27 21 20 20 20
20 20 20 20 20 20 20
12 6 18 19 20 17 18
21 20 20 20 20 20 20
20 20 20 0 20 20 18
D
Spring92
Summer92
Fall92
Spring93
Summer93
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▨ Moss mine
▤ Wayland mine
☐ Wayland ref.
☐ Blackwell ref.

Kidney tissue
Concentration Cd (ug/g)
20
15
10
5
0
24 27 21 20 20 20
20 20 20 20 20 20 20
12 0 18 19 20 17 18
21 20 20 20 20 20 20
20 20 20 0 20 20 18
A
Spring92
Summer92
Fall92
Spring93
Summer93
*
**
***
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▨ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.


Kidney tissue
Concentration Cu (ug/g)
0
2
4
6
8
***
***
***
**
*
24 27 21 20 20 20
20 20 20 20 20 20 20
12 6 18 19 20 17 18
21 20 20 20 20 20 20
20 20 20 0 20 20 18
B
Spring92
Summer92
Fall92
Spring93
Summer93
Marler mine
Hamilton mine
Eufala ref.
Moss mine
Wayland mine
Wayland ref.
Blackwell ref.


Kidney tissue
Concentration Pb (ug/g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
24 27 21 20 20 20
20 20 20 20 20 20 20
12 0 18 19 20 17 18
C
Spring92
Summer92
Fall92
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▨ Moss mine
▤ Wayland mine
☐ Wayland ref.
☐ Blackwell ref.


Kidney tissue
Concentration Zn (ug/g)
0
5
10
15
20
25
30
35
**
**
**
**
**
**
**
**
***
***
**
*
24 27 21 20 20 20
20 20 20 20 20 20 20
12 0 18 19 20 17 18
21 20 20 20 20 20 20
20 20 20 0 20 20 18
D
Spring92
Summer92
Fall92
Spring93
Summer93
■ Marler mine
▨ Hamilton mine
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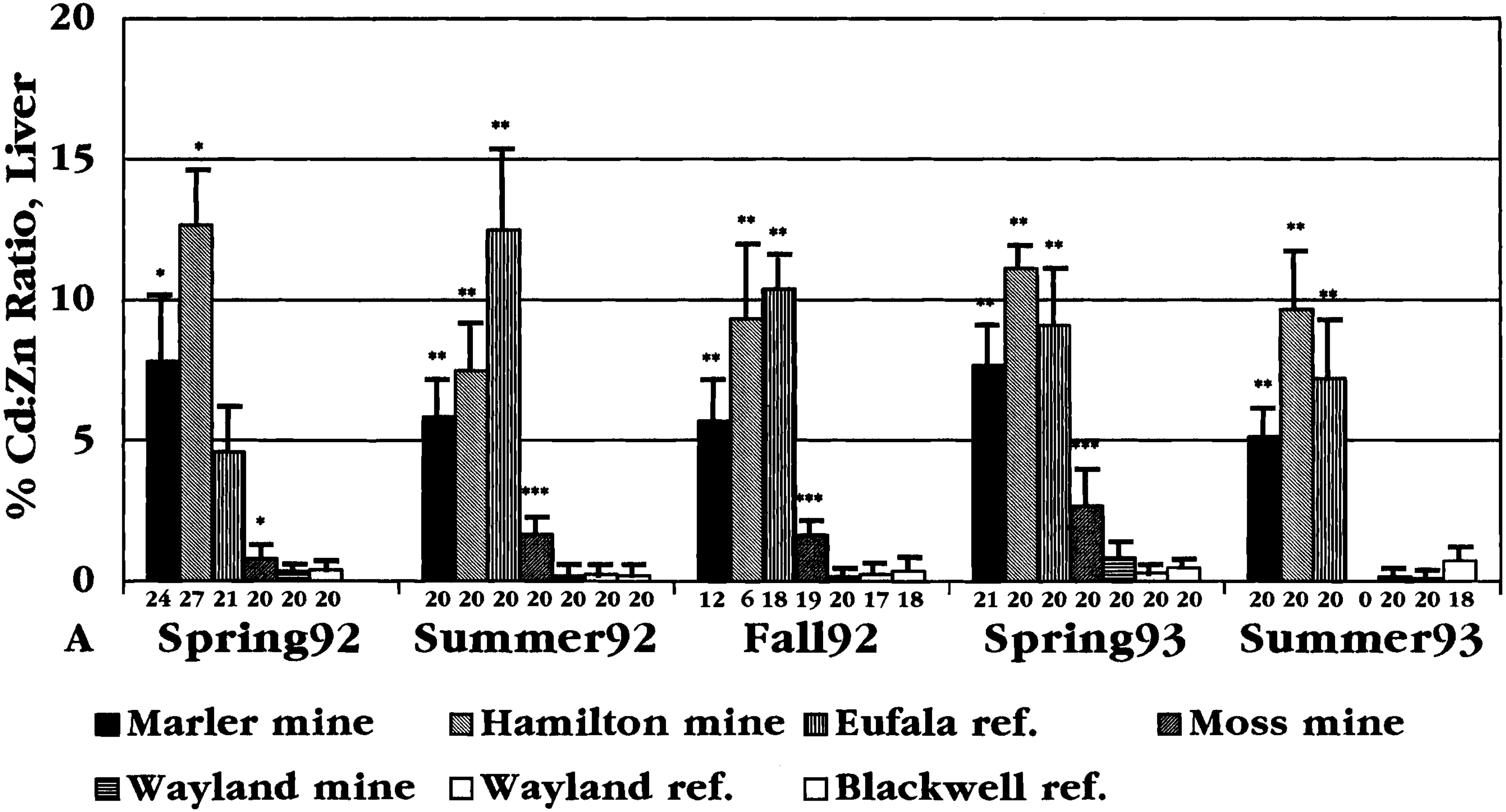
▨ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.


20
15
10
5
0
% Cd:Zn Ratio, Liver
*
*
*
**
**
**
***
**
**
**
***
**
**
**
***
**
**
**
24 27 21 20 20 20
20 20 20 20 20 20 20
12 6 18 19 20 17 18
21 20 20 20 20 20 20
20 20 20 0 20 20 18
A
Spring92
Summer92
Fall92
Spring93
Summer93
■ Marler mine
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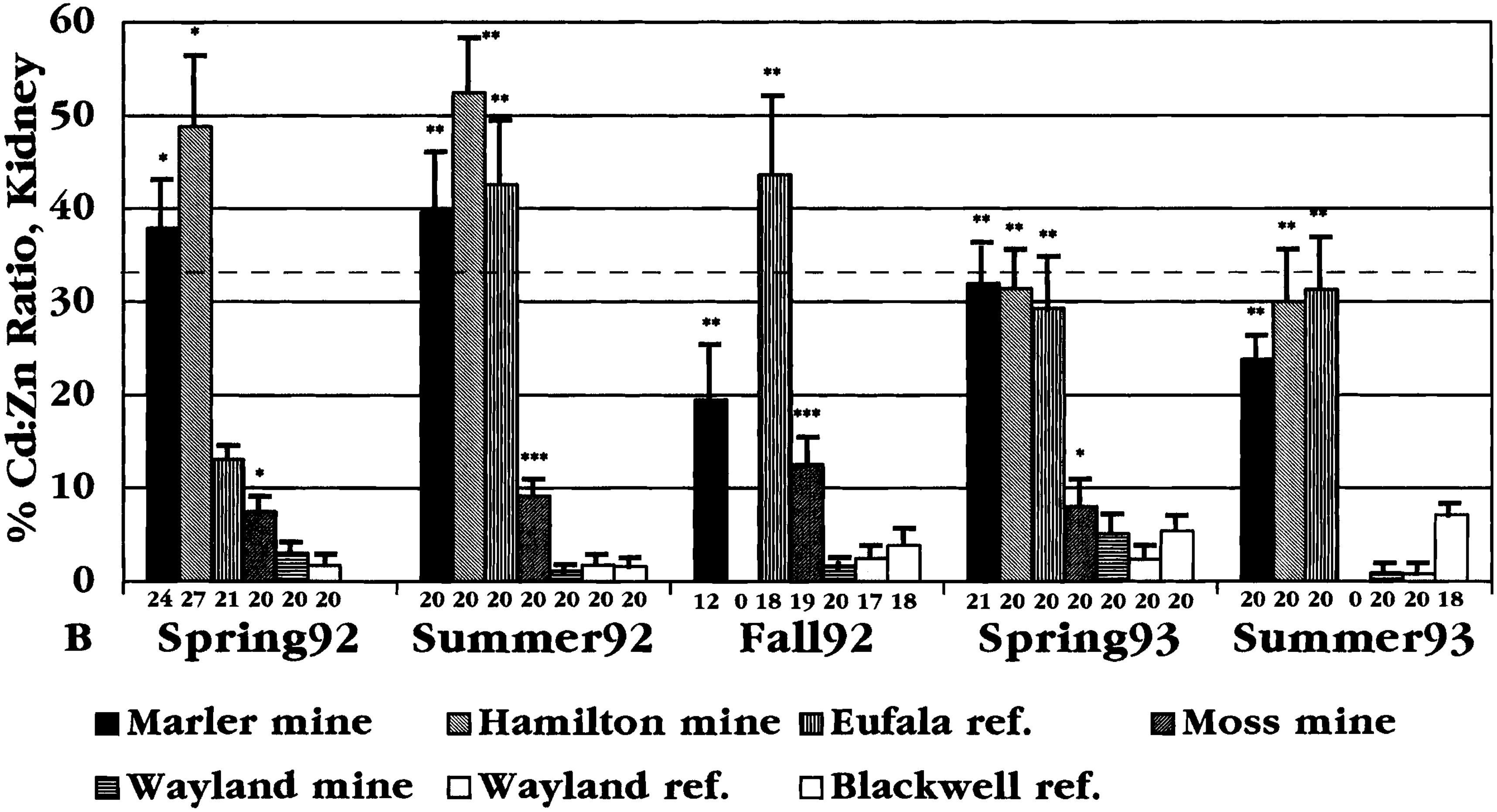
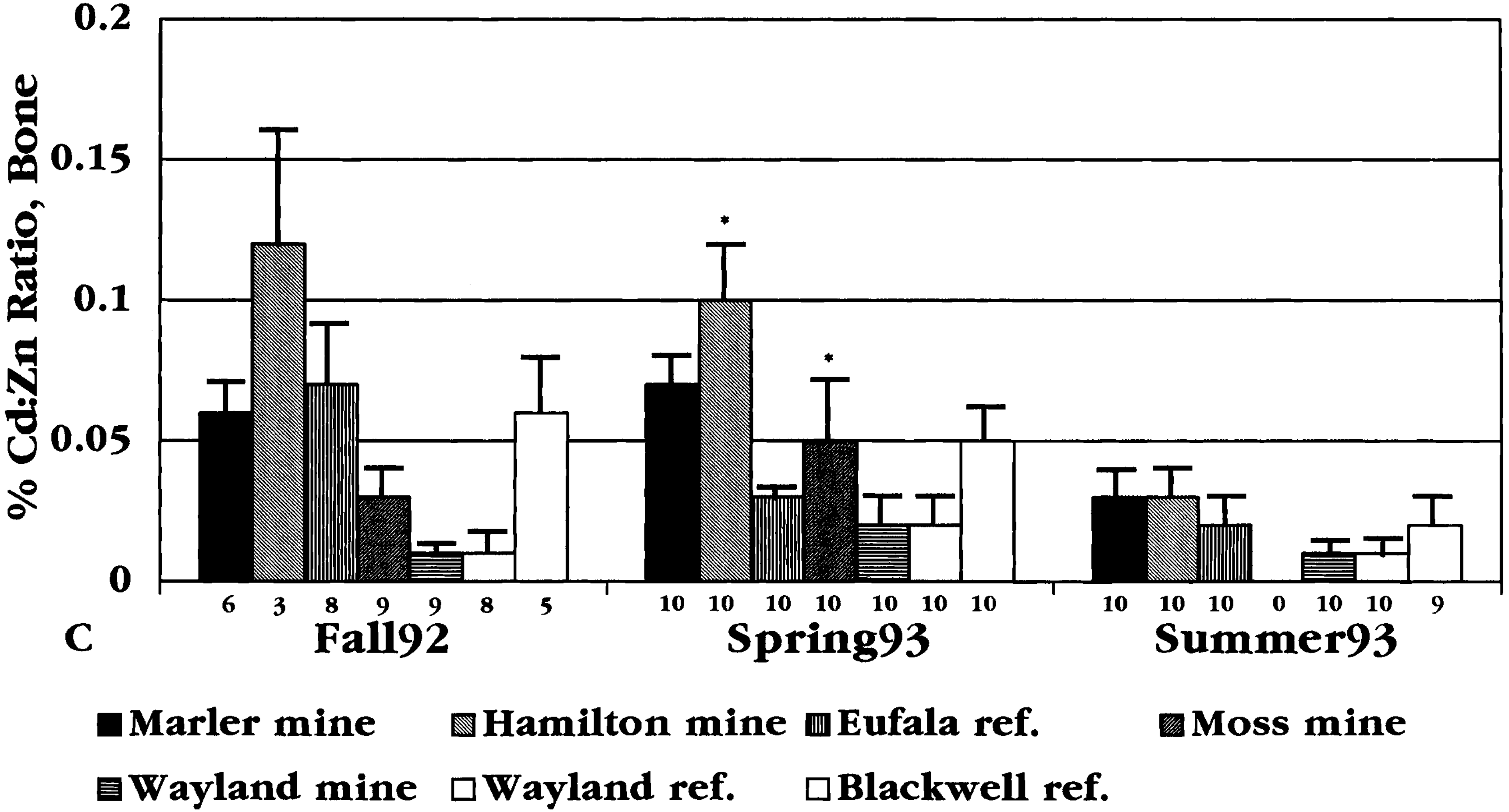
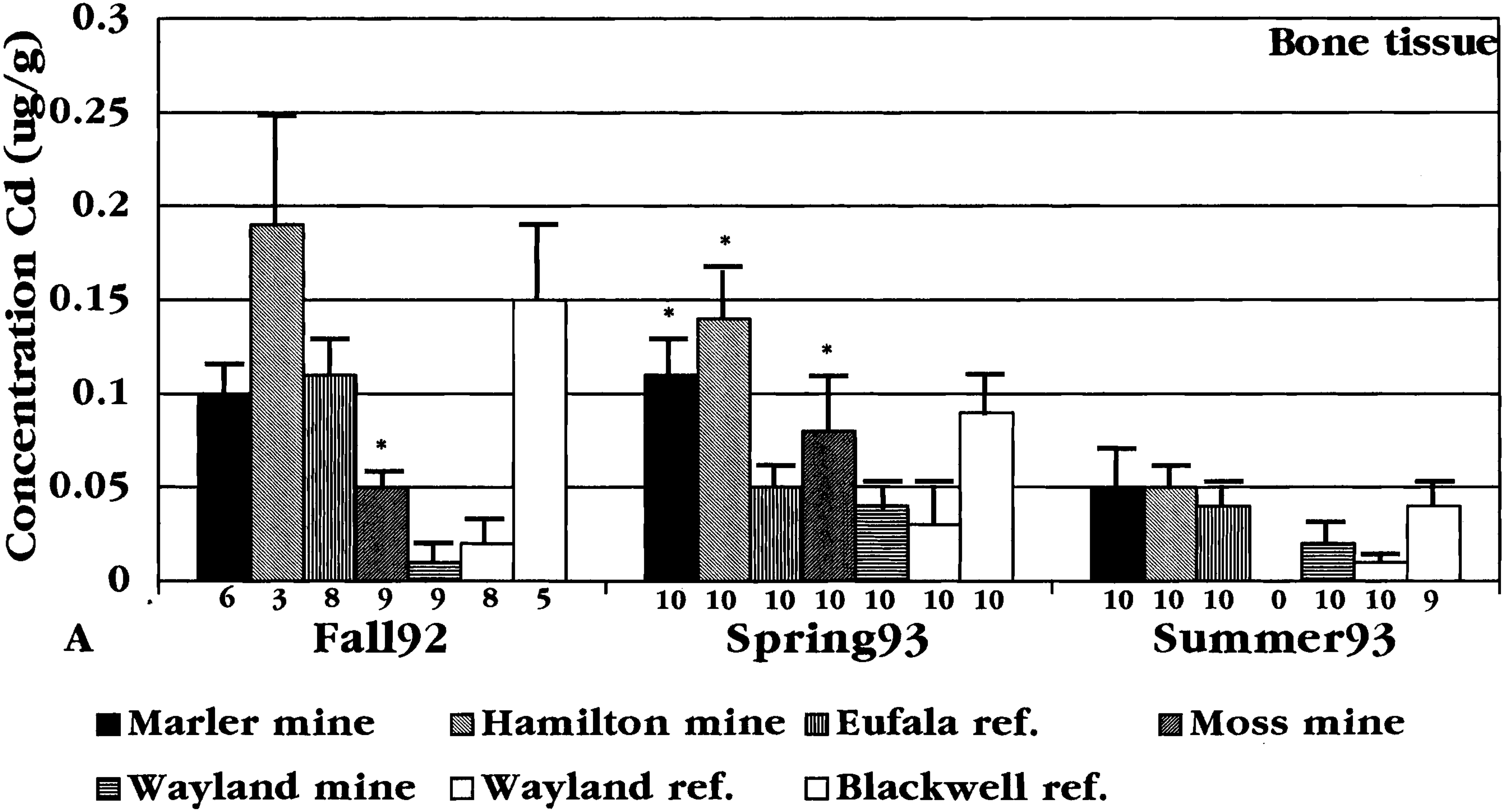
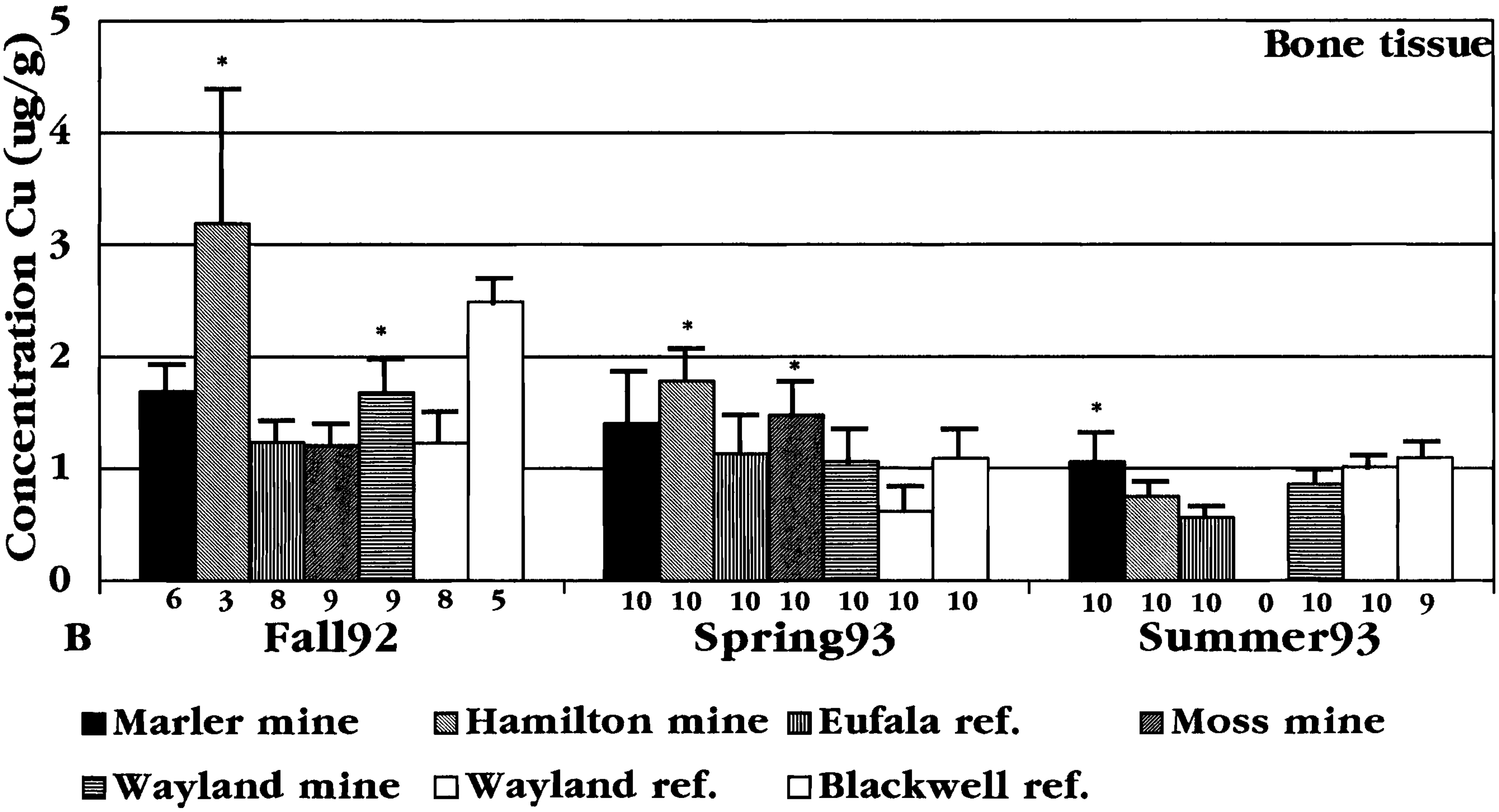
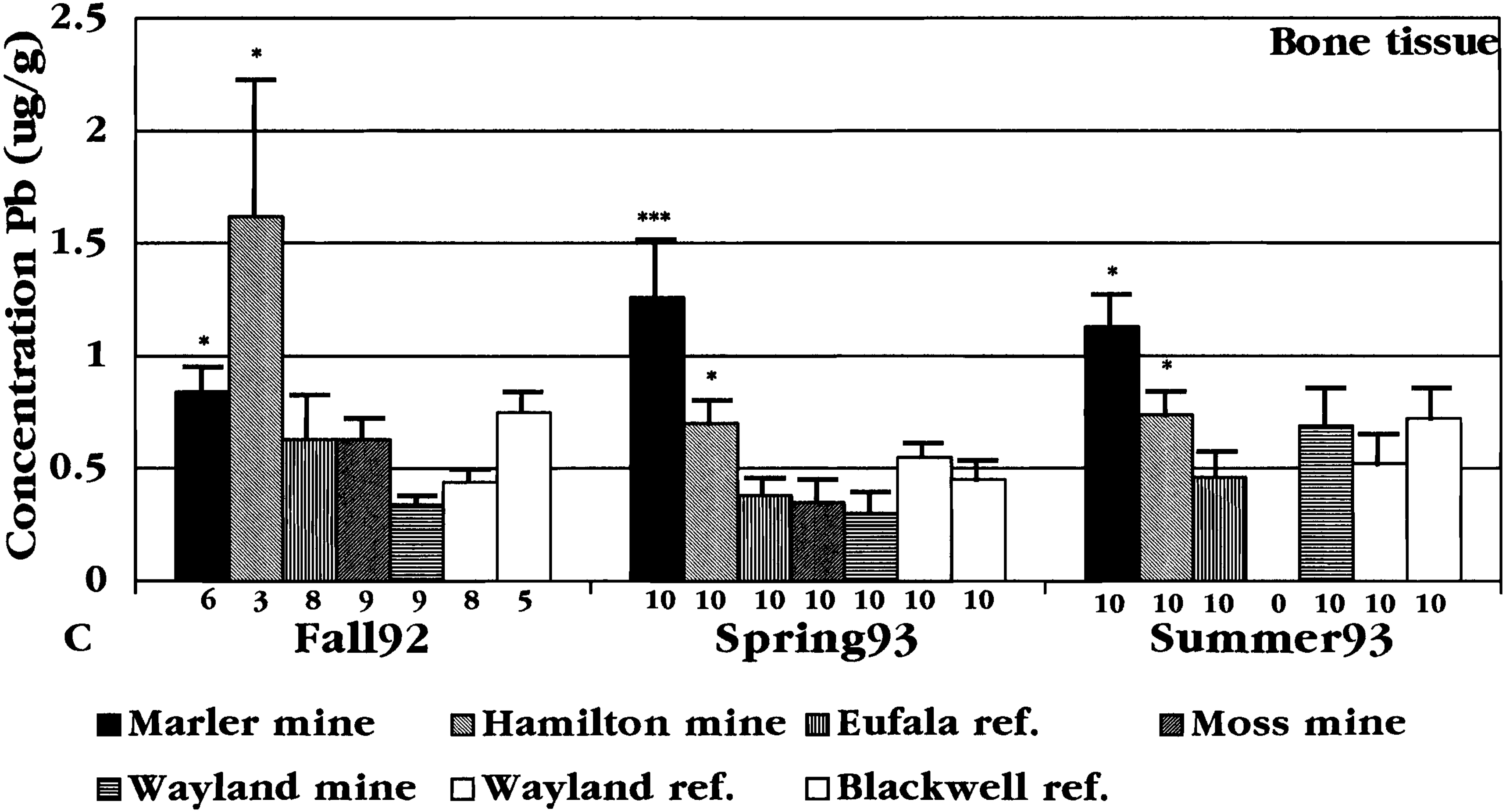
▩ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.


% Cd:Zn Ratio, Kidney
60
50
40
30
20
10
0
*
*
*
**
**
**
***
**
**
***
**
**
**
*
**
**
**
24 27 21 20 20 20
20 20 20 20 20 20 20
12 0 18 19 20 17 18
21 20 20 20 20 20 20
20 20 20 0 20 20 18
B
Spring92
Summer92
Fall92
Spring93
Summer93
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▩ Moss mine
▤ Wayland mine
☐ Wayland ref.
☐ Blackwell ref.


0.2
0.15
0.1
0.05
0
% Cd:Zn Ratio, Bone
*
*
6
3
8
9
9
8
5
10
10
10
10
10
10
10
10
10
10
0
10
10
9
C
Fall92
Spring93
Summer93
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▩ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.

Bone tissue
Concentration Cd (ug/g)
0.3
0.25
0.2
0.15
0.1
0.05
0
*
*
*
*
6 3 8 9 9 8 5
10 10 10 10 10 10 10
10 10 10 0 10 10 9
A
Fall92
Spring93
Summer93
■ Marler mine
▧ Hamilton mine
▥ Eufala ref.
▨ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.


Concentration Cu (ug/g)
0
1
2
3
4
5
Bone tissue
*
*
*
*
*
*
6 3 8 9 9 8 5
10 10 10 10 10 10 10
10 10 10 0 10 10 9
B
Fall92
Spring93
Summer93
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▨ Moss mine
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.


C
Concentration Pb (ug/g)
2.5
2
1.5
1
0.5
0
Bone tissue
*
*
***
*
*
*
6 3 8 9 9 8 5
10 10 10 10 10 10 10
10 10 10 0 10 10 10
Fall92
Spring93
Summer93
■ Marler mine
▧ Hamilton mine
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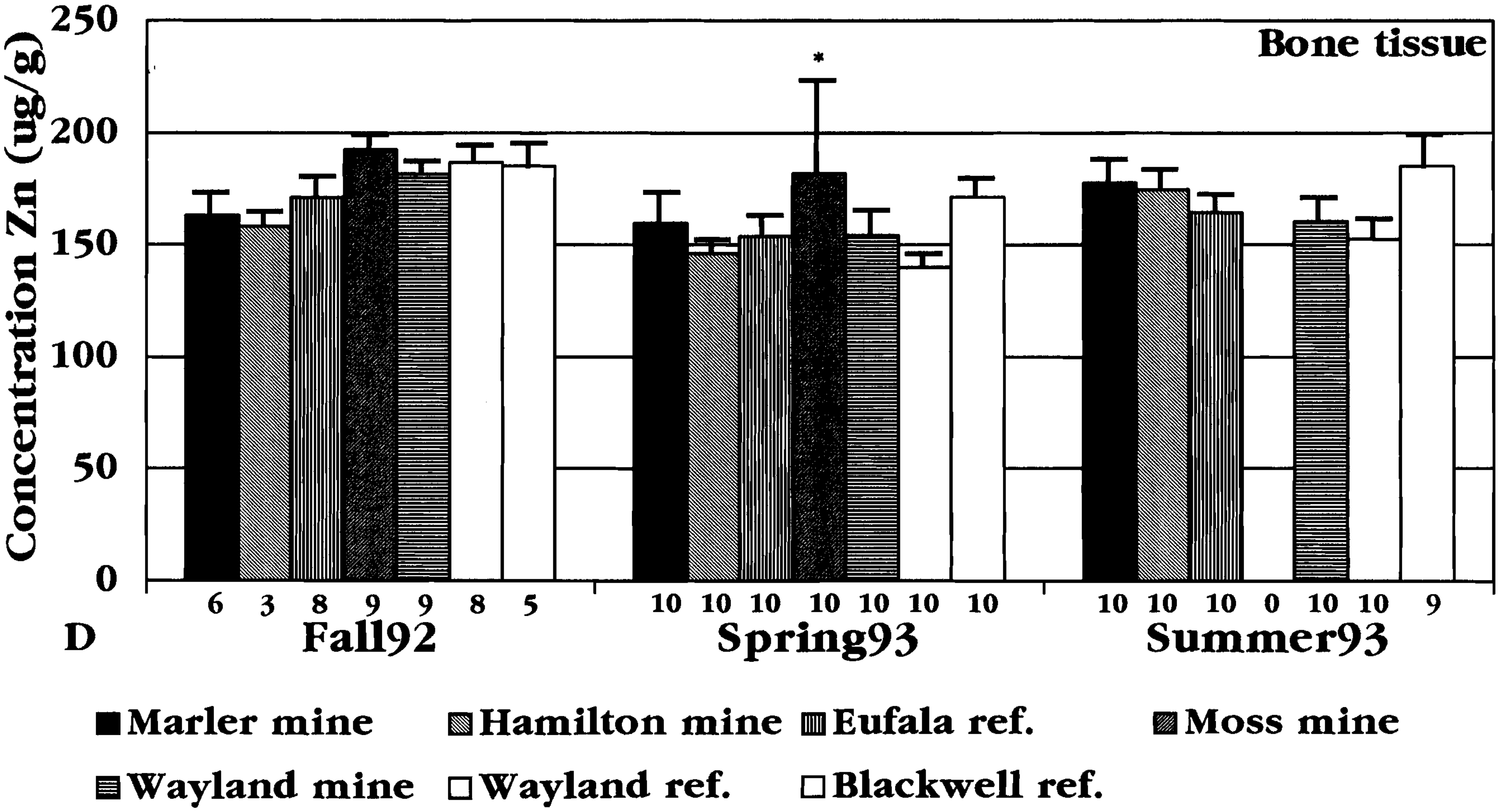
▤ Wayland mine
□ Wayland ref.
□ Blackwell ref.


Bone tissue
Concentration Zn (ug/g)
250
200
150
100
50
0
*
6 3 8 9 9 8 5
10 10 10 10 10 10 10
10 10 10 0 10 10 9
D
Fall92
Spring93
Summer93
■ Marler mine
▨ Hamilton mine
▥ Eufala ref.
▩ Moss mine
▤ Wayland mine
☐ Wayland ref.
☐ Blackwell ref.

VITA

John S. Hausbeck

Candidate for the degree of

Master of Science

Thesis: ANALYSIS OF TRACE METAL CONTAMINATION OF COAL STRIPMINES AND BIOACCUMULATION OF TRACE METALS BY *PEROMYSCUS LEUCOPUS.*

Major Field: Zoology

Biographical:

Personal Data: Born in Saginaw, Michigan, On December 19, 1969, the son of Jerry and Mary Alice Hausbeck.

Education: Graduated from Dwight D. Eisenhower High School, Saginaw, Michigan in June 1988; received Bachelor of Science degree in Biology and Fisheries and Wildlife Management from Lake Superior State University, Sault Ste. Marie, Michigan in May 1992. Completed the requirements for the Master of Science degree with a major in Zoology at Oklahoma State University in May 1995.

Professional Experience: Conducted volunteer research at Shiawassee National Wildlife Refuge, Saginaw, MI; employed as a research assistant by the Biology and Chemistry Department, Lake Superior State University; employed by Oklahoma State University, Department of Zoology as a research and teaching assistant, July 1992 to present. Research experience includes live trapping small mammals, the Standard Metaphase Chromosome Aberration Assay, and trace metal analysis of soil and biological tissue by atomic absorption spectrophotometry.

Professional Memberships: American Society of Mammalogists, Society of Environmental Toxicology and Chemistry.