# Toxicity of domestic washing bleach (Calcium hypochloride) and detergents on *Escherichia coli*

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ABSTRACT This study reveals that Washing bleach (68% Calcium hypochloride) had the highest toxicity effect (Mean $LC_{50}$ = 1.29ppm or mg/l) on Escherichia coli while Bonux had the least ($LC_{50}$ = 4122.02ppm). The toxicity quotient of the different toxicants (bleach and domestic detergent) on Escherichia coli were as follows; Bleach > Klin > Omo > Ariel > Bonux. The sensitivity of the bacterium to the different detergents shows great variation, toxic level decreases in the following order (noting that the lower the $LC_{50}$, the more toxic the toxicants): Bleach (1.96mg/l) > Omo (2708.42mg/l) > Klin (2856.42ppm) > Ariel (2985.12ppm) > Bonux (4122.02ppm). Median Lethal concentration ($LC_{50}$) percentage ratio follows the sequence: Bleach (0.01%) > Omo (21%) > Klin (23%) > Ariel (24%) > Bonux (32%).Note; the greater the $LC_{50}$ percentage ratio, the less toxic the toxicant. Standard toxicity procedures were applied using Bleach (68% Calcium hypochloride) and domestic detergents- Klin, Omo, Bonux, Ariel; prepared at concentrations of 10ppm, 100ppm, 1000ppm, 10000ppm and 100000ppm; tested for 0 h, 4 h, 8 h, 12 h, 24 h exposure for each toxicant. The high level of ammonia (0.8mg/L) and sulphide (2.5mg/L) as against the 0.2mg/L permissible limit in habitat water used as diluents in this study contributed greatly to the susceptibility of Escherichia coli to washing bleach and domestic detergents.

*Keywords:* Toxicity ratio, Bleach (68% Calcium hypochloride), $LC_{50}$

## Introduction

The increasing release of organic pollutants by industries causes many health -related problems. However, increased awareness of the harmful effects of 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 measure of the degree to which something is toxic or poisonous or Toxicity is a relative property of a chemical, which refers to its potential to have deleterious effects on a living organism. Cytotoxicity is the quality of being toxic to cells. Examples of toxic agents are a chemical substance or an immune cell. Acute toxicity is a property of a substance that has toxic effects on a living organism, when that organism is exposed to a lethal dose of a substance once. Chronic toxicity is a property of a substance that has toxic effects on a living organism, when that organism is exposed to that substance continuously or repeatedly. Genotoxicity is a property of chemical compounds capable of causing genetic mutation and contributing to the development of tumors. (Aquastel, 2007). Ecotoxicology is the study of the effects of toxic chemicals on biological organisms, especially at the population, community, ecosystem level. Ecotoxicology is a multidisciplinary field, which integrates toxicology and ecology. Aquatic toxicology is concerned with the qualitative and quantitative study of the adverse or toxic effects of chemicals and other anthropogenic materials or xenobiotic on aquatic organism (Rand and Petrocelli, 1985). According to Neuhold (1986) Aquatic toxicology emerged in the mid- 1920s as a science for testing the effect of organic chemicals on aquatic organisms. It grew principally from two disciplines: Limnology and Water Pollution Biology (Odiete, 1999).

Toxicity is generally concerned with the effect of chemicals or toxic agents at the level of the individual organism or its constituent parts and emphasies the mechanistic bases of harmful effects and the conditions under which they occur (Klaassen and Eaton, 1991).Washing bleach (Calcium hypochloride) have been proven to cause deleterious effect on some key microbial flora in aquatic ecosystems (Obire and Nrior, 2014). The widespread use and effect of washing bleach in almost all facet of both industrial and domestic (household/industrial equipment cleaning, laundry) sector has attracted serious environmental concern. Calcium hypochloride commonly named chlorine is mostly used in Nigeria for water treatment (borehole and online dosing). Detergents can have poisonous effects in all types of aquatic life if they are present in sufficient quantities, and this include the biodegradable detergents. All detergents destroy the external mucus layer that the fish from bacteria and parasites; plus they 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 is implicated in increasing the breeding ability of aquatic organisms. Obire and Nrior, 2014 reported that chlorine as low as 10ppm cause up to 95% mortality of *Pseudomonas aerogenes* and *Mucor racemosus* in four (4) hour of exposure.

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 (Lenntech, 2008).

Bacteria are particularly important because of their unique role of trophic dynamics in aquatic and terrestrial ecosystems (Jonas, 1989). Bacteria are easy to standardize for toxicity in comparism to many eucaroytic organism (Bauda and Block, 1985). They provide a source of enriched particulate organic carbon by utilization of both dissolved and particulate organic carbon (de la Cruz, 1973).

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 (NEST, 1991).

This study is designed to evaluate the effect of industrial 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.

The aim of this study is to access the permissible limit of v/v dilution usage of domestic detergent using $LC_{50.}$

## Materials and Methods

### *Collection of water samples*

Water samples were collected from Azuabie River near Zoo along Trans-Amadi Industrial Area, Port Harcourt, Rivers state, Nigeria with 100ml sterile plastic container. This was used within 1hour of the collection for isolation of bacteria employed in the study.

### *Physicochemistry of Diluent (habitat water)*

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. Kits (2008). Iron, Copper and Lead were determined using Atomic Absorption Spectrophotometer (AAS).

### *Isolation of test organisms*

The bacteria and fungi isolates was obtained from a river receiving polluted discharge in Trans-Amadi Industrial Layout, Port Harcourt. The isolate was purified on Nutrient Agar Plates with MUG (HACH, USA-Cat. No.: 28121-15). After membrane filtration of the water sample, the membrane filter is transferred unto the Nutrient Agar plate with MUG and incubated for 24hours at $45 \pm 0.5^{0}$C. Only *Escherichia coli* grew on this media. A loop full of the test organism was transferred into 10ml sterile nutrient agar broth; incubated at $45 \pm 0.5^{0}$C and stored in refrigerator at $4^{0}$C.

### *Toxicants*

Washing bleach (68% Calcium hypochloride) and domestic detergents (Klin, Bonux, Omo, Ariel) used in this study were purchased in chemical store and Mile 3 market in Port Harcourt, Nigeria.

## Toxicity Procedure

### *Preparation Test Medium*

The effluent was prepared following the procedure outlined in APHA, 1992. 10ppm, 100ppm, 1000ppm, 10000ppm and 100000ppm concentrations of the toxicants were prepared using 0.5dilution factor respectively.

*Preparation of Test Organism (Escherichia coli)*

A loopful of the test organism was transferred into 10ml sterile appropriate broth. This was incubated for 2-4days at room temperature ($45\pm2^0$C) and stored in refrigerator at $4^0$C. Aliquot (1ml) of the 24h culture was transferred into fresh sterile broth (10ml), incubated for 24h (to ensure that actively growing organisms were used for toxicity test) and preliminary standard Inoculum determined (APHA, 1998).

*Preparation of standard bacterial inoculum*

Tenfold serial dilution of the organism was made and aliquot (0.1ml) was inoculated onto Mac Conkey agar in triplicates using spread plate technique. The plates were incubated for 4 days for *Nitrobacter* sp. After the incubation periods 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.

**Evaluation Procedure**

Five milliliter (5ml) of the test organisms was added to 45ml of each toxicant concentration (10ppm, 100ppm, 1000ppm, 10000ppm and 100000ppm respectively), and plated out immediately after inoculation on appropriate media. This is known as zero hour count plating. These were incubated at room temperature ($28\pm2^0$C). Aliquot (0.1ml) of each concentration of the toxicants (drilling fluid, oil spill dispersant, degreaser and industrial detergents) was then plated out after 4 hours, 8 hours, 12 hours and 24 hours on Winogradsky agar. This was followed by incubation for 96hrs for *Nitrobacter.* The plates were then counted and average colony taken, then Colony Forming Unit per milliliter (cfu/ml) calculated.

*The percentage log survival of Escherichia coli in the toxicants*

The percentage log survival of the bacterial isolates in the toxicant used in the study was calculated using the formula adopted from Williamson and Johnson (1981). The percentage log survival of bacterial isolates in the toxicant was calculated by obtaining the log of the count in each toxicant concentration, dividing by the count in the zero toxicant concentration and multiplying by 100.

*Statistical analysis and Median Lethal Concentration ($LC_{50}$)*

The results obtained from toxicity screening were subjected to statistical analysis using Analysis of Variance (ANOVA) and student t-test at 0.05 confidence limit to determine the significant difference between the susceptibility of the *Escherichia coli* to the test toxicants (washing bleach and detergent -Klin). The median lethal concentrations were calculated using regression analysis.

**Result and Discussion**

The results revealed that *Escherichia coli* isolated from Trans-Amadi Industrial Layout River demonstrated sensitivity to the toxicity of the detergents and bleach (68% Calcium hypochloride). Washing bleach (68% Calcium hypochloride) had the highest toxicity effect (Mean $LC_{50}$ = 1.29ppm or mg/l) fig. 1-5. This was evident in the least $LC_{50}$ and the decreasing percentage log survival with increase concentration of the toxicant. Toxicity of the washing bleach and domestic detergents to *E. coli* may be due to the inhibition of the respiratory process in the organisms (Stanier *et al., 1982; Obire and Nrior, 2014).*

**Fig. 1: Percentage (%) log survival of *Escherichia coli* on washing bleach**

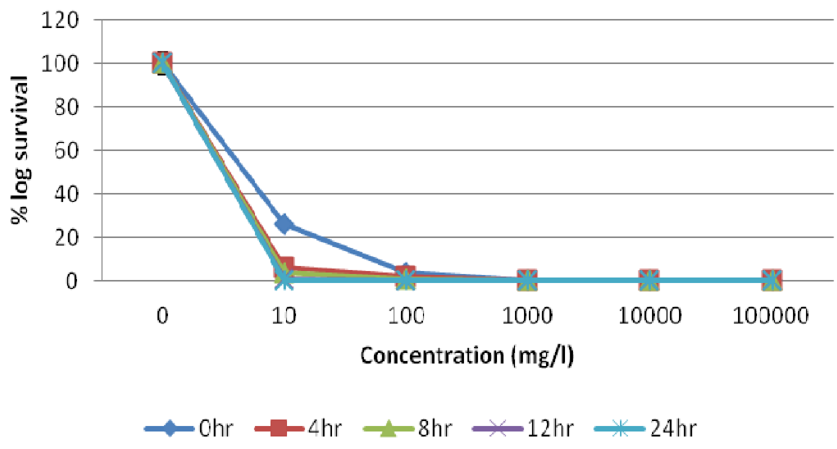


**Fig. 2: Percentage (%) log survival of Escherichia coli on domestic detergent - Bonux**

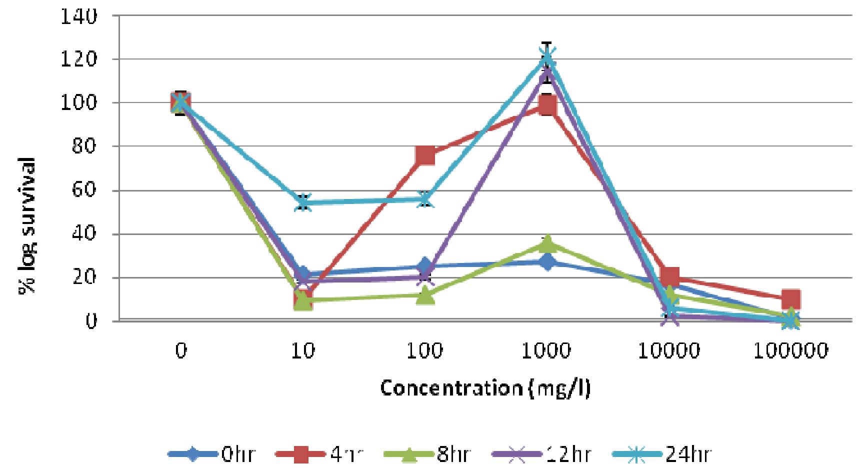



**Fig. 3: Percentage (%) log survival of *Escherichia coli* on domestic detergent - Klin**

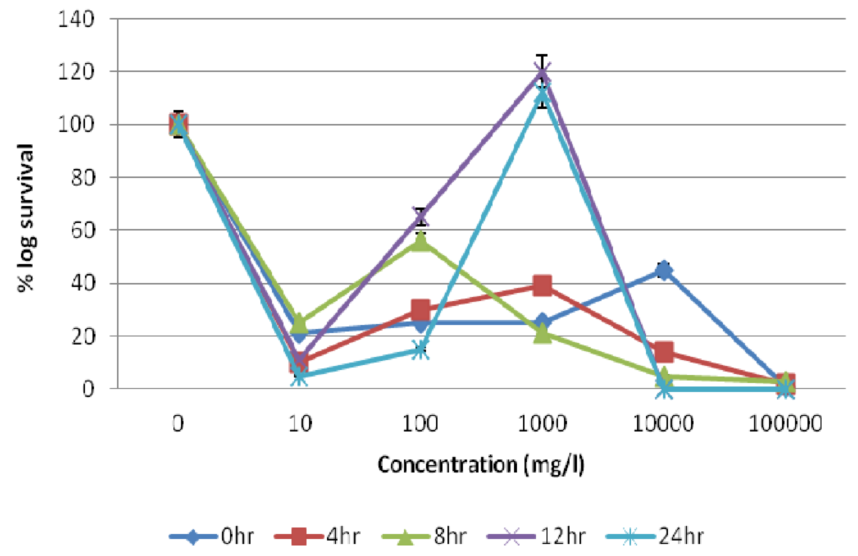


**Fig. 4: Percentage (%) log survival of *Escherichia coli* on domestic detergent - Omo**

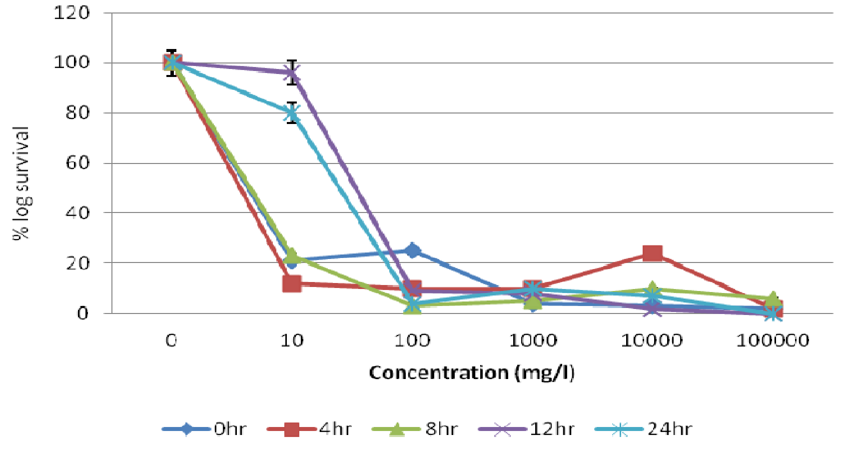
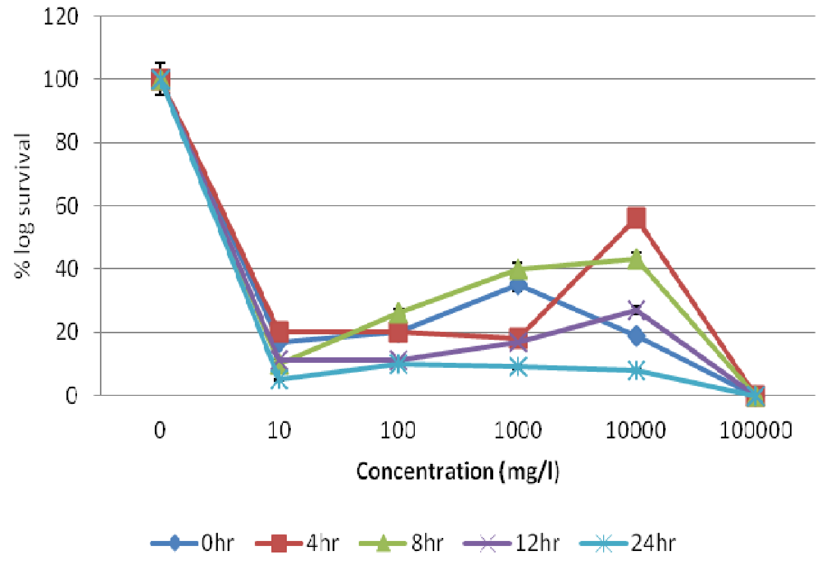
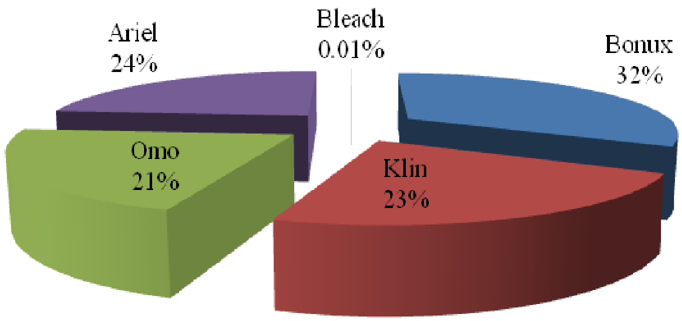



**Fig. 5: Percentage (%) log survival of *Escherichia coli* with domestic detergent - Ariel**



The toxicity quotient of the different toxicants (bleach and domestic detergent) on Escherichia coli were as follows; Bleach > Klin > Omo > Ariel > Bonux. The sensitivity of the bacterium to the different detergents shows great variation, toxic level decreases in the following order (noting that the lower the $LC_{50}$, the more toxic the toxicants): Bleach (1.96mg/l) > Omo (2708.42mg/l) > Klin (2856.42ppm) > Ariel (2985.12ppm) > Bonux (4122.02ppm). Median Lethal concentration ($LC_{50}$) percentage ratio follows the sequence: Bleach (0.01%) > Omo (21%) > Klin (23%) > Ariel (24%) > Bonux (32%).Note; the greater the $LC_{50}$ percentage ratio, the less toxic the toxicant (Fig. 6).

**Fig. 6: Median Lethal Concentration ($LC_{50}$) percentage ratio of toxicants on Escherichia coli** ***[note: the lower the $LC_{50}$, the more toxic the toxicants]***




The physio-chemical parameters result of 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 (DPR, 2002; FEPA, 2000). The ammonia concentration in the effluent is within the range causing acute and chronic lethal effect in aquatic organisms (Weis *et al.,* 1989, FEPA, 2000). 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 (Odiete, 1999). This could probably contribute to the case of the lethal effect observed in the bacterium *Escherichia coli* (Wang, 1984).

The synergistic effect of phenol and ammonia could have probably increased the toxicity effect of the effluent on the organism. Sulphide concentration observed in the effluent used for this study exceeds the maximum allowable limits (0.2mg/l) for brackish water effluents (FEPA, 2000). 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 with Salmon (Odiete, 1999).Sulphide could probably have contributed to the lethal effect in both the bacterium *Escherichia coli* used in this study.

## Conclusion

If the process of decomposition, mineralization, and nutrient regeneration are disturbed by toxicants, the nature of an ecosystem will be changed. It follows that there would be serious perturbation of important biogeochemical cycles: magnesium, sulphur, phosphorus and nitrogen as a result of pollutant stress in Trans Amadi Industrial Layout River. Above all, the overall stability of the river ecosystem will be seriously altered. This study revealed that Trans Amadi Industrial Layout River is heavily impacted with toxic substances. The brackish water effluent from concentrate detergents and Chlorine (bleach) poses great toxicity problem to the microorganisms and higher organisms living in Trans Amadi Industrial Layout River and by extension constitute serious safety/ health hazards to human beings.

## Recommendations

It is therefore recommended that: a) Bleach concentration for effluent discharge should be at most 1ppm, b) All detergent waste effluent that is mixed with ammonia should be treated before discharge into the river, c) From the research findings, it is suggested that bleach and detergent industries develop means of recovery or recycling of toxic constituents of these products.

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