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Ecotoxicity Strength of Granular Domestic Detergents on *Enterobacteraerogenes* and *Pseudomonas fluorescens*

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Original Research Article

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Abstract: Ecotoxicity strength of different granular domestic detergents on *Enterobacter aerogenes and Pseudomonas fluorescens* was evaluated to assess the response of the test organisms to different brands of domestic detergents. Standard toxicity procedures were applied using four brands of granular domestic detergents: Klin, Omo, Bonux and Ariel prepared at concentrations of 10ppm, 100ppm, 1000ppm, 10000ppm and 100000ppm; the test was carried out for 0h, 4h, 8h, 12h, and 24h for each detergent.The degree of resistance of the test organisms to the toxicants revealed *Enterobacter aerogenes* (4857±1685ppm) was more resistant to the domestic detergents than *Pseudomonas fluorescens* (117±28ppm). Mean Median lethal concentration (LC_{50}) showed that Klin was most toxic and Bonux was least toxic with toxic flow pattern (noting that the lower the LC_{50} the more toxic the toxicant) as:Klin (1354ppm) > Omo (2389ppm) > Ariel (2887.47ppm) > Bonux (3316.84ppm).Results of response of individual test organism to the different brands of detergents showed that *Pseudomonas fluorescens* was most sensitive to Bonux and least sensitive to Ariel - Bonux (94.86ppm) > Klin (104.66ppm) > Omo (109.68ppm) > Ariel (157.40ppm))while *Enterobacter aerogenes* was most sensitive to Klin and least sensitive to Bonux - Klin (2603.34ppm)> Omo (4668.68ppm) > Ariel (5617.54ppm) > Bonux (6538.82ppm). This showed that test bacteria responded differently to the toxic effect of the domestic detergents in the same aquatic environment. In mixed consortium, domestic detergent Klin had the highest toxic strength (mean LC_{50} 1354ppm); *Pseudomonas fluorescens* was more sensitive to all the test domestic detergents than *Enterobacter aerogenes.*
Keywords: Toxicity, strength, detergents, aquatic, microflora.

INTRODUCTION

The discharge of waste containing domestic and industrial detergents into the ecosystem may affect the microbiota of the receiving environment [1]. Detergents can have poisonous effects in all types of aquatic life if they are present in sufficient quantities, and this includes the biodegradable detergents. All detergents destroy the external mucus layer of fishes and can cause severe damage to gills. Most fish will die when detergents concentrations approach 15 parts per million (15ppm). Detergents concentration as low as 5ppm will kill fish eggs. Surfactant detergents are implicated in increasing the breeding ability of aquatic organisms. Detergents also add another problem to aquatic life by lowering the surface tension of the water. Organic pesticides such as pesticides and phenols are then much more easily absorbed by the fish. A detergent concentration of only 2ppm can cause fish to absorb double the amount of chemicals they would normally absorb, although that concentration alone is not enough to affect fish directly. Phosphates in detergents can lead to freshwater algal blooms that release toxins and decrease oxygen in waterways. When the algae decompose, they use up the oxygen available for life. Detergents are very widely used in both industrial and domestic premises like soaps and detergents to wash vehicles. The major entry point into water is via sewage works. They are also used in pesticide formulations for dispersing oil spills at sea. The degradation of alkylphenol polyethoxylates (non-ionic) can lead to the formulation of alkyl (particularly nonylphenols), which act as endocrine disruptors [2].

Bacteria are easy to standardize for toxicity in comparism to many eucaroytic organism [3]. The greatest impact seems to be on the aquatic environment in that our natural surface waters, ponds, streams, rivers, estuaries, lagoons, lakes, seas and oceans with inherent aquatic lives are rather “waste sinks” directly or indirectly for most Nigerian’s chemical, food, agricultural and petroleum based industries [4].

This study is designed to evaluate the effect of granular domestic detergents on the environment via aquatic degradative microflora which are simple and fast bioassay for monitoring ecosystem response to these pollutants; moreover, its eco-systemic influence in Niger Delta.

MATERIALS AND METHODS

Sample collection

Water samples were collected in 2L sterile plastic containers from Azuabie River near Port Harcourt Zoo in Port Harcourt Local Government Area in Rivers State, Nigeria. The river spans from Rumuogba via Woji, Azuabie down to Marine Base, Port Harcourt. The river does not only receive facial matters (as the coastal dwellers traditionally defecate into the water body), but other industrial chemicals, solids and domestic detergent effluents discharged from industries, landry and domestic activities.

Detergents used for toxicity test

Domestic detergents used in the study were purchased from oil mill market in Port Harcourt city, Rivers State, Nigeria. Toxicants and their sources were Klin (Eko Supreme Resources Nigeria Ltd., Ogun, Nigeria), Bonux (Proter & Gamble Nig. Ltd., Ibadan, Nigeria), Omo (Unilever Nig. Ltd., Aba, Nigeria), Ariel (Procter & Gamble Nig. Ltd., Ibadan, Nigeria) Each of the toxicant (domestic detergents) were prepared at 100000ppm, 10000ppm, 1000ppm, 100ppm, 10ppm concentrations using 0.5 dilution factor.

Isolation of Test Organisms

Pseudomonasfluorescens was isolated using two stage method: (i) Two milliliters (2ml) of *Pseudomonas* broth ampoules (Hach, USA-Cat. No. 28122-50) was clipped open and poured onto absorbent pad and placed on sterile Petri-dishes and allowed to adhere. After membrane filtration of the sample, the membrane filter was aseptically collected and placed onto the prepared *Pseudomonas* broth pad plate and incubated at 30ºC for 24h. Only Pseudomonas sp. grew on this medium. (ii) Furthermore, Morphological and biochemical identification were used for isolation of the species: *Pseudomonasfluorescens*. Identification to the generic/species level followed the scheme of APHA [5].

Enterobacter aerogenes was isolated using M-Endo agar plates (Hach,USA-Cat. No.28116-15). After membrane filtration of the sample, the membrane filter was aseptically collected from the Millipore filtration unit by sterilized forcep and carefully placed onto the M-Endo agar plates (Hach,USA-Cat. No. 28116-15). This was incubated at $35\pm0.5^{O}C$ for growth of colonies. Suspected colony was subcultured onto fresh sterile M-Endo agar plates and used for biochemical characterization tests. Identification of isolates was based on their cultural/morphology, microscopic examination, carbohydrate fermentation and other biochemical tests. References were made to *Bergey's Manuel of Determinative Bacteriology* [6], 8^{th} edition, for the identification of bacteria.

Physicochemistry of Diluent (habitat water)

Hydrogen ion concentration (pH), temperature, conductivity, and dissolved oxygen of the habitat water were determined electrometrically with a multi-parameter data logger (Hanna model H1991300). The meter was calibrated prior to use with 0.01N and 0.1N standard potassium chloride solutions (according to the manufacturer's specifications), and buffer standards (obtained from Accu standards) of pH 4, 7 and 10 at room temperature. Alkalinity was determined in accordance with ASTM D 1067B. Total Dissolved Solids (TDS) was determined electrometrically while Total Suspended Solids (TSS) was determined with a membrane filter apparatus in accordance with APHA 2540D. Chloride, Nitrite, Nitrate and Ammonia were determined by HACH, lab. Instrument [7]. Iron, Copper and Lead were determined using Atomic Absorption Spectrophotometer (AAS).

Preparation of Standard Bacterial Inoculum and its application [8]

Tenfold serial dilution of the test organisms was made and aliquot (0.1ml) was inoculated onto M-Endo agar plates (Hach, USA-Cat. No. 28116-15) for *Enterobacteraerogenes* and *Pseudomonas* broth pad plates (Hach, USA-Cat. No. 28116-15) for *Pseudomonas fluorescens* in triplicates using spread plate technique. The plates were incubated for 24 hours. After incubation, the plates were examined for discrete colonies. The dilution that gave between 200 and 300 colonies was noted and used as reference dilution to obtain the standard inoculum for the toxicity bioassay. The standard inoculum was obtained by inoculating discrete colony of the organisms in to sterile nutrient broth and incubated at about 30 - $35^{O}C$ for 18 – 24 hours; the concentrations of the bacteria in broth, after plating out of the broth culture on to the surface of differential media: M-Endo agar plates (Hach,USA-Cat. No. 28116-15) for *Enterobacteraerogenes* and *Pseudomonas* broth pad plates (Hach,USA-Cat. No. 28116-15) for *Pseudomonas fluorescens*, were 10^{8} organisms per millimeter.

Toxicity Test Procedure

Five milliliter (5ml) broth culture of each test organism (*Enterobacteraerogenes* and *Pseudomonas fluorescens*) was added to 45ml of the toxicant concentrations (10ppm, 100ppm, 1000ppm, 10000ppm and 100000ppm; that is 10% broth culture of test organism and 90%of each detergent concentration), and spread plated out immediately after inoculation on to M-Endo agar plates for *Enterobacteraerogenes* and *Pseudomonas* broth pad plates for *Pseudomonas fluorescens*; this is the zero hour testing. After which incubation was carried out at $35\pm0.5^{O}C$for *Enterobacteraerogenes* and $30^{0}C$ for *Pseudomonas fluorescens* for 24h. Aliquot (0.1ml) of each concentration of the domestic detergents was then plated out in duplicates after 4h, 8h, 12 and 24h on the appropriate agar plates and counted after incubation. The mean colony count of each test organism was taken and expressed in colony forming unit per millimeter ($CFU\ ML^{-1}$).

Percentage (%) Log Survival Evaluation of the Organism

The percentage log survival of the bacterial isolates (*Enterobacteraerogenes* and *Pseudomonas fluorescens*) in the different concentrations of the toxicants used in this study was calculated using the formular adopted from Williamson and Johnson [9], Obire and Nrior [10]. This was analyzed by obtaining the log of the counts in each toxicant concentration, divided by the counts in the zero toxicant concentration and multiplying by 100.

$$\text{Thus, \% log survival} = \frac{\text{Log C}}{\text{Log c}} \times 100$$

Percent log survival was plotted against toxicant concentration; control test (without toxicants) was also used.

Median Lethal Concentration (LC_{50}) of the test bacteria

The Median Lethal Concentration (LC_{50}) was computed from mean % log mortality and sum of dose difference using standard statistical analysis using the formula below:

$$LC_{50} = LC_{100} - \frac{\text{Sum of Dose diff. X Mean \% log Mortality}}{\text{\% Control}}$$

Statistical Analysis and Median Lethal Concentration (LC_{50})

Results obtained from toxicity screening were subjected to statistical analysis using Analysis of Variance (ANOVA) and Student t-test, at 0.005 confidence limit, to determine the significant difference ($P<0.005$) between the susceptibility of the *Mucor racemosus* to the test toxicants (Powdered and liquid detergents). The median lethal concentrations were calculated using regression analysis.

RESULTS AND DISCUSSION

The pH (7.04) of the effluent observed in the study was within the permissible limit (6-9) required by FEPA. The temperature (30^oC) also is within the permissible limit ($30\text{-}40^oC$) required by FEPA. Thus, the effect observed in this study could not be attributed to any of the above parameters. The near neutral pH may offer buffering capacity to the brackish water, which could reduce the toxicity effects that could be caused by metallic components of the brackish water. Ammonia concentration (0.8mg/l) of the brackish water sample used in this study far exceeded the maximum allowable limits (0.2mg/l) for effluent [11]. The ammonia concentration in the effluent was within the range causing acute and chronic lethal effect in aquatic organisms [12, 13]. Nrior and Odokuma [3], suggested that high ammonia concentration might be one of the major contributors of the effects they observed in fish, but were unable to correlate changes in ammonia levels with changes in toxicity, when solutions were replaced in tests. Ammonia is highly toxic; penetrates cells very rapidly and causes osmotic lysis of cells resulting in death. It is very toxic to both bacteria and fish [14, 10]. This could probably contribute to the case of the lethal effect observed in the test bacteria: *Enterobacter* species and *Pseudomonas* species [15, 16].

Sulphide concentration observed in the effluent used for this study exceeded the maximum allowable limits (0.2mg/l) for brackish water [11]. Sulphide is soluble, highly poisonous, with characteristic odour of rotten egg. Fish avoids sulphide. It is toxic to eggs, fry and adult fish and invertebrates; 1mg/L sulphide causes 100 percent mortality in 72hrs to Salmon [14]. Sulphide could probably have contributed to the lethal effect in the bacterium (*Enterobacter aerogenes*) used in this study.

The results revealed that the test bacteria: *Enterobacter aerogenes* and *Pseudomonas fluorescens* demonstrated sensitivity to the toxicity of the granular domestic detergents evident in the decreasing percentage log survival with increase concentration of the toxicant (Fig 1-4). Toxicity of the Domestic detergents to the test bacteria may be due to the inhibition of the respiratory process in the organisms [17]. This process occurs in the cell membrane of the organisms and is important in survival of the bacterial cell.

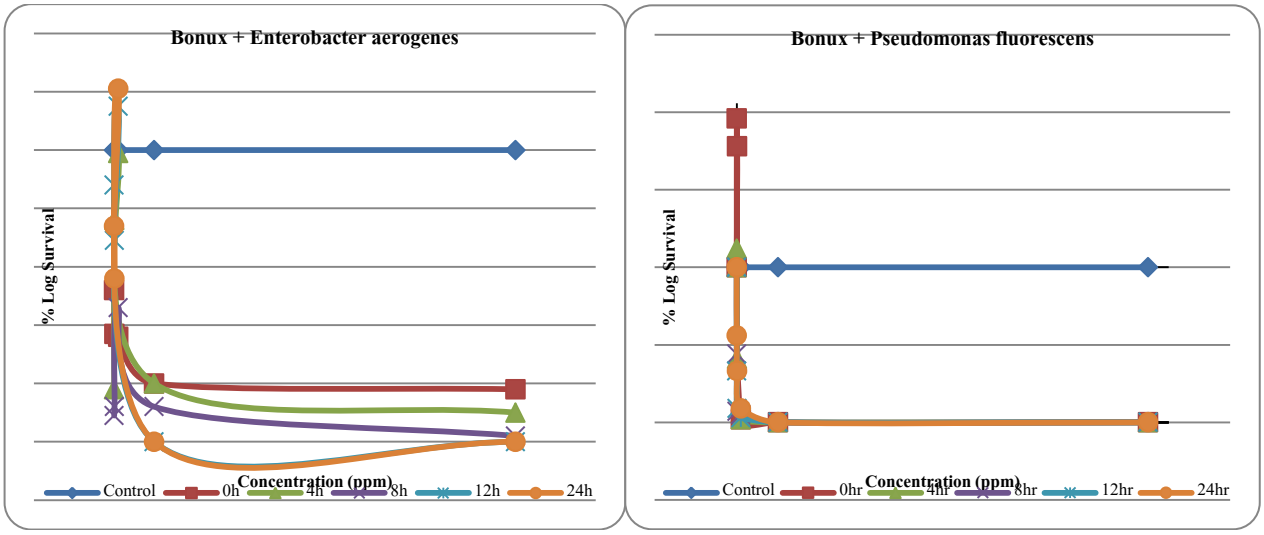

Fig-1: Lethal toxicity of domestic detergent Bonux on *Enterobacter aerogenes* and *Pseudomonas fluorescens*

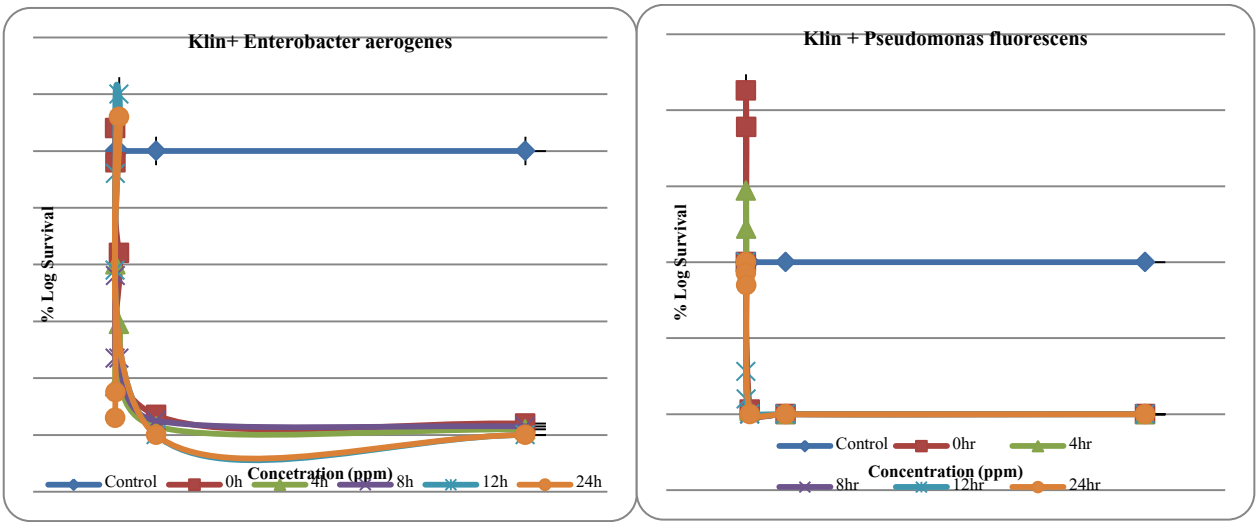

Fig-2: Lethal toxicity of domestic detergent Klin on *Enterobacter aerogenes* and *Pseudomonas fluorescens*

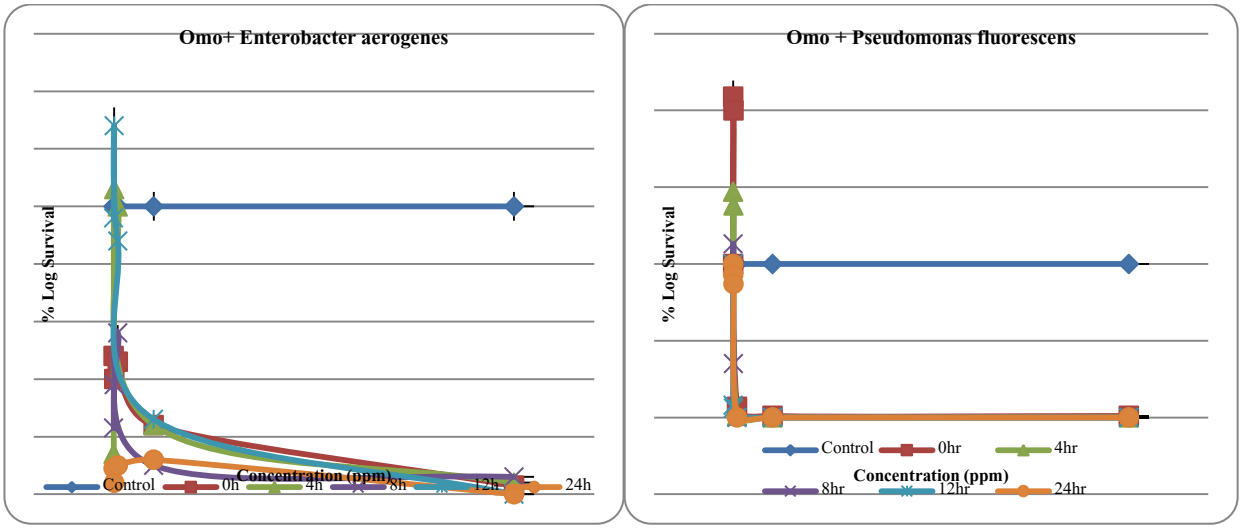

Fig-3: Lethal toxicity of domestic detergent Omo on *Enterobacter aerogenes* and *Pseudomonas fluorescens*

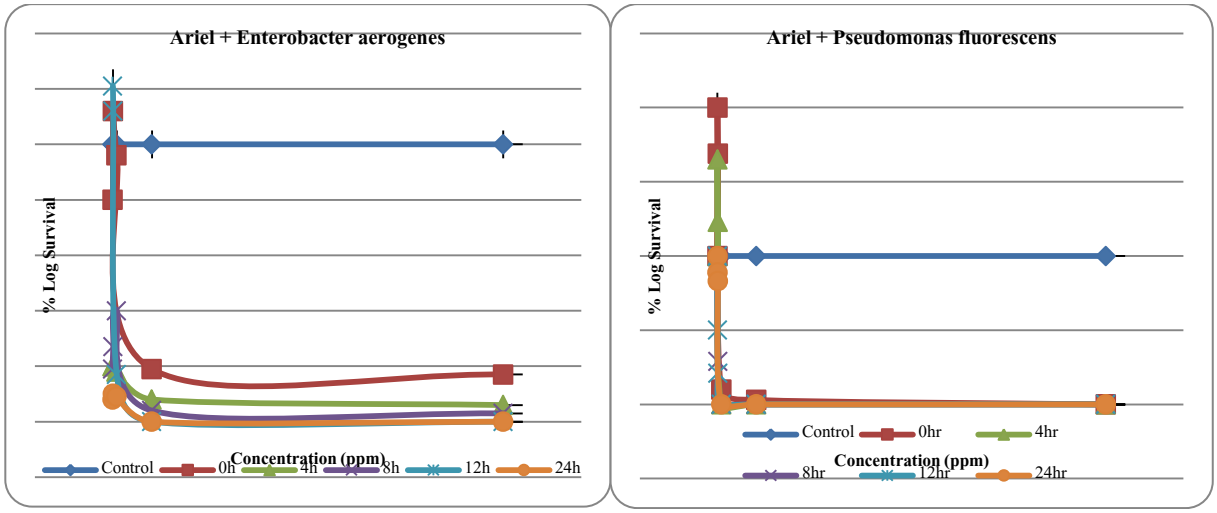

Fig-4: Lethal toxicity of domestic detergent Ariel on *Enterobacter aerogenes* and *Pseudomonas fluorescens*

Toxic chemicals present in the brackish water could come in contact with the respiratory enzymes present in the cell membrane of the organisms, thus interfering with the process.In general, the domestic detergents are toxic to the bacterial isolates used as text organisms in this study. It is interesting to note that not all species, even physiologically similar group of micro-organisms, are similarly influenced by the presence of a given pollutant in their environment. It was found among the saprophytic bacteria that many strains are resistant and others are sensitive to cadmium [18, 19. The nature of the interaction between effluent and microorganisms is complex due to various reactions taking place during a prolonged or previous exposure. Previous authors reported that after an initial period in which the toxic effects of the effluent on brackish water was evident, the microorganisms acquired some tolerance mechanisms which enabled them to repair the damaged part, and to start metabolizing and growing again at a normal rate. This "tolerance" mechanism could be tied up with a simple adaptation of cells to the presence of the toxic chemicals in their brackish water environment [3]. In a more complicated case, mutation could be suspected as was proved by Zelibor *et al.*, [20].

The sensitivity of individual organism to the different brands of detergents showed great variations; toxic level decreased in the following order: (noting that the lower the LC_{50}, the more toxic the domestic detergents: *Pseudomonas fluorescens:* Bonux (94.86ppm) > Klin (104.66ppm) > Omo (109.68ppm) > Ariel (157.40ppm); and *Enterobacter aerogenes:* Klin (2603.34ppm)> Omo (4668.68ppm) > Ariel (5617.54ppm) > Bonux (6538.82ppm) (Fig-5). *Pseudomonasfluorescens* was most sensitive to Bonux and least sensitive to Ariel while *Enterobacteraerogenes* was most sensitive to Klin and least sensitive to Bonux. Comparing the response of the two bacteria to the different brands of detergents, *Pseudomonasspecies* was more sensitive to the detergents than *Enterobacterspecies* which was less sensitive. This showed that different organisms react differently to a particular toxicant in the same aquatic environment. This could be attributed to their genetic constitution as well as inherent changes in the natural ecosystem.

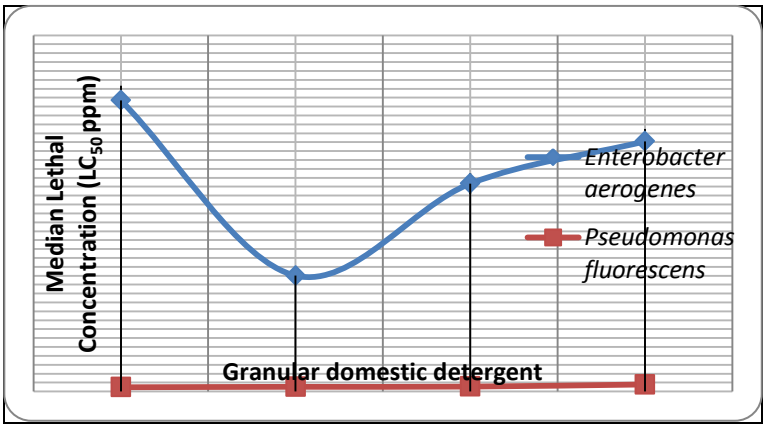

Fig-5: Median Lethal Concentration (LC_{50} - ppm) of Granular domestic detergents to *Enterobacter aerogenes* and *Pseudomonas fluorescens*

Results of mean Median Lethal Concentration (mean LC_{50}) of the toxicants showed granular domestic detergent Klin to be most toxic. The toxic flow pattern is as follows: Klin (1354ppm) > Omo (2389ppm) > Ariel (2887.47ppm) > Bonux (3316.84ppm) (Fig-6). This was evident in far above hundred percentage log survival of these organisms even with increase in the concentration of the detergents (Fig 2-4). Previous researchers have reported that the above groups of bacteria have the ability to degrade crude oil and their refined products for their metabolism [21].

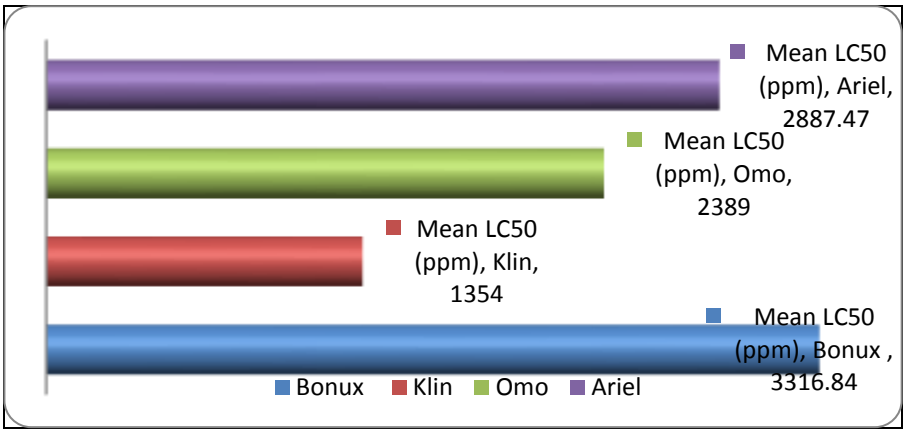

Fig-6: Mean Median Lethal Concentration (mean LC_{50} - ppm) of Granular Domestic detergents to *Enterobacter aerogenes* and *Pseudomonas fluorescens*

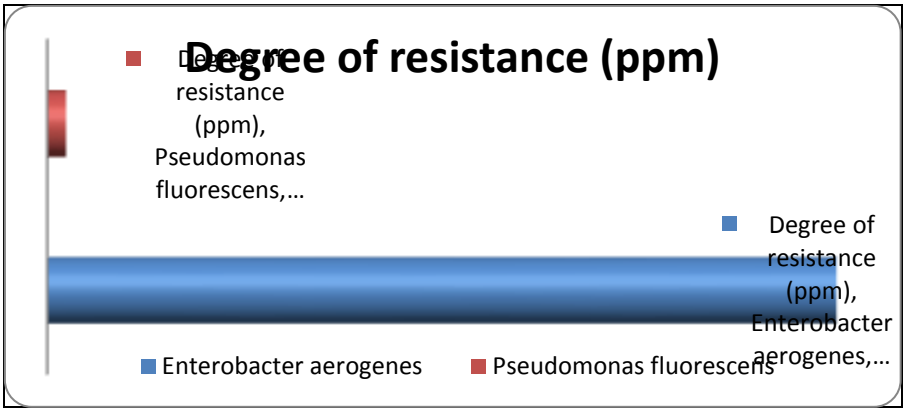

Fig-7: Degree of Resistance (ppm) of *Enterobacter aerogenes* and *Pseudomonas fluorescens to* Granular domestic detergents.

The difference in response of these bacteria to the different brands of domestic detergents could be due to differences in their genetic make-up [22], prolonged or previous exposure to the detergent effluent [16], and mutation [23] as well as relative utilization of the detergent effluent for metabolism [24, 21, 25].

CONCLUSION

The degree of resistance of the test organisms to the toxicants revealed that *Enterobacteraerogenes* (4857±1685ppm) was more resistant (being less sensitive) to the domestic detergents than *Pseudomonasfluorescens* (117±28ppm). Mean Median lethal concentration (LC_{50}) showed that granular domestic Klin was most toxic on the test bacteria. With respect to response of individual test organism; *Pseudomonasfluorescens* was most sensitive to Bonux while *Enterobacteraerogenes* was most sensitive to Kiin. This showed that the different bacteria responded differently to the toxicant in the same aquatic environment. In mixed consortium, domestic detergent Klin had the highest toxic strength (mean LC_{50} 1354ppm). *Pseudomonasfluorescens* was more sensitive to all the test domestic detergents than *Enterobacteraerogenes*. Concentrations of some physicochemical parameters influenced changes in the inherent properties of the brackish water which in turn contributed to the toxicity of the test organisms.

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