Comparative Ecotoxicological Strength of Spent Mobile Phone Batteries Blackberry and Nokia on Bioassay Evaluator *Nitrobacter* sp

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Abstract
This study was designed to evaluate the concentration of mobile phone batteries (Nokia and blackberry) and the duration of exposure that could result to potential effect on the microorganism Nitrobacter sp. in triaquatic ecosystem. Winogradsky medium was used to isolate the bacterial specie. Standard toxicity procedure were applied using spent mobile phone batteries (Nokia and Blackberry); prepared at concentrations of 6.25%, 12.5%, 25%, 50% and 75% in fresh, marine and brackish water respectively. These were tested with Nitrobacter sp for 0h, 4h, 8h, 12h and 24h separately for each toxicant. The median lethal concentration (LC_{50}) was being employed to compare the toxicities of the different toxicants on the test species. The result showed that Nitrobacter sp. demonstrated sensitivity to the toxicity of mobile phone battery (Nokia and blackberry). Using the percentage (%) median lethal concentration; the sensitivity showed variations as toxic level decreased in the following order (noting the lower the %LC_{50} the more toxic the toxicant): Blackberry mobile phone battery in fresh water (53.80%)>Nokia mobile phone battery in marine water(61.30%)>Blackberry mobile phone battery in marine water (61.40%)>. Blackberry mobile phone battery in brackish water (61.43%)> Nokia mobile phone battery in brackish water (64.51%)> Nokia mobile phone battery in fresh water (79.09%). Toxicity seems to be affected by the salinity of the medium, as the phone batteries shows to be more toxic in marine water (LC_{50} 32%) > Brackish water (33%), least with freshwater (35%). The analytical summation of Percentage LC_{50} in the three aquatic ecosystem combined revealed that spent Blackberry mobile phone battery ($\sum LC_{50}$ 58.87%) was more toxic to the test organism Nitrobacter sp. than Nokia mobile phone battery ($\sum LC_{50}$ 68.3%). Comparative evaluation of the toxicity strength gap between the two toxicants shows a significant gap between the toxicants. This may be due to chemical reactions between the compound in the lithium battery and the salts found in these waters. This marked decrease in the number of Nitrobacter sp. as the mobile battery toxicant is increase, suggests that components present in this lithium battery is highly toxic to Nitrobacter and may interfere with the nitrogen cycle if this battery are improperly disposed in the environment. In summary, Blackberry mobile phone battery proves to be more lethal to Nitrobacter than the Nokia phone battery and the longer these organisms are being exposed to these toxicants the more lethal it becomes to them as shown in the results obtained.

Keywords: *Ecotoxicological strength, Summation Median Lethal Concentration ($\sum LC_{50}$), spent mobile phone batteries (Nokia and Blackberry), Nitrobacter sp.*

INTRODUCTION

Ecotoxicology is the study of the effects of toxic chemicals on biological organisms, especially at the population, community, ecosystem level. It is a multi-disciplinary field which integrates toxicology and Ecology (Chapman, 2002). The ultimate goal of ecotoxicology is to be able to predict the effects of pollutants on population. It offers the most efficient and effective action to prevent or remediate any detrimental effect that may be identified. In those ecosystems that are already impacted by pollution, ecotoxicology studies can inform as to the best course of action to restore ecosystem services and functions efficiently and effectively (Odokuma and Nrior, 2015). Bioassays are one of the methods used to access chemicals and chemical safely (Celemedson, *et al*. 1996). Bioassays or ecotoxicology assay is an experiment in which living test species are exposed directly to an environmental sample to measure a potential biological effect due to the presence of potential contaminants (Wadhia and Thompson, 2007).

Nitrobacter is a genus of mostly rod shaped, gram negative and chemoautotrophic bacteria. Kingdom: Bacteria, Phylum: Proteobacter, Class: Alphaproteobacteria, Order: Rhizobiales, Family: Bradyrhizobiaceae, Genus: *Nitrobacter*. (Grundman, *et.al*, 2000). *Nitrobacter* plays an important role in the nitrogen cycle by oxidizing nitrite into nitrate in soil. *Nitrobacter* have an optimum pH between 7.3 and 7.5 and will die in temperatures exceeding 120^0F (49^0C) or below 32^0F (0^0C).

A mobile phone is a portable telephone that can make and receive calls over a radio frequency carrier while the user is moving within a telephone service area. All mobile phones have a variety of features: A battery which provides the power source for the phone functions; an input mechanism to allow the user to interact with the phone; Basic mobile services tec. Mobile phone battery is a member of lithium-ion-battery which belongs to the family of rechargeable battery types (Abe, *et al.* 1999).
More than 800 million people around the world currently use mobile phones and that figure is growing daily as consumers get bombarded by advertising campaigns exhorting them to upgrade to latest, most fashionable model. As a result, old mobile phone becomes out-dated and new models are introduced faster.

The old mobile phones may eventually be thrown away which poses an ever increasing problem for the environment Mobile phones and its batteries contain concentrations of toxic heavy metals or other metals including cadmium, lead, nickel, mercury, manganese, lithium, zinc, arsenic, antimony, beryllium, and copper. Metals such as these are considered as: Persistent (.i.e. don' degrade in the environment); Bioaccumulative (.i.e. build up in fatty tissue so can reach toxic levels over time). If any of these metals are leaked into the environment, it tends to create serious environmental and health problems.
The aim of this study is to be able to predict the toxic effect of mobile phones batteries on the ecosystem using aquatic micro-flora *Nitrobacter* which is a simple and fast bioassay for monitoring ecosystem response to these pollutants.

Materials and Methods

Sample Collection/Study Area

Fresh water sample was collected from Asarama stream, Asarama town in Andoni L.G.A, Rivers state with a two (2) litre sterile plastic container, marine water was collected from Bonny River in Bonny, Rivers State with a two (2) litre sterile plastic container, also, brackish water was collected from Eagle Island River in Port Harcourt Nigeria with a two (2) litre sterile plastic container. These were used within one hour of collection.

Microbiological Analysis

Isolation of *Nitrobacter* sp.

Winogradsky Agar (modified) medium composition: Agar agar 15.0g/l, King agar B base 3g/l, KNO_2 0.1g/l, Na_2CO_3 0.5g/l, $FeSO_4.7H_2O$ 0.4g/l, NaCl 0.5g/l, Distilled water 1000ml (Odokuma and Nrior, 2015) (Note: This modified medium shortens incubation period to 48h-72h (2-3days) instead of 5days).

Aliquot (0.1ml) of the water samples was transferred onto sterile Winogradsky's agar plates in duplicates. Uniformly spread with sterile glass spreader (spread plate method) and incubated in inverted position at 30^0C for 48-72hours. Creamy, mucoid, flat colonies were suggestive of *Nitrosomonas* species. Gram staining of the colonies revealed Gram negative short-rods indicative of *Nitrosomonas*. The colonies were aseptically subcultured unto fresh Winogradsky's agar plates. Grayish, mucoid, flat colonies were suggestive of Nitrobacter. Gram staining of the colonies revealed pear-shaped organism's indicatmve of Nitrobacter (Colwell and Zambruski, 1972).

Suspected *Nitrosomonas* and Nitrobacter species were used to inoculate sterile Winogradsky broth containing ammonium sulphate and sodium nitrite respectively and incubated at 30^0C for 2-3days. After 48hours of incubation, 1ml each of sulfanilic acid and dimethyl-naphthalamine, and a little zinc dust were added to the respective medium. Nitrite production from ammonium sulphate indicated by red colouration was confirmatory of *Nitrosomonas* species. Nitrate production from sodium nitrite indicated by red colouration was confirmatory of *Nitrobacter* species.

Toxicity Test

Preparation of the toxicant

Spent Nokia and Blackberry batteries were aseptically clipped open and immediately submerged in freshwater, brackish and marine water separately. The toxicants were prepared by setting up six test tubes aseptically covered with cotton wool. The test was carried out in six separate test tubes containing appropriate filtered water (fresh, marine and brackish water from the habitat of the organism separately). In each of the test tubes, the five toxicant concentrations (6.25%, 12.5%, 25%, 50% and 75%) were added separately. The test tubes were the covered with cotton wool, the control consists of fresh, marine and brackish water from the habitat of the organism.

Test procedure for *Nitrobacter* specie from freshwater, brackish and marine water

About 1ml of the test organism was added to separate toxicant concentrations in test tubes containing (6.25%, 12.5%, 25%, 50%, 75% and control respectively), and was plated out immediately after inoculation on Winogradsky agar plate. This is known as zero-hour count plating. And then was incubated at room temperature (28± 2ºC). Aliquot (0.1ml) of each of the concentrations of the effluent was then plated out after 4h, 8h, 12h and 24h on Winogradsky agar and was incubated for 48-72hours. After which the plate were counted.

Percentage log survival of the bacteria (*Nitrobacter* sp.) isolates in mobile phone batteries.

The percentage log survival of the *Nitrobacter* isolates in the mobile phone batteries effluent used in the study was calculated using the formula adopted from Williams and Johnson (1981); Odokuma and Nrior (2015). The percentage log survival of the bacteria isolates in the effluent was calculated by obtaining the log of the count in toxicant concentration, dividing by the log of the count in the zero toxicant concentration and multiplying by 100. Thus:

$$\text{Percentage (\%) log survival} = \frac{\text{LogC}}{\text{Logc}} \times 100$$

Where:
LogC = log of the count in each toxicant concentration
Logc = log of count in the control (zero toxicant concentration).
Percentage (%) log mortality = 100 - % log survival

Percentage log mortality bacteria from fresh, marine and brackish water

This study was carried out to assess the probable toxic effect, cell batteries could have in fresh, marine and brackish water (aquatic environment). The formula for calculation of percentage mortality was adopted from APHA (1992), Nrior and Obire (2015). And the percentage log mortality is calculated by subtracting the control from the % log survival. % log Mortality = 100 - % log survival.

Results and Discussion

The log survival count of *Nitrobacter sp* on mobile phone batteries (Nokia and Blackberry) is determined by adding the concentration of the battery at 6.25%, 12.5%, 25%, 50%, 75% respectively and inoculated at an interval of 4hours from the start of 0h, 4h, 8h, 12h and 24h and the colony formed were counted for each hour to show the toxicity of the battery chemical and converted into log value for simplicity as shown in Table 1.

Table 1: Log survival of *Nitrobacter* with Spent Mobile Phone batteries Nokia and Blackberry in freshwater, Brackish and Marine water

Log survival of Nitrobacter with Spent Mobile Phone batteries Nokia and Blackberry in freshwater, Brackish and Marine water

Duration/ Conc.	Nokia + Freshwater					Nokia + Brackish water					Nokia + Marine water				
	0h	4h	8h	12h	24h	0h	4h	8h	12h	24h	0h	4h	8h	12h	24h
Control	2.10	1.30	1.27	1.25	1.07	2.03	2.10	2.12	1.79	2.07	2.14	2.13	2.03	2.03	1.83
6.25%	1.98	1.93	1.88	1.78	1.66	1.97	2.04	2.06	1.71	1.99	2.00	2.07	1.98	1.93	1.90
12.5%	1.91	1.77	1.69	1.68	1.55	1.94	2.02	2.01	1.60	1.74	1.97	1.91	1.92	1.79	1.53
25%	1.78	1.50	1.67	1.46	1.30	1.92	1.92	1.86	1.68	1.60	1.93	1.88	1.80	1.74	1.47
50%	1.68	1.32	1.59	1.23	1.11	1.79	1.74	1.80	1.53	1.39	1.86	1.73	1.69	1.49	1.43
75%	1.57	1.23	1.44	1	1	1.64	1.66	1.95	1.95	1.27	1.74	1.57	1.57	1.44	1.27

Duration/ Conc.	Blackberry + Freshwater					Blackberry + Brackish water					Blackberry + Marine water				
	0h	4h	8h	12h	24h	0h	4h	8h	12h	24h	0h	4h	8h	12h	24h
Control	2.19	2.07	2.03	1.99	2.17	2.17	2.02	2.02	1.69	1.65	2.07	2.03	2.05	1.94	2.00
6.25%	1.99	1.97	1.89	1.79	1.92	2.11	1.99	1.82	1.98	1.39	1.99	1.92	2.00	1.88	1.93
12.5%	1.83	1.86	1.80	1.69	1.83	1.97	1.92	1.69	1.90	1.17	1.93	1.82	1.98	1.73	1.84
25%	1.73	1.79	1.69	1.57	1.34	1.89	1.75	1.60	1.83	1.04	1.79	1.73	1.86	1.49	1.76
50%	1.63	1.57	1.57	1.39	1.27	1.85	1.66	1.55	1.63	1	1.77	1.68	1.83	1.41	1.61
75%	1.44	1.30	1.27	1.25	1.07	1.69	1.50	1.39	1.34	1.95	1.62	1.46	1.73	1.27	1.36

The result of the log survival count shows the sensitivity of the organism (*Nitrobacter sp)* to the toxicity of mobile phone battery (Nokia, Blackberry) in triaquatic water with effect to their salinity. The log survival in Table 1 shows that the test organism showed some growth at 6.5% concentration of toxicant at 0h, 8hrs and 24hrs while at higher concentration there was relative continuous decrease in log survival at 12h and 24hrs.

Percentage (%) log mortality of *Nitrobacter sp* at different concentration of spent mobile phone battery (Nokia and Blackberry) at toxicant of 6.25%, 12.5%, 25%, 50% and 75% at 0h, 4h, 8h, 12h, 24h exposure in triaquatic water were shown in Fig. 1-3.

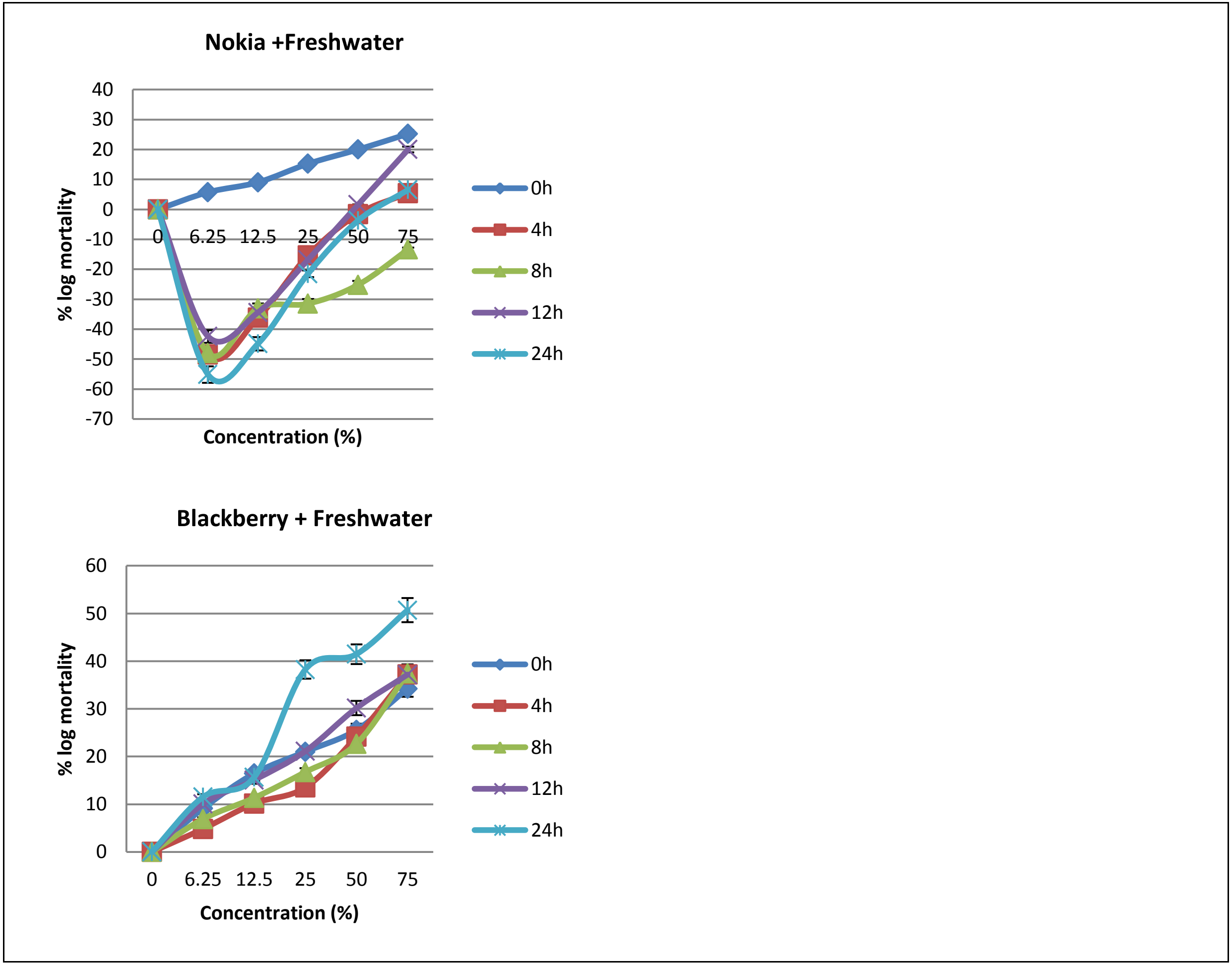


Figure 1: Percentage (%) Log Mortality of *Nitrobacter* using spent Nokia and Blackberry batteries in Fresh Water

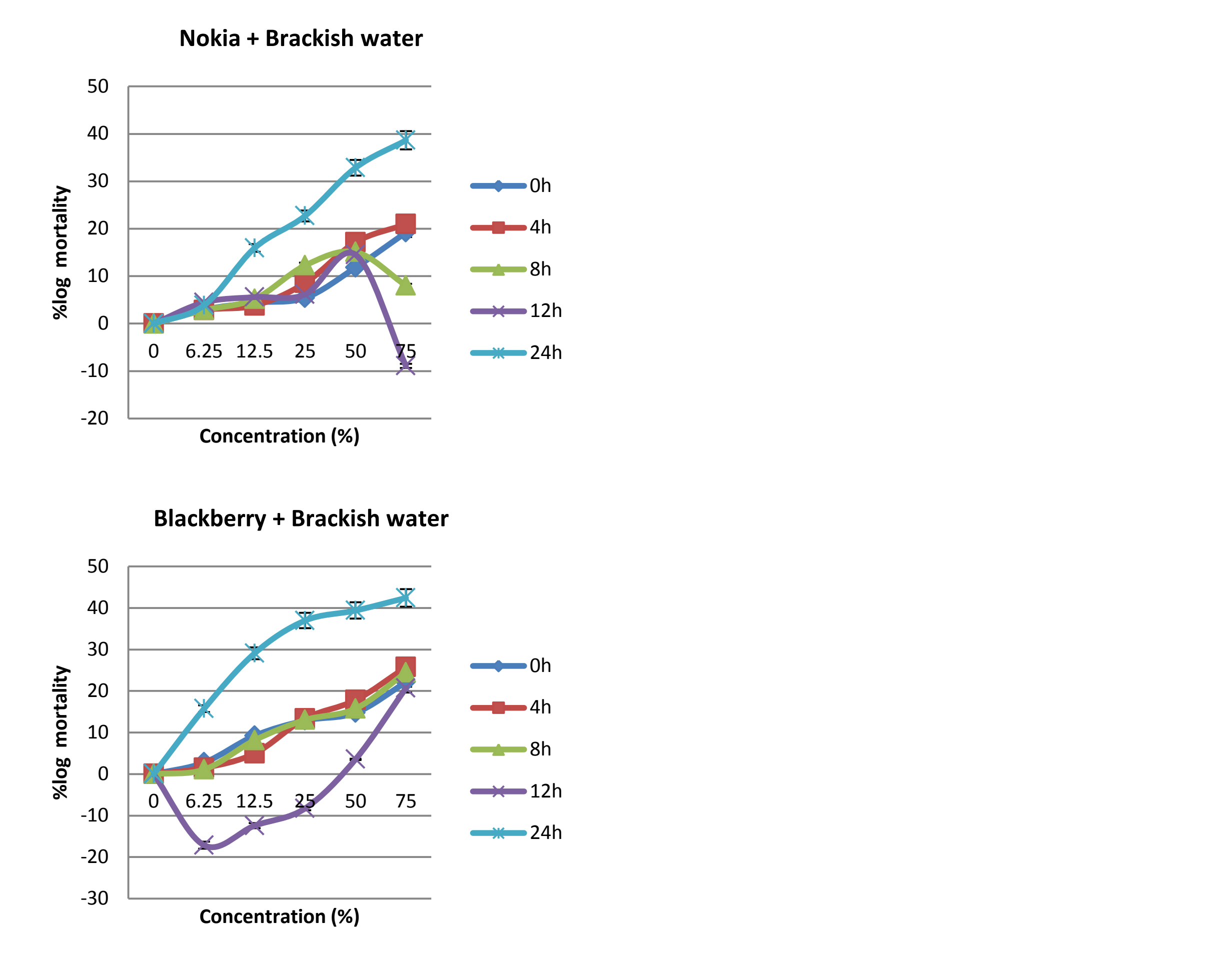


Figure 2: Percentage (%) Log Mortality of *Nitrobacter* using spent Nokia and Blackberry batteries in Brackish Water

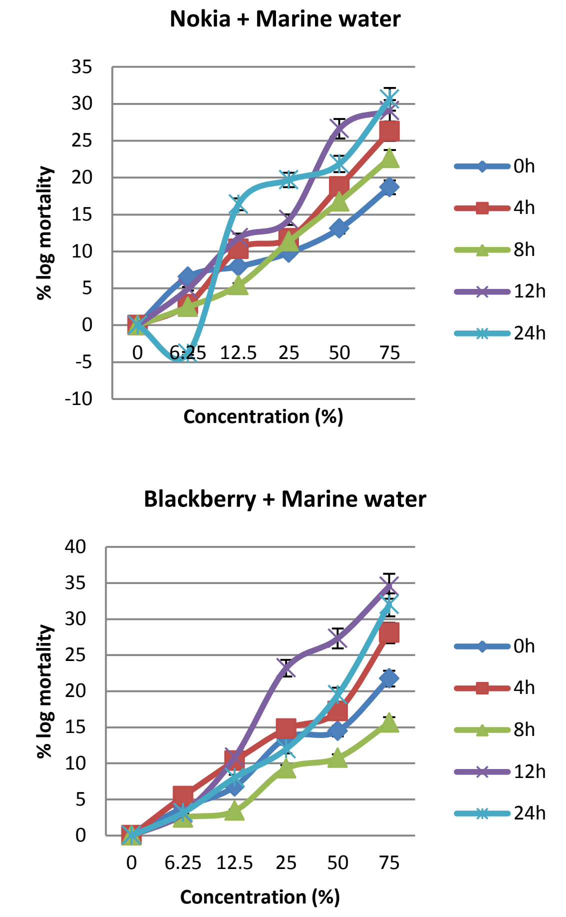
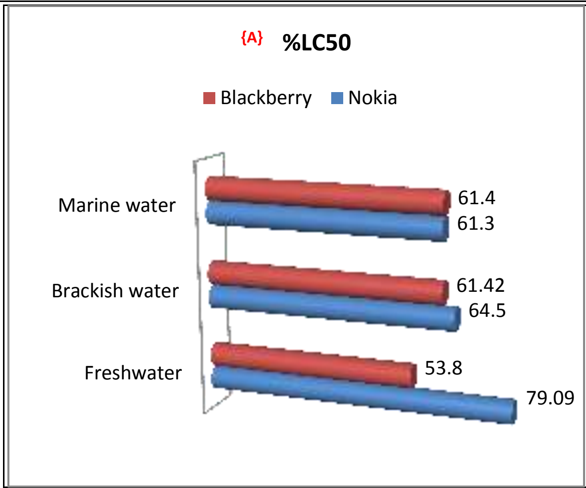
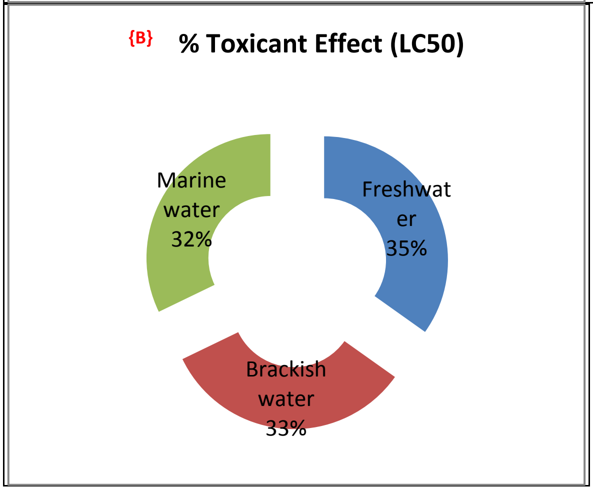


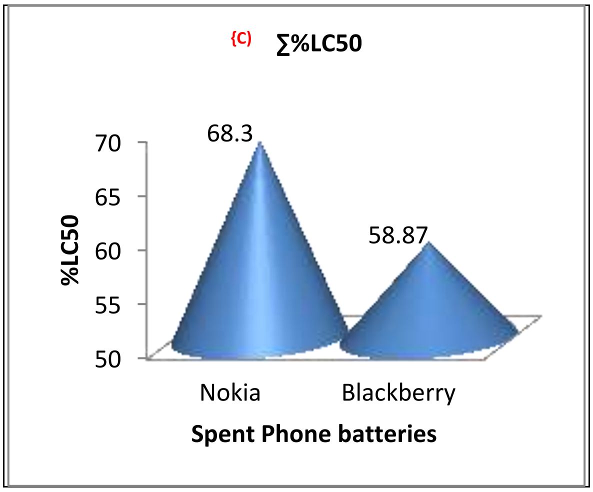
Figure 3: Percentage (%) Log Mortality of *Nitrobacter* using spent Nokia and Blackberry batteries in Marine water

In the course of this research it was shown that substances found inside the mobile phone battery can be toxic and affect the organism used (*Nitrobacter*) at certain concentration . This result confirms similar observation made by Hermann and Urbach on substances electronics (Hermann and Urbach 2000) Some advantages observed in the use of bacterial bioassay organism include; low cost, small space, simplicity and rapidity. *Nitrobacter* sp. mortality of the test organism expressed as Median Lethal Concentration (LC_{50}) was used as indices to monitor toxicity (Odokuma and Nrior, 2015).

Toxicity produces adverse biological effect on the survival, activity, growth, metabolism and reproduction of organisms. The sensitivity showed variations. Toxic level on *Nitrobacter* decreased in the following orders (noting that the lower the LC_{50} the more toxic the toxicant): Blackberry mobile phone battery in freshwater (53.80%) >. Nokia mobile phone battery in marine water (61.30%) > Blackberry mobile phone battery in marine water (61.40%) > Blackberry mobile phone battery in Brackish water (61.43%) > Nokia mobile phone battery in Brackish water (64.51%) > Nokia mobile phone battery in fresh water (79.09%). Spent Blackberry mobile phone battery in fresh water (53.80%) was seen to be more toxic in fresh water and Nokia mobile phone battery (79.09%) was found to be least toxic (Fig. 1-4}.


{A} %LC50
Blackberry
Nokia
Marine water
Brackish water
Freshwater
61.4
61.3
61.42
64.5
53.8
79.09


{B} % Toxicant Effect (LC50)
Marine water 32%
Freshwater 35%
Brackish water 33%


{C} ∑%LC50
%LC50
70
65
60
55
50
68.3
58.87
Nokia
Blackberry
Spent Phone batteries

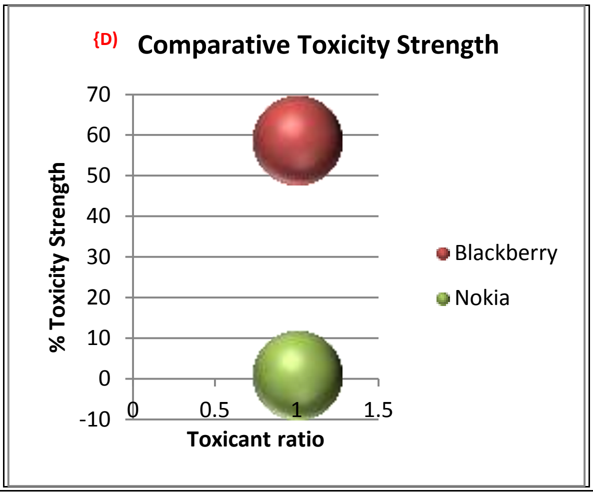


Figure 4: {A} Percentage Median Lethal Concentration (LC_{50}) of spent mobile phone batteries (Nokia and Blackberry) on *Nitrobacter* in tri-aquatic ecosystem
{B} Percentage Toxicant effect of spent mobile phone batteries (Nokia and Blackberry) on *Nitrobacter* in tri-aquatic ecosystem
{C}The Summation of Percentage LC_{50} in the three aquatic ecosystems combined
{D} Comparative evaluation of the toxicity strength gap between the two toxicants

Toxicity seems to be affected by the salinity of the medium, as the phone batteries shows to be more toxic in marine water (LC_{50} 32%) > Brackish water (33%), least with freshwater (35%); noting that the lower the LC_{50} the more toxic the toxicant. The analytical summation of Percentage LC_{50} in the three aquatic ecosystem combined revealed that spent Blackberry mobile phone battery ($\sum LC_{50}$ 58.87%) was more toxic to the test organism *Nitrobacter* sp. than Nokia mobile phone battery ($\sum LC_{50}$ 68.3%). Comparative evaluation of the toxicity strength gap between the two toxicants shows a significant gap between the toxicants. This may be due to chemical reactions between the compound in the lithium battery and the salts found in these waters. This marked decrease in the number of *Nitrobacter* sp. as the mobile battery toxicant is increase, suggests that components present in this lithium battery is highly toxic to *Nitrobacter* and may interfere with the nitrogen cycle if this battery are improperly disposed in the environment (Nrior and Gboto, 2017).

Conclusion and Recommendation

Blackberry mobile phone battery has higher toxicity potential than spent Nokia batteries especially in freshwater. The longer these organisms are being exposed to these toxicants the more lethal it becomes to them. The use of bacterial bioassay organism is now gaining wide acceptance as it offers a number of advantages which includes ease of handling, economy of space, short life cycle, low cost and it also help in the detection of potential contaminants in Eco-toxicological bioassay as it concerns the effect of potential biological contaminants or toxic chemical on humans of ecological entities.

Findings from the study revealed that prolonged presence of hearing metals from mobile phone batteries tends to greatly affect the environment. In terms of human impact and the environment in general, all batteries are harmful (hazardous waste if not disposed of properly and safely). The result of this study implies that continuous monitoring has to be carried out to ascertain the long term impact of anthropogenic inputs to take remedial measures so as to ensure a more health and safe environment.

Some steps that can be taken to reduce or minimize the damage caused to the environment due to improper battery disposal include the following: [a] Recycling of used batteries [b]

Formulation and implementation of more stringent law regarding battery disposal. [c] Providing complete information to customers at the time of purchase about the battery sustainability, safety and ways of disposal. [d] Informing the manufacturers to use eco-friendly materials thus relieving the environment's toxic load. It is noteworthy that recycling of spent mobile phone batteries offers some advantages such as pollution reduction, Saves natural resources, and Saves energy, Lessens the amount regulations, Reduces imports, Generates income, leads to innovation of other products from the recycled materials.

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