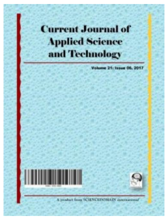

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Comparative Ecotoxicological Analyses of Spent Phone Batteries on *Pseudomonas* sp. in Tri Aquatic Environment

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Authors' contributions

This work was carried out in collaboration between both authors. Author LBK designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and literature searches under the strict supervision of author RRN. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To analyze and compare the effect of two products of spent phone batteries on *Pseudomonas* sp. in Marine, brackish and freshwater using standard toxicological bioassay.
Study Design: The study employs experimental design, statistical analysis of the data and interpretation.
Place and Duration of Study: Freshwater and Marine samples were collected from Gokana L.G.A while, brackish sample was collected from Eagle Island, all in Rivers state, Nigeria. These samples were transported with ice pack to the Microbiology Laboratory of Rivers State University, Port Harcourt, Nigeria, for analyses within 24 hours. Spent Nokia and Techno phone batteries were obtained from the main phone market, Garrison junction, Aba road, Port Harcourt.
Methodology: Toxicity testing procedures were carried out by dissolving four (4) grams of the spent phone batteries content into one hundred milliliter (100 ml) each of autoclaved water bodies separately. This served as a stock solution, from which different concentrations (%); 0, 5, 25, 50 and

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75, were made; each was inoculated with one milliliter (1 ml) of the test organism (*Pseudomonas* sp.) and tested for duration 0,4,8,12,and 24 hours respectively using spread plate techniques. The cultures were incubated at 35℃ for 18 to 24 hours. Median lethal concentration (LC_{50}) was determined using SPSS version 20.

Results: The results revealed that percentage logarithm survival of *Pseudomonas* sp decreased with increasing exposure time and concentrations. (LC_{50}) of the spent phone batteries ranging from 61.76 to 65.31%. Nokia phone battery in freshwater (65.31%) <Techno phone battery in freshwater (65.14%)<Techno phone battery in marine (64.73%)<Nokia phone battery in brackish (64.53%)<Nokia phone battery in fresh water (64.17%) < Nokia phone battery in marine (62.75%) < Techno phone battery in marine (61.76%)(noting; the lower the LC_{50} the more toxic the toxicant)

Conclusion: The effect of Techno phone battery in marine is the most toxic (LC_{50} = 61.76%) having the lowest LC_{50} while Nokia phone battery in freshwater (LC_{50}= 65.97%) has the lowest toxicity effect this is because, statistically when the median lethal concentration is high the toxicity effect is low. These results show that spent phone batteries if disposed into aquatic environments can inhibits normal biological processes within the aquatic ecosystem.

Keywords: Spent phone batteries Nokia and Tecno; ecotoxicology; Pseudomonas; median lethal concentration; marine; brackish and freshwater.

1. INTRODUCTION

According to Otsuka [1] in most developing countries, the rapid pace of urbanization is a challenge to urban environmental management. One major challenge of waste management facing some urban areas is electronic waste (e-waste). E-waste are generated from electronic devices such as television, laptop, mobile phones etc. [2]. The increasing rapid evolution of electronic technology, coupled with rapid product obsolescence, has compounded the e-waste problem. The amount of e-waste generated globally is growing at a rate nearly three times faster than the growth of overall municipal solid waste [3]. According to UNEP [4], the annual e-waste generated worldwide is estimated to be 20–50 million tons (t). Unfortunately, between 50% and 80% of such e-waste is prospectively exported to developing countries like Ghana, china, India and Nigeria [5,6]. This accumulated e-waste is poorly managed in the country, because proper systems for recycling and disposal of them are lacking [7]. The uncontrolled dumping and inappropriate recycling of e-waste poses serious threats to both micro and macro organisms and the environment at large [8]. The toxic chemicals that exist in e-waste include a wide range of heavy metals, such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As) and nickel (Ni), and also persistent organic compounds, such as brominated flame retardants (BFRs) and Phthalates. Other chemicals that appear in e-waste include the polychlorinated Biphenyls (PCBs), Nonylphenol (NP), and triphenyl phosphate (TPPs), and others [9,10]. Once released in the environment, it can accumulate in the bodies of aquatic organisms and agricultural crops. Due to its long half-life and stability, they specially can bioaccumulate in the body Continuous, low-level exposures to cadmium causes kidney disease and bone brittleness [11].

These multitude of hazardous substances contained in e-waste have the ability to inhibit normal biological processes in an environment where they are been released specially in an aquatic environment because it may affect some microorganisms such as *Pseudomonas, Nitrosomonas, Nitrobacter*, *Mucor, Penillium* species etc which play a fundamental role in the biogeochemical cycles [12]. Bacteria are ubiquitous, and capable of rapid growth when provided with nutrients and conditions favourable for metabolism and cell division, they are involved in catalysis and synthesis of organic matter in the aquatic and terrestrial environments. Many substances, such as lignin, cellulose, chitin, pectin, agar, hydrocarbons, phenols, and other organic chemicals, are degraded by microbial action [13].

The rate of decomposition of organic compounds depends upon their chemical structure and complexity and upon environmental conditions. The nitrogen cycle, including fixation of molecular nitrogen and denitrification, is mediated by microorganisms in the natural environment. other bio- geochemical cycles, including the sulphur, phosphorus, iron, and manganese cycles also depend primarily upon microbial activity [14]. Transformation and mobilization of heavy metals, degradation of pesticides, herbicides, and other man-made, allochthonous materials are left, ultimately to the microorganisms, for recycling.

The toxic effects of pollutants on the autochthonous microbial populations, therefore, become of major significance in ecotoxicology [13].

Studies conducted by Nrior and Gboto [15] Kpormon and Douglas [16] showed that spent phone batteries contained hazardous substances that are considered toxic to aquatic life. Therefore the aim of this research is to analyze and compare the level of toxic effect poses by Nokia and Techno mobile phone batteries to *Pseudomonas* species which is a key hydrocarbon degrader in the three aquatic environments.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

Fresh water sample was collected in sterile four (4) litres 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 using four (4) litres sterile plastic container from Eagle Island River in Port Harcourt City L.G.A. Rivers state Nigeria. These samples were taken to the Microbiology laboratory of Rivers State University, Port Harcourt, Nigeria, with ice pack within 24 hours of collection.

2.2 Isolation of Test the Organism

The test organism *Pseudomonas* sp. was chosen because of its importance as a key hydrocarbon degrader in crude oil polluted environment. It was isolated from the water samples using standard microbiological method (spread plate technique). An aliquot (0.1 ml) of an appropriate dilution (10^{-3}) were aseptically transferred to properly pre-dried Centrimide agar plates in duplicate, spread evenly using flamed bent glass rod and incubated for 24 to 48 hours at 37℃. After incubation, the bacterial colonies that grew on the plates were sub-cultured on fresh nutrient agar plates using the streak plate technique. Discrete colonies on the plates were aseptically transferred into 10% (v/v) glycerol suspension, well labelled and stored as stock cultures for preservation [17].

2.3 Confirmation of Test the Organisms

The isolate was confirmed according to the standard techniques in Biochemical testing of microorganisms and medical laboratory manual for tropical countries [18] and was identified base on the Bergey's Manual of Determinative Bacteriology after carrying out the morphological and various biochemical tests.

2.4 Preparation of Stock Toxicant

The phone Batteries (Nokia and Techno) were aseptically forced open and four (4) grams of each product was weighed on an electric weighing balance and dissolved into one hundred millilitre (100 ml) of each autoclaved water bodies; freshwater, brackish and marine respectively. This served as stock toxicant solution.

2.5 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. One millilitre (1 ml) 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 pre-dried Centrimide agar immediately after inoculation as zero (0) hour, inoculation and spreading continues after 4, 8, 12 and 24 hours respectively and was incubated for 24 to 48 hours at room temperature (37± 2ºC). After which the colonies on the plates were counted and converted to Logarithm base 10 ($\log_{10}$) [19,15,16].

2.5.1 Percentage log survival of *Pseudomonas* sp. in mobile phone batteries

The percentage log survival of the test organism (*Pseudomonas* sp.) exposed to the spent mobile phone batteries effluent were calculated according to the formula used by Nrior and Obire [19]. The percentage log survival of the *Pseudomonas* 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 = Logarithm count in each toxicant concentration, Log c = Logarithm count in the control (zero toxicant concentration).

2.5.2 Percentage log mortality of *Pseudomonas* sp. in mobile phone batteries

The Percentage (%) log mortality of the test organism was obtained by subtracting one hundred from the value of the Percentage (%) log survival. ie

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

2.6 Determination of the Median Lethal Concentration (LC_{50})

The median lethal concentration of the toxicant in the tri aquatic environments were determined by subtracting the value of the highest concentration value used from the sum of concentration different, multiply by mean percentage mortality divide by the control [15,16]. That is

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

3. RESULTS AND DISCUSSION

The logarithm counts of *Pseudomonas* species exposed to the toxicants of the two products of mobile phone (Nokia and Tecno) batteries in Fresh, Brackish, and Marine water are revealed in Tables 1 and 2 respectively. The median lethal concentrations for the tri aquatic environments are shown in Tables 3 to 8 while Fig. 1 show the summary of the median concentrations (Lc_{50}). Percentage logarithm mortality of the counts and Figs. 2 to 7 shows the percentage log. Survival of the test organism in the tri aquatic environments.

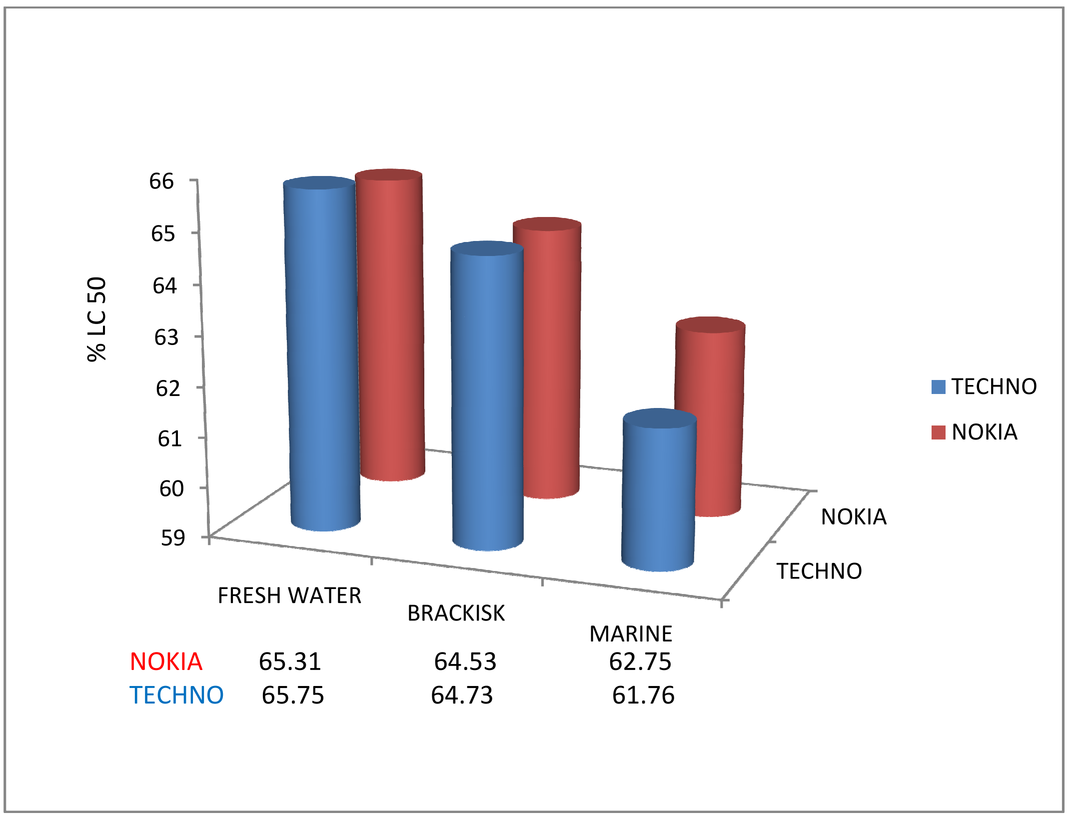

Fig. 1. Summary of median lethal concentration of mobile phone batteries (Nokia and Techno) on *Pseudomonas* sp. in freshwater, brackish and marine

Table 1. Log Counts of *Pseudomonas* Sp. with Nokia Phone Battery In the tri aquatic environments

	Fresh Water + Nokia					Brackish Water + Nokia					Marine Water + Nokia				
Conc./time	**0 h**	**4 h**	**8 h**	**12 h**	**24 h**	**0 h**	**4 h**	**8 h**	**12 h**	**24 h**	**0 h**	**4 h**	**8 h**	**12 h**	**24 h**
Control	2.30	2.34	2.37	2.41	2.45	2.26	2.31	2.39	2.45	2.48	2.29	2.38	2.42	2.46	2.49
5%	2.23	2.24	2.23	2.20	2.20	2.19	2.20	2.23	2.25	2.20	2.16	2.18	2.20	2.22	2.20
25%	2.19	2.21	2.20	2.14	2.13	2.06	2.08	2.10	2.13	2.11	2.13	2.14	2.13	2.16	2.11
50%	2.08	2.10	2.08	2.02	1.96	1.98	2.00	2.04	2.06	2.04	1.98	2.04	1.97	2.00	2.04
75%	1.97	2.01	1.99	1.95	1.84	1.79	1.97	1.99	2.01	1.97	1.89	1.97	1.91	1.88	1.84

Table 2. Log Counts of *Pseumonas* Sp. with Tecno Phone Battery In the tri aquatic environments

	Fresh Water + Nokia					Brackish Water + Nokia					Marine Water + Nokia				
Conc./time	**0 h**	**4 h**	**8 h**	**12 h**	**24 h**	**0 h**	**4 h**	**8 h**	**12 h**	**24 h**	**0 h**	**4 h**	**8 h**	**12 h**	**24 h**
CONTROL	2.08	2.17	2.26	2.37	2.44	2.17	2.23	2.34	2.43	2.53	2.26	2.34	2.37	2.40	2.50
5%	2.02	2.04	2.13	2.16	2.18	2.11	2.13	2.15	2.17	2.23	2.17	2.20	2.19	2.17	2.21
25%	1.94	2.00	1.99	2.07	2.13	2.04	2.07	2.07	2.08	2.13	2.10	2.13	2.10	2.11	2.15
50%	1.80	1.89	1.91	1.99	2.07	1.99	2.00	2.04	2.00	1.99	2.00	2.06	2.07	2.07	2.10
75%	1.68	1.67	1.88	1.92	1.77	1.97	1.93	2.00	1.99	1.93	1.94	1.95	1.95	1.92	1.98

Table 3. Median lethal conc. (LC50) from percentage (%) log mortality of Nokia battery on *Pseudomonas* sp. in fresh water

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	-		-	
5	32.15	6.43	5	32.15
25	41.78	8.36	20	167.2
50	65.42	13.08	25	327
75	88.47	17.69	25	442.25
				∑ = 968.6

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

$$LC_{50} = 75 - \frac{968.6}{100}$$

$$LC_{50} = 75 - 9.69$$

$$LC_{50} = 65.31\%$$

Table 4. Median lethal conc. (LC50) from percentage (%) log mortality of Nokia battery on *Pseudomonas* sp. in Brackish

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	-	-	-	
5	34	6.8	5	34
25	56.94	11.39	20	227.8
50	72.05	14.41	25	360.25
75	84.77	16.95	25	424.75
				∑ = 1045.8

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

$$LC_{50} = 75 - \frac{1045.8}{100}$$

$$LC_{50} = 75 - 10.56$$

$$LC_{50} = 64.54\%$$

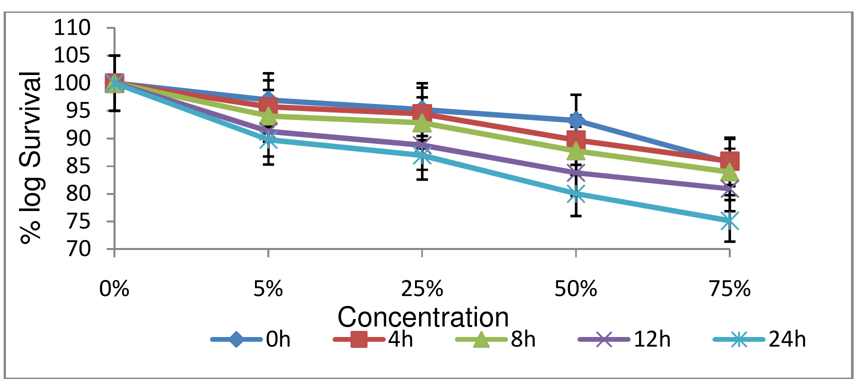

Fig. 2. Show the percentage logarithm survival of *Pseudomonas* sp. with Nokia phone battery in fresh water

Table 5. Median lethal conc. (LC50) from percentage (%) log mortality of Nokia battery on *Pseudomonas* sp. in Marine

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0			-	
5	44.59	8.92	5	44.59
25	59.51	11.90	20	238.04
50	83.19	16.6	25	415
75	105.46	21.09	25	527.3
				∑= 1224.93

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

$$LC_{50} = 75 - \frac{1224.93}{100}$$

$$LC_{50} = 75 - 12.25$$

$$LC_{50} = 62.75\%$$

Table 6. Median lethal conc. (LC50) from percentage (%) log mortality of Tecno battery on *Pseudomonas* sp. in fresh water

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	-	-	-	-
5	34.18	6.84	5	34.18
25	51.79	10.36	20	207.2
50	67.91	13.58	25	339.55
75	105.56	16.2	25	405
				∑ = 985.73

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

$$LC_{50} = 75 - \frac{985.73}{100}$$

$$LC_{50} = 75 - 9.86$$

$$LC_{50} = 65.14\%$$

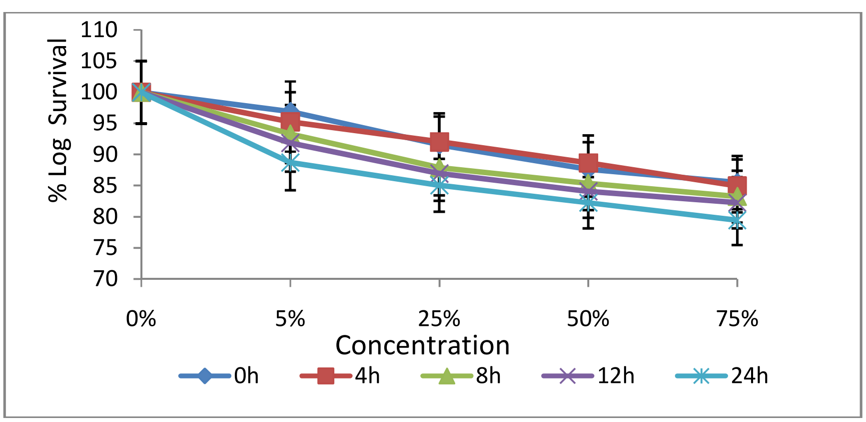

Fig. 3. Show the percentage logarithm survival of *Pseudomonas* sp. with Nokia phone battery in Brackish

Table 7. Median lethal conc. (LC50) from percentage (%) log mortality of Tecno battery on *Pseudomonas* sp. in Brackish

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0	-	-	-	-
5	37.94	7.59	5	37.94
25	54.59	10.92	20	218.36
50	70.5	14.1	25	352.5
75	83.65	16.73	25	418.25
				∑ = 1027.05

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

$$LC_{50} = 75 - \frac{1027.05}{100}$$

$$LC_{50} = 75 - 10.27$$

$$LC_{50} = 64.73\%$$

Table 8. Median lethal conc. (LC50) from percentage (%) log mortality of Tecno battery on *Pseudomonas* sp. in Marine

Concentration	% mortality	Mean % mortality	Conc. different	∑ of Conc. diff. × mean % mortality
0			-	
5	38.75	7.75	5	38.75
25	64.87	12.97	20	259.48
50	75.88	15.18	25	379.4
75	129.35	25.87	25	646.75
				∑= 1324.38

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

$$LC_{50} = 75 - \frac{1324.38}{100}$$

$$LC_{50} = 75 - 13.24$$

$$LC_{50} = 61.76\%$$

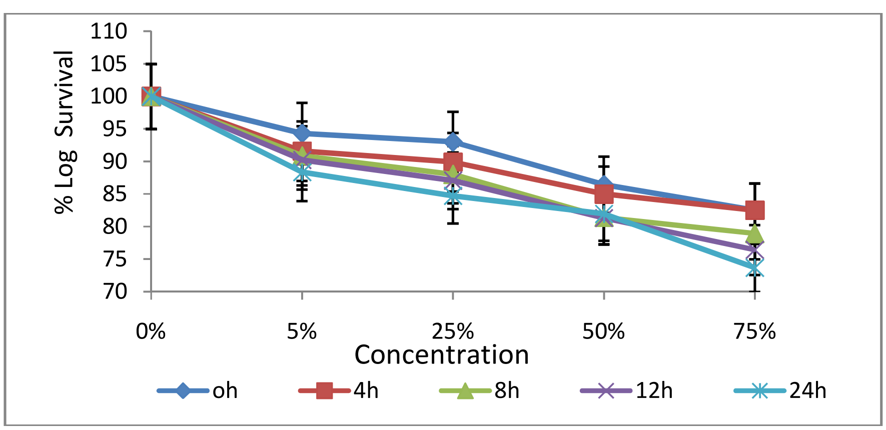

Fig. 4. Show the percentage logarithm survival of *Pseudomonas* sp. with Nokia phone battery in Marine

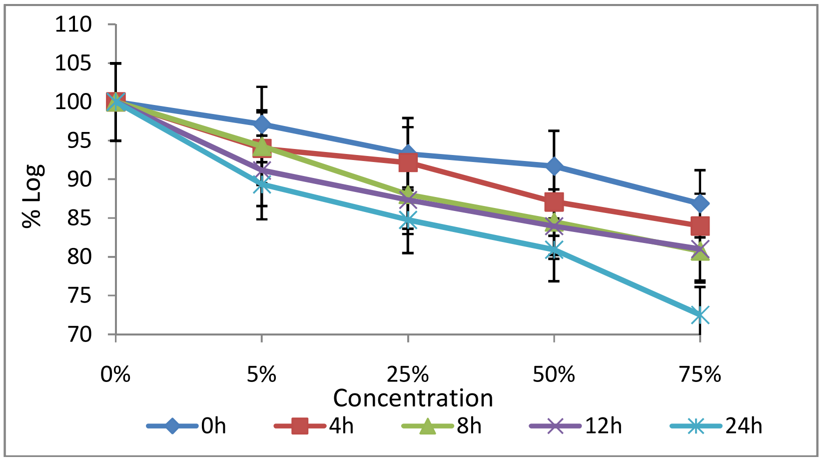

Fig. 5. Lethal toxicity of Tecno phone battery on *Pseudomonas* sp. in fresh water

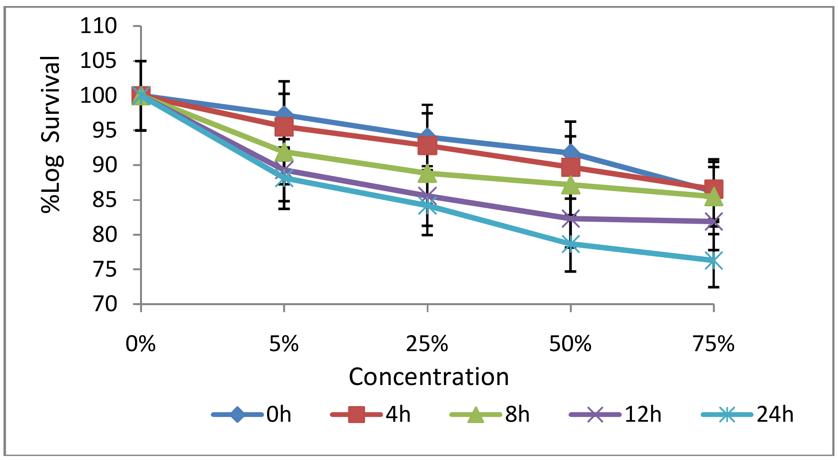

Fig. 6. Lethal toxicity of Tecno phone battery on *Pseudomonas* sp. in brackish

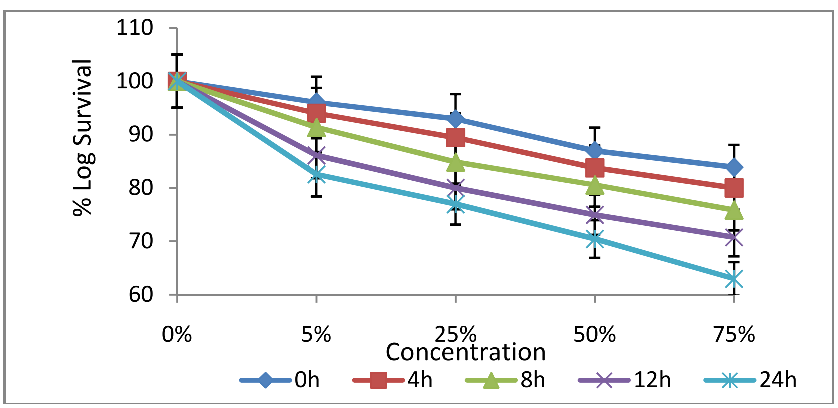

Fig. 7. Lethal toxicity of Tecno phone battery on *Pseudomonas* sp. in marine

The results obtained in this study revealed that the toxicants have the ability to affect microorganisms and in the aquatic ecosystem not only the biotic components of the environment but also the environment could be affected by the toxicants. The result concord with the observations reported by [19,15,16]. A simultaneous decrease in the percentage logarithmic survival of the test organism in the tri aquatic environments after 24 hours of exposure to the toxicant concentrations was observed (Figs. 3 to 7) respectively. This study also revealed that Techno product mobile phone battery in freshwater 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. [20] and the site of action of any toxicant depends on the nature of the toxicant.

The percent log survival of the test organism during the twenty four hours (24 hr) exposure periods to spent mobile phone batteries toxicant in the tri aquatic environments; (freshwater, Brackish and Marine) shows that both Nokia and Techno batteries exhibited toxic effect on the organism in marine than brackish followed by freshwater. This may be due to the saline nature of the marine and brackish water. The percent log survival of *Pseudomonas* species during 0 hr, 4 hr, 8 hr, 12 hr, and 24 hr exposure periods to the different concentrations of the toxicants shows that the survival rate on Techno is lower than that of Nokia batteries (Figs. 2 to 7). Hence, the results of this study suggest that both toxicants caused cell death which resulted in reduction in the viable counts. This result is in agreement with the report of Nrior and Gboto 2017, they worked on the toxicity of Samsung and Tecno mobile phone batteries on Nitrobacter species and observed similar result. Not only that but also Nrior and Owhonda 2017 Compared the strength of spent mobile phone batteries; Blackberry and Nokia on Bioassay Evaluator *Nitrobacter* sp and observed reduction in viable counts.

Median Lethal concentration (LC_{50}) was used as indices to monitor toxicity and the sensitivity of this bacterium to the toxicity of the different concentrations of spent mobile phone batteries (Nokia and Techno) with the different water bodies [15]. The median lethal Concentration (LC_{50}) of the spent mobile phone batteries decreased in the following order Nokia phone battery in freshwater (65.31%) <Techno phone battery in fresh water (65.14%) <Techno phone battery in marine (64.73%)<Nokia phone battery in brackish (64.53%)<Nokia phone battery in fresh water (64.17%) < Nokia phone battery in fresh marine (62.75%) < Techno phone battery in marine (61.76%).

(Note: the higher the $LC_{50,}$ the Lower the toxic effect and vice-vesa); Conclusively, Techno phone battery in marine (LC_{50}= 61.76%) is the most toxic; having the lowest LC_{50} while Nokia phone battery in freshwater has the lowest toxicity effect having the highest median lethal concentration (LC_{50} = 65.14%). Fig. 1 shows the summary of the median lethal concentration (LC_{50}) for the two mobile phone product used in the tri-aquatic ecosystems.

4. CONCLUSION AND RECOMMENDATION

The results revealed that, spent mobile phone batteries toxicants have negative effect on the survival rate of the test organism which indicate that these batteries is capable of causing serious environmental pollution, affecting the both biotic and abiotic component of the environment specially microorganisms such as *Pseudomonas* species and other that play vital functions in an ecosystem not only that, but also, batteries can cause divers kind of acute and chronic health challenges in humans and plants if released into the environment.

Therefore, it is recommended that proper spent mobile phone batteries management system should developed by the producers to avoid direct disposal into aquatic environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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