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Nrior Renner Renner
Department of Microbiology, Faculty of Science, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Rivers, Nigeria

Awari Victoria Ginika
1. Department of Microbiology, Faculty of Science, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Rivers, Nigeria
2. Department of Microbiology, Faculty of Natural and Applied Sciences, Tansian University Umunya, Anambra, Nigeria

Ajikwaru Glory Praisejah
A) Department of Microbiology, Faculty of Science, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Rivers, Nigeria

Correspondence
Nrior Renner Renner
Department of Microbiology, Faculty of Science, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Rivers, Nigeria

Toxicity of spent automobile battery on *Nitrobacter* and *nitrosomonas* species in soil

Nrior Renner Renner, Awari Victoria Ginika and Ajikwaru Glory Praisejah

Abstract
Aim: To evaluate the concentration of spent automobile battery that could cause potential toxic effect on the microorganisms: *Nitrobacter sp.* and *Nitrosomonas sp.* in soil.
Study Design: The study employs experimental design, statistical analysis of the data and interpretation.
Place and Duration of Study: Soil sample were collected from Agricultural, Research and Development Farm of Rivers state University, Port Harcourt, Nigeria. Spent automobile batteries were obtained from the mechanic workshop at Ikoku, Mile 1, Port Harcourt, Rivers state. These samples were transported in ice pack to the Microbiology Laboratory of Rivers State University, Port Harcourt, Nigeria, within 24 hours for analyses.
Methodology: Toxicity test procedures were carried out by submerging ten grams of automobile battery into sterile transparent plastic rubber containing 1000g of soil samples. Toxicity test was carried out using six plastic rubbers containing automobile acid battery + soil (set-up samples). Each set-up sample containing different toxicant concentration (0%, 6.5%, 12.5%, 25%, 50% and 75%) was inoculated with 1ml of test organism using spread plate technique on Winogradsky media. The cultures were incubated at 35°C for 18 to 24 hours. Thereafter, plates were counted at interval of 0hr, 4hrs, 8hrs, 12hrs and 24hrs. Median lethal concentration (LC50) was determined using SPSS version 20.
Results: The results showed increase in percentage logarithm mortality of *Nitrosomonas* species with increased toxicant concentration and exposure time. The media lethal concentration (LC_{50}) of the spent motor battery used decreased in the following order; spent automobile battery in soil environment + *Nitrosomonas* (36.80%) > automobile battery in *Nitrobacter* (47.49%). However, automobile battery in *Nitrosomonas* had lower toxicity than the *Nitrobacter*.
Conclusion: The result revealed that hazardous chemical from battery can cause environmental hazard which affect microorganisms in soil. Hence, Proper disposal of automobile spent battery should be practiced to avoid environmental pollution that can be detrimental to plant and human health.

Keywords: Nitrobacter species, Nitrosomonas species, Soil, Spent automobile battery, Toxicity

1. Introduction
Man's activity in the environment has led to the pollution of soil and environment mainly by chemical contaminants. The presence of spent automobile battery in soil can affect the quality of food, groundwater, micro-organisms' activity and plant growth etc. (Baldrain, 2003) [3]. Automobile batteries are electronic appliances used by man, in all human endeavors in order to make life and work easier. Most times, people prefer to buy new electronic device when their old device go bad, rather than repairing a faulty one, even when their devices have reusable part, (Babu *et al.*, 2007) [5]. Anually, in Nigeria, there is increase in the demand and consumption of these electrical and electronic devices due to the growing population which in turn has led to the growing volumes of wastes generated from automobile batteries (Mohan and Chaithanya, 2015) [30]. Nigeria is the highest producer of this waste in West Africa (Monhart *et al.*, 2011) [29]. These wastes referred to as E-waste or electronic waste, involves automobile battery which can be categorizes as hazardous waste including; the lead-acid batteries, and alkaline batteries, magnesium, lithium ion battery etc. (Prakash *et al.*, 2010; Otsuka *et al.*, 2012) [41, 40]. The automobile battery waste contains hazardous components which may pose serious environmental concerns when disposed without adequate treatment (Reber, 1999; Plette *et al.*, 2018) [44, 42]. Most of the batteries in use, classified as either secondary batteries are termed rechargeable and are more heavily used in commercial settings than the primary batteries which are non-rechargeable and these usually contains: Nickel-cadmium, nickel metal hydride, lithium-ion, and lead-acid batteries.

(Nrior and Gboto, 2017) [31]. Continuous exposure of battery in to the soil environment can cause detrimental effect not only to man but soil micro-organism (Amadi *et al.*, 2014) [1], this could inturn bring about ecological imbalance, biochemical activities and retard decomposition processes (Douglas and Green 2015) [8]. Although, the automobile battery industry has put in great efforts overtime to recycle and replace toxic components of these electronic materials, these batteries are burned or incinerated with other waste constantly (UNEP, 2012) [55]. The fumes pollute the air when they are released into the environment and also pollute our water bodies (Nrior and Obire, 2015) [33]. When thrown into 'dump' areas, in our environment, their toxic ingredients are left to seep into the soil, finally to groundwater, causing massive and devastating damage to our natural ecosystem (Green 2015) [19]. Unfortunately, the effects of automobile batteries on the soil and environments are negative. Battery components can inhibit the growth of certain microorganisms by interfering with enzymatic activity, like the inhibition of Nitrogenase actively involved in Nitrogen fixation (Jastrzebska, 2006). The inhibition of Nitrogenase actively can reduce the amount of nitrogen available for plants, thus reducing crop yield. Other important microbial processes in the soil like: nutrient transformation, degradation and decomposition of resistant components of plant and animal tissues, bioremediation, humus formation, surface blooming to reduce erosion losses, all which depends on the equilibrium found among the different groups of microorganisms present in the soil environment (Spain, 2003; Saviozzi *et al.*, 2017) [54, 51], which are in turn affected when high concentrations of these toxic waste are present. As the toxic components in these batteries are leached into the soil and groundwater, they contain some metals including: mercury, lithium, cadmium, chromium, and lead that are especially toxic to soil and environmental organisms as well as humans (USEPA, 2004) [56]. According to (Manhart, *et al.*, (2011) [29], introducing these wastes in large volumes, without proper environmental management system in place, could negatively impact the environment, indigenous microorganisms, plants and animals, as well as the population and the economy at large. Furthermore, the chemical components from the automobile batteries lead to the selective pressure of species which are resistant to their harmful effects. Soil contamination with batteries limits the microbial biodiversity while it increases the abundance of some bacteria species which are more resistant to changes in the environmental homeostasis, (Landi, 2013) [27].

Nitrobacter, a genus of mostly rod shaped gram negative demo-autrophic bacteria play an important role in the nitrogen cycle by oxidation of nitrate in soil. Unlike plant where electrons transfer in photosynthesis provides the energy for carbon fixation. *Nitrobacter* uses energy from oxidation of nitrite ions NO^{-}_{2} into nitrite NO^{-}_{3} to fulfill their energy needs (Nrior and Gboto, 2017) [31].

Nitrosomonas is a genus of gram negative bacteria belonging to the Beta Protein bacteria. It is one of the fine genera of ammonia oxidizing and as an obligate chemo-lithoautotroph, they use ammonia as an energy source. Various species are naturally found in our environment and also occur in soil, sewage disposal system and acquatic environments like; oceans, lake, river) (Odokuma and Ijeoma, 2013) [35].

Toxicity is the degree to which a hazardous substance or a toxic component can cause damage to an organism. Toxicity can also be referred to the effect on a whole organism such as an animal, a bacterium, plant on a structure of the organism such as the cell (cytotoxicity) or an organ such as the liver (hepatoxicity) ((Nrior and Obire, 2015) [33].

Previous studies have shown that spent phone batteries contained hazardous substances that are considered toxic to the ecosystems at large. Other researches carried out by Nrior and Gboto (2017) [31], Kpormon and Douglas (2018) [26], Awari *et al.*, (2019) [2], have also revealed that these microorganisms also have the capabilities to degrade hydrocarbon polluted soil and environments. Therefore, this research focus is to evaluate the concentration of spent automobile battery that could cause potential toxic effect on the microorganisms: *Nitrobacter sp.* and *Nitrosomonas sp.* in soil in order to find out if these battery pose any toxic effect on *Nitrobacter* and *Nitrosomonas* species.

2. Materials and Methods

A. Collection of Samples

Soil sample were collected at (1-10cm) depth from ten points using hand glove, from Agricultural, Research and Development Farm of Rivers state University, Port Harcourt, Nigeria after using sterile trowel and shovel was used to remove lumps and debris and transferred into sterile plastic polythene bag, tied immediately to minimize contamination. Whereas, spent automobile batteries were obtained from the mechanic workshop at Ikoku, Mile 1, Port Harcourt and Rivers state. Samples were transported immediately in ice pack to the Microbiology Laboratory of Rivers State University, Port Harcourt, Nigeria, within 24 hours for analyses (Gupta *et al.*, 2014; Nrior and Obire, 2015) [21, 33].

B. Pollution of Soil with Battery Acid

Before polluting the soil with automobile battery acid, about five grams of the soil (unpolluted) was weighed and used to isolate the microorganisms of the unpolluted soil samples. The soil was then polluted with automobile battery acid and mixed very well with sterile wooden spatula and kept for 21 days for proper acclimatization with the microorganisms including isolates with capabilities of utilizing chemicals and acidic components of batteries (Nrior and Obire, 2015) [33].

C. Isolation and Enumeration of Microbial Isolates

Soil samples were immediately homogenized in order to obtain a composite soil sample. **S**odium chloride (8.5g) was weighed using electronic balance and dissolved in 1000ml distilled water. Serial dilution was done by dispensing 9ml into different test tubes, autoclaved at 121^{0}C for 15 minutes (Giller *et al.*, 2008; Landi *et al.*, 20013; Nrior and Obire, 2015) [18, 27, 33].

1-Isolation and enumeration of bacterial isolates

Enumeration of microbial population from the soil sample by the standard spread plate techniques was carried out. After serially diluted samples 0.1ml aliquant of appropriate dilution (dilution that produce colony counts of between 30-300 colonies) was plated into the nutrient agar (NA) plates and spread evenly using bent glass rod. The plates were incubated inverted at 37^{0}C for 24hrs. After incubation, isolates were counted and plates yielding 30-300 colonies were enumerated (Giller *et al.*, 2008; Landi *et al.*, 2013; Odokuma and Oliwe, 2019) [18, 27, 37].

2-Isolation and enumeration of fungal isolates

Isolation and enumeration of total fungi was done by serial dilution, sterile normal saline (8.5g) of sodium chloride was used as diluent for inoculation preparation. 1g of soil was aseptically transferred into a sterile test tube containing 9 mls of diluent. This gives 10^{-1} dilution to 10^{-2} dilution. Then, 0.1ml aliquant was plated into the already prepared SDA plates and spread with glass spreader. The cultured plates were incubated at 37^0C for 3-5 days. After incubation, emerging colonies that appeared on the Sabouraud Dextrose Agar (SDA) plates were recorded as counts of total fungi count for the soil sample respectively (Giller *et al.*, 2008; Landi *et al.*, 2013; Odokuma and Oliwe, 2019) [18, 27, 37].

3-Isolation of test organisms (Nitrosomonas sp. and Nitrobacter sp.)

Aliquot (0.1ml) of the soil sample was pipetted and transferred onto already prepared sterile Winogradsky agar plates in duplicates. Uniformly spread with sterile glass spreader (spread plate method) and incubated in inverted position at 30^0C for 72-96 hours. Creamy mucoid, flat colonies were suggested of *Nitrosonomas* species. Gram staining of the colonies revealed gram negative short rods indication of *Nitrosomonas* (Griffiths *et al.*, 2007; Douglas and Nwachukwu, 2016) [20, 10]. The colonies were aseptically subcultured onto freshly prepared Winogradsky's Agar plates. Greyish, mucoid, flat colonies were suggestive of nitrobacteria gram staining of the colonies revealed pear-shaped organisms indicative of nitrobacteria (Griffiths *et al.*, 2007; Douglas and Nwachukwu, 2016) [20, 10]. 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 2-6 days. After 48hrs incubation, 1ml each of sulfuric and dimethyl-haphthalamine, 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 coloration was confirmatory of *Nitrobacter* specie (Griffiths *et al.*, 2007; Odokuma and Oliwe, 2016) [20].

D. Characterization and Identification of Pure Cultures

Sub-cultures were made on a freshly prepared Nutrient Agar (NA) plate and sabourand dextrose Aga (SDA) plate by streak plate techniques, to get discrete colonies and different morphological tests were performed on the various isolates (Cheesbrough, 2006) [7]. The different bacterial isolates obtained from the different plates were macroscopically examined and then biochemically tested, to identify the organism to the species level, using Bergey's manual of determination bacteriology (Holt *et al.*, 2004) [24]. Whereas, the growth portion of the fungal mycelia on the sabourand dextrose Agar medium was cut and placed on a grease free microscopic slide containing few drops of Lacto-phenol cotton blue, and covered with a cover slip. The mycelium was then examined under the microscope at a magnification of X10 and X40 objective lens (Holt *et al.*, 2004) [24]. Discrete colonies on the plates were aseptically preserved by transferring into 10% (v/v) glycerol suspension seperately, well labelled and stored as stock cultures for at 4^0C for further biochemical investigation/studies (Cheesbrough, 2006; Nrior and Obire, 2015) [7, 33].

E. Preparation of Toxicity Test Procedure

The automobile batteries were aseptically chipped open and ten grams was immediately submerged (toxicant) in sterile transparent plastic rubbers containing hundred grams of soil sample. The cover of the plastic rubber was placed on top in order for air to penetrate. This served as stock toxicant soil sample (Frankenberger and Tabatabai, 2001; Nrior *et al., et al.*, 2015) [13, 33].

The toxicant test was carried out with six separate small plastic transparent rubber containing (automobile acid battery + soil). Each of the small plastic rubbers containing different toxicant concentration (0%, 6.5%, 12.5%, 2.5%, 50% and 75%) was inoculated with about 1ml of the test organism separately with separate sterile Pasteur pipettes (Nrior and Obire, 2015) [33]. The 0% served as the control. Thereafter, one gram of the acid soil was transferred to 9mls test tube containing normal saline for 10-fold serial dilution. The dilution was made up to 10^{-6} dilution. Aliquot (0.1ml) was plated out immediately after inoculation on already prepared Winogradsky agar plate and spread evenly using bent glass rod and then incubated at warn temperature (28±2 ^{o}C). The plates were counted at 0hr, 4hrs, 8hrs, 12hrs and 24hrs, recorded respectively and converted to Logarithm base 10 (log10) (Frankenberger and Tabatabai, 2001; Nrior *et al., et al.*, 2015) [13, 33].

1-Percentage log survival of Nitrobacter sp. and Nitrosomonas sp. in automobile batteries

The percentage log survival of *Nitrobacter* and *Nitrosomonas* bacterial species isolate in the automobile battery effluent used in the study was calculated using the formular adopted from (Frankenberger and Tabatabai, 2001; Nrior and Obire, 2015) [13, 33]. The percentage log survival of the bacterial isolate in the soil sample was calculated by obtaining the log count in the toxicant concentration divided by the log of the count in the zero toxicant concentration and multiplying by 100 (Nrior and Obire, 2015;) [33].

$$\text{Percentage (\%) log Survival} = \frac{\text{Log C X 100}}{\text{Log c}} \quad (1)$$

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

2. Percentage log mortality of Nitrobacter and Nitrosomonas species in automobile batteries

The percentage (%) mortality of the test organism was obtained by subtracting the value of the percentage (%) survival from one hundred (100) (Nrior and Obire, 2015) [33].

Percentage (%) log Mortality = 100 - % log Survival (2)

3. Determination of the Median Concentration (LC50) of automobile batteries on Bacteria (Nitrobacter and Nitrosomonas species

The mortality of the test organisms expressed as Median Lethal Concentration (LC50) were used as a protocol to monitor toxicity.

The median lethal concentration of the toxicant in the soil environments were determined by subtracting the value of the highest concentration used from the total sum of concentration difference and multiplied by the mean percentage mortality divided by the control (Frankenberger and Tabatabai, 2001; Amadi *et al.*, 2014; Nrior and Gboto, 2017) [13, 1, 31].
Thus;

$$LC_{50} = \frac{LC_{100} \text{ - Dose Diff. X mean\% mortality}}{100} \quad (3)$$

3. Results and Discussion

A. Results of Microbial counts and identification of suspected microbial Species

The bacterial and fungal counts of soil samples are presented in table 1. The result showed that the unpolluted soil sample had higher population of total heterotrophic bacteria (THB) and total fungal (TF) counts than the polluted soil sample. Nevertheless, the hydrocarbon utilizing bacteria (HUB) counts and hydrocarbon utilizing fungal (HUF) counts were more, in the polluted soil sample than the unpolluted soil sample.

The biochemical tests of bacterial isolates and their colonial/ morphological characterization are presented in table 2 and 3 respectively. While the macroscopic and microscopic descriptions of fungal isolates are presented in table 4. This was carried out in order to observe the presence and absence of cultural characteristics like asexual reproductive structures like sporangia, conidia, head, vegetative mycelia, septate hyphae.
The bacterial isolates were identified as: *Bacillus* sp., *Staphylococus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Achromobacter* sp., *Flavobacterium* sp., *and Micrococcus* sp., whereas the fungal were identified as: *Penicillin* sp., *Rhizopus* sp., *Aspergillus sp.*, *Fusarium* sp. *and Mucor* sp. The results obtained in this study revealed that the toxicants have ability to affect microorganisms in the polluted soil environment and interchangeably, the polluted soil environment could be affected by the toxicants present in the soil samples. This results concurs with research works carried out by; Said and Lewis, (2001) [50]; Frostegard, *et al.*, (2003) [16]; Saviozzi *et al.*, (2017) [51]; Douglas *et al.*, (2018) [9]; Fagade and Adetutu, (2020) [11] who isolated similar bacterial species from soil and water samples analysed for lethal concentration.

Table 1: Microbial counts of soil samples

Soil Sample Type	Total Heterotrophic Bacteria	Total Fungi	Hydrocarbon Utilizing Bacterial	Hydrocarbon Utilizing Fungi
Polluted soil	$2.0x10^4$	$1.0x10^3$	$8.1x10^2$	$9.1x10^2$
Unpolluted soil	2.8×10^4	$7.0x10^3$	$6.0x10^2$	$2.2x10^2$

Table 2: Biochemical test of the isolates

Isolate Code	Glucose	Manitol	Lactase	Maltase	Catalase	Oxidase	Motility	Voges proskeur pPProsleur	Methyl Red	Indole	Citrate	Urease	Starch hydrolysis	Salt tolerance	Suspected organism
USS1	-	+	+	+	+	+	+	-	+	-	+	-	-	-	*Bacillus* sp.
USS2	-	+	+	+	+	+	-	+	-	-	-	-	-	+	*Micrococus* sp.
USS3	+	-	-	+	+	+	-	+	-	-	+	-	-	-	*Staphylococus* sp.
USS4	+	+	+	+	+	+	-	-	-	-	+	+	+	-	*Pseudomonas* sp.
USS5	+	+	+	+	-	+	+	-	-	-	+	+	-	-	*Achromobacter*
PSS1	+	+	+	+	+	+	-	+	-	-	+	-	-	-	*Staphylococus* sp.
PSS2	+	-	-	+	+	-	+	-	-	-		-	-	-	*Flavobactereria*
PSS3	-	+	+	+	+	+	-	+	-	-	-	-	-	+	*Micrococus* sp.
PSS4	-	+	-	+	+	+	-	-	-	-	+	+	+	-	*Pseudomonas* sp.
PSS5	+	+	+	+	+	+	+	-	+	-	+	-	-	-	*Bacillus* sp.
PSS6	+	+	-	-	-	-	+	+	+	+	+	+	-	-	*Proteus* sp.

Table 3: Colonial/Morphological characteristics of bacteria isolates

Isolate Code	Colour	Size	Elevation	Margin	Form/Shape	Surface Appearnace	Surface Texture	Organismi
USS1	Whitish gray	Large	Flat	Undulate	Irregular	Rough	Dry-wet	*Baciilus* sp.
USS2	Bright yellow	Small	Convex	Entire	Circular	Smooth	Buttery	*Micrococus* sp.
USS3	Orange – golden yellow	Small-large	Convex	Entire	Circular	Smooth	Dry-wet	*Staphylococus* sp.
USS4	Cream/yellowish green	Small-large	Convex	Entire	Circular	Smooth	Mucoid	*Pseudomonas* sp.
USS5	White- yellow	Large	Convex	Entire	Circular	Smooth	Mucoid	*Achromobacterium* sp.
PSS1	Cream - yellow	Small-large	Convex	Entire	Circular	Smooth	Mucoid	*Staphylococus* sp.
PSS2	White - yellow	Large	Convex	Entire	Circular	Smooth	Mucoid	*Flavobacterium* sp.
PSS3	Bright yellow	Small	Convex	Entire	Circular	Smooth	Dry	*Micrococus* sp.
PSS4	Greenish - yellow	Small-large	Convex	Entire	Circular	Smooth	Mucoid	*Pseudomonas* sp.
PSS5	Gray to white	Large	Flat	Undulate	Irregular	Rough	Dry-wet	*Baciilus* sp.
PSS6	Yellow	Small	Convex	Entire	Circular	Smooth	Dry	*Proteus* sp.
SSS1	Greenish-red	Small	Flat	Entire	Circular	Smooth	Mucoid	*Nitrobacater* sp.
SSS2	White -red	Small	Convex	Entire	Circular	Smooth	Mucoid	*Nitrosomonas* sp.

Table 4: Macroscopic and Microscopic description of fungi isolates

Isolates Code	Fungi	Macroscopic Description	Microscopic description
USSA	Penicillium sp.	Radically furrowed blue-green validity growth with white periphery and reverse white colour	Septate hyphae, with branched conidiophoras bearing phialides. Conidia are arranged in chains on the phialides.
USSB	Rphizophus sp.	White colony growth, with grayish to blackish spots.	Septate branched sporangiophores, with round head sporangia.
USSC	Fursarium sp.	White colony lawn like growth with reverse yellow colour	Septate hyphae with presence of banana shaped septate conidia
USSD	Mucor sp.	White fluffy growth with reverse white colour	Non-septatehyphase, with non-septate Sporangiophores
PSSA	Fusariumsp	White colony lawn like growth with reverse yellow colour	Septate hyphae with presence of banana shaped septate conidia.
PSSB	Penicillium sp.	Radically furrowed blue-green validity growth with white periphery and reverse white colour	Septate hyphae, branched conidiophoras with phialides with conidia arranged in chains.
PSSC	Mucor sp.	White fluffy growth with reverse white colour	Non-septatehyphase, with non-septate Sporangiophores

B. Results of Log Survival count of Bacterial Species with spent automobile battery in the soil environment

The log survival counts of *Nitrobacter* sp. and *Nitrosomonas* sp. were tested when exposed to the spent automobile batteries in the soil environments were determined by adding the various toxicant concentrations of the spent automobile batteries as follows; 6.5%, 12.5%, 25%, 50%, 75% which was inoculated with the test organisms at interval of 4hrs from the start of 0hr, 4hrs, 8hrs, 12hrs, 24hrs and then, colonies were counted after incubation, for each hour in order to show the toxicity of the chemical content of the spent battery. Results were further converted into logarithm value for simplicity and reported in table 5 and 6 respectively.

C. Results of toxicity testing

The percentage log survival counts of toxicity test carried on *Nitrobacter* and *Nitrosomonas* species with spent automobile battery in the soil environment were calculated and are presented in tables 7 and 8 respectively while, the median lethal concentrations (LC_{50}) of the bacterial species on the spent automobile batteries were determined and summarized in tables 9 and 10 respectively.

The result concurs with Nrior and Obire, (2015) [33]; Kpormon and Duglas, (2018) [26] observed simultaneous reduction in the percentage logarithmic survival of test organisms employed in the tri-aquatic environments after exposure to the toxicant concentrations at 24 hours.

The toxicity results obtained in this study revealed that spent automobile phone batteries can inhibit the activities of these bacterial species. Researchers including Bishop, 2000 [6]; Renella *et al.*, (2002) [45]; Douglas *et al.*, (2018) [9]; Francis (2020) [12] have also, reported in their research work on toxicity of spent phone batteries on microflora in marine, brackish and freshwater ecosystems and that *Nitrobacter* and *Nitrosomonas* species are key environmental organisms, and that their biodegradation capabilities can be negatively affected when exposed to chemicals from automobile batteries in the soil and surrounding environments (Fritze, 2002; Jastrzbska, 2006) [14, 25].

The results are also in agreement with researchers like: Odokuma and Akponah, (2010) [36]; Rayner and Sadler, (2009) [43]; Lundoteats, (2011) [28]; Nrior and Gboto, (2017) [31]; who researched on; comparative toxicity of spent mobile phone batteries (Samsung and Tecno) on bacteria, observed and reported that toxicity can be altered or affected by the chemical constituents of the medium, as well as the environments. Other researchers including: Roane, (2001) [47]; Olajure and Ayodele, (2006) [38]; Robinson, (2009) [46]; Rogers and Li, (2015) [48] further emphasized, that this may be as a result of chemical reactions between the components in the battery and the constituents found in the environments.

This study also revealed that, spent automobile phone batteries disposed into the surrounding environments could get into the soil and water bodies, reduce the biodegradation capabilities of organisms like; *Nitrobacter* sp. and *Nitrosomonas* sp. and could constantly poses great toxicity of environmental and public health concern in affected areas, in Rivers State, Nigeria.

This fact supports the research works carried out by Rether *et al.*, (2002) [49]; Sosak-Swiderska, (2010) [53]; Schierl *et al.*, (2016) [52].

Table 5: Log Survivial count of *Nitrobacter* sp. with spent automobile battery in the soil

Toxicant concentration	0 Hour	4 Hour	8 Hour	12 Hour	24 Hour
0%	1.74	1.68	1.60	1.54	1.30
6.5%	1.66	1.51	1.44	1.30	1.14
12.5%	1.60	1.56	1.47	1.39	1.30
25%	1.68	1.51	1.41	1.30	0
50%	1.36	1.17	0	0	0
75%	1.30	1.25	1.23	0	0

Table 6: Log Survivial count of *Nitrosomonas* sp. with spent automobile battery in the soil

Toxicant concentration	0 Hour	4 Hour	8 Hour	12 Hour	24 Hour
0%	2.47	2.17	2.07	2	1.74
6.5%	2.07	1.78	1.69	1.47	1.30
12.5%	2.35	2.14	2	1.80	1.60
25%	1.90	1.94	1.77	1.65	1.30
50%	2.30	1.80	1.60	1.56	1.30
75%	0.25	1.74	1.60	1.47	1.17

Table 7: % Log Survivial count of *Nitrobacter* sp. with spent automobile battery in the soil

Toxicant concentration	0 Hour	4 Hour	8 Hour	12 Hour	24 Hour
0%	100	100	100	100	100
6.5%	83.63	73.33	70	54.14	70
12.5%	80	82.22	75	71.42	100
25%	97.27	73.33	65	57.14	0
50%	41.81	33.33	25	0	0
75%	36.36	40	42.5	0	0

Table 8: % Log Survivals count of *Nitrosomonas* sp. with spent automobile battery in the soil

Toxicant concentration	0 Hour	4 Hour	8 Hour	12 Hour	24 Hour
0%	100	100	100	100	100
6.5%	40.54	40.66	41.66	30	35.71
12.5%	77.2	94	83.33	64	71.42
25%	27.02	58.66	50	44	35.71
50%	67.56	42.66	33.33	37	35.71
75%	60.81	37.33	33.33	30	26.78

Table 9: Median Lethal concentration (LC_{50}) of automobile battery on *Nitrobacter sp.* in soil

	%Mortality	Mean Mortality	Dose Difference	Dose Difference ∑ X Mean % Mortality
0%	0	-	-	-
6.5%	148.86	29.772	6.5	193.576
12.5%	91.36	18.276	6	109.656
25%	217.22	43.444	12.5	543.050
50%	399.86	79.972	25	199.3
75%	341.14	68.228	25	1705.7 Σ=2751.284

$$LC_{50} = LC_{100} - \sum \frac{\text{Dose Diff. x mean\% mortality}}{100} \quad (3)$$

$$= 75 - \frac{2751.284}{100}$$

$$= 75\text{-}27.51284$$

$$LC_{50} = 47.48716\%$$

Table 10: Median Lethal concentration (LC_{50}) of automobile battery on *Nitrosomonas sp.* in soil

	% Mortality	Mean Mortality	Dose Difference	Dose Difference ∑ X Mean % Mortality
0%	0	-	-	-
6.5%	311.49	62.298	6.5	404.937
12.5%	110.5	22.1	6	132.6
25%	284.43	56.886	12.5	711.075
50%	283.44	56.688	25	1417.2
75%	311.75	62.35	25	1558.75 Σ=3819.625

$$LC_{50} = LC_{100} = \sum \frac{\text{Dose Diff x mean\% mortality}}{100}$$

$$= 75 - \frac{3819.625}{100}$$

$$= 75 - 38.1962$$

$$LC_{50} = 36.8037\%$$

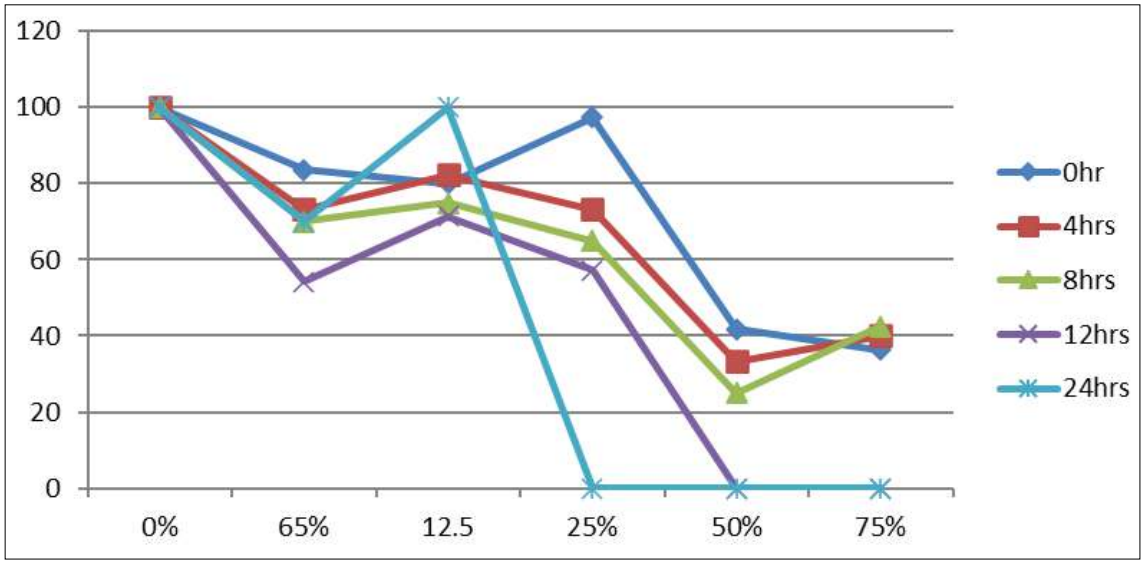

Fig 1: Percentage logarithm survival of *Nitrobacter* species with spent automobile battery in soil environment

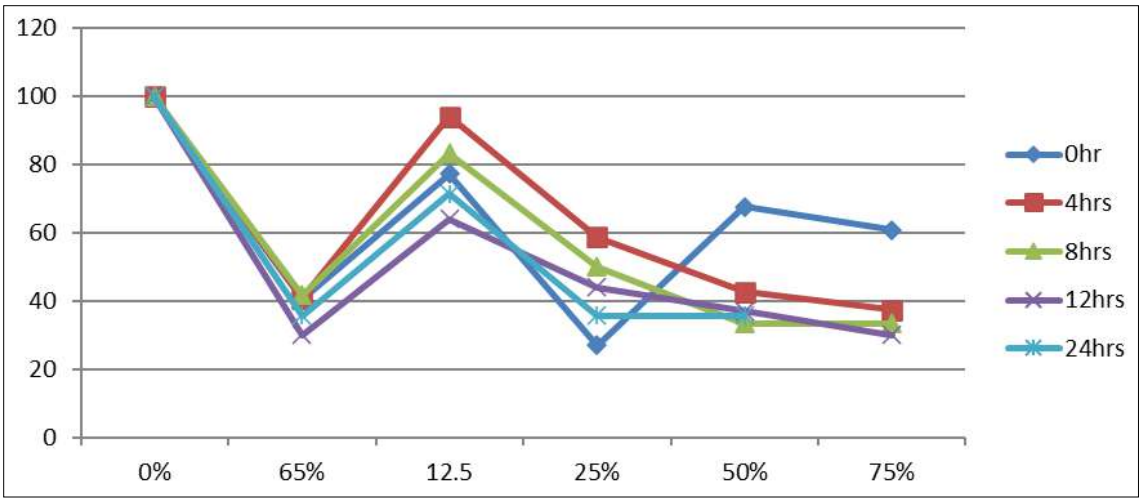

Fig 2: Percentage logarithm survival of *Nitrosomonas* species with spent automobile battery in soil environment

The result obtained during the lab work reveal that certain substances in automobile battery used to power mobile phones are relatively toxic in *Nitrosomonas* than *Nitrobacter* species. Similar observation have been reported Wang, (2004). Hence, during the research, it was observed that automobile battery proved to be more lethal to *Nitrosomonas* than in *Nitrobacter* species and that, the longer the organism are being exposed to these toxicant the more lethal it becomes to them.

The result of the log survival count shows the sensitivity of the organism (*Nitrosomonassp*) to the toxicity of automobile battery. The sensitivity tests showed variation toxic level. Spent automobile motor battery in soil environment with *Nitrosomonas* (36.80%) > automobile battery in *Nitrobacter* (47.49%). The media lethal concentration (LC_{50}) of the spent automobile battery used, decreased signifying the lower the LC50 the more toxic the toxicant concentration. Conclusively, spent automobile battery in *Nitrosomonas* has the lowest toxicity.

Figures 1 and 2 show the relationship of the lethal toxicity of *Nitrobacter* sp. and *Nitrosomonas* sp. at various toxicant concentrations (6.5%, 12.5%, 25%, 50%, 75%) when exposed to spent automobile batteries at various time interval of four hourly (0hr, 4hrs, 8hrs, 12hrs, 24hrs).

Conclusively, the toxicity of spent automobile battery on *Nitrosomonas* sp. in soil is more toxic having the lower LC50 (LC50 = 36.8037%) than the toxicity of spent automobile battery on *Nitrobacter* sp. in soil is more toxic having the higher LC50 (LC50 = 47.4871%). This result shows that the bacterial specie; *Nitrosomonas* extract higher mortality rate than the bacterial specie; *Nitrobacter*.

4. Conclusion and Recommendation

The result reveal that the hazardous chemical from the battery can cause environmental hazard which affect the *nitrobacter* and *nitrosomonas*. Exposure of these chemical can cause a variety of serious health issue in humans if released into the environment. The automobile phone battery are very toxic to *Nitrobacter* which is used as an environmental pollution monitor. And at such its concentration in the soil environment should be monitored.

Waste automobile battery are capable of inducing oxidative damage and denaturation of microorganism as well as weakening the bioremediation capacity of microbes and destructing ion regulatory, affecting the formation of DNA as well as protein.

Battery contains toxic substances such as nickel, cadmium and mercury hence, precautions such as proper disposal of automobile spent battery which involves battery recycling, should be taken.

Recycling of batteries is good for the environment as it keeps them out of landfill, where heavy metals may leak into the ground, causing soil and water pollution and endangering microorganisms. Lead acid battery waste should be returned to a local household hazardous waste collection program for management rather than to dispose in a trash or discharged directly into the environments or water bodies. Also, the manufacturer's facility should provide a strategy in monitoring and controlling of automobile battery waste such as availability of landfill should be covered with a thin layer or clay to avoid chemical in the battery to leach into the ground water thereby leading to pollution.

Furthermore, in solving e-waste problem, the government should provide an environment protection agency (EPA) for proper management of e-waste and adequate investigation possibilities for improved e-waste management systems.

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Not available

6. Author's Contribution

This work was carried out in collaboration among all authors. Author RRN designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AGP performed the statistical analyses. Authors AGP and AVG carried out the literature searches of the study. Author AVG edited and proofread the manuscript. All authors read and approved the final manuscript.

7. Conflict of Interest

Not available

8. Financial Support

Not available

9. References

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