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Determination of concentration of selected heavy metals in the organs of Guinea pigs reared at the University of Port Harcourt, Rivers State, Nigeria

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Abstract

Vegetation along major traffic roads are predisposed to heavy metals contamination due to wear and tear of vehicular components and emissions from vehicles. The concentration of ten heavy metals (Fe, Mn, Zn, Pb, Cu, Ni, Cr, Co Ag and Cd) in the leaves of four test plants (*Panicum maximum* Jacq.,*Eleusine indica* L., *Xanthosoma mafafa* Schott. and *Amaranthus spinosus* L.) growing along four busy roads (Aba road, Ikwerre road, East/West road and NTA road) in parts of Obio/Akpor Local Government Area and a control (in Ozuguru in Ikwerre Ngwo) in Etche Local Government Area, both in Rivers State, Nigeria were investigated in the study area. Along each road, four sampling points were set up at 1 km apart. The test plants were collected randomly and were pooled together to form composite sample. The collected test plants at each sample location were put in different sterile cellophane bags, labelled and served as soilage to the experimental animals. Eighteen guinea pigs were used for this investigation. Three guinea pigs were picked out of the eighteen guinea pigs using randomized block design for a preliminary test. The remaining fifteen guinea pigs were fed with the test plants from the experimental and control sites for six weeks. The concentration of the ten heavy metals were determined using Atomic Absorption Spectrophotometry (ASS). The results revealed that Aba road had the highest mean concentration value of heavy metals in Cu, Zn, Co, Cr and Cd with values (28.75±1.34), (32.46±0.9), (5.14±0.11), (4.79±0.21) and (3.17±0.12) respectively. The highest mean concentration value for Mn and Pb were observed in East/West road with values (7.57±1.48) and (5.39±1.53) respectively, while the highest mean concentration value for Fe was observed in Ikwerre road (192.84±2.64). NTA road had the least values for all heavy metals tested while Ni and Ag were not detected in all livers and kidneys of the experimental animals. The results from this findings show the health implication of roadside grazing of ruminants.

Keywords: Atomic Absorption Spectrophotometry; Heavy Metals; Guinea Pigs; Liver; Kidney

1. Introduction

Toxic heavy metals occur naturally in the Earth's crust and their concentrations vary in quantity. These heavy metals include mercury (Hg), cadmium (Cd), lead (Pb) among others. The quest for development results in the creation of more industries and vehicles which pollute our environment and leaves the biosphere foul unless properly treated. The availability of heavy metals in crop plants depend on the physiochemical features of the soil and plants predisposition to their increased absorption and organs accumulation thereby making their entrance to the food chain easier (Marschner, 2012). These heavy metals are not needed for any physiological function in animals. Hence, they are not required in the feed of animals and are referred to as contaminants (Kabata-Pendias, 2010).

Heavy metals bind to nucleic acids, enzymes and structural proteins and disturb the functioning of cells. Heavy metals may result in chronic toxicity as a result of their long biological half-life and their capacity to accumulate in human and animal bodies (Chaffai and Koyama, 2011). Effects and indications vary based on the metal and metal compound and the dosage involved. Exposure to long term toxic heavy metals might cause central and peripheral nervous system,

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carcinogenic and circulatory effects (Studziński *et al.,* 2006). Cadmium shows teratogenic, mutagenic and genotoxic effects in animals and humans (European Commission, 2006). Also, the accumulation of Cd impairs the metabolism process of vitamin D thereby reducing the absorption of calcium from the intestinal tract. The absorption of lead (Pb) from the gastrointestinal tract, is moved to the liver where it accumulates, then to the kidney, heart and the brain and later to the muscles or bone tissues. Long term intake of Cd and Pb might alter the organization of intestinal mucosa (Thirulogachandar *et al.,* 2014). The toxicity of these metals is connected to bone damage through the exchange of divalent calcium as a result of structural similarities (Tomaszewska *et al.,* 2018).

Although, there are some heavy metals that are required in minute quantities (micro-element) for both animal and human health. These vital trace elements are manganese (Mn), iron (Fe), copper (Cu) and Zinc (Zn) (Epstein and Bloom 2005; Marschner 2012). Deficiency of these vital trace elements can increase the susceptibility of heavy metal poisoning (Kabata-Pendias, 2010; Chaffai and Koyama, 2011; Studziński *et al.,* 2006). Based on the European law (European Commission, 2006)], vital heavy metals are permitted to be used in animal nutrition for the optimization of animal production.

Copper acts as enzyme activator in reduction-oxidation processes. Iron and copper partake in the initialization of the generation of reactive oxygen species (ROS), that react with polyunsaturated fatty acid residues of cell membranes, thiol-containing proteins and nucleic acids leading to oxidative stress and cytotoxic effects.

Manganese (Mn) as a vital trace element is present in all tissues and are highly concentrated in bones, liver and kidneys. Manganese is important for the healthy growth and development of the skeletal system. Large intake of manganese (Mn) results in the poisoning of the element which causes increased concentrations of manganese in the liver, severe reduction in iron absorption, change in Cu distribution and reduction in phosphorus and calcium excretion (Studziński *et al.,* 2006).

Zinc is mostly found in the bones and muscles of the body. It is a cofactor of many enzymes of which the most common are carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, glutamine, alkaline phosphatase, lactate dehydrogenase and RNA polymerase. Prolong exposure to heavy metal could cause their accumulation in organs like liver, kidneys and muscles (Marschner, 2012; Thirulogachandar, 2014).

Bioaccumulation is the process by which living organisms pick up substances and store them in their organic tissues or transfer them in the food chain because they cannot process them. Heavy metals are members of loosely defined subset of elements exhibiting metallic properties. They include some metalloids, transition metals, some actinides and lanthanides. The quest for urbanization and industrialization has led to a lot of anthropogenic and geologic activities which results to the increase in concentration of these metals to amounts that are harmful to both animals and plants. These activities include municipal waste disposal, burning of fossil fuels, sewage sludge, waste-derived fuels, the use of fertilizers and pesticides in agriculture etc (Alloway, 1990; Raskin *et al*., 1994; Shen *et al*., 2002). The toxicity of heavy metals is a problem of increasing significance for ecological, nutritional and environmental reasons and these heavy metals are non-degradable.

Roadside soils contain high level of heavy metal concentrations released from the wear and tear of tyres, exhaust fumes, corrosion of car metal parts, oil leakage and burning of fuel (Dolan, Van Bohemen and Whelan, 2006). Information on the effects of heavy metal contamination of roadside plants as it affects herbivores is largely lacking in the study area.

2. Materials and methods

2.1. Description of the Study Area

This study was conducted along four busy roadside (East/West road, NTA road, Aba road and Ikwerre road) in parts of Obio/Akpor Local Government Area and the control (Ozuguru in Ikwerre Ngwo) in Etche Local Government Area in Rivers State, in Southern part of Nigeria. Aba Road and East-West Road are part of the major roads in Obio/Akpor local government area that link parts of Northern, Western and Eastern states to Rivers State resulting to a lot of heavy vehicular and anthropogenic activities in the areas. NTA and Ikwerre roads are roads where commercial activities take place. These roads link to local roads in the city. The control site (Ikwerre Ngwo) is a rural area, inhabitants here are mostly peasant farmers.

2.2. Sample Collection

Sampling was carried out in dry season between the months of January and February, 2021. The sample collection was done between the hours of 6.30am and 10am. Four sample points along each of these roads located within a minimum of 1Km distance between sampling points were used for this study. At each sample point, four plant species (*Panicum maximum* (Jacq.), *Elusine indica* (L.), *Zanthosoma mafafa* (Schott) and *Amaranthus spinosus* (L.)) representing monocotyledon and dicotyledon were collected respectively, found flourishing within one meter by one meter of the study area, exposed to the same ecological condition with respect to soil, light and temperature were harvested. They were collected randomly from four plants of the same species in each of the study area. The plants species from the same sample point were pooled together to form composite sample. These plant species met with the criteria for species selection as biomotor which states that: the species should be represented in a large number all over the monitored area and there should be no problem with species identification (Markert, 1993). Plant specimens collected at each sample location were put in different sterile cellophane bags that were well labeled and were transported immediately to the Department of Experimental Pharmacology and Toxicology animal house research unit, Faculty of Pharmaceutical Sciences, University of Port-Harcourt, Rivers State, Nigeria.

2.3. The Experimental Animals

Eighteen (18) guinea pigs of about 8 weeks old were bought from the Department of Experimental Pharmacology and Toxicology animal house research unit, Faculty of Pharmaceutical Sciences, University of Port-Harcourt, Rivers State, Nigeria. Three guinea pigs were picked out of the eighteen guinea pigs using randomized block design. They were anesthetized with the aid of chloroform in a desiccator, sacrificed and their tissues (kidney and liver) and blood samples were used for a preliminary test to determine the concentration of heavy metals in the tissues (kidney and liver) and blood samples. The blood samples were obtained through cardiac puncture with the aid of a string. The remaining 15 guinea pigs were grouped into five different pens using randomized block design and were labeled accordingly (i.e Aba road, East-West road, Ikwerre road, NTA road and a control group). The animals were fed with the test plants (*Panicum maximum, Elusine indica, Xanthosoma mafafa* and *Amaranthus spinosus*) from the different experimental sites and the control site daily for six weeks. This was done during dry season (between January and February, 2021).

At the end of the sixth week, the animals were anesthetized using chloroform in a desicator, sacrificed and the tissues (kidney and liver) were taken to an analytical laboratory to determine the concentration of heavy metals in the tissues (kidney and liver).

2.4. Quality Assurance (QA)/Control (QC) Employed

The prescribed protocol for quality assurance (QA)/control (QC) specified by the United State Environmental Protection Agency (EPA) for analyzing heavy metals were employed. The analytical protocols were controlled by precised replications of the analytical results. The glass wares were pyrex products, they were washed with 1:4 nitric acid, rinsed twice with distilled water and oven dried at a temperature range of 105^0C for about 30 minutes. The reagents employed for this analysis were of analytical grades (BDH Chemical Limited, Poole England) standard solutions prepared from 1000µg/g stock solution of Ag, Mn, Zn, Cr, Ni, Fe, Cd, Cu, Co and Pb were used for flame atomic absorption analysis. The certified standard reference material used in this study was Accu standard USA. The obtained results are shown in Tables 4.1.26 to 4.1.29 and Tables 4.1.42 to 4.1.81 below.

2.5. Sample Digestion of the Tissues of the Experimental Animals

After isolating the tissues from the test animals, they were washed with clean distilled water and oven dried at a temperature of 105^0C for 6 to 7 hours. This was done to remove all moisture content and concentrate the heavy metal content in the sample after which the dried samples were blended into powder and homogenized. Then 2 gram of the homogenized sample was put in to silica crucibles and transferred into a muffle furnace. Samples were heated (for about 3 hours) up to 400^0 Celsius until they assumed a grayish ash white coloration. The samples were then transferred into a desiccator to cool for about 30 minutes. A solution of the ash was made by adding 5 ml of 10% HCl and 5 ml of 10% nitric acid to dissolve the ash. Also, 20 ml of distilled water was added to the acid mixture and heated on a hot plate at 95^0 Celsius. The solution was concentrated to about 10 ml. The ash solution was left to cool before being filtered into a 50 ml volumetric flask. Then the acid extract was made up to mark with distilled water. The aliquots of this were analyzed for Ag, Co, Cu, Cr, Zn, Ni, Mn, Cd, Pb and Fe using flame atomic absorption spectrophotometer (AAS) GBC Avanta Programmable A6600.

2.6. Data Analysis

The data obtained were analysed statistically using descriptive statistics, one way analysis of variance (ANOVA) and multiple comparism using Least Significant Difference (LSD) at 5% level of significance with the aid of SAS – 2007, version 9.1 statistical package

3. Results

The mean values and standard error for heavy metals in the liver and kidney of the experimental animals (guinea pigs) are shown in Tables 1 and 2 respectively.

Table 1 Concentration of Heavy Metals in the Liver

	Pre-Test	Control	Aba Road	E/W Road	Ikwerre Road	NTA Road	LSD
Cu	10.32±0.5^{c}	12.09±0.9^{c}	28.75±1.3^{a}	25.41±5.8^{ab}	22.03±1.0^{ab}	17.76±0.4^{bc}	7.771
Zn	12.76±1.0^{c}	15.58±0.7^{c}	32.46±0.9^{a}	25.86±4.8^{b}	26.82±1.0^{ab}	24±0.9^{b}	6.554
Mn	3.03±0.2^{c}	4.35±0.2^{bc}	6.15±0.2^{ab}	7.57±1.5^{a}	4.56±0.2^{ab}	3.81±0.2^{c}	1.939
Co	1.52±0.1^{d}	2.78±0.2^{c}	5.14±0.1^{a}	4.56±0.8^{ab}	4.04±0^{b}	3.76±0.1^{bc}	1.02
Ni	0.001±0	0.001±0	0.001±0	0.001±0	0.001±0	0.001±0	0
Cr	0.85±0.2^{b}	2.3±0.1^{b}	4.79±0.2^{a}	4.67±1.2^{a}	3.9±0.1^{a}	3.84±0.1^{a}	1.497
Fe	80.99±4.3^{c}	137.15±2.6^{b}	191.61±4.1^{a}	181.61±21.7^{a}	192.84±2.6^{a}	192.73±3.1^{a}	28.889
Ag	0.001±0	0.001±0	0.01±0	0.001±0	0.001±0	0.001±0	0
Pb	0.36±0.2^{c}	0.52±0.1^{bc}	5.04±0.5^{a}	5.39±1.5^{a}	4.08±0.1^{ab}	3.38±0.2^{abc}	2.062
Cd	0.38±0^{e}	0.79±0^{d}	3.17±0.1^{a}	2.55±0.5^{b}	2.25±0^{bc}	1.86±0^{cd}	0.62

Row means ± standard errors with same letters are not significantly different at 0.05 significance level

Table 2 Concentration of Heavy Metals in the Kidney

	Pre-Test	Control	Aba Road	E/W Road	Ikwerre Road	NTA Road	LSD
Cu	2.74±2.7^{d}	8.72±0.3^{c}	20.57±1.3^{b}	23.32±0.6^{a}	20.6±0.6^{b}	18.77±0.5^{b}	2.155
Zn	16.32±1.2^{c}	50.69±1.6^{a}	43.85±1.1^{b}	46.47±1.5^{b}	42.89±1.2^{b}	42.8±0.8^{b}	3.846
Mn	2.12±0.1^{d}	7.31±0.2^{b}	7.44±0.3^{b}	8.2±0.1^{a}	7.440±0.3^{b}	5.97±0.1^{c}	0.621
Co	3.73±0.6^{d}	7.25±0.2^{c}	16.9±0.6^{b}	19.14±0.3^{a}	16.14±0.3^{b}	16.72±0.2^{b}	1.229
Ni	0.001	0.001	0.001	0.001	0.001	0.001	0
Cr	1.9±0.5^{e}	3.01±0.4^{d}	8.17±0.3^{b}	9.16±0.2^{a}	8.06±0.1^{bc}	7.12±0.3^{c}	0.97
Fe	116.69±5.2^{d}	219.58±6.6^{a}	207.78±5.1^{ab}	218.98±3.9^{a}	198.73±5.7^{bc}	190.22±1.5^{c}	15.177
Ag	0.001	0.001	0.001	0.001	0.001	0.001	0
Pb	1.32±0.1^{e}	2.4±0.2^{d}	6.93±0.1^{a}	8.24±0.1^{b}	6.39±0.3^{c}	6.43±0.1^{c}	0.478
Cd	0.26±0.04^{c}	0.33±0.02^{c}	0.76±0.02^{a}	0.78±0.04^{d}	0.63±0.03^{b}	0.7±0.02^{ab}	0.093

Row means ± standard errors with same letters are not significantly different at 0.05 significance level

4. Discussion

4.1. Heavy Metals in the Liver and Kidney of the Experimental Animals

The data obtained from the analysis of heavy metals in liver and kidney of the guinea pigs are presented in Tables 1 and 2 respectively. Aba Road had the highest mean concentration value of heavy metals in Cu, Zn, Co, Cr and Cd with values

(28.75±1.34), (32.46±0.9), (5.14±0.11), (4.79±0.21) and (3.17±0.12) respectively. The highest mean concentration value for Mn and Pb were observed in East/West road with values (7.57±1.48) and (5.39±1.53) respectively, while the highest mean concentration value for Fe was observed in Ikwerre Road (192.84±2.64). NTA Road had the least values for all heavy metals tested while Ni and Ag were not detected in all livers and kidneys of the exposed animals.

Cr is a vital element that helps the body to utilize sugar, fat and protein but it is carcinogenic for organisms. Excessive amount of Cr could result in adverse health effects (Abd El-Salam *et al.,* 2013) and could lead to the reduction of the effectiveness of insulin in controlling blood sugar. Cd is distributed by water (sewage sludge) and air far over land and sea especially in the surroundings of heavy industrial plants where it is absorbed by sea organisms and plants. Report has it that Cd is toxic to almost all system in animal body (Akan *et al.,* 2010). Roga-Franc *et al.,* (1996) identified Cd levels in kidney and liver of cattle in Poland and discovered the concentration of Cd to be higher than 0.5 ppm permissible limit set by FAO/WHO (2000). Also, Doganoc (1996) discovered higher levels of zinc and cadmium in the kidneys and livers of chickens and hens, which exceeded normal tolerance levels. Cd is basically toxic to kidney particularly to proximal tubular cells. Demineralization of bone is usually indirectly affected by the toxicity of cadmium by bone damage (Solidum *et al.,* 2013). Cd could catalyze diabetes-induced effects on kidneys. Kidney damage could further develop to end stage renal disease (ESRD) and finally death if exposure is high for a long time. Research has shown that Cd could play a role in other cancer developments such as pancreatic cancer, testicular cancer, cancer of gall bladder and bladder cancer (Liu *et al.,* 2007).

Pb is a poisonous metal that has no vital effect on living organisms. The accumulation of Pb overtime in the bodies of humans and animals could cause severe ailments (Binkowski, 2012). It could enter the atmosphere during manufacturing processes, mining, refining, smelting and through the use of products containing Pb (Abd El-Salam *et al.,* 2013). Livestock get contaminated by Pb through the water they drink, air they breathe and the food they eat.

Mn is important for metabolism of lipid in animals. It is required for good health and growth of humans. Its deficiency could result to nervous system disorder (Demirezen and Uruc, 2006). The high level of Mn could be attributed to their special functions; kidney as excretory organ and liver as metabolic and storage organ (Stoyke *et al.,* 1995). Mn is contained in food in several chemical form and combination and these affect its availability to animals. It is toxic at high level to humans and animals. The Mn level obtained from this study agrees with the levels reported for kidney and liver of cow elsewhere (Iwegbue, 2008; Ubong *et al.,* 2013). Mn plays vital roles in different enzyme systems. Its deficiency in diet results in impaired growth, skeletal abnormalities and in fat and carbohydrate metabolism (Holleman *et al.,* 1985).

The elevated level of Fe in the organ of the animals could be due to the fact that Fe is one of the main constituent of blood and because it flows in these organs, its level is expected to be high (Nielsen, 2003; NAP, 2001). Fe is a vital metal that is required for metabolic activity in animals. Enzymatic reactions that involves oxidation and reduction use Fe as an agent by which electrons are transferred (Nielsen, 2002; Nielsen, 2003; NAP, 2001). Excessive Fe in the body is stored as hemosiderin and ferritin in the reticuloendothelial cells, liver and bone marrow (WHO, 1996). In this study, the concentration of Fe obtained were significantly ($P \leq 0.005$) higher than the mean levels of 125.2 – 146μg/g reported for tissue, kidney and liver of cattle from three regions of Slovakia (Korenkova, *et al.,* 2002). Gonzalez-Waller *et al.,* (2006) also observed higher level of Cd and Pb in meat exceeding recommended limits (Akan *et al.,* 2010).

5. Conclusion

Report has it that kidneys and livers are the main organs used to monitor heavy metal contamination in animals. This is because they both function in the removal and storage of toxic metals from the body and hence, they end up accumulating them (Abou-Arab, 2001; Akoto *et al.,* 2014). Kidney and liver are the preferred place for heavy metal accumulation. The findings from this study revealed the health implication of roadside farming, grazing and selling of exposed vegetables and fruits. People that buys or sells on major roadside should consider the risks associated with heavy metal accumulation in their bodies.

Compliance with ethical standards

Disclosure of conflict of interest

There was no competing of interest.

Statement of ethical approval

All applicable international, national and institutional guidelines for the care, handling and use of animals were followed.

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