Comparative Ecotoxicological Assay of E-Waste (Phone Batteries) On Some Aquatic Micro flora

A B S T R A C T

Aim: To determine and compare the effect of used phone batteries on *Nitrosomonas* spp. in tri aquatic bodies

Study design: The study employs experimental design and statistical analysis of the data and interpretation.

Place and Duration of Study: Fresh water and Marine samples were collected from Gokana L.G.A, Rivers state, brackish water was collected from Eagle Island Rivers state Nigeria. These samples were transported with ice pack to the microbiology laboratory of Rivers state university, Port Harcourt within 24 hours for microbiological and toxicity testing. The used phone batteries were purchase from Garrison Junction, Port Harcourt. The toxicity testing was done for duration of 4 hours interval for 24 hours respectively at room temperature.

Methodology: Standard microbiological techniques were used; Toxicity testing procedures were carried out by preparing mobile phone batteries at concentrations of 0%, 5%, 25%, 50% and 75%, tested for duration of 0h, 4h, 8h, 12h and 24h respectively. The cultures were incubated at 35°C for 18 to 24 hours. LC_{50} was determine using SPSS version 20

Results: The results indicate that percentage logarithm mortality of *Nitrosomonas* species increases with increased toxicants concentration and exposure time. The median lethal concentration (LC_{50}) of the mobile phone batteries increases in the following order: (Note: the higher the LC_{50} the Lower toxic the toxicant); Nokia phone battery in marine water (65.97%)<Tecno phone battery in Brackish water (65.84%)<Tecno phone battery in marine water (65.57%)<Nokia phone battery in brackish water (65.47%)<Nokia phone battery in fresh water (64.17%) Tecno phone battery in fresh water (64.13%).

Conclusion: Tecno phone battery in fresh water (LC_{50} = 64.13%) is the most toxic; having the lowest LC_{50} while Nokia phone battery in marine water (LC_{50}= 65.97%) has the lowest toxicity effect. These results show that spent phone batteries can inhibit the nitrification process in aquatic ecosystem.
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Keywords: used phone batteries, Toxicity, *Nitrosomonas*, fresh, Brackish, Marine, Nitrification, ecosystem.

1. INTRODUCTION

Ecotoxicology is the branch of toxicology concerned with the study of toxic effects caused by natural or synthetic pollutants, to the constituents of an ecosystems; animals (including human), vegetations and microorganisms, (Aquastel, 2007). Normal micro flora of an aquatic ecosystem controls the habitability of the earth through their functions in biogeochemical cycles and food webs. The oceans aquatic environments are also sensitive to durable environmental changes including those of anthropogenic origin such as; E wastes disposal, etc. Microbial activities are essential to how ecosystems transform pollutants which are reflected in biogeochemical cycles and food webs, and how microorganisms respond to toxicant in an ecosystem will partially, if not primarily, determine the fate of that ecosystem when the assimilative capacity have not been exceeded. (Aquastel, 2007)
According to Douglas and Nwachukwu (2016) the current general direction in technological advancement and latest discoveries in information and communication technology, Electronic Devices such as Laptop and Phones have become a part of day to day activities. The constant request and Use of Laptops and Phones results in the constant production of large amount of Electronic wastes yearly, these wastes are referred to as E- waste (Beata, 2014). The major components of the phone that makes it harmful to the Environment and human health when disposed is the Heavy metals contains in the battery once released into the environment, they continuously circulate therein, and can cause acute or chronic poisoning (Armstrong *et al*., 2005). “When they are released into the aquatic environment, they pollute our water bodies and when thrown into 'dump' areas their toxic

ingredients are left to seep into the soil, finally to groundwater, causing massive and devastating damage to our natural ecosystem" (Armstrong *et al*., 2005). Some metals such as mercury, lithium, cadmium, chromium, and lead are especially toxic to aquatic organisms and humans (Douglas, and Nwachukwu, 2016). But most times microorganisms are not considered when discharging wastes into water bodies and these microorganisms play vital role in an ecosystem especially *Nitrosomonas* species.

Therefore the aim of this study is to analysis and compares the toxic effect of different product of spent batteries on *Nitrosomonas* species in Fresh Brackish and Marine environments.

2. MATERIAL AND METHODS

2.1 Sample Collection/Study Area

Fresh water sample was collected in sterile (4) litre plastic container from Biara stream, while marine water was collected from Bodo city both in Gokana L.G.A, Rivers state, also, brackish water was collected from Eagle Island River in Port Harcourt L.G.A Rivers state Nigeria with a four (4) litre sterile plastic container. These samples were transported to the microbiology laboratory with ice pack within 24 hours.

2.2 Microbiological Analysis

2.2.1 Total Heterotrophic Bacteria (THB)

Total heterotrophic bacteria for each water samples were enumerated using spread plate method. An aliquot (0.1ml) of the dilution of 10^{-6} were aseptically transferred unto properly dried nutrient agar plates in duplicate , spread evenly using bent glass rod and incubate at 37□C for 24 to 48 hours, after incubation, the bacterial colonies that grew on the plates were counted and sub-cultured unto fresh nutrient agar plates using the streak plate technique. Discrete colonies on the plates were aseptically transferred into 10% (v/v) glycerol suspension, well label and stored as stock cultures for preservation and identification (Amadi *et al.,* 2014). Total Heterotrophic Bacteria (THB) Counts for each sample were then calculated using the below formula:

$$\text{THC (cfu/g)} = \frac{\text{Number of Colonies}}{\text{Dilution }(10^{-6}) \times \text{Volume plated (0.1ml)}}$$

(Nrior and Odokuma 2015).

2.2.2 Total Heterotrophic Fungi

The total fungi in each of water samples were enumerated using spread plate method. An aliquot (0.1ml) of the dilution of 10^{-4} dilution was aseptically transferred unto properly dried Sabouround Dextrose Agar plates containing antibiotic (Tetracycline and Penicillin) to inhibit bacterial growth, in duplicate , spread evenly using bent rod and incubate at 37□C for 3days , pure culture of fungal isolates were counted and sub-cultured unto Sabouround Dextrose Agar slant in bijou bottles for preservation and identification (Odokuma and Okpokwasili 1992).

Total Heterotrophic Fungi (THB) Counts for each sample were then calculated using the below formula:

$$\text{THFC (cfu/g)} = \frac{\text{Number of Colonies}}{\text{Dilution }(10^{-4}) \times \text{Volume plated (0.1ml)}}$$

2.2.3 Isolation Of *Nitrosomonas0* Species

Winogradsky Agar medium composition as modified by Odokuma and Nrior, 2015 was used: Agar agar 15.0g/l, $FeSO_4.7H_2O$ 0.4g/l, NaCl 2.0g/l, K_2HPO_4 $_{1.0g}$/l, $MgSO_4$ $.7H_2O$ 0.5g/l, and $(NH_4)_2SO_4$ 2.0g/l were dissolved in 1000ml of Distilled water and autoclaved at 121ºC for 15minutes (psi) after which it was allowed to reduce to about 40^0C and the medium was poured on the Petri-dishes. Then, the medium was allowed to solidify before progress to the hot air oven to dry the moisture. An aliquot from fresh , brackish and marine water respectively were inoculated unto the Winogradsky agar and incubate aerobically for 2 – 3days at room temperature (30± 2ºc), greyish, mucoid, flat colonies revealed pear-shaped, and Gram negative of *Nitrosomonas* (Odokuma and Nrior, 2015).

2.2.4 Confirmation of *Nitrosomonas* species

Suspected *Nitrosomonas* species were subcultured on a fresh Winogradsky agar medium and transfered into a broth containing Ammonium sulphate and sodium nitrate and incubated at about (30± 2ºc) for 2 - 3 days. 1ml of sulfanilic acid, dimethylnapthalamine and zinc dust was added to medium after (2) days of incubation. Red colouration indicated by nitrate production from ammonium sulphate was a confirmation of *Nitrosomonas* species.

2.3 Preparation of Stock Toxicant.

The phone Batteries (Nokia and Techno) were aseptically forced open and 4grams of each product was weighed on an electric weighing balance into 100ml of autoclaved fresh, brackish and marine water respectively as stock toxicant.

2.4 Toxicity Test Procedure

The toxicity tests were done by setting up fifteen test tubes aseptically covered with cotton wool. The test was carried out in five (5) separate test tubes containing appropriately autoclaved water samples from fresh, marine and brackish water from the habitat of the organism separately. In each of the test tubes, the four toxicant concentrations (5%, 25%, 50%, and 75%) were added separately. while the control consists of fresh, marine and brackish water respectively (Nrior and Gboto, 2017). One millilitre (1ml) of the test organism was added to each toxicant concentration in the test tubes containing (5%, 25%, 50%, 75% and control respectively). Then an aliquot (0.1ml) from each of the concentrations of the effluent were then plated out using spread plate technique on Winogradsky agar immediately after inoculation as zero (0) hour, inoculation and spreading continues after 4, 8, 12 and 24hours respectively and was incubated for 24 to 48 hours at room temperature (37± 2ºC). After which the colonies on the plates were counted (Odukuma and Nrior 2015).

2.4.1 Toxicity test of bacteria *Nitrosomonas* species in mobile phone batteries.

The percentage log survival of the *Nitrosomonas* species isolates in the mobile phone batteries effluent were calculated according to formula used by Nrior and Obire (2015). The percentage log survival of the *Nitrosomonas* isolates in the effluent was calculated by obtaining the log of the count in toxicant concentration, divided by the log of the count in the zero toxicant concentration and multiplying by 100. Thus:

$$\text{Percentage (\%) log survival} = \frac{\text{Log C}}{\text{Log c}} \times 100$$

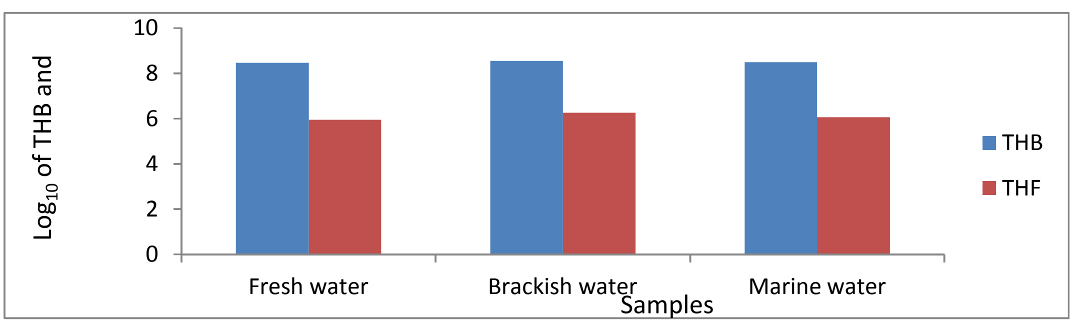
Where: Log C=log of the count in each toxicant concentration Log c = log of count in the control (zero toxicant concentration).

Percentage (%) log mortality = 100 - % log survival

3. RESULTS AND DISCUSSION

The Total Heterotrophic bacterial and fungal counts of the triaquatic bodies is presented in figure 4.1 below, the result revealed that brackish water have the highest microbial load followed by marine

Figure 4.1: Total Heterotrophic bacterial and total heterotrophic fungal counts expressed in Log_{10}.



The logarithm counts of *Nitrosomonas* species exposed to Nokia and Techno spent phone batteries toxicants in Fresh, Brackish, and Marine water are revealed in table 1 and 2 respectively. Percentage logarithm mortality of the counts is presented in the figures below.

Table 1: Log Counts Of *Nitrosomonas* Species With Nokia Phone Battery In Fresh, Brackish , And Marine Water

	Fresh Water + Nokia					Brackish Water + Nokia					Marine Water + Nokia				
Conc./time	**0H**	**4H**	**8H**	**12H**	**24H**	**0H**	**4H**	**8H**	**12H**	**24H**	**0H**	**4H**	**8H**	**12H**	**24H**
Control	**2.40**	**2.42**	**2.45**	**2.51**	**2.54**	**2.41**	**2.43**	**2.50**	**2.50**	**2.54**	**2.41**	**2.42**	**2.45**	**2.48**	**2.50**
5%	**2.39**	**2.38**	**2.14**	**2.38**	**2.40**	**2.39**	**2.38**	**2.41**	**2.42**	**2.40**	**2.38**	**2.34**	**2.33**	**2.34**	**2.31**
25%	**2.32**	**2.30**	**2.28**	**2.27**	**2.38**	**2.34**	**2.35**	**2.36**	**2.33**	**2.25**	**2.26**	**2.26**	**2.22**	**2.20**	**2.20**
50%	**2.21**	**2.19**	**2.16**	**2.14**	**2.15**	**2.27**	**2.26**	**2.24**	**2.22**	**2.02**	**2.13**	**2.15**	**2.12**	**2.04**	**2.04**
75%	**2.00**	**1.95**	**1.87**	**1.85**	**2.05**	**2.08**	**2.10**	**2.06**	**2.00**	**1.85**	**1.99**	**2.00**	**2.01**	**1.94**	**1.90**

TABLE 2: Log Counts of *Nitrosomonas* Species with Techno Phone Battery In Fresh, Brackish, And Marine Water

	Fresh Water + Nokia					Brackish Water + Nokia					Marine Water + Nokia				
CONC./TIME	**0H**	**4H**	**8H**	**12H**	**24H**	**0H**	**4H**	**8H**	**12H**	**24H**	**0H**	**4H**	**8H**	**12H**	**24H**
CONTROL	**2.40**	**2.42**	**2.45**	**2.51**	**2.58**	**2.41**	**2.43**	**2.50**	**2.50**	**2.54**	**2.41**	**2.42**	**2.45**	**2.48**	**2.50**
5%	**2.39**	**2.38**	**2.38**	**2.38**	**2.40**	**2.39**	**2.38**	**2.41**	**2.41**	**2.40**	**2.38**	**2.34**	**2.33**	**2.34**	**2.31**
25%	**2.32**	**2.30**	**2.28**	**2.27**	**2.32**	**2.34**	**2.35**	**2.36**	**2.33**	**2.25**	**2.26**	**2.26**	**2.22**	**2.20**	**2.20**
50%	**2.21**	**2.19**	**2.16**	**2.14**	**2.05**	**2.27**	**2.26**	**2.24**	**2.22**	**2.02**	**2.13**	**2.15**	**2.12**	**2.04**	**2.04**
75%	**2.00**	**1.95**	**1.87**	**1.85**	**1.85**	**2.08**	**2.10**	**2.06**	**2.00**	**1.85**	**1.99**	**2.00**	**2.01**	**1.94**	**1.90**

***TABLE 3*: Median lethal conc. (LC50) from percentage (%) log mortality of nokia battery on *nitrosomonas* sp. in fresh water**

Concentration	**% mortality**	**Mean % mortality**	**Conc. different**	**∑ of Conc. diff. × mean % mortality**
0	0	-	-	-
5	16	3.2	5	16
25	43	8.6	20	172
50	79	15.8	25	395
75	100	20	25	500
				1083

$$LC_{50} = LC_{100} - \frac{\sum \text{CONC. DIFF.} \times \text{MEAN \% MORTALITY}}{\text{\% CONTROL}}$$

$$LD_{50} = 75 - \frac{1083}{100}$$

$$LD_{50} = 75 - 10.83$$

$$LD_{50} = 64.17\%$$

Table 4: Median Lethal Conc. (LC50) from Percentage (%) log mortality of Nokia battery on *Nitrosomonas* sp. in brackish water

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	0	-	-	-
5	27	5.4	5	27
25	34	6.8	20	136
50	64	12.8	25	320
75	94	18.8	25	470
				953

$$LC_{50} = LC_{10\,0} - \frac{\sum \text{Conc. diff.} \times \text{mean \% mortality}}{\text{\% control}}$$

$$LD_{50} = 75 - \frac{953}{100}$$

$$LD_{50} = 75 - 9.53$$

$$LD_{50} = 65.47\%$$

Table 5: Median Lethal Dose (LD50) from Percentage (%) log mortality of Tecno battery on *Nitrosomonas* sp. in marine water

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	0	-	-	-
5	19	3.8	5	19
25	37	7.4	20	148
50	66	13.2	25	330
75	118	23.6	25	590
				1087

$$LC_{50} = LC_{100} - \frac{\sum \text{Conc. diff.} \times \text{mean \% mortality}}{\text{\% control}}$$

$$LD_{50} = 75 - \frac{1087}{100}$$

$$LD_{50} = 75 - 10.87$$

$$LD_{50} = 64.13\%$$

***Table 6*: Median Lethal conc. (LC50) from Percentage (%) log mortality of Tecno battery on *Nitrosomonas* sp.** in Fresh water

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	0	-	-	-
5	18	3.6	5	18
25	32	6.4	20	128
50	60	12	25	300
75	94	18.8	25	470
				916

$$LC_{50} = LC_{100} - \frac{\sum \text{Conc. diff.} \times \text{mean \% mortality}}{\text{\% control}}$$

$$LD_{50} = 75 - \frac{916}{100}$$

$$LD_{50} = 75 - 9.16$$

$$LD_{50} = 65.84\%$$

Table 7: Median Lethal Conc. (LC50) from Percentage (%) log mortality of Tecno battery on *Nitrosomonas* sp. in brackish water

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	0	-	-	-
5	17	3.4	5	27
25	34	6.8	20	136
50	64	12.8	25	320
75	94	18.8	25	470
				943

$$LC_{50} = LC_{100} - \frac{\sum \text{Conc. diff.} \times \text{mean \% mortality}}{\text{\% control}}$$

$$LD_{50} = 75 - \frac{943}{100}$$

$$LD_{50} = 75 - 9.43$$

$$LD_{50} = 65.57\%$$

Table 8: Median Lethal Dose (LD50) from Percentage (%) log mortality of Nokia battery on *Nitrosomonas* sp. in Marine water

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	0	-	-	-
5	18	3.6	5	18
25	32	6.4	20	130
50	61	12.2	25	305
75	90	18	25	450
				903

$$LC_{50} = LC_{100} - \frac{\sum \text{Conc. diff.} \times \text{mean \% mortality}}{\text{\% control}}$$

$$LD_{50} = 75 - \frac{903}{100}$$

$$LD_{50} = 75 - 9.03$$

$$LD_{50} = 65.97\%$$

FIGURE 1 : Summary of median lethal conc. (LC50) of mobile phone batteries (Nokia and Tecno) on *Nitrosomonas sp*. in freshwater, brackish water and marine.

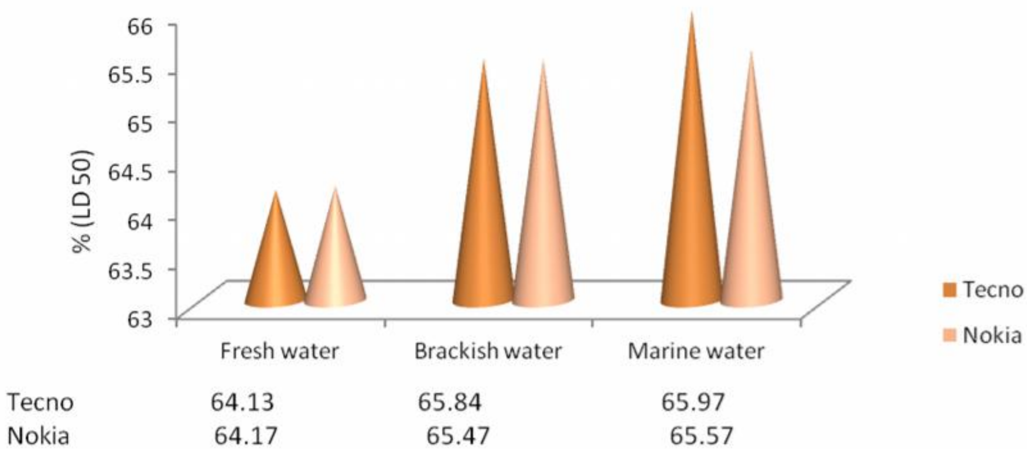


Figure 1 : Summary of Median Lethal conc. (LC50) of Moblie Phone batteries (Nokia and Tecno) on Nitrosomonas sp. in freshwater, brackish water and marine.

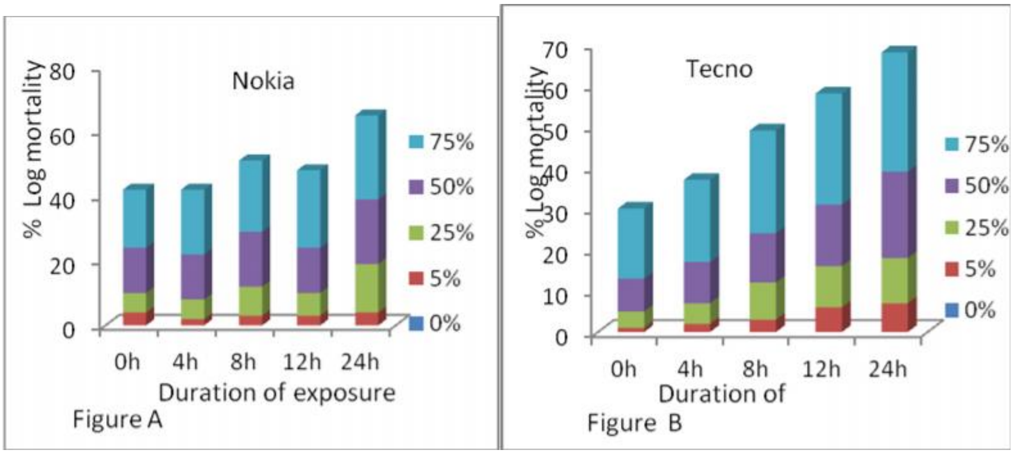


Figure A and B show the Percentage Logarithm Mortality of Nitrosomonas species with Nokia and Techno phone battery in fresh water respectively.

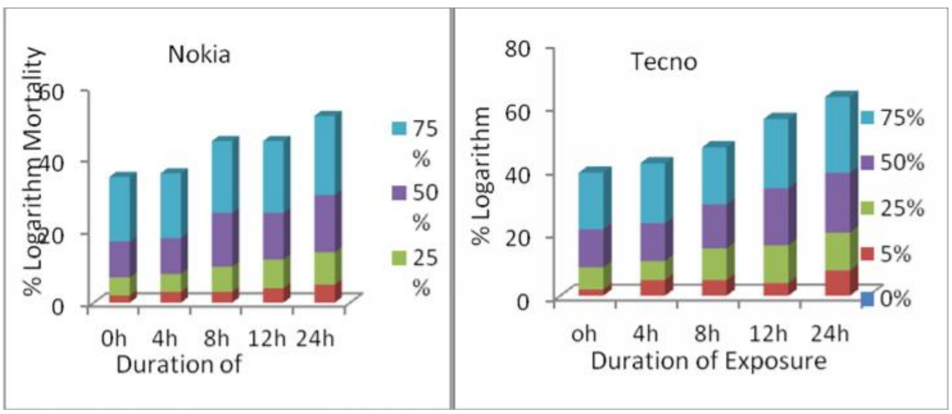


Figure C and D show the Percentage Logarithm Mortality of *Nitrosomonas* species with Nokia and Techno phone battery in brackish water respectively.

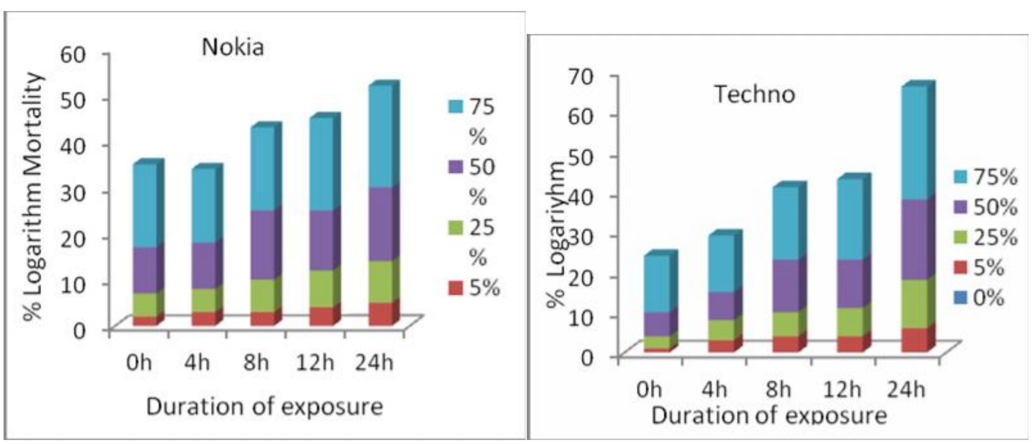


Figure E and F show the Percentage Logarithm Mortality of *Nitrosomonas* species with Nokia and Techno phone battery in Marine water respectively

The results obtained in this study revealed that the toxicants can inhibit the nitrification process by *Nitrosomonas* species. Similar observations have been reported by (Wang, 1985, Obire and Nrior, 2014, Nrior and Gboto 2017). An increase in the percentage logarithmic of mortality of *Nitrosomonas* species in Fresh, Marine and Brackish water after 24 hours of exposure to the toxicant concentrations were observed (figures A to F) respectively. This study also revealed that the toxicant (Techno product) toxicant is more toxic to the organism than the Nokia product. This may be as a result of types and level of heavy metals, according to Sander ***et al,***.(1985) and the site of action of any toxicant depends on the nature of the toxicant.

The percent log survival of *Nitrosomonas* species during 0hr, 4hr, 8hr, 12hr, and 24hr exposure periods to these phone battery products carried out in fresh, brackish water and Marine environments (Table 4.1 and 4.2) respectively shows that both Nokia and Techno batteries exhibited little effect on the test organism in fresh water than brackish water followed by Marine. This may be due to saline nature of the marine and brackish water. The percent log mortality of *Nitrosomonas* species during 0hr, 4hr, 8hr, 12hr, and 24hr exposure periods to the different concentrations of the toxicants shows that the mortality rate on Techno is higher than that of Nokia battery (figures A to F). Hence, the results of this study suggest that both toxicants caused cell death which resulted reduction in the viable counts. This may be due to inhibition of the nitrification process within the 24hour exposure period. Similar observation was reported by Nrior and Odokuma (2015) who worked on the Toxicity of domestic washing bleach (Calcium hypochloride) and detergents on *Escherichia coli.*

Nitrosomonas sp. mortality expressed as Median Lethal concentration (LC_{50}) was used as indices to monitor toxicity (Nrior and Gboto, 2017). The sensitivity of the bacterium to the toxicity of the different concentration of used mobile phone batteries (Nokia and Tecno) with the different water (freshwater, brackish water and marine water) The median lethal Concentration (LC_{50}) of the mobile phone batteries used increases in the following order: (Note: the higher the LC_{50} the Lower toxic the toxicant and vice-vesa); Tecno phone battery in marine water (65.97%) $<$ Tecno phone battery in Brackish water (65.84%) $<$ Nokia phone battery in marine water (65.57%) $<$ Nokia phone battery in brackish water (65.47%) $<$ Nokia phone battery in fresh water (64.17%) Tecno phone battery in fresh water (64.13%).Conclusively, Tecno phone battery in fresh water (LC_{50} = 64.13%) is the most toxic; having the lowest LC_{50} while Nokia phone battery in marine water (LC_{50}= 65.97%) has the lowest toxicity effect. (Table 4.3-4.8, Fig. 1)

Conclusion and Recommendation

The result revealed that, different concentrations of the toxicants have negative effect s on the survival rate of test organism which show that the content of these batteries can cause environmental pollution affecting *Nitrosomonas* species and other microorganism that play vital functions in an ecosystem not only that but also, batteries can also cause divers kind of acute and chronic health problems in humans and plants if released into the environment.

Therefore it is recommended that phone batteries should not be disposed directly into aquatic environment especially fresh water but rather it should be recycled.

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