Macrothink Institute™
Journal of Biology and Life Science
ISSN 2157-6076
2015, Vol. 6, No. 2

Heavy Metals (Cd, Pb, Cu, Fe, Cr, Mn, Zn) Contents in Ungulates of Ogun State Agricultural Farm Settlement, Ago - Iwoye, Nigeria

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Received: December 16, 2014 Accepted: January 4, 2015
doi:10.5296/jbls.v6i2.6787 URL: http://dx.doi.org/10.5296/jbls.v6i2.6787

Abstract

Heavy metals are natural components of the earth's crust which can neither be degraded nor destroyed and were known as trace elements as they occur in minute concentrations in biological systems. This study was aimed to investigate the bioaccumulation of heavy metals in the commonly consumed duikers in an agricultural farm settlement. Commercially available four duikers (*Cephalophus* spp.) were collected for four weeks and four organs (skin, lungs, kidney and liver) from each sample were used. All the organs sampled were subjected to 7 heavy metals (Fe, Cu, Pb, Zn, Mn, Cr and Cd) tests using Atomic Absorption Spectrophotometer (VGB 210 System) following standard procedure. All data were subjected to statistical analysis to find out the significant difference of heavy metal in each organ of and their accumulation effects. Heavy metals concentration in *Cephalophus spp* was in descending order of Cd (0.29±0.02 - 0.92±0.01) < Pb (0.52±0.03-1.08±0.06) < Cr (0.78±0.05-2.47±0.19) < Cu (5.05±0.30-13.35±0.54) < Mn (7.30±0.49-14.20±1.06) < Zn

(75.52±3.04-125.28±10.57) < Fe (381.52±14.25-761.40±5.76). They accumulated the metals in ascending order Fe > Zn > Mn > Cu > Cd > Cr > Pb for skin, Fe > Zn > Mn > Cu > Cr > Cd > Pb for liver, Fe > Zn > Mn > Cu > Cr > Pb > Cd for lung and Fe > Zn > Cu > Mn > Cr > Pb > Cd for kidney. Variability of heavy metals in *Cephalophus* species may not be due to the differences in the animal diet and habitat, but, the level of acute or chronic contamination of their diet and habitat.

Keywords: Bush-meat, Heavy Metals, *Cephalophus* Species, Farm Settlement

1. Introduction

Wildlife populations have been under constant threat from human activity. Even in protected areas, illegal activities have affected the populations of most wildlife species. Duiker is a medium-size antelope which were divided into two groups based on their habitat: forest and bush duikers. All forest duikers (*Cephalophus* species) are Bovids of the sub family Cephalophinae, found mainly in rainforest Sub-Saharan Africa. Meanwhile, the only known bush duiker (grey common duiker) inhabits the Savannah Africa (Grubb and Groves, 2001). The genus *Cephalophus* comprises seventeen species, making it the most species-rich group of forest ungulates (Kingdon, 1997).

They graze on wide range of diet (leaves, flowers, fruits, tubers) and can also eat insects, frogs, small birds and mammals (Eves *et al.*, 2002). Fruits and seeds constitute the bulk of their diet and they are potential important seed disperser. Many forest ungulates including duikers are intensively exploited as a source of meat over African forest zone (Gautier-Hion *et al.*, 1980; Eves *et al.*, 2002). Though, conservation status of the duikers globally is least concern, apart from Ader's and Zebra duikers (*Cephalophus adersi and Cephalophus zebra*), but their populations keep decreasing (IUCN, 2012).

Generally, they are found in habitats with sufficient vegetation cover (which allow them to hide), savanna and hilly areas, including the fringes of human settlements (Jachmann, 2008). The overall success of this animal was its ability to inhabit a wide variety of habitats, quick adaptability and generalist diet (Davies *et al.*, 2001). It grows to about 50cm in height and 12kg to 25kg in weight, while females are generally larger and heavier than the males (Wilson, 2005). Breeding is a year round with 3 to 7.5 months gestation period. They are usually active both in day and night, but become more nocturnal near human settlements (Lannoy *et al.*, 2003).

In tropical rainforest zones of Africa, people non-selectively hunt duikers for their fur, meat and horns at highly unsustainable rates (Newing, 2000). They were often captured for bush-meat, but over-exploitation of duikers affects their population as well as organisms that rely on them for survival. In fact, duikers are one of the most hunted animals both in terms of number and biomass in Central Africa (Muchaal and Ngandjui, 1999). In areas near African rain forests, majority of the people do not raise their own livestock, bush-meat is what all classes rely for their protein source (Anadu *et al.*, 1988).

Population trends for all duikers species excluding the common duiker and the smallest blue duiker, are significantly decreasing while other species are now considered endangered by the

International Union for Conservation of Nature (IUCN) red list of threatened species (IUCN, 2012). However, some species are yet to be considered 'endangered', because of the repeated damage and fragment of their habitat by human activities, such specialization of the niches are gradually becoming impaired and are contributing to the significant decrease in population (Rist *et al.,* 2009).

Many heavy metals have no known benefit for human physiology (Yousuf and El-Shahawi, 1999). Lead, mercury and cadmium are prime examples of such metals, yet, other metals are essential to human biochemical processes. For example, zinc is an important cofactor for several enzymatic reactions in human body, likewise copper, manganese, selenium, chromium and molybdenum are all trace elements which are important in the human diet (Linnik and Zubenko, 2000). Another subset of metals includes aluminum, bismuth, gold, gallium, lithium and silver which are used therapeutically in medicine (Gupta *et al.,* 2009).

Certain elements that are normally toxic for certain organisms, under certain conditions are at times beneficial. Examples include vanadium, tungsten and cadmium (Ogunfowokan *et al.,* 2009). Heavy metal pollution arises from many sources, but mostly from the purification of metals, sedimentation of dust, electroplating, smelting and precipitation of their compounds or ion into the soil which can localize and lays dormant (Blasco *et al*., 1998). Unlike organic pollutants, heavy metals do not decay and thus pose a kind of challenges for remediation (Agah *et al*., 2009).

Heavy metals enter human body through two major routes: inhalation and ingestion. Ingestion being the main route of exposure in human population, these metals enters human bodies via food (Opaluwa and Umar, 2010) which bush-meat consumption may be one of the pathways. Copper, selenium and zinc are essential trace elements to maintain body metabolism and can lead to poisoning at higher concentrations (Orebiyi *et al.,* 2010). Potentially, human exposed to these metals through commercially produced foodstuffs, dust inhalation and direct ingestion of food plants grown on metals-contaminated soil (Soewu *et al.,* 2014).

Heavy metals pollutants that usually got transferred unto man and have highly adverse effects are lead, cadmium, copper, chromium, selenium and mercury. They are cumulative poisons which cause environmental hazards and are reported to be exceptionally toxic (Dural *et al*., 2007; Orebiyi *et al.,* 2010). Heavy metals are dangerous elements of the earth crusts as they tend to bioaccumulate by increasing in concentration level in biological organisms over time (Dural *et al.,* 2007). Therefore, heavy metals can be bio-accumulated, bio-absorbed, biomagnified via the food chain and finally assimilated by human resulting in health risks (Agah *et al*., 2009).

Due to their severe accumulation in biota and toxicity, determinations of heavy metals concentration in bush-meats received attention in series of countries around the globe (Soewu *et al.,* 2014). This interest was to ensure safe consumption of bush-meat and minimizing the potential hazard effects of these metals on human health which this work also tries to validate. The need for a better understanding of heavy metal composition in this animal is essential if human communities are to be safe from the metals adverse effects. Also, the study was aimed to provide baseline information on the abundance of some heavy metals contents on forest ungulates using duiker species in an Agricultural farm settlement.

2. Materials and Methods

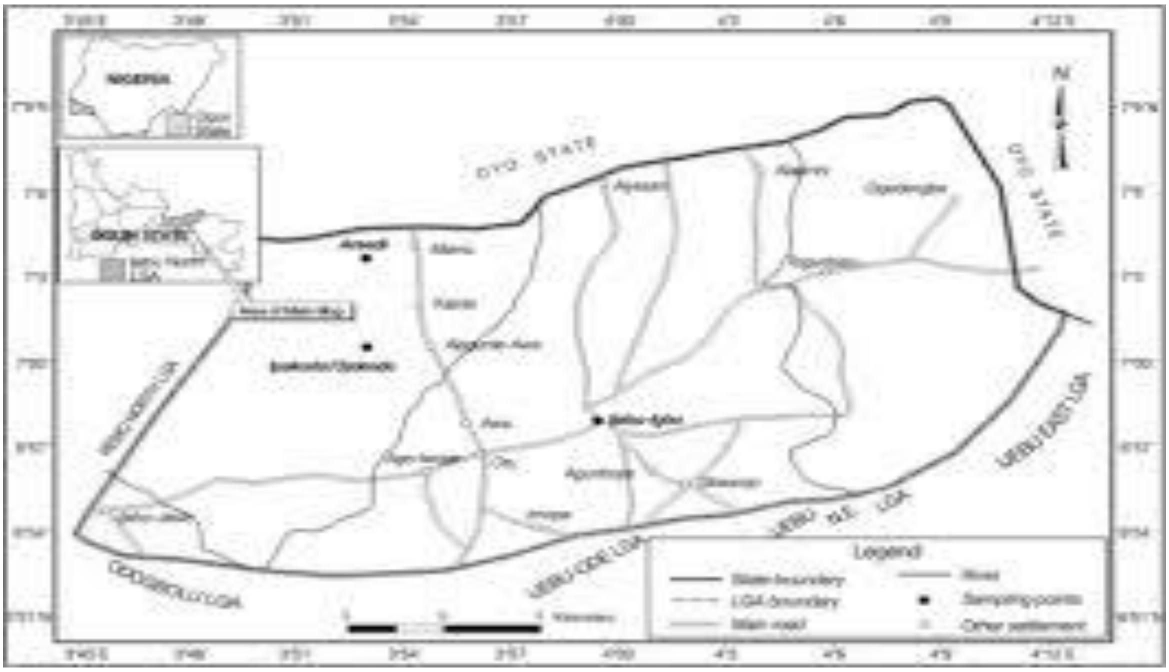

Figure 1. Political Map of Ijebu North Local Government Showing the Study Area

Ogun State is situated between Latitude 6.2 °N and 7.8 °N and Longitude 3.0 °E and 5.0 °E with estimated population of 3,486,683 people as at year 2006 (NPC, 2006). It is located in the southwest zone of Nigeria with a total land area of 16,409.26 square kilometers and entirely in tropics zone of Sub-Sahara Africa (Soewu *et al.*, 2012). Ogun State agricultural farm settlement, Ago-Iwoye (study area) is one of the state four Agricultural farm settlements across the state. The settlement was situated in Ijebu North local government which is one of the 20 local governments in the state (figure 1) with total population of 280,520 (NPC, 2006). The farm settlement was located in Ago - Iwoye along Ago - Iwoye to Ilishan road where this road has been under construction since fifteen (15) year ago.

Four daily fresh killed wild duikers (*Cephalophus* spp.) were obtained commercially from the farm hunters and four organs (liver, lung, kidney and skin) were immediately removed as to avoid autolysis of the animal organs. The abdominal part of the duikers was dissected to remove the kidney and liver organ which was labeled and coded in a separate tube. The lung was removed from the thoracic cavity, some internal skin was also sliced while this procedures were repeated for all the four samples each for a period of four weeks. Bioaccumulation of heavy metals varies in relation to the size, sex and/or age of animals (Lunt and Mhlanga, 2011).

Therefore, male adults that have average weight of 15kg and 35cm height were used to avoid differences that may be resulted from any of these factors. All the samples organs were digested in the laboratory and analyzed for seven heavy metals which are lead (Pb), copper

(Cu), iron (Fe), zinc (Zn), manganese (Mn), chromium (Cr) and cadmium (Cd) using Atomic Absorption Spectrophotometer (VGB 210 System) following the standard procedure as reported by Christian *et al*, 2010.

The data obtained were subjected to statistical analysis using SPSS (version 20.0) which analyzed for descriptive statistics and one-way analysis of variance (ANOVA) to find out the significant difference of heavy metal in each organ throughout the period. Variations due to sampling errors and differences in mean values were determined and not rejected as being significantly different if $P < 0.05$.

3. Results and Discussion

Heavy metals concentrations in the skin of *Cephalophus* species over the sampling period were presented in Table 1. Zn, Pb, Cd and Mn concentration in the skin were correlated with the liver concentration, but Fe, Cu and Cr concentration were not, which was in line with Kim and Koo (2008a) findings on heavy metals concentration in feather of Korean shore birds. There was no significant difference ($P > 0.05$) in all the metals concentration except Fe which varied in the third week of the study. However, the concentration of Pb, Cd and Cr were observed to be significantly ($P < 0.05$) higher in the fourth week than other sampling period.

Table 1. Heavy metals concentration (mg/kg) in the skin of *Cephalophus* species

Periods/Metals	Pb	Fe	Cu	Zn	Cd	Cr	Mn
WEEK 1	0.49 ± 0.03^a	395.50 ± 4.68^a	4.90 ± 0.82^a	141.36 ± 32.10^a	0.90 ± 0.04^a	0.71 ± 0.09^a	17.11 ± 1.92^a
WEEK 2	0.52 ± 0.06^a	413.96 ± 37.25^a	7.34 ± 1.29^a	115.07 ± 11.99^a	0.91 ± 0.02^a	0.83 ± 0.06^a	14.16 ± 2.06^a
WEEK 3	0.48 ± 0.11^a	319.25 ± 2.14^b	7.28 ± 0.93^a	136.27 ± 20.21^a	0.92 ± 0.02^a	0.75 ± 0.03^a	11.51 ± 0.36^a
WEEK 4	0.56 ± 0.06^a	397.36 ± 19.18^a	6.45 ± 0.43^a	108.41 ± 22.14^a	0.93 ± 0.02^a	0.83 ± 0.18^a	14.00 ± 2.94^a

Mean values (± Standard Error) in the same column with the same superscripts are not significantly different ($P > 0.05$)

Table 2. Heavy metals concentration (mg/kg) in the liver of *Cephalophus* species

Periods/Metal	Pb	Fe	Cu	Zn	Cd	Cr	Mn
WEEK 1	0.68 ± 0.05^a	668.32 ± 14.58^a	8.67 ± 0.60^b	139.17 ± 32.43^a	0.66 ± 0.05^a	1.04 ± 0.16^a	14.54 ± 3.81^a
WEEK 2	0.62 ± 0.04^a	639.55 ± 9.98^a	10.06 ± 1.03^{ab}	110.64 ± 12.72^a	0.77 ± 0.10^a	1.34 ± 0.30^a	11.39 ± 3.24^a
WEEK 3	0.64 ± 0.04^a	587.80 ± 47.81^a	12.83 ± 0.47^a	117.57 ± 21.67^a	0.83 ± 0.05^a	1.18 ± 0.47^a	11.09 ± 0.69^a
WEEK 4	0.64 ± 0.07^a	653.20 ± 30.95^a	10.62 ± 1.28^{ab}	106.11 ± 20.18^a	0.85 ± 0.11^a	1.24 ± 0.43^a	12.12 ± 2.20^a

Mean values (± Standard Error) in the same column having the same superscripts are not significantly different ($P > 0.05$)

Moreover, the liver of *Cephalophus* species in the first week recorded high concentrations in Pb, Fe, Zn, Mn and low in Cu, Cd and Cr as shown in Table 2. Meanwhile, at the fourth week, the liver was higher in Cd concentration as compared to other period. However, the differences observed in the concentration of heavy metals in the liver (Table 2) were not significantly different ($P > 0.05$). In this study, Cd concentration in the liver was found to be higher in ascending order S > L > Lu > K in line with Kim and Koo (2008a) findings which against Kim *et al.* (2010) that found Cd concentration to be higher in kidney that liver.

Pb, Cr and Cd were also recorded low in the liver as several other studies also revealed, which anthropogenic activities and influence from industrial procedure are the sources (Köck *et al.*, 1989b). Pb and Cd concentrations in the liver were within the background level or normal range which against Kim and Koo (2008b) findings, but in accordance with Kim *et al.* (2009) findings on heavy metals in tissue of some wild birds from Korea. Zn, Mn and Fe concentrations were found higher and not significantly different ($P > 0.05$) in the liver in line with Kim *et al.* (2009) findings.

All the metals were not significantly different ($P > 0.05$) in the liver except Cu which was in line with Kim and Koo (2008b) findings on heavy metals distribution in chicks of two heron species from Korea. Essential elements concentration (Fe, Zn and Mn) did not significantly differs ($P > 0.05$) in the liver and kidney as found by Kim *et al*. (2010) in monitoring of heavy metal contamination using tissue of two ardeids chicks.

Also, this study found that Pb and Cd concentrations in the liver were correlated and highly related, this indicate the contamination of the animal foraging sites which was also opined by Kim *et al.* (2010) findings. Therefore, it suggests that Pb and Cd in the liver and kidney can be used as a bio-indicator of acute and/or chronic local contamination (Kim and Koo, 2008b; Kim *et al.*, 2010). Cd and Zn concentration were recorded highest in the lungs at the fourth week (Table 3). However, no significant difference ($P > 0.05$) was observed in the metals concentration in the liver.

Table 3. Heavy metals concentration (mg/kg) in the lungs of *Cephalophus* species

Periods/Metals	Pb	Fe	Cu	Zn	Cd	Cr	Mn
WEEK 1	0.82 ± 0.04^{a}	759.16 ± 6.90^{b}	4.61 ± 0.36^{a}	72.92 ± 6.51^{a}	0.70 ± 0.14^{a}	2.17 ± 0.49^{a}	8.14 ± 1.31^{a}
WEEK 2	0.81 ± 0.03^{a}	767.98 ± 15.42^{b}	5.21 ± 0.42^{a}	67.48 ± 7.27^{a}	0.75 ± 0.10^{a}	2.35 ± 0.08^{a}	7.61 ± 1.28^{a}
WEEK 3	0.80 ± 0.07^{a}	754.43 ± 11.17^{b}	6.06 ± 0.90^{a}	76.44 ± 3.72^{a}	0.69 ± 0.10^{a}	2.17 ± 0.46^{a}	6.57 ± 0.35^{a}
WEEK 4	0.78 ± 0.06^{a}	764.04 ± 16.35^{b}	4.32 ± 0.16^{a}	85.24 ± 3.29^{a}	0.91 ± 0.01^{a}	2.09 ± 0.36^{a}	6.87 ± 1.02^{a}

Mean values (± Standard Error) in the same column having the same superscripts are not significantly different ($P > 0.05$)

The concentration of Cu in the kidney at the first week was significantly lower than subsequent weeks (Table 4), while Cr, Cd, Fe, Zn and Pb concentrations were significantly higher at the fourth week with significant difference ($P < 0.05$) occurred in Mn and Cu. Pb, Cd and Cr clearly revealed low value while the reverse was true for Fe, Zn, Mn and Cu in contrary to Köck *et al.* (1989a) finding on accumulation of heavy metals in the kidney of small mammals captured at a waste disposal site, but in line with Kim *et al.* (2009) findings.

There was no significant different ($P > 0.05$) in all the metals throughout the sampling periods except for Cu and Mn which differs in the first week for Cu, week 1 and week 3 for Mn. The result was against Köck *et al.* (1989a) findings which Zn was not significantly different ($P > 0.05$). The metals mean concentration in the lungs, skin, liver and kidneys of *Cephalophus* species from the study area was shown in Table 5.

Table 4. Heavy metals concentration (mg/kg) in the kidney of *Cephalophus* species

Periods/Metals	Pb	Fe	Cu	Zn	Cd	Cr	Mn
WEEK 1	1.07±0.20^{a}	571.15±15.79^{a}	10.76±0.35^{b}	95.19±7.10^{a}	0.24±0.04^{a}	2.27±0.38^{a}	11.41±1.16^{a}
WEEK 2	1.05±0.11^{a}	578.70±9.19^{a}	14.24±0.69^{a}	94.96±1.43^{a}	0.29±0.05^{a}	2.27±0.51^{a}	7.65±0.98^{ab}
WEEK 3	1.09±0.15^{a}	591.40±3.66^{a}	14.04±1.03^{a}	90.88±5.89^{a}	0.29±0.04^{a}	2.55±0.31^{a}	6.69±0.77^{b}
WEEK 4	1.10±0.11^{a}	549.89±18.58^{a}	14.33±0.46^{a}	91.06±4.36^{a}	0.33±0.03^{a}	2.78±0.44^{a}	7.84±1.62^{ab}

Mean values (± Standard Error) in the same column having the same superscripts are not significantly different ($P > 0.05$)

Table 5. Mean concentrations of heavy metals (mg/kg) in organs of *Cephalophus* species

Organ/Metals	Pb	Fe	Cu	Zn	Cd	Cr	Mn
Skin	0.52±0.03^{d}	381.52±14.25^{d}	6.49±0.49^{c}	125.28±10.57^{a}	0.92±0.01^{a}	0.78±0.05^{b}	14.20±1.06^{a}
Liver	0.64±0.02^{c}	637.22±15.65^{b}	10.54±0.59^{b}	118.37±10.47^{a}	0.78±0.04^{b}	1.20±0.16^{b}	12.28±1.24^{a}
Lungs	0.80±0.02^{b}	761.40±5.76^{a}	5.05±0.30^{d}	75.52±3.04^{b}	0.76±0.05^{b}	2.19±0.17^{a}	7.30±0.49^{b}
Kidneys	1.08±0.06^{a}	572.79±7.22^{c}	13.35±0.54^{a}	93.02±2.28^{b}	0.29±0.02^{c}	2.47±0.19^{a}	8.40±0.74^{b}

Mean values (± Standard Error) in the same column having the same superscripts are not significantly different ($P > 0.05$)

Generally, the concentration of heavy metals in *Cephalophus spp* was in descending order of Cd (0.29±0.02 - 0.92±0.01) < Pb (0.52±0.03-1.08±0.06) < Cr (0.78±0.05-2.47±0.19) < Cu (5.05±0.30-13.35±0.54) < Mn (7.30±0.49-14.20±1.06) < Zn (75.52±3.04-125.28±10.57) < Fe (381.52±14.25-761.40±5.76). *Cephalophus Spp* accumulated the metals in ascending order Fe > Zn > Mn > Cu > Cd > Cr > Pb for skin, Fe > Zn > Mn > Cu > Cr > Cd > Pb for liver, Fe > Zn > Mn > Cu > Cr > Pb > Cd for lung and Fe > Zn > Cu > Mn > Cr > Pb > Cd for kidney. Among all the metals tested for, concentrations of Fe and Zn were observed to be significantly higher ($P < 0.05$) than Pb, Cu, Cd, Cr and Mn in the animal (Figure 2).

The concentration of the studied metals in the organs were in ascending order K > Lu > L > S for Pb and Cr, S > L > K > Lu for Zn and Mn, while Fe, Cu and Cd also accumulated in ascending order Lu > L > K > S, K > L > S > Lu and S > L > Lu > K respectively. Pb, Cr and Cu concentrations were significantly higher ($P < 0.05$) in the kidneys than the liver, skin and lungs. Similarly, concentrations of Zn, Mn and Cd were significantly higher ($P < 0.05$) in the skin than the other organs studied (Table 5). The variability and significant difference in all the metals across the organs of *Cephalophus* species may not be as a result of differences in the animal diet and habitat as opined by Kim *et al.* (2009), but rather as to the level of acute or chronic contamination of their diet and habitat.

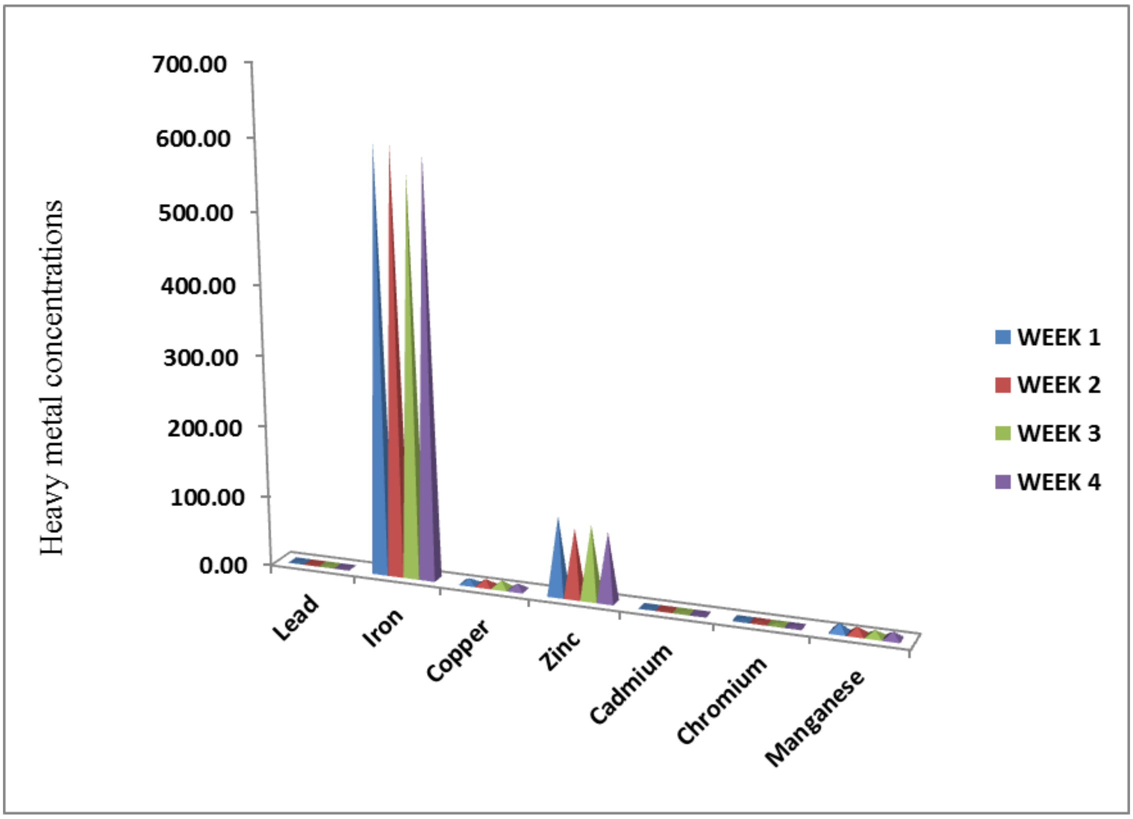

Figure 2. Heavy metals accumulation in *Cephalophus* species over the sampling periods

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