International Scholars Journals

International Journal of Agricultural Sciences ISSN: 2167-0447 Vol. 16 (4), pp. 001-006, April, 2026. Available online at www.internationalscholarsjournals.org © International Scholars Journals



*Full Length Research Paper*

# Evaluating the Impact of Industrial Effluents on Sentinel Species in Soil and Water Ecosystems

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Accepted 8 September 2025

**Lethal toxic effects of industrial detergent (Neatex) and corrosion inhibitor (Norust CR 486) commonly released into the Nigerian environment were studied using standard laboratory toxicity test. Bioindicators (fish, shrimp and earthworms) were exposed to varying concentrations of the test chemicals using the Organisation for Economic Cooperation and Development (OECD) # 203, 218 and 207 protocols respectively. The water, sediment and soil ratings indicate that both chemicals were slightly toxic to the organisms and the estimated 4, 10 and 14 days lethal concentration (LC50) showed that the corrosion inhibitor was more toxic than the industrial detergent. There was differential toxicity between organisms exposed to the test chemicals in the three media and the control groups at ($p < 0.05$). The observed sensitivity of the test organisms to both chemicals in the different media provides a basis for regular checks on chemicals discharged into the Niger Delta waters, this is because most of the chemicals released into the environment sorb to the soil and sediment particles and can cause harm to organisms in the soils, sediment and overlying waters.**

**Keywords:** lethal toxicity, fish, shrimp, earthworm, industrial chemical, surfactant.

## INTRODUCTION

The environmental concern over the use of hazardous chemicals is increasing worldwide. There are many such chemicals polluting the environment, with the damage wrought depending on their exposure, persistence in the ecosystem, as well as the characteristics of the affected organisms (Landis and Yu, 2004; Cunningham and Saigo, 1990). Considerable amounts of cleansing materials (surfactants) used in domestic and industrial domains are directly discharged into waterways and on land. These may pose environmental problems in the ecosystem including toxicity of the surfactants to fish and invertebrates, foaming and euthrophication (Abel, 2006; Hashim *et al.*, 1992).

Ecological evaluation of chemicals is important for safeguarding the environment. Nowadays, to be accepted worldwide, a product must satisfy rigorous ecological criteria in addition to having good performance and must be environmentally friendly. Under international law, before a new chemical can be used, it must first be registered and information on its environmental performance must be supplied (IPCS, 1996; Ezemonye *et al*., 2007). Ecological risk assessments are usually conducted for the purpose of defining the extent of hazardous waste contamination in the aquatic and terrestrial biota (SETAC, 1997).

Most formulated products (detergents and corrosion inhibitors) contain surfactants {linear alkylbenzene sulphonates (LAS)}. Several authors have reported that anionic surfactants (LAS) cause destruction in gill epithelium, impair chemoreceptor organs and damage epidermis and pharyngeal wall (Pozo et al., 2003). Anionic surfactants are reported to be acutely toxic to fish and other aquatic organisms at concentrations between 0.4 and 40 mg/l (Abel 2006; Tovell *et al.*, 1975). Studies also carried out by Lightowlers, 2004; Ghazali and Ahmad, 2004 showed that LAS is poorly degraded in rivers and soils and may be toxic to organisms inhabiting these environments. Other authors who have reported the harmful effects of different types of surfactants on biological indicators include; Schowanek *et al*., 2007; Madsen et *al*., 2001; Fuller *et al*., 2004 and Edward and Bohlen, 1992. Britton 1998 and Ezemonye and Enete,

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2004 have reported that the use, storage, transportation and disposal of chemicals into the environment point to a growing problem that threatens the health of the ecosystem and people.

The aim of this study was to estimate the short-term toxicity of two commonly used chemicals, {industrial detergent (Neatex) and corrosion inhibitor (Norust CR 486)} to fish (*Tilapia guineensis*); shrimp (*Desmoscaris tripsinosa*) and earthworms (*Aporrectodea longa*). These organisms were chosen based on their availability, sensitivity, ease of maintenance under laboratory conditions and consumption by many larger vertebrates (Beeby, 2001; Sandoval *et al*., 2001; Ciarelli, *et al*., 1997).

## MATERIALS AND METHODS

### Test chemicals

The test chemicals, Neatex and Norust CR 486 were obtained from the manufacturers (Manuex Nigeria Limited and Ceca Incorporated) respectively. After collection, the test chemicals were stored at 4$^{o}$C prior to the commencement of the test. The liquid water soluble chemicals were brought to room temperature before the test solutions were prepared. Both chemicals contained LAS as a major active ingredient (approximately 12 - 16% in Neatex and 25 - 27% in Norust CR 486).

### Experimental bioassay procedure for lethal toxicity of fish, shrimp and earthworm

Fresh water fish (*Tilapia guineensis*) were collected from farms at Kpakiama in the Niger Delta ecological zone. Acclimation to laboratory conditions was carried out in holding tanks for seven days before the test. Acute toxicity of fish exposed to the test chemicals was determined using the Organisation of Economic Development and Cooperation (OECD), 1992. The semi-static renewal bioassay procedure started with a range finding test. This was used to determine the range of concentrations for the definitive test. The test concentrations for the definitive test (6.25, 12.5, 25, 50 and 100 mg/l) were prepared by appropriate dilution of the stock solution (200 mg/l). A total of five (5) litres of the test medium and control (dilution water) was used to test 10 test organisms in three replicates (OECD, 1992). The test solutions were renewed daily and their physico-chemical constituents {pH, temperature, total dissolved solids (TDS), salinity and conductivity} were measured at test initiation and termination. The organisms were not fed during the 96 hours experimental period. The weight and length of the fish was 0.471 ± 0.03 g and 1.83 ± 0.12 cm respectively.

Fresh water shrimp (*Desmoscaris trispinosa*) were collected from the same environment as the fish and acclimated for seven days before starting the test. Acute toxicity of shrimp exposed to the test chemicals was determined using the OECD, # 218 (2004) method. Sediments were collected using a hand held van Veen grab and stored in the dark at 4$^{o}$C until required for the experiment. The sediment samples were removed from the refrigerator approximately 24 hours before the experiment. The sediment was allowed to equilibrate to room temperature and weighed (Whale and Worden, 1999). Stock solutions of the two chemicals (1000 mg/l) were prepared and serial dilutions were made to obtain concentrations of 500, 250, 125, 62.5 and 31.25 mg/l in three replicates. The 10-day static sediment bioassay was conducted by placing the weighed sediment into sets of 5 liters amber coloured glass tanks. The sediment in the container was spread evenly and 2000 ml of the prepared test solution was gently added. The contents of the containers were left to stand for a maximum of 3 hours before ten (10) test organisms were added. The size of organisms was 0.156 ± 0.03 g and 2.65 ± 0.36 cm in length.

Earthworms (*Aporrectodea longa*) were collected by gentle digging and hand sorting from sub surface litters and maintained in the laboratory for seven days before the test. Acute toxicity of earthworm exposed to the test chemicals was determined using the OECD # 207 protocol (OECD, 1984). Stock solutions of 1000 mg/l of the two chemicals were prepared and serial dilutions were made to obtain concentrations in the range of 500, 250, 125 and 62.5 mg/l. The soil samples were prepared by mixing clean dry soil with 20 g of cellulose and 80 ml (water) homogenized in a glass container. Thereafter, ten voided earthworms (400–600 mg) were cleaned and transferred from their holding containers with a sterilized platinum wire to the soils spiked with concentrations of the test chemicals in three replicates. The control experiment contained ten (10) organisms, cellulose, water and clean soil (Sandoval *et al*., 2001).

### Statistical Analysis

The susceptibility of fish, shrimp and earthworms to the test chemicals was determined using a computerized probit method of analysis according to Finney, (1971) for the LC50 at day 4, 10 and 14 respectively. Analysis of variance (ANOVA) in Statistical Package for Social Science (SPSS) statistical software in Version 13.0 was used to test the variables at $p < 0.05$ level of significance.

## RESULTS

The results for the fish, shrimp and earthworms bioassay are presented in Tables 1-3 and figures 1-4.

### Fish

The results of acute toxicity of the test chemicals to 14-day old *Tilapia guineensis* are presented in Table 1 and figure 1. The influence of concentration, exposure duration and environmental conditions were observed. Mean % mortality at 96 h exposure in the freshwater test and the control groups was significantly different at $p < 0.05$ for both chemicals. Mean % mortality values reported for concentrations 6.25, 12.5, 25, 50 and 100 mg/l were 37, 50, 60, 77, 87% (Neatex) and 33, 50, 73, 90, 100% (Norust CR 486) respectively.

A regular trend was generally observed in the mortality rate which increases with increased concentration. Linear alkylbenzene sulphonates (LAS) is a major active constituent, in Neatex and Norust CR 486. It could be regarded as one of the major reasons for induced mortality. At the early stage as well as lower test concentrations of 6.25 and 12.5 mg/l of the toxicants introduction, most of the fishes survived initial attack. This may be due to their protective adaptations as well as individual physiological nature of *T. guineensis*. Some damages or injuries were noticeable particularly amongst

**Table 1**: Acute toxicity profile of fish to Neatex and Norust CR 486 exposure

| | 96 hours LC50 ± SD (mg/l) | 95% CL | Probit Line Equation | Slope ± SD |
|---|---|---|---|---|
| Neatex | 17.10 ± 3.31 | 2.91 – 42.67 | Y = 3.59 + 1.13 log x | 7.33 ± 1.09 |
| Norust CR 486 | 13.58 ± 1.15 | 5.77 – 22.53 | Y = 2.89 + 1.86 log x | 3.55 ± 0.79 |

**Table 2:** Acute toxicity profile of shrimp to Neatex and Norust CR 486 exposure

| Test Chemical | 10-Day LC50 ± SD (mg/kg) | 95% CL | Probit Line Equation | Slope ± SD |
|---|---|---|---|---|
| Neatex | 139.49 ± 10.08 | 90.52 – 220.84 | Y= -0.28 + 2.46 log x | 2.55 ± 0.25 |
| Norust CR 486 | 78.61 ± 8.26 | 45.65 - 119.57 | Y= 0.444+2.402 log x | 2.61 ± 0.25 |

**Table 3:** Acute toxicity profile of earthworms to Neatex and Norust CR 486 exposure

| Test Chemical | 14-day LC50 ± SD (mg/kg) | 95% CL) | Probit Line Equation | Slope ± SD |
|---|---|---|---|---|
| Neatex | 413.82 ± 0 | 266.67 - 873 | Y= -2.066 + 2.700 log x | 2.33 ± 0 |
| Norust CR 486 | 207.61 ± 14.09 | 129.93 - 411.49 | Y= 0.25 + 2.04 log x | 3.10 ± 0.41 |

Where Y= Probit, x = concentration in mg/l
SD = Standard deviation
CL = Confidence limit

some fishes in the higher concentrations (25, 50 and 100 mg/l). These injuries are believed to weaken the organisms' resistance to the test chemicals and consequently resulting to significant death of greater than 50% within the higher concentrations of 25 and 50 mg/l of Neatex and Norust CR 486. With progressive exposure at 96-hr, deaths becomes inevitable even at lower concentrations.

Acute toxicity of both chemicals was also evaluated using estimated 96 h LC50 values in varying concentrations (Table 1). The estimated 96 h LC50 values showed that Norust CR 486 (13.58 ± 1.15 mg/l) was more toxic than Neatex (17.10 ± 3.31 mg/l). The freshwater fish were sensitive to both chemicals however; the control organisms were active and responded to stimuli throughout the experimental time.

## Shrimp

The results obtained indicate that mortality increased with increased concentrations and exposure duration for the test chemicals. Mean % mortality recorded for concentrations 31.25, 62.5, 125, 250 and 500 mg/kg at day 10 were 10, 27, 53, 70 and 100% (Neatex) and 23, 43, 70, 100 and 100% (Norust CR 486). The observed physiological changes, mortality, estimated LC50 and immobilisation showed that organisms exposed to Norust CR 486 was more sensitive than Neatex (Table 2 & 4). There was statistical significant difference between the estimated LC50 for Neatex and Norust CR 486 test at $p < 0.05$. The mean mortality for the control experiment was significantly different from the results obtained for Norust CR 486 and Neatex at levels of $p < 0.05$.

## Earthworms

The test organisms exposed to Neatex concentrations of 62.5, 125, 250 and 500 mg/kg at day 14 of the test recorded 0, 10, 37 and 50% mean mortality respectively. In Norust CR 486, mean % mortality values for the same concentrations were 20, 30, 57 and 90%. The observed results indicated that worms exposed to Neatex and

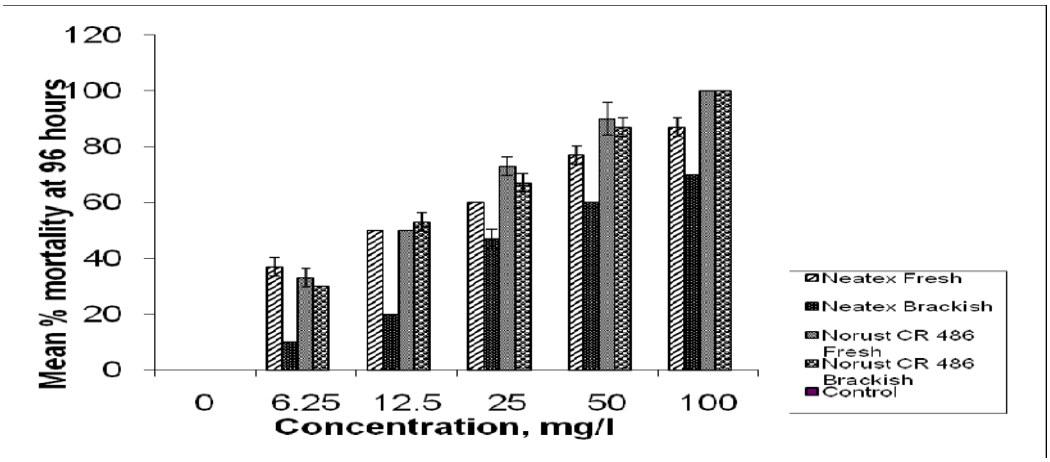


**Figure. 1**: Mean % mortality ± SEM of fish exposed to Neatex and Norust CR 486

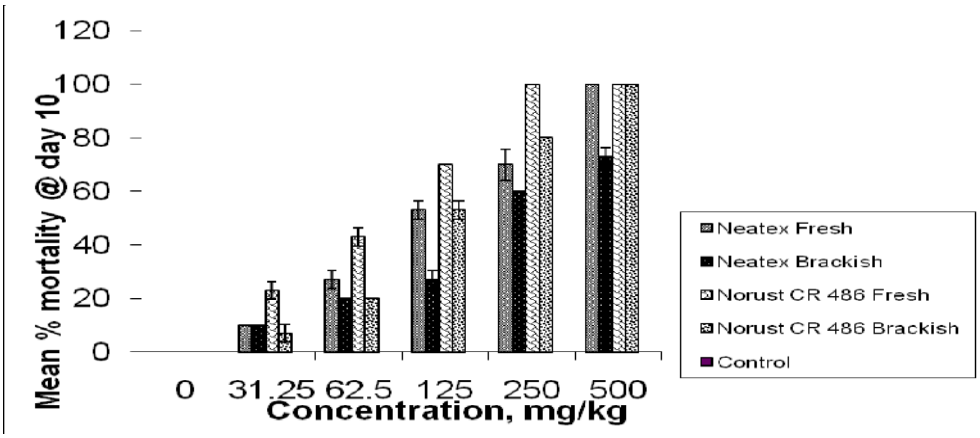


**Figure 2**: Mean % mortality ± SEM of shrimps exposed to Neatex and Norust CR 486

Norust CR 486 had mean % mortality that increased with increased concentration and exposure time. The estimated LC50 for the 14 days experiment showed that Norust CR 486 was more toxic than Neatex (Table 3). The statistical analysis also showed significant difference between the estimated LC50 for Norust CR 486 and Neatex at $p < 0.05$. However, the difference between the mean mortality rate for Norust CR 486 and Neatex were not significantly different. The mean mortality for the control experiment was significantly different from the results obtained for the test chemicals at levels of $p < 0.05$.

## DISCUSSION

Fish fingerlings exposed to the two test chemicals, which are frequently discharged into the environment of the Nigeria Niger Delta were adversely affected. The mortality values reported for *Tilapia guineensis* were influenced by toxicity modifying factors such as exposure duration, concentrations, type of chemicals and environmental conditions. The Norust CR 486 exposure was 'more toxic' than the Neatex test. This may be due to the higher surfactant content in Norust CR 486. Chemicals may have multiple effects on populations of organisms including mortality, reproductive failure, and productivity. Sensitivity of populations depends upon such factors as age groups and temporal patterns of exposure. Passage of toxins or toxicants into an organism is also highly dependant on the specific physical-chemical characteristics of a given toxicant (Ololade & Oginni, 2010; Maheswaran *et al*., 2008). Linear alkylbenzene sulphonates (LAS), an active ingredient in the test chemicals could be regarded as one of the major reasons for induced mortality due to its

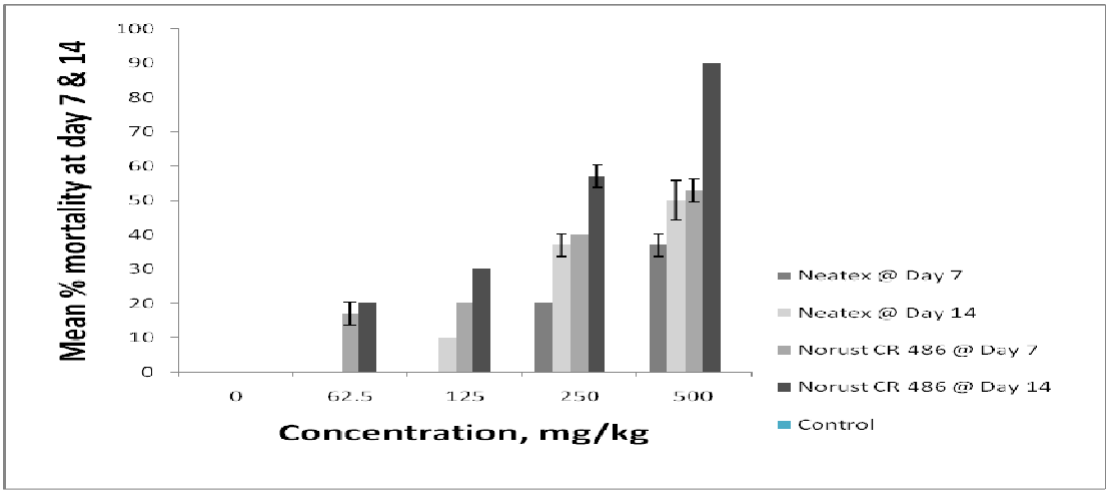


**Figure 3**: Mean % mortality ± SEM of earthworms exposed to Neatex and Norust CR 486

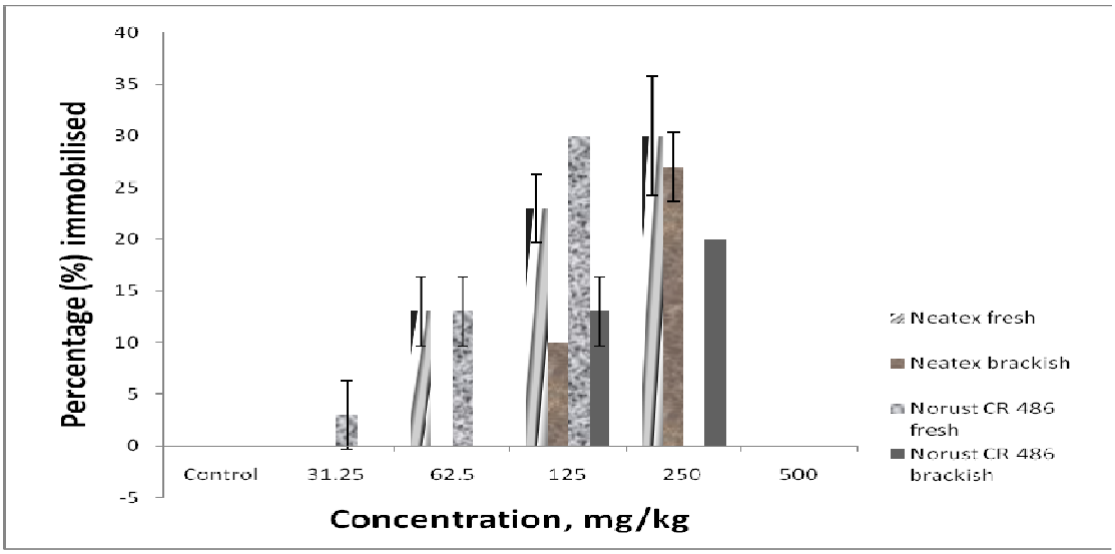


**Figure 4:** Mean % immobilised ± SEM of shrimp exposed to Neatex and Norust CR 486

lipophilic and surfactant-containing nature (Ezemonye et al., 2007). At the early stage of test initiation and the lower test concentrations of the toxicants, most of the test organisms survived initial attack. Their protective adaptations as well as individual physiological nature of *T. guineensis* may be responsible for the observed effects (Olalade & Oginni, 2010). However, as exposure progressed to 96-hr, inevitable deaths could be due to stress, individual physiology and cumulative impact of the chemical-toxicity. This results observed in this study are in agreement with other related studies (Ezemonye *et al*., 2007; Omoregie *et al*., 1995; George and Clark, 2000; Scarlett *et al*., 2005; Johnson *et al*., 2005). This study observed that the 14-day old fish were not only vulnerable to chemical contaminants but were adversely affected. The estimated 96 h LC50 values obtained in this study compared with GESAMP (1997) rating, showed that both chemicals are slightly toxic to the fish. The results reported for mean % mortality and 96 h LC50 values for the fresh water test was due to the physiology of fresh water organisms, which have a body fluid concentration (about one-third) their surrounding environment, they are constantly taking in water by diffusion through their gills and skin for osmotic balance (Delbeek, 1987). Thus in a situation where there is damage to the skin and other tissues as is the case in

exposure to high concentrations of surfactant-containing chemicals, there is an influx of not only water but also the test chemical leading to a high lethal toxicity of the chemical and death rate in the fresh water organisms (Bury *et al*., 1999; Abel, 2006).

In the shrimp bioassay, the primary toxic effect occurs as a result of the surfactant action of foam since surfactant in the water interferes with the ability of the gills to absorb oxygen from the water, thus causing the organisms to suffocate (Mckim *et al*., 1975; Abel, 2006; Soegianto *et al*., 2008). Continuous exposure to the chemicals can lead to respiratory tract damage and other body malfunction. The responses observed in the fresh water test may be related to the species response to the environment and the toxicant's mode of action similar to that observed for fish (Bury *et al*., 1999; Playle *et al*., 1992; Chindah *et al*., 2001). In the shrimp study, the ecological endpoint observed was mortality and immobilisation (inability of the organisms to move but were obviously still alive). The shrimp had the ability to recover following exposure to the test chemicals only in concentrations 31.25 and 62.5 mg/kg but effects were irreversible in the higher concentrations. The affected organisms may not be able to maintain their position in the sediment, avoid predators or feed (Willis and Ling, 2003; Bat *et al*., 1999). When exposure is due to a complex mixture of chemicals, or a mixture of chemicals sorbed to a substrate such as sediment, the concentration and composition of the mixture may vary with time and exposure. The determination of exposure and relationship of exposure to effects are more complicated. Death, though at different rates, were recorded at every test concentration as well as media (Pozo *et al*., 2003; Javed, 2003). The concentrations resulting in sediment acute toxicity indicates that shrimp populations would be adversely affected should they be exposed to high concentrations of Neatex and Norust CR 486 in real life situation.

Studies have shown that soil contaminated with organic pollutants (e.g. corrosion inhibitors, detergents etc); can be detrimental to earthworm populations. The differential acute toxicity levels of both chemicals as shown in the different LC50 values may be attributed to the toxic constituents of the chemicals (Edwards and Bohlen, 1992). This is because organisms are known to react differently to varying stressors depending on their toxicity profile. The significant difference between the control and the test concentrations is an indication that mortality may have been induced by the test chemicals. Higher concentrations of the test chemical recorded varying degrees of behavioural alterations in the surviving earthworms including lack of burrowing ability, sluggish movement and morphological changes (contraction, rigidity, and elongation). The observed signs are an indication of possible soil deterioration as well as depletion of vital terrestrial organisms resulting from contamination of the soil with the test chemicals (Bayer and Foy, 1982). Egharevba, (2002) noted that through inappropriate disposal of surfactant, the topsoil quality may be altered with the likely consequence of reduction in soil fertility and poor plant growth.

## CONCLUSION

In conclusion, the study showed that the test chemicals induced mortality in fish, shrimp and earthworms. In the three experimental media, death cause to organisms may be detrimental since this could lead to elimination of potentially reproductive organisms. The use and disposal of surfactant-containing chemicals should be prudently monitored since the test chemicals were slightly toxic to the organisms in the different environment. This would ensure amongst others that the delicate biotic components of the rich Nigerian Niger Delta biodiversity are prudently protected.

## ACKNOWLEDGEMENT

The Nigeria University Commission (NUC) Central Research Grant Fund and University of Benin Research Grant supported this study.

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