PATHO-CLINICAL EVALUATION OF *Cavia porcellus* EXPOSED TO WASTEWATER FROM OLORUNDA MINING SITE, SEPETERI, NIGERIA

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ABSTRACT
Exposure to mining wastewater, which is an undesirable consequence of mining activities, may have detrimental impacts on animal, human, and ecological health. This study evaluated heavy metal levels in mining wastewater from Olorunda Mining Site and determined their haemato-biochemical and histopathological effects on exposed Guinea pigs. Mining wastewater (MWW) samples were collected from Olorunda Mining pool in pre-cleaned sampling bottles for heavy metals determination and exposure to the experimental animals. Forty Guinea pigs were divided into five experimental groups (n=8/group), which included a control (100% pure borehole water) and four treatment levels (100%, 75%, 50%, 25% of MWW), all exposed for 30 days. Fully digested wastewater and tissue (kidney and liver) samples were subjected to Atomic Absorption Spectrophotometer for heavy metals (Lead, Cadmium, Zinc, Manganese, Iron, Uranium and Vanadium) determination. Using standard techniques, haemato-biochemical and histopathological assessments were carried out on the Guinea pigs. Data were analysed using descriptive and inferential statistics at p<0.05 level of significance. All heavy metals detected in the MWW were above the recommended permissible limits. Lead and Cadmium levels (in mg/L) in the liver [Group A (0.95±0.07, 0.05±0.01); Group B (0.97±0.09, 0.05±0.003); Group C (1.02±0.05, 0.05±0.01); Group D (1.22±0.87, 0.05±0.01)] and kidney [Group A (1.03±0.16, 0.66±0.02); Group B (0.87±0.94, 0.07±0.04); Group C (0.99±0.94, 0.07±0.01); Group D (1.26±0.19, 0.06±0.03)] tissues of treatment groups, were higher than those of the control group. However, significant differences were only observed in the kidney tissues. Haematological and biochemical alterations attributed to contaminant toxicity were observed, while histopathological architecture of the tissues evaluated showed different changes that were attributed to heavy metal toxicity. There is a need for the re-channeling of the mining wastewater away from the Ajaku River so that it does not become a source of contamination to the river water system.

Keywords: Heavy metals; Fauna health; Environmental contamination; Guinea pigs

INTRODUCTION
A peculiar problem faced by man, as a consequence of industrial and urban development, is the issue of waste generation either in gaseous, liquid or solid forms. Industrial activities such as mining of minerals often produce large quantities of waste materials with no economic value and high negative impacts on the environment (Agboola *et al*., 2020). These waste materials are known to differ in terms of their physical and chemical compositions (Falagan *et al*., 2017). Mining is a major human activity that leads to the destruction of natural ecosystems with deleterious effects on environmental quality and adverse health implications on the surrounding species population (Muhammad *et al*., 2013; Effiom *et al*., 2021). The disposal of mine wastes has become a widespread challenge, with environmental contamination risks to land

and water resources (El Khalil *et al*., 2008). The wastewater discharged from mining sites into streams and rivers alter the geochemistry, water quality and metal concentrations of these ecosystems (Ali *et al*., 2017; Wright *et al*., 2017). Most times, the processes commonly used for the treatment of the wastewater do not guarantee the quantitative removal of many contaminants, and thus result in environmental contamination after discharge (Tytla, 2019). Heavy metals are among the contaminants introduced to the environment and their concentrations have been increasing due to the chemical processes used for extraction of the metals (Muhammad *et al*., 2013; Qasem *et al*., 2021).

Although, heavy metals are natural components of the environment and are crucial for the survival of species, their bioaccumulation could be precarious for living things (Adetuga *et al*., 2020; Mitra *et al*., 2022). According to Omonona *et al*. (2019), heavy metal pollution of the environment has gained global recognition because of their persistence, expressive toxicity, longevity, and non-biodegradation properties. Prolonged exposure to heavy metals even in very low concentrations have been reported to induce morphological, histological and biochemical alterations in the tissues of animals (Kaoud and El-Dahshan, 2010).

Guinea pigs (*Cavia porcellus*) belong to the Order Rodentia and Family Caviidae (Rahmani *et al*., 2022). They were domesticated from wild species that originated from the Peruvian Highlands, following phylogenetic and morphological studies (Kimura *et al*., 2016). This species of rodents inhabit wild areas, as prey species, based on their life history and do not show early clinical signs of infection, unless the infection or ailment becomes austere (Cernochova *et al*., 2020; Fitria *et al*., 2022). Aside from being kept as pet animals, they are also often used as laboratory animal models in biochemical, pathological and toxicological tests (Minarikova *et al*., 2015; Rahmani *et al.*, 2022).

The wastewater from the Olorunda Mining Site flows into Ajaku River that traverses the Old Oyo National Park, a conservation area for wildlife. Such wastewaters, contain heavy metals which are detrimental to the environment. Therefore, this study evaluated the heavy metal levels in the wastewater from Olorunda Mining Sites. In addition, the haemato-biochemical and histopathological alterations in Guinea pigs exposed to this wastewater were determined.

MATERIALS AND METHODS

Study Area

The wastewater samples were collected from Olorunda Mining Site which is within the Lukutu Community in Sepeteri Area of Saki East Local Government Area in Oyo State, Southwestern Nigeria (Figure 1). It is approximately 5 – 6 km to the boundary of Old Oyo National Park (Wahab *et al*., 2017).

Source of Experimental Animal Subjects

Forty Guinea pigs (*Cavia porcellus*) of 3-4 months old were obtained from Makarfi Market, Zaria in Northern Nigeria. They included 20 males and 20 females. The health status of the animals were ascertained by screening their blood and evaluating their faeces for parasites. The animals were stabilized for 14 days with food and clean water supplied *ad-libitum*. Animal weights were taken with sensitive weighing scale (Pelouze, model SP5) while experimental protocol was carried out to meet the requirements of ethical guidelines on the care and use of laboratory / experimental animals.

Collection of Water Samples and Experimental Design

Wastewater samples were collected from the mining site pool into pre-cleaned sampling bottles for heavy metal determination.

The animals were divided into five experimental groups (made up of 4 males and 4 females each) consisting of one control group and four treatment groups [based on the percentage of mining wastewater (MWW) they were exposed to]. The control group was given pure borehole water (PBW) while the four treatment groups were exposed to different levels of MWW (Table 1), for 30 days. All the experimental animals were given the same measure of food, which included wheat and maize shaft, *Amaranthus*, Guinea grass, and pawpaw leaves.

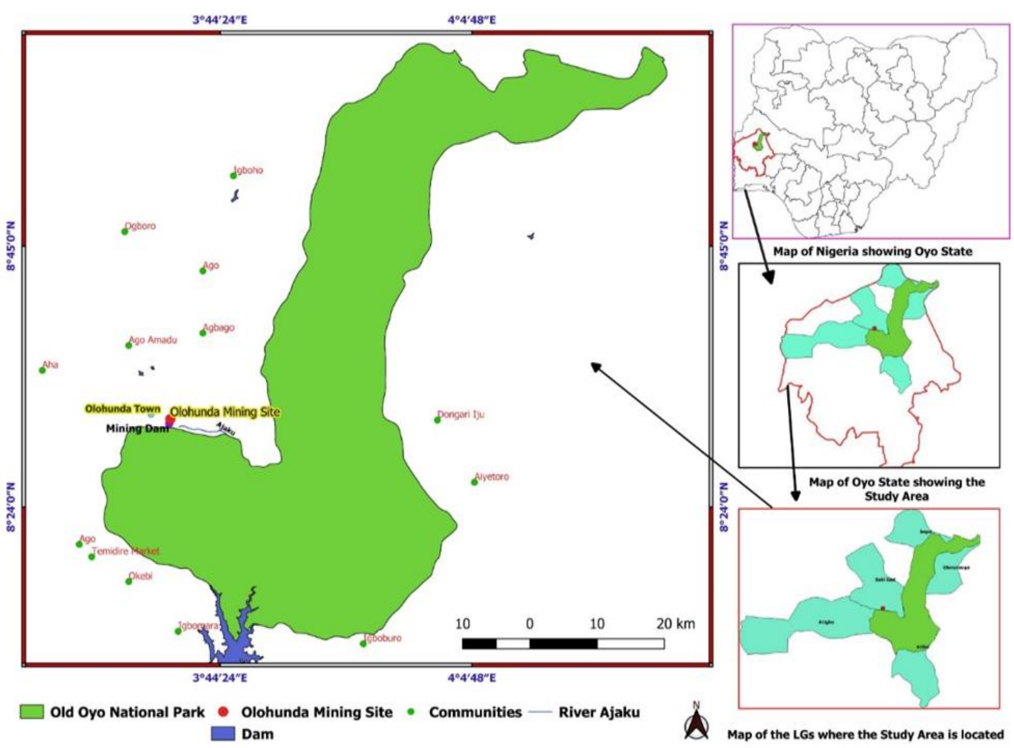

Figure 1: Map of Olorunda Mining Site, Sepeteri, Nigeria (inset: Maps of Nigeria, Oyo state and Local Government Areas)

Table 1. **Experimental animal groups and level of contamination with wastewater from Olorunda Mining Site, Sepeteri, Oyo State, Nigeria**

Experimental Group	Number of Animals	Type and Percentage of Contaminated Water
Control Group	8	PBW (100%)
Treatment Group A	8	MWW (100%)
Treatment Group B	8	MWW (75%) + PBW (25%)
Treatment Group C	8	MWW (50%) + PBW (50%)
Treatment Group D	8	MWW (25%) + PBW (75%)

Note: PBW = Pure Borehole Water; MWW = Mining Wastewater

Collection of Blood and Tissue Samples

Blood sample (2 ml) was collected from each animal within 72 hours of post exposure to mining wastewater through the ocular sinus, with the aid of a capillary tube. The blood was transferred into 1 mg of anticoagulant Ethylene Diamine Tetra Acetate (EDTA). This was used for haematological evaluation. Similarly, 3 ml of blood sample was collected into plain sample bottles for serum biochemical evaluation. Tissues were collected from the liver, kidney, brain and lungs of representative animals from each experimental group (humanely euthanized in chloroform chamber). These samples were immersed in 10% buffered formalin and subsequently processed for histopathology. After collection, blood and tissue samples were immediately taken to the Clinical Pathology Laboratory, Department of Veterinary Pathology, University of Ibadan, Nigeria for haematological, serum biochemical and histopathological evaluation. In addition, the heavy metal contents of the samples were determined at the Soil Chemistry Laboratory, Department of Soil Resources Management, University of Ibadan, Nigeria.

Laboratory Analysis of Wastewater, Blood and Tissue Samples

The heavy metal content in the wastewater, were determined to ascertain the concentrations that experimental animals were to be exposed to. Heavy metals such as Lead (Pb), Cadmium (Cd), Zinc (Zn), Manganese (Mn), Iron (Fe), Uranium (U) and Vanadium (V), which are associated with mining were determined (Muhammad *et al.*, 2013; Ghazaryan *et al.*, 2016). Then, 50 ml of the wastewater was acidified with 10% HNO_3 to prevent analyte loss and allow proper wet acid digestion. Heavy metals were assessed in the tissue samples to ascertain the concentrations that were bioaccumulated. Tissue samples were digested following the procedure described by Akan *et al.* (2012). All fully digested water and tissue samples were subjected to Atomic Absorption Spectrophotometer analysis to determine the levels of the heavy metals. Haematological and serum biochemical parameters were determined using standard techniques as described by Coles (1986); Omonona and

Emikpe (2011) as well as Bartley (2001). Microscopic evaluation of the changes in the animal tissues (histopathology) was done using light microscope (CX21) (Titford, 2009).

Statistical Analysis

Data collected were subjected to descriptive (mean, standard deviation) and inferential (ANOVA) statistics using Statistical Package for Social Sciences (version 25). Statistically significant means were separated at $p<0.05$ level of significance.

RESULTS

Body Weight and Heavy Metal Concentration in Wastewater

The mean body weights (g) before and after exposure to different treatments were 202.97±10.21 and 215.97±7.46 (Group A), 200.47±8.55 and 210.35±12.72 (Group B), 214.39±15.14 and 221.78±9.44 (Group C), 222.18±11.12 and 245.18±10.98 (Group D), while that of the control group were 196.86±8.27 g and 208.56±12.55 g, respectively (Figure 2). All treatment groups had increased weights before their exposure to mining wastewater.

The concentration of heavy metals in the wastewater: Lead (1.225 mg/L), Cadmium (0.08 mg/L), Zinc (12.28 mg/L), Manganese (26.00 mg/L), Iron (401.50 mg/L), Uranium (385.96 mg/L) and Vanadium (292.40 mg/L); were above the WHO (2011) recommended environmental permissible limits for drinking water (Table 2).

Haematological Profile of Experimental Animals Groups

There were significant differences in the Red Blood Cells (RBC; p = 0.03), White Blood Cells (WBC; p = 0.02) and lymphocytes (p = 0.01) counts across the experimental groups (Table 3). The Packed Cell Volume (PCV), RBC, Platelets, Haemoglobin (Hb), WBC Neutrophils, lymphocytes and eosionophils counts of the treatment groups were lower than that of control. However, the monocytes count of Groups A (96.14±60.30%), C (97.86±38.95%), and D (95.33±30.86%) were higher than those of the control group.

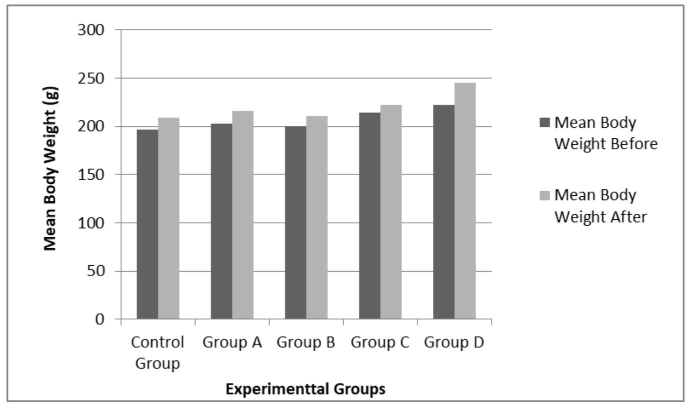

Figure 2. Body weights of *Cavia porcellus* exposed to varying levels of wastewater from Olorunda Mining Site, Sepeteri, Oyo state Nigeria

Serum Biochemical Profile of Experimental Animals Groups

The blood urea nitrogen (BUN) decreased in Groups A (15.85±0.2 mg/dl) and D (15.60±0.60 mg/dl), while those of Groups B (16.50±0.2 mg/dl) and C (16.15±0.90 mg/dl) were higher than control group (16.60±0.57 mg/dl). There was little or no change in the creatinine values between the control and treatment groups. Nevertheless, no significant differences were observed for values of BUN and Creatinine across the experimental groups.

Heavy Metal Concentrations in Tissues of Experimental Animal Groups

The concentrations of Lead and Cadmium in the livers and kidneys of treatments were higher than those of the control group (Table 5). There were no significant differences in the Lead and Cadmium concentrations in the liver tissues, but they differed significantly in the kidneys. Cadmium concentrations in liver tissues were similar across treatment groups, but was highest in the kidney tissues of Group A treatment (0.66±0.02 mg/kg).

Histopathological Evaluation of Tissues in the Experimental Animal Groups

Histopathological analysis revealed no observable lesion in the testis and epididymis (though tubular lumen was devoid of spermatozoa) in the control group. In Group A, swelling of sertoli cells, retention of round spermatids and spermatogenic arrest were observed in the testis (Figure 3a), while patchy tubular epithelial degeneration and necrosis were present in the kidney (Figure 3b). In Group B, there was a disruption of germinal epithelium in the testis (Figure 4a) and hepatocellular vacuolar degeneration (Figure 4b). Animals in Group C, experienced villi atrophy and denudation of villi tips in their intestine (Figure 5a) as well as moderate hyperplasia of lymphoid follicular cells (Figure 5b). In Group D, no hepatic lesion was observed (Figure 6a), while patchy renal tubular epithelial degeneration, coagulation necrosis, esinophilic casts and inflammation were observed (Figure 6b).

Table 2. Heavy metal concentrations in wastewater from Olorunda Mining Site, Sepeteri, Oyo state Nigeria

Heavy Metals	Concentration (mg/L)	Recommended Permissible Limit
Lead	1.23	0.01
Cadmium	0.08	0.003
Zinc	12.28	5.00
Manganese	26.00	0.40
Iron	401.50	0.30
Uranium	385.96	0.002
Vanadium	292.40	0.0051

Table 3. Haematological profiles of *Cavia porcellus* exposed to varying levels wastewater from Olorunda Mining Site, Sepeteri, Oyo state Nigeria

Blood Parameter	Control	Treatment Group Group A	Group B	Group C	Group D	Reference Value	P-value
PCV (%)	52.00±2.31	49.43±1.72	47.00±3.85	48.71±4.23	48.00±3.23	31 – 45	0.08[ns]
Hb (g/dl)	16.93±0.81	17.00±1.17	36.00±47.28	16.47±1.47	15.80±1.25	10 – 17.4	0.37[ns]
RBC (10^6)	8.66±0.20[a]	8.27±0.43[b]	7.45±0.88[b]	8.15±0.81[ab]	7.96±0.67[ab]	4 – 7	0.03
Platelets (10^4)	12.71±2.66	11.10±0.83	10.42±0.22	12.08±2.66	11.68±14.23	250 – 850	0.21[ns]
WBC (10^3)	5.71±1148[a]	4.92±1.30[ab]	3.88±0.49[b]	5.63±1.38[b]	4.49±0.51[b]	6 – 17	0.02
Neutrophils (%)	0.99±0.28	1.10±0.43	0.90±0.07	1.22±0.39	0.91±0.24	30 – 80	0.33[ns]
Lymphocyte (%)	4.56±0.97[a]	3.65±0.89[b]	2.86±0.43[b]	4.24±0.10[b]	3.40±0.38[b]	30 – 80	0.01
Monocytes (%)	81.71±33.87	96.14±60.30	51.33±19.14	97.86±38.95	95.33±30.86	1 – 12	0.23[ns]
Eosinophil (%)	106.55±58.39	77.29±49.80	66.83±54.46	91.29±79.69	83.67±41.42	0 – 5	0.79[ns]

Note: Means with the same superscript were not significantly different. PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cells; WBC = White Blood Cells; ns = not significant

Table 4. Serum biochemical profile of *Cavia porcellus* exposed to varying levels of wastewater from Olorunda Mining Site, Sepeteri, Oyo state Nigeria

Serum Biochemical Parameter	Control	Treatment Group Group A	Group B	Group C	Group D	Reference Value	p-value
BUN (mg/dl)	16.00±0.57	15.85±0.2	16.50±0.14	16.15±0.90	15.60±0.60	9.00 – 32.00	0.69[ns]
Creatinine (mg/dl)	2.00±0.70	2.00±0.65	2.00±0.65	2.00±0.60	2.00±0.70	0.60 – 2.20	0.05[ns]

BUN = Blood Urea Nitrogen, ***Reference values were adapted from Hrapkiewicz and Medina (2007) and Riggs (2009)

Table 5. Heavy metal concentrations in tissues of *Cavia porcellus* exposed to varying levels of heavy metal contaminated wastewater from Olorunda Mining Site, Sepeteri, Oyo state Nigeria

Animal Tissue	Heavy Metal	Treatment Group Control	Group A	Group B	Group C	Group D	P-value
Liver	Lead (µg/dl)	0.76±0.07[a]	0.95±0.07[a]	0.97±0.09[a]	1.02±0.05[a]	1.22±0.87[a]	0.49[ns]
	Cadmium (µg/dl)	0.05±0.00[a]	0.05±0.01[a]	0.05±.003[a]	0.05±0.01[a]	0.05±0.01[a]	0.98[ns]
Kidney	Lead (µg/dl)	0.83±0.14[a]	1.03±0.16[a]	0.87±0.94[a]	0.99±0.94[a]	1.26±0.19[b]	0.03
	Cadmium (µg/dl)	0.03±0.02[a]	0.66±0.02[b]	0.07±0.04[b]	0.07±0.01[b]	0.06±0.03[b]	0.02

Note: Means with the same superscripts were not significantly different, ns = not significant

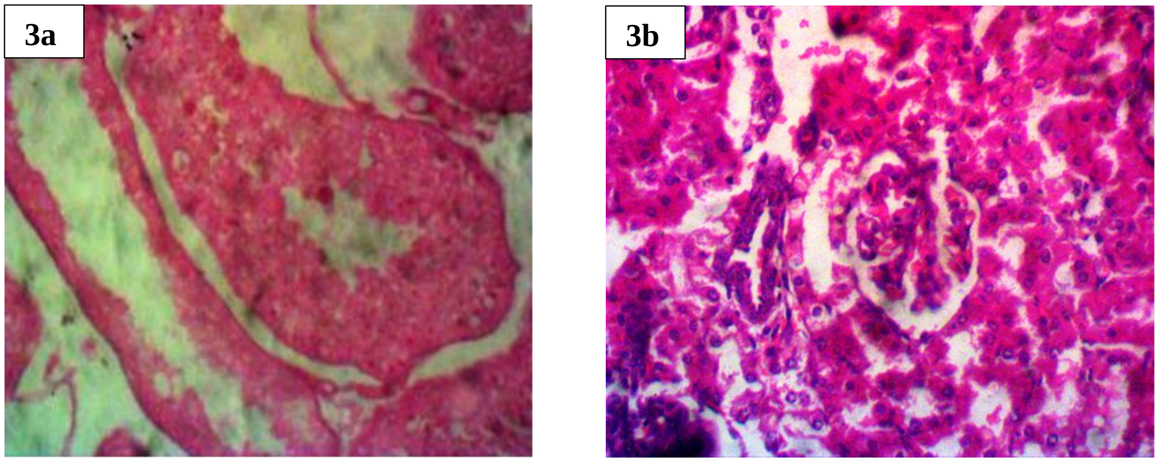

Figure 3a and b. Photomicrograph of tissues of *Cavia porcellus* in Group A (HE x100)
(a: Swelling of sertoli cells and spermatogenic arrest in the testis; b: Patchy tubular epithelial degeneration and necrosis in the kidney)

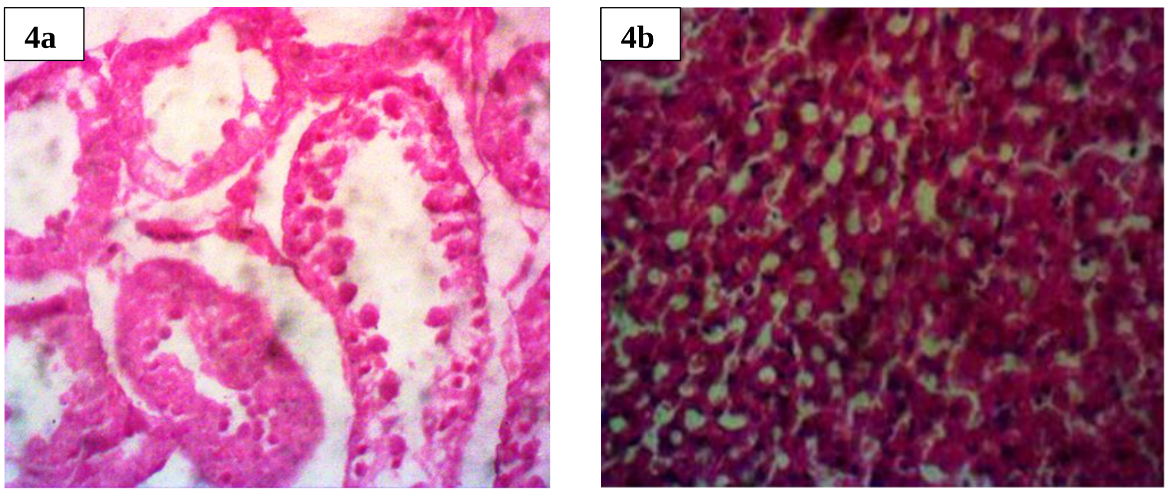

Figure 4a and b. Photomicrograph of tissues of *Cavia porcellus* in Group B (HE x100)
(a: Disruption of germinal epithelium and round spermatids in the testis; b: Hepato-cellular vacuolar degeneration and coagulation necrosis retention in the liver)

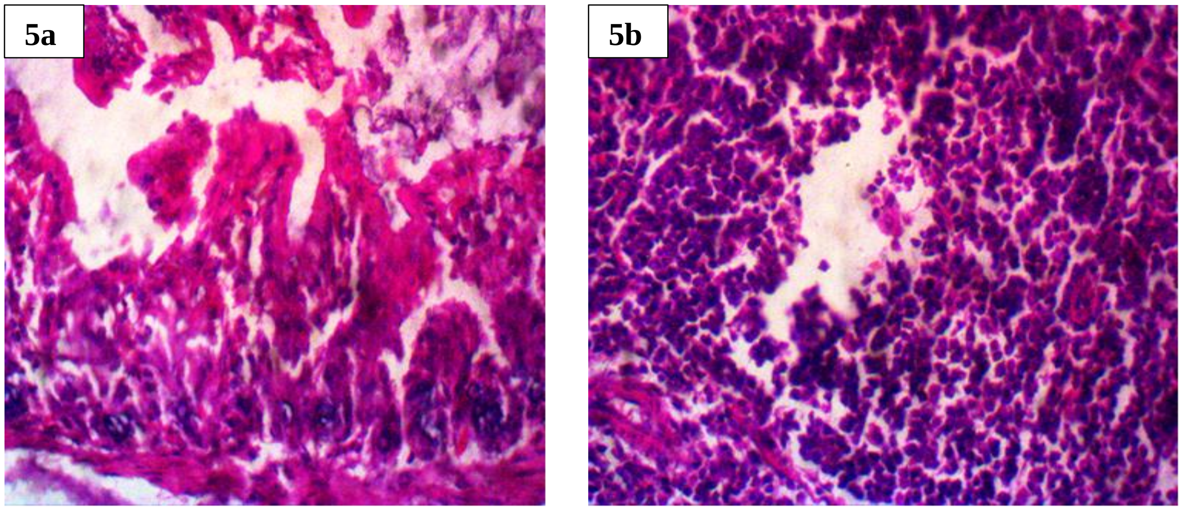

Figure 5a and b. Photomicrograph of tissues of *Cavia porcellus* in Group C (HE x100)
(a: Villi atrophy and denudation of villi tips in the intestine; b: Moderate hyperplasia of lymphoid follicular cells in the spleen)

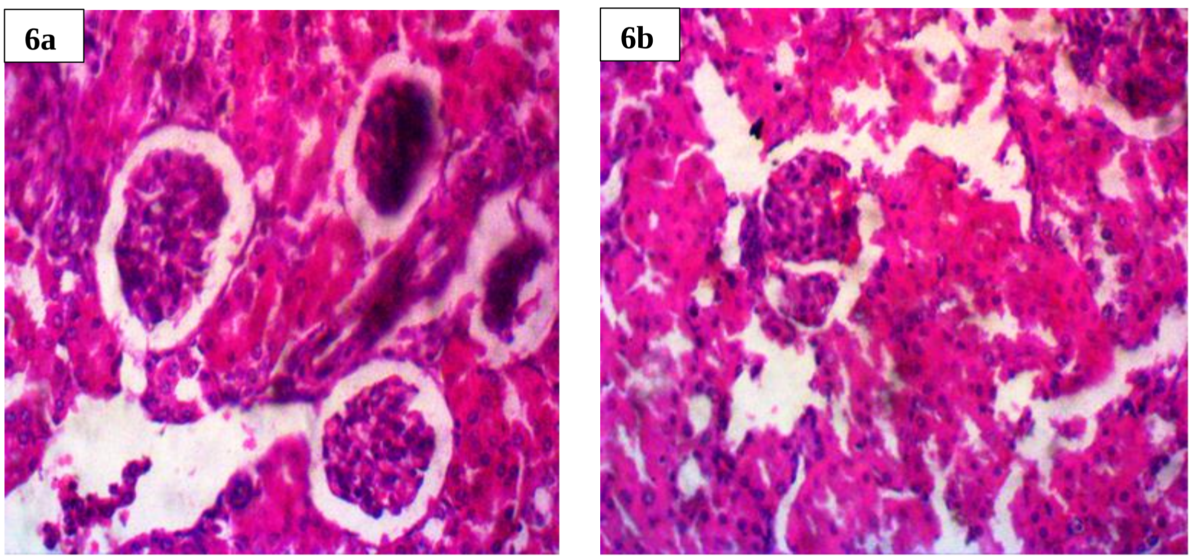

Figure 6a and b. Photomicrograph of tissues of *Cavia porcellus* in Group D (HE x100)
(a: No observable lesion in the liver; b: Patchy renal tubular epithelial degeneration, coagulation necrosis, eosinophilic casts and inflammation in the kidney)

DISCUSSION

Environmental contamination is one of the major challenges facing humanity in this contemporary age and has become a severe threat to species survival (Omonona *et al*., 2019). Mining activities are often major culprits in environmental contamination because of the toxic heavy metals produced during the operational stages (Ebenebe *et al*., 2017). These toxic heavy metals, especially those above the permissible limits, could lead to critical health implications on living organisms (Adetuga *et al*., 2020). In this study, the concentrations of heavy metals in the wastewater were above the WHO recommended permissible limits. This is a public health concern because the wastewater from Olorunda Mining Site flows into Ajaku River, which traverses the Old Oyo National Park. Hence, the water source (Ajaku River) for wild animals is contaminated and un-safe.

Haematological and serum biochemical studies are used to evaluate the stress or disease state prompted by contaminants and environmental changes (Alghamdi and El-Ghazaly, 2012; Effiom *et al*., 2021). As such, a change in the haemato-biochemical profile values designates specific physiological conditions in animals (Etim *et al*., 2014). This method is also utilized for pathological diagnosis and screening of disease conditions (Keohane *et al*., 2016). On the haematological profile of the *Cavia porcellus* exposed to mining wastewater, the RBC count and PCV values of the treatment groups were lower than that of control. This is an indication of slight anaemia probably caused by haemolysis or decrease in production of RBCs as a result of heavy metal toxicity. The toxic constituents in the mining wastewater might have caused metabolic disorder in the RBC, resulting in decreased haemoglobin carrying capacity (Lee *et al*., 1999). The alterations to the RBC and its indices could also be attributed to the toxic effect of wastewater on the bone marrow leading to the damage of its haemopoietic cells (Alabi, 2021). The decline in the PCV values may be due to the decrease in the number of circulating RBC and cell shrinkage. A decrease in the haemoglobin concentration in C and D is an indication of macrocytosis and hypochromia haematopoiesis in the liver. High concentrations of toxic metals have been reported to inhibit heme synthesis of red blood cells (Lodia and Kansala, 2012). Other clinico-pathologic parameters such as platelets, WBC, lymphocytes and eosinophils, also decreased, when compared with the control group. The reductions in the different parameters may be attributed to bone marrow suppression due to heavy metal contamination of the mining wastewater. A decrease in the WBC count is an indication of leukopenia which can possibly affect the immune system of the animals. The decrease in the WBC count may also be attributed to the decrease in eosinophil counts (Huang *et al*., 2022). The decreased neutrophil counts in Groups B and D may imply impairment in the immune system of the *Cavia porcellus* because of metal toxicity. So it is possible the bodies of the animals used immune cells faster than they produced or their bone marrows were not producing enough. Increase in mining wastewater caused an increase in the monocyte counts, which was a defense mechanism against the effects of heavy metal toxicity.

The serum creatinine and BUN have been used as indicators of renal dysfunction and biomarkers of renal injury (Robertson and Seguin, 2006; Alimba *et al*., 2012). The serum biochemical parameters of the different treatments showed no statistical significant differences, with BUN and creatinine being within the reference range (Hrapkiewicz and Medina, 2007). This indicated that at least 50-75% of renal function had not been lost (Finco, 1989). Nevertheless, the increase in BUN values in Groups B and C may be attributed to impairment of renal function by the heavy metal toxicity, especially Cadmium, which was above the environmental permissible limit

(WHO, 2011). The kidney has been noted as the primary target organ of Cadmium toxicity, particularly at chronic exposure (Hu, 2002). Creatinine is a more precise indicator of renal function than BUN, which is easily affected by non-renal factors like hydration and diet.

The Lead levels in the liver of treatment groups were higher than that of the control group while Cadmium concentrations did not differ. This indicated that Lead bioaccumulated in the liver of the Guinea pigs. The liver is the organ responsible for maintaining the body metabolic homeostasis and has been considered as the target organ for the toxic effect of Lead (Patra *et al*., 2001). Celechovska *et al*. (2008), Jarzynska and Falandysz (2011) as well as Dzugan *et al*. (2012) reported high bioaccumulation of these heavy metals in fallow deer, red deer, and wild pheasants, respectively. Nordberg *et al*. (2007) earlier asserted that more than 75% of the total burden of Cadmium was deposited in the kidney.

The histopathological architecture of tissues showed different changes, which could be used as indicators of heavy metal contamination in the *Cavia porcellus*. Histopathological investigations are thought to be a consistent and comprehensive bio-monitoring tool for analyzing animal health (Deore and Wagh, 2012). The Guinea pigs in Group A, had patchy tubular epithelial degeneration and necrosis in their kidney, while the testis exhibited the swelling of sertoli cells and spermatogenic arrest. Bibi *et al*. (2021) reported hepatocytes necrosis and degeneration in the liver, as observed in Group B. This observation was ascribed to the cumulative effects of metals and an increase in their concentrations in the liver.

The pathological alterations in the kidney of the animals may be connected to the high concentration of Lead in the treatment groups. Omonona *et al*. (2015) and Goma and Tohamy (2016) asserted that Lead is one of the major heavy metals known to be toxic to mammals, with renal lesions due to Lead toxicity being characteristic in the interstitial, glomerular and tubular damage. Also, the intensity of pathologic changes have been reported to be directly related to the quantitative accumulation of lead in various organs of animals (El-Hameed *et al*., 2008).

CONCLUSIONS

Heavy metal contamination is an after-effect of inappropriate mining practices with waste materials being sources of contamination. In this study, heavy metal concentrations in the wastewater from Olorunda Mining Site were above recommended permissible limits. The potential health implications on wildlife were evident in the haemato-biochemical alterations and histopathological changes that occurred in *Cavia porcellus*. As such, the Olorunda Mining site being close to the boundary of the Old Oyo National Park is of great conservation and ecological concern. There is need to re-channel the mining wastewater away from the Ajaku River, to ensure it does not become a source of contamination to the ecosystem.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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