TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2025, vol. 10, No 1(18): 44–53

HEAVY METALS LEVELS IN WATER AND FAECAL SAMPLES OF WILD ANIMAL SPECIES AT THE UNIVERSITY OF IBADAN ZOOLOGICAL GARDEN

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(Submitted: 3 January 2025; Accepted: 2 April 2025; Published: 25 June 2025)

ABSTRACT

Urban wildlife including those in zoological gardens may be at risk of heavy metal exposure eliciting from anthropogenic sources. Determining heavy metal levels especially through non-invasive techniques is important in comprehending their ecological effects. The goal of this study was to evaluate heavy metals levels in water and faecal samples collected from enclosures of wild animals [carnivores (n=16), herbivores (n=10) and omnivores (n=11)] at the University of Ibadan zoological garden as a biomarker of exposure. Samples collected were subjected to heavy metals determination using Atomic Absorption Spectrophotometer sequel to wet acid digestion. Statistical significant difference was only observed in the concentrations of Zinc (P=0.009) in the faecal samples across the animal groups. Results from the study confirmed exposure to heavy metals by the studied animal species. Also, the heavy metals above permissible limits can cause acute and chronic toxicities in the wild animals and may threaten their long-term health.

Key words: Biomagnification, wild animals, heavy metals, pollution, anthropogenic activities.

Introduction

Zoological gardens are institutions or facilities in which various wild animals are confined within specific enclosures based on their ecological requirements and systematically displayed to the public for viewing and recreation. These institutions offer enormous opportunities for education and entertainment whilst contributing to wildlife conservation (Abd Rabou 2011). Wild animals in zoological gardens have to be kept be kept securely (Omonona *et al.*, 2018) especially from anthropogenic threats which are gradually becoming a global concern. Amongst these threats is the issue of environmental contamination, and heavy metals are one of the most ubiquitous and toxic environmental contaminants in the world. These metals are naturally-occurring elements with a high density and atomic weight which can also be released into the environment through anthropogenic activities (Masindi *et al.* 2021). Natural sources of heavy metals include metallic deposits from volcanic eruptions, weathering, erosion and atmospheric condensation (Tesser *et al.* 2021) while anthropogenic sources include those from industrial and mining activities, fossil fuel combustion, waste disposal sites, automobile exhaust, application of agrochemicals, and so on (Adetuga *et al.* 2020). Some of the consequences of exposure to heavy metals may include immunosuppression, endocrine disruption, oxidative stress damage, reproductive impairment, and so on.

Urban wildlife including those in zoological gardens may be at risk of heavy metal exposure eliciting from anthropogenic sources as they similarly utilise human-transformed habitats. Wildlife species are typically exposed to heavy metals through dietary pathways and this has made toxic levels in wildlife to be linked to food webs especially in urban areas (Gall *et al.* 2015). Specifically, wild animal species can be exposed to heavy metals through ingestion, inhalation and dermal contact (Sardar *et al.* 2013). Different methods have been used to evaluate and bring up a concentration profile of an array of contaminants that might impact wildlife (Gupta et Bakre 2013). Traditional methods used for toxicological risk assessment in wildlife are typically invasive, involving the sacrifice of live animals in laboratory studies (Kenston *et al.* 2018). Preferably, it is best advised to use a method of contamination assessment which is non-invasive so as not to stress the animals considering the nature of the study area. Even though investigations of the effects of pollutants including heavy metals helps in improving our understanding of their ecological effects on wildlife (Saaristo *et al.* 2018), determining heavy metal levels through non-invasive techniques as a biomarker of exposure is equally sacrosanct. Dietary intake is closely related to faecal concentrations, reflecting actual utilisation of resources (Böswald *et al.* 2018) and making faeces a practical, non-invasive analytical matrix for assessment (Webster *et al.* 2022). Hence, the study investigated heavy metal levels in water and faecal samples of wild animal species at the University of Ibadan Zoological Garden, Ibadan, Nigeria.

Materials and Methods

Study Area

The University of Ibadan Zoological Garden is located along the Appleton road, University of Ibadan. It lies between latitude 7° 26’37.1’’N and longitude 3°53’43.8’’E coordinates (Adetola *et al.* 2016) and was established primarily as a menagerie to support teaching and research before it became a full-fledged zoo in 1974 due to increase in collection’s size and diversity of animals. The animals in the zoo’s collection are housed in six different sections including reptiles, carnivores, primates, herbivores, avian, as well as small animal sections. The zoological garden enjoys the tropical climate with a mean total rainfall of about 1420.06 mm and an average temperature of 26.46^{o}C (Omonona *et al.* 2018).

Ethical Consideration

Ethical approval was sought from the University of Ibadan’s Animal Care and Use Research Ethics Committee (ACUREC) prior to the commencement of the study. The research complied with all relevant national regulations as well as institutional guidelines and was given unconditional approval by the ethics committee with reference UI-ACUREC/014-0223/23.

Study Animals and Population

For this study, the wild animal species at the University of Ibadan zoological garden were grouped into three (3) based on their feeding classification. They include **carnivores** [birds (n=3), mammals (3) and reptiles (n=10)], **Herbivores** [granivores (n=6), folivores (n=4)], and **omnivores** [mammals (n=6) and birds (n=5)]. In all, a total of thirty-seven (37) species were sampled (Fig. 1).

Figure 1: Wild Animal Species Sampled at the University of Ibadan Zoological Garden

Sample Collection and Technique

About 50 ml water samples were collected randomly from the water troughs in the sampled animals' enclosure into self-cleaned adequately labelled sample bottles. The samples were immediately acidified with 10% HNO_3 to prevent analyte loss and release of metals present (David *et al.* 2012), kept in an ice bath and thereafter taken to the laboratory for heavy metal determination as a biomarker of exposure. Freshly-deposited faecal samples were collected randomly from the cages of the selected animal species into properly-labelled plastic sample bottles and evaluated as a biomarker of exposure to heavy metal contamination. All sample collection was done in triplicates between January, 2023 and April, 2023 with the assistance of the zookeepers.

Heavy Metals Analysed

For heavy metals assay, Iron (Fe), Copper (Cu), Zinc (Zn), Lead (Pb), Chromium (Cr) and Cadmium (Cd), were analyzed in water and animals' faecal samples from the study area. These heavy metals were purposively selected due to the fact that they constitute part of the eleven heavy metal elements of utmost wildlife protection concern (Beyersmann et Hartwig 2008) and also based on the observed anthropogenic activities within and around the study area such as automobile emissions, improper disposal and burning of waste, usage of generators, and so on.

Laboratory Analytical Procedures

For the water samples, about 25 ml of the water sample were transferred into 250 ml beakers and about 10 ml of acid mixture [concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$)] were added to each of the samples in 2:1 ratio. The sample was placed on a hot plate under a fume cupboard for 5–10 minutes until digestion was completed as shown by a light-coloured, clear solution (samples were not allowed to evaporate to dryness during digestion). The beaker was allowed to cool down and distilled water was added to the sample to make 25 ml and mixed thoroughly before being taken for heavy metal level determination. For the faecal samples, about 0.5 gm of dry faecal was properly weighed and put in a hard glass tube. Concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) was added to each sample in 4:1 ratio (Gaumat et Bakre 2012). Samples were thereafter kept in water bath for 5 to 6 hours until they were digested completely and became clear. Once the samples became clear, about 3 to 4 drops of H_2O_2 (30%) was added to neutralize and dissolve the fat content. After cooling, each sample was now diluted up to 50 ml with deionized water to make final volume of each sample (USEPA 2007) and transferred to sterilized borosil glass vial and stored at room temperature prior to analysis. After all the samples were properly digested, they were subjected to metal determination using Buck Scientific 210 VGP model Atomic Absorption Spectrophotometer. Samples were analysed at the Soil Chemistry Laboratory in the Department of Soil Resources Management, University of Ibadan.

Statistical Analysis

Data collected were subjected to descriptive (mean ± standard deviation) and inferential (ANOVA, T-test) statistics. Post-hoc test (LSD) was used to determine significant differences in the mean concentrations of heavy metals with statistical significance set at $\alpha_{0.05}$. All the statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 20).

Results

Heavy Metals Concentration in the Water Samples of Sampled Species

The mean heavy metal concentrations in the water samples collected from the water troughs of the carnivorous wild animal species is presented in Table 1. The result shows that the mean concentration of Fe in [mammals (0.44±0.554), birds (1.52±1.321) and reptiles (0.53±0.631)], Pb in [mammals (0.06±0.035), birds (0.69±0.644) and reptiles (0.18±0.349)], as well as Cd in [mammals (0.07±0.048), birds (0.10±0.032) and reptiles (0.21±0.0.295)] were above the permissible limit and statistically significantly different. Likewise in Table 2, the mean heavy metal concentrations in the water samples collected from the water troughs of herbivores (granivores and folivores) shows that Fe in [granivores (0.056±0.021) and folivores (0.557±0.964)], Pb in [granivores (0.253±0.420) and folivores (0.090±0.028)], and Cd in [granivores (0.148±0.235) and folivores (0.085±0.031)] were above permissible limit and statistically not significant. Similarly, the mean heavy metal concentrations in the water samples collected from the water troughs of omnivores is presented in Table 3. The result shows that Fe in [mammals (0.75±0.993)], Pb in [mammals (0.07±0.021) and birds (0.05±0.032)], and Cd in [mammals (0.12±0.175) and birds (0.23±0.239)] were above permissible limit while only Pb concentrations showed statistical significance. As shown in Table 4, across the animal groups, Fe in [carnivores (0.784±0.874) and omnivores (0.626±0.866)], Pb in [carnivores (0.250±0.424), herbivores (0.182±0.327) and omnivores (0.062±0.028)], Cr in herbivores (0.058±0.037), as well as Cd in [carnivores (0.232±0.268), herbivores (0.167±0.243) and omnivores (0.115±0.153)] were above the comparable permissible limit.

Table 1: Mean Concentration of Heavy Metals in the Water Samples of the Carnivores

Heavy metals	Mammals	Birds	Reptiles	*F*-value	*P*-value	Inference	Permissible Limits
Fe	0.44±0.554[a]	1.52±1.321[ab]	0.53±0.631[b]	6.030	0.005	Significant	0.3
Cu	0.53±0.740	0.09±0.047	0.70±1.290	1.083	0.347	Not Significant	2.0
Zn	1.07±1.449[a]	0.08±0.049[a]	0.70±1.023	2.191	0.046	Significant	5.0
Pb	0.06±0.035[a]	0.69±0.644[ab]	0.18±0.349[b]	7.217	0.002	Significant	0.01
Cr	0.05±0.040	0.04±0.039	0.05±0.031	0.559	0.576	Not Significant	0.05
Cd	0.07±0.048	0.10±0.032	0.21±0.295	2.712	0.077	Not Significant	0.03

Note: Means with the same alphabet are significantly different

Table 2: Mean Concentration of Heavy Metals in the Water Samples of the Herbivores

Heavy metals	Granivores	Folivores	*F*-value	*P*-value	Inference	Permissible Limits
Fe	0.056 ± 0.021	0.557 ± 0.964	-1.038	0.375	Not Significant	0.3
Cu	0.020 ± 0.393	0.305 ± 0.483	-0.353	0.733	Not Significant	2.0
Zn	0.255 ± 0.190	1.116 ± 0.558	-0.765	0.466	Not Significant	5.0
Pb	0.253 ± 0.420	0.090 ± 0.028	0.761	0.469	Not Significant	0.01
Cr	0.038 ± 0.020	0.028 ± 0.004	1.251	0.260	Not Significant	0.05
Cd	0.148 ± 0.235	0.085 ± 0.031	0.528	0.612	Not Significant	0.03

Table 3: Mean Concentration of Heavy Metals in the Water Samples of Omnivores

Heavy metals	Mammals	Birds	*t-value*	*P*-value	Inference	Permissible Limits
Fe	0.75 ± 0.993	0.28 ± 0.481	1.677	0.104	Not Significant	0.3
Cu	0.45 ± 0.523	0.51 ± 0.868	-0.246	0.807	Not Significant	2.0
Zn	0.26 ± 0.450	0.06 ± 0.021	1.661	0.107	Not Significant	5.0
Pb	0.07 ± 0.021	0.05 ± 0.032	2.065	0.047	Significant	0.01
Cr	0.05 ± 0.020	0.05 ± 0.022	0.278	0.782	Not Significant	0.05
Cd	0.12 ± 0.175	0.23 ± 0.239	-1.463	0.153	Not Significant	0.03

Table 4: Mean Concentration of Heavy Metals in the Water Samples across the Animal Groups

Heavy metals	Carnivores	Herbivores	Omnivores	*F*-value	*P*-value	Inference
Fe	0.784 ± 0.874[a]	0.057 ± 0.020[a]	0.626 ± 0.866	3.038	0.061	Not Significant
Cu	0.553 ± 1.099	0.243 ± 0.404	0.474 ± 0.712	0.417	0.662	Not Significant
Zn	0.656 ± 1.064	0.356 ± 0.465	0.170 ± 0.353	1.360	0.270	Not Significant
Pb	0.250 ± 0.424	0.182 ± 0.327	0.062 ± 0.028	1.068	0.355	Not Significant
Cr	0.045 ± 0.020	0.058 ± 0.037	0.047 ± 0.021	0.821	0.449	Not Significant
Cd	0.232 ± 0.268	0.167 ± 0.243	0.115 ± 0.153	0.851	0.436	Not Significant

Note: Means with the same alphabet are significantly different

Heavy Metals Concentration in the Faeces of Sampled Species

The mean heavy metal concentrations in the faecal samples collected from the enclosures of the carnivorous wild animal species is presented in Table 5. The result shows that the mean concentration of Fe in [mammals (26.62±32.444)], Cu in [birds (20.92±29.320)], Zn in [mammals (5.45±3.175)], Pb in [reptiles (9.84±19.664)], Cr in [mammals (20.57±22.615)], and Cd in [birds (29.74±42.998)] were noted to be highest while statistical significant differences were only observed in the concentrations of Fe (P=0.043), Cr (P=0.008) and Cd (P=0.001). As presented in Table 6, the mean heavy metal concentrations in the faecal samples collected from the enclosures of the herbivores shows that the mean concentration of Fe in [granivores (31.283±45.730)], Cu in [granivores (24.863±47.819)], Zn in [granivores (4.648±3.930)], Pb in [folivores (28.628±30.537)], Cr in [folivores (25.288±21.274)], and Cd in [granivores (26.080±37.290)] were respectively the highest while no statistical significant difference was observed in the concentrations of the heavy metals. Furthermore, the mean heavy metal concentrations in the faecal samples collected from the enclosures of omnivores is presented in Table 7. The result shows that the mean concentration of Fe in [birds (93.60±86.509)], Cu in [mammals (12.99±16.709)], Zn in [birds (117.42±184.542)], Pb in [birds (89.60±100.774)], Cr in [birds (106.52±124.212)], and Cd in [birds (69.61±56.262)] were observed to be the highest while statistical significant differences were only observed in the concentrations of Fe (P=0.000), Pb (P=0.000), Cr (P=0.001) and Cd (P=0.002). Across the animal groups, Fe in [omnivores (47.223±75.919)], Cu in [herbivores (17.511±36.950)], Zn in [omnivores (33.652±45.923)], Pb in [omnivores (42.221±82.449)], Cr in [omnivores (38.683±96.242)], and Cd in [omnivores (33.757±51.615)] were observed to be the highest while statistical significant difference was only observed in the concentrations of Zn (P=0.009) (Table 8).

Table 5: Mean Concentration of Heavy Metals in the Faecal Samples of the Carnivores

Heavy metals	Mammals	Birds	Reptiles	*F*-value	*P*-value	Inference
Fe	26.62 ± 32.444[a]	1.88 ± 0.803[a]	11.68 ± 20.909	4.322	0.043	Significant
Cu	6.14 ± 6.765	20.92 ± 29.320	13.52 ± 21.319	1.082	0.347	Not Significant
Zn	5.45 ± 3.175[a]	2.26 ± 1.095[a]	3.59 ± 2.930	4.607	0.037	Significant
Pb	1.99 ± 0.595	3.47 ± 1.778	9.84 ± 19.664	1.157	0.323	Not Significant
Cr	20.57 ± 22.615[ab]	2.103 ± 1.479[a]	5.18 ± 4.034[b]	7.742	0.008	Significant
Cd	3.27 ± 1.859[a]	29.74 ± 42.998[ab]	4.12 ± 5.030[b]	13.335	0.001	Significant

Note: Means with the same alphabet are significantly different

Table 6: Mean Concentration of Heavy Metals in the Faecal Samples of the Herbivores

Heavy metals	Granivores	Folivores	*t*-value	*P*-value	Inference
Fe	31.283 ± 45.730	2.930 ± 0.800	1.518	0.189	Not significant
Cu	24.863 ± 47.819	6.483 ± 3.823	0.752	0.474	Not significant
Zn	4.648 ± 3.930	3.710 ± 3.347	0.391	0.706	Not significant
Pb	13.593 ± 24.408	28.628 ± 30.537	-0.867	0.411	Not significant
Cr	23.447 ± 27.728	25.288 ± 21.274	-0.112	0.914	Not significant
Cd	26.080 ± 37.290	2.255 ± 2.214	1.561	0.179	Not significant

Table 7: Mean Concentration of Heavy Metals in the Faecal Samples of the Omnivores

Heavy metals	Mammals	Birds	*t*-value	*P*-value	Inference
Fe	7.46 ± 8.137	93.60 ± 86.509	-4.721	0.000	Significant
Cu	12.99 ± 16.709	11.37 ± 9.643	0.337	0.738	Not significant
Zn	28.78 ± 42.142	117.42 ± 184.542	-2.136	0.040	Not significant
Pb	2.74 ± 1.262	89.60 ± 100.774	-3.973	0.000	Significant
Cr	3.03 ± 2.457	106.52 ± 124.212	-3.840	0.001	Significant
Cd	17.80 ± 39.088	69.61 ± 56.262	-3.266	0.002	Significant

Table 8: Mean Concentration of Heavy Metals in the Faecal Samples of the Animal Groups

Heavy metals	Carnivores	Herbivores	Omnivores	*F*-value	*P*-value	Inference
Fe	12.644± 23.062	19.939 ± 37.101	47.223 ± 75.919	1.772	0.185	Not Significant
Cu	9.949 ± 18.046	17.511 ± 36.950	12.875 ± 15.039	0.308	0.737	Not Significant
Zn	3.692 ± 2.922[a]	4.273 ± 3.541[b]	33.652 ± 45.923[ab]	5.449	0.009	Significant
Pb	7.168 ± 16.206	19.607 ± 26.496	42.221 ± 82.449	1.746	0.190	Not Significant
Cr	7.491 ± 12.054	19.681 ± 22.936	38.683 ± 96.242	1.083	0.350	Not Significant
Cd	7.776 ± 21.201	16.548 ± 30.420	33.757 ± 51.615	1.803	0.180	Not Significant

Note: Means with the same alphabet are significantly different

Discussion

Zoological gardens are now becoming more crucial than ever especially in the conservation of wild animal species. Species in environments like zoological gardens that are not too anthropogenically-disturbed may as well be exposed to pollution since pollutants can be transported even over long distances (Zhang *et al.* 2019). Particularly, anthropogenic activities around the zoological gardens may release quantities of metals into the environment, where they may bioaccumulate and threaten wildlife health (Parker *et al.* 2023). From the study, the mean concentration of heavy metals in the water samples showed that the levels of Fe were above the comparable permissible limits across the animal groups. This may have elicited from possible rusts in the water pipes where the water given to the animals were sourced from. Corrosion of water pipes can lead to the deterioration of water quality and increased Fe levels. The contamination of ground water with Fe which is the source of the water is of concern (Kamel 2012) and this may affect its physicochemical and biological characteristics. Even though Fe is an essential metal necessary for body metabolism, a high concentration of Fe in the body system of animals may result in muscular and neurological dysfunction in animals, as well as hemochromatosis (Sullivan *et al.* 2020). Furthermore, high mean concentrations of Pb were noted in the water samples of the animal groups. Vehicular emissions around the zoological garden and/or inhalation of dust or fumes containing Pb particles may have contributed to the high mean concentration which was above the comparable permissible limit. Even at low concentrations, Pb is toxic and can have harmful effects on wild animal health. According to (Assi *et al.* 2016), the health implications of Pb toxicity are mostly on haematology, peripheral neuropathy, as well as renal, cardiovascular and hepatotoxicities. Similarly, Cd was observed to be above the comparable permissible limit in the water samples of sampled animal groups. Just like Pb, emissions from vehicles that regularly traverse the road beside the zoological garden may have contributed to the high level. Despite having no role in biological systems (Has-Schon *et al.* 2007), Cd alongside Pb have been documented to be of utmost concern (Stankovic *et al.* 2014). The bio-accumulation of Cd has been reported to be greater than most metals since it is assimilated rapidly and excreted slowly depending on the rate of excretion (Bhat 2013). Cadmium has no bio-importance in wild animal physiology and biochemistry while exposure to it even at extremely low concentrations can be highly toxic and have health effects. For instance, amongst others, exposure to cadmium has been reported to cause tissue toxicosis (Liu *et al.* 2009), effects associated with carcinogenesis and teratogenesis (Sarkar *et al.* 2013), tissue injury through oxidative stress (Karimi *et al.* 2014). In all, the high concentrations of heavy metals especially above permissible limits in the sampled water imply that the water is unsafe for animal consumption, as these metals may bioaccumulate in their tissues resulting in possible health implications. As such, in as much as animals depend on water for survival, it is very sacrosanct not to compromise water quality through anthropogenic practices that are unsustainable and not eco-friendly as animal health equally depends greatly on the quality of drinking water.

The degree to which wild animal species are exposed to heavy metal contamination can be assessed using faecal samples as bio-indicators (Gaumat et Bakre 2012). From the study, the mean concentration of heavy metals in the faecal samples showed that the levels of all the metals analysed were highest in omnivores except Cu which was highest in the herbivores. This may be attributed to their position on the food chain as metals have the tendency to biomagnify as they move from one trophic level to the other. Even though trophic transfer of metals in the ecological food chain of wildlife results from the animal's diet and environmental exposure (Soliman *et al.* 2019), dietary intake has been reported as the predominant pathway of heavy metal bioaccumulation in organisms (Wu *et al.* 2023). Considering the complex nature of ecological food webs and with respect to the high position omnivores occupy in the food chain, trophic transfer of metals through bio-magnification poses a deleterious risk to wildlife (Rodríguez-Estival et Mateo 2019). The presence of metals in the faeces of animals implies that the extent at which individual animal species bio-concentrated the metals actually differ. Many metal elements are detectable in their original form within excreta (Das *et al.* 2019) while prolonged exposure to them may result in their bio-concentration in tissues (Ortiz-Santaliestra *et al.* 2015) with possible sub-lethal and lethal effects (Sonne *et al.* 2020). As such, toxicity of heavy metal contaminants including the extent of damage may differ in wildlife depending on nature of exposure as well as animal characteristics such as physiology, nutritional status, behavior and feeding pattern (Tesser *et al.* 2021).

Conclusion

Based on the results of the current study, heavy metal levels especially those of cadmium (Cd), lead (Pb) and iron (Fe) in the water samples of the studied animal species which were above the comparable permissible limits may threaten their long-term health as well as persistence in their captive population. The concentrations of heavy metals in the faecal samples of wild animals indicate exposure to them. When permissible limits are exceeded, heavy metals can cause acute and chronic toxicities, disrupt biological processes, and impair organ function. The findings from this study underscore the contribution of anthropogenic activities to environmental contamination by heavy metals in captive terrestrial wildlife. Hence, there is need to check anthropogenic activities around the zoological garden which are thought to be the major source of heavy metal contamination to the captive wild animal species.

Acknowledgments

The authors wish to thank personnel from the University of Ibadan zoological garden for their assistance during sample collection. The authors also appreciate the critical evaluations of the anonymous reviewers.

Conflict of Interest

The authors have not declared any conflict of interests.

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