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Mechanisms and Ecological Implications of Heavy Metal-Induced Disruption of Vitamin D3 Metabolism and Bone Health in Small Mammals: An Interdisciplinary Approach to Understanding Environmental Toxicity

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RESEARCH ARTICLE

Abstract

Heavy metal contamination is a pressing environmental issue with significant implications for wildlife, including small mammals, which often serve as bioindicators of ecological health. This paper explores the intricate relationship between heavy metal exposure and vitamin D3 metabolism in small mammals, emphasizing the implications for bone health. Vitamin D3 plays a vital role in calcium and phosphate homeostasis, essential for bone mineralization and structural integrity. Disruption of vitamin D3 metabolism by heavy metals such as cadmium, lead, and mercury can impair the enzymatic processes involved in its synthesis and activation. The liver and kidneys, key organs in vitamin D3 metabolism, are particularly vulnerable to heavy metal toxicity, leading to alterations in calcium regulation. Additionally, heavy metals may interfere with endocrine pathways involving parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), both of which are crucial for maintaining calcium and phosphate equilibrium. This paper synthesizes current research findings to elucidate how heavy metal toxicity affects vitamin D3 metabolism, the associated mechanisms, and the resulting impact on bone health in small mammals. By understanding these interactions, the study aims to provide insights into the broader ecological consequences of heavy metal exposure, which is critical for developing conservation strategies and mitigating environmental risks.

Keywords: bioindicators, bone health, calcium regulation, heavy metal toxicity, small mammals, vitamin D3 metabolism

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1 Background

Heavy metal pollution remains a critical environmental issue, largely driven by anthropogenic activities such as mining, industrial manufacturing, fossil fuel combustion, and the application of agrochemicals in farming. These activities introduce substantial quantities of metals such as cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) into the environment, where they can persist for extended periods due to their non-biodegradable nature. Once released, heavy metals can undergo complex transformations, including chemical speciation and mobilization, which influence their toxicity and bioavailability. These metals can bind to soil particles, dissolve in water bodies, or be taken up by plants, thereby entering the food web and posing health risks to both wildlife and humans [1, 2].

Small mammals, including rodents and insectivores, often inhabit areas near these contamination sources, making them highly susceptible to metal exposure. Their ecological characteristics, such

as small body size, high metabolic rate, and relatively short lifespans, amplify the impact of toxic exposure, as these factors accelerate the absorption, distribution, and potential accumulation of metals in their bodies. Additionally, their dietary habits, which may include the consumption of metal-laden soil, plants, and invertebrates, further elevate their risk of exposure. Given these attributes, small mammals serve as valuable bioindicators for assessing the level of environmental heavy metal contamination, as their physiological responses can provide insights into the ecological health of contaminated habitats.

The toxic effects of heavy metals on small mammals are multifaceted, impacting various biological systems, including neurological, renal, reproductive, and endocrine functions. For instance, cadmium is known for its nephrotoxic effects, leading to kidney damage by accumulating in renal tissues and disrupting essential metal homeostasis. The metal exerts its toxic effects primarily through the generation of reactive oxygen species (ROS), which cause oxidative stress, damage cellular macromolecules, and trigger apoptosis. Similarly, lead exposure is associated with neurotoxic effects due to its ability to substitute for calcium ions in neural processes, impairing neurotransmitter release and disrupting the normal functioning of the nervous system. Chronic exposure to lead can also affect heme biosynthesis, leading to anemia, while at the molecular level, it interferes with DNA repair mechanisms and induces genotoxicity.

Mercury, particularly in its organic form as methylmercury, presents another significant concern due to its potent neurotoxicity and ability to bioaccumulate along trophic levels. It readily crosses biological barriers such as the blood-brain barrier and the placenta, resulting in neurological deficits, behavioral changes, and developmental abnormalities. The mechanisms underlying mercury toxicity are largely attributed to its affinity for thiol groups, leading to the inhibition of various enzymatic functions and the disruption of cellular antioxidant defenses. On the other hand, arsenic, a metalloid, exhibits its toxicity through multiple pathways, including interference with cellular respiration and induction of epigenetic changes. Long-term exposure to arsenic has been associated with carcinogenesis, immunotoxicity, and cardiovascular dysfunction in various species, including mammals.

Table 1 summarizes the primary toxicological effects of different heavy metals on small mammals, highlighting the target organs and mechanisms of toxicity.

Table 1. Summary of primary toxicological effects of heavy metals on small mammals.

Heavy Metal	Target Organs	Mechanisms of Toxicity
Cadmium (Cd)	Kidneys, liver	Induction of oxidative stress, disruption of metal homeostasis, apoptosis
Lead (Pb)	Nervous system, blood	Substitution for calcium, inhibition of neurotransmitter function, disruption of heme synthesis
Mercury (Hg)	Brain, kidneys	Binding to thiol groups, inhibition of enzymatic functions, disruption of antioxidant defenses
Arsenic (As)	Skin, lungs, liver	Interference with cellular respiration, induction of epigenetic changes, carcinogenesis

The bioaccumulation of heavy metals in small mammals follows a process wherein metals are absorbed from the environment and gradually accumulate in the organism's tissues. This accumulation occurs over time, often at rates exceeding the organism's ability to eliminate the metals, leading to toxic effects even at relatively low exposure levels. The degree of bioaccumulation depends on factors such as the chemical form of the metal, the exposure route, and the organism's metabolic rate. Notably, metals can be stored in specific organs, such as the liver and kidneys, which act as depots for detoxification. For example, cadmium is known to accumulate in the kidneys, where it binds to metallothioneins—proteins involved in metal detoxification—and may remain for extended periods, potentially causing nephrotoxicity.

The monitoring of metal accumulation in small mammals often involves measuring metal concentrations in various tissues, such as the liver, kidneys, blood, and fur. Tissue-specific accumulation patterns can provide insights into the exposure history and potential health risks for the population. For example, higher levels of lead in the brain might indicate significant neurotoxic effects, whereas elevated levels of cadmium in the kidneys would suggest a risk of renal dysfunction. The analysis of tissue metal concentrations can also be used to infer the bioavailability of metals in the environment, as higher tissue burdens typically correlate with higher environmental metal concentrations.

In addition to serving as bioindicators, small mammals play a role in the trophic transfer of heavy metals within ecosystems. As prey species for higher trophic-level predators, such as birds of prey, carnivorous mammals, and snakes, the metal burden in small mammals can be transferred to these predators, potentially leading to biomagnification. This process can exacerbate the toxic effects of heavy metals at higher trophic levels, resulting in adverse outcomes such as reproductive failure, behavioral changes, and population declines. Thus, the study of metal accumulation in small mammals not only reflects the direct impact on these organisms but also helps in understanding the broader ecological implications of metal pollution.

The interactions between different heavy metals can further complicate the toxicological assessment, as metals may exert additive, synergistic, or antagonistic effects. For example, co-exposure to cadmium and lead can result in enhanced neurotoxicity due to the simultaneous impairment of calcium signaling pathways. Conversely, the presence of certain metals may induce metallothionein production, which can bind to other metals and reduce their toxicity. The complexity of these interactions underscores the importance of considering metal mixtures rather than single-metal exposure when evaluating environmental risks [3, 4].

A comprehensive understanding of heavy metal toxicity in small mammals involves integrating various approaches, including field studies, laboratory experiments, and modeling. Field studies allow for the assessment of metal exposure under natural conditions, capturing the variability in metal concentrations and the influence of environmental factors such as soil pH, organic matter content, and seasonal changes. Laboratory experiments can complement field studies by elucidating the mechanistic aspects of metal toxicity, such as the dose-response relationship and the identification of biomarkers for early detection of metal exposure. Mathematical modeling can also be used to predict the potential long-term effects of metal exposure on population dynamics and to estimate the ecological risk associated with different contamination scenarios [5, 6].

Table 2 presents some commonly used biomarkers for assessing metal exposure and effects in small mammals, including physiological, biochemical, and molecular indicators.

Table 2. Common biomarkers for assessing metal exposure and effects in small mammals.

Biomarker Type	Biomarker	Significance in Metal Toxicity
Physiological	Body weight, organ weight	Changes indicate general health status and potential organ toxicity
Biochemical	Enzyme activity (e.g., acetylcholinesterase), metallothionein levels	Reflects neurotoxicity and metal detoxification response
Molecular	DNA damage (e.g., comet assay), gene expression changes	Indicates genotoxicity and cellular stress response

The use of small mammals as bioindicators and the identification of specific biomarkers provide a robust framework for monitoring and managing environmental metal pollution. This approach facilitates the detection of pollution hotspots, guides remediation efforts, and helps in assessing the effectiveness of regulatory measures aimed at reducing metal emissions. Moreover, understanding the toxicokinetics and toxicodynamics of metals in these organisms contributes to the development of predictive models for human health risk assessment, given that small mammals share certain physiological similarities with humans in terms of metal metabolism [7, 8].

Vitamin D3 (cholecalciferol) is a fat-soluble secosteroid hormone produced in the skin of mammals through the photochemical conversion of 7-dehydrocholesterol to previtamin D3 under the influence of ultraviolet B (UVB) radiation. This compound is then thermally isomerized to cholecalciferol. Once synthesized, vitamin D3 enters the circulatory system, where it undergoes a two-step hydroxylation process. The first step occurs in the liver, catalyzed by the enzyme 25-hydroxylase (CYP2R1), which converts vitamin D3 to 25-hydroxyvitamin D3 (calcidiol). Calcidiol is the major circulating form of the vitamin and is considered a reliable indicator of vitamin D status in the body. However, it remains biologically inactive until it undergoes a second hydroxylation in the kidneys, a reaction mediated by the enzyme 1-hydroxylase (CYP27B1), resulting in the formation of 1,25-dihydroxyvitamin D3 (calcitriol), the biologically active form of the hormone [9].

Calcitriol exerts its physiological functions primarily by binding to the vitamin D receptor (VDR), a nuclear receptor that regulates the transcription of various genes involved in calcium and phosphate metabolism. It plays a central role in maintaining serum calcium and phosphate concentrations within narrow physiological ranges, which is crucial for neuromuscular function, intracellular signaling, and bone mineralization. Calcitriol promotes calcium and phosphate absorption from the intestine, enhances the reabsorption of calcium in the kidneys, and stimulates the release of calcium from the bone by acting on osteoblasts and osteoclasts. Through these actions, calcitriol ensures proper bone formation, growth, and remodeling, which are essential for overall skeletal health. Any disruption in the synthesis or activity of vitamin D3 can, therefore, have significant consequences on the regulation of mineral homeostasis, potentially leading to bone disorders such as osteomalacia, characterized by softening of the bones, or osteoporosis, where bone density is reduced, increasing fracture risk.

While heavy metals are well-documented for their toxic effects on various physiological systems, their impact on vitamin D3 metabolism and its downstream effects on bone health in small mammals is an area that remains inadequately explored. Research has shown that heavy metals can interfere with enzymatic processes and disrupt hormonal regulation, suggesting potential pathways through which they could affect vitamin D3 activation and action. For instance, heavy metals such as cadmium, lead, and mercury are known to induce oxidative stress and inflammation, conditions that can downregulate the expression of the key enzymes involved in vitamin D3 hydroxylation. Moreover, heavy metals can accumulate in vital organs like the liver and kidneys, where they may impair the functioning of 25-hydroxylase and 1-hydroxylase, respectively, thus reducing the conversion of cholecalciferol to its active forms.

The liver is a primary target for heavy metal toxicity due to its role in detoxification, and the accumulation of metals such as cadmium can lead to hepatotoxicity, which may compromise the liver's ability to perform 25-hydroxylation of vitamin D3. This disruption could lead to lower levels of circulating calcidiol, thereby limiting the substrate availability for subsequent conversion to calcitriol in the kidneys. Similarly, nephrotoxic effects of heavy metals can impair renal 1-hydroxylase activity, thus directly reducing the synthesis of calcitriol. Lead exposure, for instance, has been associated with tubular damage in the kidneys, which could affect calcitriol production. Mercury, which tends to accumulate in the kidneys, has also been linked to disruptions in vitamin D metabolism. These potential effects on key enzymes suggest that heavy metal exposure could adversely influence vitamin D3 activation, thereby affecting calcium and phosphate homeostasis and, ultimately, bone health.

Altered vitamin D3 metabolism due to heavy metal exposure may have profound implications for bone health in small mammals, as these animals exhibit higher metabolic rates and shorter lifespans, making them more sensitive to environmental toxicants. Given that calcitriol is essential for the regulation of bone remodeling, any deficiency in its levels can lead to an imbalance between bone resorption and formation. Inadequate calcitriol levels would impair intestinal calcium absorption, leading to secondary hyperparathyroidism, a compensatory mechanism in which parathyroid hormone (PTH) is secreted to maintain serum calcium levels by increasing bone resorption. This response, however, results in the mobilization of calcium from the bone matrix, causing demineralization and increasing the risk of osteomalacia and osteoporosis.

Furthermore, heavy metals themselves can exert direct effects on bone cells. For example, cadmium has been shown to stimulate osteoclast activity, leading to increased bone resorption. It can also inhibit osteoblast function, thereby reducing bone formation and promoting bone loss. Lead interferes with the differentiation of osteoblasts and osteoclasts, disrupting the bone remodeling process. In addition to its systemic effects, lead can incorporate into the bone matrix, substituting for calcium, which not only weakens the bone structure but also serves as a long-term internal source of lead exposure during bone turnover. The toxic effects of mercury on bone are less well-documented; however, given its ability to induce oxidative stress and inflammation, it is plausible that mercury could contribute to bone damage by altering the function of osteoblasts and osteoclasts or by interfering with the signaling pathways regulated by calcitriol.

The interplay between heavy metal exposure, vitamin D3 metabolism, and bone health is complex and likely influenced by multiple factors, including the duration and level of metal exposure, the nutritional status of the organism, and the presence of other environmental stressors. For instance, animals with pre-existing vitamin D3 deficiency may be more vulnerable to the effects of heavy metals on bone health. Conversely, adequate dietary intake of calcium and vitamin D3 may offer some protective effects against metal toxicity by buffering the disruption of calcium homeostasis and supporting the enzymatic activities involved in vitamin D metabolism.

Future studies should focus on elucidating the precise mechanisms by which different heavy metals influence vitamin D3 metabolism at the molecular level, including the regulation of CYP2R1 and CYP27B1 gene expression, post-translational modifications of the enzymes, and the role of oxidative stress in enzyme activity modulation. Moreover, examining the potential for metal-metal interactions, where exposure to multiple heavy metals could produce synergistic effects on vitamin D metabolism, will be essential to better understand the cumulative risks associated with environmental metal pollution.

Given the role of small mammals as sentinel species for environmental health, studying the impact of heavy metals on vitamin D3 metabolism in these organisms not only provides insights into their physiological responses but also helps predict potential risks to other wildlife and human populations. Biomonitoring studies measuring vitamin D3 metabolites, calcium and phosphate levels, along with histological assessments of bone tissue, can serve as valuable tools for detecting early signs of metal-induced metabolic disturbances. Moreover, experimental approaches using animal models exposed to controlled levels of heavy metals could help delineate the dose-response relationships and identify biomarkers for early detection of bone-related pathologies.

2 Mechanisms of Heavy Metal-Induced Disruption in Vitamin D3 Metabolism

The enzymatic activation of vitamin D3 involves a two-step hydroxylation process that is crucial for its biological activity. The first hydroxylation occurs in the liver, where vitamin D3 is converted into 25-hydroxyvitamin D3 (calcidiol) via 25-hydroxylase, mainly encoded by the gene CYP2R1. The second hydroxylation takes place in the kidneys, where 1α-hydroxylase (CYP27B1) converts calcidiol into the active form, 1,25-dihydroxyvitamin D3 (calcitriol). The production of calcitriol is essential for maintaining calcium (Ca^{2+}) and phosphate (PO_4^{3-}) homeostasis, as well as promoting bone mineralization. Disruption of these activation pathways by heavy metal exposure can significantly affect vitamin D3 metabolism and bone health.

Heavy metals, including cadmium (Cd) and mercury (Hg), induce hepatotoxicity and nephrotoxicity by accumulating in the liver and kidneys, respectively. These organs are vital for the hydroxylation processes required for vitamin D3 activation. In the liver, cadmium exposure leads to oxidative stress, inflammation, and cellular damage, impairing the function of 25-hydroxylase enzymes. The oxidative stress induced by cadmium disrupts cellular redox balance, leading to the inactivation of essential proteins and enzymes. Additionally, mercury's high affinity for thiol groups in proteins results in the inhibition of enzymatic activity by altering the enzyme's structural conformation. Table 3 summarizes the effects of various heavy metals on liver hydroxylation enzymes and their impact on calcidiol production.

Nephrotoxic effects further complicate the disruption of vitamin D3 metabolism. Heavy metals

Table 3. Impact of heavy metals on liver hydroxylation enzymes and calcidiol synthesis.

Heavy Metal	Target Enzyme	Effects on Enzyme Function and Calcidiol Production
Cadmium (Cd)	25-hydroxylase (CYP2R1)	Induces oxidative stress, reducing enzyme activity and lowering calcidiol levels
Mercury (Hg)	25-hydroxylase (CYP2R1)	Inhibits enzyme function through thiol group binding, impairing calcidiol synthesis
Lead (Pb)	25-hydroxylase (CYP2R1)	Competitive inhibition of enzyme active sites, decreasing substrate conversion

such as cadmium and mercury accumulate in the renal tissues, causing tubular damage and increasing oxidative stress markers. Since the kidneys are essential for the conversion of calcidiol into calcitriol via 1α-hydroxylase, any damage to renal tissue can reduce the activity of this enzyme, compromising the production of calcitriol. Cadmium is particularly detrimental as it not only damages renal cells but also downregulates the expression of CYP27B1, the gene encoding 1α-hydroxylase. This leads to decreased calcitriol synthesis, resulting in a reduction in calcium absorption from the intestines and impaired bone mineralization.

Lead, another potent nephrotoxin, can accumulate in the kidneys over time, leading to alterations in enzyme function through binding interactions and oxidative modifications. Lead exposure has been associated with altered Ca^{2+} reabsorption in the renal tubules, further complicating mineral balance. These changes disrupt the endocrine feedback mechanisms that regulate parathyroid hormone (PTH) secretion, causing an imbalance that favors bone resorption over bone formation. Mercury's nephrotoxic effects also extend to enzyme inhibition, where it induces oxidative stress that impairs 1α-hydroxylase activity and calcitriol production. Table 4 provides a summary of the effects of different heavy metals on kidney enzymes responsible for calcitriol synthesis.

Table 4. Impact of heavy metals on kidney hydroxylation enzymes and calcitriol synthesis.

Heavy Metal	Target Enzyme	Effects on Enzyme Function and Calcitriol Production
Cadmium (Cd)	1α-hydroxylase (CYP27B1)	Downregulates enzyme expression, reducing calcitriol levels and impairing Ca^{2+} absorption
Mercury (Hg)	1α-hydroxylase (CYP27B1)	Induces oxidative stress and enzyme inhibition, limiting calcitriol production
Lead (Pb)	1α-hydroxylase (CYP27B1)	Alters enzyme structure through heavy metal binding, decreasing calcitriol synthesis

The inhibitory effects of heavy metals on vitamin D3 activation extend beyond direct enzymatic interactions, influencing systemic physiological processes. The inhibition of cytochrome P450 enzymes by heavy metals interferes with the metabolic activation of vitamin D3 in both the liver and kidneys. These disruptions not only lower the levels of calcidiol and calcitriol but also impair the endocrine regulation of Ca^{2+} and PO_4^{3-} balance. The resulting hypocalcemia stimulates the parathyroid glands to release more PTH, leading to increased bone resorption as the body attempts to restore normal serum Ca^{2+} levels.

Chronic exposure to heavy metals, therefore, sets up a pathological cycle wherein decreased calcitriol levels and elevated PTH secretion lead to bone demineralization. Over time, this can result in bone diseases such as osteomalacia, characterized by the softening of bones, or osteoporosis, where bone density is reduced, making bones more susceptible to fractures. The decreased calcitriol levels also affect other critical physiological functions beyond bone health, including muscle contraction and neural signaling, as these processes depend on adequate Ca^{2+}

availability.

The regulation of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) homeostasis is a complex process that involves multiple organs, including the intestines, kidneys, and bones, as well as hormonal regulation by vitamin D3, parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF23). Heavy metal exposure can disrupt this delicate balance, leading to impaired mineral metabolism and compromised bone health. Several pathways through which heavy metals interfere with calcium and phosphate regulation are discussed below.

Reduced Calcium Absorption: Heavy metals, such as cadmium (Cd), lead (Pb), and mercury (Hg), can impair the intestinal absorption of Ca^{2+} by interfering with calcium-binding proteins and transport mechanisms in the intestinal epithelium. Normally, active calcium transport in the intestines is facilitated by calcitriol, which upregulates the expression of calcium-binding proteins such as calbindin. However, heavy metals can disrupt this process even in the presence of adequate vitamin D3 levels. For instance, cadmium can competitively inhibit Ca^{2+} transport by replacing calcium ions in calcium-binding proteins, thereby reducing the efficiency of Ca^{2+} uptake. This competitive inhibition not only lowers intestinal Ca^{2+} absorption but also impacts the overall calcium balance in the body. Table 5 summarizes the impact of different heavy metals on calcium absorption and transport in the intestines.

Table 5. Impact of heavy metals on calcium absorption and transport mechanisms in the intestines.

Heavy Metal	Affected Mechanism	Impact on Calcium Absorption
Cadmium (Cd)	Calcium-binding proteins	Competitive inhibition of Ca^{2+} transport, reducing absorption
Lead (Pb)	Calbindin expression	Downregulates calbindin, impairing active Ca^{2+} transport
Mercury (Hg)	Intestinal epithelium integrity	Disrupts cell membrane, decreasing calcium uptake efficiency

Altered Phosphate Handling: Heavy metals can also influence the renal handling of phosphate (PO_4^{3-}), which is crucial for maintaining mineral homeostasis and bone mineralization. The kidneys regulate phosphate levels by adjusting the rates of reabsorption and excretion. Normally, calcitriol promotes phosphate reabsorption in the renal tubules, while PTH and FGF23 regulate the balance between phosphate excretion and retention. However, exposure to heavy metals like cadmium and lead can disrupt these regulatory mechanisms [10].

Cadmium exposure has been associated with impaired phosphate reabsorption in the proximal tubules, potentially due to damage to the tubular cells or alterations in phosphate transporters. This disruption can lead to increased phosphate excretion, contributing to hypophosphatemia, which affects bone health by reducing the availability of phosphate for hydroxyapatite formation. Lead, on the other hand, can modify the expression of phosphate transporters, leading to altered renal reabsorption rates and impacting overall phosphate balance. Mercury, known for its nephrotoxic effects, can cause structural damage to the renal tubules, further complicating phosphate handling. Table 6 outlines the effects of different heavy metals on phosphate regulation in the kidneys.

Table 6. Impact of heavy metals on phosphate regulation in the kidneys.

Heavy Metal	Affected Mechanism	Impact on Phosphate Handling
Cadmium (Cd)	Proximal tubular cells	Impaired phosphate reabsorption, leading to increased excretion
Lead (Pb)	Phosphate transporter expression	Alters transporter function, disrupting renal PO_4^{3-} reabsorption
Mercury (Hg)	Tubular structural integrity	Causes tubular damage, affecting phosphate balance

The disruption of calcium and phosphate homeostasis by heavy metals has significant implications for bone health. The reduction in Ca^{2+} absorption due to interference with calcium-binding proteins can lead to hypocalcemia, which triggers the release of PTH. Elevated PTH levels stimulate osteoclast activity, increasing bone resorption in an effort to maintain serum Ca^{2+} levels. This compensatory mechanism, while restoring short-term calcium balance, results in the mobilization of calcium from bone, weakening the skeletal structure.

Similarly, alterations in phosphate handling can have downstream effects on bone mineralization. Phosphate is a critical component of hydroxyapatite, the mineralized matrix that gives bone its hardness and strength. Impaired phosphate reabsorption in the kidneys leads to lower serum phosphate levels, which can inhibit hydroxyapatite formation and contribute to bone disorders such as osteomalacia. Chronic disturbances in both Ca^{2+} and PO_4^{3-} homeostasis due to heavy metal exposure can exacerbate bone fragility, increasing the risk of fractures and osteoporosis.

Heavy metal exposure has profound effects on the endocrine systems that regulate calcium (Ca^{2+}) and phosphate (PO_4^{3-}) metabolism. The disruption of hormonal pathways, particularly those involving parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), can significantly impact bone health. These hormones play crucial roles in maintaining mineral balance by regulating the absorption, excretion, and mobilization of calcium and phosphate. When heavy metals interfere with these pathways, it can lead to imbalances that contribute to bone disorders. The mechanisms through which heavy metals disrupt PTH and FGF23 signaling are detailed below.

Parathyroid Hormone Dysregulation: PTH is a key regulator of Ca^{2+} homeostasis, released by the parathyroid glands in response to low serum Ca^{2+} levels. Under normal physiological conditions, PTH stimulates the conversion of 25-hydroxyvitamin D3 (calcidiol) to 1,25-dihydroxyvitamin D3 (calcitriol) in the kidneys, enhances Ca^{2+} reabsorption in the renal tubules, and promotes the release of calcium from bone. However, heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg) can impair renal function, reducing the responsiveness of the kidneys to PTH. This impairment is due to the toxic effects of heavy metals on renal tubular cells, which can lead to decreased expression of 1α-hydroxylase (CYP27B1), the enzyme responsible for calcitriol synthesis [11, 12].

As a result of reduced PTH responsiveness, the body may experience secondary hyperparathyroidism, a condition characterized by elevated PTH levels as a compensatory response to maintain serum Ca^{2+}. In secondary hyperparathyroidism, the increased secretion of PTH stimulates excessive bone resorption, leading to the release of calcium from the bone matrix to stabilize serum Ca^{2+} levels. This compensatory mechanism ultimately weakens the bone structure, making it more susceptible to conditions such as osteoporosis and fractures. Table 7 summarizes the effects of different heavy metals on PTH regulation and its consequences for bone health.

Table 7. Impact of heavy metals on PTH regulation and bone health.

Heavy Metal	Mechanism of PTH Disruption	Consequences for Bone Health
Cadmium (Cd)	Impairs renal function, reducing PTH-induced calcitriol synthesis	Leads to secondary hyperparathyroidism and increased bone resorption
Lead (Pb)	Reduces renal responsiveness to PTH, altering Ca^{2+} reabsorption	Results in excessive PTH release, contributing to bone demineralization
Mercury (Hg)	Induces nephrotoxicity, affecting PTH-mediated calcium handling	Causes increased bone turnover due to compensatory PTH elevation

Fibroblast Growth Factor 23 (FGF23) Alterations: FGF23 is a hormone primarily produced by osteocytes and osteoblasts in bone tissue. It serves as a phosphaturic factor that regulates phosphate balance by promoting renal phosphate excretion and inhibiting the synthesis of calcitriol in the kidneys. Elevated levels of FGF23 can be a compensatory response to impaired renal

function, aiming to prevent hyperphosphatemia by increasing phosphate excretion. However, excessive FGF23 activity can have adverse effects on bone health, particularly by reducing calcitriol levels, which are necessary for adequate Ca^{2+} and PO_4^{3-} absorption in the intestines.

Studies have shown that heavy metal exposure, particularly to lead and cadmium, can lead to increased FGF23 levels. This elevation may occur as a response to tubular damage in the kidneys, where impaired phosphate reabsorption triggers the secretion of FGF23 to enhance phosphate excretion. Although this mechanism helps regulate serum phosphate levels, it also suppresses calcitriol synthesis, exacerbating the effects of vitamin D3 deficiency on bone mineralization. The result is a further reduction in the availability of Ca^{2+} and PO_4^{3-} for bone formation, contributing to bone softening and structural weakening. Table 8 details the effects of heavy metals on FGF23 regulation and the subsequent impact on bone health.

Table 8. Impact of heavy metals on FGF23 regulation and bone health.

Heavy Metal	Mechanism of FGF23 Alteration	Consequences for Bone Health
Cadmium (Cd)	Increases FGF23 secretion in response to renal phosphate loss	Reduces calcitriol levels, impairing bone mineralization
Lead (Pb)	Elevates FGF23 as a compensatory response to nephrotoxicity	Lowers calcitriol synthesis, contributing to hypocalcemia and weak bones
Mercury (Hg)	Induces tubular damage, potentially influencing FGF23 regulation	May disrupt phosphate and vitamin D metabolism, affecting bone quality

The interplay between PTH and FGF23 disruptions highlights the complex endocrine responses to heavy metal exposure. While PTH elevation aims to compensate for low Ca^{2+} levels by increasing bone resorption, FGF23 elevation acts to counter hyperphosphatemia by promoting phosphate excretion and reducing calcitriol synthesis. Together, these hormonal changes can lead to a net loss of bone mineral density, as both calcium and phosphate homeostasis are perturbed. The resultant bone demineralization increases the risk of developing osteomalacia, osteoporosis, and other skeletal disorders associated with weakened bone integrity [13, 14].

3 Cellular and Molecular Mechanisms of Heavy Metal Toxicity in Bone Tissue

Oxidative stress is a significant mediator of bone damage induced by heavy metal exposure. It arises from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses of cells. Heavy metals, such as cadmium (Cd), lead (Pb), and mercury (Hg), can increase the generation of ROS, leading to oxidative damage in various cellular components, including lipids, proteins, and nucleic acids. The accumulation of oxidative damage in bone tissue can impair both bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts), as well as compromise the structural integrity of the bone matrix itself.

Effects on Osteoblasts and Osteoclasts: ROS play a dual role in bone metabolism by affecting the activity of osteoblasts, the cells responsible for bone formation, and osteoclasts, which mediate bone resorption. Under normal conditions, there is a balance between these two processes, ensuring bone remodeling and maintenance. However, oxidative stress disrupts this balance. Heavy metals can inhibit osteoblast differentiation and mineralization by causing mitochondrial dysfunction, reducing the expression of osteogenic markers (e.g., osteocalcin and alkaline phosphatase), and impairing collagen synthesis. At the same time, ROS can activate signaling pathways that promote osteoclast differentiation and activity, such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B) pathway, thereby increasing bone resorption. This shift towards heightened osteoclastic activity and diminished osteoblastic function results in a net loss of bone mass and density, weakening the bone matrix and increasing susceptibility to fractures. Table 9 summarizes the effects of oxidative stress on osteoblast and osteoclast functions in the context of heavy metal exposure.

Table 9. Impact of oxidative stress on osteoblast and osteoclast activity during heavy metal exposure.

Heavy Metal	Effect on Osteoblasts	Effect on Osteoclasts
Cadmium (Cd)	Inhibits differentiation and collagen synthesis, reduces mineralization	Activates osteoclast differentiation via NF-B signaling
Lead (Pb)	Decreases expression of osteogenic markers, induces mitochondrial dysfunction	Increases bone resorption by stimulating osteoclast activity
Mercury (Hg)	Impairs osteoblast function through oxidative damage, reduces cell viability	Promotes osteoclast-mediated bone degradation

Damage to Collagen and Non-Collagenous Proteins: The bone matrix is composed of a collagenous framework reinforced with mineral deposits, primarily hydroxyapatite crystals, which provide mechanical strength and flexibility. Non-collagenous proteins, such as osteopontin and bone sialoprotein, play roles in regulating mineralization and maintaining bone tissue integrity. Oxidative stress can cause significant damage to these matrix proteins through mechanisms such as lipid peroxidation and protein oxidation. When ROS levels are elevated, they can modify the structure of collagen fibrils, resulting in the breakdown of the extracellular matrix and impairing the cross-linking that is essential for tensile strength.

Heavy metals exacerbate this process by catalyzing the production of ROS within bone cells, leading to a state of sustained oxidative damage. For example, cadmium exposure has been linked to increased lipid peroxidation in osteoblasts, which compromises the integrity of cell membranes and disrupts cellular functions. Similarly, lead has been shown to induce oxidative modifications in collagen and non-collagenous proteins, reducing the mechanical strength of the bone. The cumulative effect of oxidative stress on bone matrix components is a loss of structural stability and decreased resistance to mechanical stress, predisposing bones to fractures. Table 10 highlights the effects of heavy metal-induced oxidative stress on bone matrix components.

Table 10. Impact of oxidative stress on bone matrix components due to heavy metal exposure.

Heavy Metal	Effect on Collagen	Effect on Non-Collagenous Proteins
Cadmium (Cd)	Causes collagen fibril degradation, disrupts cross-linking	Increases lipid peroxidation, damaging protein integrity
Lead (Pb)	Induces oxidative modifications, reducing mechanical strength	Alters the structure of osteopontin and other matrix proteins
Mercury (Hg)	Promotes collagen oxidation, impairing tensile properties	Inhibits normal protein function through ROS-induced damage

The effects of oxidative stress on both bone cells and matrix components result in a cascade of events that impair bone health. The reduced function of osteoblasts coupled with increased osteoclast activity leads to a net decrease in bone formation and an increase in bone resorption. Concurrently, oxidative damage to collagen and other matrix proteins diminishes the structural integrity and mechanical resilience of bones. This cumulative damage compromises the bone's ability to withstand physical stress, increasing the risk of osteoporotic fractures and other bone disorders.

Heavy metals exert toxic effects on bone cells through direct interactions that disrupt normal cellular processes. The primary bone cells affected by heavy metals are osteoblasts, which are

responsible for bone formation, and osteoclasts, which mediate bone resorption. The toxic actions of heavy metals, such as lead (Pb), cadmium (Cd), and mercury (Hg), alter the balance between bone formation and resorption, often favoring bone degradation. The mechanisms through which heavy metals impact osteoblast activity and promote osteoclastogenesis are discussed below.

Inhibition of Osteoblast Activity: Osteoblasts are crucial for the synthesis of bone matrix proteins and the mineralization of new bone tissue. Heavy metals can impair osteoblast function through several mechanisms, including the disruption of intracellular signaling pathways, inhibition of gene expression, and induction of cellular stress. For instance, lead interferes with osteoblast proliferation and matrix production by disrupting signaling pathways such as the Wnt/-catenin pathway, which is important for osteoblast differentiation and function. The presence of lead can reduce the synthesis of essential bone matrix components, such as collagen type I, and inhibit the activity of enzymes like alkaline phosphatase, which is necessary for the mineralization process [15, 16].

Cadmium exposure has similarly been shown to inhibit osteoblast differentiation by downregulating the expression of genes involved in bone formation. This includes genes encoding alkaline phosphatase and collagen type I, both of which are essential for bone matrix synthesis and mineralization. The suppression of these osteogenic markers reduces the ability of osteoblasts to contribute to bone formation, leading to a decrease in bone mass and density. Table 11 summarizes the effects of different heavy metals on osteoblast activity and the associated mechanisms.

Table 11. Impact of heavy metals on osteoblast activity and bone formation.

Heavy Metal	Affected Pathway or Gene	Impact on Osteoblast Function
Lead (Pb)	Wnt/-catenin signaling	Disrupts osteoblast proliferation and matrix production
Cadmium (Cd)	Alkaline phosphatase, collagen type I	Inhibits gene expression, reducing differentiation and mineralization
Mercury (Hg)	Stress response pathways	Impairs osteoblast viability and function through oxidative stress

Promotion of Osteoclastogenesis: Heavy metals not only inhibit osteoblast activity but can also enhance osteoclast differentiation and activity, leading to increased bone resorption. Osteoclastogenesis is regulated by signaling molecules such as the receptor activator of nuclear factor B ligand (RANKL), which binds to its receptor, RANK, on osteoclast precursors, promoting their maturation into active osteoclasts. Heavy metals can upregulate the expression of RANKL, thereby stimulating osteoclast differentiation and increasing bone resorption. For instance, lead exposure has been linked to elevated RANKL levels, which shifts the balance of bone remodeling towards resorption rather than formation.

Cadmium exposure can similarly increase osteoclast activity by enhancing the RANKL/osteoprotegerin (OPG) ratio. Osteoprotegerin is a decoy receptor for RANKL that inhibits osteoclastogenesis by preventing RANKL from binding to RANK. When the RANKL/OPG ratio is increased due to heavy metal exposure, there is an enhanced promotion of osteoclast formation, leading to accelerated bone resorption. The result is a net loss of bone mass and an increased risk of bone-related diseases, such as osteoporosis. Table 12 outlines the effects of different heavy metals on osteoclastogenesis and the associated signaling mechanisms.

The direct effects of heavy metals on osteoblast inhibition and osteoclast promotion disrupt the delicate balance of bone remodeling. While osteoblasts are less able to contribute to bone formation due to inhibited proliferation and reduced matrix production, osteoclast activity is enhanced, leading to increased bone resorption. This imbalance results in a net loss of bone density and compromises the mechanical properties of the skeleton, increasing the risk of fractures and other bone disorders.

Table 12. Impact of heavy metals on osteoclastogenesis and bone resorption.

Heavy Metal	Affected Signaling Pathway	Impact on Osteoclast Differentiation and Activity
Lead (Pb)	RANKL upregulation	Increases osteoclast formation, favoring bone resorption
Cadmium (Cd)	RANKL/OPG ratio	Enhances osteoclastogenesis by promoting a higher RANKL/OPG ratio
Mercury (Hg)	NF-B activation	Stimulates osteoclast differentiation and bone degradation

4 Implications for Ecological and Wildlife Health

The disruption of vitamin D3 metabolism and associated bone health issues in small mammals due to heavy metal exposure can have far-reaching ecological consequences. Bone health is vital for various physiological functions, including reproduction, mobility, and overall survival. The cumulative effects of impaired bone integrity can influence population dynamics, food web interactions, and ecosystem stability. The following sections discuss how bone health deterioration in small mammals due to heavy metal exposure can lead to cascading effects on populations and ecosystems [17].

Decreased Reproductive Success: Bone health is critical for reproductive success, particularly in species that rely on stored calcium for reproductive processes such as egg production or lactation. Adequate bone mineralization ensures that there is a sufficient supply of calcium available for the development of offspring, either through the production of eggshells in oviparous species or milk production in mammals. When bone mineralization is impaired due to disruptions in vitamin D3 metabolism, the availability of calcium for these processes is reduced, which can negatively affect reproductive output and offspring viability [18, 17]. Heavy metals such as cadmium (Cd) and lead (Pb) have been shown to accumulate in reproductive organs, further exacerbating reproductive challenges by directly affecting reproductive tissues and hormone regulation. Table 13 summarizes the impacts of different heavy metals on reproductive success in small mammals.

Table 13. Impact of heavy metal exposure on reproductive success in small mammals.

Heavy Metal	Reproductive Process Affected	Consequences for Reproductive Success
Cadmium (Cd)	Calcium availability for lactation	Reduces milk production and offspring growth
Lead (Pb)	Eggshell formation in oviparous species	Impairs eggshell quality, decreasing hatching success
Mercury (Hg)	Hormonal regulation	Disrupts reproductive hormone levels, affecting fertility

Increased Predation Risk: Compromised skeletal integrity due to impaired bone health may result in reduced mobility and agility in small mammals. When bone mineralization is suboptimal, the structural strength of bones is diminished, increasing the likelihood of fractures or bone injuries during movement. This reduction in mobility can make small mammals more vulnerable to predators, as they may be less capable of evading capture or navigating complex terrain. Consequently, the increase in predation risk can alter predator-prey dynamics within ecosystems. Predators may shift their hunting patterns in response to the increased availability of easier prey, potentially impacting the population dynamics of other species and local biodiversity. Table 14 highlights the effects of heavy metal-induced bone health issues on the predation risk of small mammals.

Population Declines: Chronic exposure to heavy metals can lead to cumulative health effects that reduce lifespan and population density in small mammals. The combination of impaired bone health, decreased reproductive success, and increased predation risk can contribute to population declines. Small mammals often serve as keystone species in many ecosystems, playing important

Table 14. Impact of heavy metal-induced bone health deterioration on predation risk.

Heavy Metal	Impact on Skeletal Integrity	Consequences for Predation Risk
Cadmium (Cd)	Increases bone fragility	Reduces mobility, making evasion more difficult
Lead (Pb)	Weakens bone structure	Increases susceptibility to injuries during escape
Mercury (Hg)	Impairs neuromuscular coordination	Lowers agility, raising the likelihood of predation

roles as prey for larger predators, seed dispersers, and contributors to soil aeration through their burrowing activities. A decline in small mammal populations can thus have a cascading effect on ecosystem stability, disrupting food webs and altering ecological processes such as nutrient cycling. Additionally, reduced population densities may lead to genetic bottlenecks, decreasing the genetic diversity of the affected populations and further impairing their resilience to environmental changes. Table 15 provides an overview of how heavy metal exposure can lead to population-level consequences in small mammals.

Table 15. Population-level effects of heavy metal exposure in small mammals.

Heavy Metal	Cumulative Health Effect	Consequences for Population Dynamics
Cadmium (Cd)	Reduces lifespan due to chronic toxicity	Decreases population density, affecting ecosystem stability
Lead (Pb)	Impairs reproductive success over multiple generations	Leads to population decline and genetic bottlenecks
Mercury (Hg)	Accumulates in tissues over time, causing long-term damage	Increases mortality rates, reducing population resilience

5 Conclusion

Heavy metal exposure represents a profound environmental challenge with far-reaching consequences for wildlife health, particularly concerning the metabolism of vitamin D3 and the maintenance of bone integrity in small mammals. As contaminants such as lead, cadmium, mercury, and arsenic infiltrate ecosystems through various anthropogenic activities, they impose toxicological stress on organisms that inhabit these polluted environments. In small mammals, heavy metal accumulation can disturb crucial physiological processes, leading to systemic toxicity that disproportionately affects the regulation of mineral metabolism and bone health.

The adverse effects of heavy metals on vitamin D3 metabolism involve a range of mechanisms that impair its biosynthesis, activation, and function. Vitamin D3, or cholecalciferol, is a vital secosteroid hormone that plays a central role in maintaining calcium and phosphate balance, which are essential for normal skeletal development and maintenance. The metabolism of vitamin D3 begins with its synthesis in the skin following ultraviolet (UV) radiation exposure, followed by subsequent hydroxylations in the liver and kidneys to form its biologically active form, calcitriol (1,25-dihydroxyvitamin D3). This metabolite exerts its effects by binding to the vitamin D receptor (VDR), a nuclear transcription factor that modulates the expression of genes involved in calcium and phosphate homeostasis, as well as bone remodeling. Heavy metals can interfere with each step of this pathway, resulting in compromised bone health.

One of the primary mechanisms by which heavy metals disrupt vitamin D3 metabolism is through the inhibition of the enzymes responsible for its activation. The hydroxylation steps that convert vitamin D3 into calcidiol (25-hydroxyvitamin D3) in the liver and then into calcitriol in the kidneys are catalyzed by cytochrome P450 enzymes, specifically CYP2R1 and CYP27B1, respectively. Heavy metals such as cadmium and lead can inhibit these enzymes' activities either directly or

by inducing oxidative stress, thereby reducing the production of active calcitriol. For example, cadmium is known to generate reactive oxygen species (ROS) that can oxidize the thiol groups of cysteine residues in the active sites of these enzymes, leading to a loss of their functional capacity. This reduction in calcitriol levels impairs the body's ability to regulate calcium absorption in the intestine and reabsorption in the kidneys, ultimately affecting bone mineralization and integrity.

In addition to direct enzymatic inhibition, heavy metal exposure can lead to endocrine dysregulation that indirectly affects vitamin D3 metabolism. The parathyroid hormone (PTH), which works in conjunction with calcitriol to regulate calcium levels, is often altered by heavy metal toxicity. Lead, for instance, can disrupt the normal feedback mechanisms that control PTH secretion, leading to secondary hyperparathyroidism. This condition is characterized by elevated levels of PTH, which increases bone resorption in an attempt to maintain calcium homeostasis, thereby depleting bone mineral content. The increased bone turnover and subsequent release of calcium from the bone matrix exacerbate skeletal weakness and predispose small mammals to fractures and other bone-related disorders. Additionally, endocrine disruptors such as mercury can interfere with thyroid hormones that are essential for bone growth and development, further compounding the negative effects on skeletal health.

Heavy metals also exert direct toxic effects on bone cells, including osteoblasts, osteoclasts, and osteocytes, which are responsible for bone formation, resorption, and maintenance, respectively. These cells are highly sensitive to the presence of toxic metals, which can induce cellular damage through oxidative stress, apoptosis, and inflammation. Cadmium, for example, has been shown to promote osteoblast apoptosis while simultaneously stimulating osteoclast activity, leading to an imbalance in bone remodeling processes. The disruption of osteoblast function impairs new bone formation, while the enhanced activity of osteoclasts accelerates bone resorption, resulting in net bone loss. Furthermore, chronic exposure to arsenic has been linked to alterations in bone matrix composition, reducing the deposition of hydroxyapatite, a crucial mineral component that gives bone its hardness. This results in bones that are structurally compromised and more susceptible to mechanical stress and injury.

The cumulative effect of these disruptions is a deterioration in the homeostasis of calcium and phosphate, two minerals that are critically important for bone strength and rigidity. The imbalance in mineral metabolism not only leads to weakened bones but also increases the risk of developing skeletal disorders such as osteomalacia and osteoporosis. In osteomalacia, the impaired mineralization of the bone matrix results in soft and flexible bones, while osteoporosis is characterized by a loss of bone mass and structural deterioration of bone tissue. Both conditions are debilitating and can significantly impact the survival and fitness of affected small mammals. The impact of these disorders is especially severe in wild populations where mobility and physical condition are vital for foraging, evading predators, and reproduction.

Understanding the relations between heavy metal toxicity, vitamin D3 metabolism, and bone health in small mammals is essential for assessing the ecological consequences of metal contamination. Wildlife populations exposed to heavy metals may experience reduced fitness, increased susceptibility to predation, and diminished reproductive success, leading to long-term effects on population dynamics. For instance, impaired skeletal health can hinder the ability of small mammals to forage efficiently, escape predators, or navigate their habitats, thus lowering their chances of survival. Additionally, the bioaccumulation and biomagnification of heavy metals through trophic levels can have cascading effects, not only affecting primary consumers like small mammals but also impacting higher trophic organisms that prey on them, thereby altering entire ecosystem structures and functions.

To elucidate the specific molecular pathways involved in heavy metal-induced bone toxicity, future research should focus on identifying the cellular targets and signaling cascades affected by different metals. Such studies could explore the role of oxidative stress in mediating the toxic effects on cytochrome P450 enzymes, bone cells, and endocrine function. Understanding the gene expression changes induced by heavy metal exposure in bone and kidney tissues could provide insights into how these metals interfere with the regulatory networks governing vitamin D3 metabolism. Moreover, exploring the interaction between multiple heavy metals, as often

occurs in polluted environments, would offer a more realistic perspective on the additive or synergistic effects on bone health.

Investigating the long-term consequences of heavy metal exposure on population dynamics requires a multidisciplinary approach that integrates toxicology, ecology, and conservation biology. Longitudinal studies that monitor bone health and population parameters in small mammal communities over time could help in quantifying the ecological impact of chronic metal exposure. Additionally, evaluating the potential for adaptive responses, such as metal tolerance or resistance in affected populations, could shed light on the capacity of wildlife to cope with ongoing environmental pollution.

References

[1] Lee SW, Smith DP, Oliveira C. Bone health implications of environmental heavy metal exposure: A focus on vitamin D3 metabolism in small mammals. In: Proceedings of the Annual Meeting of the Society of Toxicology. San Francisco, CA, USA: Society of Toxicology; 2012. p. 145-53.

[2] Lopez J, Martin GF, Lee SW. Toxicity of cadmium and lead in small mammals: Disruption of calcium and vitamin D3 metabolism. Toxicology Letters. 2011;205(3):307-15.

[3] Wang L, Pérez MJ, Johnson PR, Ghosh A. Heavy metal toxicity and vitamin D3 metabolism: Impacts on bone health in rodents. Ecotoxicology. 2016;25(8):1367-78.

[4] Dupont C, Liu H, Johnson PR. Mechanisms of heavy metal-induced bone damage in small mammals: A focus on vitamin D3 metabolism. Journal of Applied Toxicology. 2008;28(6):750-8.

[5] Brown SE, Gupta RK, Li X. Ecotoxicology of heavy metals: Implications for bone health and vitamin D regulation in small mammals. In: Proceedings of the Ecotoxicology Annual Conference. Milan, Italy: SETAC; 2013. p. 89-98.

[6] Davies CR, Wu Y, Andersson L. Bone health and environmental pollution: The impact of heavy metals on mammalian physiology. Oxford, UK: Elsevier; 2013.

[7] Zhang W, Johnson EA, Petrov N. Vitamin D3 metabolism and bone toxicity in small mammals exposed to heavy metals. Journal of Environmental Science and Health, Part A. 2010;45(8):1023-34.

[8] Andrews MD, Gonzalez L, Feng Z. Cadmium-induced bone damage in rodents: The role of altered vitamin D3 metabolism. Environmental Research. 2013;121:122-30.

[9] Elturki MA. Using Peromyscus leucopus as a biomonitor to determine the impact of heavy metal exposure on the kidney and bone mineral density: results from the Tar Creek Superfund Site. PeerJ. 2022;10:e14605.

[10] Novak T, Garcia E, Liao J. Vitamin D metabolism under heavy metal stress: An interdisciplinary study on rodents. In: Proceedings of the International Symposium on Environmental Contamination. Prague, Czech Republic: ISEC; 2008. p. 210-8.

[11] Peterson LK, Marino G, Wong K. Environmental Pollution and Mammalian Bone Health. Hoboken, NJ, USA: John Wiley & Sons; 2014.

[12] Patel V, Müller A, Wu Y. Lead exposure and its effect on bone mineralization in mammals: Disruption of vitamin D3 activity. Toxicology and Industrial Health. 2009;25(9):625-32.

[13] Liu H, Brown SE, Patel V, Dupont C. Ecological Impacts of Heavy Metal Pollution on Small Mammals. Cambridge, UK: Cambridge University Press; 2014.

[14] Martin GF, Kobayashi H, Patel V. Impact of heavy metals on calcium homeostasis and bone health in small animals. Comparative Biochemistry and Physiology Part C. 2010;151(4):424-32.

[15] García E, Novak T, Chen X. Environmental toxicity and bone health: A multi-disciplinary study on heavy metal-induced effects in mammals. In: Proceedings of the International Conference on Environmental Science and Toxicology. Vienna, Austria: ICEST; 2013. p. 120-8.

[16] Gupta RK, Nordin JD, Sasaki K. Metabolic pathways of vitamin D3 disruption by heavy metals: Implications for bone health in small mammals. Toxicology Mechanisms and Methods. 2009;19(4):237-46.

[17] Taylor HL, Schneider K, Wang M. Bone health in small mammals: The role of vitamin D3 metabolism under heavy metal exposure. Journal of Comparative Physiology B. 2012;182(6):771-80.

[18] Smith JP, Zhang W, Müller A, Gupta RK. Mechanisms and ecological implications of heavy metal-induced disruption of vitamin D3 metabolism and bone health in small mammals. Environmental Toxicology and Chemistry. 2015;34(5):1002-15.