Useing *Peromyscus leucopus* as a biomonitor to determine the impact of heavy metal exposure on the kidney and bone mineral density: Results from Tar Creek Superfund Site

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Abstract

Background. Human population growth and industrialization contribute to increased pollution of wildlife habitats. Heavy metal exposure from industrial and environmental sources is still a threat to public health, increasing disease susceptibility. In this study, I investigated the effects of heavy metals (cadmium (Cd), lead (Pb) and zinc (Zn)) on the kidney and bone density.

Objective. This study aims to determine the concentrations of Cd, Pb, and Zn in soil and compare them to the levels of the same metals in *Peromyscus leucopus* kidney tissue. Furthermore, the study seeks to investigate the impact of heavy metals on bone density and fragility using the fourth lumbar vertebra (L4) of *P. leucopus*.

Methods. Cd, Pb, and Zn concentrations in soil specimens collected from Tar Creek Superfund Site (TCSFS), Beaver Creek (BC) and two reference sites (Oologah Wildlife Management Area [OWMA] and Sequoyah National Wildlife Refuge [SNWR]). Heavy metals concentrations were analyzed using inductively coupled plasma-mass spectroscopy (ICP-MS). Micro-computed tomography (μCT) was used to assess the influence of heavy metals on bone fragility and density.On the one hand, soil samples revealed that Pb is the most common pollutant in the sediment at all of the investigated sites (the highest contaminated site with pb was TSCF). Pb levels in the soil of TCSFS, BC, OWMA, and SNWR were found to be 1132±278, 6.4±1.1, and 2.3 ±0.3 mg/kg in the soil of TCSFS, BC and OWMA and SNWR, respectively. This is consistent with the fact that Pb is one of the less mobile heavy metals, causing its compounds to persist in soils and sediments and being barely influenced by microbial decomposition. On the other hand, the kidney samples revealed greater Cd levels, even higher than those found in the soil samples from the OWMA and SNWR sites. Cd concentrations in the kidney specimens were found to be

Commentato [a1]: divide methods and results

4.62±0.71, 0.53±0.08, and 0.53±0.06 μg/ kg, respectively. In addition, micro-CT analysis of L4 from TCSFS showed significant Pearson's correlation coefficients between Cd concentrations and trabecular bone number (-0.67, $p \leq 0.05$) and trabecular separation (0.72, $p \leq 0.05$). The results showed no correlation between bone parameters and metal concentrations at reference sites. This study confirmed some earlier research by demonstrating substantial levels of heavy metal contamination in soil samples, kidney samples, and *P. leucopus* L4 trabecular bone separations from TCSFS.

Keywords.
Lead, Cadmium, Zinc, Microarchitecture, Heavy metal, soil, *Peromyscus leucopus.*

Introduction

Commentato [a2]: Provide more references and more recent bibliography. See suggestions below

Tar Creek Superfund site (TCSFS), Ottawa, OK is in northeastern Oklahoma near the Kansas-Oklahoma border. TCSFS covers a 40-square mile area and is one of the Tri-State Mining District (Oklahoma, Kansas, and Missouri) sites. These sites include territories of ten tribal nations such as Quapaw Nation and several other communities such as Picher, Cardin, North Miami, and Commerce (Agency for Toxic Substances and Disease Registry, 2013). Lead and zinc ores were mined at TCSFS from the early 1900s to the late 1970s.

Peromyscus. leucopus has been the subject of several different studies of the effects of environmental contaminants.

The Agency for Toxic Substances and Disease Registry (ATSDR) lists the major pathways of exposure to lead contamination at TCSFS as contaminated air, contaminated water, contaminated food resources, and contaminated soil. Such human health problems as respiratory illness, liver dysfunction, and reproductive and renal failure can occur after exposure via these pathways (Agency for Toxic Substances and Disease Registry, 2013).

Heavy metals such as cadmium, lead and zinc at TCSFS were studied because of their effects on human health and their accumulation in small mammals' bodies (Sanchez-Chardi et al., 2007). Numerous human health issues have been documented after exposure to cadmium because of its ability to substitute for other metals and nutrients such as zinc (Beyersmann & Hartwig, 2008). Small amounts of free cadmium ions are more toxic than bound cadmium ions, and cadmium can cause toxicity in different organs including the pancreas, testis, and nervous system. Elimination of cadmium by the kidneys is slow. Chronic exposures to cadmium ions can result in proteinuria and tubular dysfunction in the proximal tubules (Godt et al., 2006). Renal toxicity from cadmium exposure is correlated with the number of cadmium ions in kidney tubule cells, reabsorption, degradation of cadmium metallothionein complexes, and excess production of metallothionein by renal tubules. Cadmium toxicity observed in the renal cortex of laboratory rats (*Rattus rattus*) resulted in cytosolic damage and renal malfunction after oral administration of cadmium chloride (CdCl2) (Siddiqui, 2010).

Commentato [a3]: Provide more specific references on the effects of heavy metal on human bones

Some suggestions:

Scimeca et al. 2017 Heavy metals accumulation affects bone microarchitecture in osteoporotic patients

Bjørklund et al. 2020 Long-term accumulation of metals in the skeleton as related to osteoporotic derangements

Lead is a non-essential metal and is one of the abundant toxic metals at TCSFS.

Lead exposure can result in acute and chronic toxic effects. Lead accumulates in wild mammals in tissues such as the kidney, liver, and bone. In smelter and mining sites, lead and zinc were recorded in wood mice (*A. sylvaticus*), bank voles (*C. glareolus*), and field voles (*M. agrestis*).

The results showed high lead concentrations in bones; 42-68% of total lead found in body tissues was contained in bone (Johnson et al., 1978).

Lead exposure decreases bone mineral density (BMD) which can cause osteoporosis (Campbell et al., 2004). In addition, lead exposure inhibits osteoblast function. Zinc is another trace metal that was measured in this study. Zinc has important functions in bone formation, turnover, and metabolism (Bekheirnia et al., 2004). Zinc as a cofactor plays essential roles in other tissue and enzyme functions that are important for bone mineralization and development such as alkaline phosphate and collagenase (Bekheirnia et al., 2004).

Commentato [a4]: Same as the previous comment. More recent literature

Bone as a connective tissue has different sizes, shapes, and structures that serve important functions. Mineralized bone is the osseous tissue that gives bone rigidity. Bone tissue accumulates heavy metals such as cadmium and lead. Furthermore, bone tissue is one of the tissue markers that indicate xenobiotic and metal exposure (Marks & Popoff, 1988). *P. leucopus* as bioindicator species recorded as the most common species of the small mammals at TCSFS is the white-footed mouse (*Peromyscus leucopus*) (Phelps & McBee, 2009; Phelps & McBee, 2010).

Soil as a large natural source can contain any contaminant in the environment. Soil samples were used to analyze metal concentrations in TCSFS, BC and reference sites. The use of soil in the current study is to determine the presence of environmental contaminants in a biological source and to examine the alteration in physiological parameters of *P. leucopus* as a biomonitoring species.

Commentato [a5]: Aims of the study should be clearer. Rewrite

Studying bone microarchitecture helps to evaluate the toxic effects on bone after exposure to heavy metals. Bone dysfunction and osteoporosis are reported as toxic effects of exposure to cadmium (Youness et al., 2012). Significant decrease in bone density and the presence of osteopenia has been recorded in women who are exposed to cadmium from environmental sources (Engström et al., 2012).

Commentato [a6]: more recent literature

Materials & Methods

Study site

Soil samples were collected from the Beaver Creek area of the Tar Creek Superfund site (TCSFS, BC) and two reference sites, Sequoyah National Wildlife Refuge (SNWR) and Oologah Wildlife Management Area (OWMA) following the procedure that is described by USEPA (United States Environmental Protection Agency, 2005). A random design was used to collect soil samples in each site separately. Position for collection sites was detected with the help of Eterx Vista CX Garmin and a Google. Samples were collected to a depth of 18-20 cm from each position (Figure.1). Field experiments were approved by the Oklahoma State University Institutional Animal Care and Use Committee (ACUP No. AS 066).

Soil digestion

Soil samples were digested using a microwave digestion protocol (Milestone, Inc, Shelton, Connecticut) specified by USEPA Method Number 3051A (Kingston et al., 1997; United State Environmental Protection Agency, 1986).

Commentato [a7]: Different matrices might require corrections in the method? Provide more detail about the digestion as you did for tissue digestion

ICP-MS analysis

Cd, Pb, and Zn concentrations in soil specimens from both contaminated and uncontaminated sites were determined by ICP-MS according to USEPA Method 6010 (United States Environmental Protection Agency, 1996). Terbium was used as an internal standard (Perkin Elmer, Shelton, CT).

Kidney sampling

Frozen kidney samples of *Peromyscus leucopus* were provided from the OSU Collection of Vertebrates, Department of Integrative Biology. The *P. leucopus* had been collected from TCSFS, BC and the two reference sites, OWMA and SNWR. Oklahoma State University Animal Care and Use provided full approval for this research (Protocol AS056).

Commentato [a8]: Provide more details about the process:
how did you dissect?
how did you store the kidneys if so (temperature) etc.

Tissue digestion

Kidney samples were digested using a microwave system (Milestone, Inc, Shelton, CT) and USEPA Method Number 3051A (United State Environmental Protection Agency, 1986). One ml of concentrated high-purity HNO3 and 0.15 ml of H2O2 were added to the microwave digestion vessel and the sample was digested for 50 minutes at a temperature of 100°C. One ml of acid solution was then diluted with Millipore water.

Metal analysis

Cd, Pb, and Zn concentrations in kidney specimens were determined by inductively coupled plasma mass spectrometry (ICP-MS) according to USEPA method 6010 (United States Environmental Protection Agency, 1996). Terbium was used as an internal standard (Perkin Elmer, Shelton, CT).

Commentato [a9]: 1) The method USEPA 6010 was written for ICP-OES. Is this similar to ICP-MS? If not, you should describe differences in the method. Be more detailed.

2) Report the ICP-MS model

Bone microarchitecture:

µCT analysis:

Eight skeletons of *P. leucopus* from each site were provided from the vertebrate collection from the Department of Integrative Biology at OSU. The lumbar 2, 3, 4, and 5 vertebrae section of each skeleton was excised and the L4 was scanned using a high-resolution computed tomography system or micro-CT scanner (µCT 40, Scano Medical AG, Zurich, Switzerland). The fourth lumbar vertebra was detected in each skeleton sample and saved as a 3-D image. The trabecular bone in the 3-D images of L4 was contoured in a 300-400 µCT slide image. L4 slices were contoured every 10 slices beginning 10 slices below the detection of the spongiosa and ending 10 slices from the growth plate. The threshold for evaluation was set as 350 (gray scale, zero-1000) for all slides. The trabecular bone was contoured to measure the trabecular thickness (mm), trabecular number (mm-1), and trabecular volume as a percent of bone volume fraction (bone volume/tissue volume) for individual lumbar vertebra. The 3D images of the results were evaluated, and the data set was exported to evaluate and analyze the results.

Statistical analyses

This study examined soil and kidney samples collected from a contaminated site, TCSFS, BC and compared them with reference sites (SNWR and OWMA). In addition to soil and kidney samples, bone parameters from the contaminated site were compared with reference sites. Metal concentrations in the kidney were correlated with bone parameters. Pearson's correlation coefficients were determined for all samples taken together and by individual sites; Proc GLM, Proc Corr, SAS, V 9,4 were used and values of $P<0.05$ were taken as significant.

Results

Soil analysis:

Soil samples from the contaminated site (TCSFS, BC), and two reference sites (SNWR and OWMA) were compared. Mean concentrations of Cd, Pb, and Zn mg/kg in soil samples are presented in Table 1. The results showed that cadmium concentrations in soil samples in TCSFS, BC and the two reference sites were sharply different. In TCSFS, BC as a contaminated site, soil cadmium concentrations (mean±SE) at 48±04 mg/kg were recorded significantly higher than those at the two reference sites (0.06±0.01, and 0.15±0.03 mg/kg).

As expected, lead concentrations in the TCSFS, BC soil samples (1132±278 mg/kg) were higher (P<0.0001) than in the two reference sites (2.3±0.33, 6.4±1.1 mg/kg). Zinc concentrations in TCSFS, BC (14083±1826) were also much higher (P<0.0001) than in the two reference sites (20±2 and 53±5 respectively) (Table.1).

Metal concentrations (Cd, Pb, and Zn) in kidney samples (µg/ Kg) are presented by the site in Table 2. The zinc concentrations in kidney samples (µg/ Kg) were compared between contaminated TCSFS, BC and reference sites OWMA and SNWR, and the results showed no significant differences.

Cadmium concentrations (µg/ Kg) in kidney samples from TCSFS, BC and reference sites were significantly different (Table 2). In TCSFS, BC, higher cadmium concentrations (4.62±0.71 µg/ Kg) were recorded than at the two reference sites (P≤0.0005), but the results showed no differences between the two reference sites, OWMA (0.53 ±0.10) and SNWR (0.53 ±0.06) µg/ Kg. Likewise, Pb concentrations in kidney samples from TCSFS, BC (0.57±0.10 µg/ Kg) were higher than in the two reference sites, OWMA and SNWR (0.04±0.01 and 0.05±0.01 µg/ Kg respectively).

Commentato [a10]: Better to divide the results for kidney from those for soils

Bone microarchitecture relation to heavy metals at TCSFS, BC site

Correlation between bone parameters and kidney mineral concentrations by individual site was also examined and Pearson's correlation coefficients are presented in Table 4. In TCSFS, BC, Cd concentration was positively correlated with trabecular bone separation (r=0.72, P ≤ 0.05). Cadmium concentrations were negatively correlated with trabecular bone number (r=-0.67, P ≤ 0.05), and Pb concentration was positively correlated with trabecular bone separation (r=0.72, P ≤ 0.05).

Bone microarchitecture parameters in relation to heavy metals for uncontaminated sites

Trabecular bone microarchitecture parameters for the lumbar vertebrae (L4) and kidney metal concentrations (Cd, Pb, and Zn) in *Peromyscus leucopus* were analyzed to detect 1) the differences between the contaminated site TCSFS, BC site (Table 4) and 2) the and the reference sites (n=16) (Table 5). Micro-computed tomography evaluation results of bone parameters showed no correlations with kidney Cd, Pb, and Zn. Kidney lead positively correlated with kidney Zn (0.85, ≤ 0.05).

Discussion

Commentato [a11]: Enrich with more recent literature on the topic

This environmental toxicology field study showed several impacts and physiological alterations in *Peromyscus leucopus* due to their contact with the contaminants Cd, Pb, and Zn (Tables 1-5). The present study used specimens collected from the TCSFS, BC contaminated area and two reference sites (OWMA and SNWR). As expected, the concentrations of heavy metals (Cd, Pb, and Zn) in soil and kidney at TCSFS, BC were higher than at the reference sites. This study also analyzed the correlations between mineral concentrations (Cd, Pb, and Zn) of the kidney and the biomarkers such as bone parameters in the biomonitor species *Peromyscus leucopus*.

Several studies have determined that TCSFS is a site highly contaminated with Cd, Pb, and Zn. Mineral analysis of soil sample results confirmed heavy metal contamination at TCSFS, BC compared to reference sites (OWMA and SNWR). recorded the elevation of cadmium, lead, and zinc in soil sediments at Beaver Creek and Douthat Settling Pond at TCSFS. Lead concentrations were 440-540 mg/kg and cadmium concentrations were 20-56 mg/kg while zinc concentrations were 3000-9300 mg/kg. Large amounts of chat at TCSFS and extensive amounts of Cd, Pb, and Zn from mining and acid water were reported from the 1900s through the 1960s (Oklahoma Department of Environmental Quality, 2003). Heavy metals Cd, Pb, and Zn in tailings and yard soil at Tar Creek National Priorities List Superfund site in Oklahoma were analyzed in order to reduce metal and restore vegetation in this area (Brown et al., 2007). Soil chemical analysis showed unequal and extend distribution of heavy metals at Tar Creek superfund site at chat near Pitcher Oklahoma. The highest concentration of the contaminants was zinc > 4000 ppm, lead >1000ppm, and cadmium > 40 ppm (Beattie et al., 2017).

Bone is one of the most targeted tissues by lead (Pounds et al., 1991). Lead toxicity effects on bone cellular levels cause alterations. These effects include changes in circulating hormone 1, 25-dihydroxy vitamin D3 that regulates bone functions (Pounds et al., 1991). Also, Martiniaková et al. (2010) recorded significant heavy metal concentrations in *Apodemus flavicollis* and *Apodemus sylvaticus* at another polluted site in Slovakia. Although slight heavy metal accumulations were recorded in the femora, the study observed no changes in the femora's bone weight and the length of both species.

The present study affirms previous studies which have documented major effects on bone density and osteoporosis resulting from cadmium and lead exposure, including humans (Youness et al., 2012). In addition, the findings of this study are also consistent with other studies which have shown that heavy metals can cause liver and renal damage. For example, Lavery et al. (2009) investigated heavy metal effects on bone density, other bone parameters, renal damage, and metallothionein (MT) concentrations of South Australian bottlenose dolphins (*Tursiops aduncus*). The results showed Cd, Zn, and Cu in Tursiops aduncus caused liver as well as renal damage. Bone parameters of two individuals of Tursiops aduncus showed dysfunctions, renal damage, and high levels of MT (Lavery et al., 2009).

The findings of this study regarding cadmium contamination at TCSFS, BC have important implications for human health. Bone resorption and negative health effects have been shown to increase in women after middle age due to exposure to even low levels of cadmium in the diet (Åkesson et al., 2006). According to a study of women in southeast China in an area heavily polluted by cadmium, cadmium affected the bone formation and turnover through indirect effects on vitamin D3 metabolism (Wang et al., 2003). Heavy metal toxicity reduces the function of micro and macronutrients such as Zn, phosphate, and calcium which are the main components for bone strength and density.

Conclusions

This study recorded higher metal concentrations in soil and kidney samples from TCSFS compared with the two reference sites. However, the bone microarchitecture analyses of *Peromyscus leucopus* L4 vertebra of contaminated and uncontaminated sites did not show a strong correlation between bone parameters and metal concentration in the kidney. The lack of significant differences could have resulted from a limited sample size (n=8 from each site). Correlations between bone microarchitecture variables appeared to be higher in TCSFS, and BC samples than in reference sites.

In addition, this study used only adult mice, which are more exposed to the contaminants than younger mice due to their age. However, we did not know the exact age of the mice in this study. Bone mineral density and other bone parameter changes can be influenced by age. Legrand et al. (2000) recorded several vertebra fractures in a male patient of 52 years of age with lumbar osteopenia. These findings are evaluated through X-ray absorptiometry and bone microarchitecture changes of L2 and L4 trabecular bone. Bone resorption is associated with the inhibition of osteoblast function, and the studies reported this inhibition associated with the lead effects on cellular functions and regulation such as 1, 25–dihydroxy vitamin D3 (Pounds et al. 1991). Variations in bone parameters were expected due to the specimens' habitat, environmental contaminants, and variable ages.

In the current study, the contaminated site (TCSFS) showed significant elevation in metal concentrations in soil, kidney, and L4 trabecular bone separations of *P. leucopus*. This study showed metal accumulation in a small mammal, *P. leucopus*. The analysis of heavy metal concentrations in the kidney may elucidate issues of environmental quality and physiological alteration.

Commentato [a12]: This part is more appropriate in the discussions and supported by more recent literature

The results of this study reflected actual environmental contamination, but due to the field conditions, this study had several uncontrolled variables such as contaminant levels, duration of exposure to the heavy metals, and animal age. The contaminated site TCSFS, BC location was far from reference sites OWMA & SNWR (SNWR is located substantially further than OWMA from TCSFS). The results are a very important approach to determine the specific endpoint of concern and to reach conclusions that help human health and environmental sustainability. The fact that these mice had such high mineral concentrations in their kidneys and were still alive raises the possibility that this species, *P. leucopus* adapted to the heavy metal exposure. Moreover, this is the first study to record information regarding bone microarchitecture parameters in *P. leucopus* in North America.

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References

Agency for Toxic Substances and Disease Registry. 2013. Tar creek superfund site-Ottawa, Ok. Available: http://www.atsdr.cdc.gov/sites/tarcreek/.

Åkesson A, Bjellerup P, Lundh T, Lidfeldt J, Nerbrand C, Samsioe G, et al. 2006. Cadmium-induced effects on bone in a population-based study of women. Environ Health Perspect 114:830-834.

Beattie RE, Henke W, Davis C, Mottaleb MA, Campbell JH, McAliley LR. 2017. Quantitative analysis of the extent of heavy-metal contamination in soils near Picher, Oklahoma, within the tar creek superfund site. Chemosphere 172:89-95.

Bekheirnia R, Shamshirsaz A, Kamgar M, Bouzari N, Erfanzadeh G, Pourzahedgilani N, et al. 2004. Serum zinc and its relation to bone mineral density in β-thalassemic adolescents. Biol Trace Elem Res 97:215-224.

Beyersmann D, Hartwig A. 2008. Carcinogenic metal compounds: Recent insight into molecular and cellular mechanisms. Arch Toxicol Suppl 82:493-512.

Brown S, Compton H, Basta N. 2007. Field test of in situ soil amendments at the tar creek national priorities list superfund site. J Environ Qual 36:1627-1634.

Campbell JR, Rosier RN, Novotny L, Puzas JE. 2004. The association between environmental lead exposure and bone density in children. Environ Health Perspect 112:1200-1203.

Engström A, Michaëlsson K, Vahter M, Julin B, Wolk A, Åkesson A. 2012. Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. Bone 50:1372-1378.

Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A, et al. 2006. The toxicity of cadmium and resulting hazards for human health. J Occup Med Toxicol 1:22-22.

Johnson M, Roberts R, Hutton M, Inskip M. 1978. Distribution of lead, zinc and cadmium in small mammals from polluted environments. Oikos:153-159.

Kingston H, Walter P, Chalk S, Lorentzen E, Link D. 1997. Environmental microwave sample preparation: Fundamentals, methods, and applications. Microwave-enhanced chemistry: Fundamentals, sample preparation and application Am Chem Soc, Washington, DC:223-349.

Lavery TJ, Kemper CM, Sanderson K, Schultz CG, Coyle P, Mitchell JG, et al. 2009. Heavy metal toxicity of kidney and bone tissues in south Australian adult bottlenose dolphins (*tursiops aduncus*). Mar Environ Res 67:1-7.

Legrand E, Chappard D, Pascaretti C, Duquenne M, Krebs S, Rohmer V, et al. 2000. Trabecular bone microarchitecture, bone mineral density, and vertebral fractures in male osteoporosis. J Bone Miner Res 15:13-19.

Marks SC, Popoff SN. 1988. Bone cell biology: The regulation of development, structure, and function in the skeleton. Am j anat 183:1-44.

Martiniaková M, Omelka R, Jancova´ A, Stawarz R, Formicki G. 2010. Heavy metal content in the femora of yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (Apodemus sylvaticus) from different types of polluted environment in slovakia. Environ Monit Assess 171:651-660.

Oklahoma Department of Environmental Quality. 2003. Summary report of washed and unwashed mine tailings (chat) from two piles at the tar creek superfund site, Ottawa county, Oklahoma. Oklahoma Department of Environmental, Oklahoma City, OK.

Phelps, McBee K. 2009. Ecological characteristics of small mammal communities at a superfund site. Am Midl Nat 161:57-68.

Phelps KL, McBee K. 2010. Population parameters of *Promyscus leucopus* (white-footed deermice) inhabiting a heavy metal contaminated superfund site. Southwest Nat 55:363-373.

Pounds JG, Long GJ, Rosen JF. 1991. Cellular and molecular toxicity of lead in bone. Environ Health Perspect 91:17-32.

Sanchez-Chardi A, Penharroja-Matutano C, Oliveira Ribeiro CA, Nadal J. 2007. Bioaccumulation of metals and effects of a landfill in small mammals. Part ii. The wood mouse, apodemus sylvaticus. Chemosphere 70:101-109.

Siddiqui MF. 2010. Cadmium induced renal toxicity in male rats, *Rattus rattus*. East J Med 15:93-96.

United State Environmental Protection Agency U. 1986. Method 3051a, microwave assisted acid digestion of sediments, sludges, soils and oils. Washington DC, USA

United States Environmental Protection Agency. 1996. Method 6010b, inductively coupled plasma-atomic emission spectrometry.

United States Environmental Protection Agency. 2005. Tcsfs fact sheet, tar creek (Ottawa county). Washington DC, USA.

Wang H, Zhu G, Shi Y, Weng S, Jin T, Kong Q, et al. 2003. Influence of environmental cadmium exposure on forearm bone density. J Bone Miner Res 18:553-560.

Youness ER, Mohammed NA, Morsy FA. 2012. Cadmium impact and osteoporosis: Mechanism of action. Toxicol Mech Methods 22:560-567.