Toxicity of VAT BLUE 1 (INDIGO DYE) to Nile Tilapia *Oreochromis Niloticus* Fingerlings and Effects on Some Target Organs

Adekanye J. Ayobanji., *Adeniyi F. Adedamola., Adelusi O. Modupeoluwa., and Olaoye S. Oluwaremilekun

Department of Fisheries and Aquaculture Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ondo state, Nigeria

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ABSTRACT

The Discharge of effluents containing textile dye into aquatic environments is a major concern because of the potential to alter water quality and adversely affect aquatic organisms. This study investigated the acute toxicity of **VAT BLUE 1 (INDIGO DYE)** to *Oreochromis niloticus* fingerlings and its effects on selected target organs. *Oreochromis niloticus* fingerlings were acclimatized for 48 hours and exposed to graded concentrations of Indigo dye (vat blue dye 1) (200, 220, 240, 260, and 280 mg/L) using a static bioassay system. Mortality and behavioural responses were monitored over 96 hours, while temperature, dissolved oxygen, pH and electrical conductivity were determined during the exposure period. Histological examinations of the liver, gills and kidney were carried out after exposure. The results showed a concentration- and time-dependent increase in mortality. Mortality at 96 hours was 50% at 200 mg/L, 60% at 220 mg/L and 100% at 240, 260, and 280 mg/L. Probit analysis produced an estimated 96-h LC50 of approximately 199 mg/L (95% Confidence level). Exposed fish exhibited progressive behavioural abnormalities, including reduced swimming activity, sluggishness, erratic swimming, reduced responsiveness, severe stress and loss of equilibrium, particularly at higher concentrations. Vat dye addition also caused immediate changes in dissolved oxygen, pH and electrical conductivity, while temperature remained relatively stable. Histological examination revealed progressive tissue alterations in the liver, gills and kidney with increasing dye concentration. Liver lesions included vacuolar degeneration, hepatocellular degeneration, congestion and necrosis, while gill tissues showed epithelial erosion, disruption and destruction of the secondary lamellae. Kidney tissues exhibited varying degrees of tubular erosion, vacuolation, degeneration and necrosis at the higher concentrations. The study demonstrated that Vat textile dye is acutely toxic to *O. niloticus* fingerlings and caused significant behavioural and histological changes at sufficiently high concentrations.

Keywords: Textile Acute toxicity, *Oreochromis niloticus* Histopathology, Aquatic pollution, Textile Dye, LC_{50}

INTRODUCTION

Water pollution resulting from industrial activities has become one of the major environmental problems affecting aquatic ecosystems globally. Textile effluents are considered highly hazardous because they contain large quantities of synthetic dyes, heavy metals, suspended solids, and other toxic chemicals that may adversely affect aquatic organisms when discharged into water bodies without adequate treatment (Sharma *et al*., 2019). The textile industry is one of the largest consumers of water in manufacturing processes, and large volumes of wastewater generated during dyeing and finishing operations are often released into rivers, lakes, and reservoirs. These pollutants can alter the physicochemical properties of water, reduce dissolved oxygen levels, impair light penetration, and negatively influence the survival and productivity of aquatic organisms (Forgacs *et al*., 2004).

Textile dyes are designed to possess high stability against environmental conditions such as light, temperature, and microbial degradation, making them persistent contaminants in aquatic environments. Many dyes contain complex Vatmatic molecular structures that may exhibit toxic, mutagenic, or carcinogenic effects on living

organisms (Kant, 2012). When introduced into aquatic ecosystems, these dyes may accumulate in water and sediments, thereby exposing fish and other aquatic organisms to harmful substances over prolonged periods. Exposure to toxic dyes can lead to physiological stress, behavioral abnormalities, reduced growth, impaired reproduction, tissue damage, and mortality in fish species (Pathan *et al*., 2010). Consequently, the contamination of aquatic habitats by textile effluents has become an important issue in environmental toxicology and aquaculture research.

Fish are widely used as bioindicators in aquatic toxicological studies because they respond sensitively to changes in water quality and environmental contamination. Among cultured fish species, *Oreochromis niloticus*, is highly valued due to its fast growth rate, adaptability to diverse environmental conditions, economic importance, and widespread distribution in aquaculture systems (El-Sayed, 2006). However, despite its relative tolerance to environmental stress, prolonged exposure to toxic substances such as textile dyes may significantly affect its survival and physiological performance. Fingerlings are particularly vulnerable to toxicants because of their delicate developmental stage and high metabolic activity. Therefore, investigating the toxicity of textile dye on *Oreochromis niloticus* fingerlings is important for understanding the potential ecological risks associated with textile wastewater discharge and for developing appropriate environmental management strategies to protect aquatic life and sustain fish production.

MATERIALS AND METHODS

Study Area

The study was conducted at the Research Laboratory of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. The laboratory was used for the acclimatization, exposure, and monitoring of *O. niloticus* fingerlings during the acute toxicity experiment. The experimental tanks were prepared and maintained under controlled laboratory conditions to ensure uniformity throughout the study. Water quality parameters, including temperature, pH, dissolved oxygen, and electrical conductivity, were monitored periodically during the experimental period to ensure suitable conditions for the fish and to evaluate the effects of textile dye exposure. The histological examination of the gill, liver, and kidney tissues of the exposed fish was carried out after the completion of the toxicity test using standard histological procedures.

Source and Procurement of Experimental Fish

Apparently healthy *O. niloticus* fingerlings were obtained from Idowu's Fish Farm, Zion City Estate, Wesco, Akure. A total of 250 fingerlings were procured and had a mean body of 8.54±0.01 g and a mean total length of 8.90±0.01 cm. The fish were carefully selected to ensure they were healthy, active, and free from visible signs of disease, injuries, or deformities before being transported to the Research Laboratory of the Department of Fisheries and Aquaculture Technology, FUTA, where they were acclimated for 48 hours prior to the commencement of the study.

Acclimation of Experimental Fish

The experimental fish were transferred into holding tanks containing clean water and acclimatized under laboratory conditions for 48 hours before the commencement of the experiment. During the acclimatization period, the fish were closely monitored, and only healthy and active fingerlings were selected for the toxicity test. The water in the holding tanks was renewed as necessary to maintain good water quality throughout the acclimation period.

Experimental Fish

The experimental fish used in this study were *O. niloticus* fingerlings with a mean body weight of 8.54 ± 0.01 g and a mean total length of 8.90 ± 0.01 cm. The fish were selected based on their active behavior and absence of visible signs of disease or physical deformities. Following acclimatization for 48 hours, the fingerlings were randomly distributed into the experimental tanks for the acute toxicity test.

Textile Dye (Vat) Characterization

The toxicant evaluated in this study was a commercial-grade water-insoluble synthetic blue vat dye, specifically identified as Vat Blue 1 (Indigo dye), sourced from an industrial textile supplier in Akure, Ondo State, Nigeria. The dye batch possessed a chemical purity of 94.0% with a registered Chemical Abstracts Service (CAS) Registry Number of 482-89-3 and a molecular formula of $C_{16}H_{10}N_2O_2$. Stock solutions were prepared via chemical reduction in an alkaline medium, utilizing sodium hydrosulphite ($Na_2S_2O_4$) as the reducing agent and sodium hydroxide (NaOH) to dissolve the dye into its soluble leuco form. Exposure concentrations reported in this study represent nominal calculated weights prepared inside independent exposure units. To maintain nominal concentration integrity and prevent localized precipitation of the dye complex back into its insoluble oxidized form, a 50% static-renewal protocol was executed every 24 hours across all experimental units during the 96-hour bioassay

Range Finding Test

The range-finding test (which served as a preliminary test) was conducted prior to the definitive acute toxicity test. For the range-finding test, six treatments (including the control treatment) were used, with each treatment containing ten (10) *Oreochromis niloticus* fingerlings in 10 L of water. The fingerlings were acclimatized in the laboratory for 48 hours prior to the introduction of the textile dye. The textile dye was introduced at concentrations of 300 mg, 350 mg, 400 mg, 450 mg and 500 mg, while the control contained no textile dye. Fish mortalities were recorded at intervals and at the end of the 96-hour exposure period, the following were determined:

a. Total number of fish deaths (mortality) after 96 hours.

b. Percentage mortality after 96 hours.

c. The 96-hour LC_{50}, which is the concentration at which 50% of the exposed test organisms died.

The LC_{50} was determined from the graph of percentage mortality plotted against textile dye concentration. The response of the fish to slight external stimuli was used as an index of toxicity, while failure to respond to external stimuli was taken as an indication of death. Dead fish were removed immediately from the experimental tanks to prevent deterioration of water quality during the exposure period. The results obtained from the range-finding test were used in selecting the concentrations for the definitive acute toxicity test.

Definitive Test

The definitive acute toxicity test was conducted based on the results obtained from the range-finding test to determine the 96-hour median lethal concentration (LC_{50}) of Vat textile dye on *O. niloticus* fingerlings. Six treatments, including the control, were used. The test concentrations consisted of 200 mg, 220 mg, 240 mg, 260 mg, and 280 mg of Vat textile dye dissolved separately in 10 L of water, while the control contained no textile dye. Each treatment was replicated ten times, with one healthy *O. niloticus* fingerling stocked in each replicate.

To ensure absolute statistical independence and avoid the confounding effects of pseudoreplication, a completely randomized design was implemented using ten physically distinct, spatially isolated plastic replicate units per exposure concentration (10 exposure units × 5 concentrations + 10 control units = 50 independent experimental tanks). Exactly one healthy *O. niloticus* fingerling was stocked into each separate 10-litre replicate tank. There was zero water sharing or visual/physical contact between replicate units throughout the 96-hour exposure window.

Fish Behavioral Responses

The behavioural responses of *O. niloticus* fingerlings exposed to different concentrations of Vat textile dye were observed and recorded throughout the 96-hour exposure period. Observations were made at regular

intervals to detect any behavioural changes resulting from exposure to the toxicant. The behaviour monitored included swimming activity, opercular movement, and loss of equilibrium, surfacing behaviour, erratic swimming, discoloration, mucus secretion, and response to external stimuli. Fish that exhibited normal swimming patterns, active movement, and rapid response to slight external stimuli were regarded as healthy, whereas reduced swimming activity, frequent surfacing, excessive mucus secretion, loss of equilibrium, sluggish movement, and failure to respond to external stimuli were considered signs of toxic stress.

Water Quality Parameters

The water quality parameters of the experimental media were monitored throughout the exposure period to ensure suitable environmental conditions for the experimental fish and to assess the influence of the textile dye on water quality. The parameters measured included water temperature (°C), dissolved oxygen (DO), hydrogen ion concentration (pH), and electrical conductivity (EC).

Histological Examination of the Liver, Kidney and Gill of *Oreochromis niloticus*

At the end of the experiment, one *O. niloticus* fingerling was randomly selected from each of the five treatment groups and one fish was selected from the control group, making a total of six fish for histological examination. The selected fish were dissected, and the liver, kidney, and gill tissues were carefully excised. The organs were fixed in 10% formalin for three days to preserve the tissue structure. Following fixation, the tissues were subjected to standard histological tissue processing involving dehydration through graded concentrations of alcohol and subsequent embedding in paraffin wax.

The organs were embedded in melted paraffin wax and sectioned into thin sections of approximately 5–7 µm thickness using a rotary microtome. The tissue sections were subsequently stained with Harris haematoxylin and eosin (H&E) stain following the method described by Bancroft and Cook (1994). The prepared tissue sections were mounted on clean glass slides and examined under a light microscope at ×100 magnification. The liver, kidney, and gill tissues were examined for histological alterations and structural abnormalities associated with exposure to the test substance. Photomicrographs of the examined tissues were taken using an Olympus BH2 microscope fitted with a photographic attachment (Olympus C35 AD4), an Olympus C40 AB-4 camera, and an automatic light exposure unit (Olympus PM C5SP).

Animal Welfare and Ethics Statement

Animal Welfare and Ethical Compliance

All experimental protocols involving live fish in this investigation were conducted in strict compliance with globally recognized guidelines for the care and use of laboratory animals. Every effort was made to minimize suffering and reduce the total number of fingerlings used during the acute bioassays. Following the termination of the 96-hour exposure window, surviving fish scheduled for histopathological analysis were immediately and humanely euthanized via rapid thermal shock (immersion in ice-chilled water at below 4°C) followed by cervical dislocation prior to targeted organ dissection.

RESULTS

Water Quality Determination

Table 4.1 shows the water quality values recorded before toxicant addition, immediately after toxicant addition, and after 96 hours of exposure during the definite toxicity test. The parameters measured were temperature, dissolved oxygen (DO), pH and conductivity at toxicant concentrations of 200, 220, 240, 260 and 280 mg/L. Before the addition of the toxicant, the temperature values were relatively similar across the experimental concentrations, ranging from 26.8 to 26.9°C. The dissolved oxygen values ranged from 4.4 to 5.2 mg/L, while pH values ranged from 7.48 to 7.74. Conductivity values ranged from 149.0 to 156.2 µS/cm. The relatively narrow ranges recorded for the parameters before toxicant addition indicate that the experimental groups had generally similar initial water quality conditions.

Overall, the results show that the addition of the toxicant was accompanied by immediate changes in dissolved oxygen, pH and conductivity, whereas temperature showed comparatively little variation immediately after addition. After 96 hours, dissolved oxygen and conductivity generally returned towards their initial values, while pH also decreased towards the initial range**.**

Table 4.1: Water Quality Parameters Before, After and 96 Hours after Addition of Toxicant during the Definite Test

Definitive concentration (mg/L)	**Parameter**	**Before toxicant addition**	**Immediately after addition**	**After 96 h exposure**
0 (Control)	Temperature (°C)	26.8 ± 0.1^{a}	26.9 ± 0.1^{a}	28.2 ± 0.2^{b}
	Dissolved oxygen (mg/L)	5.3 ± 0.2^{a}	5.3 ± 0.2^{a}	5.8 ± 0.3^{a}
	pH	7.52 ± 0.05^{a}	7.53 ± 0.04^{a}	7.53 ± 0.06^{a}
	Electrical conductivity (µS/cm)	155 ± 4^{a}	156 ± 5^{a}	159 ± 6^{a}
200	Temperature (°C)	26.8 ± 0.1^{a}	27.1 ± 0.2^{a}	28.2 ± 0.1^{b}
	Dissolved oxygen (mg/L)	5.2 ± 0.1^{a}	3.0 ± 0.2^{b}	5.2 ± 0.2^{a}
	pH	7.74 ± 0.04^{a}	8.64 ± 0.09^{b}	7.11 ± 0.08^{c}
	Electrical conductivity (µS/cm)	156.2 ± 3.1^{a}	343 ± 12^{b}	163 ± 5^{a}
220	Temperature (°C)	26.8 ± 0.1^{a}	27.0 ± 0.1^{a}	28.2 ± 0.1^{b}
	Dissolved oxygen (mg/L)	4.7 ± 0.2^{a}	2.6 ± 0.1^{b}	4.7 ± 0.1^{a}
	pH	7.73 ± 0.05^{a}	8.71 ± 0.11^{b}	7.35 ± 0.07^{c}
	Electrical conductivity (µS/cm)	152.0 ± 2.8^{a}	355 ± 15^{b}	152 ± 4^{a}
240	Temperature (°C)	26.9 ± 0.1^{a}	26.9 ± 0.1^{a}	28.2 ± 0.2^{b}
	Dissolved oxygen (mg/L)	4.4 ± 0.1^{a}	2.5 ± 0.2^{b}	4.4 ± 0.2^{a}
	pH	7.63 ± 0.03^{a}	8.82 ± 0.14^{b}	7.63 ± 0.05^{a}
	Electrical conductivity (µS/cm)	150.2 ± 3.4^{a}	384 ± 18^{b}	150.2 ± 3.9^{a}
260	Temperature (°C)	26.8 ± 0.1^{a}	26.8 ± 0.1^{a}	28.2 ± 0.1^{b}
	Dissolved oxygen (mg/L)	4.9 ± 0.2^{a}	2.7 ± 0.1^{b}	4.9 ± 0.1^{a}
	pH	7.55 ± 0.06^{a}	8.70 ± 0.12^{b}	7.55 ± 0.04^{a}
	Electrical conductivity (µS/cm)	149.6 ± 2.9^{a}	436 ± 22^{b}	147.6 ± 4.1^{a}
280	Temperature (°C)	26.9 ± 0.1^{a}	27.0 ± 0.2^{a}	28.2 ± 0.1^{b}
	Dissolved oxygen (mg/L)	5.1 ± 0.1^{a}	2.5 ± 0.1^{b}	5.1 ± 0.3^{a}
	pH	7.48 ± 0.04^{a}	9.01 ± 0.15^{b}	7.48 ± 0.06^{a}
	Electrical conductivity (µS/cm)	149.0 ± 3.2^{a}	416 ± 19^{b}	147.0 ± 3.7^{a}

Temp. = temperature; DO = dissolved oxygen. Conductivity values are expressed in µS/cm.

Range-Finding Test

Table 4.2 shows the results of the first range-finding test conducted at toxicant concentrations of 500, 1000, 1500, 2000 and 2500 mg/L. The observation period for all the tested concentrations was recorded as less than 24 hours. The results show that all the tested concentrations had the same observation period of less than 24 hours. Therefore, the table indicates that the first range-finding test was conducted using concentrations ranging from 500 to 2500 mg/L, with observations made within the first 24 hours of exposure. There was no recorded survival among the five concentrations in the table. Overall, the outcome of this preliminary test was subsequently relevant to determining the concentration range used for the next stage of the toxicity assessment.

Table 4.2: First Range-Finding Test

Concentration (mg/L)	Total Fish Exposed	Observation Window	Recorded Cumulative Mortality (%)
500	10	< 24 h	100%
1000	10	< 24 h	100%
1500	10	< 24 h	100%
2000	10	< 24 h	100%
2500	10	< 24 h	100%

Second Range-Finding Test

Table 4.3 shows the results of the second range-finding test conducted at toxicant concentrations of 300, 350, 400, 450 and 500 mg/L over an exposure period of up to 96 hours. Mortality and survival were recorded at 24, 48, 72 and 96 hours. At 300 mg/L, mortality was recorded progressively with increasing exposure time. After 24 hours, 6 fish died, representing 60% mortality, while 4 fish survived, representing 40% survival. At 48 hours, mortality increased to 8 fish (80%), with the number of surviving fish decreasing to 2 (20%). By 72 hours, mortality had increased to 10 fish (100%), while survival decreased to 0 (0%). No mortality or survival value was recorded at 96 hours because all the fish had already died by 72 hours. For the 350 mg/L concentration, no survival values were recorded at 24, 48, 72 or 96 hours. Similarly, no survival was recorded for the 400, 450 and 500 mg/L concentrations throughout the 96-hour observation period.

Table 4.3: Second Range-Finding Test Results

Concentration (mg/L)	Fish Exposed	24 h Mortality (%)	48 h Mortality (%)	72 h Mortality (%)	96 h Mortality (%)
300	10	6 (60%)	8 (80%)	10 (100%)	0
350	10	10 (100%)	0	0	0
400	10	10 (100%)	0	0	0
450	10	10 (100%)	0	0	0
500	10	10 (100%)	0	0	0

Definite Test

Table 4.4 shows the mortality and survival of fish exposed to different concentrations of the toxicant (200, 220, 240, 260 and 280 mg/L) during the 96-hour definite toxicity test. Mortality and survival were recorded at 24, 48, 72 and 96 hours of exposure. At 200 mg/L, no mortality was recorded at 24 and 48 hours, with all 10 fish surviving during both observation periods, representing 100% survival. At 72 hours, mortality increased to 5 fish (50%), with 5 fish (50%) surviving. The same mortality and survival values were recorded at 96 hours, indicating that no additional mortality occurred between 72 and 96 hours at this concentration.

Table 4.4: Mortality and Survival of Fish at Different Toxicant Concentrations during the Definite Test

Concentration (mg/L)	Exposure Time	Mortality	Mortality (%)	Survival	Survival (%)
0 (Control)	24 h	0	0	10	100
	48 h	0	0	10	100
	72 h	0	0	10	100
	96 h	0	0	10	100
200	24 h	0	0	10	100
	48 h	0	0	10	100
	72 h	5	50	5	50
	96 h	5	50	5	50
220	24 h	0	0	10	100
	48 h	2	20	8	80
	72 h	6	60	4	40
	96 h	6	60	4	40
240	24 h	0	0	10	100
	48 h	3	30	7	70
	72 h	8	80	2	20
	96 h	10	100	0	0
260	24 h	0	0	10	100
	48 h	5	50	5	50
	72 h	8	80	2	20
	96 h	10	100	0	0
280	24 h	2	20	8	80
	48 h	6	60	4	40
	72 h	10	100	0	0
	96 h	10	100	0	0

Behavioral Responses of Fish during Toxicant Exposure

Table 4.5 shows the behavioral responses of fish exposed to different concentrations of the toxicant (200–280 mg/L and the control) at 24, 48, 72 and 96 hours of exposure. The observed responses were based mainly on swimming activity, movement, responsiveness to external stimuli, equilibrium and general activity level. The control group recorded 0% mortality throughout the 96-hour exposure period, with all 10 fish surviving at 24, 48, 72 and 96 hours. This indicates that mortality observed in the treatment groups was associated with exposure to the test substance rather than mortality occurring naturally under the experimental conditions.

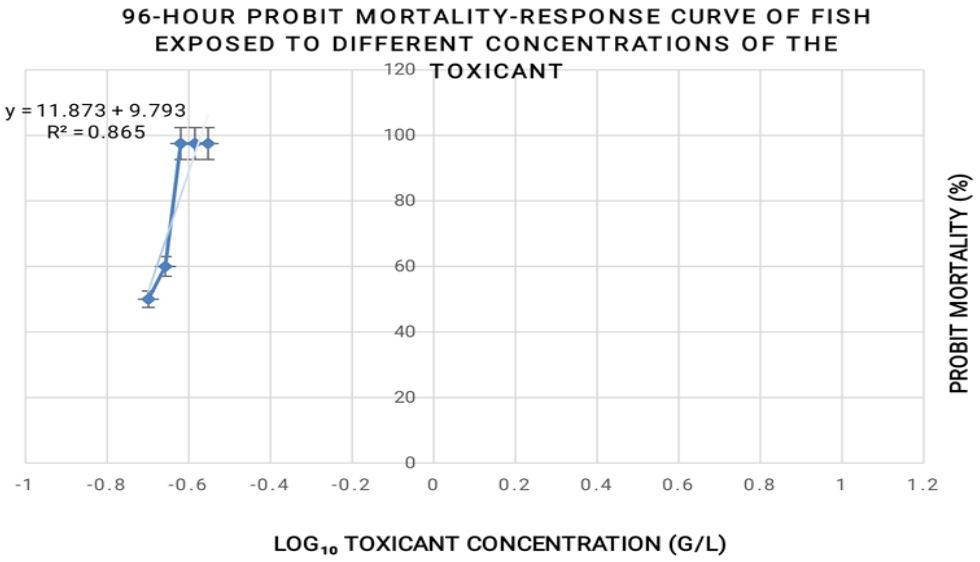
Table 4.5: behavioral Responses of Fish Exposed to Different Concentrations of the Toxicant

Concentration (mg/L)	24 Hours	48 Hours	72 Hours	96 Hours
0 (control)	Normal	Normal swimming	Normal swimming	Normal swimming

	swimming activity	activity	activity and responsiveness	activity and normal response to external stimuli
200	Normal swimming activity	Reduced swimming activity and sluggish movement	Marked reduction in activity and responsiveness	Severe sluggishness and reduced response to external stimuli
220	Normal to slightly reduced activity	Reduced activity and slower movement	Sluggish movement and reduced responsiveness	Increased inactivity and reduced response
240	Slight reduction in swimming activity	Erratic swimming and sluggish movement	Pronounced erratic movement and reduced responsiveness	Severe stress and very limited movement
260	Slight reduction in activity	Sluggish and irregular swimming	Pronounced sluggishness and erratic movement	Severe impairment of swimming activity and reduced responsiveness
280	Noticeable reduction in activity	Strongly reduced activity and erratic swimming	Severe stress, loss of equilibrium and immobility	Total Mortality

Probit Analysis and Concentration–Mortality Relationship

Concentration (mg/L)	**Number exposed (n)**	**Number of dead fish**	**Observed mortality (%)**	**Adjusted mortality (%)**	**Probit**	**Log_{10} concentration**
0 (Control)	10	0	0	0	0	0
200	10	5	50	50	5.000	-0.6990
220	10	6	60	60	5.253	-0.6576
240	10	10	100	95	6.645	-0.6198
260	10	10	100	95	6.645	-0.5850
280	10	10	100	95	6.645	-0.5528



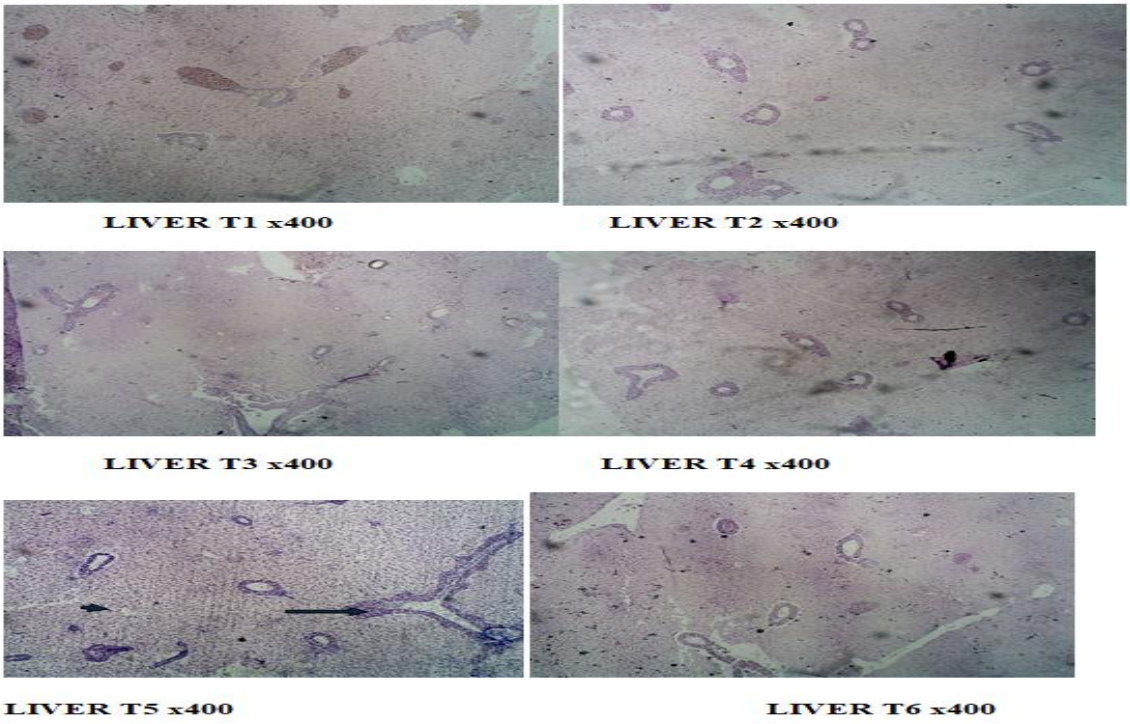
The 96-hour LC_{50} of the test substance was estimated at approximately 200 mg/L using probit regression of

mortality response against log_{10} concentration.

Histology of the Liver

The Plates (photomicrographs) show histological alterations in the liver of *O. niloticus* fingerlings exposed to graded concentrations of vat dye for 96 hours stained with Haematoxylin and Eosin (H and E). T1 represents the control group (0 mg/L), showing normal hepatocyte parenchyma and well-preserved hepatic architecture. T2 (200 mg/L) shows mild vacuolar degeneration of the hepatocytes. T3 (220 mg/L) exhibits slight erosion and disruption of hepatocellular architecture. T4 (240 mg/L) shows multiple foci of vacuolar degeneration of hepatocytes.

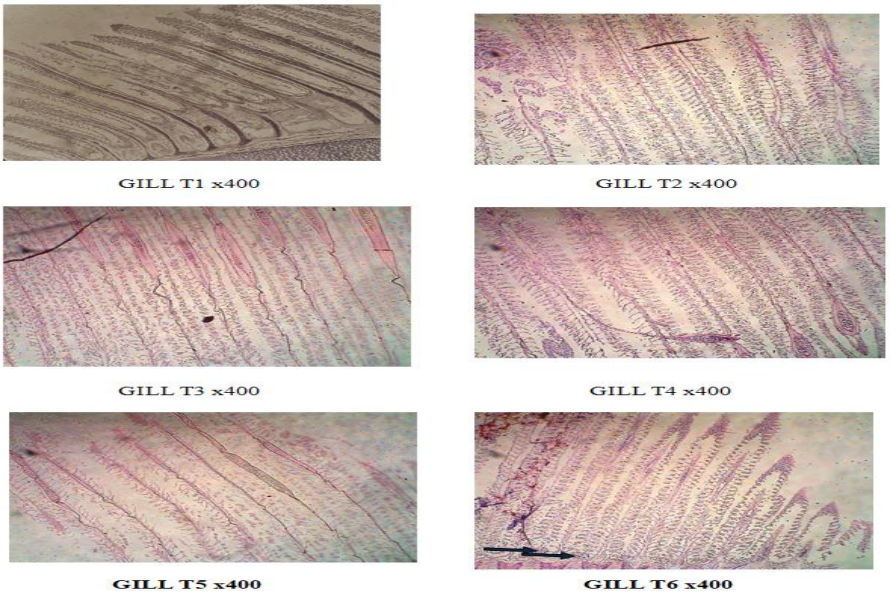
T5 (260 mg/L) displays severe vacuolation of hepatocytes, indicating marked hepatocellular degeneration. T6 (280 mg/L) shows severe hepatocellular necrosis accompanied by marked congestion, erosion, extensive cellular degeneration, and pronounced disruption of the normal hepatic architecture, representing the most severe histopathological alteration observed across the exposure groups.



Photomicrographs representing histology of the liver sections of *O. niloticus* fingerlings exposed to varying concentrations of vat dye (200 – 280 mg/l).

Histology of the Gills

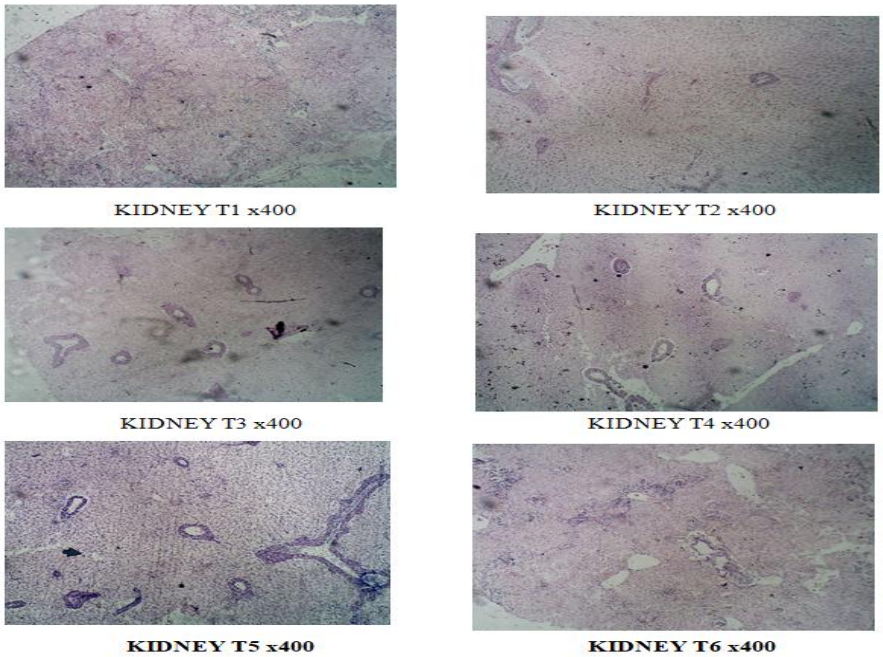
Figure 2: Photomicrographs showing histological alterations in the gills of *Oreochromis niloticus* fingerlings exposed to graded concentrations of vat dye for 96 hours stained with Haematoxylin and Eosin (H and E). T1 represents the control group (0 mg/L), showing regular and well-organized secondary lamellae with preserved gill architecture. T2 (200 mg/L) shows slight erosion of epithelial cells along the secondary lamellae. T3 (220 mg/L) exhibits increased erosion and disruption of the epithelial cells of the secondary lamellae. T4 (240 mg/L) shows severe erosion and marked disruption of the secondary lamellar epithelium. T5 (260 mg/L) displays complete erosion and destruction of the secondary lamellar epithelial cells, indicating severe structural damage to the gill tissue. T6 (280 mg/L) shows extensive destruction and complete loss of the secondary lamellar epithelium, accompanied by severe disruption of the gill architecture and pronounced degeneration of the lamellar structures, representing the most severe histopathological alteration observed across the exposure groups.



Photomicrographs representing histology of the gill sections of *O. niloticus* fingerlings exposed to varying concentrations of vat dye (200 – 280 mg/l).

Histology of the Kidney

Figure 3: Photomicrographs showing histological alterations in the kidney of *O. niloticus* fingerlings exposed to graded concentrations of vat dye for 96 hours stained with Haematoxylin and Eosin (H and E). T1 represents the control group (0 mg/L), showing normal renal architecture with well-organized clusters of renal tubules and interstitial tissue. T2 (200 mg/L) shows renal architecture comparable to the control, with no clearly demarcated pathological alteration. T3 (220 mg/L) exhibits slight erosion and disruption of the renal tubules. T4 (240 mg/L) shows noticeable vacuolation of the renal tubules, indicating early degenerative changes. T5 (260 mg/L) displays severe erosion and degeneration of the renal tissue, indicating marked renal injury. T6 (280 mg/L) shows extensive tubular degeneration and necrosis, severe erosion, and pronounced disruption of the normal renal architecture, representing the most severe histopathological alteration observed across the exposure groups.



Photomicrographs representing histology of the kidney of *O. niloticus* fingerlings exposed to varying concentrations of vat dye (200 – 280 mg/l).

DISCUSSION

The present study investigated the acute toxicity of Vat textile dye to *Oreochromis niloticus* fingerlings under laboratory conditions, with emphasis on water-quality characteristics, mortality response, behavioural changes and the determination of the 96-hour median lethal concentration (LC_{50}). The results demonstrated that exposure to the dye produced changes in the physicochemical characteristics of the test water and a concentration-dependent increase in fish mortality. In the definitive test, 50% mortality was recorded at 200 mg/L, 60% at 220 mg/L, while 100% mortality occurred at 240, 260 and 280 mg/L after 96 hours.

The present findings also agree with the broader observations of Kassaye Balkew and Balkew (2013), who reported increasing mortality of Nile tilapia exposed to increasing concentrations of textile effluent. The similarity is important because both studies involved *O. niloticus* and textile-related contaminants. However, textile effluent contains a mixture of dyes, salts, metals, surfactants and other chemicals, whereas the present experiment focused on Vat dye. Consequently, differences in toxicity may reflect the complexity of the contaminant mixture as well as the characteristics of the individual dye.

The estimated 96-hour LC_{50} of approximately 199 mg/L represents the concentration expected to cause mortality in 50% of the exposed fish under the conditions of the present experiment. The estimate is strongly supported by the experimental observation because 50% mortality was recorded at 200 mg/L. The close agreement between the observed concentration and the probit-derived LC_{50} suggests that the estimate adequately represents the acute lethal response of the fish to Vat dye. The relatively narrow transition from 50% mortality at 200 mg/L to 60% at 220 mg/L and 100% at 240 mg/L indicates that the lethal response occurred within a narrow concentration interval. This may be associated with the sensitivity of the fingerlings, the characteristics of the dye and the exposure conditions. Ayebidun and Ajibare (2023) similarly demonstrated that concentration and exposure duration strongly influenced the response of *O. niloticus* to dye exposure. *Sharma et al.,* (2009) also showed that mortality could serve as a sensitive endpoint for assessing acute toxicity of textile-related contaminants.

However, the concentration-response relationship observed in this study aligns with general trends reported for textile colorants in fish, such as the observations by Ayadi *et al.* (2015) regarding *O. niloticus* exposed to reactive Red 195. However, a major toxicological distinction must be made regarding the chemical behavior of the colorants. While standard azo and reactive dyes are water-soluble and directly cause cellular toxicity through their degradation byproducts, Vat Blue 1 is naturally water-insoluble. Consequently, its toxicity profile is uniquely compounded by the severe alkaline environment (pH > 9.0) and chemical reducing agents required to make the dye solution stable, as shown by the sudden spike in pH and conductivity metrics in Table 4.1. This distinct chemical combination causes immediate damage to the gill epithelium and reduces available dissolved oxygen upon addition. This explains why the calculated 96-h LC_{50} value in this study (201.3 mg/L) is higher than values reported for completely water-soluble dyes, yet it induces rapid structural damage and mortality once these environmental thresholds are crossed.

The present findings agree with Ayebidun and Ajibare (2023), who observed irregular swimming, hyperventilation, rapid opercular movement and restlessness in *O. niloticus* exposed to indigo dye. Their study further reported that these responses became more pronounced with increasing concentration and exposure duration. Kassaye Balkew and Balkew (2013) similarly reported abnormal swimming and respiratory-related behavioural responses in Nile tilapia exposed to textile effluent. These similarities suggest that behavioural abnormalities are useful early indicators of physiological stress in fish exposed to textile-related contaminants.The present study therefore provides evidence that uncontrolled release of Vat textile dye into aquatic environments could pose a significant risk to fish populations and, consequently, to fisheries and aquaculture resources.

Histology of the Liver

The liver, as the principal organ of xenobiotic biotransformation in teleosts, is consistently identified as one of the earliest and most sensitive targets of aquatic pollutant exposure, owing to its direct exposure to absorbed

toxicants via the hepatic portal circulation and its central role in detoxification (Bernet *et al.,* 1999). The present findings in *O. niloticus* fingerlings exposed to vat dye (200 mg – 280 mg/l) revealed two closely related but distinct hepatic responses such as vacuolar (fatty) degeneration of hepatocytes, observed from T2 through T4, and melanomacrophage centre (MMC) proliferation, most prominent at T5 and T6.

The vacuolar degeneration observed at T2 and T4 aligns closely with findings reported by Ayadi *et al.* (2015) which found that Nile tilapia exposed to Red 195 dye developed increased cytoplasmic vacuolization, disruption of endothelial lining, metabolic zonation, necrotic cells, and rupture of the hepatocyte membrane, with damage severity linked to both dye concentration and exposure duration. The concentration-dependent and time-dependent nature of that damage supports the present study's core finding that hepatocellular injury scales with vat dye concentration. However, the absence of necrosis or membrane rupture in this present study both reported by Ayadi *et al.,* (2015) at their higher concentrations and longer durations suggests the exposure conditions used here produced sub-lethal, reversible-to-early-degenerative hepatocellular stress, without progressing to the more advanced necrotic injury documented in that comparative study.

The pronounced MMC response at T5 and T6 warrants separate mechanistic discussion, since melanomacrophage centres are functionally distinct from the vacuolar degeneration pathway. melanomacrophage centres are pigmented macrophage aggregates involved in the storage, detoxification, and recycling of endogenous and exogenous material, including cellular debris and lipid peroxidation by-products, and their increased number or size is widely used as a biomarker of chronic toxicant exposure in fish reported by Agius and Roberts, 1981; Fournie *et al.,* 2001). The proliferation of MMCs alongside declining vacuolation at T5 is consistent with a macrophage-mediated clearance process acting on hepatocytes already damaged by the degenerative injury observed at T2–T4, rather than an independent or unrelated pathological process.

Histology of the Gill

The gill is the primary site of contact between waterborne toxicants and the fish, given its direct and continuous exposure to the surrounding medium during respiration, making it one of the first organs to register histopathological change following aquatic pollutant exposure (Fernandes and Mazon, 2003). In this present study, gill filaments of *O. niloticus* fingerlings exposed to vat dye (200 – 280 mg/l) showed a less uniform severity progression than the liver, with early epithelial oedema at T3, an apparent reduction in severity at T4, and the most marked lesion club-shaped swelling of filament tips at the highest concentration, T5 and T6.

The pattern of injury observed here such as epithelial oedema, swelling, and lamellar clubbing is consistent with established gill lesion categories reported for Red Aro dye exposure in tilapia. According to Ayadi *et al.* (2015) reported that Nile tilapia gills exposed to Red 195 aro dye exhibited necrotic cells, oedema, lifting of the filament and lamellar epithelium, and vascular disorders including extreme vasodilatation, a lesion spectrum broadly consistent with, though more advanced in oedema and epithelial swelling recorded at T5 and T6 in the present study. The absence of overt necrosis or vasodilatation in the present findings again suggests that vat dye exposure at the concentrations and duration used here produced early-to-moderate branchial stress rather than advanced tissue destruction.

The club-shaped swelling observed at T5 and T6 specifically corresponds to lamellar clubbing, a well-recognized toxicant-induced gill lesion. In a biomonitoring study of Nile tilapia from Lake Tana, oedema and lamellar clubbing were attributed to waterborne toxin exposure, with this departure from normal gill structure noted to compromise the functional efficiency of the gills for both gas exchange and ionic regulation. These reports interpret with T5 and T6 finding as a genuine marker of toxic stress on respiratory and osmoregulatory function, rather than an incidental or artefactual change, and provides a physiological rationale for why lamellar clubbing is considered a more advanced lesion than simple epithelial oedema: it reflects a structural response to sustained irritation that directly compromises gill function.

Histology of the Kidney

The histological evaluations of *Oreochromis niloticus* trunk kidney sections exposed to lower concentrations of the Vat textile dye (T_2 at 200 mg/L) revealed a well-preserved renal architecture, presenting regular clusters

of renal tubules and intact glomeruli comparable to the control (T_1) group. However, as the concentration of the toxicant escalated from T_3 (220 mg/L) to T_6 (280 mg/L), a concentration-dependent progression of structural degeneration was observed, characteristic of nephrotoxicity. This structural degeneration manifested as marked tubular vacuolation, structural disruption of the nephrons, and focal zones of advanced tubular necrosis at the highest exposure tier (T_6). This pathological progression closely mirrors the findings of tank-exposure tests by Hussain *et al*. (2019), who observed a graded spectrum of renal injury in freshwater teleosts exposed to industrial pollutants, moving from initial epithelial hypertrophy to severe glomerular necrosis and tubular degradation under elevated pollutant loads. The structural degradation observed in the renal tubules at higher dye concentrations directly accounts for the systemic collapse of homeostatic and osmoregulatory functions in the fingerlings, as damaged filtration units can no longer regulate internal electrolyte balances or eliminate accumulated waste products effectively."

Taken together, these two studies support the interpretation that renal architecture in freshwater fish, including *O. niloticus* specifically, is capable of remaining substantially intact under lower or threshold-level contaminant burden, with structural damage emerging in a graded, exposure-dependent manner and becoming most severe only at higher pollutant loads or more prolonged exposure. This pattern is consistent with the preserved kidney histology observed across the vat dye concentrations tested in the present study, and offers two non-mutually-exclusive explanations for it. First, the vat dye concentrations and exposure duration used here, while sufficient to produce clear hepatic and branchial lesions, may not have reached the threshold required to produce overt renal histology in this species. Second, an organ-specific hierarchy of vulnerability may apply, whereby the gill (as the first point of contact with the waterborne toxicant) and the liver (as the principal site of systemic biotransformation) sustain injury before the kidney under comparable exposure conditions consistent with the general toxicological principle that the sequence and severity of organ damage reflect both the route of exposure and the pathway of toxicant distribution and metabolism within the body. The species-dependent susceptibility reported by Hussain *et al.* (2019) also raises the possibility that *O. niloticus* itself may show comparatively higher renal resilience to waterborne contaminants than some other freshwater species, though this would need to be confirmed by direct comparative studies using the same toxicant.

Limitations of the Study

While this study successfully established a definitive baseline for the acute toxicity and primary organ damage caused by Vat Blue 1 dye in *Oreochromis niloticus* fingerlings, several limitations are acknowledged. First, due to field facility constraints, toxicant levels throughout the 96-hour window were tracked via nominal concentrations rather than continuous analytically measured exposure values. Second, the static renewal configuration represents an acute surge model; it does not fully simulate the dynamic, continuous dilution vectors typical of natural river basins or open receive waters.

CONCLUSION

The study established that Vat textile dye has significant acute toxic effects on *Oreochromis niloticus* fingerlings under laboratory conditions. The mortality response increased with both concentration and duration of exposure. At the end of the 96-hour exposure period, mortality was 50% at 200 mg/L, 60% at 220 mg/L, and 100% at 240, 260 and 280 mg/L. Probit analysis estimated the 96-hour LC_{50} of the Vat textile dye at approximately 199 mg/L, indicating that relatively small increases in dye concentration within the tested range produced a marked increase in mortality.

The addition of Vat dye temporarily altered the physicochemical characteristics of the experimental water. Dissolved oxygen decreased immediately following toxicant addition, while pH and electrical conductivity increased considerably. However, most of these parameters moved closer to their initial values after 96 hours, while temperature remained comparatively stable. The changes in water quality may have contributed to the stress experienced by the exposed fish, although the mortality pattern and control response indicate that the toxicant was an important cause of the observed effects.

The histological examination further demonstrated that Vat dye exposure caused structural damage to the examined organs. The liver showed progressive vacuolar degeneration, hepatocellular degeneration, congestion and severe necrosis at the highest concentration. The gills exhibited increasing epithelial erosion and disruption, progressing to extensive destruction and loss of the secondary lamellar epithelium at the highest exposure level. The kidney also showed concentration-related alterations, ranging from slight tubular erosion and vacuolation to severe tubular degeneration, necrosis and disruption of normal renal architecture at 280 mg/L.

Overall, the findings demonstrate that Vat textile dye represents a potential threat to *O. niloticus* and other aquatic organisms when present in sufficiently high concentrations. The combined mortality, behavioural, water-quality and histopathological responses indicate that uncontrolled discharge of Vat dye-containing textile wastewater into aquatic environments may adversely affect fish health, survival and the sustainability of aquatic ecosystems.

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