*Asian Journal of Biochemistry, Genetics and Molecular Biology*

*5(1): 17-27, 2020; Article no.AJBGMB.57693*
*ISSN: 2582-3698*

# Experimental Assessment of the Toxicity Effects of Phone Battery Wastes on Aquatic and Terrestrial Bioindicators

**Bright Obidinma Uba[1*], Chisom Judith Okonkwo[1] and Odera Richard Umeh[2]**

*[1]Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, P.M.B. 02, Uli, Anambra State, Nigeria.*
*[2]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B. 5025 Awka, Anambra State, Nigeria.*

***Authors' contributions***

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

***Article Information***

DOI: 10.9734/AJBGMB/2020/v5i130117
Editor(s):
(1) Dr. Ahmed Medhat Mohamed Al-Naggar, Cairo University, Egypt.
Reviewers:
(1) Swarnali Nath Choudhury, The ICFAI University Tripura, India.
(2) Endah Rita Sulistya Dewi, Universitas PGRI Semarang, Indonesia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57693

***Original Research Article***

***Received 02 April 2020***
***Accepted 08 June 2020***
***Published 22 August 2020***

## ABSTRACT

**Aims:** The present study aimed at assessing the toxicity effects of phone battery wastes on aquatic and terrestrial bioindicators.
**Study Design:** Five treatments and the controls designs designated as 6.25%, 12.5%, 25%, 50%, 100%, CTRL and 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, CTRL were set up in triplicates and incubated for 24 h, 72 h and 20 days at 25 ± 2°C.
**Place and Duration of Study:** Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Nigeria during May, 2019 - August, 2019.
**Methodology:** The growth inhibition and mortality were analyzed using *Aspergillus terreus* toxicity test, *Allium cepa* toxicity test and *Limicolaria flammea* toxicity test.
**Results:** The results revealed that marine water (7.12 logCFU/mL) was shown to had more fungal

**Corresponding author: E-mail: ubabright4real@yahoo.com;*

count than the fresh water (7.07 logCFU/mL) ecosystem. On *A. terreus*, Itel in fresh water sample had the highest $LC_{50}$ values of 30.49% while Gionee in fresh water sample had the lowest $LC_{50}$ values of 21.74% after 24 h, respectively. The Itel battery sample had higher $EC_{50}$ value (86.08%) than Gionee battery sample with $EC_{50}$ value of 65.46% after 72 h on *A. cepa*. On *L. flammea*, Itel phone battery sample had lower (5.11%) $LC_{50}$ value than Gionee phone battery sample with higher (6.20%) $LC_{50}$ value at 6 mg/kg concentration after 20 days' exposure, respectively.
**Conclusion:** The results indicate that indiscriminate release of E- wastes into the aquatic and terrestrial environments should be discouraged.

*Keywords: Environmental health; cytogenototoxic; Itel; Gionee; Aspergillus terreus; Allium cepa; Limicolaria flammea.*

## 1. INTRODUCTION

Mobile phones have turn out to be a crucial accessory in the daily activities of numerous Nigerians. These devices permeate virtually all open shops and electronic shopping mall across key cities and municipal areas around the country. As a result of its growing telecommunications business and ever-increasing mobile phones ownership, the country has experienced the influx of several brands of mobile phone from different manufacturers in Europe and Asia. It has also been estimated that there are more than 110 million subscribers in the country making Nigeria to be the largest mobile market in Africa [1].

Several investigators have postulated that the remarkable development in material technology, mobile telecom approval and relatively short lifecycles of mobile phones have led to the production of bulk amounts of electronic waste (e - waste) in the country [2]. Annually, it has reported that electronic users generate about 20 to 50 million metric tonnes of e - wastes globally [1]. Studies in Nigeria have revealed that twice a year, an average mobile phone consumer would usually change a mobile phone battery and charger amounting to a total e-waste generation of 9, 500 tonnes [2]. These large e-waste productions eventually constitute an environmental and public health challenge as the hazardous metallic components of these wastes are leached, absorbed and translocated across different trophic levels in the aquatic and terrestrial ecosystems with their attendant toxicity problems.

A lot of short - term bioassays such as *Aspergillus* spp., *Allium cepa* and *Limicolaria flammea* bioassays have been applied in the assessment of chemical toxicity by several researchers [3,4,5]. *Aspergillus terreus* is one of the several species of tube - like fungi in the family Trichocomaceae. It is one of the few species whose colonies are typically pseudo-like and cinnamon-buff to sand-brown colour with a yellow to deep dirty brown vegetative mycelia. The conidia heads are in the form of compact columns and chains that are round, smooth brown walled. The conidiophores are hyaline, smooth walled with biseriate phialides that are limited mainly to the upper part of the vesicle surface [6]. *A. terreus* was used in this study as bioindicator because it has fast mitotic and meiotic life cycles, easily available in the fresh and marine aquatic environments and possesses fast response to the presence of small concentration toxic chemicals in the environments.

*A. cepa* assay is generally used as a short-term and cheap indicator of toxicity for monitoring aquatic and terrestrial contamination in various parts of the world [7]. The *Allium* test has been applied in assessing the likely synergistic effects of a mixture of contaminants including heavy metals, and hydrophilic and lipophilic chemicals [8]. *A. cepa* test has also been applied in assessing cytotoxic and genotoxic biomarkers such as mitotic index inhibition, root growth inhibition et cetra. The valuable data from this assay could suggest the possible presence of mutagenic or genotoxic mixtures in sediments and surface waters of aquatic ecosystems [7]. *A. cepa* was used in this study because it is rapid, easily available throughout Nigeria and has cost benefit than the mammalian systems.

Mollusc have been considered as an effective and resourceful model for assessing the toxic potential of contaminants. Among the mollusc models used for toxicity assessment of terrestrial ecosystem, the gastropod *Limicolaria flammea* was selected for this study because of its common availability in the Eastern Part of Nigeria, its considerable economic importance as an edible species and evidence of its sensitivity

to environmental stress. *L. flammea* are herbivores and feed on leaves of pawpaw (*Carica papaya)*, water leaf (*Talinum triangulare*), dead and rotting leaves, cereal grains such as guinea corn and vegetables without any significant damage [5,9].

Furthermore, in Nigeria, very few studies were aimed at evaluating the cytotoxicity and mortality effects of e – wastes on model microbes, plants and animals. However, Nrior et al. [3] reported the toxicity of spent mobile phone batteries (Samsung, Tecno and Nokia) on *A. nidulans* in freshwater. Bakare et al. [4] reported the cytogenotoxic effects of electronic waste leachate in *A. cepa* root. Douglass et al. [10] reported that spent phone batteries when disposed into the aquatic environment, affect *Pseudomonas* sp. more than the *Mucor* sp. The present study was aimed at assessing the toxicity effects of phone battery wastes on aquatic and terrestrial bioindicators. It is hope that valuable data obtained from the study would suggest the likely toxins present in the phone battery wastes with a view of demonstrating the ability of such substances to induce mortalities and cell abnormalities in the cell populations of *A. terreus, A. cepa* and *L. flammea.*

## 2. MATERIALS AND METHODS

### 2.1 Site Description, Sample and Specimen Collection

The study areas were Onne Light Flows Terminal Seaport located in Eleme Local Government Area of Rivers State and River Niger in Onitsha South Local Government Area of Anambra State. They are located between latitude 4°22'50"N and latitude 7°65'56.5"N and longitude 7°11'6.77"E and longitude 7°11'16.2"E for the former and latitude 6°7'50"N and latitude 6°9'30"N and longitude 6°45'47"E and longitude 6°46'20"E for the latter. Fresh and marine water samples were aseptically collected randomly from five different sampling points using a sterile labelled 10 L containers and mixed to have a representative sample. The samples were brought ice packed coolers to Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University and stored in the refrigerator for subsequent processing. The spent phone batteries (Itel and Gionee brands) were bought at Emeka Offor Plaza, Main Market Onitsha, Anambra State and placed into clean sterile polyethylene bags. The medium sized onion bulbs of 1.50 ± 2.00 cm in diameter) (2n = 16) were purchased at Chukwuemeka Odumegwu Ojukwu University, Uli Campus Market, in Ihiala Local Government Area, Anambra State and placed into clean sterile polyethylene bags while the snails were collected during the rainy season at different farmlands within the premises of Chukwuemeka Odumegwu Ojukwu University, Uli Campus. The collected specimens were then adapted in the laboratory for one week before the start of the test to curtail differences in the snail' physiology [5,8,10].

### 2.2 Laboratory Experimental Research Design

Five treatments and the controls designs designated as 6.25%, 12.5%, 25%, 50%, 100%, CTRL for *Aspergillus terreus* toxicity test, *Allium cepa* toxicity tests and 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, CTRL for *Limicolaria flammea* toxicity test. The experimental designs were set up in triplicates and incubated for 24 h, 72 h and 20 days at 25 ± 2°C.

### 2.3 Sample Preparation

The water samples were filtered using clean sterile Whatman No. 1 filter paper while the contents of the spent phone battery samples were obtained by forcefully opening of the battery lids and placed into clean and labelled aluminum foil. The contents were prepared by setting up five glass test tubes aseptically covered with cotton wool containing the appropriate filtered fresh and marine water as described above from the habitat of the fungal isolates. The first four test tubes contained concentrations (6.25%, 12.5%, 25% and 50%) while the fifth tubes contained only the filtered fresh and marine water separately and the test organism and finally serves as the negative control [10].

### 2.4 Estimation of Heavy Metal

The heavy metals of the spent phone battery samples were estimated using oxyacetylene flame atomic absorption spectrometry by adopting the method of APHA [11].

### 2.5 Quantification of Microorganisms from the Water Samples

The spread plate method was used for the quantification of the total heterotrophic fungal counts. One millilitre (1 mL) of each water samples was pipetted aseptically and placed into 9 mL of sterile distilled water in glass test tubes. After serially diluting of the samples up to $10^{-4}$,

0.1 mL aliquot was aseptically pipetted and dispensed on the surfaces of sterile Sabouraud's Dextrose Agar (SDA) (chloramphenicol 0.005%) plates, respectively. The inoculants were evenly spread using a sterile glass rod and plates were incubated for 72 h at 25 ± 2°C for fungi. The experiments were carried out in duplicates. After incubation, the colonies on the respective plates were counted and the mean values of total heterotrophic fungal counts were determined and used to calculate the colony forming units per milliliter (CFU/mL) [10].

## 2.6 Characterization of the Isolates

The suspected *Aspergillus terreus* fungal isolates were selected, purified by sub-culturing into new SDA plates and identified based on colonial and microscopic features such as colonial growth outline, conidial structure, and pigmentation. The technique described by Uba et al. [12] was also adopted for the identification of the isolated fungi by comparison with those of known taxa from a fungal key of identification atlas.

## 2.7 Toxicity Assessment

### 2.7.1 *Aspergillus terreus* toxicity test

The toxicity of the spent phone battery samples on *A. terreus* was assessed by adopting the method of Douglass et al. [10]. In this study, the pure and stored culture of *A. terreus* were prepared by inoculating a loopful into sterile Sabouraud's Dextrose Broth (SDB) until late exponential phase. After inoculum development, 1 mL of the *A. terreus* was pipetted and dispensed into the five test tubes with the respective concentrations above and 0.1 mL was plated out immediately after inoculation on the surfaces of sterile SDA plates and serve as 0 h plate counting. Subsequently, aliquot (0.1 mL) of the respective inoculated test tubes were then plated out after 4 h, 8 h, 12 h and 24 h of toxicant exposure on sterile SDA plates and incubated by inversion for 72 h at 25 ± 2°C. After incubation, the plates were counted and CFU/mL determined. The respective percentage survivals and mortalities were derived and used to determine the $LC_{50}$ of each spent phone battery samples in the fresh and marine water set ups.

### 2.7.2 *Allium cepa* toxicity test

The toxicity of the spent phone battery samples on *A. cepa* was assessed by adopting the method of Radic et al. [8] with little modifications. At the start of the experiment, the outer parts of the onion bulbs and the dry bottom plate were detached after washing and rinsing with detergent and running tap water. For each spent phone battery samples, a series of ten bulbs were inserted in sterile distilled water for 48 h to allow for proper sprouting of *Allium* root cells. The bulbs with satisfactory root lengths (2 – 2.5 cm) were used in the study. Therefore, six sets of ten bulbs were used for each spent phone battery samples. Tap water (pH 7.04) was used as a negative control. After 48 h exposure period, numerous root tips were cut away from the bulbs, ethanol glacial acetic acid fixed for 24 h and rinsed in running tap water. The 3 - 4 washed root tipped cells were placed on microscope slides, stained with aceto-carmine solution, squashed and examined using bright - field binocular microscopy (Stereo OF0533 microscope, China) at a total magnification of 1000×. To obtain mitotic indices (MI), approximately 500 cells were observed for each spent phone battery sample. Furthermore, after 72 h of exposure to the spent phone battery samples, the root lengths were measured using a metre rule and recorded. The results for mitotic index and root length were expressed as percent of the negative control.

### 2.7.3 *Limicolaria flammea* toxicity test

The toxicity of the spent phone battery samples on *L. flammea* was assessed by adopting the method of Amusan et al. [5] with little modifications. After one week of adaptation, 1 kg of guinea corn was weighed, grounded and moistened with water. They were rolled into pellets, placed inside sterilized glass Petri dishes and dried in an electric oven at 80°C for 2 h. The dried grain was removed from the oven and reweighed. The sterile dried grains were then mixed with 1 mg - 6 mg doses of spent phone battery samples and placed inside seven 10 L plastic containers (dimension: 43 cm X 32 cm x 25 cm). Snails weighing average of 23.73 ± 0.21 g in body weight were then used. Twenty each were placed into each container and kept under laboratory condition with temperature (25 ± 2°C) and photoperiod of 12 h light: 12 h dark. Animals were daily moistened by sprinkling of water and mortality was recorded by examining the animal's behaviours every day for 20 days. The experiment was replicated twice for each spent phone battery samples.

## 2.8 Biological Statistics Analysis

The data obtained were subjected to two tailed paired T - TEST and 2-way ANOVA with Dunnet

comparison test to determine the differences among the treatment set ups and their controls. Linear regression analysis was adopted to determine the 50% effect of each spent phone battery samples on each test organism. Two tailed Pearson correlation coefficients was also employed to determine the relationship between the different doses of spent phone battery samples and either mortality or cellular aberrations. Threshold values greater than 0.05 were considered non-significant at 95% confidence intervals.

## 3. RESULTS

### 3.1 Microbial Profile

The result of the microbial counts of the two aquatic ecosystems is demonstrated in Fig. 1. Marine water (7.12 logCFU/mL) was shown to had more fungal count than the fresh water (7.07 logCFU/mL) ecosystem.

### 3.2 Heavy Metal Assessment

The result of the heavy metal constituents of the spent phone battery sample is shown in Table 1. From the result, Gionee battery sample had the highest values of arsenic (1.200 ppm), cadmium (0.800 ppm), mercury (0.704 ppm) and lead (8.147 ppm) while Itel battery sample had the highest value of nickel (9.786 ppm), respectively.

### 3.3 Microbial Exposure Assessment

The results of the log count of *Aspergillus terreus* in response to spent Itel and Gionee phone battery contamination of fresh water (A) and marine water (B) samples are demonstrated in Fig. 2a – b and 3a – b. The results in Fig. 1a – b showed that the highest (6.76 logCFU/mL) and the lowest (5.86 logCFU/mL) counts of *A. terreus* were observed in control and 50% doses of Itel exposed marine water sample after 24 h while the highest (6.78 logCFU/mL) and the lowest (5.70 logCFU/mL) counts of *A. terreus* were observed in control and 50% doses of Itel exposed marine water sample after 24 h, respectively. Similarly, the results in Fig. 2a – b revealed that the lowest (5.59 logCFU/mL) and the highest (6.77 logCFU/mL) counts of *A. terreus* were observed in 50% and control doses of Gionee exposed freshwater sample after 24 h while the lowest (5.68 logCFU/mL) and the highest (6.76 logCFU/mL) counts of *A. terreus* were observed in 50% and control doses of Gionee exposed marine water sample

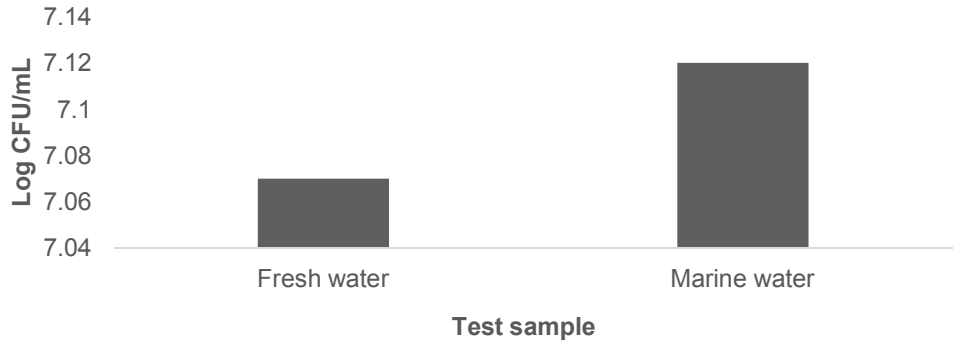


**Fig. 1. Microbial count of the two aquatic ecosystems**

**Table 1. Heavy metal constituents of the spent phone battery sample**

| Parameters | Metal concentration (ppm) | | WHO [13]/FEPA [14] standards in sediment (ppm) | WHO [13]/FEPA [14] standards in water (ppm) |
|---|---|---|---|---|
| | Itel | Gionee | | |
| Arsenic | 0.288 | 1.200 | - | 0.100 |
| Cadmium | 0.000 | 0.800 | 0.030 | 0.005 – 0.010 |
| Mercury | 0.199 | 0.704 | - | 0.050 |
| Lead | 1.41 | 8.147 | 0.010 | 0.050 |
| Nickel | 9.786 | 2.456 | 0.020 | 0.100 – 0.200 |

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

after 24 h, respectively. The result of the 24 h median lethal concentration of the spent phone battery samples on the population of *Aspergillus terreus* is shown in Fig. 4. The result showed that the Itel in fresh water sample had the highest $LC_{50}$ values of 30.49% while Gionee in fresh water sample had the lowest $LC_{50}$ values of 21.74% after 24 h, respectively. Two factor ANOVA with Dunnett comparison test revealed significant differences ($P < 0.05$) were detected among the doses and their controls. Two tailed Pearson correlation coefficients showed there were strong positive non - significant ($P > 0.05$) correlations among the doses, freshwater and marine water contamination by spent Itel ($r = 0.8490$; 0.9453) and Gionee ($r = 0.7121$; 0.6960) phone battery.

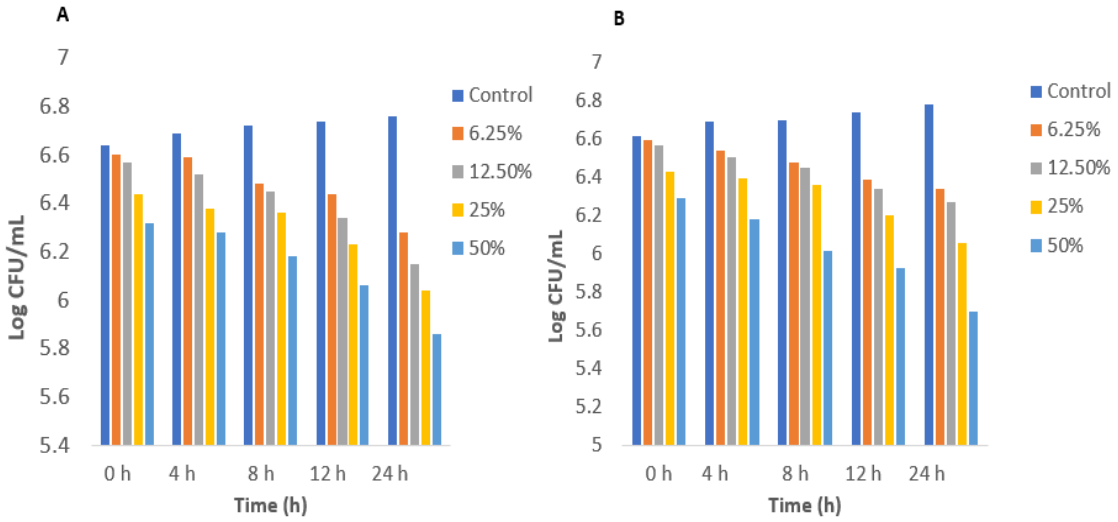


**Fig. 2. Log count of *Aspergillus terreus* in response to spent Itel phone battery contamination of fresh water (A) and marine water (B) samples. LogCFU/mL = Logarithmic colony forming unit per millilitre; h = Hour; % = Percent**

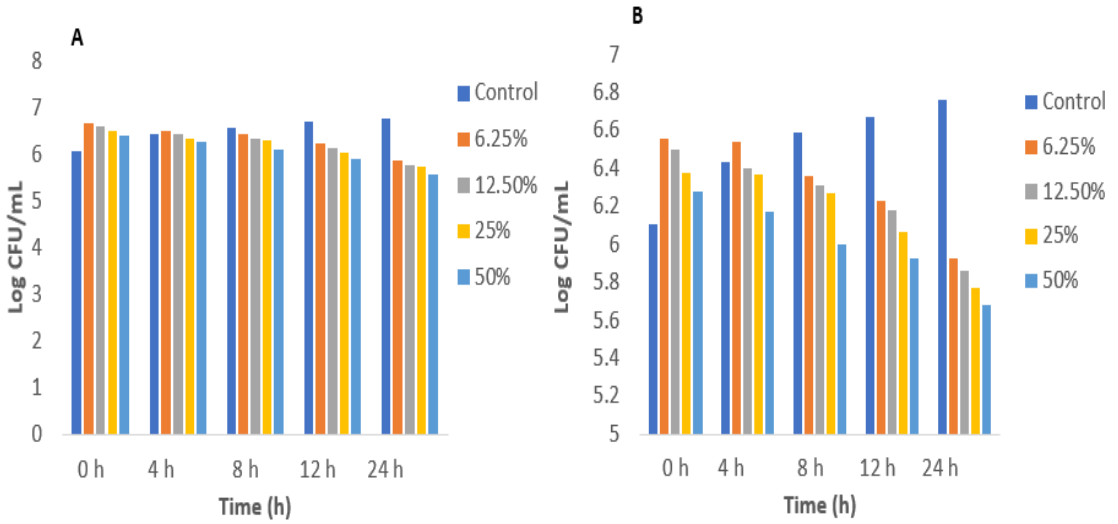


**Fig. 3 Log count of *Aspergillus terreus* in response to spent Gionee phone battery contamination of fresh water (A) and marine water (B) samples. LogCFU/mL = Logarithmic colony forming unit per millilitre; h = Hour; % = Percent**

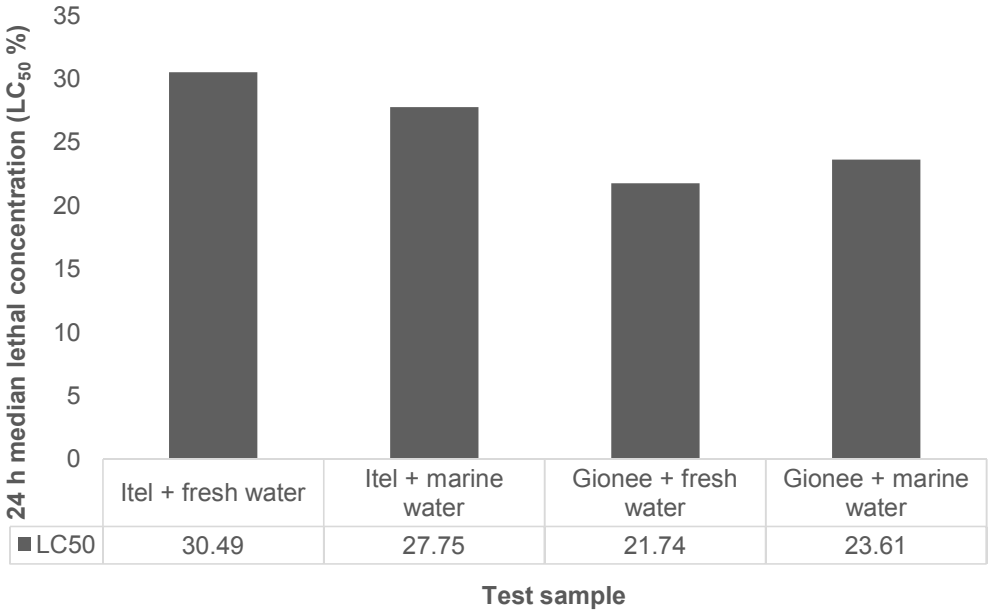


**Fig. 4. 24 h median lethal concentration of the spent phone battery samples on the population of *Aspergillus terreus.* LC50 = 50 percentage lethal concentration; % = Percent**

### 3.4 *Allium cepa* Exposure Assessment

The result of the root growth features of *Allium cepa* in response to the different concentrations of spent Itel and Gionee phone battery is displayed in Table 2. From the results, 100% concentration had the lowest and highest percentage root length and root inhibition of 41.20% and 51.00% as well as 38.00% and 62.00% for both Itel and Gionee phone battery samples, respectively. The Itel battery sample had higher $EC_{50}$ value (86.08%) than Gionee battery sample with $EC_{50}$ value of 65.46%. The result of the mitotic aberration induced by different concentrations of the spent phone battery samples on *Allium cepa* root tips is illustrated in Fig. 5. From the result, Itel had the lowest values of mitotic index (15.3%) and mitotic inhibition (94.63%) while Gionee had the highest values of mitotic index (18.0%) and mitotic inhibition (97.53%), respectively. Two tailed paired T - TEST revealed no significant differences ($P > 0.05$) was observed in the root inhibitions of *A. cepa* between spent Itel and Gionee phone battery. Two tailed Pearson correlation coefficients revealed there was strong positive significant correlations among the doses of spent Itel ($r = 0.8498$) and Gionee ($r = 0.8528$) phone battery and root inhibitions of *A. cepa*.

**Table 2. Root growth features of *Allium cepa* in response to the different concentrations of spent Itel and Gionee phone battery**

| Sample | Concentration (%) | Mean root length | % Root length | % Root inhibition | $EC_{50}$ (%) |
|---|---|---|---|---|---|
| Itel | Control | 8.50 | 100.00 | - | |
| | 6.25 | 6.30 | 74.12 | 26.60 | |
| | 12.50 | 6.30 | 74.12 | 26.60 | |
| | 25.00 | 6.00 | 71.00 | 29.00 | 86.08 |
| | 50.00 | 5.00 | 59.00 | 41.10 | |
| | 100.00 | 3.50 | 41.20 | 51.00 | |
| Gionee | Control | 8.50 | 100.00 | - | |
| | 6.25 | 6.50 | 76.50 | 24.00 | |
| | 12.50 | 6.30 | 74.10 | 26.00 | |
| | 25.00 | 4.50 | 53.00 | 47.10 | 65.46 |
| | 50.00 | 4.30 | 51.00 | 49.10 | |
| | 100.00 | 3.20 | 38.00 | 62.00 | |

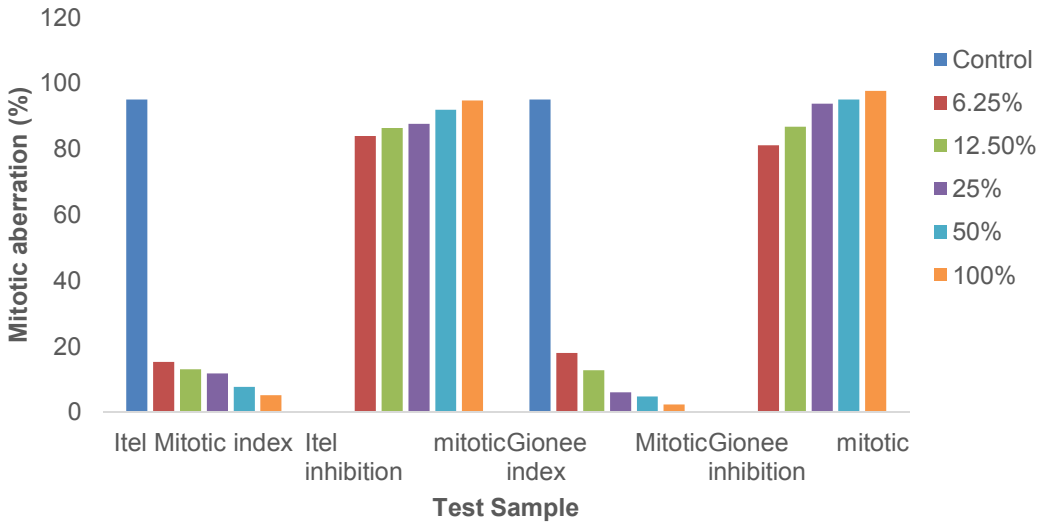


**Fig. 5. Mitotic aberration induced by different concentrations of the spent phone battery samples on *Allium cepa* root tips. % = Percent**

### 3.5 *Limicolaria flammea* Exposure Assessment

The results of the toxic response of *Limicolaria flammea* to different concentrations of spent Itel and Gionee phone battery are presented in Tables 3 and 4. The results showed that Itel phone battery sample had higher (65.00%) and lower (5.11%) percentage mortality and $LC_{50}$ value at 6 mg/kg dose than Gionee phone battery sample with lower (55.00%) and higher (6.20%) percentage mortality and $LC_{50}$ value at 6 mg/kg dose after 20 days' exposure, respectively. Similarly, two factor ANOVA with Dunnett comparison test revealed significant differences ($P < 0.05$) among the doses and their controls. Two tailed Pearson correlation coefficients showed there was strong positive significant correlations among the doses of spent Itel ($r = 0.9896$) and Gionee ($r = 0.9673$) phone battery and percentage mortalities of *L. flammea*.

**Table 3. Toxic response of *Limicolaria flammea* to different concentrations of spent Itel phone battery**

| Parameter | Treatment period (day) | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **4** | **8** | **12** | **16** | **20** |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 mg/kg | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 5.00 | 5.00 | 5.00 |
| 2 mg/kg | 0.00 | 0.00 | 0.00 | 2.00 | 2.00 | 3.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 10.00 | 10.00 | 15.00 |
| 3 mg/kg | 0.00 | 0.00 | 0.00 | 3.00 | 3.00 | 5.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 15.00 | 15.00 | 25.00 |
| 4 mg/kg | 0.00 | 0.00 | 0.00 | 4.00 | 5.00 | 7.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 20.00 | 25.00 | 35.00 |
| 5 mg/kg | 0.00 | 0.00 | 0.00 | 6.00 | 8.00 | 10.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 30.00 | 40.00 | 50.00 |
| 6 mg/kg | 0.00 | 0.00 | 2.00 | 8.00 | 11.00 | 13.00 |
| % Mortality | 0.00 | 0.00 | 10.00 | 40.00 | 55.00 | 65.00 |
| $LC_{50}$ (%) | - | - | 75.84 | 8.43 | 6.50 | 5.11 |

**Table 4. Toxic response of *Limicolaria flammea* to different concentrations of spent Gionee phone battery**

| Parameter | Treatment period (day) | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **4** | **8** | **12** | **16** | **20** |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 mg/kg | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.00 |
| 2 mg/kg | 0.00 | 0.00 | 0.00 | 2.00 | 1.00 | 2.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 10.00 | 5.00 | 10.00 |
| 3 mg/kg | 0.00 | 0.00 | 0.00 | 3.00 | 2.00 | 3.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 15.00 | 10.00 | 15.00 |
| 4 mg/kg | 0.00 | 0.00 | 0.00 | 4.00 | 3.00 | 5.00 |
| % Mortality | 0.00 | 0.00 | 0.00 | 20.00 | 15.00 | 25.00 |
| 5 mg/kg | 0.00 | 1.00 | 1.00 | 5.00 | 5.00 | 8.00 |
| % Mortality | 0.00 | 5.00 | 5.00 | 25.00 | 25.00 | 40.00 |
| 6 mg/kg | 0.00 | 2.00 | 2.00 | 6.00 | 7.00 | 11.00 |
| % Mortality | 0.00 | 10.00 | 10.00 | 30.00 | 35.00 | 55.00 |
| $LC_{50}$ (%) | - | 36.49 | 36.49 | 9.67 | 9.30 | 6.20 |

## 4. DISCUSSION

The electronic industry is among the major industries in Nigeria that contributes its economy. Also, they pose severe and long -lasting impacts on the environment by the release of their hazardous E – wastes. The importance of using *Aspergillus terreus*, *Allium cepa* and *Limicolaria flammea* as model indicators in the assessment of chemical toxicity has been documented by diverse investigators [3,4,5,7,8,10]. In this study, an attempt was made to assess the possible environmental health implications linked to indiscriminate release of spent laptop battery into the aquatic and terrestrial environments.

Earlier study by Douglas et al. [10] revealed higher quantities of harmful heavy metals in their analyzed spent phone battery samples and corroborates with the result in this study as shown in Table 1 which also revealed higher values of arsenic (Ar), cadmium (Cd), mercury Hg), lead (Pb) and nickel (Ni) and hence above the standard limits of WHO [13] and FEPA [14].

The result in Fig. 1 revealed that there more diversity of fungi in the marine ecosystem than the fresh water ecosystem and the reason is due to the nutrient rich nature of the marine ecosystem. Furthermore, the cell number of *A. terreus* was negatively and significantly ($P < 0.05$) inhibited by the doses of the spent phone battery samples as well as the days of exposure (Figs. 2a – b and 3a – b). Higher doses of the studied spent laptop battery samples induced reductions in microbial counts compared to lower doses as demonstrated by the logarithmic colony forming unit per millilitre (logCFU/mL) values. The reason for the general inhibition in the *A. terreus* count could be due to the presence of the selected and analyzed metallic components especially lead and nickel from the spent battery wastes as they have been widely implicated among the list of toxic and dangerous substances [1,2]. There was continuous counts of *A. terreus* in the control setups throughout the 24 h study period and the possible reason could be due to the absence of the toxicants. The result was similar to the publication of Nrior et al. [3] who reported that certain substances in lithium battery used to power mobile phones are relatively toxic at certain concentrations to *Aspergillus nidulans*. However, the $LC_{50}$ as evaluated and shown in Fig. 4 revealed the order of toxicity of the spent phone battery to *A. terreus* population thus: Gionee + fresh water > Gionee + marine water > Itel + marine water > Itel fresh water. Previous studies reported that *Aspergillus* spp. are resistant to the spent battery toxicant in the fresh water ecosystem [3,10].

In order to widen our research on terrestrial eukaryotic organisms, *A. cepa* test was carried out to determine the probable cytotoxic effects of spent phone battery sample as the data obtained could assist in predicting the likely cytogenotoxic effects in other eukaryotic organisms. The result in Table 2 and Fig. 5 showed that significant ($P < 0.05$) reduction in root growth and mitotic division of the *A. cepa* root tips by the different doses of the phone battery samples. Morphological

abnormalities such as short, curled and scanty root tips as the doses of the samples advanced with time were observed. These observed abnormalities could be as a result of heavy metals components of the battery wastes which tend to trigger cellular and genetic variations in the root tips of *A. cepa.* Gionee battery sample had more toxic effect than Itel battery sample as it had lower values of mitotic index. This could possibly be due to its high contents of Ar, Cd and Hg in Gionee battery than Itel battery samples as they are regarded as dangerous and toxic substances [1,2]. Chemical substances with mitotic index less than 22 were considered to be toxic according to Wijeyaratne and Wadasinghe [7] and hence our studied Itel and Gionee phone battery samples were regarded as cytotoxic to *A. cepa* and could possess genotoxic and mutagenic substances. Previous study by Radić et al. [8], Caritá and Marin-Morales [15], Fatima and Ahmad [16] and Glińska et al. [17] reported that trace metals, pesticides and other pollutants were considered responsible for the diminished mitotic index of the *A. cepa* roots and the findings of these researchers upheld the results obtained in this study.

Further study was carried out to determine the environmental health impacts of the spent phone battery sample using *L. flammea* (African giant snail) as bioindicators for soil monitoring. The standardization and reason of using this native mollusc species for ecotoxicological assay are also applicable mainly for West Africans where dogmas and guidelines concerning the environments are relatively developed compared to other countries. The result in Tables 3 and 4 showed that continuous significant ($P < 0.05$) progression in mortalities of *L. flammea* as the period of exposure and dose of spent phone battery samples increased. There was no mortality recorded in both control setups throughout the 20 - day study period and the possible reason could be due to the absence of the toxicants. The probably reason for these high mortalities effects could be as a result of the accumulation of some hazardous metals from the spent phone battery sample into the body tissues of *L. flammea* and similar results observations was obtained by previous studies [5,9]. Also, the $LC_{50}$ result revealed that the Itel phone battery was more toxic and lethal to *L. flammea* population than the Gionee phone battery. Previous research by Dhiman [18] reported the $LC_{50}$ for chromium was 4.027 mg/L for 96 h period of exposure while lead $LC_{50}$ values was 1.352 mg/L for 96 h period of exposure, respectively on terrestrial snail (*Helix aspersa*).

## 5. CONCLUSION

This study demonstrated that toxic substances are present in the phone battery wastes. The order of toxicity of the spent phone battery to *A. terreus* population was Gionee + fresh water > Gionee + marine water > Itel + marine water > Itel fresh water. Itel and Gionee phone battery samples were regarded as cytotoxic to *A. cepa* and could possess genotoxic and mutagenic substances while Itel phone battery was more toxic and lethal to *L. flammea* population than the Gionee phone battery. The indiscriminate release of phone batteries commonly called e-wastes into the aquatic and terrestrial environments should be discouraged by government, non - governmental organizations and environmental policy makers.

## ACKNOWLEDGEMENTS

We are grateful to Confidence Sunday of the Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University Uli Campus for her technical support to this study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Babatunde OA, Eguma CA, Oyeledun BT, Igwilo OC, Awosanya OG, Adegbenro O. Mobile phone usage and battery disposal in Lagos, Nigeria. Int J Appl Psych. 2014;4(4):147–154.
2. Osibanjo O, Nnorom IC. Materials flow of mobile phones and accessories in Nigeria: Environmental implications and sound end-of-life management options. Env Impact Assess Rev. 2008;28(2-3):198–213.
3. Nrior RR, Ugboma JC, Kpalap D. Ecotoxicological percentage assessment of spent mobile phone batteries (Samsung, Tecno and Nokia) to *Aspergillus nidulans* in freshwater. Int J Geog Env Manag. 2018;4(1):51–64.
4. Bakare AA, Adeyemi AO, Adeyemi A, Alabi OA, Osibanjo O. Cytogenotoxic effects of electronic waste leachate in *Allium cepa.* Caryol. 2012;65(2):94–100.

5. Amusan AAS, Anyaele OO, Lasisi AA. Effects of copper and lead on growth, feeding and mortality of terrestrial gastropod *Limicolaria flammea* (Muller, 1774). Afr J Biomed Res. 2002;5:47–50.
6. Okoye EL, Uba BO, Dike UC, Eziefule CJ. Growth rate and antifungal activities of acetone extracts of *Ocimum gratissimum* (Scent Leaf) and *Allium sativum* (Garlic) on cassava and banana peels formulated media. J. Adv Microbiol. 2020;20(4):19–29.
7. Wijeyaratne WMDN, Wadasinghe LGYJG. *Allium cepa* bio assay to assess the water and sediment cytogenotoxicity in a tropical stream subjected to multiple point and nonpoint source pollutants. J Toxicol. 2019;5420124:1-10.
8. Radić S, Stipaničev D, Vujčić V, Rajčić MM, Širac S, Pevalek-Kozlina B. The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. Sci Total Env. 2010;408:1228–1233.
9. Amusan AAS. Feeding and growth response to copper and lead in the terrestrial gastropod *Limicolaria flammea* (Muller 1774). B. Sc. Project Department of Zoology University of Lagos; 1990.
10. Douglas SI, Nrior RR, Kpormon LB. Toxicity of spent phone batteries on microflora in marine, brackish and freshwater ecosystems. J Adv Microbiol. 2018;12(2):1–10.
11. American Public Health Association. Standard methods for examination of water and wastewater (22nd Edn). Washington DC (USA): American Public Health Association; 2012.
12. Uba BO, Okoye EB, Anyaeji OJ, Ogbonnaya OC. Antagonistic potentials of actinomycetes isolated from coastal area of Niger Delta against *Citrus sinensis* (Sweet Orange) and *Lycopersicum esculentum* (Tomato) fungal pathogens. Res Rev A J Biotech. 2019;8(3):4–15.
13. WHO. Environmental health criteria: Guidelines on studies in environmental epidemiology. Geneva, Switzerland: World Health Organization Publication. 1983;27.
14. FEPA. Guidelines and standards for industrial effluent, gaseous emissions and hazardous waste management in Nigeria. National Environmental Protection Regulations, Federal Republic of Nigeria. 1991;78(42):B15–B3.
15. Caritá R, Marin-Morales MA. Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chem. 2008;72:722–725.
16. Fatima RA, Ahmad M. Genotoxicity of industrial wastewaters obtained from two different pollution sources in Northern India: A comparison of three bioassays. Mutat Res. 2006;609:81–91.
17. Glińska S, Bartczak M, Oleksiaka S, Wolska A, Gabara B, Posmyk M, Janas K. Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of *Allium cepa* L. roots treated with heavy metals. Ecotox Env Safe. 2007;68:343–350.
18. Dhiman V. Preliminary toxicity assessment of chromium (Cr) and lead (Pb) on terrestrial snail (*Helix aspersa*). Arch Agric Env Sci. 2020;5(1):67–72.




*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sdiarticle4.com/review-history/57693*