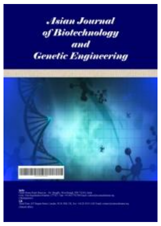

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Evaluation of the Ecotoxicity Potentials of E-Waste Using *Selenastrum capricornutum* (Microalga), *Eisenia fetida* (Earth Worm) and *Allium cepa* (Onion Bulb) as Bioindicators

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BOU, ELO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BGN, CPI managed the analyses of the study. Authors BGN, CPI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This research work was designed to evaluate the ecotoxicity potentials of e-waste using *Selenastrum capricornutum*, *Eisenia fetida* and *Allium cepa* as bioindicators.
Study Design: *Selenastrum capricornutum* and *Allium cepa* bulbs were exposed for 72 h at concentration ranging from 0 mg/L to 100 mg/L while *Eisenia fetida* were exposed for 48 h and 14 days at sub - lethal concentrations ranging from 0 mg/L to 8.54 mg/Lfor Hp laptop battery and 0 mg/L to 9.34 mg/L for Toshiba battery sample, respectively.
Place and Duration of Study: Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University (COOU), Uli Anambra State, Nigeria during May, 2019 - December, 2019.

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Methodology: A laboratory scale study was carried out on spent Hp and Toshiba laptop battery samples using microalgal toxicity test, earthworm mortality test and *Allium cepa* tests.
Results: The result showed that the spent Toshiba battery sample had the most hazardous toxic effect (ErC_{50} 19.58 mg/L; EC_{50} 34.54%; LC_{50} 3.60 mg/kg and 3.16 mg/kg) on the growth rate of *S. capricornutum,* root growth of *A. cepa* and *E. fetid a* survival after the treatment periods. Morphological abnormalities were also observed on the exposed roots of *A. cepa.* Inhibition (%), biomass change and mortality (%) of all species used were found to be concentration dependent with significant ($P < 0.05$) strong positive correlation at increasing concentrations.
Conclusion: Thus, the government should enforce strict regulations and heavy fines on industries, which do not practice E - waste prevention and recovery in the production facilities.

***Keywords**: Electronic-waste; mortality test; rhizo-toxicity; genotoxicity; ecological contamination.*

1. INTRODUCTION

Ecotoxicity is the branch of toxicology concerned with the study of lethal effects caused by natural or man -made contaminants to the inhabitants of the ecosystem which includes man, animals, microorganisms and plants. Ecotoxicity testing involves measuring the toxicity of polluted ecological samples, which may involve the practise of animal model such as *Eisenia fetida* (earthworm), plant model such as *Allium cepa* and microbial model such as microalgal *Selenastrum capricornutum* as bio - indicators in order to evaluate the toxicity of substances in the environment as these pollutants could be chemicals, electronic wastes (E – waste), pesticides, oil spills which when released into the environment result into harmful effects on humans, plants and micro-organisms [1] .

Electronic-waste is described as discarded electrical or electronic devices or used electronics which are considered for repair, reprocess, resale and salvage recycling through material recovery, or discarding. Informal processing of e-waste in developing countries like Nigeria can result to adverse human health impacts and ecological contamination[2].

Algae are amongst the species of microorganisms frequently used in biotest batteries for hazard assessment of chemically polluted wastes and leachates. As one of the key producers, changes in the morphology and efficiency of the algal community may bring about direct morphological changes in the entire ecosystem and/or indirectly disturb the ecosystem by altering water quality. It is then critical to evaluate the toxicity of chemicals to algae as the harzard is likely to end up in water bodies through industrial or domestic wastes 3]. *Selenastrum capricornutum* was selected in this study because it is a model organism recommended by ISO [4].

E. fetida **(**earthworm) are viewed as one of the key terrestrial animals for measuring the toxicity of chemicals in soils and have been adopted as model organism for ecotoxicological analysis. In numerous procedures regarding earthworm toxicity tests, *E. fetida* was selected because it is easy to cultivate in the laboratory and a wide-ranging database on the toxicities of all categories of chemicals are documented and described for this species. According to Lee et al. [5] the conventional methods of evaluating pollutants toxicity in earthworms are acute and chronic toxicity tests by measuring mortality and growth behavioural features as biomarkers. Furthermore, the earthworm avoidance behaviour test which records the capability of earthworms to select or evade a particular soil is under progress and standardization [5].

A. cepa test are mostly used for evaluating the quality of drinking water and water-causing contamination. Certainly, it is an effective method for chemical screening and on -site monitoring of the genotoxicity effect of environmental pollutants. The test is also generally used to study the toxic effects of e-wastes as all facts and literatures have shown that *A. cepa* test is more sensitive for detecting toxicity and cytotoxicity than the rest. This claim plays a significant part in bio-monitoring since roots of onions were sensitive for any toxic substance. Additionally, plant roots are very suitable in biological testing since root tips are the first to be in contact with the toxicants released in soil or water. Besides, the root tip chromosomal abnormality assays establish fast and sensitive approaches for bio-monitoring from the level of contamination and also assess the effects of toxic and mutagenic materials in the natural environment [6 – 9].

There abound literatures on the hazardous effects of e -wastes on the terrestrial and aquatic ecosystems in Nigeria using a battery of assays but to the best our knowledge, there is paucity of information regarding the toxicity of spent laptop batteries to microalgal species, earthworm species and onion bulbs. Consequent upon this, there is need to investigate the toxicities of these pollutants in order to fill the gap of knowledge and widen our horizon on the existing data and literatures on the above subject. Therefore, the objective of our study is to evaluate the ecotoxicity of e-waste using *S. ccapricornutum, E. fetida*and *A. cepa*as bioindicators.

2. MATERIALS AND METHODS

2.1 Source of Toxicant Sample

The spent laptop batteries (Hp and Toshiba) were bought from Emeka Offor Plaza, Main Market Onitsha, Anambra State, packed into clean and disinfected polyethylene bags and brought to Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University Uli.

2.2 Preparation of the Toxicant Sample

The spent Hp and Toshiba laptop batteries were aseptically forced open and contents stored separately into well labeled 1 L round bottomed plastic containers for further processing.

2.3 Measurement of Heavy Metals

The heavy metal contents of the spent Hp and Toshiba laptop batteries were determined by atomic absorption spectrometry using standard analytical methods of APHA [10].

2.4 Ecotoxicity Bioassay

2.4.1 Algal growth inhibition test

The algal growth inhibition test was carried out according to the methods of ISO [4] with little modifications. The growth medium was prepared by adding an appropriate volume of the nutrient stock solution to water and was inoculated with 1 mL of prepared broth culture of the microalga *S. capricornutum* containing an algal density of 5 x 10^4 cells/mL suspension. The microalga cells in 25 mL test tubes were exposed to different concentrations of the toxicant sample in a geometric series with a ratio not exceeding 2.0 as follows: 6.25 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L with 0 mg/L acting as negative control. A quality control test was carried out with the reference chemical potassium dichromate ($K_2Cr_2O_7$) having the same toxicant dilution series. Three replicate batches for each test sample concentration and six replicates control batches were prepared by adding the appropriate volume of inoculum to growth medium. The pH of a replicate batch at each test concentration and in one control replicate were measured at the beginning and end of experiment. The test vessels were covered with lids to avoid airborne contamination and to reduce water evaporation and then incubated at 25 ± 2℃ under aeration and continuous white fluorescent lamps for 72 h. The cell density in each test batch (including the controls) were measured at 0, 24, 48 and 72 h by mixing the test batches prior measurement. The percent growth inhibition and the effective concentration (E_rC_x) were calculated from the mean growth rate values.

2.4.2 Earthworm mortality test

2.4.2.1 Source

The *Eisenia fetida* (earth worm) was obtained from the moist and wet parts of Farmlands during rainy season within the premises of Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State. The specimens were brought to Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University, Uliin 1.5 L sterile plastic containers. The *Eisenia fetida* (earthworm) weighed between 0.96 - 1.25 g with well -developed clitella.

2.4.2.2 Acclimatization of the specimen

The earth worms were maintained in the natural soil (5% decomposed cattle manure and 35 % moisture) at 25 ± 2℃ for at least two weeks prior to use for the toxicity experiments. After two weeks, they were incubated for 24 h on moist filter paper contained in 9 cm Petri dishes at 25 ± 2℃ in the dark to empty their gut contents. In each toxicity test, a primary trial was conducted to find out the range concentrations that resulted in 0 - 100% mortality rate of each toxicant [11].

2.4.2.3 Acute investigational test

2.4.2.3.1 Assessment with contact filter paper assay

This test was performed according to OECD guideline [12]. Petri dishes with 9 cm in diameter were lined each with a piece of Whatman filter paper. After toxicant preparations, 2 mL solution of toxicant containing 2.14 mg/L, 4.0 mg/L, 8.54 mg/L and 2.34 mg/L, 4.60 mg/L, 9.34 mg/L concentrations of Hp and Toshiba laptop battery samples, respectively were added on the surface of the filter paper. Equal volume of distilled water was dispensed into the control treatments. One earthworm was placed per dish and each dish was covered with plastic lid containing small openings and incubated in the dark at 25 ± 2℃. The experiment was carried out in 10 replicates and the biomass change and mortality rate were noted after 24 h and 48 h of toxicant exposure.

2.4.2.3.2 Assessment with artificial soil assay

The Artificial soil test was carried out according to OECD guideline [12] with little modifications. In the artificial soil test, soil consist of 70% fine sand, 20% kaolin clay, 10% composted and 10% non - composted coco peat, 25 % cow dung with small amount of calcium carbonate ($CaCO_3$) to adjust the test soil pH to 6.0 ± 0.5,whereas sterile distilled water was added to adjust water content to 35%. Five hundred grams of the test soil described above were added into 1.5 L of plastic container and the desired amount of toxicant solutions as previously described were thoroughly mixed into the soil to allow a homogenous distribution of toxicant. Ten earthworms were placed into each of the 1.5 L plastic containers and were covered with aluminium foil containing small opening to allow aeration. Equal volume of distilled water was dispensed into the control treatments and the experiment was carried out in triplicates. The set ups were incubated at 25 ± 2℃ in a 12:12h light – dark photo period and the biomass change and mortality rate were noted after 7th and 14th days of toxicant exposure.

2.4.3 *Allium cepa* assay

2.4.3.1 Adaptation of specimen

Fresh and healthy equal sized *Allium cepa* (Onion bulbs;1.50 ± 2.00 cm in diameter; diploid 2n = 16) were commercially obtained from Chukwuemeka Odumegwu Ojukwu University, Uli Campus Market, in Ihiala Local Government Area, Anambra State. The experiments were conducted as described by Dada et al. [13] and Babatunde et al. [14] with minor modifications. The onion bulbs were sun - dried for one week before start of experiment. Bulbs were easily stored at variations of room temperature (25 ± 2°C).

2.4.3.2 Experimentalprocedure

The outer scales of the bulbs were carefully removed and the brownish bottom plates scraped away using dissecting knife. After proper washing and rinsing of the bulbs with detergent and running tap water, ten bulbs were randomly selected and plantedin 100 mL plastic beakers containing 6.25%, 12.5%, 25%, 50% and 100% concentrations of the spent Hp and Toshiba battery samples and grown at room temperature in the dark to avoid effect from light. Tap water was used as negative controls. Test solutions were changed every day and left for 72 h. Five of the exposed healthy onion bulbs were randomly picked and the roots measured while two were also picked from each concentration and their root tips cut, fixed in 3:1 v/v glacial acetic acid solution and stored at 4ºC for cytological analysis. The fixed roots were later viewed under the light microscope (Stereo OF0533 microscope, China) after root tips were squashed, stained with acetocarmine for 10 minutes and the cover slips sealed with clear fingernail polish against the slides. The values obtained were used to determine the root and mitotic changes as well as EC_{50} values of the respective spent Hp and Toshiba battery samples in comparison to the control set up [13, 14].

2.5 Bio statistical Data Management

The data obtained in this study were subjected to one factor analysis of variance with Dunnet multiple comparison test and regressional relationship among the samples, controls and concentrations at 95% confidence intervals such that values lower than 0.05 probability level are considered statistically significant ($P < 0.05$) using GraphPad Prism Version 8.0.

3. RESULTS

3.1 Heavy Metal Content

The result of the heavy metal content of the spent laptop batteries is presented in Table 1. From the result, Toshiba battery has the highest values of lead (6.405 ppm), nickel (22.802 ppm) and mercury (1.532 ppm) while Hp laptop battery had the highest values of cadmium (0.019 ppm) and arsenic (0.179 ppm), respectively.

3.2 Microalgal Inhibition Assessment

The result of the percentage growth inhibition of the microalgal *Selenastrum capricornutum* (microalga) in response to the presence of toxicant sample is presented in Fig. 1. The result showed that 100 mg/L concentration of spent

Toshiba laptop battery sample had the highest percentage (%) inhibition of 88.09% while $K_2Cr_2O_7$ sample had the lowest percentage (%) inhibition of 68.81%. In Fig. 2, the result of the 72h effective concentration (ErC) of spent laptop batteries and potassium dichromate on *Selenastrum capricornutum* presented shows that the positive control $K_2Cr_2O_7$ had the lowest ErC_{10} (-86.68 mg/L) and the highest ErC_{90} (130.71 mg/L) while the spent Hp battery sample had the highest ErC_{10} (-34.19 mg/L) and the lowest ErC_{90} (55.3 mg/L), respectively. There were no statistically significant differences detected (P > 0.05) among the samples and control with R^2 value of 0.0043.

3.3 *Eisenia fetida* Growth and Mortality Assessment

The result of the effect of spent Hp and Toshiba laptop battery sample on *Eisenia fetida* (earthworm) biomass and percentage mortality using contact plate and artificial soil tests are shown in Figs. 3 and 4. From the contact filter paper test result, 8.54 mg/L HP dose concentration and control had the highest and lowest biomass changes of 61.00% and 3.00% at 48 and 24 h, while 9.34 mg/L and 8.54 mg/L of Toshiba and HP dose concentrations as well as control had the highest and lowest mortality rate of 100.00% and 0.00% at 7th and 14th days (Fig. 3), respectively. Also, from the artificial soil test result, 9.34 mg/L and 2.35 mg/L of Toshiba dose concentrations had the highest and lowest biomass changes of 101.00% and 6.00% at 48 and 24 h, while 9.34 mg/L of Toshiba dose concentration and control had the highest and lowest mortality rate of 86.67% and 10.00% at 7th and 14th days (Fig. 4), respectively. Significant differences (P < 0.05) were detected among the treatment group and controls on the biomass change and percentage mortality in both contact plate and artificial soil tests. The results of the 48 h and 14 day lethal concentration (mg/L) of the spent Hp and Toshiba laptop battery sample on *Eisenia fetida* are presented in Tables 2 and 3. From the results, the Toshiba laptop battery sample had the highest LC_{10} (1.53 (0.06 – 2.55) mg/L), LC_{50} (3.60 (3.31 - 3.88) mg/L) and LC_{90} (19.83 (19.50 - 20.06) mg/L) while spent Hp battery samples had the lowest LC_{10} (0.39 (0.19 - 0.59) mg/L), LC_{50} (2.25 (2.04 - 2.45) mg/L) and and LC_{90} (9.83 (9.05 - 10 .06) mg/L)at48 hin the contact plate test. Also, the Toshiba laptop battery sample had the highest LC_{10} (1.54 (1.16 - 1.91) mg/L), LC_{50} (3.16 (2.78 - 3.53) mg/L) andLC_{90} (18.48 (17.42 - 19.52) mg/L)while spent Hp battery samples had the lowest LC_{10} (0.39 (0.19 - 0.59) mg/L), LC_{50} (2.25 (2.04 - 2.45) mg/L) and LC_{90} (11.22 (10.93 - 11.50) mg/L)at 14 days, in the artificial soil test.

Table 1. Heavy metal content of the spent laptop batteries sample

Parameters	Metal concentration (ppm) Hp	Toshiba	WHO (1983)/FEPA (1991) standards in sediment (ppm)	WHO (1983)/FEPA(1991) standards in water (ppm)
Arsenic	0.179	0.167	-	0.100
Cadmium	0.019	0.000	0.030	0.005 – 0.010
Mercury	0.357	1.532	-	0.050
Lead	4.171	6.405	0.010	0.050
Nickel	11.491	22.802	0.020	0.100 – 0.200

Legend: WHO = World Health Organization; FEPA = Federal Environmental Protection Agency; ppm = Part Per Million

Table 2. 48 h lethal concentration (mg/L) of the spent Hp and Toshiba laptop battery sample on *Eisenia fetida*

Time (h)	Sample	LC_{10} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)
24	Hp	0.39 (0.19 - 0.59)	2.25 (2.04 - 2.45)	9.83 (9.05 - 10 .06)
48	Hp	1.44 (1.32 - 1.55)	5.60 (4.90 - 5.70)	11.50 (11.22 - 10.93)
24	Toshiba	1.38 (0.09 - 1.66)	3.52 (2.29 - 4.65)	14.12 (13.91 - 14.32)
48	Toshiba	1.53 (0.06 – 2.55)	3.60 (3.31 - 3.88)	19.83 (19.50 - 20.06)

Legend: h = hour; LC = Lethal concentration; mg/L = Milligram per litre; % = Percentage; CI = Confidence interval

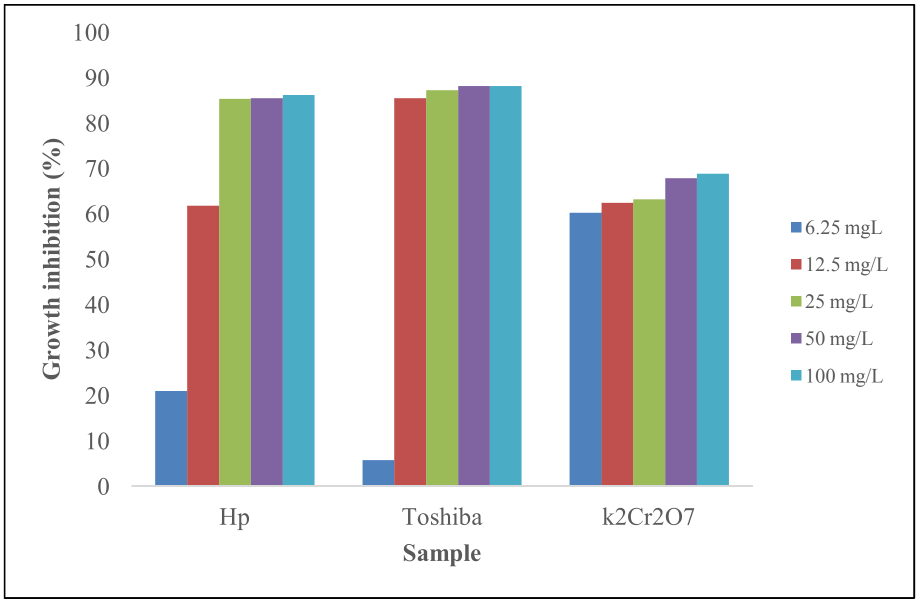

Fig. 1. Percentage growth inhibition of the microalgal *Selenastrum capricornutum* (microalga) in response to the presence of toxicant sample

Legend: $K_2Cr_2O_7$ = Positive control potassium dichromate; mg/L = milligram per litre; % = Percent

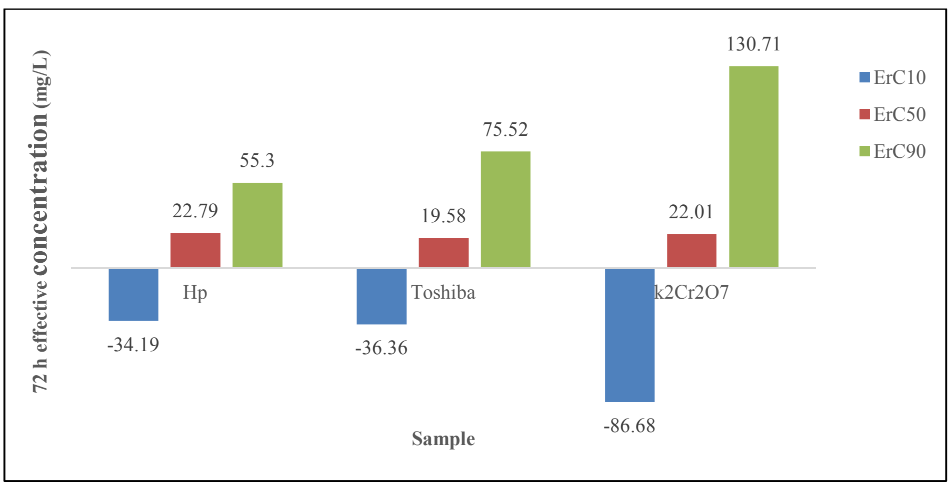

Fig. 2. 72-h effective concentration (ErC) of spent laptop batteries and potassium dichromate on *Selenastrum capricornutum*

Legend: $K_2Cr_2O_7$ = Positive control potassium dichromate; ErC = Effective growth rate concentration

Table 3.14 day lethal concentration (mg/L) of the spent Hp and Toshiba laptop battery sample on *Eisenia fetida*

Time (h)	Sample	LC_{10} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)
7	Hp	0.39 (0.19 - 0.59)	2.25 (2.04 - 2.45)	11.22(10.93 - 11.50)
14	Hp	1.38 (1.09 - 1.66)	3.60 (3.31 - 3.88)	13.91 (12.40 - 14.32)
7	Toshiba	0.40 (0.24 - 0.55)	2.08 (1.92 - 2.23)	11.23 (11.04 - 11.35)
14	Toshiba	1.54 (1.16 - 1.91)	3.16 (2.78 - 3.53)	18.48 (17.42 - 19.52)

Legend: LC = Lethal concentration; mg/L = milligram per litre; % = Percentage; CI = Confidence interval

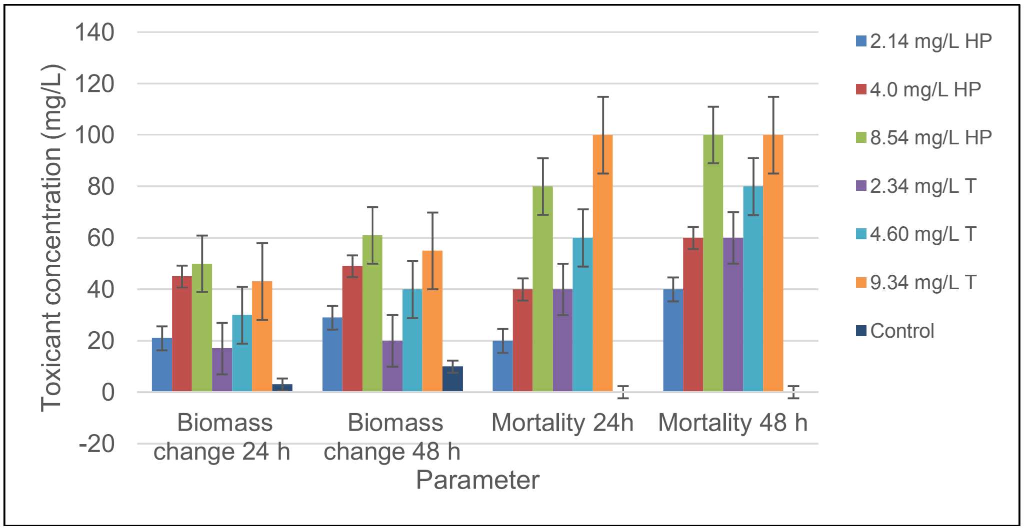

Fig. 3. Effect of spent Hp and Toshiba laptop battery sample on *Eisenia fetida* (Earthworm) biomass and percentage mortality using contact plate test

Legend: T = Toshiba battery; mg/L = milligram per litre; Error bar = Standard error in mean; h = hour

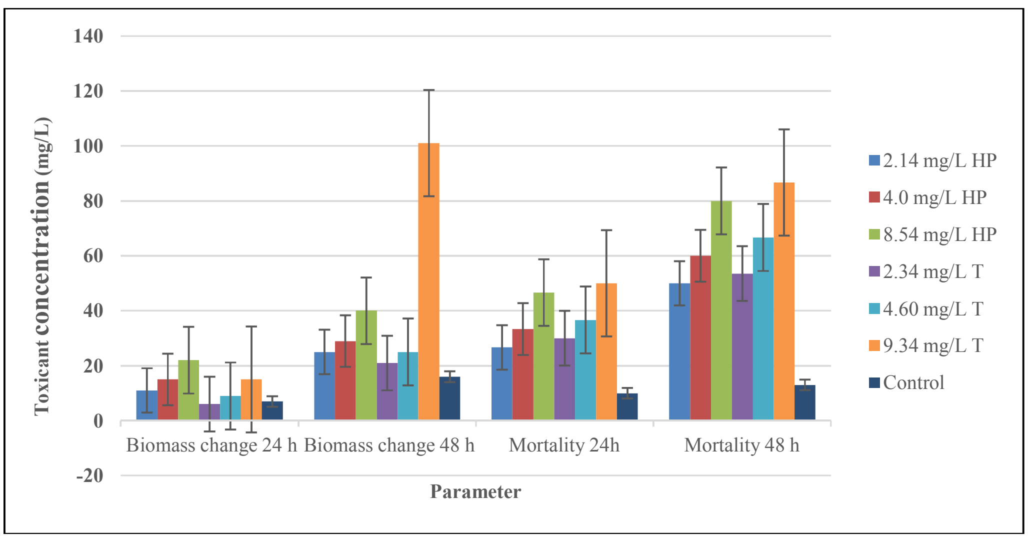

Fig. 4. Effect of spent Hp and Toshiba laptop battery sample on *Eiseniafetida* (Earthworm) biomass and percentage mortality using artificial soil test

Legend: T = Toshiba battery; mg/L = milligram per litre; Error bar = Standard error in mean; h = hour

3.4 Onion Bulb Inhibition Assessment

The result of the root changes of the *Allium cepa* root exposed to different concentrations of spent laptop test sample is illustrated in Fig. 5. From the result, the root length increases as the concentration decreases with the highest concentration (100%) having the lowest and highest percentage root length and root inhibition of 36.90% and 38.10% as well as 61.90% and 63.10% for Hp and Toshiba battery samples, respectively. The result of mitotic changes of the *Alliumcepa* root exposed to different concentrations of spent laptop test sample is illustrated in Fig. 6. From the result, the mitotic index (%) and mitotic inhibition (%) were found to be lowest and highest with 3.84% and 85.72% in Toshiba and HP samples, respectively. Also, it was observed that increase in concentration of the effluents led to decrease in number of dividing cells. The result of the 72 h effective concentration of spent laptop test samples on root growth inhibition of the *Allium cepa* is also illustrated in Fig. 7. The result revealed that the Hp battery sample had the highest EC_{10} (-11.90%) and lowest EC_{90} (153.69%), while the Toshiba laptop battery sample had the lowest EC_{10} (-104.74%) and highest EC_{90} (173.82%), respectively.The mitotic index (%) and mitotic inhibition (%) were found to have non – significant ($P > 0.05$) concentration - dependent effects with R^2 value of 0.2499 and 0.4130 while the root inhibition (%) were found to have significant ($P < 0.05$) concentration dependent effects with R^2 values of 0.8101 on Hp and Toshiba spent battery samples, respectively.

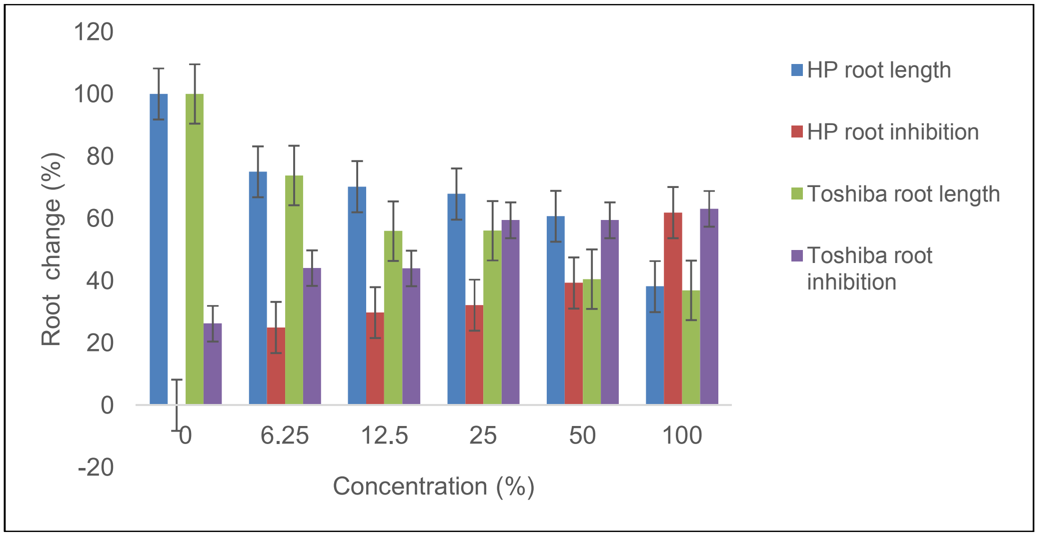

Fig. 5. Root changes of the *Allium cepa* root exposed to different concentrations of spent laptop test sample
Legend: Error bar = Standard error in mean; % = Percentage

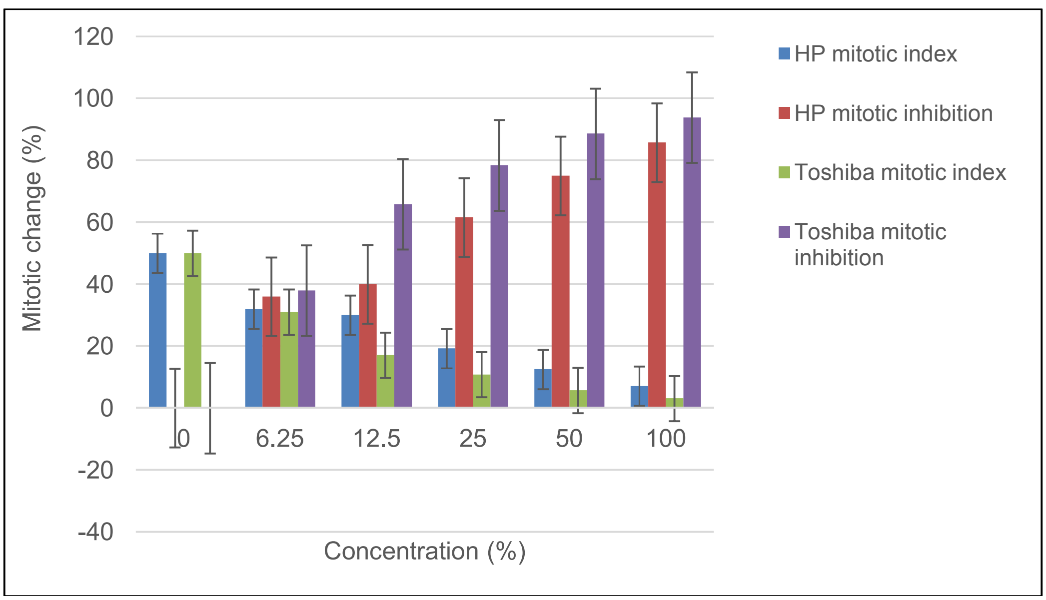

Fig. 6. Mitotic changes of the *Allium cepa* root exposed to different concentrations of spent laptop test sample
Legend: Error bar = Standard error in mean; % = Percentage

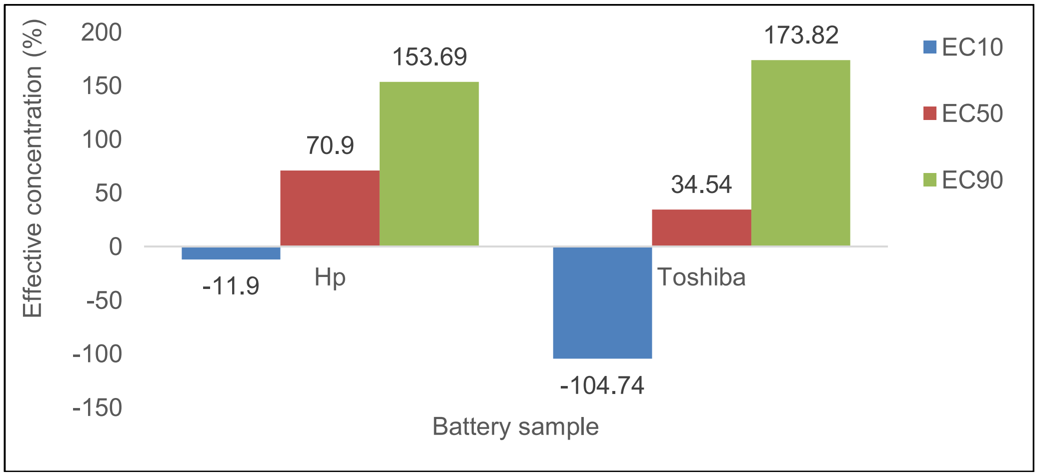

Fig. 7. 72 h effective concentration of spent laptop test samples on root growth inhibition of the *Allium cepa*

Legend: EC = Effective concentration; % = Percentage

4. DISCUSSION

The growing demand and consumption of laptop electronic devices in Nigeria has led to ever increasing generation of large volumes of their wastes commonly called E - waste. If proper treatment measures and recycle practices are not put in place, this could lead to dangerous outcomes on the living populations of microorganisms, macro-organisms, plants and animals. Hence, the need to screen these E-wastes inorder to classify and predict their probable effects on the community structures within the aquatic and terrestrial ecosystems. In this study, the result in Table 1 showed that all the analysed heavy metals from the toxicant samples were above the WHO [15] and FEPA [16] guidelines. Similar trends were observed by Douglas et al. [17] who published higher values of some of these metals in the phone batteries sample as they contain relatively high concentrations of hazardous metals in their electrodes [18].

The results in Figs. 1 and 2 demonstrated that there was remarkable decline in the algal cell density which increased as the time of exposure increased (24 - 72 h). This decline in the algal cell density as a result of the contact with the toxicant samples further revealed that the rate of metabolism was negatively affected making the cells of *S. capricornutum* incapable of withstanding the dynamic nature of the spent laptop batteries and potassium dichromate. The reason for this metabolic decrease could be as a result of high concentrations of heavy metals in the analyzed samples. The average growth rate (3.61), coefficient of variation (4.7%) and pH (1.1) of our control replicates met the validity criteria of ISO [4] which states that the average growth rate, variation coefficient (CV) and pH in the control replicates shall be at least 1.4 d^{-1}, not more than 5% and 1.5 relative to the pH of the growth medium. This growth rate corresponds to an increase in cell density by a factor 67 in 72 h. Furthermore, the spent Toshiba battery sample has demonstrated to be the most hazardous toxicant samples (ErC_{50} 19.58 mg/L) followed by $K_2Cr_2O_7$ (22.01 mg/L) and spent Hp laptop battery sample (22.79 mg/L) on the growth rate of the *S. capricornutum* and is in line with GESAMP [19] and CSP [20] toxicity classification system which states that test samples are harmful/hazardous to aquatic environment if LC_{50}/EC_{50} values ranges from 10 mg/L - 100 mg/L. Comparatively, our spent battery samples were comparable to the positive control sample $K_2Cr_2O_7$ in terms of toxicity levels on *S. capricornutum*. Similar trends were observed by Lukavsky et al. [21] who reported that cadmium was the most toxic metal to all tested algae as the toxicity hierarchy for all organism indicates: Cd > Co > Cr > Cu > Ni > Zn > Al > Fe while the sensitivity hierarchy indicates: *S. capricornutum>Scenedesmus subspicatus> Chlorella > Scenedesmus quadricauda.*

The results in Figs. 3 and 4 demonstrated that there was general increasein *E. fetida* biomass change and percentage mortality as the concentration of the toxicant samples and period of exposure advanced. The reason for this decrease could be attributed to the high levels of metalloids in the spent Hp and Toshiba laptop battery samples. There was more toxic response in the contact plate test than in the artificial soil test and the reason could be due to the fact that some concentrations of the toxicant samples could be leached and adsorbed into the soil particles thereby exposing the *E. fetida*to little concentration of the samples unlike the full dose exposure in the contact plate. Similar observation was discovered by Wang et al. [11] who reported that low toxicity was observed in artificial soil test than contact plate test during a study on the comparative acute toxicity of twenty - four insecticides to earthworm (*E. fetida*). The result in Tables 2 and 3 revealed that both spent laptop battery samples are toxic and is in line with GESAMP [19] toxicity classification system which states that test samples are toxic to the environment if LC_{50} values ranges from 1 mg/L - 10 mg/L. The Toshiba laptop battery sample (3.60 (3.31 - 3.88) mg/L) and (3.16 (2.78 - 3.53) mg/L) was found to be more toxic than the Hp laptop battery sample (5.60 (4.90 - 5.70) mg/L) and (3.16 (2.78 - 3.53) mg/L) after 48 h and 14th day treatments because the lower the LC_{50} of a substance, the higher the toxicity of that substance. Previous study by Bradham et al. [22] corroborates with the result of this study in which they reported the bioavailability and toxicity of lead to earthworm in soil as our spent laptop test samples contained a high dose of lead in them.

The result in Fig. 5 showed that the spent laptop battery sample were rhizo-toxic to the *A. cepa* root with continuous decrease in the average length, increase in root inhibition and morphological abnormalities such as short, curled and scanty root tips as the concentration of the samples increased with time. Similarly, the result in Fig. 6 suggested the possibility of occurrence of mito - depressive and cytological effect as the mitotic index decreased andmitotic inhibition increased in both samples. The reason for these occurrences on the root tips of *A. cepa* root could be due to the presence of inorganic metalloids that characterized both spent laptop batteries. The mitotic index is regarded as reliable guide in detecting the existence of cytotoxic chemicals in the environment [23]. Glińska et al. [24] suggested that the decreased mitotic index in *A. cepa* roots treated with metals is probably due to either disturbances in the cell cycle or chromatin dysfunction induced by metal–DNA interactions. Previous study by Wijeyatne and Wadasinghe [25] reported that the mitotic index of < 22% is considered to be toxic to *A.cepa* and that the significant decreases observed in root length and mitotic activity were regarded as indicators of rhizotoxicity, being a common phenomenon produced by most chemicals. In the present study, the mitotic index ranges of 50.00 -7.14% and 50.00 – 3.08% were obtained for spent HP and Toshiba laptop battery samples, respectively. Thus, we could infer that the spent laptop battery samples could be lethal to living organisms in aquatic and terrestrial ecosystems.

5. CONCLUSION

The study revealed that the spent laptop test samples contained high concentrations of heavy metals which were above WHO and FEPA limit standards. The spent samples had hazardous effect on *S. capricornutum* comparable to the positive control sample $K_2Cr_2O_7$ in terms of toxicity levels after 72 h exposure. The Toshiba laptop battery sample was found to be more toxic to *E. fetida* and *A. cepa* root than the Hp laptop battery sample after 48 h,14th day and 72 h of treatments. Thus, there is an urgent need to plan preventive strategy in relation to health hazard of e-waste handling among the general public and Federal Government of Nigeria should encourage the formation of centralized facilities for the treatment of hazardous e- wastesin order to minimize their environmental hazards.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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