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Testing for the Environmental Fate and Safety of E-Waste using Nitrobacter and Mice Model

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Abstract**—** *The present study aimed to test for the environmental fate and safety of e-waste using Nitrobacter sp. and mice model assays. The Nitrobacter sp. toxicity test was designed in four treatments and control set-ups namely 6.25, 12.5, 25, 50 % and control separately in both fresh and marine water for 24 h at 25 °C. The Wister albino mice were separated into three set-ups of 8 animals each (4 females and 4 males) in which 1 ml/kg bw of normal saline which serves as control set-up was orally administered to Group 1 while 500 mg/kg bw doses of Products A and B preparations were administered to Groups 2 and 3, respectively once daily for 14 days at 25 °C. The result revealed higher values of heavy metals in products A and B. Products A and B had the highest and lowest EC_{50} values of - 111.19 % and - 922.26 % in fresh and marine water, respectively. The oral administration of 500 mg/kg bw of products A and B resulted in non-significant (P > 0.05) body/tissue weights decrease and increase in the levels of AST, ALT, ALP, urea and creatinine in the biochemical profile of the exposed mice. There were more serious but non-cancerous histopathological injuries to the kidney tissue structures than liver tissue structures. The study demonstrated the possible high toxicity of unregulated disposal of these e-wastes into the environments and animals and therefore recommends proper treatment or recycling of these wastes before disposal.*

Keywords**— E-waste toxicity, mice model, Nitrobacter sp., pollution, public health monitoring.**

I. INTRODUCTION

The growth and usage of electronic devices such as laptop of diverse brands especially in Nigeria has over the last two decades widely increased. The very possible reason for this increase could be due to high population density. As a result, there has numerous reports of unused and spoilt parts of these of devices containing poisonous heavy metals, metalloids, polyaromatic hydrocarbons, polychlorinated biphenyls scattered in major electronic waste dumps across significant cities and hence constitutes wastes (Manhart *et al*. 2011; Alabi *et al.* 2012; Uba *et al*. 2020a). These wastes commonly termed as E-wastes if not properly treated or managed could poised serious danger to public and animal health.

Evaluation of these wastes to determine their safeties when release either to aquatic or terrestrial ecosystems is usually carried out using chemical assays. This assay has been found to be limited in that it cannot detect the minutest concentration of these wastes and mostly not rapid. Bioassays using biological monitors has been found to overcome these demerits and as a result, deployed to complete the chemical toxicity assay (Uba *et al.* 2020b).

Previous studies by Nrior and Owhonda (2017); Nrior and Gboto (2017) and Uba *et al*. (2020a) reported significant lethal and inhibitory effects of spent phone and laptop battery wastes on *Nitrobacter* sp., *Phaseolus vulgaris* (common bean), *Sorghum bicolor* (guinea corn), *Allium cepa*, *Eisenia fetida* and *Selenastrum capricornutum* when these wastes are discharged into aquatic and terrestrial ecosystems. Alabi and Bakare (2014) reported a significant concentration-dependent induction of micronucleated polychromatic erythrocytes, sperm abnormalities and

decrease in sperm count across the treatment groups of mice exposed orally with e-waste contaminated underground water five weeks. In a study carried out by Andjelkovic *et al*. (2019), they reported that the acute exposure of adult Wistar rats to Cd and/or Pb induced toxic effects in their blood, liver and kidney tissues.

There is paucity of information on toxicity effects of spent laptop batteries on Nitrobacter sp. and mice model. To the best of our knowledge, the literatures available as at the period of this study, focused their scope of studies on toxicity effects of spent phone batteries only on *Nitrobacter* sp. and therefore demand the study. The current study was therefore aimed to test for the environmental fate and safety of E-waste using *Nitrobacter* and mice model assays.

II. MATERIALS AND METHODS

2.1 Collection of sample, processing and site description

The studied sites were Nigerian fresh water: River Niger (latitude 4°22'50"N - latitude 7°65'56.5"N and longitude 7°11'6.77"E - longitude 7°11'16.2"E) Anambra State and marine water: Onne Light Flows Sea water (latitude 6°7'50"N - latitude 6°9'30"N and longitude 6°45'47"E - longitude 6°46'20"E) Rivers State, respectively. The water samples were collected and processed as described in our previous study (Uba *et al.* 2020c). The spent laptop battery wastes (Dell brand was designated as product A and Lenovo brand designated as product B due to ethical issue) were bought at Onitsha Market, Nigeria and transported to Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University, Nigeria. They were finally processed using by force rupturing of the battery lid and emptying of the contents into sterile plastic 1 L containers.

2.2 Specimen collection and adaptation

Healthy adult Wister albino mice (Equal sex, 23.2- 27.9 g, 16 – 17 weeks old) purchased from Green Stone Farm Okohio, Otolo Nnewi, Anambra State were placed, fed, cared and adapted in medium and spacious aluminum cages at 25 °C with 12 h light and 12 h darkness cycles for two weeks before carrying out the experiment according to the protocol of Organization for Economic Cooperation Development (OECD) (2009).

2.3 Heavy metal evaluation

The method of APHA (2012) and as previously described by Uba (2018) were employed for the determination of metal contents of products A and B using atomic absorption spectrophotometry.

2.4 Isolation of *Nitrobacter* sp.

The test organism *Nitrobacter* sp. was isolated from the fresh and marine water samples using Modified Winogradsky' Agar (MWA) as previously described by Odokuma and Nrior (2015). The organism was biochemically characterized and identified using Bergey's Manual for Determinative Bacteriology by Holt *et al.* (1994).

2.5 Toxicity evaluation

2.5.1 Toxicity evaluation using *Nitrobacter* sp.

The method of Uba *et al.* (2020c) was adopted in evaluating the toxicity of the laptop battery wastes on *Nitrobacter* sp. Pure culture of *Nitrobacter* sp. was prepared by inoculating a loopful into sterile Modified Winogradsky' Medium (MWM) until late log phase. After development of inoculum, 1 mL of the test organism was added to separate toxicant (Products A and B) concentrations (6.25, 12.5, 25, 50 and 0 %) in test tubes containing sterile fresh and marine water as separate diluents. The 0 % represents the control set-ups without the toxicant samples. After inoculation, zero-hour count plating was carried out on sterile Nutrient agar plates and incubated at 25 °C. Subsequently, 0.1 mL of each concentration of the toxicants was plated out and incubated after 4, 8, 12 and 24 h on sterile Nutrient agar plates for 48-72 h. The experiment was carried out in triplicates and the plates were later counted after emergence of colonies.

2.5.1 Toxicity evaluation using mice model

2.5.1.1 Acute toxicity testing

Acute toxicity testing of the laptop batteries wastes was carried out at oral dose administrations of 5, 50 and 500 mg/kg body weight (bw) for 24 h intervals in order to determine the safe dose in accordance with the guidelines of OECD (2009). Toxicity signs and mortality were monitored on each toxicant (Products A and B) set ups once daily for at least 14 days.

2.5.1.2 Sub-chronic toxicity testing

Repeated-dose of 500 mg/kg bw of the laptop batteries preparations was used to evaluate the sub-chronic toxicity testing as no mortality was recorded after the acute test. The Wister albino mice were separated into three set-ups of 8 animals each (4 females and 4 males). Then, 1 ml/kg bw of normal saline which serves as control set-up was orally administered to Group 1 while 500 mg/kg bw doses of Products A and B preparations were administered to Groups 2 and 3, respectively daily for 14 days. During the study, regular intake of water and food (TOP VITAL FEEDS, Kano State, Nigeria) were monitored. After the 14th day of the study, the mice were sacrificed after chloroform vapour sedation and whole blood was collected after dissection

through cardiac puncture and placed into labeled sample bottles.

2.5.1.3 Weight analysis

The body/tissue weight of mice in each set-up were determined at the beginning and end of the 14 days study.

2.5.1.4 Evaluation of biochemical indices

By adopting the method of Egurefa *et al*. (2020) and Uba *et al*. (2020b), the following biochemical profile were analyzed: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea and creatinine.

2.5.1.5 Histopathological assay

As described by Egurefa *et al.* (2020) and Uba *et al*. (2020b), the liver and kidney tissues were fixed on slides, stained and counter-stained with hematoxylin and eosin (H and E stains) and finally viewed under a compound microscope (CX 23 Olympus, Japan). All the abnormalities from the normal architectural tissue structures were observed and noted.

2.6 Statistical Study

Mean of the triplicate values were determined at different concentrations and expressed in Tables and Figures. The mean values were further analyzed to determine the effects of products A and B on the growth survival of the *Nitrobacter* sp. using linear regression. Analysis of variance was used to compare the effects of products A and B on the weight and biochemical indices of the exposed Wister Albino mice using GraphPad Prism Software version 8.0.2.

III. RESULTS

3.1 Heavy metal profile

Table 1 showed the heavy metal profile of the products A and B. Product B had the highest values of mercury 1.532 ppm, nickel 22.802 ppm, lead 6.405 ppm while product A had the highest value of arsenic 0.179 ppm and cadmium 0.019 ppm, respectively.

Table 1: Heavy metal profile of the products A and B

Parameter	Concentration (ppm)		USEPA 2009/NESRA 2009 standards in water (ppm)
	Product A	Product B	
Mercury (Hg)	0.284	0.407	0.002
Nickel (Ni)	13.874	21.898	NA
Arsenic (As)	0.119	0.111	0.010
Cadmium (Cd)	0.191	0.000	0.005
Lead (Pb)	2.691	2.028	0.015 – 0.050

Key: Ppm = Part Per Million; NESRA = National Environmental Standards and Regulation Enforcement Agency (2009) permissible limits for drinking water; USEPA = US Environmental Protection Agency (2009); NA = Not available.

3.2 Nitrobacter toxicity profile

Figs. 1 and 2 showed the inhibitory response of *Nitrobacter* sp. to different concentrations of the toxicants (Products A and B). From the Figs., there were decrease in log count as the concentration and exposure period increased with fresh water having the highest log count of 5.48 logCFU/mL while marine water had the lowest log count of 5.11 logCFU/mL after 24 h, respectively. Similarly, Fig. 3 showed the 24 h toxic response of *Nitrobacter* sp. to products A and B. Products A and B had the highest and lowest EC_{50} values of - 111.19 % and - 922.26 %, respectively.

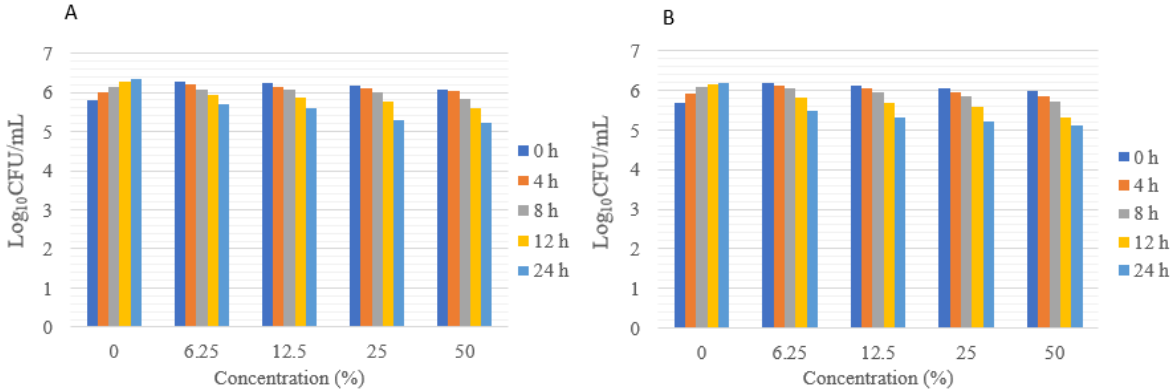


Fig. 1: Inhibitory response of Nitrobacter sp. to different concentrations of the Product A. A = Fresh water; B = Marine water; LogCFU/mL = Logarithmic colony forming unit per millilitre; h = Hour; % = Percent

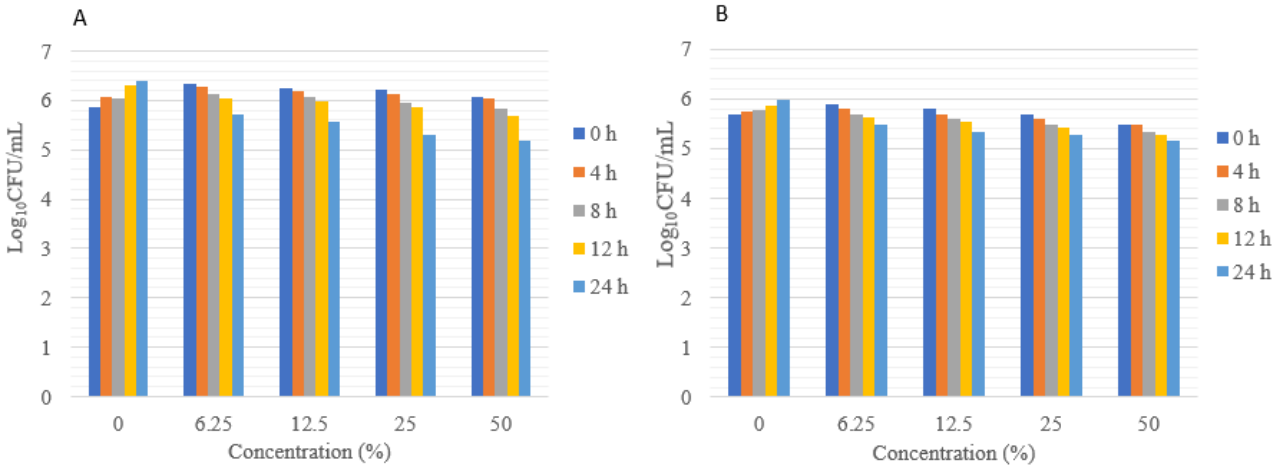


Fig. 2: Inhibitory response of Nitrobacter sp. to different concentrations of the Product B. A = Fresh water; B = Marine water; LogCFU/mL = Logarithmic colony forming unit per millilitre; h = Hour; % = Percent

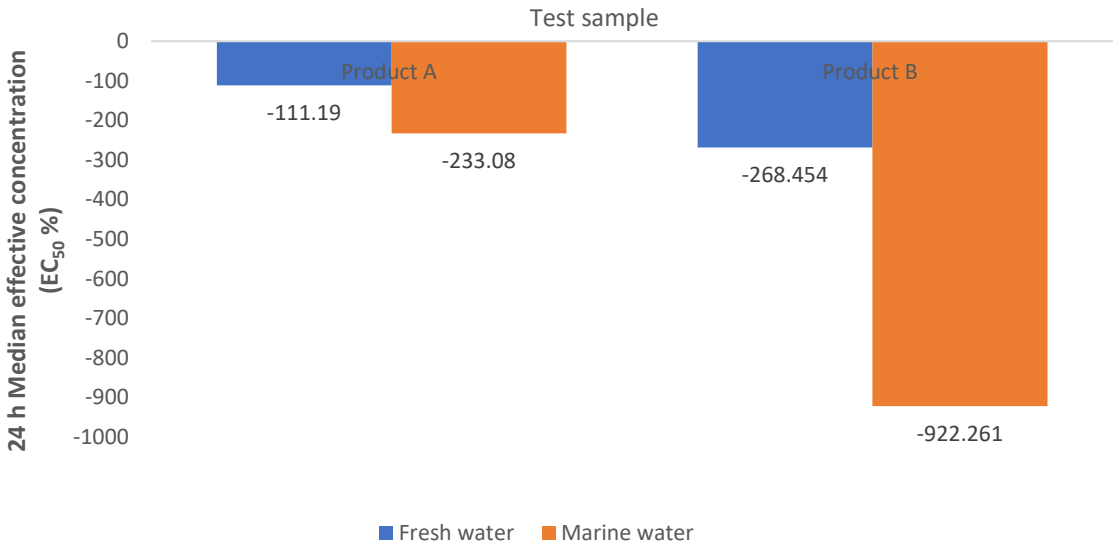
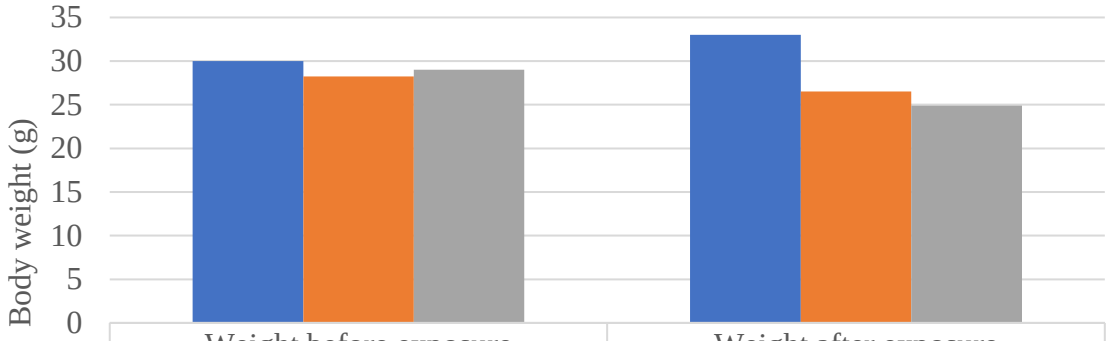


Fig. 3: 24 h toxic response of Nitrobacter sp. to products A and B. EC_{50} = 50 percentage median effective concentration; % = Percent; h = Hour

3.3 Mice toxicity profile

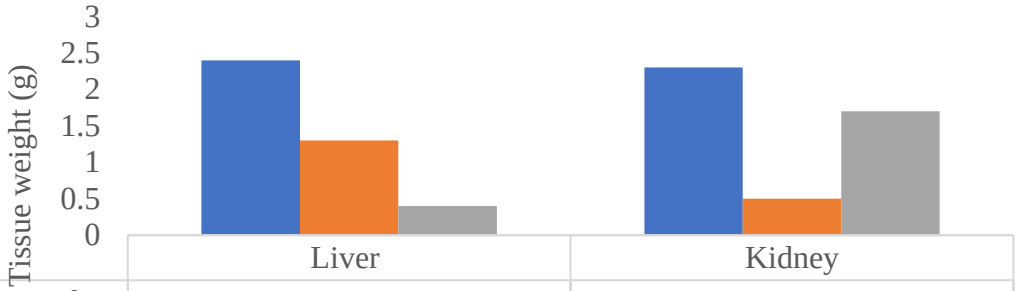
Figs. 4 and 5 displayed the body/tissue weights of the Wister albino mice before and after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. The control set-up had the highest body weight values of 30.00 g before exposure while product B set-up had the lowest body weight values of 24.90 g after the 14 days exposure (Fig. 4). Similarly, control set-up had the highest liver and kidney weights of 2.40 g and 2.30 g; while product B and A set-ups had the lowest liver and kidney weights of 0.40 g and 0.50 g after the 14 days exposure (Fig. 5). Fig. 6 showed the biochemical indices of the Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. Product A set-up had the highest values of AST (20.00 U/L), ALT (28.00 U/L), ALP (84.00 U/L), urea (15.60 U/L) and creatine (240.00 U/L); while control set-up had the lowest values of AST (10.00 U/L), ALT (15.00 U/L), ALP (44.00 U/L), urea (7.80 U/L) and creatine (72.00 U/L) after 14 days exposure. Figs. 7a – c and 8a – c showed the micrograph of haematoxylin and eosin stained liver and kidney tissues of Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. Morphological aberrations of the product A liver tissues showed presence of inflammatory cells; while product B liver showed focal inflammatory and congestion of the central vein (Fig. 6a – c). The product A kidney tissue showed inflammatory cells emasculating the tubule while

product B kidney tissue showed vascular congestion in the medulla (Fig. 7a – c). The control mice set-ups revealed normal architecture of the liver and kidney structures.



	Weight before exposure	Weight after exposure
Control	30	33
Product A	28.23	26.5
Product B	29	24.9

Fig. 4: Body weight of the Wister albino mice before and after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. g = Gram



	Liver	Kidney
Control	2.4	2.3
Product A	1.3	0.5
Product B	0.4	1.7

Fig. 5: Tissue weight of Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. g = Gram

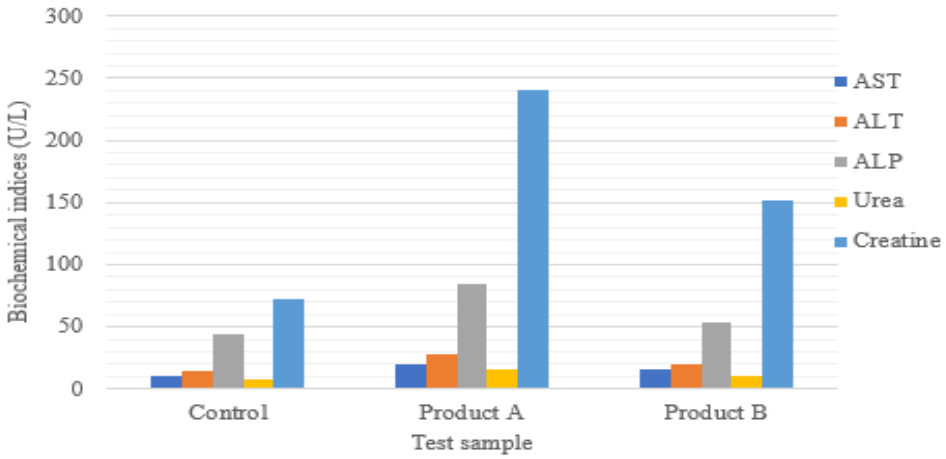


Fig. 6: Biochemical indices of the Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up
Key: AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase; BW = Body weight; mg/kg = milligram per kilogram; U/L = Unit per litre.

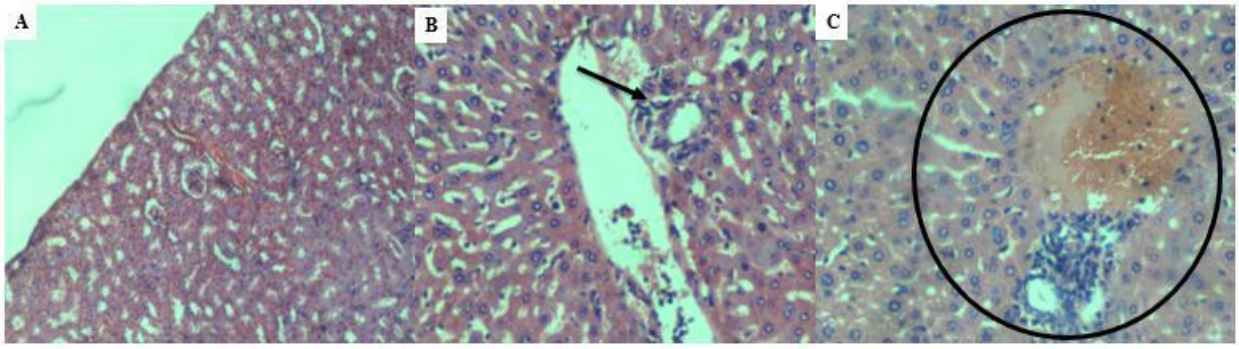


Fig. 7a – c: Micrograph of haematoxylin and eosin stained liver tissues of Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. A = Control; B = Product A; C = Product B.

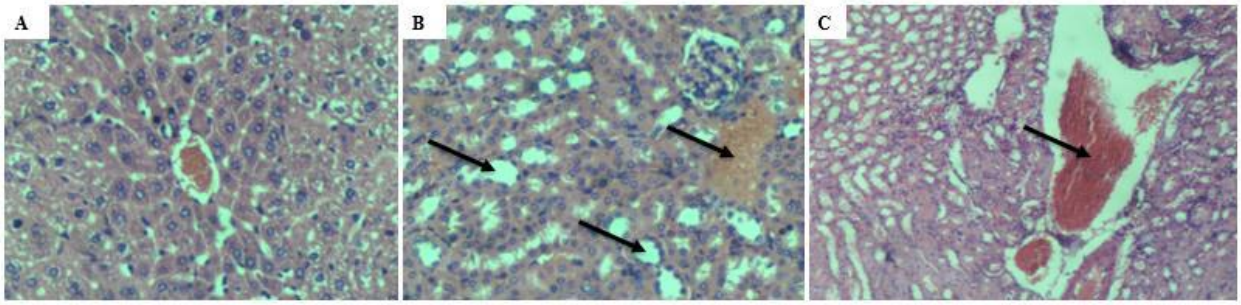


Fig. 8a – c: Micrograph of haematoxylin and eosin stained kidney tissues of Wister albino mice after 14 days exposure to 5000 mg/kg bw of products A and B preparations and control set-up. A = Control; B = Product A; C = Product B.

IV. DISCUSSION

The wide application of *Nitrobacter* sp. and mice model for assessing pollutant and chemical substances have been largely reported by numerous researchers (Odokuma and Nrior 2015; Nrior and Gboto 2017; Nrior and Owhonda 2017; Uba et al. 2020b; Alabi and Bakare, 2014; Andjelkovic *et al.* 2019). Earlier studies by Uba et al. 2020a and Alabi and Bakare, 2014 revealed higher levels of Ni, Pb and Cd heavy metals in their e-wastes preparations and leachates and similar findings were obtained in the heavy metal profile of products A and B e-wastes in this study.

In order to determine the fate of these wastes on the fresh and marine aquatic ecosystems, *Nitrobacter* sp. a key ecological test organism commonly found and isolated in both ecosystems was studied by exposing it to products A and B leachate preparations. The results in Figs. 1 and 2 revealed non - significant ($P > 0.05$) concentration-dependent reduction in the logarithm count of *Nitrobacter* sp. after 24 h exposure in all the set-ups except the control set-up with increase which could be as a result of the absence of e-waste preparations in them. Product B was found to be more toxic to *Nitrobacter* sp. than product A after 24 h exposure in both fresh and marine waters (Fig. 3) and the probable reason could be due to the higher profile of metals and metalloids present in product B than product A. Products A and B were classified as very acutely toxic according to Verma (2007) toxicity classification scheme because their EC_{50} values were < 1 %. Previous studies by Nrior and Gboto (2017); Nrior and Owhonda (2017) and Uba *et al.* (2020a) reported reduction in the percentage logarithmic survival of *Nitrobacter* sp. and bacteria in two aquatic ecosystems after exposure for 24 h and therefore upheld the findings in this study.

Furthermore, to widen our knowledge on the safety on these wastes on animals, mice model was studied by also exposing them to products A and B leachate preparations at 500 mg/kg bw. Figs. 4 and 5 demonstrated non-significant ($P > 0.05$) body/tissue weight reductions in the mice treated set ups in comparison to their controls. This revealed the

probable developmental health impacts of these wastes if disposed without treatment. There was non-significant ($P > 0.05$) elevated levels of ALT, AST, ALP, urea and creatine in the product A exposed groups than the product B exposed groups in comparison to the control unexposed groups (Fig. 6). These elevated features could suggest hepatic and renal functional impairment and injuries in both treated groups and the possible reasons could be linked to the presence of hazardous metal substances in the e-wastes. The results are similar to the findings of Yuan *et al*. (2014) and Cobbina *et al.* (2015) who reported increase in the hepatic and renal biomarkers in heavy metal exposed Wistar rats but contradict the findings of Zhu *et al*. (2014) and Andjelkovic *et al.* (2019) who reported decrease in these biomarkers. There were more serious injuries to the kidney structures than liver structures as depicted by the histopathological micrographs (Figs. 7a - c and 8a -c) which led to the secretions of more renal biomarkers than hepatic biomarkers. Andjelkovic *et al*. (2019) reported no serious disturbance on the liver structure exposed to single and combined heavy metal doses.

V. CONCLUSION

The study showed that product B was more toxic to *Nitrobacter* sp. than product A if discharged in the fresh and marine aquatic ecosystems. There was non-significant decrease in body/tissue weights of exposed Wistar mice. The hepatic and renal enzymes were found to be elevated with more serious but non-cancerous features in the kidney than the liver tissues. Hence, further study on reproductive, haematological and genetic fates of these wastes are recommended.

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