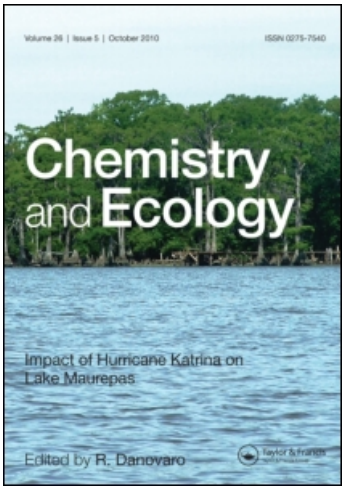

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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To cite this Article Ezemonye, L. I. N. , Ogeleka, D. F. and Okieimen, F. E.(2007) 'Biological alterations in fish fingerlings (*Tilapia guineensis*) exposed to industrial detergents and corrosion inhibitors', Chemistry and Ecology, 23: 5, 373 — 382
To link to this Article: DOI: 10.1080/02757540701587715
URL: http://dx.doi.org/10.1080/02757540701587715

Chemistry and Ecology
Vol. 23, No. 5, October 2007, 373–382

Taylor & Francis
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Biological alterations in fish fingerlings (*Tilapia guineensis*) exposed to industrial detergents and corrosion inhibitors

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(Received 30 January 2007; in final form 2 July 2007)

The common cichlid (*Tilapia guineensis*) of the Niger Delta was exposed to lethal (6.25, 12.5, 25.0, 50.0, and 100 mg l^{-1}) and sublethal (1.56 and 3.13 mg l^{-1}) concentrations of Neatex and Norust CR 486 for four and twenty eight days, respectively. The rate of mortality, level of glycogen reserves in the muscle tissues, and bioaccumulation of surfactants were measured as the ecotoxicological end-point. Estimated 96-h LC_{50} values were 82.42 mg l^{-1} (Neatex) and 20.21 mg l^{-1} (Norust CR 486), indicating that Norust CR 486 was more toxic. The levels of glycogen reserves in muscle tissues decreased significantly ($P < 0.05$) in fish exposed to sublethal concentrations of the chemicals compared with the levels in the control groups. The decreases in glycogen levels in the muscle tissues in the highest concentrations (3.13 mg l^{-1}) were 67% (Neatex) and 75% (Norust CR 486). The percentage reduction correlated with the increase in concentrations of chemicals and exposure duration. Surfactant bioaccumulation in the gill, gut and muscle tissues of fish increased significantly with increasing concentrations. The absence of mortality and surfactant accumulation in the control group may be an indication that the observed effects on the exposed fish may have been due to the chemicals. This study demonstrates the lethal and sublethal effects of surfactant-containing industrial chemicals on mortality and muscle glycogen in *Tilapia guineensis*.

Keywords: *Tilapia guineensis*; Chemicals; Muscle glycogen; Mortality; Bioaccumulation; Ecotoxicology; Surfactants

1. Introduction

Petroleum exploration and production activities have led to considerable environmental problems in the Niger Delta area of Nigeria in the past 30 yr [1]. These environmental problems include, oil spillage, emission of toxic gases, loss of biodiversity, and discharge of untreated sewage and wastewaters. High levels of toxic heavy metals and persistent organic pollutant are released into the aquatic environment from point and diffuse sources. Most of the present water-pollution problems in the area result from very small amounts of many different kinds of toxic chemicals discharged into surface water. The discharge of effluent into aquatic ecosystem is continuous and not just sporadic with attendant cumulative effects.

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Chemistry and Ecology
ISSN 0275-7540 print/ISSN 1029-0370 online
http://www.tandf.co.uk/journals
DOI: 10.1080/02757540701587715

Characterization of effluents from industries operating in the area showed that they contained chemicals and toxic substances such as biocide, dispersant, corrosion inhibitors, oil and grease, heavy metals, polyaromatic hydrocarbons (PAH), and polychlorinated biphenyls (PCBs) [2–7]. The discharge of chemicals of high toxicity into the environment is usually regulated so that the levels of chemicals do not exceed the amount that will harm organisms.

Detergents and corrosion inhibitors are formulated products containing surfactants, which decrease surface tension, facilitate dirt, stain, and soil removal from surfaces or equipments, suspend materials (solubilize and emulsify), and act as biocides [8]. Their use and disposal often contaminate the aquatic ecosystem, which usually acts as a receptacle of effluent. As a result of their chemical properties, detergents and surfactants can enhance the capacity of toxic substances to pass cellular membranes, playing a possible role for bioavailability of pollutants.

Linear alkylbenzene sulfonates (LAS), a class of chemical compounds known as surfactants, are found in water bodies in several orders of magnitude depending on the type of environment. By volume, they are the most widely used group of anionic surfactants today in synthetic laundry detergent and corrosion inhibitor formulation. They are discharged into the environment through industrial/domestic or launderette effluents into waterways and by sludge disposal [9]. Kimerle [10] compared reported ranges of LAS toxicity (0.1–100 mg l^{-1}) to reported ranges of LAS environmental concentrations and found safety margins (toxic effect concentration/water concentration) of 10–1000 for freshwater algae, invertebrates and fish. They affect the aquatic environment and alter the physiological properties of water and the exchange rate of oxygen across the gills of fish [9]. Fish react to acutely toxic concentrations of surfactants with a sequential pattern of increased activity, inactivation, and immobilization, and if not removed from exposure, death occurs. The cause of death is suffocation, probably as a result of both physical and chemical disruption of the gill epithelium [11]. Most fish will die when anionic detergent concentrations approach 15 parts per million [12].

Sublethal concentrations of LAS have also been found to deplete the glycogen reserves in aquatic organisms. Misra *et al.* [13] reported that exposure of fish fingerlings (*Cirrhina mrigala*) to a concentration of 0.005 mg l^{-1} LAS caused alterations in glycogen in muscles of fish. Significant decreases in the level of glycogen demonstrated impairment of carbohydrate metabolism [14]. The reduction in glycogen levels is associated with increases in lactic acid along with inhibition of acid and alkaline phosphatase activity. Glycogen is considered the principal storage form of glucose and is found mainly in liver and muscle, with kidney and intestines adding minor storage sites. The body of organisms obtains glucose from diet, amino acids, and lactate via gluconeogenesis. Glucose obtained from these sources either remains soluble in the body fluids or is stored in a polymeric form, glycogen. Glycogen plays a major role in supporting the energy demands of skeletal muscles during intense activity or stress. Despite its importance, the amount of glycogen stored in skeletal muscles is so small that a large fraction of it can be depleted in response to high intense swimming or opercular activity [15].

A relatively new pollution concern is that some chemicals have a tendency to bioaccumulate. Bioaccumulation is the 'building-up' of a chemical to a toxic level in an organism's body. Bioaccumulation becomes an environmental problem when chemicals accumulated are toxic, where this will lead to an elevated amount in the organism's body. Bioaccumulation of chemicals is an important factor in the assessment of environmental hazards. It has been accepted as a trigger factor for decisions of administrative relevance [16, 17].

LAS have hydrophobic components, which may partition into lipids of organisms and bioaccumulate. If the surfactants are not catabolized, the possibility exists for magnification of potential toxicological effects up the food chain [18]. Only minimal experimental and monitoring information has been gathered on the bioaccumulation properties of currently used

surfactants [19]. Sublethal (chronic) concentrations of surfactants can affect aquatic species. Sub-acute studies in fish show that the gills and the locomotive muscles are the sites most vulnerable to surfactant toxicity. Low levels of LAS induce behavioural changes, such as a disruption in avoidance response and attraction. Early developmental stages, particularly the feeding sac-fry, are also very susceptible to LAS [8].

Neatex as a detergent is very widely used in both industrial and domestic premises to wash equipment, installations, heavy-duty machines, vehicles, and oil-soiled materials. They are used in pesticide formulations and dispersal of oil spills at sea. Hazardous effects of cationic, anionic, and non-ionic detergents on aquatic organisms have been reported [19, 20]. Similarly, Norust CR 486, are corrosion inhibitors with a wide range of applications, such as oil pipelines, domestic central heating systems, industrial water-cooling systems, and metal-extraction plants. Their toxic effects on aquatic resources such as fish have necessitated the need for regular monitoring of water bodies that receive effluent containing these chemicals

Since fish are known to be affected by exposure to industrial chemicals in the ambient environment to levels that could be deleterious to the organisms, this study aimed to demonstrate the effects of lethal and sublethal concentrations of Neatex and Norust CR 486 on mortality and muscle glycogen in *Tilapia guineensis*, which is of economic significance in the Niger Delta of Nigeria [21].

2. Materials and methods

2.1 *Collection of test organisms*

Tilapia guineensis from the Niger Delta were collected from a farm at Kpakiama in the Niger Delta area. The characteristics of the organisms include the following: age, 28 d old; weight $1.19\,g \pm 0.04$ and size $3.82\,cm \pm 0.15$. They were not sexually mature. Acclimatization to laboratory conditions was carried out in holding tanks with dimensions length $\times$ height $\times$ width $= 100\,cm \times 100\,cm \times 100\,cm$ for 7 d prior to commencing the test.

2.2 *Test chemicals*

The test chemicals were purchased from the manufacturers (Manuex Nigeria Limited and Ceca Incorporated, Lagos, Nigeria) with the trade names Neatex and Norust CR 486 respectively. Both chemicals are currently used by oil-industry operators in the Nigeria Niger Delta area. The constituents for the two industrial chemicals used for the bioassays are reported in table 1.

Table 1. Characteristics of the chemicals as contained in the Materials and Safety Data Sheet (MSDS).

Properties	Neatex	Norust CR 486
State or form	Liquid	Liquid
Colour	Light brown	Colourless
Odour	Pleasant	Pungent
Composition	Linear alkyl benzene sulphonate, sodium hydroxide, sodium carbonate, and ammonium oxalate	Heterocyclic derivatives, surfactant, and alkaline sulfide in ethylene glycol
Solubility	Soluble	Soluble
Specific gravity	1.04	1.09
pH	10.62	1.97

2.3 *Bioassay procedure*

The Organization for Economic Cooperation and Development recommended semi-static renewal bioassay procedure started with a range-finding test. Stock solutions of 200 mg l^{-1} were prepared by dissolving the chemicals in the dilution water.

The physico-chemical condition of the dilution water from the fish habitat in the test experiment includes: temperature at 27 ± 2 °C with a 16:8 h light:darkness photoperiod. The pH was 5.4 ± 0.4 while dissolved oxygen (DO) had a range of 6 ± 0.8 mg l^{-1}. The mean pH and salinity were 5.9 ± 0.2 pH units and 59.72 ± 3.4 mg l^{-1}, respectively. Total dissolved solids (TDS) and conductivity were 86.81 ± 2.5 mg l^{-1} and 176.23 ± 6.8 μS cm^{-1}, respectively. Although Norust CR 486 had a pH value of 1.97 in the manufactured state, when added to the dilution water (pH ~ 5.9), the pH of the resulting test solution increased to 5.7 ± 0.2. The test organisms *Tilapia guineensis* are known to survive in a pH range of 5–9. These physico-chemical constituents are similar to that of the Niger Delta waters.

The semi-static with renewal bioassays were carried out in amber-coloured wide-mouth glass tanks measuring 40 cm × 25 cm × 25 cm. A total of 5 l of the test medium and controls (dilution water) was used to test 10 test organisms of *Tilapia guineensis* for the fresh environment in three replicates [22–24].

Fifteen aquaria were filled with 75 l of dilution water, and chemical stock solutions were added to each aquarium to make the final concentrations of 6.25, 12.5, 25.0, 50.0, and 100 mg l^{-1} for the acute toxicity test. The sublethal test had six aquaria with final concentrations of 1.56 and 3.13 mg l^{-1}. Three aquaria were kept as controls. Ten fish were added to each aquarium, and the ecological end-points were investigated after 96 h (acute test) and 28 d (sublethal test).

The fish were not fed during the 96 h lethal test. In the sublethal bioassay, organisms were fed once every week on 3% body weight on commercial fish food (40% carbohydrate), and the glycogen levels were investigated a day prior to the next feeding on the 14th and 28th days. The test solutions were renewed daily, and their physico-chemical constituents were measured throughout the duration of the experiment.

2.4 *Mortality*

Observations were made on a daily basis and mortality recorded. The dead organisms were removed immediately on detection. Fish were considered dead when they failed to show any evidence of opercula activity and did not respond to gentle prodding [24].

2.5 *Muscle glycogen determination*

Muscle glycogen in the fish was determined by the anthrone method. Glycogen was extracted into potassium hydroxide (KOH), purified by precipitation, hydrolysed to glucose, and quantified with anthrone reagent [25, 26].

The fish samples were dissected, and the muscle tissues collected. These were then stored in the refrigerator prior to analysis. The muscle tissues of fish to be analysed for glycogen levels were first weighed (approximately 100 mg) and then placed directly into centrifuge tubes containing 3 ml of KOH solution (30%). The centrifuge tubes were kept in a hot-water bath for 30 min. Then, 0.5 ml of saturated Na_2SO_4 and 3.5 ml of ethyl alcohol (95% pure) were added, followed by boiling for a further 15 min. After being cooled, all samples were centrifuged at 3500 rpm, and the supernatants were discarded. The precipitations in the tubes were dissolved in 2 ml of distilled water, followed by the addition of 2.5 ml of ethyl alcohol (95% pure).

The tubes were then centrifuged at 3500 rpm for a further 10 min, and the supernatants were discarded. The final precipitations in the tubes were then dissolved in 2 ml of HCl (5 M) and neutralized with 0.5 M NaOH, followed by dilution to 50 ml with distilled water before analysis. The glycogen levels in the samples were determined by the anthrone method [25, 26].

2.6 *Bioaccumulation of surfactants in fish*

2.6.1 Water determination. The test solutions and standard (0.25, 0.50, 1.00, and 2.00 mg l^{-1}) were accurately measured (100 ml) into a separator funnel. The solution was made alkaline by dropwise addition of 1 N NaOH, using a phenolphthalein indicator. The pink colour was discharged by dropwise addition of 1 N of H_2SO_4 to a pH level of 7.45. To this solution, 10 ml of $CHCl_3$ and 25 ml of Methylene Blue reagent were added [27]. The funnel was rocked vigorously for 30 s, and the phases were allowed to separate. The $CHCl_3$ layer was drawn off into a second separator funnel. The extraction was repeated twice using 10 ml of $CHCl_3$ each time. All $CHCl_3$ extract was combined in the second separator funnel. Then, 50 ml of wash solution was added and the mixture shaken vigorously for 30 s. The mixture was allowed to settle, and the $CHCl_3$ layer was drawn off through a funnel containing a plug of glass wool into a 100-ml volumetric flask. The wash solution was extracted twice with 10 ml of $CHCl_3$. The washing was collected in volumetric flask and diluted to the mark with $CHCl_3$. The surfactant content in the solutions was determined by the Methylene Blue Active Substances method (the absorbances of the samples and standards were determined at 652 nm against a blank of chloroform). The concentrations of surfactant in the samples were extrapolated from the calibration graph [27].

2.6.2 Organ and tissue determination. The organs and tissues of the fish were weighed (100 mg) and placed in an extraction container. The surfactant was extracted over 16 h with 30 ml of methanol. The methanol extract was then evaporated until dryness in the extraction vessel, and the dry residue was redissolved with 100 ml of warm water in a bath [28]. The redissolved solutions containing the extract of the fish organs and muscle tissues were used to determine the surfactant levels following the procedure described above.

2.7 *Statistical analysis*

The susceptibility of fish to both chemicals was determined using the probit method of analysis [29] for the 96-h lethal toxicity test. This is the concentration that would kill 50% of the total number of test organisms used. Significant differences within a group in the lethal and sublethal bioassays were assessed with the two-factor ANOVA (analysis of variance) in Microsoft Excel. Bar and line graphs were also used in this study for the pictorial representation of assessment end-points.

3. Results

3.1 *Acute toxicity*

3.1.1 Mean percentage mortality. The observed mean percentage (%) mortality increased with increasing chemical concentrations (figure 1). Higher levels of mortality were observed in the fish exposed to Norust CR 486. The influence of exposure duration was also observed, and no mortality was observed in any of the control aquaria. The mean percentage (%) mortality

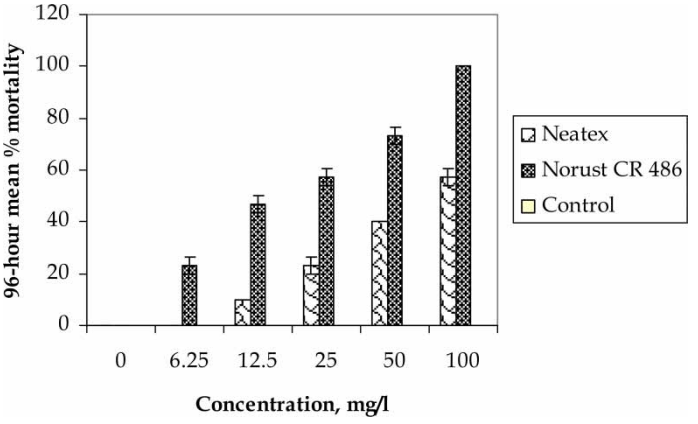

Figure 1. Mean percentage mortality ± SEM for 96 h in freshwater fish (28 d old) exposed to Neatex and Norust CR 486.

was statistically significant in the Neatex and Norust CR 486 aquaria (at $P < 0.05$; t-value 4.53). There was also a significant difference between the control and the test experiments (at $P < 0.05$; t-values = 2.30, 3.45).

3.1.2 Estimated 96-h LC_{50}. The estimated 96-h LC_{50} values at different concentrations were used to evaluate the lethal toxicity of both chemicals. For Neatex, the estimated 96-h LC_{50} was 82.42 mg l^{-1} (95% confidence limit 47.56–93.16 mg l^{-1}), whereas for Norust CR 486, the value was 20.21 mg l^{-1} (95% confidence limit of 10.39–34.98 mg l^{-1}; table 2). Comparing the estimated 96-h LC_{50} values, Norust CR 486 was found to be more toxic than Neatex, and differences were significant ($P < 0.05$; t-value = 81.5). Probit analysis revealed that LC_{50} values decreased with increasing chemical concentrations.

Table 2. Acute toxicity profile of freshwater fish to Neatex and Norust CR 486 exposure.

Chemical	LC_{50} (mg l^{-1})	Confidence limit	Probit equations	Slope
Neatex	82.42 ± 1.66	47.56–93.16	$y = 1.40 + 1.88 \log x$ conc.	3.39 ± 0.32
Norust CR 486	20.21 ± 2.98	10.39–34.98	$y = 2.68 + 1.78 \log x$ conc.	3.14 ± 0.33

Table 3. Bioconcentration factor of surfactant after continuous intake of Neatex and Norust CR 486 for 14 and 28 d.

			BCF			
Concentration	Log of dose	No. tested	Neatex exposure day 14	Neatex exposure day 28	Norust CR 486 exposure day 14	Norust CR 486 exposure day 28
1.56 mg l^{-1}, gill	0.19	10	75.16 ± 4.3	98.14 ± 9.4	50.73 ± 3.5	94.89 ± 7.3
1.56 mg l^{-1}, gut	0.19	10	80.12 ± 1.9	118.01 ± 12.1	86.13 ± 0.6	106.93 ± 7.1
1.56 mg l^{-1}, tissue	0.19	10	115.58 ± 7.5	147.83 ± 2.2	113.14 ± 4.1	137.23 ± 0.6
3.13 mg l^{-1}, gill	0.49	10	99.14 ± 1.5	125.43 ± 3.4	81.6 ± 3.8	96.48 ± 0.9
3.13 mg l^{-1}, gut	0.49	10	122.41 ± 0.8	144.83 ± 3.4	109.98 ± 5.3	138.94 ± 3.9
3.13 mg l^{-1}, tissue	0.49	10	134.05 ± 2.7	160.78 ± 4.5	136.59 ± 1.2	253.42 ± 3.0

3.2 *Chronic* (*bioaccumulation*)

3.2.1 Surfactant bioaccumulation. Surfactants residues were measured in the gills, guts, and muscle tissues of the fish at day 0, 14, and 28 for both chemicals at concentrations of 1.56 mg l^{-1} and 3.13 mg l^{-1}. Our results showed that the fish organs and muscle tissues showed high surfactant residue concentrations (3.13 mg l^{-1}), and the bioconcentration factor was higher in the Norust CR 486 than in the Neatex treatment. The mean surfactant concentrations in the gills, gut, and muscle tissues at the end of the 28-d experiment were significantly different for both the chemicals and the control tests ($P < 0.05$).

3.2.2 Muscle glycogen. The muscle glycogen level was highest in the control experiment (figures 2 and 3). The levels of muscle glycogen showed a reduction in the test experiments (67% for Neatex and 75% for Norust CR 486) at the end of the 28 d and were dependent on concentration and exposure duration. The reduction in muscle glycogen level was more evident in Norust CR 486 and was related with increasing concentration of surfactant residues. Significant differences were observed between the control and test experiments ($P < 0.05$).

4. Discussion

4.1 *Acute toxicity*

The results of the present study showed that the predetermined concentrations of Neatex and Norust CR 486 induced mortality in the fish *Tilapia guineensis* fingerlings. Our results are consistent with data reported in previous studies [30–33]. The composition of the Neatex and Norust CR 486 suggested the presence of surfactants, in particular the LAS. Surfactants are toxic at high concentrations, and this characteristic may be responsible for the levels of mortality reported in this study. Our data suggested that Norust CR 486 was found to be 'more

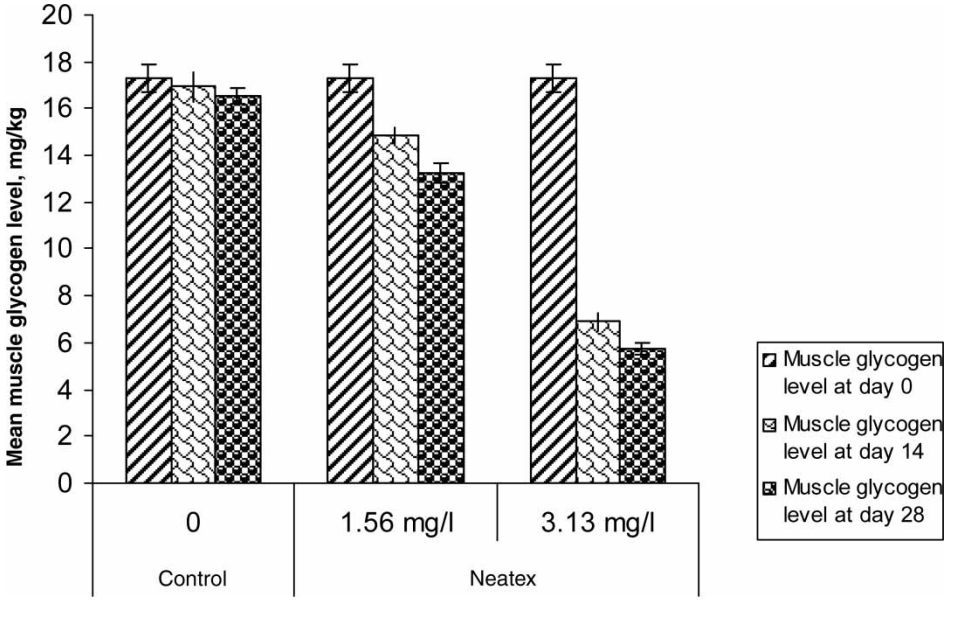

Figure 2. Mean ± SEM of muscle glycogen levels in fish exposed to Neatex at day 0, 14, and 28.

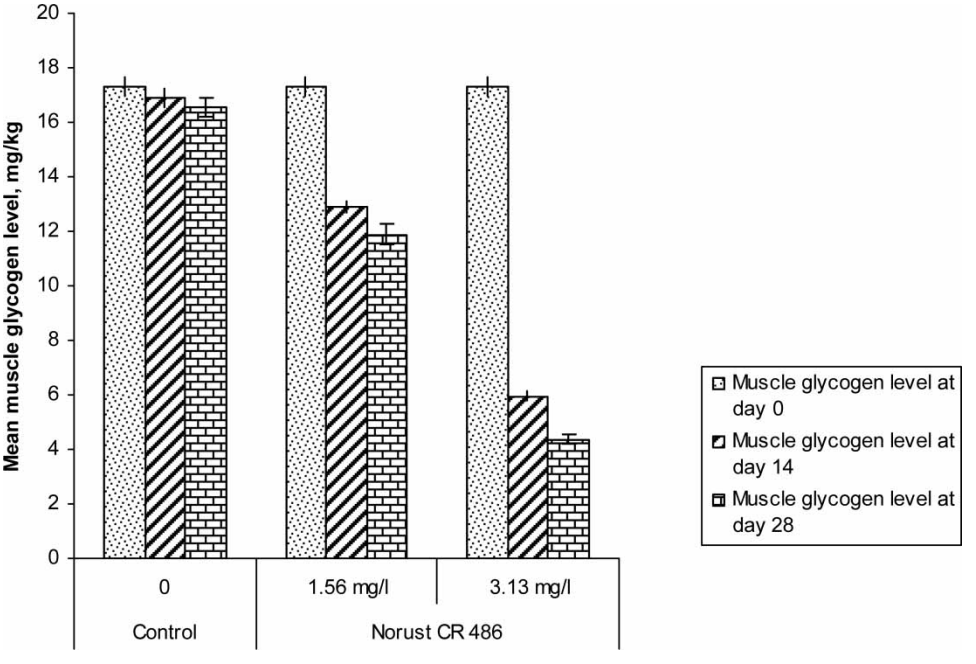

Figure 3. Mean ± SEM of muscle glycogen levels in fish exposed to Norust CR 486 at day 0, 14, and 28.

toxic' than Neatex. Estimated 96-h LC_{50} values obtained in this study when compared with GESAMP, rating [34], showed that both chemicals were slightly toxic to the fish.

4.2 *Chronic toxicity*

4.2.1 Surfactant bioaccumulation. Although the LC_{50} is a commonly used measure of environmental toxicity, it does not usually provide substantial information about the long-term effects of sublethal concentrations on aquatic environment [35]. Surfactants can be toxic at concentrations as low as $0.025\,mg\,l^{-1}$ [35]. The concentration inside the organism is an important consideration to assess and evaluate surfactants' behaviour in the environment. The results obtained in this study are consistent with those reported by Whitehouse *et al.* [36].

The degree to which a contaminant will concentrate in an organism is expressed as a bioconcentration factor (BCF), which is defined as the concentration of a chemical in an organism's tissues divided by the exposure concentration. Whitehouse *et al.* [36] found the bioconcentration factors for LAS in aquatic organisms to be *c*. 300 and that a bioconcentration factor >1000 tends to bioaccumulate [37]. In this study, different bioconcentration values were reported for Neatex and Norust CR 486. Although values were less than 1000 for both chemicals, the sublethal effects indicate the real effects of the chemicals on the tested organisms. Therefore, surfactants should always be scrutinized for potential bioaccumulation effects [38, 39]. Understanding the dynamic process of bioaccumulation is very important in protecting humans and other organisms from the adverse effects of chemical exposure, and it has become a critical consideration in the regulation of chemicals [40].

4.2.2 Muscle glycogen depletion. The interactions of chemicals with organisms are frequently associated with depletion in stored muscle glycogen, evident in decreased energy production [26]. Percentage reductions in the organism's muscle glycogen were observed with

Neatex and Norust CR 486 exposures. In this study, there was an inverse relationship between the concentration of the chemicals and decrease in muscle glycogen [41]. This depletion in the organisms exposed to the chemicals compared with the control experiment is an indication of probable toxicological effect. Glycogen reserves in the muscle tissues of fish under stressful conditions can be used as an emergency energy supply, and any change observed in the tissues and organs acts as an index of the organism's health status, especially in the aquatic community.

Carbohydrates are stored as glycogen in fish tissues and organs such as the muscle and liver to supply the energy needs when there are hypoxic conditions, intensive swimming activity, opercular activity, and lack of food. It has been found that chemicals could reduce glycogen reserves in fish by affecting the activities of enzymes that have roles in the carbohydrate metabolism such as gluconeogenesis and glycolysis [26, 41]. The muscle glycogen depletion in the present experiment might have been affected by the chemicals, since changes in the control experiments were not significant. Under stressful conditions or chemical contaminations, glucose levels usually drop in the organism, thereby impairing metabolic processes. Fatigue usually sets in, making the organism vulnerable to attack and injury. Prolonged fish exposure to surfactant makes adaptation difficult and creates weakness in the organisms. Weakness is characterized by decreases in muscle glycogen levels, subsequently resulting in death of the organisms [42].

5. Conclusions

This study has shown that Neatex and Norust CR 486 induced mortality and altered the carbohydrate metabolism in fish by affecting the levels of glycogen reserves in the muscles. Bioaccumulation of surfactants, which were positively correlated with glycogen reduction, could impair vital energy-requiring processes and affect the health status of the fish. There is a need to regulate the discharge of these chemicals in the fragile Niger Delta ecological zone of Nigeria with a view to protecting the fish population.

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