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Metal Accumulation In Soils, Invertebrates, And Whiptail Lizards In The Northern Chihuahuan Desert

Allyson Benson-Pedraza
University of Texas at El Paso

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ALLYSON BENSON-PEDRAZA
Master's Program in Biological Sciences

APPROVED:

Vicente Mata-Silva, Ph.D., Chair

Jerry D. Johnson, Ph.D.

Lin Ma, Ph.D.

Stephen Crites, Ph.D.
Dean of the Graduate School

DEDICATION

I would like to dedicate this work to my parents, Donald and Yolanda Benson, brother, Derek Benson, and to my husband, Ivan Pedraza who have provided their continued love and support throughout my academic career. Thank you for always encouraging me to be the best I can be, and to always follow my dreams.

METAL ACCUMULATION IN SOILS, INVERTEBRATES, AND WHIPTAIL LIZARDS IN THE NORTHERN CHIHUAHUAN DESERT

by

ALLYSON BENSON-PEDRAZA, B.Sc.

THESIS

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ABSTRACT

Smelting activities by the American Smelting and Refining Company (ASARCO) lead to the substantial release of metal pollution into the El Paso, Texas, and border cities region. To-date few studies have examined metal accumulation within vertebrate taxa in these areas. As reptiles are currently experiencing population declines globally for a variety of reasons including pollution, lizards were chosen to be evaluated for trace element (As, Cu, Cr, Cd, Pb, and Zn) concentrations. These metals were measured within soils, invertebrate prey items, and the livers of three species of whiptail lizards (*Aspidoscelis*) from the un-remediated University of Texas at El Paso (UTEP) campus, and a control site, the Indio Mountains Research Station (IMRS), in Hudspeth County, TX. Invertebrate and lizard samples were collected from each study site over the course of two field seasons in 2020 and 2021. Soils were analyzed for metal concentrations using a portable x-ray fluorescence (p-XRF) machine and underwent pH testing to address metal bioavailability. Inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS) was used to analyze metals within pooled invertebrate groups and individual lizard livers. Soils demonstrated persistently high metal concentrations at UTEP, whereas IMRS exhibited significantly lower As, Cu, Pb, and Zn concentrations. Soil pH across all study sites were found to be alkaline (pH $<$ 7) indicating a reduced potential for metal bioavailability. Metal concentrations observed in each trophic level were highest in soils as compared to invertebrates and whiptail lizard livers, apart from cadmium which was highest in lizard livers. Invertebrate samples collected for this study included the primary diet items of whiptail lizards which include the orders Orthoptera, Coleoptera, Araneae, Isoptera, and the family Cicadidae. Metal concentrations within invertebrates varied by metal, taxonomic group, and study site. Among the whiptail lizards only *A. neomexicana* was encountered at the UTEP site, while *A. marmorata* and

A. tesselata were found at IMRS. Lizard liver concentrations of As, Cd, Cu, and Pb at UTEP were significantly higher than at IMRS. Generally, at the UTEP site whiptail lizards exhibited lower concentrations of metals than invertebrates, with the exception of lead and cadmium. Overall, this study demonstrated how whiptail lizards inhabiting a historically polluted urban Chihuahuan Desert environment are accumulating higher quantities of toxic metals as compared to their remote counterparts. Further research is necessary to understand the full extent of the physiological, biological, and ecological impacts that this metal pollution event has caused whiptail lizards to endure in the El Paso, TX region.

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INTRODUCTION

Global ecosystems and the organisms within them have been severely impacted by the release of anthroprogenic pollutants into the environment. Sources of these toxic pollutants include products such as pesticides and herbicides released during agricultural and landscaping practices, as well as naturally occurring metals that are largely brought to the surface from mining and smelting activities (Sparling, 2016a). Records of metal pollution dates back to the Roman Empire with the advent of ancient mines and smelters. In the 18th century it became a widespread issue with the beginning of the Industrial Revolution where the development of furnaces with tall stacks lead to higher demand for metals and consequently increased metal emissions (Nriagu, 1990, 1996). Today, metal emission regulations vary by country but industrial practices that cause metal pollution still persist. With increases in the human population, urbanization, technological advancements, and agricultural expansion, pollutants continue to be released into the environment (Tilman et al., 2001; Izatt et al., 2014). Furthermore, metal pollutants have known roles as carcinogens and enzyme diruptors, and a number of other toxic properties are still being discovered (Sparling, 2016a). The study of toxicology aims to address these issues as well as the effects that toxicants have on organisms, including humans, to develop remediation potential (Singh, 2012). Ecotoxicology, defined as the toxic effects agents can have on living organisms, populations, and communities, merges the study of ecology and toxicology (Connell et al., 1999; Sparling, 2016b). Metals as environmental pollutants have been examined extensively in ecotoxicological studies, ranging from the analysis of soils, plants, and invertebrates, to various vertebrate taxa (Heikens et al., 2001; Peralta-Videa et al., 2009; Sparling et al., 2010). Metals are naturally found in the lithosphere and are often brought to the earth's crust by volcanic activity (Sparling, 2016a). However, the mining and smelting of metals have become the main source of these metals in the environment (Sparling, 2016a). Heavy metals are considered those that have higher molecular weights and densities than iron and include lead, copper, zinc, chromium, cadmium, nickel, and mercury (Sparling, 2016a). Metal bioavailability is a complex process which is dependent on three major factors. The environmental availability which is the amount of metal that is able to dissolve from the soil matrix into pore water, the environmental bioavailability, the amount of dissolved metal within the water that may be taken up by plant roots or other organisms, and lastly, the toxicological bioavailability, the amount of metal that can then be physiologically bioaccumulated within the organism (Kim et al., 2015). Many physio-chemical factors of the environment as well as the type of metal and metal species influence the potential bioavailability and fate of each metal within the environment (Ali et al., 2019). In low quantities some metals like copper and zinc are beneficial to plant life by providing them with essential micronutrients, however, when found in higher quantities these metals become toxic (Peralta-Videa et al., 2009). Other metals such as arsenic, lead, cadmium, and chromium are considered harmful to both plant and animal life regardless of their quantities (Peralta-Videa et al., 2009). Another issue with metals includes their persistence in the environment. Metals are inorganic, have high melting points, and do not break down easily (Sparling, 2016b). Consequently, as metals are toxic and can persist in the environment for long periods of time, it is important to consider their impacts on particularly vulnerable organisms.

Many reptile populations are currently declining globally for a variety of reasons, most of which are anthropogenic, including habitat loss, climate change, disease/parasitism, invasive species, and environmental pollution (Gibbon et al., 2000; Saha et al., 2018). A polluted environment can have both direct and indirect impacts on reptiles. Pollution that is bioaccumulated can directly affect the health and overall fitness of an organism, and indirectly affect organims by altering the biotic and abiotic environment (Sasaki et al., 2016). In comparison to other classes of organisms, reptiles, and especially squamates (snakes and lizards), are considerably understudied in ecotoxicology. A survey of 17,375 animal contaminant-related papers from 1996-2008, revealed that only 0.8% (152) were on reptiles (Sparling et al., 2010). As reptiles play important roles in ecosystems, it is crucial to understand the factors that are contributing to their decline. Squamates are a highly diverse group of reptiles that can provide a central link in ecosystems and also are crucial prey items for larger predators (Campbell and Campbell, 2002; Gardner, 2005). This middle trophic status occupied by squamates may result in the bioaccumulation of pollutants released into the environment and can lead to the biomagnification of pollutants to predator species (Todd et al., 2010). Reptiles experience the most considerable exposure to pollutants through trophic transfer of contaminated food and respiration (Grillitsch and Schiesari, 2010). While these are the known main routes of exposure, it is currently unknown whether the fate of the metal relies on one of these routes specifically (Grillitsch and Schiesari, 2010). It has been demonstrated in several laboratory studies that the ingestion of contaminated prey leads to significant metal accumulation within reptile predators thus indicating that trophic transfer is an important route of exposure (Grillitsch and Schiesari, 2010). Trophic transfer of pollutants in squamates has been demonstrated in several studies performed either in a historically polluted field setting or within simulated laboratory experimentation (Grillitsch and Schiesari, 2010). These mechanisms of metal uptake can vary with the ecology of each group of organisms. Lizards for example, vary in foraging behavior (active forager vs sit-and-wait forager), diet (generalist vs specialist and herbivorous vs carnivorous/insectivorous), and reproductive features (oviparous, viviparous, parthenogenetic, gonochoristic) (Gardner, 2005). In oviparous (or egg-laying) species, maternal transfer of metals could occur through the internal environment including during ovulation and fertilization, and from deposition to hatching in the external incubation environment (Gardner, 2005; Grillitsch and Schiesari, 2010). Overall metal bioaccumulation strongly depends on various ecological and biological factors that will further determine the burden put on the lizard.

There are several potential fates of a metal once it has entered an organism. In vertebrate species, organotropism occurs when specific metals accumulate in specific organs and tissues. Once metals enter the blood stream, they can become distributed into tissues and cells via unselective carrier systems such as albumin for zinc and cadmium, and metallothionein for zinc, cobalt, copper, silver, cadmium, and mercury (Grillitsch and Schiesari, 2010). Metallothionein is a peptide that can be found in all tissues, yet has the greatest potential for induction in organs associated with metal processing such as the liver, kidneys, and intestine (Grillitsch and Schiesari, 2010). Previous research on reptiles demonstrated that the liver and kidneys do accumulate the highest amounts and diversity of metals (Hopkins, 2005; Grillitsch and Schiesari, 2010). Additionally, chronic exposure to high amounts of lead and strontium resulted in the accumulation within muscle tissue and bone (Hopkins, 2005; Grillitsch and Schiesari, 2010). Accumulated metals may also be bio-transformed resulting in either toxification or detoxification of the metal which is dependent on changes in oxidation states (Grillitsch and Schiesari, 2010). Bioaccumulation of metals has been documented in a number of different studies covering several groups of reptiles including snakes and lizards. Cadmium, even in small amounts, has been found to be toxic to the Wall Lizard (*Podarcis sicula*) as it accumulated in the liver, kidneys, ovaries, and brain (Trinchella et al., 2006). Another study looking at cadmium, lead, and zinc concentrations in wild caught Bosc's Fringe-toed Lizards (*Acanthodactylus boskianus*) from polluted sites found varying results of metal concentrations within stomach contents, liver, kidney, and tail tissues. This study showed that lead accumulated most in the kidneys followed by the tail and then liver; cadmium accumulated most in the liver followed by the kidneys and then tail; and zinc accumulated most in the kidneys, followed by the tail and then liver (Nasri et al., 2017). These results reveal the potential for metals to accumulate differently within these tissues which is important to consider when trying to determine the fate of metals within lizards. While metals that accumulate in organs can and often do have detrimental and long-lasting effects on the body, they may not stay within the organism permanently. Generally in reptiles, metals can potentially be eliminated in the form of feces, eggs, and shed skin (Burger, 1992; Hopkins et al., 2001; Xu et al., 2006). Reptile skin is high in keratin and is known to have metal-binding capabilities and could therefore help regulate the burden of certain metals on the body (Grillitsch and Schiesari, 2010). As previously stated, maternal transfer of metals has been shown to occur in reptiles and as such, represents another form of elimination from the mother. Whether they can be eliminated or not, the impacts of metal pollution on reptiles as well as within their food chain and environment needs further examination, as pollutants are one of the major drivers of their decline (Gibbon et al., 2000). It is also important to consider that reptiles could be enduring multiple stressors simultaneously, such as disease, parasitism, habitat loss, etc., alongside metal pollution that could have further negative impacts on their populations (Gibbon et al., 2000; Campbell and Campbell, 2002).

The City of El Paso, situated in far west Texas, previously had a smelting plant from 1887 to 1999 known as The American Smelting and Refining Company (ASARCO). Located in western El Paso near the Rio Grande (also the international United States/Mexico border), ASARCO released over 1,000 tons of lead, 1 ton of arsenic, and 573 tons of other heavy metals into the region from 1969-71 alone (Perales, 2008). This facility smelted copper, lead, and zinc; in which zinc smelting activities ceased in 1982, lead in 1985, and copper in 1999 when the facility was shut down (Pingitore Jr. et al., 2005). The smoke stacks were eventually demolished in 2013 (Robinson, 2017). Remediation both onsite and offsite began in the early 2000's but was limited to mostly residential areas within a three mile radius from the site and largely occurred on the U.S. side of the border (Darby, 2012). Adults were excluded from the population of concern and offsite cleanup was mostly addressed to areas with children. The University of Texas at El Paso was not included for cleanup due to the low chance of children experiencing exposure, even though it is near the smelting site and exhibited high quantities of metals in soils (Darby, 2012). Considering the quantities of metals released, several studies were performed to evaluate the environmental impacts these metals have had locally. Copper, lead, zinc, arsenic, cadmium, and chromium concentrations have been evaluated within soils in the ASARCO area, and all have been detected in high quantities at the surface confirming smelting activities as the source of contamination (Barnes, 1993). Many studies have explored the aftermath of the local smelting by examining not only soil, but also air quality, plant and animal life, and of course humans. Nearby flora experienced a reduction in species richness as a result of this severe pollution (Worthington, 1989; Mackay et al., 1998). The Creosote Bush (*Larrea tridentata*) growing closest to the smelting facility exhibited the highest concentrations of lead while those furthest from the smelter had decreased amounts (Robinson, 2017). Creosote Bush within these areas had the highest metal quantities within the roots as compared to the stems and leaves, suggesting the roots as the primary route of bioaccumulation (Polette, 1997; Sharma and Dubey, 2005). Invertebrates in the ASARCO area have also been studied for metal bioaccumulation. Rough Harvester Ants (*Pogonomyrmex rugosus*) collected near the ASARCO area were found to contain high metal concentrations compared to those collected farther away, thus indicating that metal bioaccumulation through trophic transfer is occurring (Del Toro et al., 2010). Another study near the ASARCO area examined arsenic, lead, and cadmium concentrations in four different trophic levels including Creosote Bush (*L. tridentata*), herbivorous ants *(P. rugosus)*, detritivorous beetles (*Eloedes sp*.), and carnivorous scorpions (*Centruroides vittatus)* (Mackay et al., 1997). In this study, the beetles exhibited the highest metal concentrations, and metals did not appear to bioaccumulate in the scorpions (*C. vittatus*) (Mackay et al., 1997). Nonetheless, bioaccumulation of heavy metals in local vertebrate species remains unknown.

Due to El Paso having experienced heavy metal pollution, it is important to address the effects these metals have had on local species, including their continued potential bioavailability to squamates within the area. The main objective of this study was to assess the current concentrations of heavy metals in a food chain containing soils, invertebrates, and lizards living in Chihuahuan Desert landscapes that have been both historically polluted and undisturbed. The University of Texas at El Paso Campus was regarded as the polluted site and the Indio Mountains Research Station-Hudspeth County, Texas was considered the undisturbed site. Sampling from the Indio Mountains Research Station allowed for the determination of heavy metal concentrations found in components of Chihuahuan Desert ecosystems regarded as undisturbed and thus served as a control site. The heavy metals of focus include lead, copper, chromium, cadmium, arsenic, and zinc as that they have been found in high concentrations as a result of local smelting activities (Barnes, 1993; Pingitore Jr. et al., 2005). Heavy metal concentrations in soils, invertebrates, and lizard livers were expected to be higher in samples collected from the urban site (UTEP) as compared to the remote site (IMRS). Among the samples, metal concentrations were expected to be highest in soils as compared to invertebrates or lizards. These results were predicted as metals are known to persist in the environment for long periods of time (Wu et al., 2016) but are not always bioavailable for uptake, UTEP is in close proximity to the old ASARCO smelting facility, and previous research indicates that metals have bioaccumulated in plants and several groups of invertebrates in the ASARCO area (Mackay et al., 1998; Del Toro et al., 2010; Robinson, 2017).

MATERIALS AND METHODS

Study Sites

The study sites included The University of Texas at El Paso (UTEP) campus, specifically UTEP owned property behind the Student Recreation Center, and the Indio Mountains Research Station (IMRS) (See Figure 1). The UTEP Campus is located on the westside of El Paso TX, within El Paso County. The UTEP sampling area covers approximately 36 hectares and has an elevation of around 1,200 m (Google Earth; accessed on 19 August 2020). Geologically, the UTEP area is characteristic of andesitic plutons dating back to the Tertiary with inclusions of shale, limestone, quartz from the Cretaceous, and masses of hornblend, hornblend-mica, and hornblend-biotite-feldspar with debated origin (Hoffer, 1970). The vegetation at this site is characteristic of Chihuahuan Desert scrubland, although is noticeably sparse, containing mainly Creosote Bush (*Larrea tridentata*), Ocotillo (*Fouquieria splendens*), Yucca (*Yucca sp.*), and Lechuguilla (*Agave lechuguilla*) (Hoffer, 1970), and various other shrubs. Eleven pit-fall traps as the main route of lizard capture were established at UTEP in May 2020 and five more were added in February 2021 (See Figure 2a & 2b).

The IMRS site is comparably larger containing approximately 16,187 hectares and is located ~40 km south of Van Horn, Texas in Hudspeth County, with an elevation range of 900-1600 m (Worthington et al., 2022).The Indio Mountains began their formation in the Cretaceous period and consists of sandstone, limestone, and conglomerate rock, as well as volcanic tuffs and trachytes (Worthington et al., 2022). The vegetation at IMRS is also characteristic of Chihuahuan Desert scrubland including the aforementioned species as well as Catclaw Acacia (*Senegalia greggii*), Western White-Thorn Acacia (*Vachellia constricta*), Honey Mesquite (*Prosopis glandulosa*), and various other cacti such as the Prickly Pear Cactus (*Opuntia sp.*) and various grasses including the Tobosa Grass (*Hilaria mutica*) and Black Gramma (*Bouteloua eriopoda*) (Mata-Silva et al., 2013; Franco, 2015). IMRS was acquired by UTEP in 1937 with the primary intent of conducting biological and geological research (Worthington et al., 2022). Attempts of mining ore from the research station occurred in the 1900's, however, the initiative was abandoned as no significant sources of ore were found and did not outweigh the costs of transportation (Worthington et al., 2022). The IMRS site was regarded as a non-polluted control site for this study considering its remoteness, no processing of ore occurred, and all activities remained exploratory. At IMRS, 78 pit-fall traps were used from two subsites: Ranch House (RH, headquarters of the station) and Prospect Pits (PP, ~ 1 km SE of RH) (See Figure 3a & 3b).

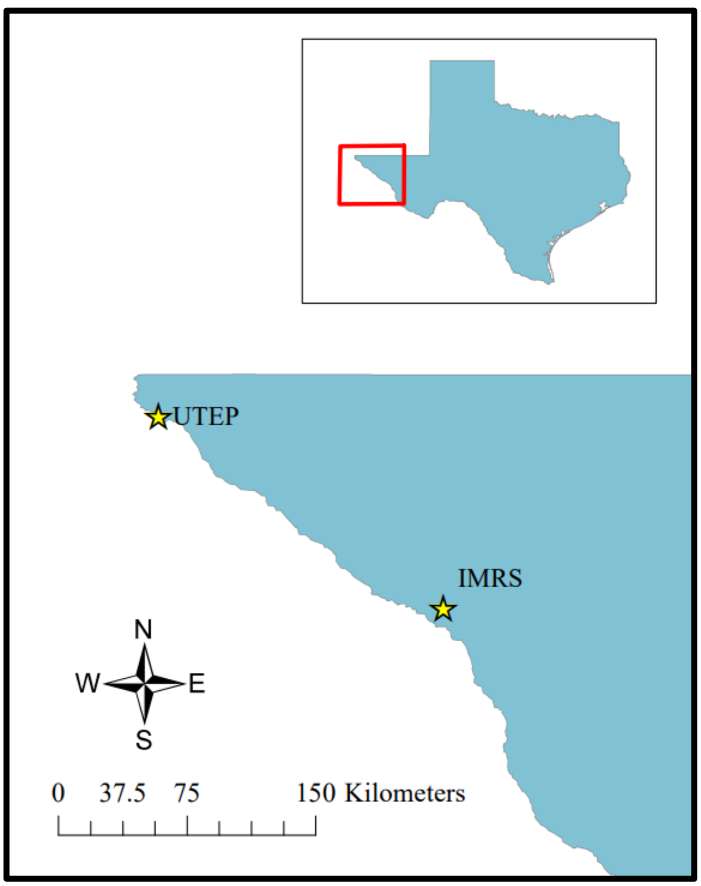

Figure 1. Map depicting study sites: The University of Texas at El Paso (UTEP) in El Paso County, and the Indio Mountains Research Station (IMRS) in Hudspeth County.

Study Organisms

This study focused on three lizard species of the genus *Aspidoscelis, A. tesselata, A. marmorata*, and *A. neomexicana*. *Aspidoscelis tesselata* and *A. marmorata* are local to both proposed study sites, while *A. neomexicana* is only present at the UTEP site. *Aspidoscelis tesselata* and *A. neomexicana* are all-female parthenogenetic species, while *A. marmorata* is gonochoristic containing males and females. Lizards in the genus *Aspidoscelis* often occur sympatrically, are all active foragers, and exhibit similar dietary patterns (Scudday and Dixon 1973; Mata-Silva et al., 2013). These lizards are also known to feed opportunistically and may exhibit seasonal variation in diet in response to wetter or dryer conditions (Scudday and Dixon 1973). Previous diet studies on Whiptail Lizards have shown that the main components of their diet includes Isopterans, Orthopterans, Coleopterans, Araneaens, and Cicadas (suborder of Hemipterans) (Scudday and Dixon, 1973; Paulissen et al., 2006; Mata-Silva et al., 2013). All target species of interest are abundant within their range and are currently evaluated as Least Concern according to the International Union for Conservation of Nature's Red List of Threatened Species (IUCN Red List) with the exception of *A. marmorata* which has not been evaluated (Hammerson et al., 2007, 2019). Metal bioaccumulation studies within this genus have not yet been performed. As these lizards are abundant and exhibit very similar dietary patterns, they present a unique opportunity to examine the potential effects of long-term metal exposure from an urban population of whiptail lizards as compared to its remote counterpart.

Invertebrates have been identified as reliable bioindicators of pollution, and therefore can be examined to determine levels of contamination throughout time and subsequently reveal pollutant persistence and mobility (Hansson et al., 2019). As the physiological, behavioral, and dietary features of invertebrates are highly variable, the potential for specific metal bioaccumulation may be higher for certain groups of invertebrates than others (Heikens et al., 2001; Gall et al., 2015). The invertebrates of focus for this study include termites (Isoptera), grasshoppers (Orthoptera), beetles (Coleoptera), cicadas (Hemiptera), and spiders (Araneae) as they are the groups that are most consumed by the target lizards (Scudday and Dixon, 1973; Paulissen et al., 2006; Mata-Silva et al., 2013).

Each invertebrate group of focus for this study exhibits unique potential pathways for the bioaccumulation of metals from the environment. Subterranean termites, local to the area, play a crucial role in the Chihuahuan Desert as they aid in the decomposition of plants and wood material as well as perform nutrient cycling (Whitford et al., 1982). As detritivores, termites ingest and process soils and plant material (mostly wood) and therefore exhibit a potential for metal uptake via substrate and plant material. Spiders vary considerably in ecological characteristics such as web building, habitat type, size, diet, and others. These features as well as age (juvenile vs adult) have been found to influence metal bioaccumulation within this taxonomic group (Hansson et al., 2019). Wolf spiders are active hunters and have been proposed as useful bioindicators and bio-monitors of pollution as their frequent movement would expose them to a range of contaminants. This ecological feature can provide a better representation of the condition of the environment rather than relying on single-point soil analysis (Hansson et al., 2019). In a study comparing metal concentrations in two different species of spiders which varied in web usage, higher quantities of metals were found in the actively hunting wolf spider (*Pardosa lugubris*) than in the web-building spider (*Agelena labyrinthica*) (Wilczek et al., 2004). Spiders occupy a higher trophic status as carnivorous invertebrates and could bio-magnify metals to other predator species. Orthopterans such as grasshoppers, katydids, and crickets, are largely herbivorous and can exhibit several different dietary patterns such as monophagy, oligophagy, and polyphagy (Joern, 1979). Various studies demonstrated evidence of heavy metal bioaccumulation within the order, examining biomarkers, DNA damage, and enzyme activities associated with metal exposure (Warchałowska-Śliwa et al., 2005; Yousef et al., 2010). Some Orthopterans are highly specialized like the Creosote Bush Grasshopper (*Bootettix argentatus*) which strictly feeds on the Creosote Bush (Chapman et al., 1988). Beetles are another highly diverse order of invertebrates, varying in diet, size, habitat, and behavior (Heikens et al., 2001). Some invertebrate groups in the El Paso/ASARCO area have already been examined for heavy metal concentrations and the results of each study indicate varying signs of metal bioaccumulation occurring (Mackay et al., 1997; Del Toro et al., 2010). In this study, metal concentrations examined within the target invertebrates provides insight on whether present populations have experienced bioaccumulation of heavy metals and transferred them to the target lizard species.

Soil sampling

Metal concentrations within soils were measured at two depths to monitor downward travel of metals from the surface. Depths chosen for metal analysis included 0 cm (surface) and 10 cm below the surface. Soil samples were taken from randomly generated GPS points within polygons created in QGIS™ at the IMRS and UTEP campus locations (See Figure 2a & 2b and Figure 3a & 3b). At each point and at each depth, *in situ* metal concentrations were measured by two 60-second readings of a Portable X-Ray Fluorescence (P-XRF) machine. A plastic shovel was used to dig out the soil and a ruler was used to make accurate depth measurements. Metal Portable X-Ray Fluorescence was chosen as it is a practical new technique for rapid and reliable analysis of heavy metals within soils. A recent study compared the use of p-XRF equipment to the use of mass spectrometry on heavy metal analysis in soils and found that the p-XRF was just as accurate as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Pb, Cu, and Zn, and Atomic Fluorescence Spectrometry (AFS) for As (Wan et al., 2019). Using the p-XRF as an *in situ* soil screening procedure for heavy metals can be achieved by placing the p-XRF in the standing position with the probe window placed flatly in direct contact with the soil according to Method 6200 (U.S. EPA, 2007a). Previous research performed in the El Paso and ASARCO area have found that metals are found in higher concentrations within the first 2.5 cm of surficial soil, confirming the source as the smelting facility rather than a natural source (Barnes, 1993). Therefore, it was determined that using the p-XRF *in situ* would be sufficient for the goals of this project. A corer was also used to collect soils at depths of approximately 0-10 cm and 0-20 cm to be analyzed for pH as this factor often influences the toxicity and bioavailability of metals (Sparling, 2016a; Wan et al., 2019), as well as their retention (Harter, 1983). Soils from each level were placed into individual plastic bags and taken to the laboratory for pH testing. Soils were filtered using a 2 mm sieve and weighed to achieve 15 g. Then 30 ml of distilled water (DI) were added (or a 1:2 ratio of soil:DI water when 15 g could not be achieved) and stirred. The sample then rested for 30 minutes, was stirred again and the pH was measured using Hach HQ430d flexi pH meter. Prior to pH testing the pH meter was calibrated with a certified buffer solution pH 7 and a certified buffer solution pH 10.

Invertebrate Sampling

As the main diet of the target lizard species consists of the orders Isoptera (termites), Araneae (spiders), Coleoptera (beetles), and Orthoptera (grasshoppers, katydids, and crickets), and the family Cicadidae (cicadas) (Scudday and Dixon, 1973; Paulissen et al., 2006; Mata-Silva et al., 2013), all encountered specimen were collected as there is no known specific species in any of these target groups that the whiptail lizards specialize on. While there is a range and variety of invertebrates being used for this study, they all represent the most numerous and largest volumes of the diet of each whiptail species. These orders of invertebrates were collected from all study sites upon encounter. Target invertebrates were removed from established pit-fall traps, captured by hand, or with a net. Collected specimens were placed in sterilized containers and transported to the UTEP lab B220 for identification to the lowest taxonomic unit possible, rinsed with DI water, and frozen (at -20° C) until analysis.

Lizard Sampling

The three target species within the genus *Aspidoscelis* (*A. marmorata, A. tesselata,* and *A. neomexicana*) were collected from both study sites using pit-fall traps as the main route of capture. There were two subsites utilized at the Indio Mountains Research Station, Prospect Pits (PP) and Ranch House (RH), which exhibited a total of 13 transects and 78 useable pit-fall traps. At The University of Texas at El Paso, 11 pit-fall traps were established during the early summer of 2020 and five more were added in February of 2021 (See Figure 2a & 2b). Pit fall traps were opened during periods of highest lizard activity between May and August 2020 and 2021. Lizards were collected each morning, evening, and before trap closure, placed into individually sealed containers and taken to the lab B220 for respective processing. Each lizard was assigned a unique ID number and morphometric data was collected including snout-vent length, tail length, weight, sex, tail status (freshly broken, original, or regenerated), and any other general notes on the status of the lizard.

Lizards from both study sites were euthanized following methods approved by the UTEP IACUC (ID:1559526-2). An overdose of isoflurane was administered in an open drop method followed by decapitation and pithing (Leary et al., 2020). The chamber designed for euthanasia included a plastic tube, a small bottle, cotton balls, and tape. The cotton ball was placed inside the bottle and soaked with 0.5 cc of isoflurane. The rim of the bottle was then taped to the rim of the tube to secure it in place. The lizard was placed in the open side of the tube and secured. After the lizard had reached a surgical plane of anesthesia, it was removed from the tube and placed on a sterilized dissection board. All surgical equipment was sterilized with 70% ethanol and allowed to air dry prior to use. Lizards were decapitated using butcher scissors followed by pithing the vertebral canal to destroy spinal tissue and complete the euthanasia process. Following euthanasia, the lizard liver tissue was removed. The wet weight (in mg) was recorded for each whole tissue and was placed in an individual Eppendorf™ tube and frozen (at -20° C) until further analysis. Lizard bodies were fixed in 10% formalin, stored in 70% ethanol, and catalogued in the University of Texas at El Paso Biodiversity Collections (Catalogue numbers UTEP 22462-22534).

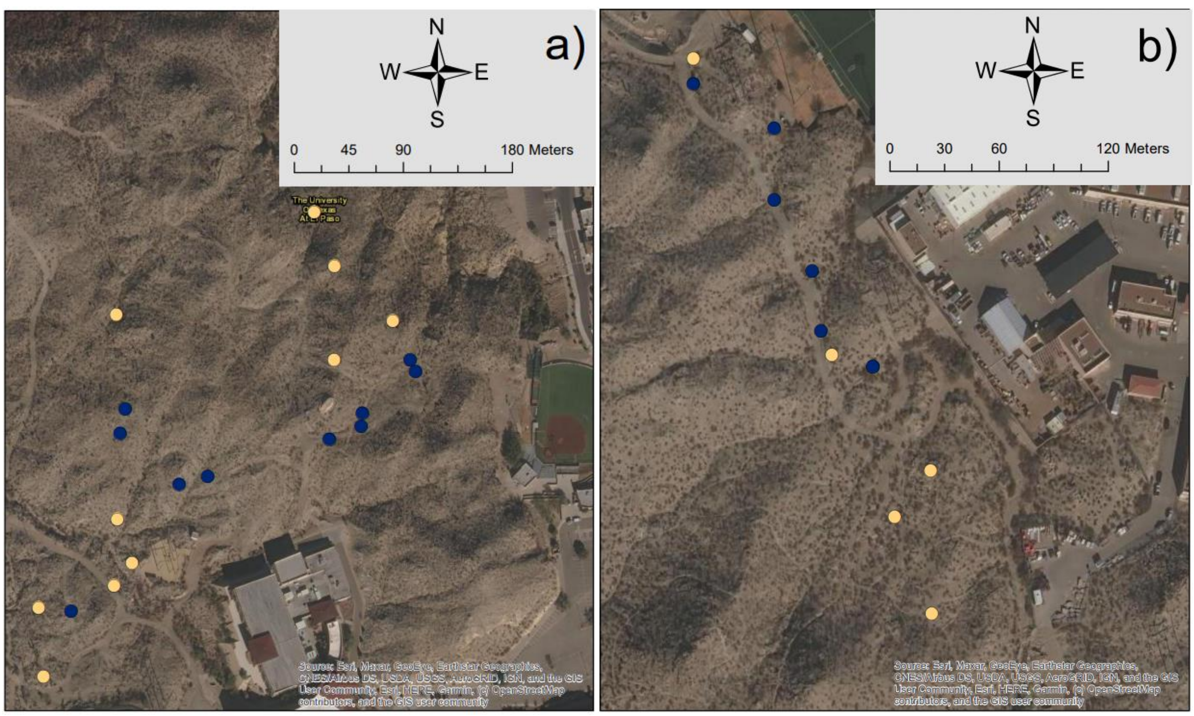

Figure 2. Pit-fall trap locations (blue) and Randomized GPS points for soil sampling (orange) on the UTEP campus (a) north and (b) south of the Student Recreation Center.

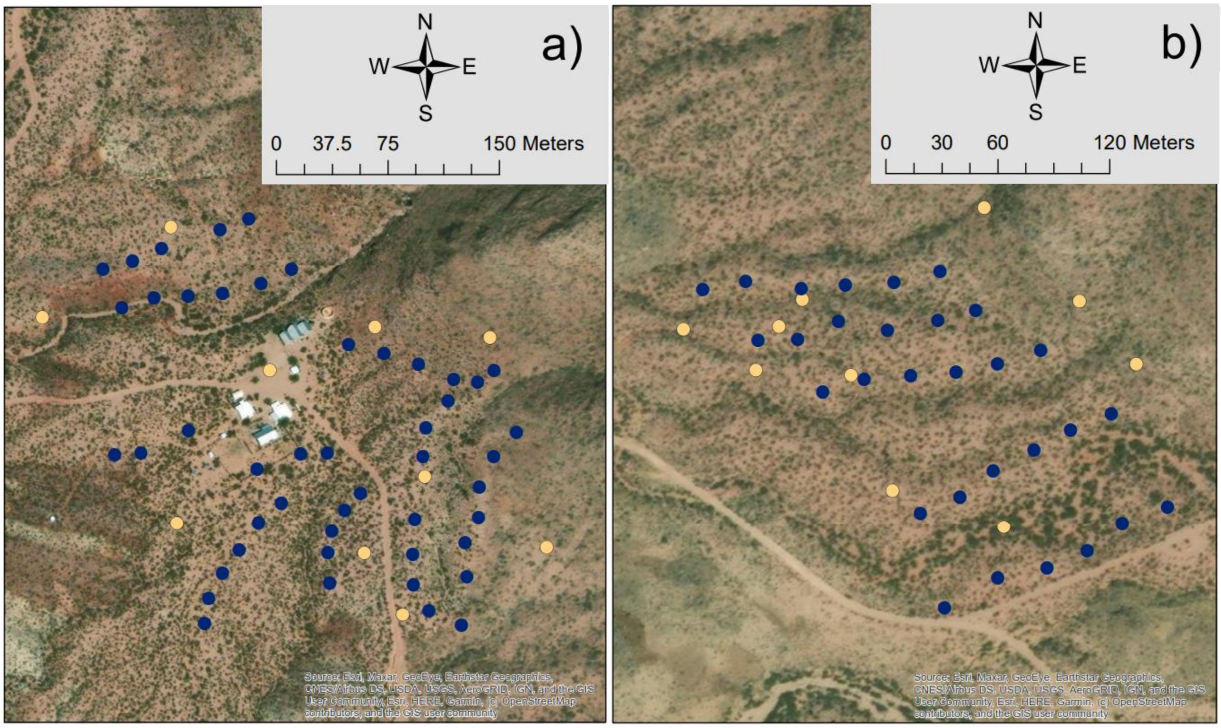

Figure 3. Pit-fall trap locations (blue) and Randomized GPS points for soil sampling (orange) at IMRS (a) Ranch House and (b) Prospect Pits.

Sample processing and metal analysis

Prior to metal concentration analysis, invertebrate and lizard samples were lyophilized to achieve the proper state for mass spectrometry analysis. For lizard livers, the lyophilization process was carried out individually; while for invertebrates, the samples were pooled by taxonomic group and site. Samples were weighed (in mg) within their Eppendorf tube, then frozen in a -80° C freezer for a minimum of 30 minutes. After this time, the samples were removed from the freezer, lids were removed, and a small piece of aluminum foil was placed tightly around each individual rim. Several small holes were made in the aluminum foil using a sterile needle to allow for moisture to escape during lyophilization. Samples were placed inside the lyophilizer's chamber set to auto settings, which achieved a temperature of around -100° C and a vacuum level of around 200 mT. Time for sample completion was determined by regularly checking samples after 24 hours and then again after 48 hours. It was determined that samples were completely lyophilized after approximately 48 hours from the start time. Once samples were complete, dry weights were then obtained and stored again in -20° C until metal analysis. Pooled invertebrate tissues needed to be further processed and were crushed into a powder using a mortar and pestle. In between each use the mortar and pestle were rinsed with Milli-Q water, gently scrubbed using Formula 409® all-purpose cleaner, rinsed again with Milli-Q, rinsed with 5% nitric acid, followed by a final rinse with Milli-Q water. The mortar and pestle were then allowed to dry before next use. Whole lizard livers and 300 mg of each invertebrate group were then analyzed for heavy metal concentrations via Inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS) at Brooks Applied Labs (BAL), Bothell, Washington. At BAL samples were acid digested following a modified EPA method 3050B protocol in which a mixture of concentrated nitric acid, hydrochloric acid, and hydrogen peroxide we used. Spiked blanks as well as standard reference material standards were also digested and analyzed for quality control purposes.

When data did not follow a normal distribution, non-parametric Kruskal-Wallis analysis and post hoc comparisons were performed to determine statistical differences in soils, soil pH, invertebrates, and lizard livers. In the cases that data were normal, an ANOVA and post hoc Tukey HSD were used. All statistical analyses were performed in RStudio. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Soil Analysis

Soil metal concentrations did not fit a normal distribution even after transformation and therefore nonparametric analyses were performed. Kruskal-Wallis and corresponding post hoc analyses were performed to determine statistical differences in total soil metal concentrations between each study site. Average and standard deviations of total soil metal concentrations for each study site are presented in Table 1. No statistical differences were found between metal concentrations in soils between IMRS – PP and IMRS – RH. Concentrations did differ significantly between UTEP and both IMRS sites for arsenic, copper, lead, and zinc (p-values <0.0001; Table 2). No significant differences were found between any study site for chromium. Cadmium was not detected at either of the IMRS sites. At UTEP, cadmium results were highly variable between each GPS location in which ten out of the fifteen samples also resulted in no detection of the metal. Differences between surface and subsurface soil metal concentrations for each study site were evaluated using paired t-tests. There were no significant differences found between soil depths at the UTEP site or IMRS – RH for any metal; however, IMRS – PP demonstrated significant differences in copper. At IMRS – PP, all other metals did not exhibit significant differences between the depths (Table 1).

Table 1. Average ± standard deviation of soil metal concentrations at surface (0 cm) and subsurface (10 cm) depths. P-values determined by paired t-tests performed between metal concentrations at each depth.

Study Site	**Depth (cm)**	**As**	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
UT	0	110.3 ± 162.4	20.3 ± 46.8	73.9 ± 24.1	1029.6 ± 1433.5	674 ± 992.2	782.4 ± 1027.7
UT	10	84.9 ± 69.3	3.4 ± 9	72.1 ± 17.5	603.3 ± 542.9	336.4 ± 351.5	423.4 ± 356.7
p-value		0.56	0.22	0.82	0.28	0.23	0.2
PP	0	3.7 ± 2.7	<MDL	72.1 ± 19	9.7 ± 3.2	9 ± 3.1	28.5 ± 12.7
PP	10	4.3 ± 1.3	<MDL	80.8 ± 15.4	13.9 ± 4.3	8.3 ± 4.9	26.9 ± 11.8
p-value		0.48	N/A	0.22	<0.05*	0.49	0.49
RH	0	4.2 ± 1	<MDL	55.5 ± 23.3	10.8 ± 4.9	10.7 ± 4.9	34.6 ± 13.3
RH	10	4.8 ± 1.6	<MDL	65.1 ± 27.2	11.7 ± 4	9.1 ± 4.4	31.7 ± 6.3
p-value		0.22	N/A	0.2	0.6	0.37	0.5

MDL – Method Detection Limit

* Indicates significant differences ($p < 0.05$)

Table 2. P-values determined by Kruskal-Wallis and corresponding post hoc analysis for total soil metal concentrations between each study site. Cadmium was excluded from analysis as it was not detected at either IMRS site.

	As	Cd	Cr	Cu	Pb	Zn
UT-RH	<0.0001*	N/A	0.1	<0.0001*	<0.0001*	<0.0001*
UT-PP	<0.0001*	N/A	0.99	<0.0001*	<0.0001*	<0.0001*
RH-PP	0.87	N/A	0.11	0.96	0.95	0.75

* Indicates significant differences ($p < 0.05$)

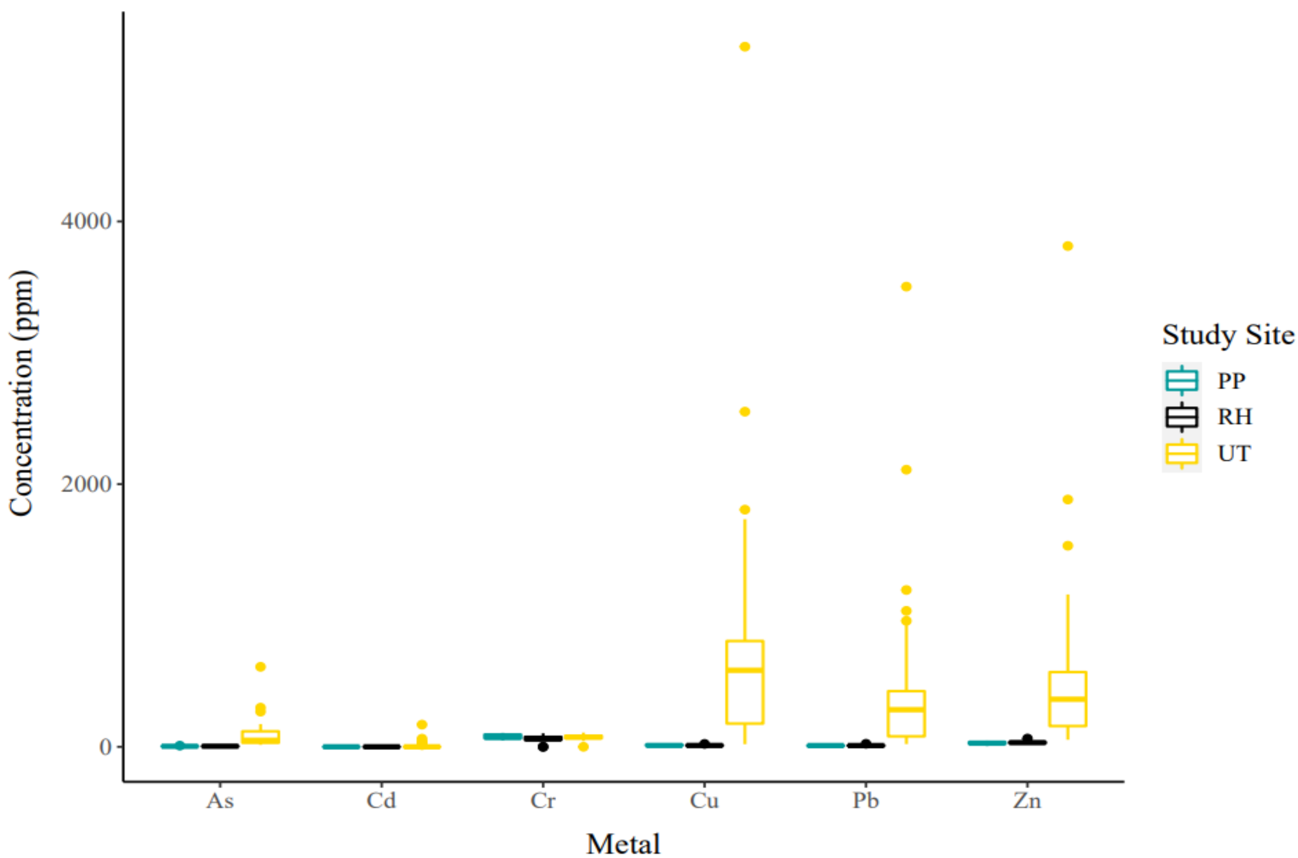

Figure 4. Soil metal concentrations (ppm) of As, Cd, Cr, Cu, Pb, and Zn from both UTEP and IMRS (RH and PP) p-XRF soil readings. Standard error bars and outlier points shown.

Soil pH at UTEP and both IMRS sites were alkaline. Average pH of the first 10 cm and 0-20 cm of soil at UTEP were both 8.5. At IMRS, average soil pH of the first 10 cm and 0-20 cm at RH were 8.9 and 8.8 respectively and at PP was 8.7 for both depths. Kruskal-Wallis tests were performed to determine differences between pH at each test site as data were not normal for either 10 cm or 20 cm depths. No significant differences were found in soil pH between RH and PP for 10 cm and 20 cm measurements ($p = 0.39$ and 0.16 respectively). Soil pH did not differ significantly between PP and UTEP at 10 cm or 20 cm either ($p = 0.19$ and 0.63 respectively). Soil pH did differ significantly between RH and UTEP at both 10 cm and 20 cm depths ($p < 0.01$). Paired t-tests were used to evaluate differences between pH at each depth for each study site. No significant differences were found between depths for IMRS – RH, IMRS – PP, or UTEP ($p = 0.29$, $p = 0.84$, $p = 0.85$ respectively).

Invertebrate Analysis

Invertebrates were collected during active periods of 2020 and 2021. Number of individuals that comprised each invertebrate order collected at each study site is shown in Table 3. Furthermore, a summary of the families that comprised each pooled invertebrate order for metal analysis is included (Appendix Table 7). Metal analyses were performed on a single pooled sample for each taxonomic group and site, as such statistical analysis was limited. Metal concentrations found in each group are presented in Table 4 and depicted in Figures 5a – f. Results from each site and each metal were averaged to demonstrate overall values of each metal found within invertebrates generally. Invertebrate tissues from the UTEP site were highest in zinc, followed by copper, lead, arsenic, cadmium, then chromium. The highest metal concentration found in IMRS – RH invertebrates was zinc, followed by copper, chromium, lead, cadmium, then arsenic. Invertebrates from IMRS – PP exhibited the same pattern as IMRS – RH.

Average invertebrate metal concentrations were evaluated for statistical differences through non-parametric analyses. Invertebrate arsenic concentrations were not significantly different between PP and RH ($p = 0.99$) nor PP and UT ($p = 0.06$), but were significantly different between RH and UT ($p = 0.04$). Cadmium concentrations were not significantly different between RH and PP ($p = 0.99$) but did significantly differ between RH and UT ($p = 0.02$) and PP and UT ($p = 0.036$). Chromium concentrations did not differ significantly between RH and PP ($p = 0.98$), RH and UT ($p = 0.80$), or PP and UT ($p = 0.91$). Copper metal concentrations also did not demonstrate significant differences between any study site (RH – PP: $p = 0.93$, UT – PP: $p = 0.27$, and UT – RH: $p = 0.45$). Invertebrate lead concentrations did not differ significantly between PP and RH ($p = 0.87$) but did differ significantly between UT and PP ($p = 0.01$) as well as UT and RH ($p = 0.04$). Zinc concentrations did not differ significantly between any study site (RH – PP: $p = 0.91$, UT – PP: $p = 0.91$, and UT – RH: $p = 1.00$).

Table 3. Number of invertebrates captured within each taxonomic group at each study site.

	Araneae	**Cicadidae**	**Coleoptera**	**Orthoptera**	**Isoptera**
UT	16	7	46	30	30
RH	30	5	44	23	105
PP	30	5	35	15	66

Table 4. Metal concentrations in parts per million detected in each invertebrate group by study site. Average ± standard deviations were calculated for each metal per study site to demonstrate overall concentrations found within invertebrates generally.

	Invertebrate Metal Concentrations (ppm)					
	As	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
UTEP						
Isoptera	20	8.12	12.4	373	217	323
Coleoptera	58.73	2.462	0.273	34.475	16.255	99.11
Orthoptera	1.986	4.262	0.216	118.7	12.92	141.65
Araneae	5.19	15.1	0.248	243	6.54	358
Cicadidae	0.934	10.6	0.195	43.9	7.49	104
Average ± SD	17.37 ± 24.36	8.11 ± 5.04	2.67 ± 5.44	162.62 ± 144.19	52.04 ± 92.30	205.15 ± 125.26
IMRS-RH						
Isoptera	1.58	0.174	8.02	25.3	5.49	194
Coleoptera	0.625	0.045	0.146	29.79	0.173	101.2
Orthoptera	0.129	0.081	0.186	58.4	0.255	118
Araneae	0.235	0.966	0.693	96.9	0.359	439
Cicadidae	0.014	2.8	0.054	77.4	0.117	120
Average ± SD	0.52 ± 0.64	0.81 ± 1.17	1.82 ± 3.47	57.56 ± 30.63	1.28 ± 2.36	194.44 ± 141.32
IMRS-PP						
Isoptera	1.73	0.196	5.76	19.6	3.79	154
Coleoptera	1.235	0.032	0.481	12.275	0.144	75.315
Orthoptera	0.088	0.187	0.18	53.4	0.17	129
Araneae	0.359	1.61	0.299	107	0.203	445
Cicadidae	0.012	2.33	0.049*	76.4	0.127	97.2
Average ± SD	0.68 ± 0.76	0.87 ± 1.04	1.35 ± 2.47	53.74 ± 39.48	0.89 ± 1.62	180.10 ± 151.09

* Value is ≤ method detection limit (MDL) so the MDL is reported

SD – Standard Deviation

Isoptera and Orthoptera from UTEP exhibited higher concentrations for all metals than both IMRS sites. Coleoptera from UTEP were higher in arsenic, cadmium, copper, and lead concentration than both IMRS sites while chromium was highest at IMRS – PP and zinc was highest at IMRS – RH. Araneae from UTEP were also higher in arsenic, cadmium, copper, and lead than both IMRS sites while chromium was higher at IMRS – RH and zinc was higher at both IMRS sites than the UTEP site. Cicadas at UTEP were higher in arsenic, cadmium, chromium, and lead than both IMRS sites, while copper was higher at both IMRS sites and zinc was higher at IMRS – RH (See Table 4).

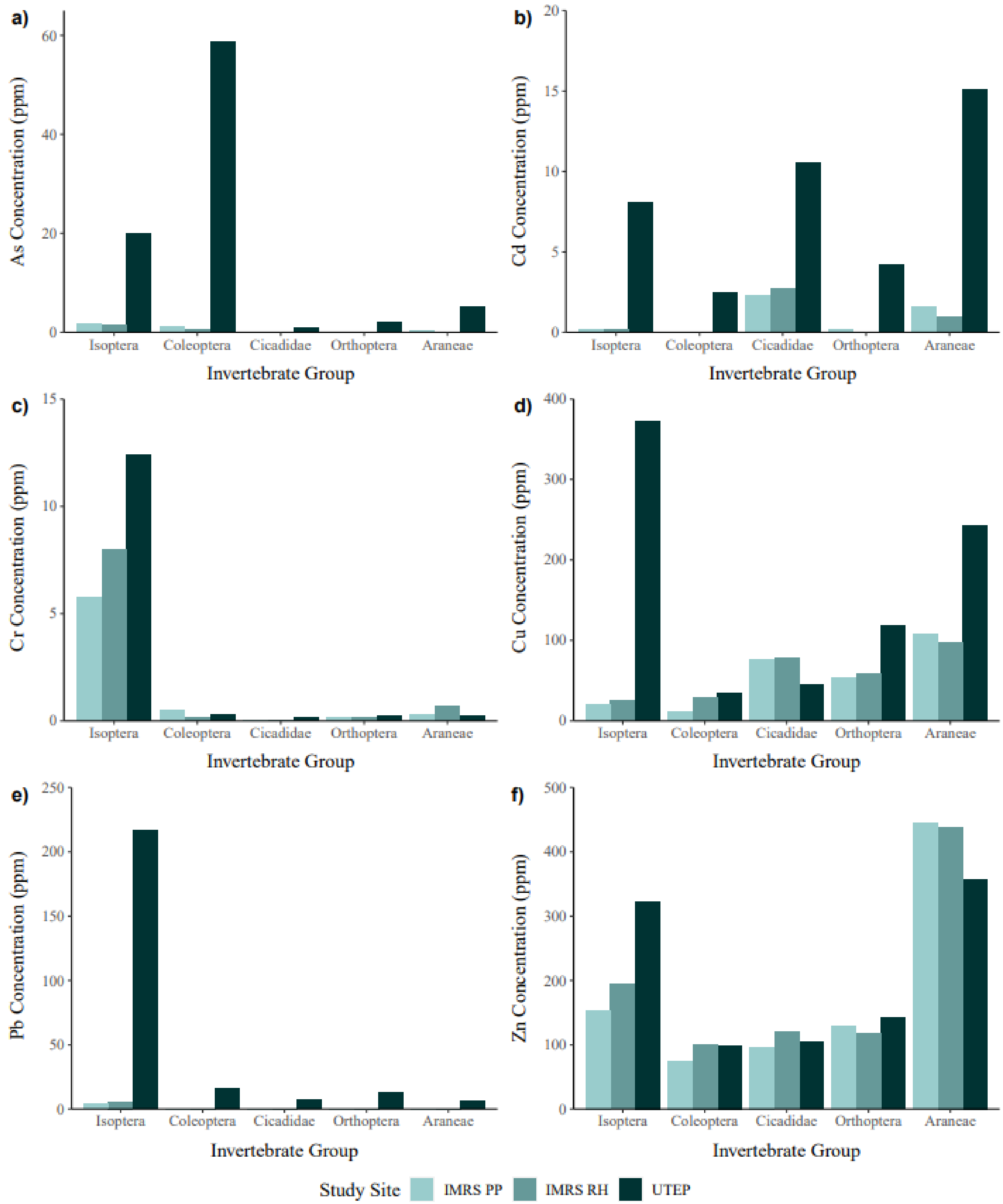

Figure 5. Concentrations in parts per million (ppm) of (a) arsenic, (b) cadmium, (c) chromium, (d) copper, (e) lead, and (f) zinc measured in each pooled invertebrate group at UTEP, IMRS – PP and, IMRS – RH.

Lizard Analysis

At the UTEP site, only *Aspidoscelis neomexicana* was encountered; whereas at IMRS, both *A. marmorata* and *A. tesselata* were found. Metal concentrations were analyzed in thirty-three whiptail lizards, which included ten *A. neomexicana* from the UTEP site, four *A. tesselata* and nine *A. marmorata* from IMRS – RH, and six *A. marmorata*, and four *A. tesselata* from IMRS – PP. Both male and female *A. marmorata* were analyzed in which five males and four females were from RH and two males and four females were from PP.

Average and standard deviations of metal concentrations were determined for all lizard species, for each sex, and at each study site (See Table 5). Lizard metal concentrations were not normally distributed for all metals except zinc. Therefore, to determine statistical differences between metal concentrations and study site, an ANOVA was only used to evaluate zinc concentrations, while all others were subject to nonparametric Kruskal-Wallis analyses. Chromium concentrations for all lizards at each study site were below method detection limits and therefore excluded from any further analysis. Lizard livers from UTEP were highest in zinc, followed by copper, lead, cadmium, and then arsenic. Livers from IMRS – RH were highest in zinc, followed by copper, cadmium, arsenic, then lead. At IMRS – PP lizard livers were highest in zinc, followed by copper, cadmium, lead, then arsenic. Non-parametric analysis of arsenic, cadmium, copper, and lead revealed statistically significant differences between UT and both IMRS – RH and IMRS – PP, however no differences between PP and RH were found (see Table 6 for p-values and Figures 6a – d). Analysis from a one-way ANOVA and Tukey HSD post hoc test revealed that zinc concentrations were not statistically significantly different between RH and UT, and RH and PP (p-values = 0.08 and 0.88 respectively). Zinc concentrations did differ significantly between PP and UT (p-value = 0.04; See Figure 6e).

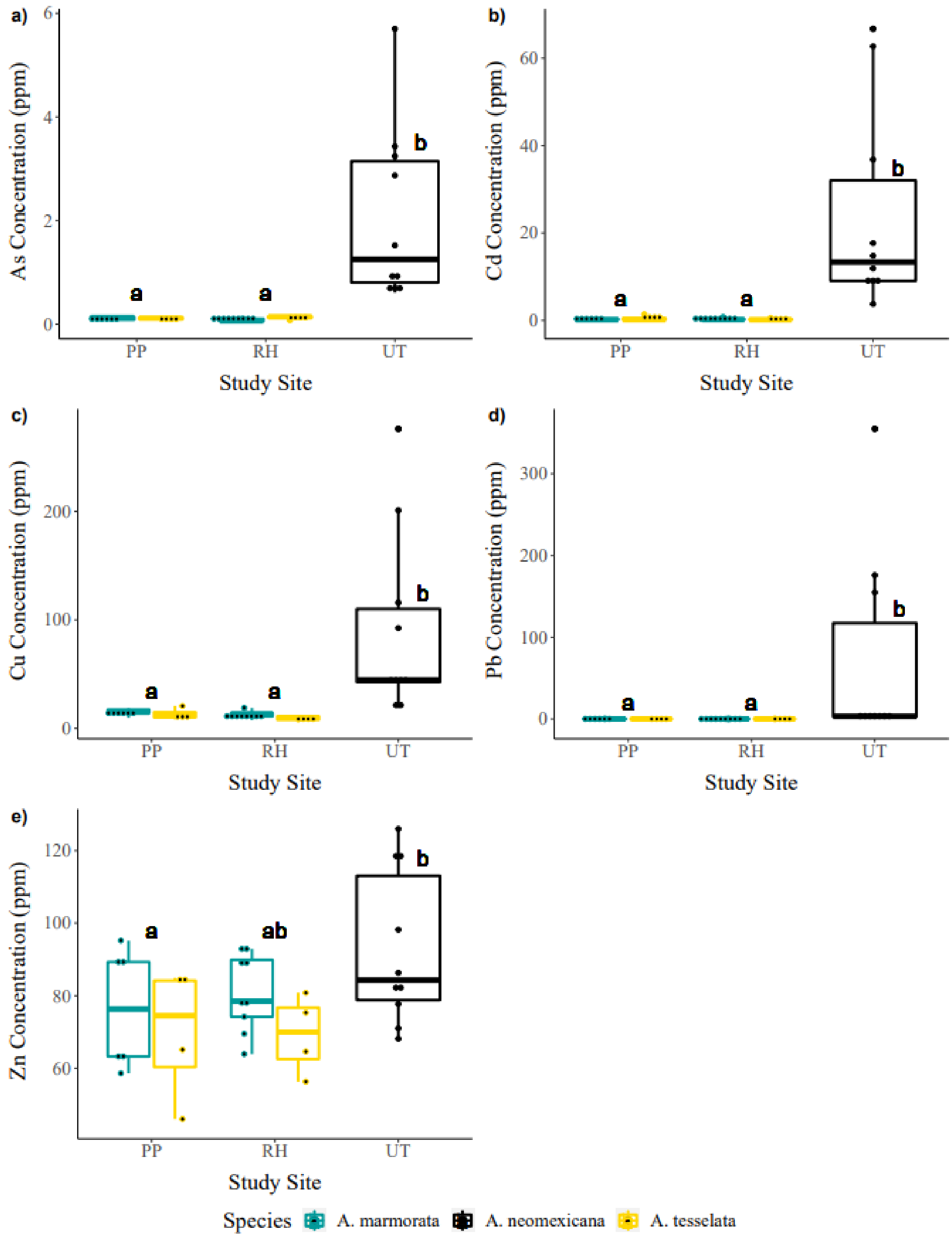

Figure 6. Concentrations of (a) arsenic, (b) cadmium, (c) copper, (d) lead, and (e) zinc, within each lizard species from UTEP, IMRS – PP and IMRS – RH. Letters indicate significant differences between study sites.

Table 5. Average ± standard deviations of metal concentrations measured within each lizard species by sex captured at each study sites. MDL – Method Detection Limit.

			Average Lizard Metal Concentration (ppm)					
Species	**Sex**	**Site**	**As**	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
A. neomexicana	F	UT	2.07 ± 1.69	24.13 ± 23.2	≤MDL	90.34 ± 85.36	70.82 ± 120.62	92.87 ± 21.17
A. marmorata	M	RH	0.06 ± 0.01	0.19 ± 0.1	≤MDL	13.8 ± 0.88	0.11 ± 0.03	82.28 ± 7.89
A. marmorata	F	RH	0.12 ± 0.05	0.37 ± 0.27	≤MDL	11.32 ± 5.33	0.08 ± 0.01	79.05 ± 14.49
A. tesselata	F	RH	0.13 ± 0.04	0.26 ± 0.19	≤MDL	9.33 ± 2.81	0.11 ± 0.07	69.25 ± 10.94
A. marmorata	M	PP	0.09 ± 0.04	0.11 ± 0.05	≤MDL	14.75 ± 1.06	0.1 ± 0.03	76.25 ± 18.46
A. marmorata	F	PP	0.12 ± 0.03	0.37 ± 0.24	≤MDL	15.23 ± 4.03	0.13 ± 0.11	76.6 ± 18.33
A. tesselata	F	PP	0.11 ± 0.03	0.45 ± 0.64	≤MDL	13.26 ± 5.34	0.1 ± 0.04	69.98 ± 18.4

Table 6. P-values determined by one-way ANOVA and post hoc Tukey HSD tests for zinc and Kruskal-Wallis and corresponding post hoc analysis for As, Cd, Cu, and Pb on lizard livers.

	As	**Cd**	**Cu**	**Pb**	**Zn**
UT-RH	<0.0001*	<0.001*	<0.0001*	<0.001*	0.08
UT-PP	<0.001*	<0.001*	0.004*	<0.001*	0.04*
RH-PP	0.94	0.96	0.55	0.99	0.88

* Indicates statistical significance

Interaction plots were created to observe patterns of metal concentrations across soils, invertebrates, and lizard livers. Arsenic and chromium concentrations decreased with increasing trophic level at all study sites (See Figures 7 and 9). Cadmium at the UTEP site was highest in lizards followed by soils then invertebrates. Whereas at both IMRS sites, although not detected within soils, cadmium was higher in invertebrates than lizards (See Figure 8). Copper concentrations at UTEP decreased with increasing trophic level, while at both IMRS sites increased from soils to invertebrates but decreased from invertebrates to lizards (See Figure 10). Lead concentrations at UTEP decreased from soils to invertebrates but then increased from invertebrates to lizards. At both IMRS sites, lead decreased with increasing trophic level (See Figure 11). Lastly, zinc concentrations decreased with increasing trophic level at the UTEP site, whereas at both IMRS sites, experienced sharp increases from soils to invertebrates and a decrease from invertebrates to lizards (See Figure 12).

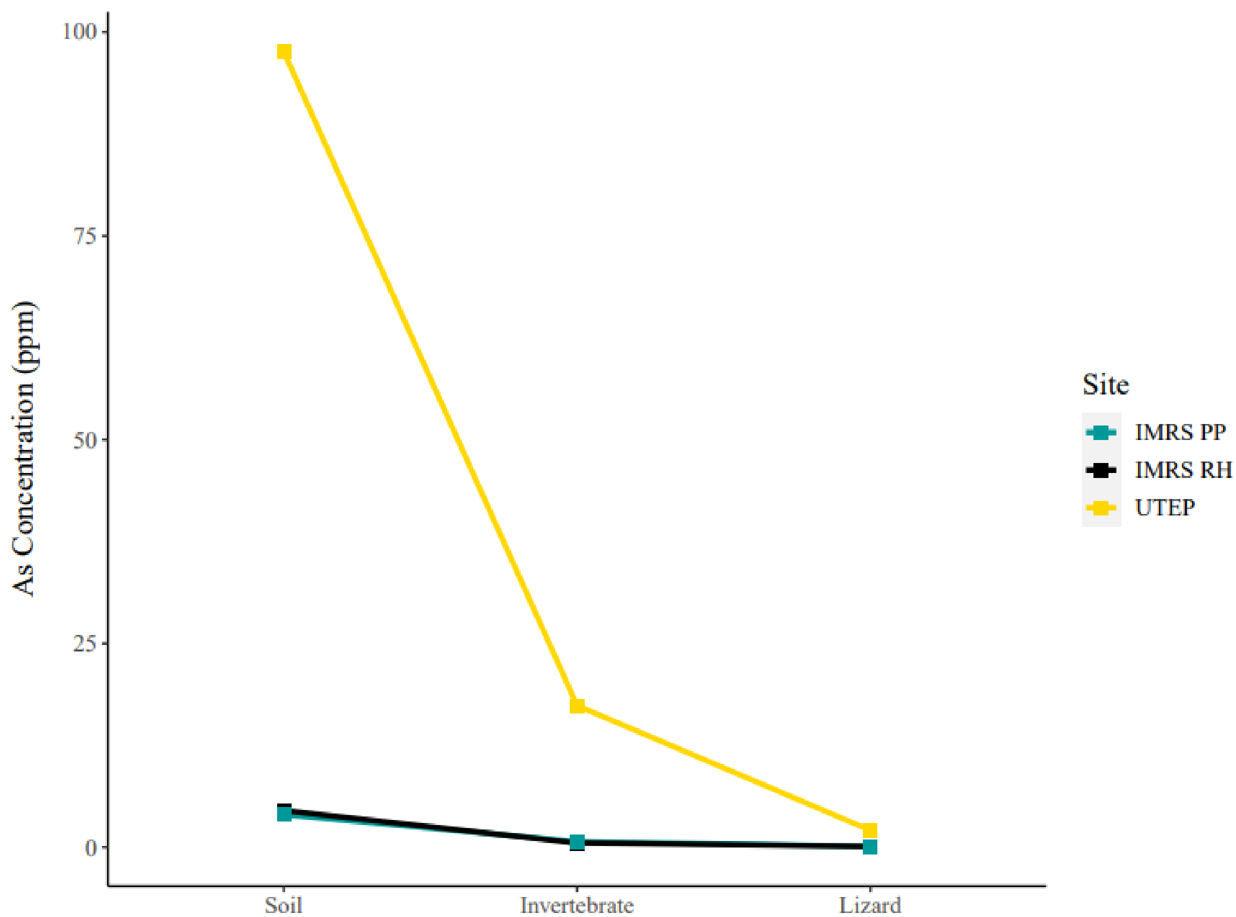

Figure 7. Interaction plot depicting trends in average arsenic concentrations between soils, invertebrates, and lizards at each study site.

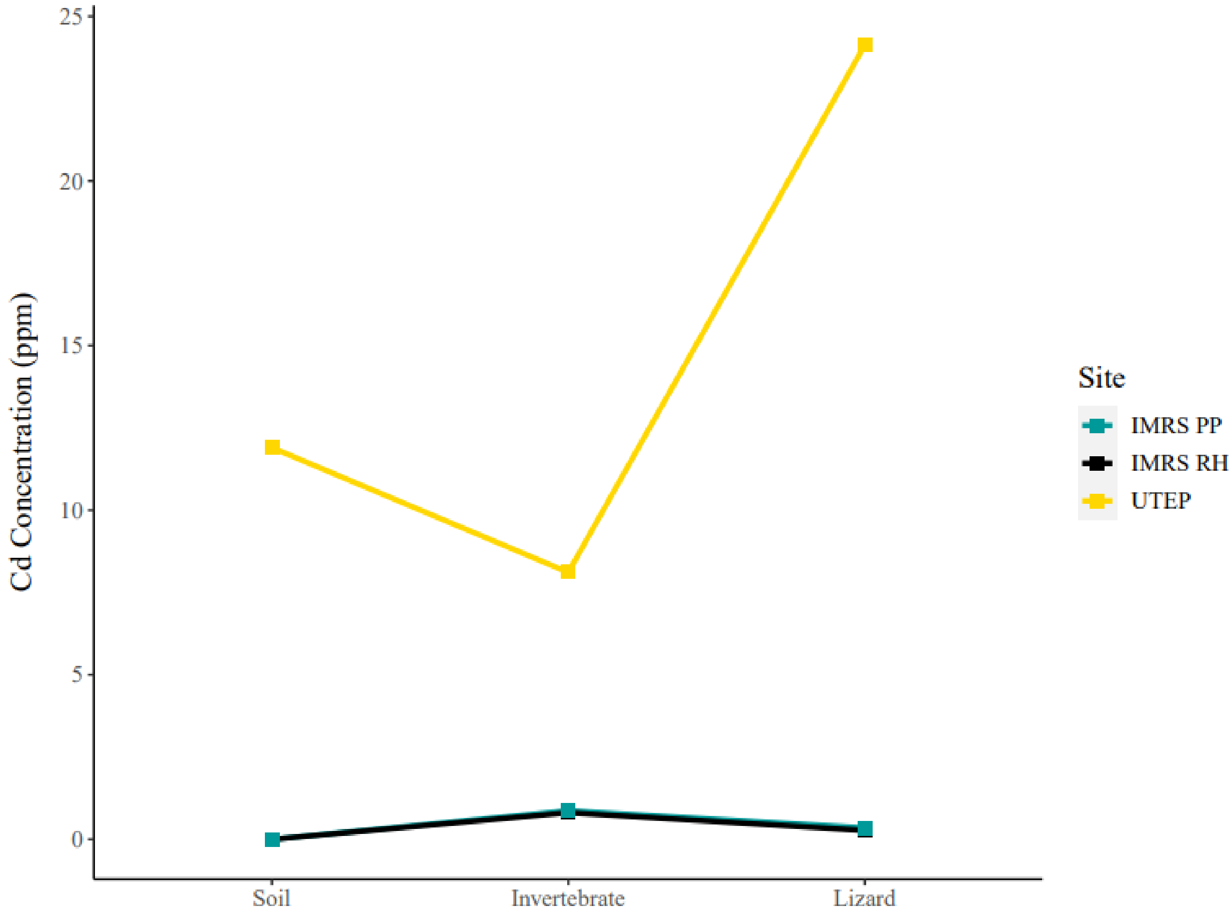

Figure 8. Interaction plot depicting trends in average cadmium concentrations between soils, invertebrates, and lizards at each study site.

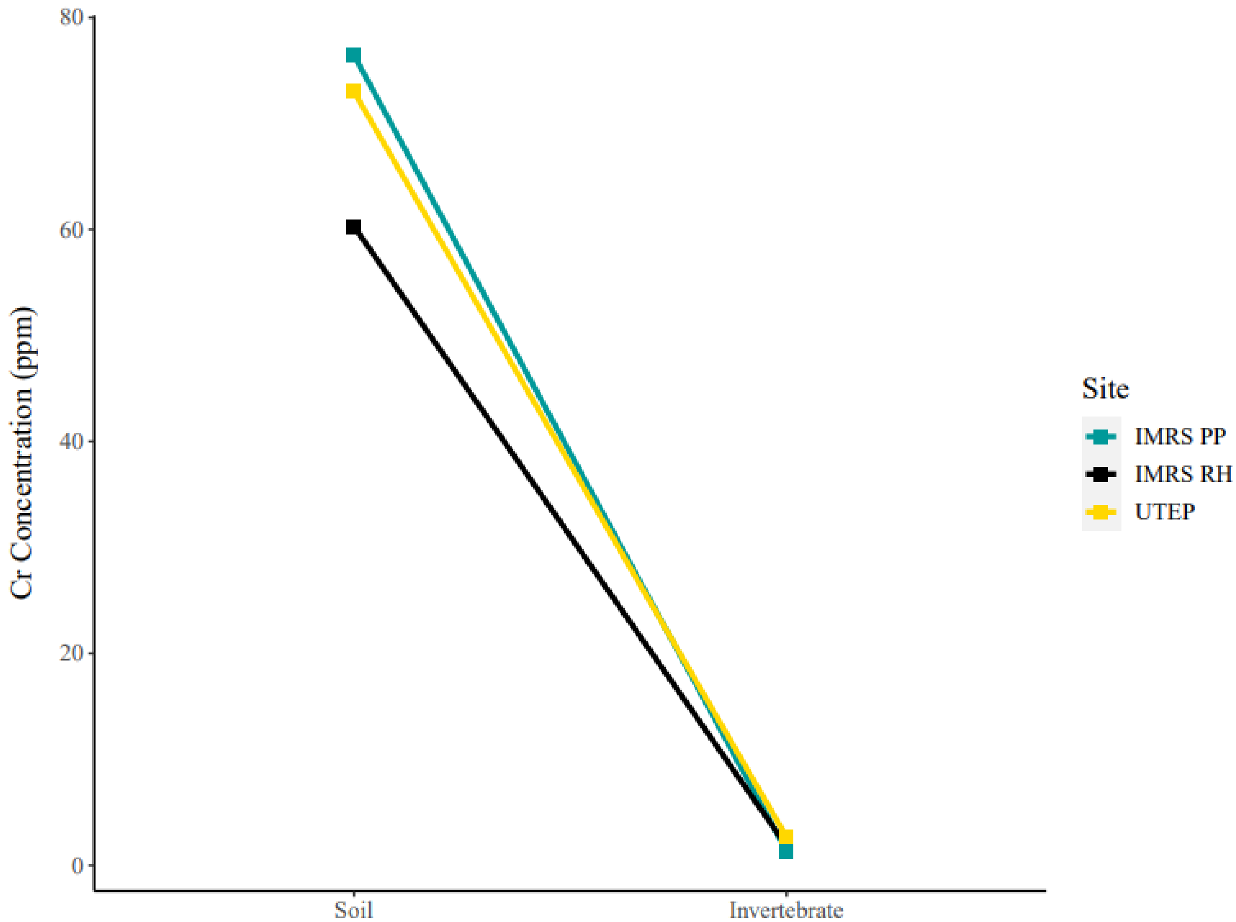

Figure 9. Interaction plot depicting trends in average chromium concentrations between soils, invertebrates, and lizards at each study site.

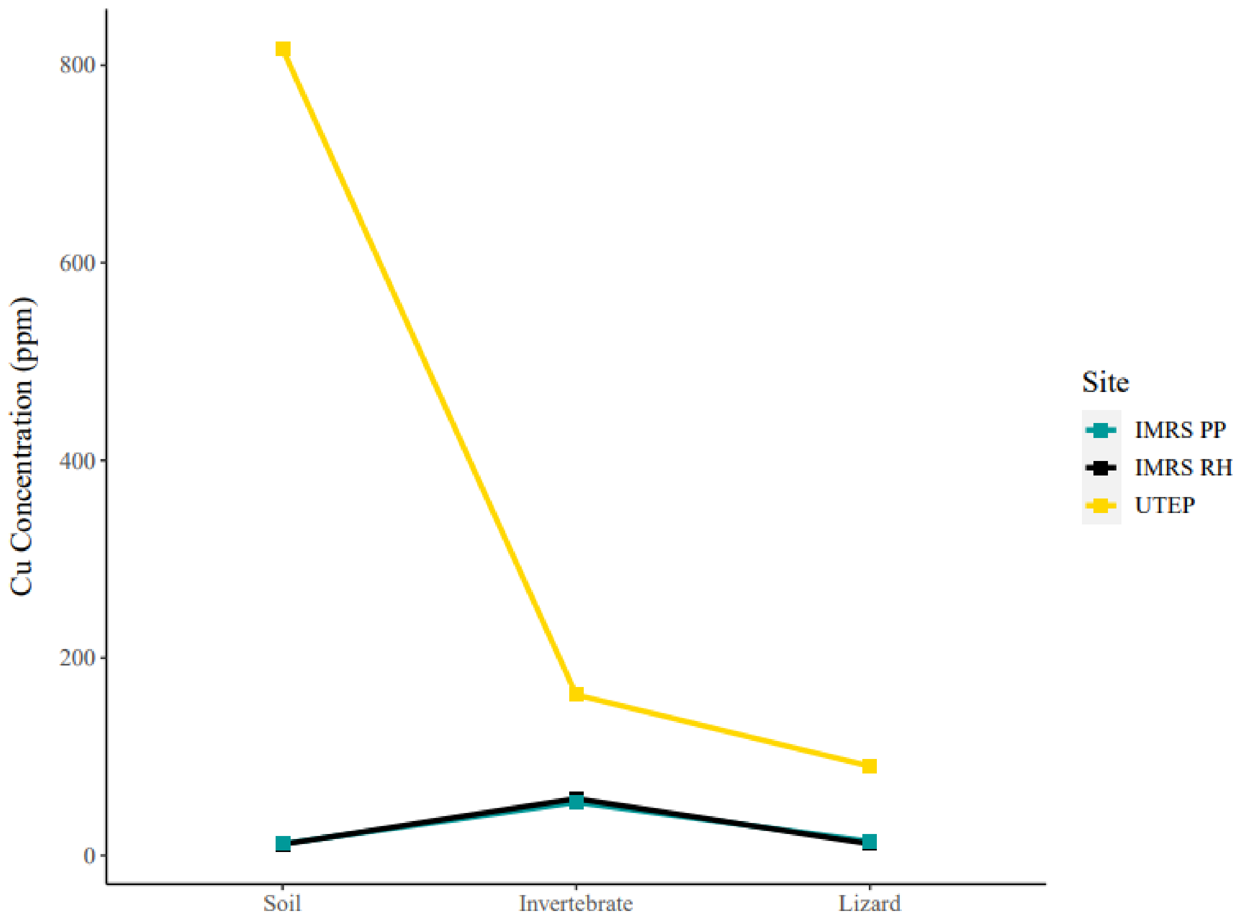

Figure 10. Interaction plot depicting trends in average copper concentrations between soils, invertebrates, and lizards at each study site.

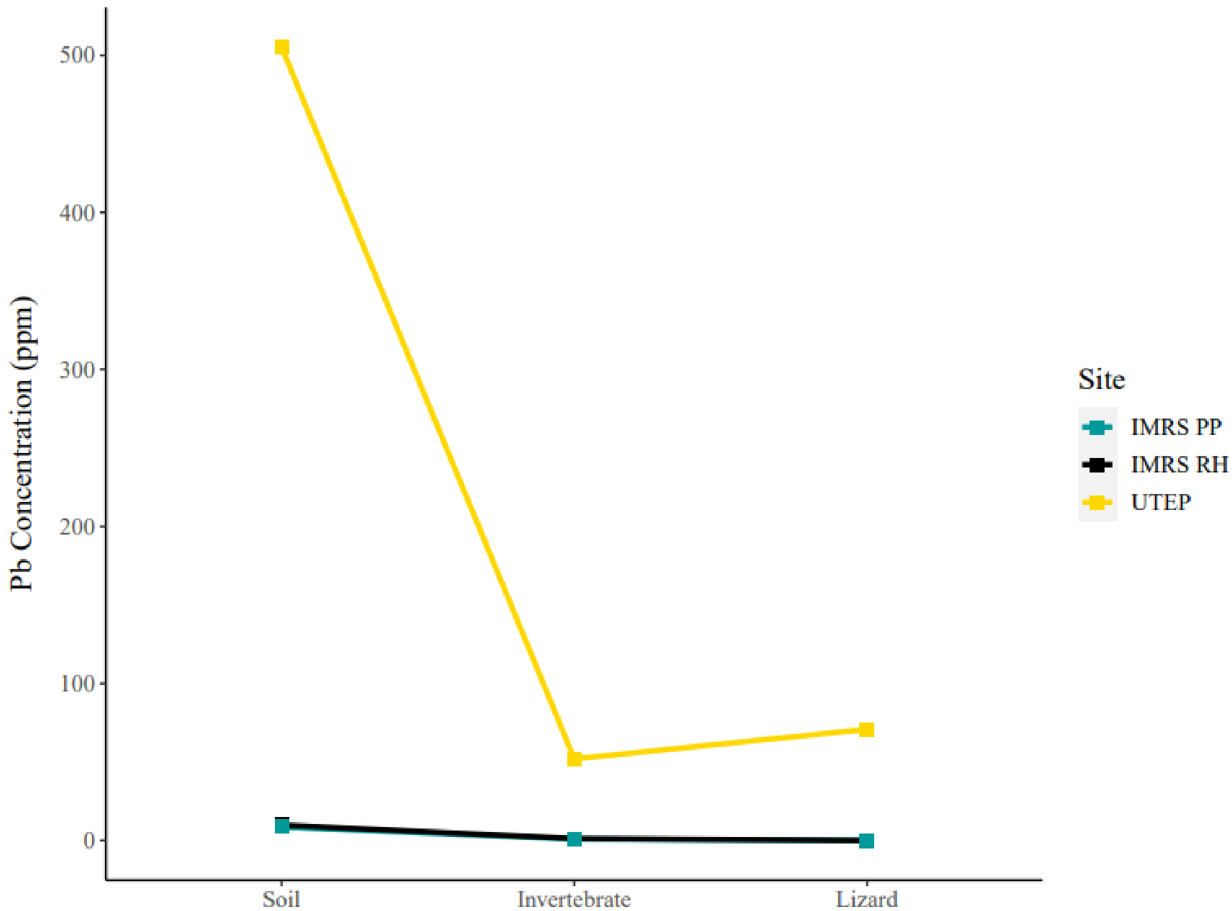

Figure 11. Interaction plot depicting trends in average lead concentrations between soils, invertebrates, and lizards at each study site.

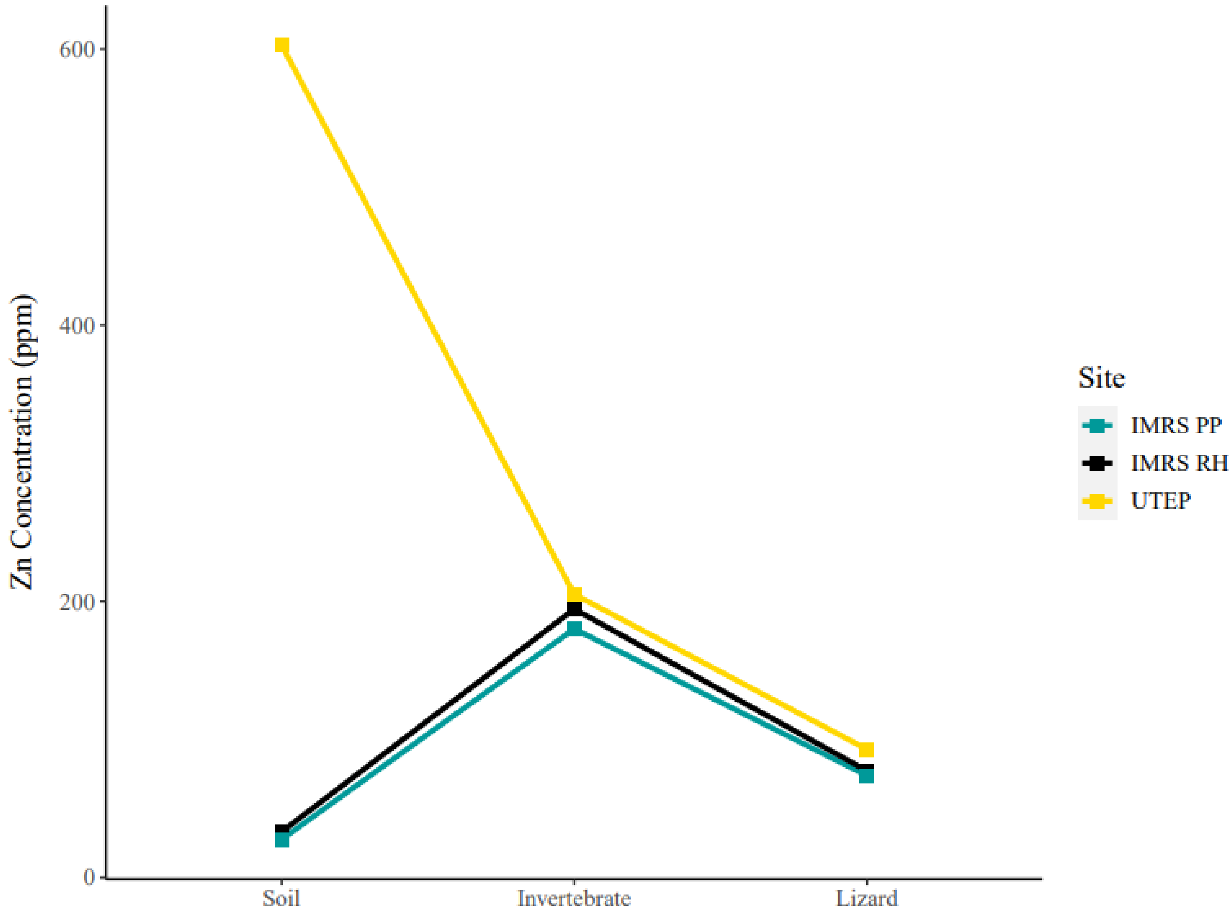

Figure 12. Interaction plot depicting trends in average zinc concentrations between soils, invertebrates, and lizards at each study site.

DISCUSSION

El Paso, Texas and border cities, have experienced a century of severe metal pollution from the American Smelting and Refining Company (Pingitore Jr. et al., 2005; Perales, 2008). As a result, many children in close proximity to the smelter experienced elevated blood lead levels (Landrigan and Baker, 1981) and vegetation close to the smelter experienced reduced species richness (Worthington, 1989; Mackay et al., 1998). Several studies have examined the bioaccumulation of this metal pollution into vegetation and invertebrate species (Mackay et al., 1997; Del Toro et al., 2010; Robinson, 2017). To date, few studies have examined the trophic transfer of metals to vertebrate taxa in the El Paso area. This study demonstrated the persistence of high metal concentrations within soils near the ASARCO site and their potential to transfer to invertebrates and whiptail lizards.

Measuring soil metal concentrations was crucial to determine the current levels of metals within soils at UTEP. There were significant differences between all soil metal concentrations, except for chromium and cadmium, between the polluted UTEP site and the undisturbed IMRS sites. Lead concentrations measured at the UTEP site were comparable to those found in previous studies in the area (Robinson et al. 2017). Several outliers were present for lead, copper, and zinc soil concentrations at the UTEP site. These outliers exhibited the highest concentrations found of these metals within UTEP soils, indicating heterogeneity of metals at this site. Subsurface metal concentrations were evaluated along with surficial concentrations to identify levels of metals that organisms may be exposed to underground. Previous research in El Paso has revealed higher surficial soil copper, lead and arsenic concentrations than subsurface concentrations; however depths evaluated ranged from 10 cm – 60 cm (Barnes, 1993). In the present study, subsurface concentrations did not differ significantly from surficial concentrations (except for copper at IMRS – PP), but on many occasions surficial metal concentrations were higher than those reported at the subsurface. These results suggest the possibility that invertebrates and lizards inhabiting more shallow burrows may still be exposed to high concentrations while underground. Another important aspect to consider is the bioavailability of metals. While soils at UTEP were found to have very high metal concentrations, they may not all be bioavailable for uptake. Many factors that can influence metal bioavailability include soil pH, soil organic matter, percent soil moisture, clay content, and soil salinity, among others (Stigliani et al., 1991; Wang et al., 2013; Sparling, 2016b). Soil pH is an important parameter to monitor as it can determine metal mobility within soils (Houben et al., 2013) and some studies indicate that pH is one of the most important factors in metal accumulation (Bradham et al., 2006; Wang et al., 2013). In this study, soil pH at all study sites were found to be alkaline (pH > 7). Previous research suggests that with increasing soil pH, metal bioavailability and mobility may be reduced as metals are more likely to become adsorbed to soils (Harter, 1983; Anguissola Scotti et al., 1999; Hou et al., 2019). The relationship between pH and metal bioaccumulation in plants has been examined previously. It has been demonstrated that the bioaccumulation of metals in plants is negatively correlated with pH (Hu et al., 2013; Wang et al., 2013). Del Toro et al. (2010), measured soil moisture alongside soil pH in El Paso and found moisture to be less than 3%. More alkaline pH as well as low soil moisture may be a major contributing factor in explaining the lower metal concentrations found within invertebrates and lizards as compared to the high metal concentrations found within soils. Our prediction that soils would exhibit higher metal concentrations than organisms was supported for each metal except cadmium at the UTEP site, while at IMRS there were varying results. Zinc, copper, and cadmium at IMRS displayed lower soil metal concentrations than invertebrates and lizards. Zinc and copper are known to be essential micronutrients to plants (Peralta-Videa et al., 2009), and certain plants are known to hyperaccumulate metals (Baker et al., 2000; Baker and Brooks, 1989). As such, metals could be moderately biomagnifying into herbivorous invertebrates at IMRS. One study that evaluated lead, cadmium, and mercury within soils, plants, and both herbivorous and carnivorous terrestrial invertebrates found evidence of biomagnification with increasing trophic level except to the carnivorous invertebrates (Zhang et al., 2009). Vegetation at IMRS should be further investigated for metal concentrations to better understand the trophic mechanisms taking place. Biomagnification factors were not calculated between trophic levels in this study due to limitations of the data, especially for the invertebrates, but should be considered in future research.

Invertebrate metal concentration data is considered preliminary as no replicates were performed for each group. Despite this limitation, several groups of invertebrates displayed higher concentrations of metals at UTEP compared to the IMRS sites. At the UTEP site, termites were only encountered once after a rain event in September 2021. Although extensive searches were conducted throughout active periods, it was unclear why they were only encountered once. Even though only 30 termites were pooled and analyzed for metals from UTEP, they had high concentrations of copper (373 ppm) and lead (217 ppm), while at both IMRS sites, which had larger sample sizes, were below 30 ppm for copper and 10 ppm for lead. Termites at UTEP had higher chromium, copper, and lead concentrations than any other invertebrate group at this site. Termites feed on dead plant material and even soils, and use their digested materials to build mounds (Eggleton, 2010). This ecological feature may be a contributing factor in explaining why termites had such high levels of lead and copper which are among the metals that demonstrated particularly high concentrations within UTEP soils. Coleopterans exhibited much higher arsenic concentrations as compared to IMRS sites. A majority of the beetles collected at all study sites belonged to the family Tenebrionidae (Appendix Table 6) which are highly speciose and widely distributed (Bousquet et al., 2018). These beetles vary in their feeding habits but are known to be important detritivores in arid regions, and some larvae are known to consume plant roots (Bartholomew and El Moghrabi, 2018; Ayal, 2007; Crawford, 1979). Other beetles collected belonging to the families Meloidae, Cantharidae, Scarabaeidae, Elateridae, Cleridae, Coccinellidae, and Curculionidae vary in polyphagous, insectivorous, detritivorous, nectivorous, and herbivorous feeding habits and some species may have larval stages with further dietary specializations including insectivorous and parasitic habits (Linsley, 1958; Selander, 1981; Arnett et al., 2002). In this study, metals detected within Coleopterans therefore could have been accumulated in a variety of ways. Orthopterans from UTEP had approximately double the amount of copper and much higher levels of lead as compared to the IMRS sites. The most frequently captured family of Orthopterans was Acrididae, the short-horned grasshoppers. Orthopterans generally are predominantly herbivorous (Bidau, 2014) but have species specific specializations including monophagous, oligophagous, and polyphagous feeding habits (Joern, 1979). Therefore, metals accumulated within this group would mainly be a result of plant consumption. Araneae from UTEP demonstrated similarly higher patterns of arsenic, copper, lead, and cadmium as compared to IMRS. Spiders exhibited the highest diversity of invertebrates collected with a total of 17 different families. Although UTEP and IMRS sites only shared six families in common. The order Araneae is a widely diverse group of predaceous invertebrates and many exhibit polyphagous feeding habits (Pekár and Toft, 2015). Spiders also vary in their feeding techniques as some are active hunters while others build webs to catch their prey causing further variation in the pathways of metal uptake within spiders collected for this study. Spiders as secondary consumers would have the potential to experience increased biomagnification of metals. At the UTEP site, spiders exhibited the highest concentrations of cadmium and zinc and were second highest in copper. Metals accumulated in this group, aside from accidental ingestion, would primarily have come from consumed prey items. All cicadas collected were adults. Only one species of cicada was encountered at the UTEP site while two were found at IMRS. Cicadas did not have noteworthy differences between UTEP and IMRS sites except for cadmium which was 10.6 ppm at UTEP and less than 3 ppm at the IMRS sites. Cicadas are xylem feeders and are particularly unique as they spend time underground as nymphs and live above ground as adults (Cheung and Marshall, 1973; Harvey and Thompson, 2006; Moriyama and Numata, 2019). A previous study examined the patterns of metal concentrations in exoskeletons, nymphs, and adult 17-year periodic cicadas (*Magicicada* spp.). This study indicates that arsenic and lead may sequester in nymphal exoskeletons, and results varied between nymph and adult concentrations (Robinson et al., 2007). Adult cicadas had mostly low concentrations of metals. As such, nymphs and nymph exoskeletons should be evaluated at UTEP as metals may be eliminated through ecdysis in the transition from nymph to adult stages.

Metal concentrations have been evaluated previously within herbivorous Rough Harvester Ants, detritivorous beetles belonging to the genus *Eleodes*, and scorpions captured near the ASARCO site (Mackay et al., 1997; Del Toro et al., 2010). Current UTEP arsenic, lead, and cadmium concentrations were lower in detritivorous invertebrates (Isopterans and Coleopterans – partly) in comparison to detritivorous beetle concentrations found in Mackay et al. (1997). One group of herbivorous invertebrates measured for metal concentrations within Mackay et al. (1997) and Del Toro et al. (2010) were Rough Harvester Ants. In Mackay et al. (1997), ants exhibited insignificant concentrations of arsenic whereas in this study herbivorous invertebrates (Orthoptera) did present arsenic concentrations. Cadmium and lead concentrations in herbivores were lower than previously found in Mackay et al. (1997). Copper in Orthoptera showed slightly higher levels than those found in ants in Del Toro et al. (2010). Arsenic, lead, zinc, and cadmium concentrations were very similar between ants and Orthopterans (Del Toro et al. 2010). Scorpions represented a carnivorous trophic level within the Mackay et al. (1997) study and demonstrated insignificant metal accumulation for arsenic and lead. In this study, spiders represented a carnivorous trophic level and exhibited higher arsenic, cadmium, and lead concentrations than those found within the scorpions by about an order of magnitude.

Invertebrates collected for this study covered a wide range of species that have differing ecological features. Variations in species specific interactions with plants and other organisms are not accounted for when considering the variable pathways for metal uptake and transfer to lizards. Differences in species collected between study sites may also have influenced the results. Furthermore, metal tolerance as well as genetic and behavioral adaptations have been examined in many invertebrate groups before (Posthuma and Van Straalen, 1993). Invertebrates also may have ways of coping with metal pollution other than tolerance and behavioral adaptations. Excretion is another potential fate of a metal that has entered an invertebrate (Posthuma and Van Straalen, 1993). As invertebrates at UTEP demonstrated lower metal concentrations than soils, they should be evaluated for these types of adaptations and the possibility of excretion, as it would provide better insight on further trophic transfer potential.

This study is one of the first to measure metal concentrations within vertebrate species generally, and lizards specifically, from the El Paso, TX region. Metal concentrations did not appear to biomagnify from invertebrates to lizards at either study site, with the exception of cadmium. As invertebrates can exhibit behavioral adaptations and tolerances to metals, as well as have the ability to reduce the overall body burden of metals through ecdysis and excretion (Posthuma and Van Straalen, 1993), this may provide less of an opportunity for metals to transfer to lizards. Additionally, dietary patterns have been examined across multiple species of whiptail lizards. Other invertebrates that these lizards may consume include coleopteran larva, lepidopterans (larva and adults), among other insects (Scudday and Dixon, 1973; Paulissen et al., 2006; Mata-Silva et al., 2013). As these potential prey items are consumed to a lesser degree, they were not collected for metal evaluation which could present gaps in our full understanding of further potential trophic transfer to whiptail lizards. It is also possible that dietary shifts to or between particular groups of invertebrates may occur to help cope with the metal pollution. Despite a lack of biomagnification, lizards at UTEP did exhibit significantly higher concentrations for all metals, except for zinc, than those from IMRS therefore supporting our prediction. Cadmium was the only metal that was higher within lizard livers as compared to soils and invertebrates at the UTEP site. Lizard livers were evaluated for metals as it is one of the main organs thought to accumulate the highest amounts and diversity of metals. However, the potential for kidneys, muscle tissue, skin, and bone to accumulate high amounts of metals has also been documented (Hopkins, 2005; Grillitsch and Schiesari, 2010). Previous research on metal bioaccumulation within lizards have demonstrated variations in tissue metal accumulation (Trinchella et al., 2006; Mann et al., 2007; Oyekunle et al., 2012; Nasri et al., 2017). A prior study on the Wall Lizard (*Podarcis sicula*) examined patterns of distribution of cadmium within different tissues over time after injection of a cadmium solution (Trinchella et al., 2006). The results of this study found that cadmium initially sequestered the most within the kidneys and ovaries but then after several days, the most cadmium was found within the liver (Trinchella et al., 2006). Nasri et al. (2017) observed lead, cadmium, and zinc concentrations within stomach, liver, kidneys, and tail tissues of Bosc's Fringe-toed Lizard (*Acanthodactylus boskianus*) inhabiting a polluted site. The results of this study showed that cadmium also sequestered the most within lizard livers, while lead and zinc were found to be highest within the kidneys (Nasri et al., 2017). In our study, it is possible that while cadmium was highest within lizard livers, arsenic, lead, copper, chromium, and zinc may be found in higher concentrations in other organs and tissues. Lizards also have the ability to regulate metals through feces, shedding, and maternal transfer (Burger, 1992; Hopkins et al., 2001; Xu et al., 2006). Future studies in the UTEP area should evaluate various tissues as well in order to increase our understanding of the tissue specific relationships with metals as well as the overall body burden lizards at UTEP are enduring.

While more recent studies have attempted to address metal bioaccumulation patterns within various lizard tissues, unfortunately, few studies have examined the overall pathological impacts of metals on lizards including synergistic effects (Silva et al., 2020). One study investigated the health impacts of acute and chronic doses of varying amounts of lead administered to Western Fence Lizards (*Sceloporus occidentalis*) (Salice et al., 2009). This study reported important outcomes regarding lizard health after varying doses of lead were administered, including changes in behavior, reduced body weight, increased kidney weight, lead induced anemia, and an approximate lethal dose of lead was determined to be 2,000 mg Pb/kg lizard body mass (Salice et al., 2009). Several studies have also evaluated genotoxic effects on lizards after metal exposure (Sargsyan et al., 2019; Simonyan et al., 2018). One study demonstrated that there were significantly positive correlations between DNA damage and metal concentrations (Simonyan et al., 2018). Soil metal concentrations in Simonyan et al. (2018) were lower than those found at our UTEP study site, therefore future research is warranted to investigate if lizards at UTEP have experienced genotoxic effects.

Body condition is an important indicator of the overall health and fitness of an organism and can be impacted by a variety of factors that come along with living in an urban environment including pollution (Lazić et al., 2017). As lizard species collected from UTEP differed from IMRS, addressing the effects of metal contaminants on body condition was not possible. Although *Aspidoscelis marmorata* and *A. tesselata* have ranges within the El Paso area, these species were not encountered at the polluted UTEP site. Along with *A. neomexicana*, the Greater Earless Lizard (*Cophosaurus texanus*), Side-Blotched Lizard (*Uta stansburiana*), Mediterranean House Gecko (*Hemidactylus turcicus*) and the Desert Spiny Lizard (*Sceloperus magister*) were encountered at the UTEP site. It is possible that due to the UTEP site being heavily polluted, having reduced vegetation (Worthington, 1989; Mackay et al., 1998), and potentially less termite activity, reduces the ability to support multiple species of whiptail lizards especially as they are known to consume the same types of invertebrate prey items. The effects of metal contamination on lizard body condition have been evaluated in other studies. For example, the Giant Sungazer Lizard (*Smaug giganteus*) exhibited a negative relationship between copper concentrations within tail tissue and body condition, although this relationship was not statistically significant (McIntyre and Whiting, 2012). McIntyre and Whiting (2012) did find however that lizards collected from one mining site were in significantly poorer body condition than one of their control sites. Future studies in the El Paso, TX region should be performed on the same lizard species in order to address body condition and be able to determine if lizard health has been compromised due to the pollution.

The United States Environmental Protection Agency has determined Ecological Soil Screening Levels (Eco-SSLs) for arsenic, cadmium, chromium, lead, copper, zinc, and a number of other toxic soil contaminants. An Eco-SSL refers to the concentration of a soil contaminant in which an organism that lives among soil or consumes organisms that live among soil are still protected from the contaminant (U.S. EPA, 2008, 2007b, 2007c, 2005a, 2005b, 2005c). While these Eco-SSLs have been evaluated for plants, soil invertebrates, birds, and mammals, currently, there are no set standards for reptiles. Average arsenic and lead concentrations in UTEP soils were higher than Eco-SSLs derived for plants, birds, and mammals (U.S. EPA, 2005b, 2005a). Arsenic Eco-SSLs have not been determined for soil invertebrates while lead concentrations within soil invertebrates were lower than Eco-SSLs (U.S. EPA, 2005b, 2005a). Average cadmium soil concentrations at UTEP were higher than Eco-SSLs for birds and mammals but lower than those for plants and soil invertebrates (U.S. EPA, 2005c). Average chromium concentrations were higher than the Eco-SSL's for chromium III for mammals and birds but lower than Eco-SSLs derived for chromium VI for mammals (U.S. EPA, 2008). UTEP copper and zinc soil concentrations were higher than those derived for plants, soil invertebrates, birds, and mammals (U.S. EPA, 2007c, 2007b). As the UTEP campus exhibits soil concentrations for several metals that are higher than those determined to be safe for birds and mammals, it is possible that lizards living in this area are being negatively impacted. With the persistence of metal pollution into the environment (Izatt et al., 2014), and reptile populations experiencing decline (Saha et al., 2018), it is increasingly important to study bioaccumulation processes and the impacts on an organism's fitness in areas such as UTEP which have been left un-remediated after severe historical smelting activities. This is also important as higher trophic predators may also be negatively impacted. This study provided crucial preliminary insight on metal bioaccumulation processes and trophic transfer potential occurring within a diverse group of invertebrates and an abundant group of generalist lizards inhabiting a historically polluted Chihuahuan Desert environment.

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APPENDIX

Table 6. Summary of the invertebrate families collected at each study site.

		Study Site		
Order	**Family**	**UT**	**PP**	**RH**
Isoptera	Rhinotermitidae	**30**	**66**	**105**
Hemiptera	Cicadidae	**7**	**5**	**5**
Orthoptera	Acrididae	24	9	12
	Gryllidae	6	1	2
	Mogoplistidae	0	2	1
	Tettigonidae	0	0	7
	Rhaphidophoridae	0	3	1
	TOTALS	**30**	**15**	**23**
Coleoptera	Tenebrionidae	39	35	23
	Cantharidae	0	0	2
	Cerambycidae	0	0	5
	Elateridae	1	0	0
	Meloidae	1	0	8
	Scarabaeidae	0	0	2
	Cleridae	0	0	2
	Coccinellidae	3	0	1
	Curculionidae	2	0	1
	TOTALS	**46**	**35**	**44**
Aranea	Lycosidae	6	8	5
	Agelenidae	0	1	0
	Araneidae	0	10	4
	Diguetidae	1	0	0
	Philodromidae	1	0	2
	Salticidae	2	0	1
	Scytodidae	1	0	0
	Gnaphosidae	1	0	1
	Oxyopidae	1	0	0
	Filistatidae	0	1	2
	Phlocidae	0	3	3
	Sparassidae	0	0	1
	Thomisidae	2	0	1
	Sicariidae	0	4	5
	Theraphosidae	0	1	0
	Selenopidae	0	2	4
	Theridiidae	1	0	1
	TOTALS	**16**	**30**	**30**

CURRICULUM VITA

Allyson Benson-Pedraza completed her Bachelor of Science in 2015 at the University of Texas at El Paso in Ecology and Evolutionary Biology. As an undergraduate, she was funded under an NSF grant to participate in an Undergraduate Research Mentorship program in Dr. Jerry Johnson's lab. In this position, she spent a year and a half conducting a unique field work-based project on whiptail lizards. In the Fall of 2019, Allyson began her master's degree at UTEP. For her master's thesis project, she secured internal funding through the Dodson Grant. As a graduate student, she has taught several semesters of the Topics in Study of Life Biology 1107 laboratory course as well as two semesters of the Organismal Biology 1108 course. Allyson presented her thesis research at the Southwestern Association of Naturalists Spring 2021 Conference and was awarded 3rd place in the Clark Hubbs student poster competition. In the summer of 2021 Allyson also worked a temporary four-month Field Biologist position at Ft. Bliss in which she spent the summer conducting vegetation and raptor surveys. In her time as a graduate student, Allyson also took on extra coursework in order to obtain a Geographic Information Science and Technology (GIST) Certificate. In her spare time, she volunteered with the UTEP Biodiversity Collections and 500 Women Scientists in attending and teaching at local outreach events. Allyson's future career goals include securing a position in the biological sciences.

Email address: ally_benson21@yahoo.com

This thesis was typed by Allyson Benson-Pedraza