Acute Toxicity of Green Liquid Detergent to the Guppy *Poecilia reticulata* and Nile Tilapia Fingerlings *Oreochromis niloticus*

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Abstract
The acute toxicity of green liquid detergent was investigated using *Poecilia reticulata* and *Oreochromis niloticus* fingerlings in a 96 h static renewal laboratory bioassay. The $96hLC_{50}$ values were 0.06 mL/L against guppy and 0.09 mL/L against tilapia respectively. On the basis of $96hLC_{50}$ liquid detergent was 1.5 times more toxic to *P. reticulata* than to *O. niloticus* fingerlings. Symptoms of toxicosis observed in fish after their exposure include lack of balance, difficulty in breathing, erratic swimming, restlessness, skin discolouration and bleeding through gills. Results are discussed on the basis of data available on the damage caused by the exposure of teleosts to green liquid detergent. Hence, the concept of safe disposal of wastewaters into the aquatic environment should be encouraged.

Keywords: Toxicity, green liquid detergent, *P. reticulata*, *O. niloticus*

Introduction

Most Nigerian industries discharge their untreated or partially treated effluents through drains or canals into the nearest water body such as streams, rivers, and lagoons, relying on disposal by dilution, thereby causing water pollution (FEPA, 1991). Increase in pollution of water bodies has led to serious deleterious effects on the aquatic organisms (Oyewo, 1986; Otitoloju, 2006). The indiscriminate discharge of detergents into the aquatic environment, primarily as a result of its commercial and household use has resulted in pollution of our water bodies (Edwards *et al*., 2003). All detergents destroy the external mucus layers that protect the fish from bacteria and parasite infection (Belanger *et al*., 1993; Madsen *et al*., 2001). They are known to cause severe damage to the gill, and fish deaths have occurred at concentrations of 15 ppm and above (Passino and Smith, 1987).

Exposure to low-levels of some contaminants such as detergent can cause disruption of endocrine functions in fish, such as reproduction (Barber *et al*., 2007). Sublethal concentrations of an anionic detergentwas reported to have damaged the catfish barbell taste buds (Zeni and Caligiuri, 1992; Zeni *et al*., 1995).

Fish have often been considered as the sentinel organism for health of aquatic environment and respond to chemicals in a manner similar to that of higher vertebrates (Solbe, 1995; Beey, 2001). Guppy and Nile tilapia occupy a wide range of aquatic habitats, such as estuaries, lakes, ponds, weedy ditches and canals (Page and Burr, 1991; Skelton, 1993; Khallaf *et al.,* 1994; 1998; Lawal, 2010). They are sensitive, easy to maintain in the laboratory and tolerate a wide range of environmental conditions.

Poecilia reticulata has been used as a test species for metals such as zinc (Malagrino and Mazzilli, 1994), cadmium chloride (Mehmet *et al*., 2004), a wide spectrum organophosphorus insecticide (Mahmut *et al*., 2005), spent lubrication oil and laundry detergent (Otitoloju, 2006), and actellic insecticide (Lawal and Samuel, 2010). *O. niloticus* also, has been used as a test species for pesticides and heavy metals (Khallaf *et al.*, 1994; 1998). Thus, the aim of this study is to evaluate the acute toxicity of green liquid detergent to *P. reticulata* and *O. niloticus* with a view to assess the environmental hazards from man-made chemicals that contaminate aquatic ecosystems.

Materials and Methods

Test Animals

Two hundred and fifty *P. reticulata* (mean weight = 0.30 g; mean length = 1.75 cm) were collected (July, 2009) with scoop net (mesh size = 20 mm) from a drainage canal at the Army Barracks off Herbert Macaulay Road, Yaba, Lagos and carried in a plastic bucket filled with water from source to the laboratory. Two hundred fingerlings of *O. niloticus* (mean weight = 0.70 g; mean length = 2.10 cm) were obtained (July, 2009) from a fish farm in Lagos. The fish were kept in stock aquarium tanks (80 x 45 x 30 cm) one quarter filled with dechlorinated tap water and allowed to acclimatise to laboratory conditions ($26.0 \pm 0.8\ ^oC$, 78 ± 2% R.H, pH = 7.0) for one week before using them in bioassays. Fish were fed daily at 10% of their body weight. Unconsumed feed and wastes of the animals were removed regularly and water replenished twice a week to prevent accumulation of toxic waste metabolites in the medium.

Test Compound
Green Liquid detergent is an effective grease and stain remover used frequently in the kitchen. This product was manufactured by Liby-Tidi Nigeria Limited. Ingredients used in its production were sodium lauryl sulphate, salt, fatty acid, water, diethanolamide, perfume and preservatives.

Acute Toxicity Test
A static renewal bioassay technique was adopted in which the test media was renewed at the same concentration once every 24hours (ASTM, 1990). Preliminary screening was carried out to determine the appropriate concentration range for testing chemicals as described by Solbe (1995). Depending on the test concentrations, a given volume of water from the test organism's natural environment was measured into bioassay glass tanks (45 x 30 x 30 cm) and a predetermined volume of detergent was added into the water to make up 1000 mL to achieve the desired concentration.
Ten (10) active animals form each of the test species were introduced into the test medium containing the liquid detergent. Each treatment was in triplicate, giving a total of 30 animals per treatment including untreated media (control). The concentrations of the test chemical were as follows:

- 0.10, 0.20, 0.40, 0.60 and 0.80 mL/L and control (*P. reticulata*)
- 0.085, 0.090, 0.095, 0.10 and 0.20 mL/L and control (*O. niloticus*)

Assessment of Quantal Response (Mortality)
Mortality assessment was carried out every 24 hours over a 96hour experimental period. Fish were assumed dead when there was no body or operculum movement, even when probed with a glass rod.

Physico-chemical Parameters of the Test Media
Physico-chemical parameters such as temperature, hydrogen ion concentration (pH) and dissolved oxygen (DO) of the test media were measured during the experimental period. Temperature and pH were measured with Hanna instrument (Model H1991301) while dissolved oxygen was measured with a Jenway DO meter (Model 9071).

Statistical Analysis
The quantal response (mortality) data were analysed by Probit analysis after Finney (1971), using SPSS 15.0 for windows. The indices of toxicity measurement derived from the analysis were:

- LC_{50} = The concentration that kills 50% of the population
- LC_{95} = The concentration that kills 95% of the population
- TF = Toxicity factor for relative potency measurements

Results
Physico-chemical conditions of the test media during the experimental period were fairly constant. The temperature ranged between 25.0 ^{0}C and 27.0 ^{0}C, pH ranged between 6.0 and 7.0 while dissolved oxygen ranged between 7.0 and 8.0 mg/L over the 96 hours acute toxicity investigation.
Results of the acute toxicity of Green liquid detergent against *P. reticulata and O. niloticus* at 24 h, 48 h, 72 h and 96 h of exposures are shown in Table 1. The analysis of dose response data of green liquid detergent when tested against guppy and tilapia revealed that LC_{50} values ranged from 0.06 mL/L at 96h LC_{50} to 0.09 mL/L at 24 h LC_{50} (Table 1; Figure 1) and 0.09 mL/L at 96 h LC_{50} to 0.19 mL/L at 24 h LC_{50} (Table 1; Figure 1) respectively. The toxicity of green liquid detergent was found to be relatively similar over the experimental period with toxicity factor (TF) ranging from 1.0 to 1.5 for guppy and 1.0 to 2.1 for tilapia between 24 h LC_{50} and 96 h LC_{50} data (Table 1).
Comparison of 96 h LC_{50} of green liquid detergent against guppy and tilapia showed that the detergent was 1.5 times more toxic to guppy (Figure 2) hence; *P. reticulata* is more susceptible to green liquid detergent.
There was no adverse behavioural change or any mortality recorded in the control fish throughout the period of bioassay. Symptoms of toxicosis observed

Table 1: Relative Acute Toxicity of Green Liquid Detergent against *P. reticulata and O. niloticus*

	Exposure Time (h)	LC_{50} (95% CL) (mL/L)	Slope ± SE	Probit line equation	TF
P. reticulate	24	0.09 (0.08 – 0.10)	8.82 ± 1.88	Y = 14.8 + 8.82x	
	48	0.08 (0.07 – 0.09)	9.02 ± 1.75	Y = 15.3 + 9.02x	
	72	0.07 (0.06 – 0.08)	9.12 ± 1.72	Y = 15.7 + 9.12x	
	96	0.06 (0.05 – 0.07)	10.4 ± 1.86	Y = 17.6 + 10.4x	1.0
O. niloticus	24	0.19 (0.16 – 0.21)	3.99 ± 1.01	Y = 7.90 + 3.99x	
	48	0.14 (0.13 – 0.15)	4.95 ± 1.05	Y = 9.30 + 4.95x	
	72	0.11 (0.10 – 0.13)	5.33 ± 1.17	Y = 10.03 + 5.33x	
	96	0.09 (0.07– 0.10)	37.8 ± 7.21	Y = 42.9 + 37.8x	1.0

CL = Confidence limit, SE = Standard error, TF = Toxicity factor = 96hLC_{50} value of green liquid detergent

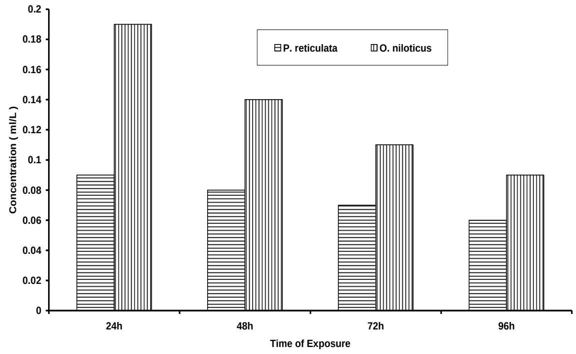

Figure 1: Relative Acute Toxicity of Green Liquid Detergent against *P. reticulata* and *O niloticus* at 24 h, 48 h, 72 h and 96 h LC_{50} exposures

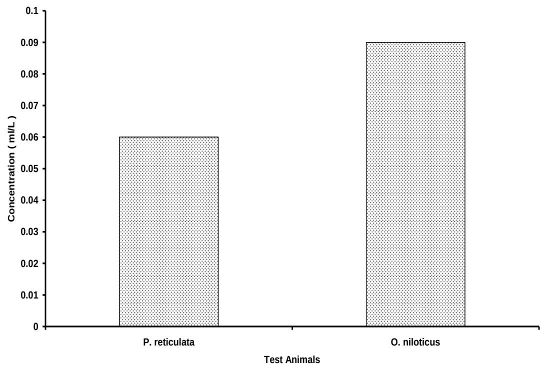

Figure 2: Relative Acute Toxicity of Green Liquid Detergent against *P. reticulata* and *O. niloticus* at 96 h LC_{50} Exposure

in the behaviour of fish exposed to green liquid detergent before the eventual death include lack of balance, difficulty in breathing, erratic swimming and restlessness. In addition, skin discolouration and bleeding through gills were noticed in *O. niloticus*.

Discussion

The behavioural responses of fish to most toxicants and difference in reaction times have been observed to be due to the effect of chemicals, their concentrations, the reactions of the organism receiving the toxicant, its size and specific environmental conditions (Aguigwo, 2002; Ezemonye *et al.*, 2007). Indiscriminate discharges of chemicals such as detergents into the aquatic environment are bound to expose organisms living and breeding there in addition, to multiple stressors of varying sources and intensity (Madsen *et al*., 2001; Belanger, 1993). Accordingly, the exposure of *P. reticulata* to liquid detergent resulted in erratic swimming movement, rapid gulping of water and increased opercula movement. Mehmet *et al*. (2004) and Mahmut *et al*. (2005) reported similar behavioural responses in *P. reticulata* when exposed to alpha-cypermethrin and chlorpyrifos-methyl respectively. Nile tilapia exhibited varying degrees of stressful behaviour in different concentrations of detergent such as erratic movement, bleeding through gills and skin discolouration, this agreed with Ezemonye *et al*. (2007) who reported that *Tilapia guineensis* showed varying degrees of stress to Neatex and Norust exposure. Respiratory impairment observed was due to the deterioration of the gill epithelium caused by the detergent. The deterioration of the gill epithelium alters the respiratory function and the hydro mineral balance in fish (Alcaraz *et al*., 1993). Similar results were reported by Passino and Smith (1987) and Rahman *et al*. (2002).

In this study, the acute toxicity level based on the 96h LC_{50} value of green liquid detergent against *P. reticulata and O. niloticus* was found to be 0.06 mL/L and 0.09 mL/L respectively while its toxicity is shown to increase with increasing concentration. This result agreed with the observations of other related studies (Alcaraz *et al*., 1993; Lawal and Samuel, 2010). However, guppy is more susceptible to green liquid detergent compared to tilapia, this could be due to differential in size of the two test organisms based on the toxicity modifying factors such as exposure duration, concentrations, life stages, and environmental conditions (Ezemonye *et al.*, 2007).

Conclusion

In conclusion, the result obtained showed that green liquid detergent causes stress, interfere with fitness and eventual death of aquatic organisms. Hence, this study would increase the scientific communities' understanding of the potential risks of waste-water in aquatic ecosystem and this information can be used to make better decisions regarding the discharge of waste-water to the environment.

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