

Brief Report

Acute Toxicity and Stress Behaviour of *Heterobranchus bidorsalis* Exposed to the Detergent Nittol[®] NTL

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Abstract: The acute toxicity of the detergent Nittol[®] 0.8, 1.0, 1.2, 1.4, 1.6, and 0.0 mg NTL/L of clean water on *Heterobranchus bidorsalis*, 5.5 ± 0.3 g, 6.4 ± 0.5 cm were investigated, using semi-static bioassay, for 96 h in 50 L capacity plastic test bowls. The fingerlings of the same brood stock and age were collected from Onose Farms Limited, Ughelli, Delta State to the University Research Laboratory, Enugu Lat. 7.4 N; 8°7'5 and long 6°8' E. 7°6' W. The test fish were acclimatized for 14 days, and fed at 3% body weight once daily, on a 40% CP commercial diet. Feeding was suspended 24 h before and during the range finding and acute tests. The whole set-up was replicated three times, and no death was recorded during the acclimatization period and in the control. A total of 180 fingerlings were used, and 10 fingerlings were assigned to each replicate. The test set-up was monitored daily for water quality parameters, opercular ventilation, tail fin beat frequency, and mortality. Dose and time-dependent behavioural patterns exhibited by the test fish, during the exposure periods include rapid swimming, air gulping, loss of balance, and a period of convulsion before death. Significant elevation in pH and temperature, reduction of DO compared to the control ($p < 0.05$) in the water quality, and dose-dependent early elevation of the tail and fin movements declined towards the end of the experiment. The 96 h LC₅₀ was determined to be 1.41 mg/L, indicating that the detergent NTL is toxic to the test fish. The haematological parameters were significantly ($p < 0.05$) reduced in the treated ranges of RBC 5.20 ± 0.07 – $8.00 \pm 0.02 \times 10^6 \text{ mm}^3$, HB 7.53 ± 0.50 – $10.72 \pm 0.14 \text{ g/dl}$, PCV 13.20 ± 0.85 – $18.00 \pm 0.43 \%$ below their elevated respective controls of $10.50 \pm 0.01 \times 10^6 \text{ mm}^3$, $11.00 \pm 0.01 \text{ g/dl}$, and $23.48 \pm 0.26 \%$. The white blood cells (WBC) recorded a significant ($p < 0.05$) increase in ranges of 23.72 ± 0.14 – $51.80 \pm 1.9 \times 10^3 \text{ mm}^3$ above the control value of $11.00 \pm 0.01 \times 10^3 \text{ mm}^3$. Therefore, values greater than the safe amount of 0.014 mg/L should not be allowed in the receiving culture waters for *Heterobranchus bidorsalis* fingerlings.



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1. Introduction

Detergents generally contain a mixture of a wide variety of chemical substances including, water softeners, processing acids, cleaning agents, optical brighteners, perfumes, and colouring agents [1–3]. These chemicals may act individually or collectively as a build-up of a more complex compound, that becomes too difficult to degrade, or cause eutrophication, in the event of trying to bring about their breakdown [4–6]. The entry point of detergent effluents from sewages, washing factories, hospitals, refineries, detergent-making industries, and agro-allied industries into aquatic waters is a major challenge to fish farmers and scientists [7–9]. Nittol is composed of linear alkyl benzene sulphonate (LABS), sodium tripolyphosphate (SIPP), sodium carbonate, sodium sulphate, sodium perborate, and sodium silicate, widely used in many homes, washing premises, and industries which channel their effluents into the receiving waters reserved for fisheries and culture of

Heterobranchus bidorsalis, an important fish in Africa [10,11]. There is a scarcity of reports on the acute toxicity and safety level of detergents in Nigeria, and therefore the objective of this study was to determine the behaviour, acute toxicity, and safety concentration of the detergent Nittol[®], on exposed fingerlings of *Heterobranchus bidorsalis*. It is considered to be an important culture fish in West Africa due to its fast growth, easy adaptation, and hardy nature [12]. Blood parameters are important physiological indicators of animals undergoing stressful conditions such as the presence of toxicants since blood acts as a pathophysiological reflector for the whole body [13]. Haematological parameters have been recognized as valuable tools for monitoring fish health [14].

2. Materials and Methods

2.1. Experimental Fish and Nittol Detergent

A total of one hundred and eighty (180) fingerlings of *Heterobranchus bidorsalis* (mean weight 5.5 ± 0.3 g, mean length 6.4 ± 0.5 cm), was obtained from Onose Farms, Ugheli, Delta State Nigeria, and transported to the Fisheries Laboratory of the Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology Enugu Lat. 7.4° N; $8^{\circ}7'5$ and long $6^{\circ}8' E$. $7^{\circ}6' W$. The experimental fish were maintained in four fibre-reinforced plastic (FRP) tanks, containing 600 L of de-chlorinated tap water. Aeration was provided to all tanks around the clock to maintain dissolved oxygen contents. Before the commencement of the study, the fish were acclimatized for 14 days and were fed a commercial fish diet composed of 40% crude protein. The faecal matter and other waste materials were siphoned off daily to reduce the ammonia content in the water. Nittol[®] was collected from a local market and prepared according to the standard procedures of Ivon et al. (2020) [14]. It was dissolved in distilled water to make a stock solution for the study. Ethical clearance from the Enugu State University of Science and Technology Committee on Experimental Animal Care ESUST/CEAC/12-08-2022/015 was obtained and followed throughout.

2.2. Acute Toxicity Test

The toxicity of NTL to *H. bidorsalis* was carried out according to the Organization for Economic and Development OECD guideline for testing chemicals No. 203 in a semi-static renewal system by using 200 L capacity rectangular glass aquaria $100 \times 40 \times 50$ cm for 10 fish. Five different concentrations (0.8, 1.0, 1.2, 1.4, 1.6), and a control 0.00 mgL^{-1} were selected and prepared in triplicates for definitive exposures after a range-finding test, and the 10 fish were exposed to each replicate. Feed was not offered to the fish for the 96 h of the test period. Dead fish were immediately removed to prevent deterioration of water quality. The exposure solution was renewed each day and was conducted under the natural photoperiod of a 12:12 light:dark cycle and the physicochemical parameters of the test water were analysed daily, using standard methods APHA (2005) [15]. The test fish were sampled on hours 24, 48, 72, and 96 in each replicate, to determine the toxic effects of the detergent on the fish. The behavioural responses in the exposed and control fish were observed and recorded daily as Little et al. 1990 [16]. The LC_{50} was determined by Probit analysis as Finney (1971) [17]. The safe level was estimated by applying the safety application factor (AF), suggested by CCREM (1991) [18].

2.3. Haematological Assay

The blood samples were collected by cardiac puncture from juveniles of *Clarias gariepinus* into ethylene tetra-acetic acid (EDTA) bottles and estimated for red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), and packed cell volume (PCV), using the methods of Zutshi et al. (2010) [19], Micronucleus assay, Farag & Alagawany 2018 [20] and blood cell damages assay, Cozzolino et al., 2009 [21].

2.4. Statistical Analysis

Data were expressed as a mean ± standard error and were analysed using the statistical package SPSS 20.0 computer program (SPSS Inc., Chicago, IL, USA). Differences in the test concentrations and control were subjected to a one-way analysis of variance (ANOVA), followed by Duncan range tests to determine the significant mean differences.

3. Results

The behavioural changes of the test fish exposed to the acute doses of NTL are shown in Table 1, while Tables 2 and 3 represent the mortality rate and cumulative probit mortality, and Table 4 represents mean water quality. Figure 1 represents the logarithmic probit line for 96 h LC₅₀ during the acute exposure of NTL on the experimental fish and Figure 2 indicates the mean opercular ventilation OVR and tail fin beat frequency TBF of exposed fish to NTL

Table 1. The behaviour of *Heterobranchus bidorsalis* exposed to acute doses of NTL.

| Behavioural Parameters | Concentration mg/L | Period (h) | | | |
|------------------------|--------------------|------------|------|------|------|
| | | 24 | 48 | 72 | 96 |
| Rapid swimming | 1.60 | ++++ | ++++ | +++ | ++ |
| | 1.40 | +++ | ++ | ++ | + |
| | 1.20 | - | ++ | + | + |
| | 1.00 | - | - | + | + |
| | 0.80 | - | - | - | + |
| | 0.00 | - | - | - | - |
| Air gulping | 1.60 | ++ | ++ | ++++ | ++++ |
| | 1.40 | + | + | ++ | +++ |
| | 1.20 | - | + | + | ++ |
| | 1.00 | - | - | + | ++ |
| | 0.80 | - | - | - | + |
| | 0.00 | - | - | - | - |
| Loss of balance | 1.60 | ++ | ++ | ++++ | ++++ |
| | 1.40 | + | + | ++ | +++ |
| | 1.20 | - | + | + | ++ |
| | 1.00 | - | - | + | ++ |
| | 0.80 | - | - | - | + |
| | 0.00 | - | - | - | - |
| Period of convulsion | 1.60 | ++ | ++ | ++++ | ++++ |
| | 1.40 | + | + | ++ | +++ |
| | 1.20 | - | + | + | ++ |
| | 1.00 | - | - | + | ++ |
| | 0.80 | - | - | - | + |
| | 0.00 | - | - | - | - |

Key: -none, + mild, ++ moderate, +++strong, ++++ very strong.

Table 2. Cumulative and probit mortality of experimental fish exposed to acute doses of the detergent NTL.

| Concentration mgL ⁻¹ | Log Concentration | No Exposed Fish | Replicate1 | Replicate2 | Replicate3 | Cumulative Mortality | % Mortality | Probit Mortality |
|---------------------------------|-------------------|-----------------|------------|------------|------------|----------------------|-------------|------------------|
| 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.8 | -0.09 | 30 | 1 | 2 | 2 | 5 | 16.67 | 4.01 |
| 1 | 0 | 30 | 2 | 2 | 3 | 7 | 23.33 | 4.23 |
| 1.2 | 0.07 | 30 | 3 | 4 | 4 | 10 | 33.33 | 4.56 |
| 1.4 | 0.14 | 30 | 4 | 5 | 4 | 13 | 43.33 | 4.82 |
| 1.6 | 0.2 | 30 | 6 | 7 | 7 | 20 | 66.66 | 5.41 |

Table 3. Mean water quality parameters.

| Parameters | Toxicant Concentration (mgL ⁻¹) | | | | | |
|-------------------------|---|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| | 1.6 | 1.4 | 1.2 | 1.0 | 0.8 | 0.0 |
| Temp. (°C) | 27.50 ± 0.50 ^b | 27.50 ± 0.35 | 26.75 ± 0.32 | 26.50 ± 0.54 | 26.75 ± 0.48 | 26.38 ± 0.50 ^a |
| DO (mgL ⁻¹) | 4.99 ± 0.06 ^a | 5.98 ± 0.05 ^b | 5.94 ± 0.05 ^b | 6.99 ± 0.09 ^c | 6.96 ± 0.07 ^c | 8.03 ± 0.07 ^d |
| pH | 9.88 ± 0.18 ^c | 9.70 ± 0.15 ^c | 9.60 ± 0.15 ^c | 7.48 ± 0.13 ^b | 7.43 ± 0.17 ^b | 6.19 ± 0.32 ^a |

Differences in letter superscript indicate significant differences at $p < 0.05$.

Table 4. Mean Blood parameters ± SEM of *Hetrobranchus bidorsalis* exposed to acute concentrations of Nittol for 96 h.

| Parameters | Toxicant Concentration (mgL ⁻¹) | | | | | |
|--------------------|---|--------------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| | 1.6 | 1.4 | 1.2 | 1.0 | 0.8 | 0.0 |
| RBC 106/mm6 | 5.2 ± 0.07 ^e | 6.4 ± 0.08 ^d | 7.2 ± 0.06 ^c | 7.4 ± 0.05 ^c | 8.0 ± 0.2 ^b | 10.5 ± 0.1 ^a |
| WBC 103/mm3 | 51.80 ± 1.9 ^b | 50.15 ± 1.4 ^c | 45.58 ± 0.75 ^c | 38.30 ± 0.16 ^a | 23.72 ± 0.14 ^a | 11.00 ± 0.1 ^a |
| Haemoglobin (g/dl) | 7.53 ± 0.5 ^e | 8.24 ± 40.3 ^d | 8.95 ± 0.25 ^c | 9.32 ± 0.16 ^c | 10.72 ± 0.14 ^b | 11.00 ± 0.1 ^a |
| PCV(%) | 13.2 ± 8.5 ^d | 15.4 ± 1.2 ^c | 16.5 ± 1.01 ^c | 17.1 ± 1.01 ^b | 18.0 ± 0.44 ^b | 23.48 ± 2.6 ^a |

Differences in letter superscript indicate significant differences at $p < 0.05$.

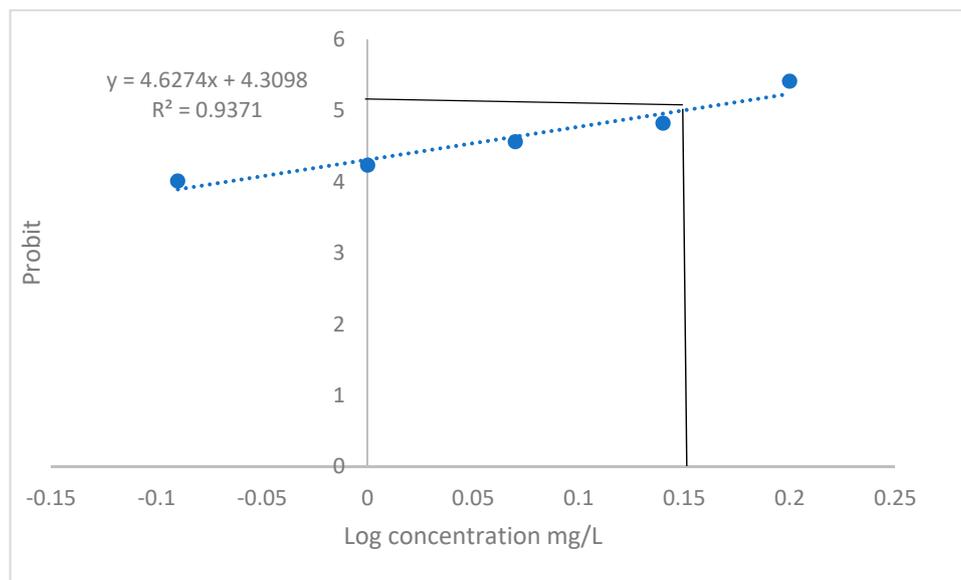


Figure 1. Logarithmic probit line to determine the 96 h LC 50 of detergent NTL exposed to the fish.

Dose and time-dependent behavioural patterns exhibited by the test fish during the exposure periods include rapid swimming, air gulping, loss of balance, and a period of convulsion before death (Table 1). Significant elevation in pH and temperature and reduction of DO compared to the control ($p < 0.05$) in the water quality (Table 4), and insignificant early elevation of the OVR and TBF movements ($p > 0.05$), declined towards the end of the experiment (Figure 3). The 96 h LC₅₀ determined to be 1.41 mg/L (Tables 2 and 3; Figure 1) indicated that the detergent NTL was toxic to the test fish.

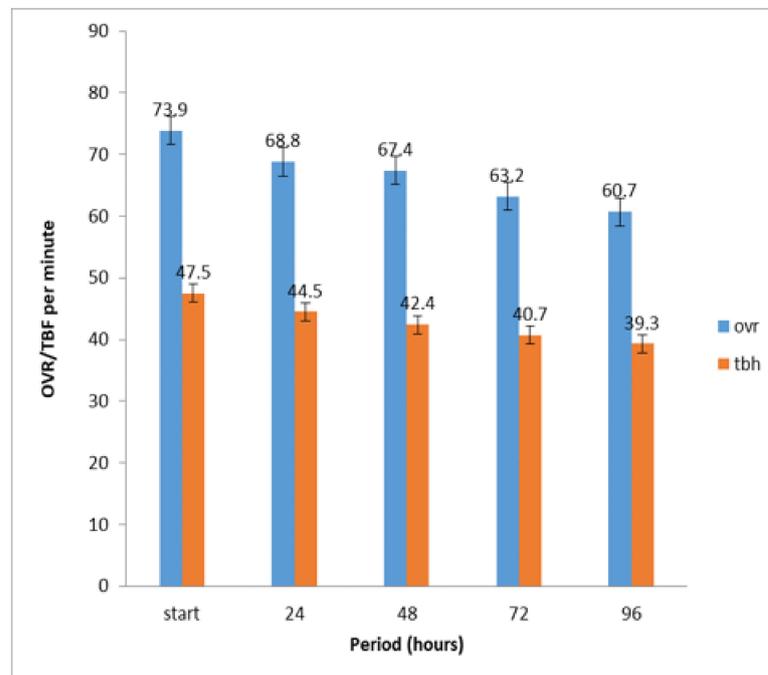


Figure 2. Mean OVR/TBF of exposed fish on acute doses of NTL.

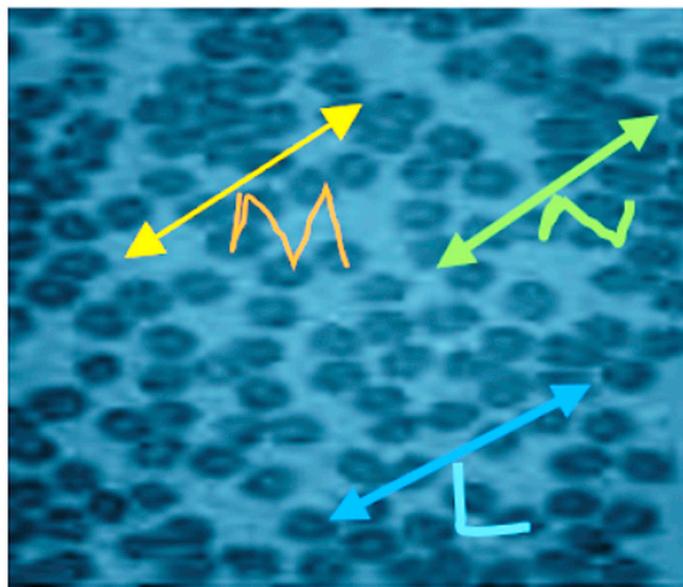


Figure 3. Photomicrograph of the blood cells of control fish 0.00 mg/L Nittol showing monocytes M, neutrophils N and lymphocytes L.

Haematology

The haematological parameters were significantly ($P < 0.05$) reduced in the treated ranges of RBC 5.20 ± 0.07 – $8.00 \pm 0.02 \times 10^6 \text{ mm}^3$, HB 7.53 ± 0.50 – $10.72 \pm 0.14 \text{ g/dl}$, PCV $13.20 \pm 0.8.50$ – $18.00 \pm 0.43\%$ below their elevated respective controls of $10.50 \pm 0.01 \times 10^6 \text{ mm}^3$, $11.00 \pm 0.01 \text{ g/dl}$ and $23.48 \pm 0.2.6\%$. The white blood cells recorded a significant ($p < 0.05$) increase in ranges of 23.72 ± 0.14 – $51.80 \pm 1.9 \times 10^3 \text{ mm}^3$ above the control value of $11.00 \pm 0.01 \times 10^3 \text{ mm}^3$ (Table 4). The exposed fish to the Nittol detergent suffered various damages to the blood cells (Figure 4).

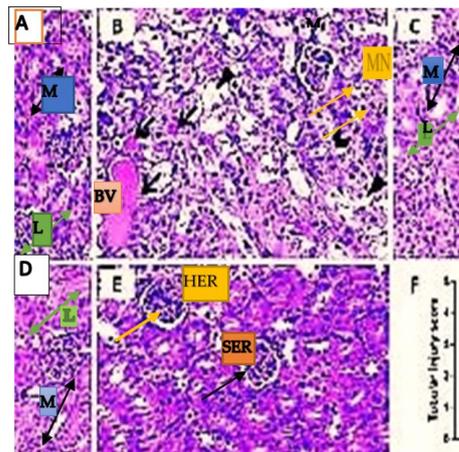


Figure 4. Photomicrographs of damaged blood cells (A) = 0.80 mg/L Nittol showing monocytes M, (B) = 1.00 mg/L Nittol showing: blood vessels BV and Lymphocytes L and micro nucleus MN, (C) = 1.20 mg/L Nittol showing monocytes and lymphocytes M,L, (D) = 1.40 mg/L Nittol showing monocytes and lymphocytes M,L, (E) = 1.60 mg/L Nittol haemolysed erythrocytes HER and swollen erythrocytes SER, (F) = The titular injury score of the blood cells.

4. Discussion

The display of stressful responses by the test fish corroborates with the reports of other workers [22,23]. The corroborated report of oxygen reduction in this study along with other works [24,25] with increased alkalinity death point, [26,27] and temperature [28,29], may suggest that the presence of the detergent in the aquatic environment is responsible for the significant water quality disruption since the control water maintained normal ranges. This development may have elicited insignificant opercula and tail movements to make up for the oxygen tension, due to respiratory dysfunction [30,31] and an attempt to move out of the non-conductive containers, which resulted in the exhaustion of its energy [32,33] and eventual death of the exposed fish. The median lethal value obtained for the detergent with a positive linear logarithmic probit line correlation signifies a dose-dependent mortality rate in agreement with [34] who reported that detergent has poisonous effects on all types of aquatic life if present in sufficient quantities. The study in [35] found that most fish will die when detergent concentration approaches 15 parts per million. The median lethal dose in this study was higher than 0.9 mg/L reported by [14] in sub-adults of *C. gagepinus*.

The effects of toxicants on fish can be assessed using haematological indices as this has been reported to be a routine procedure in toxicological research, environmental monitoring, and fish health conditions [13,36]. Fish that inhabit a detergent polluted environment are particularly susceptible to contaminants that can damage their haematology which causes deleterious changes in their various cellular structures and blood cells [18,37]. The inhibition of the hybrid catfish erythrocytes and its index component PCV and haemoglobin in this investigation agrees with other workers' studies on exposed fish to detergents [38,39], which may result in haemodilution of the blood by the pollutant, erythropoietic damage, anaemic responses, or as a result of reduced gill osmoregulation [40]. Increased catfish WBC observed in the present investigation agrees with other workers on exposing fish to pollutants [40] to restore the immune system of the stressed fish. Fish that inhabit a detergent polluted environment are particularly susceptible to contaminants that can damage their haematology causing deleterious changes in their various cellular structures and blood cells [41].

The acute toxicity of nonionic detergent oleyl-cetyl alcohol-ethylene oxide condensate (ekaline FI liquid) was reported to range from <0.1–7.9 ppm for the plankton *Diaptomus forbesi*, <20.0–7880.0 ppm for the worm *Branchiura sowerbyi*, and 19.9–308.0 ppm for the fish *Tilapia mossambica*. Chronic toxicity of detergents on fish was reported to significantly affect feeding intake, growth rate and maturity, fecundity, and egg maturity [42].

5. Conclusions

The 96 h LC₅₀ value of 1.41 mg/L of Nittol detergent on *Heterobranchus bidorsalis* fingerlings caused behavioural responses, mortality, and significant effects on the haematology of the test fish. Therefore, values greater than the safety amount of 0.014 mg/L should not be allowed in the receiving culture water for *Heterobranchus bidorsalis* fingerlings. Although the observed acute effect was in amounts greater than the limit, chronic studies are needed to ensure the safety of aquatic ecosystems.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Data is unavailable due to privacy and ethical restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

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