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Toxicity of Spent Phone Batteries on Microflora in Marine, Brackish and Freshwater Ecosystems

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Authors' contributions

This work was carried out in collaboration between all authors. Author SID designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RRN and LBK managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To analyse and compare the effect of two products of spent phone batteries on bacteria (*Pseudomonas* sp.) and fungi *(Mucor* 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 was collected from Biara and Marine samples were collected from Bodo City both of Gokana L.G.A, while brackish water sample was collected from Eagle Island, all in Rivers State, Nigeria. These samples were transported with ice pack to the Microbiology Laboratory of the Rivers State University, for analyses within 6 hours. While Spent phone batteries (product A and B) were obtained from the phone market, Garrison junction, Aba road, Port Harcourt.

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Methodology: Toxicity testing procedures were carried out by preparing stock toxicant solution (four (4) grams of the spent phone battery content put into one hundred milliliter (100 ml) each of sterilized water samples separately), from which different concentrations (%); 0, 5, 25, 50 and 75, were made; each was inoculated with one milliliter (1 ml) of the test organisms (*Pseudomonas* sp. and *Mucor* sp.) in a separate set-up and tested for duration 0, 4, 8, 12, and 24 hours respectively using the spread plate techniques. The bacterial cultures were incubated at 37°C for 24 hours while fungal cultures were incubated for three (3) days at 35°C. The logarithms of total viable counts were used as a directory to determine the percentage survival and mortality. Median lethal concentration (LC_{50}) was determined using the formulae; $LC_{50} = LC_{100} - \sum$ conc. Diff. × mean % mortality / % control. Data obtained were analyzed statistically using SPSS version 22.
Results: The results revealed that percentage logarithm survival of *test* organisms decreased with increasing exposure time and concentrations. The median lethal concentration (LC_{50}) of the mobile phone batteries increases in the following order: (Note: the higher the LC_{50}, the Lower the toxic effect) for *Pseudomonas* sp. Product B in freshwater (57.54%) < Product B in Brackish water (57.99%) < Product A in freshwater water (58.22%) < in brackish water (58.68%) < Product A in brackish water (58.88%) Product A in marine water (58.99%). While for *Mucor* sp.; Product A in freshwater (61.33%) < Product B in freshwater (61.55%) < Product B in brackish water (65.66%) < Product A in brackish water (71.88%)< Product A in marine water (71.88%) Product B in fresh water (74.22%).
Conclusion: The effect of Product B in fresh water is the most toxic having the lowest while Product A in marine has the lowest toxicity effect. These results show that if spent phone batteries are disposed into the aquatic environment, *Pseudomonas* sp will be more affected than the *Mucor* sp.

Keywords: Spent phone batteries; environmental; Pseudomonas sp.; Mucor sp.; mortality; tri-aquatic ecosystem.

1. INTRODUCTION

According to Douglas and Nwachukwu [1] the demands on charged batteries used in different electronic devices, such as cellular phones, are growing rapidly worldwide. In the last decades. the discarded spent cellular phone batteries cause serious environmental problems because it contains relatively high concentrations of hazardous metals in their electrodes. Various types of batteries, such as; Li -ion (libs), nickel–cadmium (Ni–Cd), and nickel-metal hydride (Ni-Mh) batteries, are used for different electronic products due to its good performance compared to the other batteries, are becoming the most dominant powerful source [2]. These batteries are composed of a cathode, an anode, an electrolyte, and a separator [3]. Thus, the production of phone batteries and consequently, discarded waste is increased dramatically.

For example, the worldwide production of lib unit was nearly 2044 million in 2007 [4], and was up to about 4.6 billion unit in 2010 [5]. Spent phone batteries are defined as hazardous waste. If not handled properly, it will cause very serious harmful effect to the environment, microorganisms, animals and human health [6]. Therefore, recycling of these spent batteries is necessary and important from both economical aspect as well as environmental protection. Recycling processes make economic sense where the recovered materials are chemically important, quite valuable, and to avoid disposal costs. Furthermore, the metal value in spent phone batteries when recovered represents an important secondary source for these metals with a higher grade than those found in natural minerals and ores [7].

Unfortunately, between 50 and 80% of such e-waste is prospectively exported to developing countries like Ghana, China, India and Nigeria [8,9]. This accumulated e-waste is poorly managed in the country, because proper systems for recycling and disposal of them are lacking. The uncontrolled dumping and inappropriate recycling of e-waste poses serious threats to both micro and macro organisms and the environment at large [10].

These multitude of hazardous substances contained in spent phone batteries have the ability to inhibit normal biological processes in an environment where they are being released specially in an aquatic environment because it may affect some key environmental microorganisms such as , *Pseudomonas* sp.,

Mucor sp., which play a fundamental role in the biogeochemical cycles [11]. Microorganisms are ubiquitous, and capable of rapid growth when provided with nutrients and conditions favorable 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 [12].

Obviously, the introduction of the e-waste to the local water bodies poses hazards to the local aquatic organisms. A study conducted by the Institute for Applied Ecology (the Öko-Institute) indicated that local residents living near the lagoon, where uncontrolled dumping and e-waste recycling activities occur, lamented the adverse impacts of the site on the aquatic life of nearby water bodies [10]. This study revealed that the lagoon, which used to be a common fishing ground for the residents of the local communities until a few years ago, is now heavily polluted. As a result, many aquatic species in the lagoon have been eliminated. Amoyaw-Osei et al. [13] also observed that the Odaw River, which was formerly an important fishing ground, has become dead because of the extensive pollution caused by uncontrolled dumping and the crude processing of e-waste in the area.

Studies conducted by Nrior and Gboto [14], Nrior and Kpormon [11] also showed that spent phone batteries contained hazardous substances that are considered toxic to aquatic life. Therefore the aim of this research is to analyse and compare the level of toxic effect poses by spent mobile phone batteries in the tri-aquatic ecosystem on bacteria and fungi using species of *Pseudomonas* and *Mucor* as case study due to their role in the biogeochemical cycles.

2. MATERIALS AND METHODS

2.1 Sample Collection/Study Area

Freshwater and Marine water samples were collected in sterile (4) litre plastic container from Biara and Bodo city respectively both of Gokana L.G.A while Brackish sample was collected from Eagle Island Port Harcourt, all in Rivers state, Nigeria. These samples were taken in ice pack to the Microbiology Laboratory of Rivers State University, Port Harcourt, Nigeria, for analyses within 24 hours. Spent phone batteries A and B were obtained from the main phone market, at Garrison junction, Aba road, Port Harcourt.

2.2 Microbiological Analysis

2.2.1 Total heterotrophic bacteria count (THBC)

Total heterotrophic bacterial counts for each water samples were enumerated using spread plate method as described by Prescott et al. [15]. An aliquot (0.1 ml) of the dilution of 10^{-4} were aseptically transferred unto properly dried nutrient agar plates in duplicate, spread evenly using bent glass rod and incubate at 37°C for 24 hours. After incubation, the bacterial colonies that grew on the plates were counted and an average taken. The colony forming unit for the THBC of water samples were then calculated using the formula; [THFC (cfu/g) = {Number of Colonies/ Dilution (10^{-4}) x Volume plated (0.1 ml)}] [16].

Discrete colonies on the plates were sub-cultured unto fresh nutrient agar plates using the streak plate technique to obtain a pure culture of the bacterial isolates. The pure cultures were aseptically transferred into 10% (v/v) glycerol suspension, well label and stored at -4°C as stock cultures [17,18].

2.2.2 Total *Pseudomonas* species count

Total *Pseudomonas* sp. counts in the tri aquatic ecosystem was enumerated using standard microbiological method [15]. The samples were diluted serially up to 10^{-3} then an aliquot of 0.1 milliter from 10^{-3} dilutions of each the samples were inoculated on a dried Centrimide agar in duplicates and spread evenly using flamed bent glass rod. After incubation for twenty four hours (24 h) at 37°C, the colonies that grew on the plates were counted and an average taken and colony forming unit was calculated using an appropriate formula.

2.2.3 Total heterotrophic fungal count

The total fungi in each of water samples were enumerated using spread plate method. An aliquot of 0.1 ml from the dilution of 10^{-2} dilution was aseptically transferred unto properly dried Sabouraud Dextrose Agar plates containing antibiotic (Tetracycline) to inhibit bacterial growth, in duplicate, spread evenly using bent glass rod and incubated at 28°C for 3 days, the fungal isolates which developed, were counted

and sub-cultured unto Sabouraud Dextrose Agar slant in bijou bottles for preservation and identification [18].

Total Heterotrophic Fungal (THF) Counts for each sample were then calculated using the below formula: [THFC (cfu/g) = {Number of Colonies/ Dilution (10^{-4}) x Volume plated (0.1 ml)}]

2.2.4 Isolation and identification of the test organisms

2.2.4.1 Pseudomonas sp.

Pseudomonas species was isolated from the water samples using standard microbiological method (spread plate technique). An aliquot (0.1 ml) of each samples were aseptically transferred unto properly pre-dried Centrimide agar plates in duplicate, spread evenly using flamed bent glass rod and incubate at 37°C for 24 to 48 hours. After incubation, the bacterial colonies that grew on the plates were 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 labelled and stored as stock cultures for preservation [19]. The pure cultures were identified based on standard techniques in Biochemical testing of microorganisms and medical laboratory manual for tropical countries [20].

2.2.4.2 Mucor sp.

Mucor sp. was isolated from the water samples using standard microbiological method as described by Prescott et al. [16] An aliquot (0.1 ml) of each samples were aseptically transferred unto properly pre-dried Sabouraud Dextrose Agar plates containing antibiotic in duplicates, spread evenly using flamed glass bent rod and incubate at 28°C for 3 days.

The fungal isolates were identified based on cultural and morphological characteristics such as colony growth pattern, conidial morphology, and pigmentation. The technique described by Oyeleke and Manga [21] was also adopted for the identification of the isolated fungi using cotton blue in lactophenol stain.

2.3 Analyses of Physiochemical Parameters of the Water Samples

Physiochemical parameters such as colour, pH, conductivity, total hardness, conductivity, turbidity, total alkalinity, chloride, total suspended solids, total dissolved solids, total solid, nitrate, sulphate, calcium, magnesium, BOD COD, total iron, lead, copper, of the tri-aquatic ecosystems were determined using standard methods [22].

2.4 Toxicity Testing

2.4.1 Preparation of stock toxicant

The phone batteries (product A and B) 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 samples; freshwater, brackish and marine respectively. This served as stock solution (Toxicant).

2.4.2 Heavy metal analysis of the toxicants

The heavy metals present in products A and B phone batteries was analysed using atomic absorption spectrophotometer (UNICAM 929 AAS).The metals analysed include: Nickel, Cadmium, Chromium, Lead and Copper [22].

2.4.3 Toxicity test procedure

The toxicity tests were done by setting up fifteen flask aseptically covered with cotton wool. The test was carried out in five (5) separate test tubes containing sterile water samples from fresh, marine and brackish water from the habitat of the organisms separately. In each of the test tubes, the four toxicant concentrations (5%, 25%, 50%, and 75% of the stock toxicant) were added separately. While the control contains no toxicant. 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 set-up were then plated out using spread plate technique on an appropriate growth medium (Centrimide and SDA), immediately after inoculation as zero (0) hour. This was repeated after 4, 8, 12 and 24hours respectively. Centrimide plates were incubated for 24 to 48 hours at the temperature of (37± 2ºC). While SDA plates were incubated for three (3) days at 35°C. After incubation, the total viable colonies on the plates were counted and expressed as colony forming unit then converted to Logarithm base 10 (log_{10}) [23,14,24]

2.4.4 Percentage log survival of the test organisms exposed to the spent mobile phone batteries

The percentage log survival of the test organisms (*Pseudomonas* and *Mucor* sp.) exposed to the spent mobile phone batteries were calculated according to the formula used by Nrior and Obire [23].

Percentage (%) log survival = (Log **C**/ Log c) × 100

Where: Log **C** = Logarithm count in each toxicant concentration, Log c = Logarithm count in the control (zero toxicant concentration).

2.4.5 Percentage log mortality of the test or-organisms

The Percentage (%) log mortality of the test organisms were obtained using the formula adopted by Nrior and Obire [23] by subtracting one hundred from the value of the Percentage (%) log survival.

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

2.4.6 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 difference multiply by mean percentage mortality then divide by the control [14,24] That is LC_{50} = LC_{100} - ∑ conc. Diff. × mean % mortality / % control

2.5 Statistical Analysis

Statistical analysis was carried out on the data obtained during the study using a computer based program SPSS version 20 for Analysis of variance (ANOVA) of the data in the respective ecosystems.

3. RESULTS AND DISCUSSION

3.1 Microbiological Results

The Total Heterotrophic bacterial counts which ranges from 6.9 to 7.1 log_{10} cfu/ml, fungal 4.5 to 4.7 log_{10} cfu/ml and *Pseudomonas* sp counts 4.6 to 4.9 log_{10} cfu/ml in the tri-aquatic bodies (Fresh, Brackish and marine water) respectively, are presented in Fig. 1. The results show that microbial load in brackish water is higher than followed by marine and fresh water respectively. This may be as results of physiochemical parameters of the respective water bodies which are subject to variation based on the nature and types of anthropogenic activities within the area where water is located.

3.2 Results of the Physiochemical Parameters

The physiochemical parameters of the tri-aquatic ecosystem are revealed in Table 1. The pH of the three water bodies ranges from 5.60 to 6.60 which are favorable for microbial growth [24].

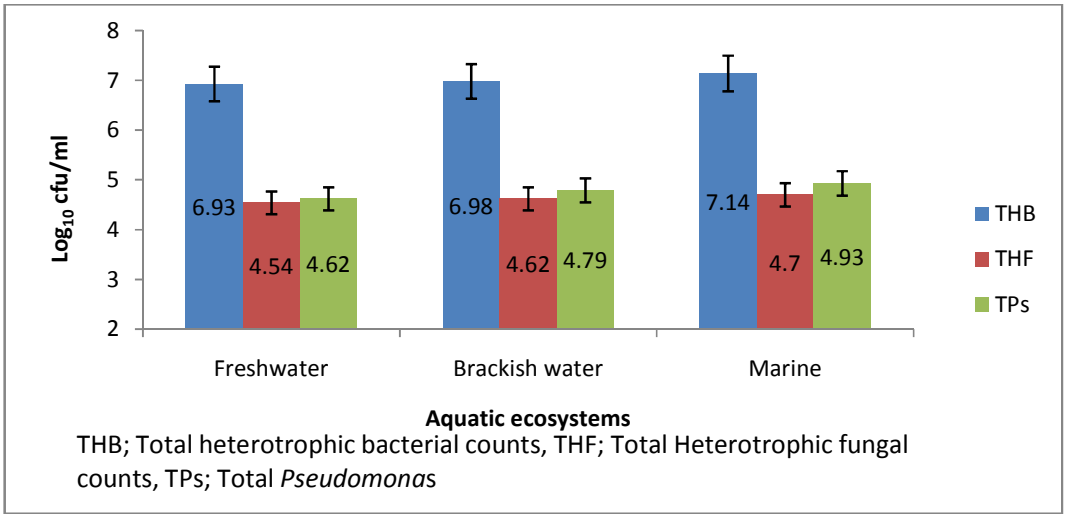

Fig. 1. Microbial counts of tri-aquatic ecosystem

Table 1. Results of physiochemical parameters of the tri- aquatic ecosystem

S/N	Parameter	Freshwater	Brackish water	Marine water	Unit
1	Colour	1.00	2.50	3.00	Hazen units
2	pH	5.60	6.00	6.60	
3	Conductivity	40.00	1430.00	2850.00	uS/cm
4	Turbidity	<1.00	1.50	3.00	NTU
5	Total hardness	19.40	20.10	24.70	mg/L
6	Total Alkalinity	16.20	19.50	26.75	mg/L
7	Chloride	7.50	143.55	293.00	mg/L
8	Total suspended solid	54,00	30,00	42.00	mg/L
9	Total dissolved solid	6.0	98.96	2150.00	mg/L
10	Total solid	60.00	134.87	2192.00	mg/L
11	Nitrate	1.45	1.00	0.95	mg/L
12	Sulphate	1.30	6.54	13.40	mg/L
13	Calcium	9.40	10.00	11.65	mg/L
14	Magnesium	2.00	1.83	2.00	mg/L
15	BOD	6.20	18	39	mg/L
16	COD	20.55	39	113	mg/L
17	Lead	0.02	<0.01	0.01	mg/L

3.3 Toxicity Testing Results

The percentage survival of *Mucor* species exposed to the batteries (products A and B) in Fresh, Brackish, and Marine water are revealed in Tables 3 and 4 while Tables 5 and 6 respectively show the percentage survival of *Pseudomonas* species. The summary of the median concentrations (Lc_{50}) of the two mobile phone batteries tested in the tri-aquatic ecosystems on the bacterial and fungal species are shown in Fig. 2.

The toxicity results obtained in this study revealed that spent mobile phone batteries can inhibit the activities of these key environmental organisms if disposed into the aquatic ecosystem. The results are in agreement with observations reported by Obire and Nrior [22] that worked on the Toxicity of domestic washing bleach (Calcium hypochlorite) and detergents on *Escherichia coli* and observed a decreased in percentage survival of the test organism with increased concentration and exposure time. Nrior and Gboto, [14] who worked on Comparative

Table 2. Results of heavy metal analysis of the battery content

S/N	Parameters	Unit	Product A	Product B	WHO 2011 standard
1	Chromium (Cr)	ppm	0.19	0.20	0.05
2	Cadmium (Cd)	ppm	0.09	0.09	0.003
3	Lead (Pb)	ppm	0.77	0.77	0.01
4	Copper (Cu)	ppm	0.99	1.00	2.00
5	Nickel (Ni)	ppm	76. 8	77.9	0.07

Key: Cr; Chromium, Cd; Cadmium, Pb; Lead,, Cu; copper, Ni; Nickel

Table 3. Effect of various concentrations of product A in fresh, brackish and marine water on percentage survival of *Mucor* sp. population during 24 hour exposure period

Toxicants Conc. (%)	Treatments		
	F+A+M	Br+A+M	Ma+A+M
0	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
5	98.57 ± 1.27^{cd}	98.84 ± 1.33^{cd}	99.34 ± 1.35^{cd}
25	96.970 ± 1.11^{c}	97.18 ± 1.57^{bc}	99.34 ± 1.35^{cd}
50	95.11 ± 1.65^{b}	96.39 ± 1.32^{ab}	95.16 ± 2.75^{b}
75	93.08 ± 1.88^{a}	94.90 ± 1.98^{a}	91.04 ± 2.00^{a}

**Means with the same alphabet across the column shows no significant difference at (p≥0.05)*
F; Fresshwater, B; Product A, M; Mucor sp, Br; Brackish water, Ma; Marine water

Table 4. Effect of various concentrations of product B in fresh, brackish and marine water on percentage survival of *Mucor* sp. population during 24 hour of exposure period

Toxicants Conc. (%)	Treatments		
	F+B+M	Br+B+M	M+B+M
0	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}
5	98.40±1.45^{d}	99.61±2.40^{cd}	99.34±1.35^{cd}
25	95.97±0.98^{c}	96.81±1.87^{bc}	97.29±1.60^{bc}
50	93.42±1.59^{b}	94.06±2.84^{ab}	95.16±2.75^{b}
75	90.58±3.13^{a}	91.28±2.51^{a}	91.04±2.00^{a}

**Means with the same alphabet across the column shows no significant difference at (p≤0.05)*
F; Fresshwater, B; Product B, M; Mucor sp, Br; Brackish water, M; Marine water

Table 5. Effect of various concentrations of product A in fresh, brackish and marine waters on percentage survival of *Pseudomonas* sp. population during 24 hour exposure period

Toxicants Conc. (%)	Treatments		
	M+A+PS	F+A+PS	Br+A+PS
0	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}
5	93.57±2.99^{c}	93.20±3.16^{c}	91.08±2.18^{c}
25	91.64±3.62^{bc}	88.69±3.00^{b}	88.55±3.11^{c}
50	86.92±5.16^{ab}	85.59±2.59^{ab}	83.22±2.36^{b}
75	82.31±4.49^{a}	83.09±2.42^{a}	78.82±3.86^{a}

**Means with the same alphabet across the column shows no significant difference at (p≤0.05)*
F; Freshwater, A; Product A, Ps; Pseudomonas sp, Br; Brackish water, M; Marine water

Table 6. Effect of various concentrations of product B in fresh, brackish and marine water on percentage survival of *Pseudomonas* sp. population during 24 hour exposure period

Toxicant Conc. (%)	Treatments		
	F+B+PS	B+B+PS	M+B+PS
0	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}
5	93.16±3.01^{c}	92.41±3.91^{c}	95.68±2.53^{c}
25	89.12±3.53^{bc}	89.08±4.31^{bc}	92.74±3.12^{bc}
50	85.64±4.03^{ab}	85.90±5.36^{ab}	90.39±3.11^{ab}
75	81.92±3.71^{a}	83.27±4.32^{a}	87.55±3.22^{a}

Means with the same alphabet across the column shows no significant difference at (p≤0.05)
F; Freshwater, B; Product B, Ps; Pseudomonas sp, B; Brackish water, M; Marine water

toxicity of spent mobile phone batteries on *Nitrobacter* species also reported similar observations. Decrease in the percentage logarithmic survival of test organisms in tri-aquatic ecosystems after 24 hours of exposure to the toxicant concentrations was observed. A simultaneous decrease in the percentage logarithmic survival of the test organism in the tri aquatic environments after 24 hour of exposure to the toxicant concentrations were observed (Tables 3 to 6) respectively which revealed that both toxicants caused cell death which resulted in a reduction in the total viable counts.

Decrease in the percentage logarithmic survival of test organisms in tri-aquatic ecosystems after 24 hours of exposure to the toxicant concentrations was observed (Tables 3-4) respectively. This study also revealed that product B is more toxic to the test organisms than product A. This may be as a result of the heavy metal concentrations in the respective products. According to Sander et al. [25], the site of action of any toxicant depends on the nature of the toxicant and the environment.

The percent log survival of the test organisms during the twenty four hour (24 h) exposure periods to spent mobile phone batteries in the tri aquatic environments shows that the toxicant exhibited more toxic effect on the bacterial isolate (*Pseudomonas* sp.) than the fungal isolate (*Mucor* sp.) in marine than brackish followed by freshwater. This may be due to the nature of their cell wall. According to Leonard et al. [7], heavy metal cell toxicity molecular

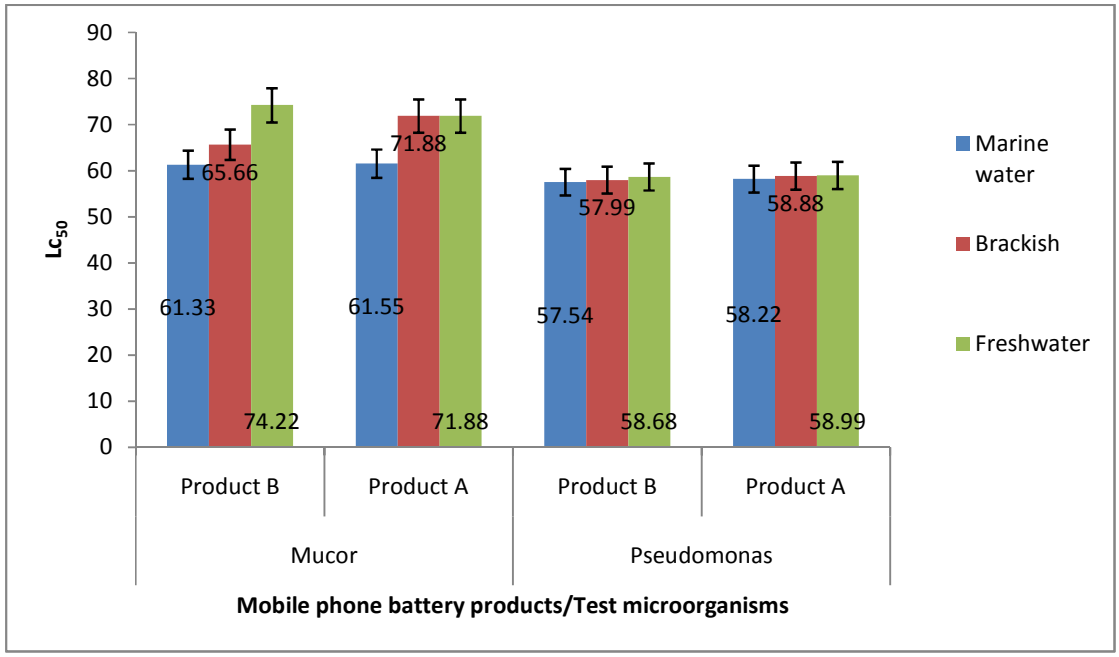

Fig. 2. Summary of median lethal concentrations of products A and B on the test organisms in the tri aquatic ecosystem

mechanisms include: binding to proteins and phospholipids, damage to plasma membranes, inhibition of transmembrane, enzyme inhibition; lipid peroxidation oxidative DNA damage.

Median Lethal concentration (LC_{50}) was used as indices to monitor toxicity and the sensitivity of the test organisms to the toxicant at different concentrations of spent mobile phone batteries [14]. The median lethal concentration (LC_{50}) of the mobile phone batteries increases in the following order: (Note: the higher the LC_{50}, the Lower the toxic effect) for *Pseudomonas* sp. Product B in marine water (57.54%) < Product B in Brackish water (57.99%) < Product A in marine water (58.22%) < Product B in brackish water (58.68%) < Product A in brackish water (58.88%) > Product A in freshwater (58.99%). While for *Mucor* sp; Product A in marine water (61.33%) < Product B in marine water (61.55%) < Product B in brackish water (65.66%)< Product A in brackish water (71.88%) < Product A in freshwater (71.88%) Product B in fresh water (74.22%). Summary of the median lethal concentrations (LC_{50}) for the two mobile phone product used in the tri-aquatic ecosystems on the test organisms are presented in Fig. 2. These results are in agreement with report of mean lethal concentration reported by Nrior, and Owhonda [25], who worked on the comparative strength of spent mobile phone batteries (Blackberry and Nokia) on Bioassay Evaluator *Nitrobacter* sp.

4. CONCLUSION AND RECOMMENDATIONS

The results obtained in this research revealed that, spent mobile phone batteries have the ability to inhibit biological processes that are mediated by key environmental microorganisms such as *Pseudomonas* sp and *Mucor* sp in an aquatic ecosystem due the effect on the survival rate of these organisms which indicates that these batteries are capable of causing serious environmental pollution, affecting the biotic component of the environment not only that, but also, spent mobile phone 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 be developed by the producers to avoid direct disposal into aquatic environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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