International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 3 issue 3 Sept, 2024 Research Article

Comparative Study of Eco-Toxicity of Domestic Detergents on *Nitrobacter* Species in Triaquatic Ecosystem

Williams, J. O., Nrior, R.R. and Caleb, U.T*
Department of Microbiology, Rivers State University,
Nkpolu-Oroworukwo, P.M.B. 5080, Port Harcourt, Nigeria.
***Corresponding Author:** *calebugochinyere807@gmail.com*

ABSTRACT

Detergents including biodegradable detergents can be toxic to aquatic life and hazardous to human health and the environment due to their properties. The aim of this study was to evaluate the acute toxicity of domestic detergents on *Nitrobacter* sp. (NS) isolated in marine water (MW), brackishwater (BW) and freshwater (FW). Fresh water was collected from Chokocho stream in Etche local Government Area; brackish water from Eagle Island, Port Harcourt; and marine water from Bonny River, all in Rivers State, Nigeria. Microbiological and physicochemical parameters of the water samples were analysed using standard methods. The pH of the water ranged from 5.96 to 8.23 while the temperature of the samples ranged from 27.9^{o}C to 31.2^{o}C. The total dissolved solid (TDS) ranged from 520mg/l to 26700mgl and the total soluble solid (TSS) ranged from 0.01 to 0.003mg/l. The electrical conductivity of the samples ranged from 1040-12840 uS/cm. The toxicity of some domestic detergents including Branded Liquid soap - Morning fresh (MF), mama lemon (ML); Unbranded Liquid Soap: green soap (GS), and orange soap (OS) at 0%, 1.625%, 3.26%, 12.5%, 25%, 50% and 75% were tested against *Nitrobacter* species in fresh, marine, and brackish water for 0, 2,8 ,12, 24, and 48 hours. The Median Lethal Concentration (LC_{50}) was employed to compare the toxicities of the detergents on the test organism. The process was monitored to determine frequency of mortality and survival for 48 hours using standard ecotoxicological methods. Results of Total heterotrophic bacterial count ranged from 9.6 x10^{6} to 1.15 x10^{7} cfu/ml with marine water recording the lowest counts; while *Nitrobacter* counts ranged from 2.5x10^{4} to 7.3 x10^{4} cfu/ml. The percentage log survival of *Nitrobacter* sp decreased in marine water followed by fresh water and then brackish water. The Median Lethal Concentration (LC_{50}) (%) for *Nitrobacter* decreased in the following order (noting that the lower the LC_{50}, the more toxic the toxicant). For branded liquid soap; Morning Fresh: BW (33.27) > FW (42.75) > MW (47.25), Mama Lemon: FW (23.82) > MW (32.57) > BW (33.10) while Unbranded Soap; Orange soap: BW (26.86) > FW (28.97) > MW (33.82), Green Soap: MW (22.94) > BW (26.77) > FW (36.10). The result of the mean lethal concentration showed the highest toxicity of soap/ detergent to test organism (*Nitrobacter*) in brackish water (30.00%) followed by Freaswater (32.91%) and marine water (34.15%). The use of detergents in aquatic ecosystems should be regulated to reduce their toxicity and to ensure safety of aquatic ecosystem.

Keyword: *Nitrobacter*, Domestic detergent, aquatic ecosystem, toxicity.

Introduction

An aquatic ecosystem is an ecosystem found in and around a body of water, in contrast to land-based terrestrial ecosystems. The two main types of aquatic are marine ecosystems and freshwater ecosystems. Freshwater ecosystems may be lentic (slow moving water, including pools, ponds, and lakes); lotic (faster moving water, for example streams and rivers); and wetlands (areas where the soil is saturated or inundated for a while) (Vaccari *et al*., 2005).

Marine ecosystems are the largest of Earth's aquatic ecosystems and exist in waters that have a high salt content. These systems contrast with freshwater ecosystems, which have a lower salt content.

Marine waters cover more than 70% of the surface of the Earth and account for more than 97% of Earth's water supply and 90% of habitable space on Earth.

Seawater has an average salinity of 35 parts per thousand of water. Actual salinity varies among different marine ecosystems.

Fresh water ecosystems are a subset of Earth's aquatic ecosystems. They include lakes, ponds, rivers, streams, springs, bogs, and wetlands (Robert *et al*., 2001). They can be contrasted with marine ecosystems, which have a larger salt content. Freshwater habitats can be classified by different factors including temperature, light penetration, nutrients and vegetation.

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

Aquatic environments being the largest part of the biosphere, contains many species of microorganisms in both marine and freshwater. Open oceans contain salt and many marine bacteria, referred to as oligotrophic psychrophiles, which have a requirement for salt and can grow at relatively low temperatures. Fresh water from lakes and rivers has a complex flora, which includes genuinely aquatic microbial species as well as those introduced from terrestrial, animal, and plant sources. *Nitrosomonas* and *Nitrobacter* species are chemoautotrophic organisms found in soil and water, and are responsible for the oxidation of ammonium to nitrite (*Nitrosomonas*) sp and nitrite to nitrate (*Nitrobacter*) sp. This process known as nitrification is important because it can affect plant growth beneficially.

Detergents being organic compounds, which have both polar and non-polar characteristics, tend to exist at phase boundaries, where they are associated with both polar and non-polar media. Detergents are of three types: anionic, cationic and non-ionic. Anionic and cationic have permanent negative or positive charges, attached to non-polar (hydrophobic) C-C chains. Non-ionic detergents have no such permanent charge; instead, they have a number of atoms which are weakly electropositive and electronegative. This is due to the electron-attracting power of oxygen atoms. The increasing release of organic pollutants by industries causes many health-related problems. However, increased awareness of the toxicity of these environmental pollution has led to a dramatic increase in research on various strategies that may be employed to clean up the environment (Olusola and Benjamin, 2009).

Toxicity is a relative property of a chemical, which refers to its potential to have deleterious effects on living organisms. The potential of acute aquatic toxic effects due to the release of secondary or tertiary sewage effluents containing the breakdown products of laundry detergents may frequently be low. However, untreated or primary treated effluents containing detergents may pose a problem.

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours). To be described as *acute* toxicity, the adverse effects should occur within 14 days of the administration of the substance. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years).

It is widely considered unethical to use humans as test subjects for acute (or chronic) toxicity research. However, some information can be gained from investigating accidental human exposures (e.g., factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, *in vitro* testing methods and inference from data on similar substances.

The harmful effects that chemicals have upon individual organisms depend on many different factors. Not only the difference between the freshwater species, but also the form in which pollutants occur, and if the pollutant shows up in lentic or lotic systems. To measure the toxicity, some toxicity tests will be carried out after which the lethal dose will be expressed in a LC_{50} or LD_{50}.

The effects of pollutants on the whole organism are considered under three main headings, namely neurophysiological, behavioural and reproductive effects. These effects can often be inter-related: neurological changes can affect behaviour; changes in behaviour can affect reproduction and so on. A compound does not always exert an effect on a target organism or a community. It always depends on the concentration of that compound and the time of exposure to it. These effects eventually can be either acute or chronic. Acute toxicity occurs rapidly, are clearly defined, often fatal and rarely reversible. Chronic effects develop after long exposure to low doses or long after exposure and may ultimately cause death.

A poison is lethal when it causes death, or sufficient to cause it, by direct action. A poison is sub lethal when it is below the level that directly causes death. Which results in the regression of the physiological or behavioural processes of the organism, and its overall fitness is reduced. The effects of pollution on freshwater species are registered in the loss of some species, with maybe some profits for some of them. There normally is a reduction in diversity but not necessarily numbers of individual species, and a change in the balance of such processes as predation, competition and materials cycling. Due to the complexity of pollution, the effects of take-up in the aquatic life are also depended on the pollutants characteristic feature. If two or more poisons are present together in an effluent they may exert a combined effect to an organism, which can be additive, antagonistic or synergistic. Thus, the aim of this study is to evaluate a comparative eco-toxicological bioassay of domestic detergent on *Nitrobacter* species in triaquatic ecosystem.

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

Materials and Methods

Sample Collection/ Study Area

Freshwater was collected from Chokocho stream in Etche Local Government Area, brackish water sample was collected from Eagle Island, Port Harcourt; and Marine water samples from Bonny local Government Area, all in Rivers State, Nigeria, These samples were collected in sterile plastic containers and transported within 24hours to the Microbiology Laboratory Department of Rivers State University, Port Harcourt, Nigeria, for analyses.

Toxicants Used

National Agency for Food and Drug Administration Comission (NAFDAC) registered domestic detergents are classified as Branded liquid soap: morning fresh (MF) and Mama lemon (ML) were purchased from Everyday Supermarkets GRA Port Harcourt; while Unbranded liquid soap: Orange soap (OS) and Green soap (GS) used in this study were purchased from Mile 3 Market, Port Harcourt.

Microbiological Analysis of the water samples

Isolation and Enumeration of Total Heterotrophic Bacteria counts (THBC)

The total heterotrophic bacterial count for the water samples was determined using standard microbiological methods (spread plate method) (Prescott *et al.,* 2005). An aliquot (0.1ml) from 10^{-4} dilutions (after serial dilution) were aseptically transferred unto properly dried nutrient agar plates in duplicate, spread evenly using flamed bent rod and incubated at 37ºC for 24 hours, after incubation, the bacterial colonies that grew on the plates were counted and average taken (Prescott *et al*., 2005).

The colony forming unit for the THB of water samples were then calculated using the (Equation 1) according to Nrior and Odokuma (2015).

$$\mathbf{CFU/ml} = \frac{\boldsymbol{Number\ of\ colonies}}{\boldsymbol{Dilution\ x\ Volume\ plated}}$$

Discrete colonies on the plates were sub-cultured unto fresh nutrient agar plates using the streak plate technique to obtain pure culture of the bacterial isolates.

The pure cultures were aseptically transferred into 10% (v/v) glycerol suspension, well label and stored at -4ºC as stock cultures (Amadi *et al.,* 2014).

Isolation of *Nitrobacter* species

The total *Nitrobacter* species in the tri aquatic ecosystem was isolated and enumerated using microbiological standard methods (Prescott *et al.,* 2005). 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 (psi) for 15minutes after which it was allowed to cool to about 40^0C and aseptically transferred to sterile Petridshes. The Petridishes were then inoculated with the river water and incubated aerobically for 4days at room temperature (30± 2°C). Cultural and morphological characteristics revealed; grayish, mucoid, flat colonies and Gram's reaction of the colonies revealed pear shaped, and other biochemical tests for identification of *Nitrobacter* were carried out as earlier reported (Colwell and Zambuski, 1972; Okpokwasili and Odokuma, 1996a,b). The colonies were aseptically streaked on fresh Winogradsky agar and incubated for 2 days at 30±2°C.

Furthermore, the grayish, mucoid and flat colonies were aseptically transferred from the Petridishes into 200ml Erlenmeyer flasks containing the growth medium and incubated for 24hours at room temperature.

Confirmation of *Nitrobacter* species

Suspected *Nitrobacter* species were subcultured on a fresh Winogradsky agar medium and transferred 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 the medium after (2) days of incubation.

A red colouration indicated by nitrate production from ammonium sulphate was a confirmation of *Nitrobacter* species according the method adopted by Nrior *et al.* (2017) and Kpormon *et al.* (2018).

Toxicity testing of domestic detergents on *Nitrobacter* species

The method adopted by Nrior *et al.* (2017), Williams and Ogolo (2018) and Kpormon *et al.,* (2018) were adopted for testing the toxicity of the detergents on *Nitrobacter* sp in the triaquatic ecosystem. The test was carried out in eight (8) separate test tubes containing appropriately habitat water (Marine, fresh and brackish), for the toxicant and test organism.

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

The following concentrations were used 0% (control) 1.62%, 3.25%, 6.25%, 12.5%, 25%, 50% and 75% in a final volume of 10ml. each of the test tube containing a different concentration of the detergent and 1ml of the 0.5 MacFarland standardized of the bacterial suspension was added. The process was monitored to determine frequency of mortality and survival at a constant interval of 4 hours for 48 hours using microbiological methods as adopted by Nrior and Kpormon (2018), and Williams *et al.* (2020), which involve the inoculation of an aliquot (0.1 ml) from each concentration's of the corresponding set-up on Winogradsky agar media, using the spread plate technique. The inoculation was then repeated after 4, 8, 12, 24 and 48 hours, respectively, after which the colonies on the plate were counted. The formula below was used to estimate the percentage log survival and mortality of the test organism.

Determination of Percentage log survival of the test organisms

The percentage log survival of *Nitrobacter* sp. in the detergent/soap effluent used in the study was calculated using the formula adopted by (Williamson and Johnson 1981; Nrior and Obire, 2015). The percentage log survival of the *Nitrobacter* in the detergent/soap effluent was calculated by obtaining the log of the count in each toxicant concentrations (*Log C*), divided by the log of the count in the zero toxicant concentration (*Log c)* and multiplying by 100.

Thus:

$$\textbf{Percentage (\%) logarithmic survival} = \frac{\textbf{Log C}}{\textbf{Log c}} \textbf{ x100}$$

Where;

Log C = logarithmic count of the toxicant,

Log c = logarithmic count of the control

Percentage log mortality: The Percentage (%) log mortality of the test organisms exposed to the toxicant were determined by subtracting the one hundred from the value of the percentage log survival Percentage (%) = 100 - % log survival

Median lethal concentration (LC_{50}): The median lethal concentration of the two spent mobile phone batteries on the test organisms in tri-aquatic ecosystems were determined by subtracting the value of the highest concentration used (75%) from the sum of concentration difference, multiply by mean percentage mortality and divide by the control (100).

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

Statistical Analysis

The data obtained during the study was analyzed statistically using a computer-based program, SPSS version 22 for analysis of variance (ANOVA) of the data in the respective ecosystem.

Results

Physicochemical Parameter of the samples

Table 1 shows the physicochemical parameters of the water samples. The pH of the water ranged from 5.96 to 8.23 with the fresh water sample having the least value and the highest pH was recorded in the marine sample. The temperature of the samples ranged from 27.9^{o}C to 31.2^{o}C with the fresh water having the least temperature 27.9^{0}C and the highest temperature value 31.2^{O}C was recorded in the brackish water. The total dissolved solid (TDS) ranged from 520mg/l to 26700mgl with the marine water having the highest value 26700mg/l and the lowest value 520mg/l was observed in the fresh water sample. The total soluble solid (TSS) ranged from 0.01 to 0.003mg/l with the brackish water and marine water having the lowest value 0.01mg/l and the highest value 0.03mg/l was observed in the fresh water. The electrical conductivity of the samples ranged from 1040-12840 uS/cm with the freshwater having the lowest concentration 1040uS/cm and the highest value 12840uS/cm was observed in the marine sample. The total solid (TS) was recorded to be of the range, 520.03mg/l to 26700mg/l with the freshwater sample having the least total solid 520.03mg/l and highest total solid 26700mg/l was observed in the marine water. The amount of chlorine and bromine was not detected in the freshwater and brackish water however; the marine water recorded the value of 0.01mg/l and 0.02mg/l for chlorine and bromine respectively. The dissolved oxygen (DO) value ranged from 0.6 to 1.2mg/l with the marine water having the least value 0.6mg/l and highest value 1.2mg/l was recorded in the fresh water sample. The BOD value ranged from 0.1 to 1.9mg/l with the brackish water having the least value 0.1mg/l and the highest value 1.9mg/l was recorded in the marine water sample. The nitrate value ranged from 0.367mg/l to 3.701mg/l with the brackish water sample having the least nitrate concentration 0.367mg/l and the highest 3.701mg/l was observed in the freshwater. The sulphate concentration was not detected in the fresh water and brackish water while the marine water recorded the sulphate concentration of 0.022mg/l.

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

Table 1: Physico-chemical Properties of the Tri-aquatic Ecosystem

S/N	Parameters	Unit	Freshwater	Brackish water	Marine water
1	pH	-	5.96±0.014^{c}	7.08±0.035^{c}	8.23±0.014^{c}
2	Temperature	^{0}C	31.0±0.707^{e}	31.2±0.070^{e}	27.9±1.41^{e}
3	Total Dissolved Solids (TDS)	mg/L	520±0.707^{f}	6423±1.414^{f}	26700±0.707^{f}
4	Total Suspended Solids (TSS)	mg/L	0.03±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}
5	Electrical Conductivity (EC)	µS/cm	1040±1.414^{g}	12840±1.414^{g}	4400±2.121^{g}
6	Total Solids	mg/L	520.03±0.00^{f}	6423.01±0.00^{f}	26700.01±1.40^{f}
7	Chlorine	mg/L	0.01±0.07^{a}	0.01±0.07^{a}	0.01±0.07^{a}
8	Bromine	mg/L	0.01±0.07^{a}	0.01±0.07^{a}	0.02±0.07^{a}
9	Dissolved Oxygen (DO)	mg/L	1.2±0.1^{a}	1.1±0.1^{a}	0.6±0.14^{a}
10	Biochemical Oxygen Demand (BOD)	mg/L	0.2±0.07^{a}	0.1±0.1^{a}	1.9±0.1^{a}
11	Nitrate	mg/L	3.701±0.00^{b}	0.367±0.00^{b}	0.513±0.00^{b}
12	Sulphate	mg/L	<0.01±0.00^{a}	<0.01±0.00^{a}	0.022±0.00^{a}
13	Phosphate	mg/L	0.006±0.00^{a}	0.003±0.00^{a}	0.611±0.388^{a}
14	Total Hydrocarbon Content (THC)	mg/L	10±1.41^{d}	24±0.71^{d}	96±1.41^{d}

The phosphate concentration ranged from 0.003mg/l to 0.611mg/l with the brackish water having the least value 0.003mg/l while the highest 0.611mg/l was recorded in the marine water. The concentration of total hydrocarbon content (THC) of the sample ranged from 10mg/l to 96mg/l with the freshwater having the least value 10mg/l and the highest value 96mg/l was observed in the marine water sample.

The result of the bacteriological counts obtained from the fresh and brackish and marine water samples is presented in Table 2. Total heterotrophic bacterial count ranged from 9.6 x10^{6} to 1.15 x10^{7} cfu/ml. Marine water sample recorded lowest counts of total heterotrophic bacteria, while *Nitrobacter* counts ranged from 2.5x10^{4} to 7.3 x10^{4} cfu/ml.

Table 2: Microbial Counts of the Tri-aquatic Ecosystem

Parameter	Freshwater	Brackish water	Marine water
THBC (CFU/ml)	1.15±0.01 x10^{7}	1.04±0.01x10^{7}	9.6±0.01 x10^{6}
Nitrobacter count (CFU/ml)	2.5±0.00x10^{4}	7.3±0.01 x10^{4}	3.9±0.01x10^{4}

Key: THBC = Total Heterotrophic Bacterial Count, CFU = Colony Forming Unit

Effect of various concentrations of branded liquid soap (morning fresh) on percentage log survival of *Nitrobacter* sp. during 48 hours exposure period

The results of the log survival count show the sensivity of the organism (*Nitrobacter sp)* to various concentrations of morning fresh in fresh, brackish and marine ecosystems. The mean log percentage survival of the organism is presented in Table 3.

It indicates that the test organism showed a decrease in percentage log survival as concentration increased with increasing time. More percentage log survival was observed in marine water (96.44%), followed by Brackish water (84.81%) while fresh water had the lowest percentage log survival (83.65%) at 1.62% concentration while at 75% concentration; FW (41.45%) > MW (39.30%) > BW (23.76).

Table 3: Effects of Various Concentration of Branded Liquid Soap (Morning Fresh) in Marine, Brackish and Freshwater on Percentage Survival of *Nitrobacter* sp. during 48 Hours of Exposure Period

Conc. (%)	Treatment		
	FW+BLS (MF)+NB	BW+BLS (MF)+NB	MW+BLS (MF)+NB
Control (0)	100.00±0.00^{h}	100.00±0.00^{h}	100.00±0.00^{h}
1.62	83.65±5.25^{g}	84.81±7.32^{g}	96.44±1.95^{g}
3.25	76.62±6.64^{f}	70.83±6.07^{f}	92.74±2.90^{f}
6.25	71.92±9.48^{e}	60.19±13.07^{e}	89.42±2.97^{e}
12.5	63.97±12.24^{d}	50.07±15.57^{d}	83.20±9.62^{d}
25	52.61±12.34^{c}	38.45±16.19^{c}	74.48±17.60^{c}
50	46.53±10.13^{b}	32.40±18.48^{b}	58.46±31.54^{b}
75	41.45±9.55^{a}	23.76±18.31^{a}	39.30±39.42^{a}

*Means with different alphabet along the column shows a significant difference ($p \leq 0.005$) **FW;** Freshwater, **BW;** Brackish water, **MW;** Marine water, **BLS;** Branded Liquid Soap, **MF;** Morning Fresh, **NB;** *Nitrobacter*

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

Effect of Various Concentrations of Branded Liquid Soap (Mama Lemon) on Percentage Survival of *Nitrobacter Sp* During 48 Hours of Exposure Period.

The result of the log survival count shows the sensitivity of the organism *(Nitrobacter*) to various concentrations of morning fresh in fresh, brackish and marine ecosystems. The mean log percentage survival of the organism is presented in Table 4. It indicates that the test organism showed a decrease in log percentage survival as concentration increased with increasing time. Higher percentage log survival was observed in marine water (84.19%), followed by brackish water (83.17%) at 1.62% concentration; while fresh water (59.44) had the lowest percentage log survival; while at 75% concentration; BW (22.50%) > MW (21.19%) > FW (9.26%).

Effect of various concentrations of unbranded liquid soap 1 (orange soap) on percentage log survival of *Nitrobacter sp* during 48 hours of exposure Period

The result of the log survival count shows the sensitivity of the organism (*Nitrobacter sp)* to various concentrations of morning fresh in fresh, brackish and marine ecosystems. The mean log percentage survival of the organism is presented in Table 5. It indicates that the test organism showed a decrease in log percentage survival as concentration increased with increasing time. Higher percentage log survival was observed in marine water (82.92%), followed by fresh water (79.28%) while brackish water had the lowest percentage log survival (70.95%) at 1.62% concentration; while at 75% concentration; MW (22.51%) > FW (14.06%) > BW (10.64%).

Table 4: Effects of Various Concentration of Branded Liquid Soap (Mama Lemon) in Marine, Brackish and Freshwater on Percentage Survival of *Nitrobacter* sp. during 48 Hours of Exposure Period

Conc. (%)	Treatment		
	FW+BLS (ML)+NB	BW+BLS (ML)+NB	MW+BLS (ML)+NB
Control (0)	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
1.62	59.44±9.30^{b}	83.17±6.14^{b}	84.19±5.61^{b}
3.25	50.65±11.72^{c}	72.93±11.27^{c}	71.36±7.29^{c}
6.25	42.76±10.83^{d}	59.44±11.21^{d}	59.00±4.64^{d}
12.5	32.44±11.79^{e}	49.12±13.91^{e}	49.31±6.60^{e}
25	27.22±12.43^{f}	39.08±16.25^{f}	38.07±8.65^{f}
50	17.76±13.44^{g}	32.91±15.91^{g}	31.51±8.66^{g}
75	9.26±9.07^{h}	22.50±15.07^{h}	21.19±8.81^{h}

*Means with different alphabet along the column shows a significant difference ($p \leq 0.005$) **FW;** Freshwater, **BW;** Brackish water, **MW;** Marine water, **BLS;** Branded Liquid Soap, **ML;** Mama Lemon, **NB;** *Nitrobacter*

Table 5: Effects of Various Concentration of Unbranded Liquid Soap 1 (Orange colour) in Marine, Brackish and Freshwater on Percentage Survival of *Nitrobacter* sp. during 48 Hours of Exposure Period

Conc. (%)	Treatment		
	FW+ULS (OS)+NB	BW+ULS (OS)+NB	MW+ULS (OS)+NB
Control (0)	100.00±0.00^{h}	100.00±0.00^{h}	100.00±0.00^{h}
1.62	79.28±8.51^{g}	70.95±17.30^{g}	82.92±6.27^{g}
3.25	68.56±13.83^{f}	60.39±19.62^{f}	73.29±6.98^{f}
6.25	55.37±13.45^{e}	52.31±18.82^{e}	62.87±8.36^{e}
12.5	45.78±13.32^{d}	39.64±18.17^{d}	53.02±8.85^{d}
25	34.29±15.76^{c}	30.92±18.81^{c}	41.89±9.60^{c}
50	26.28±16.26^{b}	21.72±20.26^{b}	33.41±10.46^{b}
75	14.06±16.65^{a}	10.64±14.06^{a}	22.51±10.80^{a}

*Means with different alphabet along the column shows a significant difference ($p \leq 0.005$) **FW;** Freshwater, **BW;** Brackish water, **MW;** Marine water, U**LS;** Unbranded Liquid Soap 1, **OS;** Orange Soap, **NB;** *Nitrobacter*

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

Effect of Various Concentrations of Unbranded Liquid Soap 2(Green Soap) on Percentage Survival of *(Nitrobacter* Sp During 48 Hours of Exposure Period.

The result of the log survival count shows the sensitivity of the organism *(Nitrobacter*) to various concentrations of morning fresh in fresh, brackish and marine ecosystems. The mean log percentage survival of the organism is presented in Table 6.

It indicates that the test organism showed a decrease in log percentage survival as concentration increased with increasing time.

More percentage log survival was observed in fresh water (75.32%), followed by brackish water (67.39%) while marine water (61.34%) had the lowest percentage log survival at 1.62% concentration; while at 75% concentration; FW (22.92%) > BW (12.42%) > MW (7.16%).

Table 6: Effects of Various Concentration of Unbranded Liquid Soap 2 (Green colour) in Fresh, Brackish and Marine Water on Percentage Survival of *Nitrobacter* during 48 Hours of Exposure Period

Conc. (%)	Treatment		
	FW+ULS (GS)+NB	BW+ULS (GS)+NB	MW+ULS (GS)+NB
Control (0)	100.00±0.00^{ab}	100.00±0.00^{ab}	100.00±0.00^{a}
1.62	75.32±11.62^{bc}	67.39±14.54^{bc}	61.34±12.17^{b}
3.25	79.54±11.62^{d}	61.37±14.82^{d}	52.74±14.39^{c}
6.25	68.27±14.16^{e}	50.04±13.86^{e}	43.34±9.73^{d}
12.5	61.27±14.38^{f}	39.49±13.43^{f}	33.95±9.95^{e}
25	50.36±14.44^{g}	31.46±9.83^{g}	25.52±8.77^{f}
50	36.67±11.94^{h}	22.50±10.29^{h}	15.95±9.14^{g}
75	22.92±11.79^{i}	12.42±7.91^{i}	7.16±8.17^{h}

*Means with different alphabet along the column shows a significant difference ($p \leq 0.005$) **FW;** Freshwater, **BW;** Brackish water, **MW;** Marine water, **ULS;** Branded Liquid Soap, **GS;** Green Soap, **NB;** *Nitrobacter*

Table 7: Median Lethal Concentration (LC_{50}) with Comparative Mean values of Branded and Unbranded Liquid Soap in Freshwater, Brackish and Marine Water on *Nitrobacter* during 48hours of exposure period

Toxicants	FW LC_{50} (%)	BW LC_{50} (%)	MW LC_{50} (%)	Mean LC_{50} (%)
BLS(MF)	42.75	33.27	47.25	41.09
BLS(ML)	23.82	33.10	32.57	29.83
ULS(OS)	28.97	26.86	33.82	29.88
ULS(GS)	36.10	26.77	22.94	28.60
Mean LC_{50} (%)	**32.91**	**30.00**	**34.15**	

Key: FW; Freshwater, **BW;** Brackish water, **MW;** Marine water, **BLS**; Branded Liquid Soap, **MF**; Morning Fresh, **ML**; Mama Lemon, **ULS;** Branded Liquid Soap, **OS**; Orange Soap, **GS;** Green Soap, **NB;** *Nitrobacter*

Median Lethal Concentration (LC50) of Branded and Unbranded Liquid Soap on *Nitrobacter s*p. In the tri-aquatic ecosystem.

The Median Lethal Concentration (LC_{50}) (%) for *Nitrobacter* decreased in the following order (noting that the lower the LC_{50}, the more toxic the toxicant). For branded liquid soap; Morning Fresh: BW (33.27) > FW (42.75) > MW (47.25), Mama Lemon: FW (23.82) > MW (32.57) > BW (33.10) while Unbranded Soap; Orange soap: BW (26.86) > FW (28.97) > MW (33.82), Green Soap: MW (22.94) > BW (26.77) > FW (36.10) as presented in Figure 1. The result of the mean lethal concentration showed the highest toxicity of soap/ detergent to test organism (*Nitrobacter*) in brackish water (30.00%) followed by Freaswater (32.91%) and marine water (34.15%) was presented in Figure 2.

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

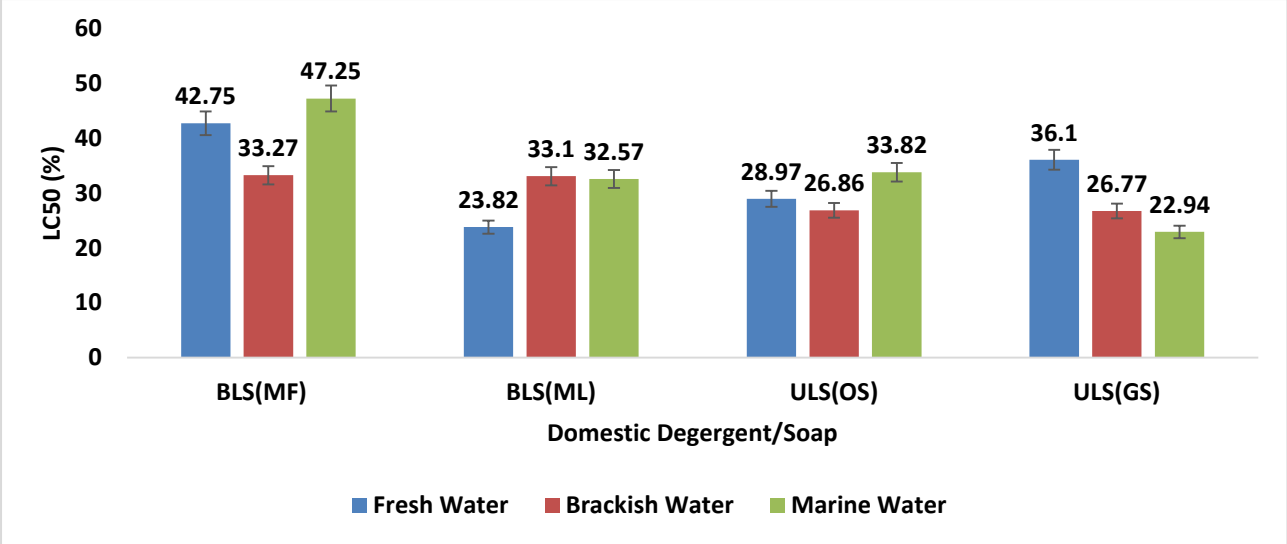

Figure 1: Median Lethal Concentration (LC_{50}) (%) of Detergent/Soap on *Nitrobacter* species in the Tri aquatic Ecosystem

Key: BLS = Branded Liquid Soap, ULS = Unbranded Liquid Soap, MF = Morning Fresh, ML = Mama lemon, OS = Orange Soap, GS = Green Soap

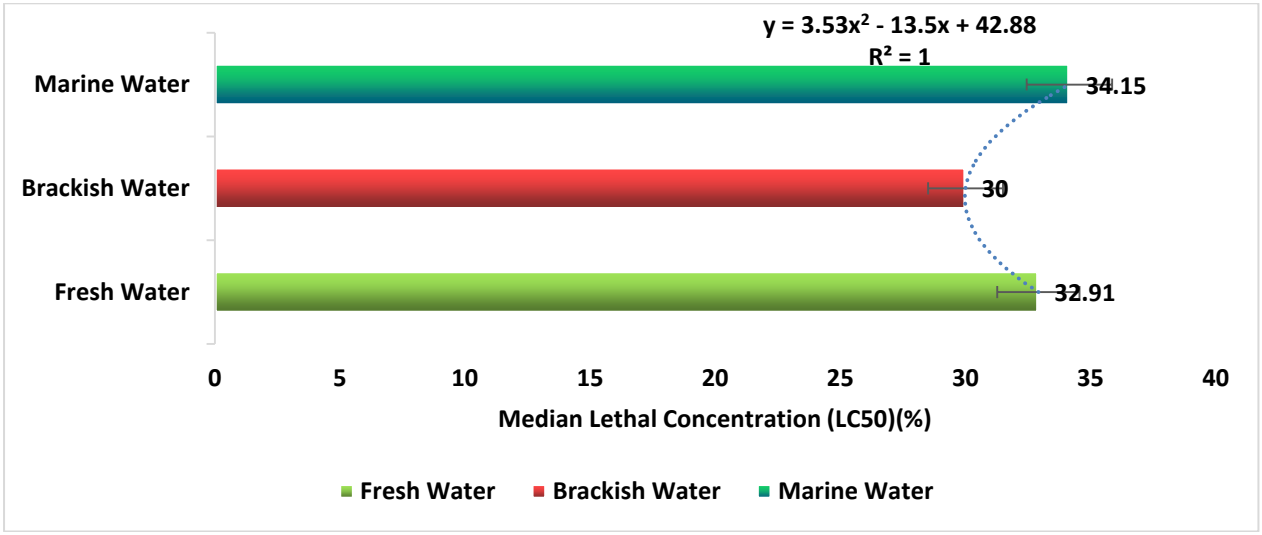

Figure 2: Comparative LC_{50} of Detergent/Soap on *Nitrobacter* species in the Tri aquatic Ecosystem

Discussion

The result of the bacteriological counts obtained from the fresh and brackish and marine water samples is presented in Table 1. Total heterotrophic bacterial count ranged from 9.6 $x10^6$ CFU/ml to 1.15 $x10^7$ CFU/ml. Marine water sample recorded lowest counts of total heterotrophic bacteria, while *Nitrobacter* counts ranged from $2.5x10^4$ CFU/ml to 7.3 $x10^4$ CFU/ml. The microbial count in the study showed that the highest count of total heterotrophic bacteria was observed in the freshwater sample followed by the brackish water.

This range of count is not in line with the findings of Nrior *et al*. (2022) in which higher count of total heterotrophic bacteria was reported in brackish water followed by fresh water and followed by marine water (Nrior *et al.*, 2022). The highest nitrifying bacteria count was recorded in the brackish water sample followed by the marine water and the least counts were observed in the fresh water sample.

This is similar to the findings of Kpormon and Douglas (2018) in which least count of *Nitrobacter* species was recorded in fresh water samples. The count of *Nitrobacter* species in this study is slightly lower than those recorded in the study of Ekanem and

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

Udosen (2023) in the microbial determination of aquatic ecosystems. The differences in bacterial counts of the water samples can be attributed to the activities going on within the water bodies (Ekanem and Udosen, 2023). As recorded in other studies, salinity might have been a factor in the microbial population of the different microbial group (Nrior *et al.,* 2022). Previous studies have shown that total heterotrophic bacteria are the most abundant and diverse group within aquatic systems and in relation to nitrifying bacteria, somehow contribute to maintaining suitable water quality by eliminating nitrogenous compounds from the environment through assimilation into microbial biomass in a single-step process called denitrification (Pan *et al.*, 2015). In this process, heterotrophic denitrifying bacteria use organic matter coming from uneaten food and fish faeces to metabolize nitrogen oxides (NO_2, NO_3) to nitrogen gas (N_2), producing a large amount of bacterial biomass (Ruiz *et al.*, 2019).

Effect of Detergent on Percentage Survival of *Nitrobacter Species* During 48 Hours of Exposure Period.

The result of the log survival counts shows the sensitivity of the organism *(Nitrobacter sp*) to various concentrations of morning fresh in fresh, brackish and marine ecosystems. The mean log percentage survival of the organism is presented in Table 3-6 It indicates that the test organism showed a decrease in log percentage survival as concentration increased with increasing time.

Comparative LC_{50} of Detergent on *Nitrobacter* species in the Tri aquatic Ecosystem

From the study, the increase in the mortality of the test microorganism (*Nitrobacter* sp) and) in the aquatic ecosystems by the soap with increase in the exposure time. This is in line with the study of Williams and Dilosi (2018) in which similar trend of mortality was observed in the study of toxicity of pesticides to *Nitrobacter* and *Nitrosomona* bacteria in aquatic ecosystems. The percentage log survival of the test organisms *Nitrobacter* species used in this study to the detergent in the three aquatic ecosystems was observed to be highest in the fresh water followed by the brackish water and the least percentage log survival was observed in the marine water. The mortality of the test organism in response to the soap in this study can be attributed to the enzyme's inactivation and susceptibility of the microorganism to soap (a xenobiotic) which might have hampered their ability to recovered gradually within the period of study (Nrior *et al.*, 2017).

The mean lethal concentration (LC_{50}) which is used to determine the amount or concentration of material or toxicant that is expected to kill 50% of the test organism when there is exposure to the environment (Yuniari *et al.,* 2018). The lower the LC_{50} of a substance, the more toxic the substance and *vice versa* (Williams and Dilosi, 2018). As shown in Figure 1, the median lethal concentration (LC_{50}) of the local liquid and solid soap on the test organism revealed that the local liquid and solid soap were more toxic to marine followed by the brackish water and fresh water. The higher toxicity to to the *Nitrobacter* iin marine water is in line with the study of Kpormon and Douglas (2018) in which higher toxicity was of used phone battery was recorded in marine water compared to brackish and fresh water respectively.

In conclusion, the result of the mean lethal concentration showed the highest toxicity of detergent to the test microorganism (*Nitrobacter*) in marine water followed by brackish water and fresh water. Low median lethal concentrations was observed in the three aquatic ecosystems especially in in marine water which revealed the ability of the detergent to inhibit biological processes such nitrification that are mediated by this key environmental microorganisms such (*Nitrobacter* species). Therefore the use of soap and detergent in the aquatic ecosystem should be regulated to reduce their toxicity to the aquatic ecosystem and to ensure the environment safety of the aquatic ecosystem also the public and industries who discharge detergent and soap into the water body should be educated on the impact to beneficial role of the nitrifying microorganisms to aquatic ecosystems.

References

Amadi, E.N., Kiin, K.D., Kpormon, L.B. and Robinson, V.K.K. (2014) Microbial flora and nutritional composition of adult palm-wine beetle. *International Journal of Current Microbiology and Applied Science, 3(11),* 189-192.

American Public Health Association (1992). Standard Methods for the Examination of Water and Waste Water, 20th edition, Washington DC.

Colwell RR, Zambruski MS (1972). Methods of Aquatic Microbiology, University Park Press, Baltimore.

Ekanem, A. M. and Udosen, N. I. (2023). Evaluation of groundwater potentiality and quality in Ikot Ekpene - Obot Akara Local Government Areas, Southern Nigeria. *IResearch Square,* 1-28

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.

Kpormon, L. B. and Douglas, S. I. (2018), Comparative Ecotoxicological Assay of E- waste (Phone Batteries) on Some Aquatic Micro Flora. *Journal of Advances in Biology & Biotechnology, 17(2),* 1-10.

Nrior, R R, Ngeeregbara N. N, Barol, R. T. and Amadi, L. (2017). Ecotoxicity of local and industrial refine kerosene. *International Research Journal of Public and Environmental Health, 4(9),* 199-204..

Nrior, R. R. & Kpormon, L. B. (2018), Comparative Ecotoxicological Analyses of Spent Phone Batteries on *Pseudomonas* sp. in Tri Aquatic Environment, Current *Journal of Applied Science and Technology, 27(6),* 1-11.

Nrior, R. R. and Obire, O. (2015). Toxicity of domestic washing bleach (Calcium hypochloride) and detergents on *Escherichia coli. Journal of International Society of Comparative Education, Science and Technology*. *2(1),* 124-135

Nrior, R. R., Douglas, S. I. and Igoni, Y. G. (2022). Toxicity of Herbicide, Paraquat Dichloride and Insecticide, Lambda-cyhalothrin on Phosphate Solubilizing Bacteria, *Pantoa dispersa* in Aquatic Ecosystems. *Microbiology Research Journal International*, 32(4), 13-21.

Odokuma, L. O. and Nrior, R. R. (2015). Ecotoxicological evaluation of industrial degreaser on *Nitrobacter* sp. *Journal of International Society of Comparative Education, Science and Technology 2(2),* 356-365

Odokuma, L. O. and Nrior, R. R. (2015). Ecotoxicological Evaluation of Industrial Degreaser on *Nitrobacter* sp. *Current Studies in Comparative Education, Science and Technology, 2(2),* 356-365

Okpokwasili, G. C. and Odokuma, L. O. (1996a). Response of *Nitrobacter* to toxicity of drilling chemicals. *Journal of Petroleum Science and Engineering. 16,* 81-87.

Okpokwasili, G. C. and Odokuma, L. O. (1996b). Tolerance of *Nitrobacter* to toxicity of hydrocarbon fuels. *Journal of Petroleum Science and Engineering. 16,* 89-93.

Olusola, L. and Benjamin, H. (2009). Determination of physicochemical parameters of effluents at discharge points into the New Calabar River along Rumuolumeni axis, Niger Delta, Nigeria. *Journal of Environmental and Analytical Toxicology, 8(3),* 2161-052.

Pan, B, Sou, M. & Hijikata, N. (2015). Effect of detergents from grey water on irrigated plants: case of okra (Abelmoschus esculentus) and lettuce (*Lactuca sativa*). *Journal of Arid Land, 24,* 117–120.

Prescott, L. M., Harley, J. P. & Klein, D. A. (2005). *Microbiology. 6th ed. McGraw Hill*, London. 33-50.

Robert, Z., Darnault, C. J., Tian, F., Philippe, C. B. & Hu, H. (2001) Influence of anionic surfactant on saturated hydraulic conductivity of loamy sand and sandy loam soils. *Water, 9(6),* 433.

Ruiz, A. J., Carlson, A. K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T., Kidd, K.A., MacCormack, T.J., Olden, J.D., and Ormerod, S.J., (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Review, 94 (3),* 849–873.

Vaccari, D. A., Strom, Peter F. & Alleman, J. E. (2005*). Environmental Biology for Engineers and Scientists. Wiley-Interscience.ISBN0-471-74178-7*

Williams, J. O. & Dilosi, L. B. (2018). Response of Chemolithotrophic *Nitrobacter*, *Nitrosomonas* to Toxicity of Organophosphate and Pyrethroid Pesticides. *Asian Journal of Biology, 7(1),* 1-8

Williams, J. O. & Ogolo, E. (2018). Physicochemical and Microbial Quality of a Creek in the Niger Delta Region of Nigeria. *Journal of Advances in Microbiology, 10(1),* 1-10.

Williamson, K. J. and Johnson, O. G. (1981). A bacterial bioassay for assessment of wastewater toxicity. *Water Resources 15,* 83-190

Yuniari, S. H., Hertiko, A. M. S. & Leksono, A. S. (2018). Lethal concentration 50 (LC_{50}-96) Nile Tilapia (*Oreochromis niloticus*) exposed to cypermethrin-based pesticide. *Journal of Experimental Life Sciences, 6(2),* 58-61.

Citation: Williams *et al*. (2024). Comparative study of eco-toxicity of domestic detergents on *Nitrobacter* species in triaquatic ecosystem. *International Journal of Microbiology and Applied Sciences*. *3(3)*: 37 - 46.