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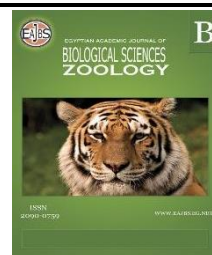


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Bioaccumulation of Metals in African Brush-tailed Porcupine, *Atherurus africanus* (Gray, 1842) in the Tropical Rainforests of Ibadan, Oyo State, Nigeria

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ABSTRACT

Wildlife meat is an important source of protein in tropical and subtropical regions. Samples of African brush-tailed porcupine (*Atherurus africanus*), a wildlife meat source, were procured from Oluwo Market, Epe, Epe Local Government Area (L.G.A.), Lagos and Odo Ona Kekere in Oluyole L.G.A. Ibadan, Nigeria. Trace metals such as zinc, cadmium, vanadium, barium, nickel, copper, lead, cobalt, chromium, and manganese in the liver and intestine and the associated implications on the lipid profile, and antioxidant biomarkers were investigated. The concentrations of all the metals in the liver and intestine of the porcupines were below the established FEPA standard of 100mg/kg. Zinc > barium > vanadium was the order of significant bioaccumulation of metals in the liver of *A. africanus*.

The other metals poorly bioaccumulated in the organs, with the liver having bioaccumulation factors lower than 1. In the intestine, only barium was significantly bioaccumulated, while the other metals bioaccumulation factors lower than 1. The levels of triglyceride and superoxide dismutase (SOD) in the intestine were much higher than the level detected in the liver. The bioaccumulation rates of zinc and vanadium, coupled with the correlation of the metals with the indices of oxidative stress and dyslipidemia might be a prognosis worthy of monitoring. In the intestine, protein had strong negative correlations relationships with SOD (-0.73), and catalase (-0.84). This indicated the tendencies of damage of the cell membrane proteins due to an increased antioxidant defense system. The results presented did not implicate barium in the intestine. In natural environment, multiple factors might be responsible for the concerns detected in the intestine of the animal.

Further study is recommended to determine the leading factors in the multi-stress conditions of the porcupines. This shall be of paramount benefit to the conservation of the animal and the protection of the consumers.

INTRODUCTION

Africans in the sub-urban areas who live in close proximity to forest usually hunt wild animals and their products as major sources of dietary protein. Such animal products sourced from the wild are referred to as bush meat. They are considered important delicacies that constitute vital parts of several cultural meals (Igene *et al.*, 2016). In Nigeria for

example, bush meat constitutes a significant proportion of total animal protein consumed, particularly among dwellers of communities in proximity to large forest reserves. The unsustainable modes of hunting adopted by the uninformed poachers such as the use of explosives, hunting guns, poisons, and chemicals often contaminate the environment with heavy metals, causing secondary ecophysiological challenges (Igene *et al.*, 2016). These heavy metals are incorporated in the exposed animals through the food chain and may bioaccumulate to toxic levels that may impact the health of game meat consumers. Despite these factors, bush meat remains an important source of protein to many Nigerians in both rural and urban areas (Davies, 2002) mainly because it is a relatively available source of protein compared to livestock, fish, and cattle which are relatively scarce or more expensive (Igene *et al.*, 2016). They provide protein for poor rural communities who lack access to arable lands or agricultural markets. Furthermore, wild animals that provide bush meat such as grasscutter, rabbits, and antelopes that are hunted as bush meat have been successfully domesticated particularly in the humid and sub-humid tropics (Draulans and Krunkelsven, 2002).

On the whole, game meat represents 80% of all animal protein consumed in appreciable parts of Central Africa (Sharkawy and Amal, 2003). Bush-tailed porcupine (*Atherurus africanus*) constitutes a significant part of this percentage, particularly in Cameroon, Gabon and Nigeria where the animal is abundant (Igene *et al.*, 2016). In Nigeria, porcupines live in most habitats from rain forests of the South to the semi-arid regions of the extreme north (Happold, 1987). This makes them one of the bush meats that could be found on sale across an alarge part of the country.

As habitat degradation and fragmentation encroached conservation areas, the well-being of wildlife is progressively impacted by poaching (Olajesu *et al.*, 2019). Their vulnerability to poaching is influenced by the fact that they are terrestrial, ground mammal animals with great preference for deserts, grasslands, and forests, often foraging on cultivated crops, tree bark and fleshy tissues (Draulans and Krunkelsven, 2002).. *A. africanus* is hunted in large quantities and prices are often higher than that of other game meat or domesticated animals. The market value per kilogram of bush meat obtained from porcupines is markedly higher than prices-of other game animals; consequently, the hunting pressure on the animals is relatively higher. This may be a threat to the population of the animals and may mean a greater application of the aforementioned unsustainable hunting techniques by desperate poachers who seek attractive monetary rewards (Igene *et al.*, 2016). Worse still bush meat may be further contaminated by additional sources including animal drugs, pesticides, feed and other agricultural or industrial chemical substances.

A number of anthropogenic activities in the tropical rain forests of Ibadan, Oyo State include the use of agrochemicals, build-up of automobile emissions on the highways, along the boundaries of the forest may contaminate the entire land, air, and water and thus having negative impact on the wild animals. Durojaye *et al.* (2014) reported high concentrations of Fe, Cu, Cd, Pd, Mn, Cr, and Zn in organs of game animals sampled in Omo forest reserve of Ogun State. Heavy metals from these various sources may build up to toxic concentrations within unexpectedly short periods. Heavy metals being considered persistent, bioaccumulative, and toxic micropollutants, may rise to even greater levels in higher animals through biomagnification till it ultimately elicits severe health consequences in humans who consumes bush meat products (Igene *et al.*, 2016; ATSDR, 2010).

Logging and clearance of vegetation canopy may change the ecosystem structure and function, thereby rendering the wildlife vulnerable to poaching. Furthermore, the process of hunting by the poachers may render the bushmeat unfit for consumption as some methods involve the use of chemical baits and gunpowder to incapacitate the animals. These hunting practices could expose consumers of bush meat products to trace metals such as Zn, Cd, V, Ba, Ni, Cu, Co, Pb, Cr, Mn, etc. (Hunt *et al.*, 2009). Trace metals are persistent toxic

micropollutants that bioaccumulate in exposed organisms and biomagnify up the pyramid of biomasses (Dural *et al.*, 2007).

Bushmeat may be contaminated by animal drugs, pesticides, feed and other agricultural or industrial chemical substances (Khalafalla *et al.*, 2011). Events of heavy metal contamination of bushmeat products during processing have been widely reported (Akan *et al.*, 2010; Harlia and Balia, 2010). Meat production processes may also have implications on the edibility of the meat product. For example, burning of animal hair using fossil fuel or used engine oil which is common in Sub-Saharan Africa may render the products unsafe for consumption. Methods such as burning off the hairs of the animals in flame fueled by various substances such as wood mixed with spent engine oil, plastics mixed with refuse, or tyres. These materials contain toxic substances such as heavy metals which can contaminate the meat and render the meat unfit for human consumption (Okiei *et al.*, 2009). Despite these associated issues with bushmeat, the commercial benefits and nutritional value play a direct role in the livelihoods of about 150 million people in the world (Hunt *et al.*, 2009). A significant percentage of the high demand for bush meat is met by hunting using guns, dogs, baiting with chemicals, and bush burning (Oduro and Kankam, 2000). In other cases, contaminated animal feed and the rearing of livestock in proximity to contaminated environment were reportedly responsible for heavy metal contamination in meat (Khalafalla *et al.*, 2011). Copper, nickel, chromium and iron, are essential in very low concentrations for the survival of all forms of life. Essential trace metals may however be toxic at concentrations greater than the threshold of essentiality (Isibor *et al.*, 2020 a).

Ecosystems undergo constant changes due to high rise in human population. The steady increase has been accompanied by a commensurate rise in anthropogenic perturbations that are deleterious to health and the environment. Human interferences underlie habitat degradation and fragmentation which threaten biodiversity, especially the antelope species from its common range (East, 1999; IUCN, 2009).

Olajesu *et al.* (2019) pointed out that wild consumed animals are exposed to different parasitic infestations which can be transmitted to their offspring and other mammals including humans that may come into contact with them if domesticated. In the event of high habitat alterations due to multi-stress conditions, the inherent species may suffer immunosuppression which may enhance their susceptibility to parasitic infections. Various researchers have reported ectoparasites and endoparasites in wild animals in Nigeria (Ajayi *et al.*, 2007; Opara and Fagbemi, 2008; Opara and Fagbemi, 2010; Opara, 2012). As the natural barrier between wild and man is either crossed or eroded, the likelihood of a zoonotic outbreak increases. The metal burden accumulated from the environment may be toxic to the endoparasites who may as well sequester the metal concentration in the host. However, if the concentration is not high enough to impact the endoparasite, the parasite population may rise to levels that may ultimately impact the host's health. The host may therefore either benefit from low parasitic intensity aided by the metals or reduced metal concentration aided by the parasites. These two outcomes are not classically exclusive, and they may occur simultaneously.

The width of the leaf provides a wide surface area for adsorption of air pollutants. Other plants that are resilient to desertification are not in the natural diet of the animal. The most dominant grasses in the forest include the red grass- *Themeda triandra*, spear grass- *Heteropogon contortus*, tassel three-awn- *Aristida congesta*, wool finger grass- *Digitaria pentzii*, blue buffalo grass- *Cenchrus ciliaris* and white buffalo grass- *Panicum coloratum*. and short grasses such as couch grass- *Cynodon dactylon*.

Assessing the bioaccumulation of Zn, Cd, V, Ba, Ni, Cu, Pb, Co, Cr, and Mn in the African brush-tailed porcupines and demonstrating the impacts on the liver and intestine may shed some light on the safety of the consumers of the bushmeat product. Ecotoxicological tools such as lipid indices, and oxidative stress biomarkers were employed

to determine the impacts of the accumulated heavy metals. The study aimed to assess the bioaccumulation of selected heavy metals in the African brush-tailed porcupine and the associated impacts on the liver and intestine of the animal.

MATERIALS AND METHODS

Study Location:

Samples of hunted porcupines were procured from Oluwo Market, Epe and Odo Ona Kekere in Oluyole Local Government Area Ibadan. Epe is a town and Local Government Area (LGA) in Lagos State, Nigeria located on the north side of the Lekki Lagoon and about 90 km from Ibadan; it has a road connection to Ijebu-ode and Ikorodu. It lies between latitude 6°35' 3N and 3°59' 43'E. During most months of the year, there is significant rainfall in Epe. There is only a short dry season. The average temperature is 26.3 °C and precipitation is 1990 mm per year. The climate comprises the rainy season between March to October and the dry season from November to February.

Samples of brush-tailed porcupine were procured on a monthly basis from hunters within the catchment area of Odo Ona Kekere in Oluyole Local Government Area Ibadan in Ibadan North, Oyo, Nigeria (7°14'1" N, 3°51'9" E) from May 2018 to December 2020.

Determination of Trace Metals in Environmental Media:

Collection of Samples:

The liver and intestine of fresh porcupines were excised and preserved in a chest freezer at -10°C for onward tissue digestion and analysis of trace metals.

Analysis of Samples for Trace Metals:

Analysis of Metals in the Liver and Intestine:

The frozen liver and tissues were thawed and two (2) grams of a wet sample of liver and intestine were weighed and were separately placed in a beaker and digested with 25mL of ratio 1:1 hydrogen peroxide and Nitric acid. The mixture was heated to about 5mL and allowed to cool afterwards. It was then filtered and made up with distilled water to 50mL. The concentrations of Zn, Cd, V, Ba, Ni, Cu, Pb, Co, Cr, and Mn were then determined using the flame ASS at detection limits of 0.5µg g⁻¹, 0.01µg g⁻¹, 0.01µg g⁻¹, 0.03µg g⁻¹, 0.1µg g⁻¹, 0.05µg g⁻¹, 0.1µg g⁻¹, 0.05µg g⁻¹, 0.01µg g⁻¹, and 0.5µg g⁻¹ respectively. All procedures were guided by the guidelines of Whiteside (1981).

Determination of Biochemical Biomarkers:

Determination of Cholesterol:

Total cholesterol in the liver and intestine of the porcupines were determined using the enzymatic endpoint method described by Roeschlau *et al.* (1974).

Determination of High-Density Lipoprotein-Associated Cholesterol (HDL):

The high-density lipoprotein-associated cholesterol was spectrophotometrically measured using a series of coupled reactions as described by Burstein *et al.* (1980).

Low-Density Lipoprotein-Associated Cholesterol (LDL):

The low-density lipoprotein-associated cholesterol (LDL) was determined using the method of Assman *et al.* (1984), which is a combination of polyvinyl sulphate precipitation and enzymatic method.

Determination of Protein (PRO):

The protein content of the liver and intestine was determined using the Biuret method as described by Umemoto (1966).

Triglycerides:

Triglycerides were analyzed by enzymatic method described by Tietz (1990).

Glucose:

The glucose concentrations in the liver and intestine of porcupine were determined within 30 minutes of collection using the method of Wedermeyer and Yasutake (1977).

Catalase (CAT):

Catalase (CAT) was assayed calorimetrically at 620nm and expressed as moles of hydrogen peroxide (H₂O₂) consumed /min/ mg protein as described by Quinlan *et al.* (1994).

Superoxide Dismutase (SOD):

Superoxide dismutase activity in the tissue (liver and intestine) homogenates was determined using the procedure described by Marklund and Marklund (1974). The method is based on the ability of SOD to inhibit the autoxidation of pyrogallol. One unit of SOD activity was defined as the amount of enzyme that inhibited the auto-oxidation of 50% of the total pyrogallol in the reaction.

Reduced Glutathione (GSH):

Reduced glutathione (GSH) was determined by the method of Ellman (1959). To the liver homogenate 10% TCA was added and centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8 mg of 5,5'-dithiobis nitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH8.0). The absorbance was read at 412nm.

Malondialdehyde (MDA):

Malondialdehyde (MDA) an index of lipid peroxidation was determined by adding 1.0ml of the supernatant of digested tissues to 2ml of (1:1:1) TCA-TBA HCL reagent (thiobarbituric acid 0.37%, 0.24 n HCL and 15% TCA) tricarboxylic acid-thioarbituric acid-hydrochloric acid reagent boiled at 100°C for 15mins, and allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10mins. The supernatant was removed and the absorbance read at 532 against a blank. MDA was calculated using the molar extinction coefficient for MDATBA-complex of 1.5×10^5 M/cm.

Data Analysis:

The descriptive statistics of the trace metals, lipid profile and antioxidants of the liver and intestine of the porcupines were subjected to analysis of variance (ANOVA) using Microsoft Excel 2020 to test for the significant differences between the liver and the intestine of the porcupine samples with regards to the concentration of trace metals, lipid profile, and antioxidant biomarkers. The Bonferroni posthoc test was employed in determining the actual locations of the significant difference at the probability level of 0.05.

RESULTS

Bioaccumulation of Trace Metals:

The concentrations of all the metals in the liver and intestine of the porcupines investigated were below the established FEPA limits (Fig. 1). The concentration of zinc in the liver is higher than the concentration in the intestine but lower than the established limit (100 mg/kg) of FEPA (2003). The concentrations of cadmium, nickel, copper, lead, chromium, and manganese in the liver and intestine were also all below the established limits of FEPA; which are <1, 10, 30, 2, 50, and 1 respectively.

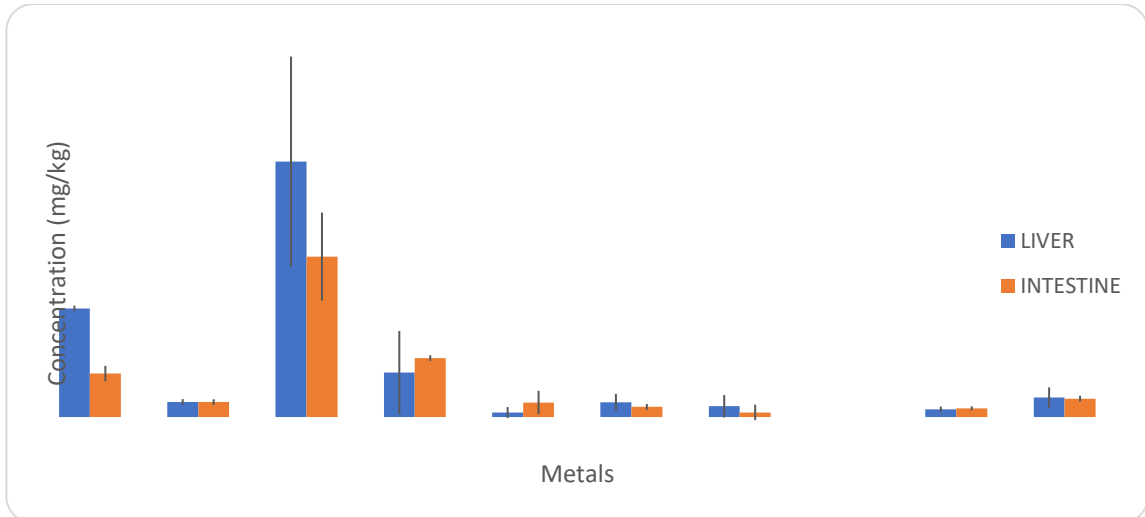


Fig. 1: Comparative trace metal concentrations in the liver and intestine of porcupine.

Zinc > barium > vanadium was the order of significant bio-accumulation of metals in the liver of *A. africanus* (Fig. 2). The other metals were poorly bio-accumulated in the organ. The poor accumulation of the metals in the liver of the African brush-tailed porcupine is characterized by bioaccumulation factors lower than 1.

In the intestine, only barium was significantly bioaccumulated, while the other metals were poorly accumulated (Fig. 3). The poor accumulation of the metals in the intestine of the African brush-tailed porcupine is characterized by bioaccumulation factors lower than 1.

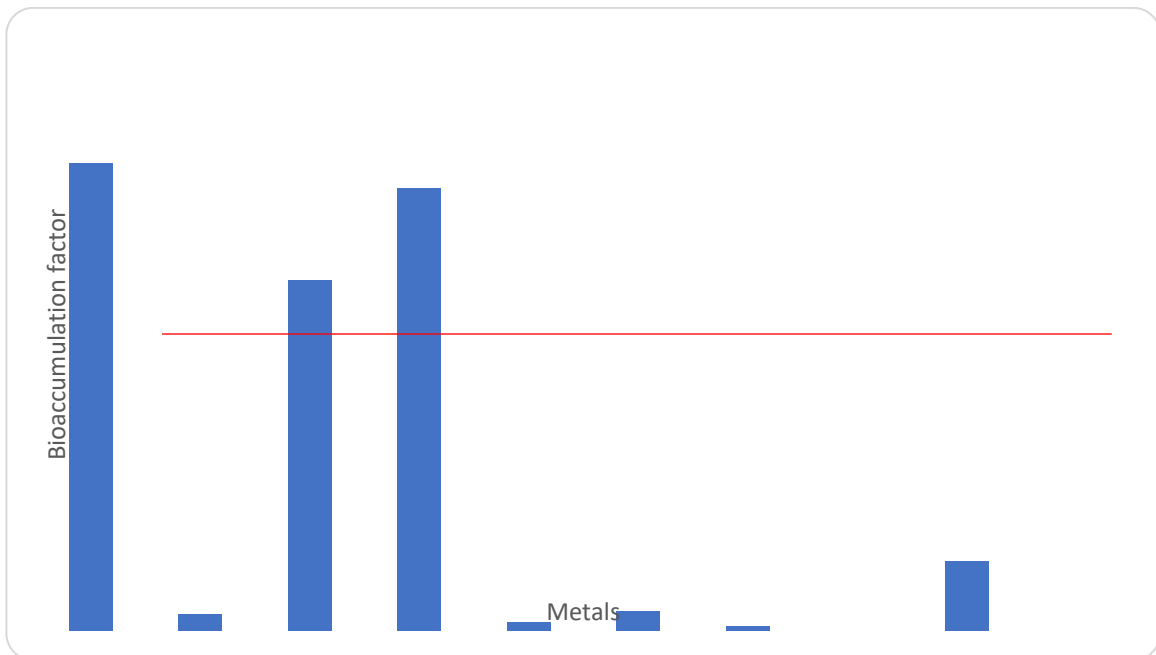


Fig. 2: Bioaccumulation factor of metals in the liver of porcupine.

Legend: Bars above the red line (1) indicate significant bioaccumulation.

Bioaccumulation of Metals in African Brush-tailed Porcupine

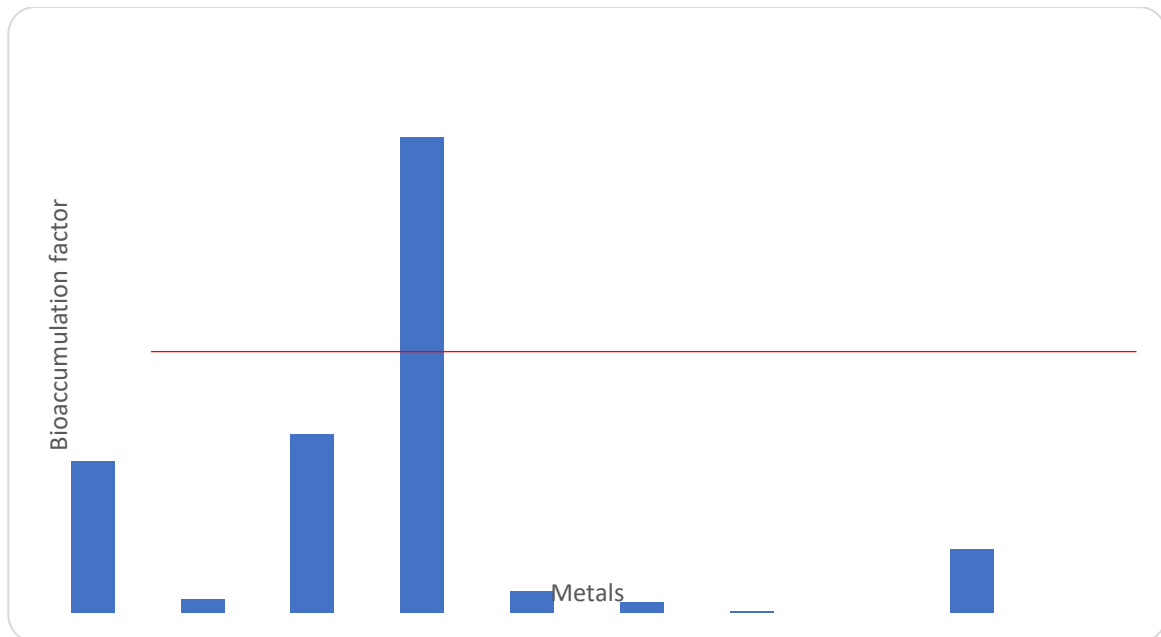


Figure 3: Bioaccumulation factor of metals in the intestine of porcupine.

Legend: Bars above the red line (1) indicate significant bioaccumulation.

Triglyceride > Cholesterol was the lipid biomarkers that exhibited marked levels in both organs (Fig. 4). The level of triglyceride in the intestine was much higher than the level detected in the liver. However, the level of cholesterol in both organs had no significant difference, while the levels of protein, glucose, and high-density lipoprotein were slightly higher in the liver than in the intestine. Conversely, the level of low-density lipoprotein was higher in the intestine than in the liver.

The level of SOD in the intestine was significantly higher than the level detected in the liver (Fig. 5). The levels of MDA, CAT, and GSH were not significantly different in the two organs.

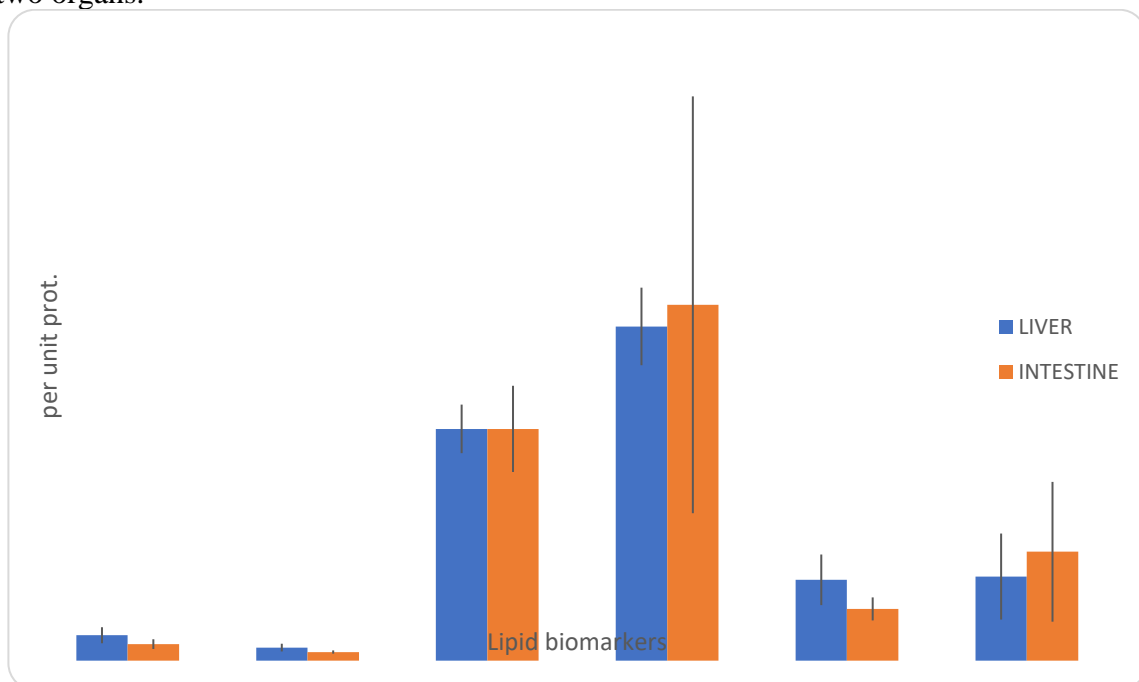


Fig. 4: Comparative lipid biomarkers in the liver and intestine of porcupine

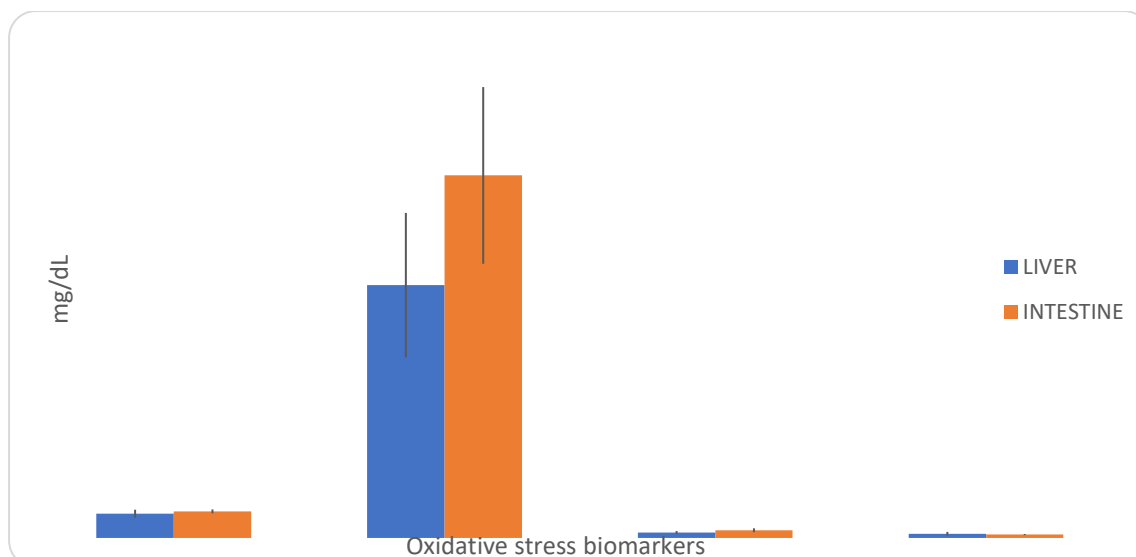


Fig. 5: Comparative oxidative stress biomarkers in the liver and intestine of porcupine

The correlation relationship among the metals, lipid indices, and oxidative stress biomarkers in the liver of the African brush-tailed porcupines showed notable trends (Table 1). Zinc concentration in the liver of the porcupine had significant positive correlations with cholesterol (0.58) and catalase (0.52). Cadmium had significant positive correlation with glucose (0.62) and SOD (0.59). Vanadium had a positive relationship with the high-density lipoprotein (0.52). Nickel had a significant negative relationship with MDA (-0.60). Copper had a significant negative correlation with protein (-0.65), a positive correlation with cholesterol (0.64), and catalase (0.62). Lead has a significant positive correlation with cholesterol (0.58). Chromium had a significant positive relationship with the high-density lipoproteins (0.55). Conversely, chromium had a highly significant negative correlation with the low-density lipoproteins (-0.77), catalase (-0.73), and glutathione (-0.70). It also had a negative correlation with superoxide dismutase (-0.51). Manganese had a strong correlation relationship with cholesterol (0.65), and catalase (0.52).

Barium and cobalt had no significant correlation with the lipid profile and the oxidative stress biomarkers. Strong positive correlation relationships occurred among the metals, while most importantly, very strong negative correlation relationships occurred between protein and superoxide dismutase (-0.70), catalase (-0.93), and glutathione (-0.54).

As expected, a negative correlation relationship (-0.90) which occurred between the high-density lipoproteins and the low-density lipoproteins is consistent with their converse relationships with other analyzed variables. For example, chromium had a significant positive relationship with the high-density lipoproteins (0.55), but a negative relationship with the low-density lipoproteins (-0.77). Glutathione also had a high negative correlation relationship with the high-density lipoproteins (-0.66), but a high positive correlation relationship with the low-density lipoproteins (0.74).

The correlation relationship among the metals, lipid indices, and oxidative stress biomarkers in the intestine of the African brush-tailed porcupines (Table 2) also showed some notable trends, but not as frequent as the observations in the liver.

The concentration of zinc had negative correlation relationships with cholesterol (-0.50), low-density lipoprotein (-0.52), and malondialdehyde (-0.54). Cadmium had a significant positive correlation relationship with malondialdehyde (0.61). Vanadium had a significant positive correlation relationship with the high-density proteins (0.57), and negative correlations with superoxide dismutase (-0.54), and catalase (-0.56). Malondialdehyde had significant negative correlation relationships with nickel (-0.73), and manganese (-0.53).

Bioaccumulation of Metals in African Brush-tailed Porcupine

Contrary to the trend observed in the liver, the high-density lipoproteins had no significant relationship with the low-density lipoproteins in the intestine. Notably, protein in the intestine also had strong negative correlations relationships with superoxide dismutase (-0.73), and catalase (-0.84), but conversely had no significant relationship with glutathione.

Table 1: Correlation of the concentrations of metals, lipid indices, and oxidative stress biomarkers in the liver of porcupines

	Zn	Cd	V	Ba	Ni	Cu	Pb	Co	Cr	Mn	PRO	GLU	CHOL	TRIG	HDL	LDL	MDA	SOD	CAT	GSH
Zn	1.00																			
Cd	-0.16	1.00																		
V	0.58	-0.28	1.00																	
Ba	0.61	-0.30	0.48	1.00																
Ni	0.31	-0.30	0.72	0.38	1.00															
Cu	0.91	0.16	0.46	0.41	0.06	1.00														
Pb	0.85	-0.14	0.66	0.56	0.15	0.85	1.00													
Co	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00												
Cr	-0.47	-0.05	0.00	0.30	0.16	-0.61	-0.34	0.00	1.00											
Mn	0.96	-0.28	0.61	0.51	0.37	0.86	0.78	0.00	-0.58	1.00										
PRO	-0.49	-0.32	-0.05	0.32	0.05	-0.65	-0.36	0.00	0.91	-0.52	1.00									
GLU	0.10	0.62	0.42	0.11	0.11	0.30	0.21	0.00	0.20	-0.01	-0.02	1.00								
CHOL	0.58	-0.11	0.25	-0.17	-0.06	0.64	0.58	0.00	-0.79	0.65	-0.74	-0.19	1.00							
TRIG	0.20	0.37	0.49	0.11	0.16	0.36	0.41	0.00	0.02	0.21	-0.08	0.56	0.32	1.00						
HDL	-0.05	0.30	0.52	0.34	0.36	0.02	0.24	0.00	0.55	-0.14	0.36	0.68	-0.33	0.69	1.00					
LDL	0.32	-0.31	-0.26	-0.31	-0.28	0.29	0.11	0.00	-0.77	0.41	-0.61	-0.61	0.70	-0.41	-0.90	1.00				
MDA	-0.06	-0.01	-0.10	0.17	-0.60	0.13	0.23	0.00	0.02	-0.09	0.22	0.18	-0.15	0.02	0.12	-0.16	1.00			
SOD	0.15	0.59	-0.05	-0.40	-0.10	0.39	0.21	0.00	-0.51	0.13	-0.70	0.18	0.61	0.53	0.07	0.21	-0.34	1.00		
CAT	0.52	0.35	0.24	-0.21	0.23	0.62	0.41	0.00	-0.73	0.52	-0.93	0.12	0.65	0.20	-0.12	0.40	-0.44	0.74	1.00	
GSH	0.37	0.02	-0.37	-0.09	-0.42	0.44	0.16	0.00	-0.70	0.45	-0.54	-0.47	0.62	-0.03	-0.66	0.74	0.04	0.33	0.31	1.00

Legend: Emboldened numbers are significant ($> \text{ or } = 0.50$)

Table 2: Correlation of the concentrations of metals, lipid index, and oxidative stress biomarkers in the intestine of porcupines

	Zn	Cd	V	Ba	Ni	Cu	Pb	Co	Cr	Mn	PRO	GLU	CHOL	TRIG	HDL	LDL	MDA	SOD	CAT	GSH
Zn	1.00																			
Cd	-0.25	1.00																		
V	0.49	-0.18	1.00																	
Ba	0.31	0.32	0.35	1.00																
Ni	0.37	-0.46	-0.17	0.01	1.00															
Cu	0.90	-0.17	0.46	0.55	0.34	1.00														
Pb	0.72	0.06	0.26	0.38	-0.04	0.84	1.00													
Co	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00												
Cr	-0.07	0.24	-0.04	-0.15	-0.35	-0.02	0.10	0.00	1.00											
Mn	0.93	-0.20	0.51	0.50	0.34	0.92	0.76	0.00	-0.26	1.00										
PRO	-0.03	-0.05	0.57	0.12	-0.44	0.07	0.21	0.00	-0.05	0.18	1.00									
GLU	-0.29	0.00	0.13	0.20	-0.12	-0.28	-0.33	0.00	-0.44	-0.01	0.58	1.00								
CHOL	-0.50	0.12	-0.12	-0.08	-0.38	-0.46	-0.31	0.00	0.31	-0.37	0.52	0.63	1.00							
TRIG	0.28	0.09	-0.07	0.36	-0.21	0.33	0.43	0.00	0.22	0.18	-0.41	-0.52	-0.29	1.00						
HDL	0.26	-0.07	0.08	-0.17	-0.30	0.05	0.24	0.00	-0.36	0.33	0.21	0.29	-0.07	0.04	1.00					
LDL	-0.52	0.03	-0.05	-0.23	-0.06	-0.49	-0.49	0.00	0.11	-0.39	0.53	0.65	0.80	-0.78	-0.24	1.00				
MDA	-0.54	0.61	-0.27	0.05	-0.73	-0.47	-0.14	0.00	0.11	-0.53	-0.16	-0.12	-0.01	0.42	0.13	-0.28	1.00			
SOD	-0.12	0.33	-0.54	-0.05	0.09	-0.31	-0.31	0.00	0.16	-0.28	-0.73	-0.23	0.02	0.43	-0.07	-0.23	0.30	1.00		
CAT	0.01	-0.12	-0.56	-0.13	0.30	-0.10	-0.23	0.00	0.17	-0.15	-0.84	-0.31	-0.21	0.42	0.03	-0.39	0.09	0.68	1.00	
GSH	0.19	-0.03	-0.24	-0.26	-0.04	0.27	0.42	0.00	0.43	0.06	-0.27	-0.61	-0.41	0.21	0.12	-0.39	0.10	-0.19	0.32	1.00

Legend: Emboldened numbers are significant (< 0.05).

DISCUSSION

The concentrations of the metals analyzed were mostly higher in the liver than in the intestine due to the physiological function of detoxification carried out by the liver. Although the intestine is the first to make contact with heavy metals ingested, this study shows a higher susceptibility of the liver. All the heavy metals analyzed may be lower than the established regulatory limit of FEPA (2003), Zinc > barium > vanadium will be the first set of metals to exhibit ecological concerns if the exposure of the liver increases (Walker and Morgan, 2014). Zinc is an essential element in the body system of vertebrates, being

the leading metal bioaccumulated in the liver of the animal. However, excess zinc may elicit gastrointestinal disorder, nausea, vomiting, epigastric pain, lethargy, and fatigue at extremely high zinc intakes (Fosmire, 1990). The reported health implications of barium include cardiovascular complications, kidney diseases, metabolic disruptions, neurological disorders, and cognitive impairment. These complications are however influenced by intrinsic factors such as age, race, lifestyle, dietary intake, and excessive use or abuse of medications that interfere with absorbed barium in humans. Vanadium was another metal that exhibited a significant bioaccumulation factor in the liver of the porcupine. The continuous bioaccumulation of zinc, vanadium and barium by the brush-tailed porcupine may threaten the health of the animals in the future by contributing to multi-stress conditions, therefore there is a need for constant monitoring and early detection of the health effects of chronic low-level and moderate-level exposures to these metals in porcupines and their consumers in the higher trophic levels, especially humans (Olajesu *et al.* 2019; Abara *et al.* 2021). Hence, further research is needed to understand the bioaccumulation patterns of vanadium, barium and zinc in order to mitigate their potential health impacts on the exposed porcupine and the dependent populace.

As technologies advance and industrialization progresses, the use of vanadium has increased, and its application has been favored by diverse industries. Due to the wide applicability of vanadium, the potential for occupational exposure to vanadium remains a concern. Similarly, there is an increased risk of environmental contamination by vanadium agents or the by-products released into the environment. The use of vanadium in sulfuric acid production results in the release of soot rich in vanadium pentoxide. Petroleum refinery, smelting, welding, and cutting of vanadium-rich steel alloy, the cleaning and repair of oil-fired boilers, and catalysis of chemical productions are other sources of increased airborne vanadium-bearing particles at far and near destinations, including forest ecosystems. Studies have demonstrated associations between exposure to airborne vanadium-bearing particles may elicit increased risks of hypertension, dysrhythmia, systemic inflammation, hyper-coagulation, cancers, and bronchial hyper-reactivity. Therefore, exposure of the porcupines to trace metals can be regulated through mitigation of the predominant anthropogenic activities, which includes application of agrochemicals and artificial fertilizers. Mitigation of poaching and illegal hunting methods, especially those that involve the use of chemical poisons.

In the intestine, barium was the only metal that exhibited significant bioaccumulation factor in the porcupine. Small amounts of water-soluble barium may cause a person to experience breathing difficulties, increased blood pressure, increased heart rhythm, irritation of the gastrointestinal tract, muscle spasm, neurological disruption, hepatomegaly and swelling of the brain, ultimately nephrotoxicity and hepatotoxicity (ATSDR, 2010).

In stress conditions, some physiological reactions occur, including changes in levels of hormones and components in the blood. These events might lead to higher cholesterol levels which may result in dyslipidemia. As seen in this study, although the levels of bioaccumulation of zinc, vanadium and barium in the liver of the porcupine were higher than the intestine, the latter however exhibited some signs that causes suspicion of dyslipidemia, characterized by significantly higher levels of triglyceride and low-density lipoproteins in the intestine than the liver. This observation in the porcupine was corroborated by the higher superoxide dismutase detected in the intestine than in the liver. Oxidative damage might have occurred in the intestine due to the fact that the reactive oxygen species (ROS) generated from multi-stress, particularly barium exposure might have overwhelmed the antioxidant defense system of the parasites. The upregulation of SOD is strongly linked to oxidative stress (Akinsanya *et al.* 2020) as it is one of the foremost responsive antioxidants in the event of exposure of organisms to stressors. The antioxidant

defense system releases SOD to mop-up the oxyradicals (Vijayavel, 2004; Nabi *et al.* 2017) by fostering superoxides' dismutation to H_2O_2 , which are destructive to biological membranes. Vanadium also exhibited a significant bioaccumulation factor in the intestine of the infected rats. Vanadium has a variety of properties that make it suitable for use in ceramics, and marbles. It is also used as an accelerator for drying paint, and the production of dye, and textiles. Furthermore, due to its hardness, resilience, ability to form alloys and resistance to corrosion, vanadium is also used in the production of tools, steel, and machinery parts (Gimba and Dawam, 2015). Vanadium has several other applications and it is almost indispensable in technology.

If cadmium exposure exceeds the regulatory limit in the consumers of bush meat due to the observed significant bioaccumulation, the unregulated exposure can lead to a variety of adverse health effects including cancer. Acute exposure, for example, to high levels of cadmium over a short period of time can result in flu-like symptoms such as chills, fever, and muscle pain, which can damage the lungs. However, exposure to low levels over an extended period of time as observed in the parasite can inflict toxicity on the kidney, lungs, and other vital organs. The current study conforms appreciably to the observations of Mustafa (2019) who discovered high concentrations of copper, chromium, cadmium, and cobalt exceeding the established/ permissible regulatory limits. Durojaye *et al.* (2014) also discovered impermissible concentrations of Fe, Cu, Cd, Pd, Mn, Cr, and Zn in the skin, liver, lung, and kidney of *Thryonomys swinderianus* sampled in Omo forest reserve of Ogun State. In the current study, although the concentrations of all trace metals investigated were below the stringent regulatory limits of established and certified bodies around the world, barium, vanadium and zinc may be metals of future concern due to their attendant bioaccumulation in the grasscutter.

Although there is currently no cause for concern in the liver of the porcupine, the bioaccumulation rates of zinc, barium and vanadium, coupled with the correlation of some of the metals with indices that indicate stress and dyslipidemia might be a prognosis worthy of monitoring. For example, zinc concentration in the liver of the porcupine had significant positive correlations with cholesterol (0.58) and catalase (0.52), while vanadium had a positive correlation relationship with the high-density lipoprotein (0.52). Manganese had a strong correlation relationship with cholesterol (0.65), and catalase (0.52).

In the intestine of the brush-tailed porcupines, barium exhibited no significant correlation relationship with the lipid indices and oxidative stress biomarkers. The protein in the intestine however had strong negative correlations with superoxide dismutase (-0.73), and catalase (-0.84). This indicates the tendencies of the destruction of the cell membrane proteins due to an overwhelmed antioxidant defense system. Results presented might not have implicated barium, in the natural environment multiple factors might be responsible for the concerns detected in the intestine of the animal.

The study demonstrated some empirical relationships in the concentrations of the heavy metals analyzed and the lipid profile, and the antioxidant defense system of the porcupine. This was well supported by the correlation of the concentrations of metals, lipid index, and oxidative stress biomarkers in the liver and intestine of the investigated porcupines.

Recommendation and Conclusion:

The liver managed the situations of the toxicokinetics due to organ-specificity. This study has demonstrated empirical evidence of toxicity in the intestine of the brush-tailed porcupines, the study however, did not determine the actual stressor. Further study is recommended to determine the leading factors in the multi-stress conditions of the porcupines. This shall be of paramount benefit to the conservation of the animal and the protection of the consumers.

Ethical Permission:

Ethical approval was obtained from the University of Lagos College of Medicine health research ethics committee with reference number CMUL/HREC/05/20/724.

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