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TOXICOLOGICAL STUDY OF CYANOBACTERIA *(MICROCYSTISAERUGINOSA)* ON HAEMATOLOGICAL AND BIOCHEMICAL RESPONSE IN O. NILOTICUS (L) OF THOMAS RESERVOIR, KANO, NIGERIA

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ARTICLE INFO	ABSTRACT
	The study assessed the acute and sub chronic toxicity of Microcystisaeruginosa on Haematology and Biochemical responses in the Kidney, Liver and gills of Nile tilapia (Oreochromis niloticus) from Thomas reservoir, Kano, Nigeria between September and December, 2018. Field and laboratory investigations were performed using standard protocols. A total of 144 samples of Oreochromis niloticus with mean weight range of 26.5 ± 0.25 -37.5 ± 0.31g, mean length 15.0 ± 0.41 -17.3 ± 0.82cm were exposed to different concentrations of the cyanobacterial extract (0.0 mg/1-control, 50.0, 100.0 and 150.0mg/kg) for 28 days. The oral LC50 of the extract was 6.14µg/ml. The results obtained indicated significant reductions ($P<0.05$) with increased concentrations of the extract in; haemoglobin (Hb), Red blood Cell (RBC) and packed cell volume (PCV). The white blood cell (WBC) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC), Erythrocyte Sedimentation Rate (ESR) and mean corpuscular volume (MCV in fish were significantly ($P<0.05$) higher than that of the control. Antioxidant biomarkers activities revealed that Glutathione Reductase (GSH) and Glutathione S-transferase (GST) increase significantly ($P<0.05$) in the fish tissues. There was significant decrease ($P<0.05$) in Catalase (CAT) and Superoxide dismutase (SOD) activities. It can be deduced that alteration in the antioxidant enzymes reflects cyanobacterial effect in the fish tissues. Uncontrolled discharge from human activities should be controlled in order to curtail degradation of the reservoir in the long run.

INTRODUCTION

Algae are group of aquatic organisms regarded as beneficial for their roles as primary producers, food for humans and indicators of water quality (Mustapha, 2008). Cyanobacteria as a taxonomic division of algae produce blooms which portray a public health attention in sources of drinking and recreational water bodies by producing toxins, causing taste and odour problems and reduce water clarity (Ferrão-Filho *et al.*, 2009). Toxins from cyanobacteria are transferred through the tropic food chain/ food web in the aquatic ecosystem and eventually to humans (Montagnolli *et al.*, 2004). These toxins have been reported to cause livestock, wildlife and pet mortalities and primary cancer in humans especially liver cancer (Molica *et al.*, 2005). Haematological parameters have been used as indicators of environmental stress and physiological response

of fish from toxic substances such as cyanotoxins, tannery effluents among other pollutants (Ural, 2013). This is due to the mutual coexistence between the circulatory system and the external environment (Fiore, 2009). Enzymatic assay is among the biomakers used for detection of toxins in aquatic ecosystem (Dutta and Areids, 2003). Changes have been observed in the activity of alkaline phosphatase, Aspartate amino transferases (AST), Alanine Aminotransferase (ALAT) in tissues, organs and blood of fish exposed to cyanotoxins (Montagnolli *et al.*, 2004). Studies have shown alterations in enzymes activity in the body and blood of fish when exposed to toxins (Dai *et al.*, 2009).These enzymes are essential amino acids which plays vital role in the body's tissues for carbohydrates and proteins metabolism (Burtis and Ashwood, 2001). Aspartate amino transferases (AST) for instance are naturally found in the cytoplasm and mitochondria of cells in

different types of tissue such as liver, heart, muscle and brain. Therefore, AST level increases, if the tissues are injured (Dai *et al.*, 2009). Prieto *et al.* (2006) studied the response of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), GST and glutathione reductase (GSH) as well as lipid peroxidation (LPO) as a biomarker of oxygen-mediated toxicity in liver, kidney and gill of *Oreochromis* sp. In Kano State, Nigeria, there is no effective monitoring programme or legislation to enhance proper management and utilization of water resource with regards to cyanotoxins. Besides, the establishment of such a programme is dependent on the availability of based line information on the occurrence and the distribution of cyanobacteria toxins; which also depend on the availability of adequate facilities or resources for the detection and quantification of cyanotoxins (Nafiu *et al.* 2017). Existing water quality assessment and monitoring strategies that employ microbial and physicochemical criteria are inadequate for comprehensive assessment of water quality especially in water bodies showing evidence of progressive eutrophication (Mustapha, 2008). In view of the foregoing this research aimed at investigating the Toxicological study of cyanobacteria (*Microcystisaeruginosa*) on Haematological and Biochemical Assay on O. *niloticus* (L) from Thomas Reservoir, Kano, Nigeria

MATERIALS AND METHODS

Study Area: Thomas Reservoir is located within Sudan savannah zone of Nigeria (12° 16 44" N - 21° 18'35N and 8°30'5''E - 8° 31'34''E) with distinct wet and dry seasons. The rainy season lasts from May to October and dry season spans from November to April. The Reservoir is about 585 square meters, while its mean depth is 30m. The Reservoir is sited near Danmarke village of Dambatta Local Government area of Kano State, 30km away from the ancient Kano City (Kutama *et al.,* 2013). The agro-metrological station nearest to the area shows the estimated average rainfall as 385 mm during months of July and August. The total annual free water evaporation was calculated as 248 mm from the station, and monthly evaporation varies from 171m in December to 270 m in May. During dry season intensive irrigation activities are carried out within the vicinity of the reservoir with a capacity to irrigate 8,000 acres of land; besides, commercial fishing is carried out by fishers on routine basis. The reservoir provides drinking water to the communities at a point where Kano State Water Works (Dambatta zone) is located.
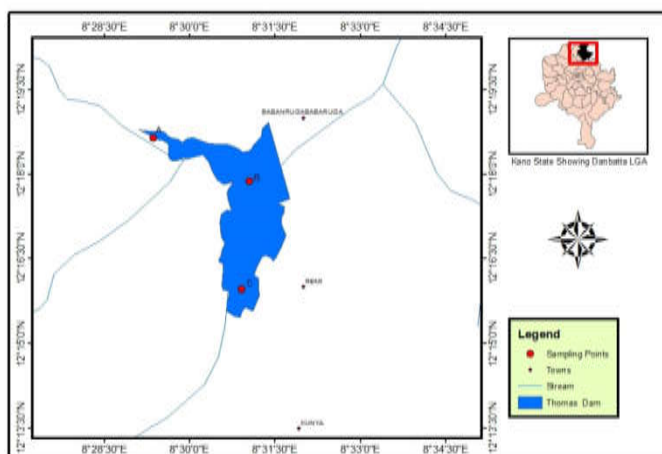

Figure 1. Thomas Reservoir Kano State with Marked Sampling Sites (Source: Cartography Lab., Bayero University, Kano, 2018)

Collection and Identification of Cyanobacterial Samples: Cyanobacterial samples were collected using plankton net of 15cm diameter and mesh size 70µm according to the procedure described by Verlencar and Desai (2004). They were collected at depth of 20cm between 7:00am-8:00am fortnightly from September- December, 2018. Three sampling sites (Figure 1) were chosen for the purpose of this study and designated as upstream (A), midstream (B) and downstream (C) on the water course of the Reservoir. The choice of the sites was based on the ecological setting of the sampling areas. The samples were maintained at 4°C in ice box and immediately transported to hydrobiology Laboratory for further analysis. The collected samples were condensed to10mI and 1ml of the cyanobacteria subsample which was withdrawn for sorting and counting using camera microscope (LEICA DM 2500 model). Identification of the phytoplankton to species level was done using keys described by Suther and Rissik (2009).

Isolation and Culturing *Microsysitisaeruginosa* in BG 11 Medium: Isolation of cyanobacteria (*Microsysitisaeroginasa)* was done using Capillary Pipette Isolation method as adopted by Ibrahim and Raneen (2018). It involved putting small droplets containing *M.aeroginasa* on a sterile glass slide. They were examined under the microscope and the one with a single algal cell was removed with a sterile capillary pipette and transferred in to a freshly prepared BG 11 medium and incubated at 24˚C. The culture was transferred in to 90 ml of medium in a 100ml conical flask and then transferred in to three replicates of 1000ml flask containing 900ml of BG 11 medium each, which was subjected into modified model Photo Bioreactor to obtained pure line biomass. The culture was maintained at 15-20°C under 37.5µmol-1m2s-1 intensity with 16:8 hours' photoperiod for 2 weeks as adopted by Ilavarasi *et al.* (2011). Pure cultures of *M. aeroginasa* were purified against contaminants using a combination of antibiotics containing Chloramphenicol 25mg/l and Penicillin 10mg/l. Daily reading of Optical Density was made at 540nm using a Beckman Coulter DU 720 General Purpose UV/Visible Spectrophotometer as described by Monad (2012).

Harvesting and Lyophilisation of Pure M. *aeruginosa:* The pure culture was harvested using filtration method as adopted by Akin-Oriola *et al.* (2006). The pH was adjusted from a range of 7.0 to 9.8- 9.9 using 1M NaOH. With aid of 80 µm sieve, 1000ml of cultured sample was harvested and freeze/dried at specimen chamber (SB4 Model) at Central Laboratory, Bayero University Kano. To maintain its viability and purity, the dried cells were kept in its fresh appearance and chemical composition at -20°.

Fourier Transform InfraRed Spectroscopy: Samples of the cyanobacterial extracts were identified for chemical compounds, molecular bonds and functional groups by infrared radiation using Fourier Transform Infrared Spectrophotometer using procedure adopted by Mukund*et al.* (2014).

Cyanobacterial Toxins Extraction and Hatching of Brine Shrimp Eggs (*Artimia salina):* Cyanobacterial toxins were extracted according to the procedure adopted by Akin-Oriola *et al.* (2006). Samples extraction was carried out for 24 h in 80% aqueous methanol at a concentration of 50 mg dry weight of cells per ml using glass rod and magnetic stirrer. Methanol was removed from the filtrate by evaporation at 42.0˚C. The

extract was used to prepare a stock solution of 1000mg/l. Brine shrimp (*Artimiasalina*) eggs were obtained from Biochemistry Laboratory, Bayero University, Kano, Nigeria. They were hatched in artificial sea water prepared by dissolving 38 g sodium chloride in 1 liter of distilled water under constant aeration and light source of 32˚C for 48 h. The hatched larvae (active nauplii) were pipette by siphoning with a plastic tube. Ten (10) nauplii were collected through a glass capillary and transferred into 250cm^3 conical flasks. Fourty five (45.0ml) of artificial sea water solution and 5.0 ml ofthe diluted cyanobacterial extract (100-0.07 mg/ml) was added and maintained at room temperature for 24 h under constant aeration and light source as adopted by Akin-Oriola *et al.*(2006).

Brine shrimp Lethality Assay: Brine Shrimp Lethality Assay was carried out using the procedure described by Jegathambigai *et al.* (2014). Exactly 75mg of M. *aeruginosa* extracts was dissolved in 300μl of filtered artificial sea water to give a concentration of 300μg/ml in terms of dry masses equivalent. This was then diluted with sea water to obtain concentrations of 60μg/ml, 40μg/ml, 20μg/ml and 0.00μg/ml as control respectively. Ten (10) Brine Shrimp larvae were introduced into test tube; 9ml of the sample was added and maintained under florescent light. After 96hrs, camera microscope (LEICA DM 2500 model) were used to identify number of dead larvae.

Determination of Lethal Concentration (LC$_{50}$): Lethality was calculated from the mean survival of larvae in extract-treated tubes and that of control. Mean percentage mortality for cyanobacterial toxin for 24, 48, 72 and 96hrs was plotted against the logarithm of concentrations in triplicate using probit analysis as adopted by Leena (2012). A concentration that kills 50 % of the larvae (LC50) was calculated from the linear equation by taking the antilogarithm.

Ethical Statement : In the present research, test fishes were procured from Kano State Polytechnic fish farm. Standard procedure for handling the test organism (fishes) has been followed. Although, there is no any formal institutional animal care and use committee is available in Kano state, Nigeria.

Experimental Design for Acute and sub chronic Toxicity Evaluation of *M. aeruginosa* Extract on *O. niloticus*

The experimental male *O. niloticus* were subjected to Completely Randomized Design (CRD) with three exposure concentrations to *M. aeruginosa* extracts (50.0, 100.0 and 150.0mg/kg) in triplicate. A total of 216 samples of *Clariasgaripinus* mean weight range of 26.5 ± 0.25 -37.5 ± 0.31g, mean length 15.0 ± 0.41 -17.3 ± 0.82cm were used for the research. They were procured and maintained in aquarium from fish farm unit of Kano State Polytechnic Entrepreneurship Study Center Jaafar Mahmud road, Kano, Nigeria. They were acclimated for a week and fed twice with pellet diet (35% crude proteins). During the treatment period, the aquarium water was renewed daily. They were divided into three groups as follows: group 1(n=72) Biochemical analysis and group 2 (n=72) haematological investigations. Fish captured were stored in ice block and immediately transported to the laboratory for further analysis. They were counted, weighted to the nearest 0.1g using weighing balance (TF220 Model). The standard lengths and total lengths were measured to the nearest 0.1cm on a measuring board. The biometric

features such as type of mouth, position of the mouth, fin count, spine count, barbell counts and position, kind of teeth, dorsal and anal fin rays count, gill raker count, body shape, size, colour and shape of the caudal fin were examined on the fish species using identification key by Olaosebikan and Raji (1998).

Acute Toxicity Test: A stock solution of the cyanobacterial extract was prepared in a sterile flask at a concentration of 1000 mg/ml. The volume of the stock solution to be administered to the fishes was calculated using the formula as adopted by John *et al.* (2014).

$$\text{Standard Dose} = \frac{\left(\frac{No}{kg}\right)x \text{ weight of experimenta fish}}{\text{Number of Algae (ml)}}$$

This was conducted out on the experimental and control fish, in which control were fed orally with normal fish feed while experimental were given the doses of 0.0ml, and 1.5ml equivalent to 0.0mg/ml and 1.5mg/ml from the stock solution containing 1000mg/ml prepared in distilled water and observed after 96hrs as described by OECD (2008). Cyanobacterial extract doses were formulated from 1/4th, 1/8th, 1/16th of the limit test dose of 500 mg/kg as adopted by John *et al.* (2014). Means values of water parameters such as temperature, pH, DO were measured and maintained daily at 29°C±0.81, 7.9±0.39 and 6.2±0.32mg/L.

Sub-chronic Toxicity Test: The juveniles of *O. niloticus* were divided into plastic aquarium 30liters containing 20 litres of water each and acclimated to laboratory conditions for 28 days, aerated and covered with mosquito nets to prevent the fish from jumping out. Fish feed (35% crude protein) was orally given to five (5) experimental fishes concurrently with cyanobacterial extract doses of 0.5, 1.0, 1.5 ml corresponding to 0.5, 1.0, 1. 5ml from the stock solution containing 1000mg/ml prepared in distilled water during the treatment. Observation on the Morphological and behavioral parameters of the fishes for 30 minutes was carried out after renewal of the water and extracts which include: number of buccal movement per minute, number of feeding attempt/minute, breathing activity/minute and number of mortality.

Biochemical Analyses of Fish Tissue: Enzymes activity of CAT, SOD, GST and GSH in the fish tissues (n=72) was carried out in Biochemistry Laboratory, Ahmadu Bello University Zaria, according to the procedure described by Nahed (2011). The fishes were kept in declorinated water before the commencement of the experiment. The liver, kidney and gills of the fish were removed, washed in ice cold buffer. The tissues were rinsed clear of blood with cold isolation buffer containing 100 mM Tris-HCl and pH of 7.20 and homogenized on ice in homogenizing vessel with a motar and a pestle. The homogenate was centrifuged at 10000 g for 8 minutes to produce the post mitochondrial fraction which was used for biochemical analysis.

Catalase Activity: The catalase (CAT) activity was determined using the method adopted by Haque *et al.* (2003). Exactly 100μl of tissues (liver, gill and kidney) homogenate was added to a 10ml test tube containing 3.0ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 1ml of 30mM H$_2$O$_2$ and results in absorbance was recorded after every 30 seconds at 240 nm in a double beam spectrophotometer.

Glutathione Reductase Activity Assay: Reduced glutathione (GSH) was measured in the post mitochondrial fraction of the liver, gills and kidney tissue of *O. niloticus* according to the method described by Habig *et al.* (1974). To 150µl of tissues homogenate (in phosphate – saline buffer pH 7.4), 1.5ml of 10% TCA was added and centrifuge at 1500g for 5 minute. 1ml of the supernatant was treated with 0.5ml of Ellman's reagent (39.6 mg of 5, 5-dithio-bis-2- nitrobenzoic acid (DTNB) in 10 ml of 1% sodium citrate) and 3ml of phosphate buffer (0.2M, pH 8.0). The absorbance was obtained at 412nm. The amount of GSH was obtained from the graph of the GSH standard curve. The activity of GSH was calculated based on tissue protein concentration.

Glutathione *S*-transferase (GST) : Glutathione *S*-transferase (GST) activity was measured at 340nm wavelength as described by Habig *et al.* (1974) adopted by Oluwatosin *et al.* (2016) using 1 -chloro 2, 4- dinitrobenzene as substrate.

Superoxide Dismutase (SOD): Activity Superoxide dismutase activity was measured as described by Magwere *et al.* (1997). During the reaction SOD inhibit auto-oxidation of epinephrine. Three (3ml) of the reaction mixture containing 2.95ml 0.05 M Sodium Carbonate buffer pH 10.2, then 0.02ml of sample tissues and 0.03ml of epinephrine, 0.005M HCl was used to initiate the reaction. Activity of the enzymes was determined by observing change in absorbance at 480nm for 5 minutes.

Haematological Studies: Haematological examination was carried out at the expiration of 28 days as described by Dahunsi and Oranusi (2013). Blood samples were collected from a total of 72 fish (three fish/ treatment) with heparinized plastic syringe, fitted with 21gauge hypodermic needle of plastic syringe from the liver behind the anal fins and stored in labeled ethylene diamine tetra-acetic acid (EDTA) bottles. The blood samples collected were analysed for packed cell volume (PCV), haemoglobin, (Hb) Red Blood Cells (RBCs), red blood indices such as mean Corpuscular Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Erythrocyte Sedimentation Rate (ESR) and Mean Corpuscular Haemoglobin (MCH). The values of haematological indices were calculated Brown (1980).

$$MCHC\ (g/dl) = \frac{Hb(g/dl) \times 100}{Pcv\ (\%)}$$

$$MCH\ (pg/cell) = \frac{Hb(G/dl) \times 10}{RBC\ count\ in\ million\ mm-6}$$

$$MCV\ (fl/cell) = \frac{PCV\ (\%) \times 10}{RBC\ count\ in\ million\ mm-6}$$

Statistical Analysis: Statistical analysis was conducted using Open Stat Statistical Software (Version 08. 12.14). Data from the 3 replicates of the experiment was subjected to one-way Analysis of Variances of (ANOVA). Treatment means was separated using Least Significant Differences (LSD) at 5% probability level. Probit analysis will be conducted and used to determine the LC50 of the different extracts

RESULTS

FT-IR Transmittance for *Microcystis aeruginosa* Extract : Figure 2 revealed the Fourier Transform Infrared spectroscopy of *Microcystis aeruginosa*. The peaks of functional groups at 2994, 3430, 1764 and 1534 wavelengths cm^1 of Amides, Alkenes, Alcohol comprising of Proteins, Pectin and Polysaccharides were identified.

Lethal Concentration (LC$_{50}$) of Algal Extracts: Result of Probit analysis revealed the values for the LC$_{50}$ at 5% level of confidence for the *Microcystis aeruginosa* extracts was 6.14µg/ml.

Biochemical Assay: The present findings revealed that all the enzymes under investigation (GSH, GST, CAT, SOD) where higher in the *Oreochromis niloticus* with exception of SOD which decrease significantly (P<0.05) in all the tissues examined when compared with control (Table 1). However, variation based on the exposure period between treatment and control did not differ significantly (P>0.05). GSH concentration was significantly higher (P<0.05) in the liver tissue of Reservoir fish with 69.3±2.43 nmol/min/mg prot when compared with kidney (46.4±1.43nmol/min/mg prot) and gills (59.0±2.83nmol/min/mg prot) (Table 1). Similarly, GST activity was significantly higher (P< 0.05) in the kidney tissues with 17.2±1.48 nmol/min/mg prot while the lowest was 11.1±1.10 nmol/min/mg prot in the gills tissue. CAT concentration decrease significantly (p<0.05) in its activity in the examined tissues in the following order: gills 81.40 ±1.61 > liver 46.30 ± 1.93> kidney 40.13 ± 1.34 Unit/mg prot. However, the above values obtained were significantly higher when compared with control which had the following pattern: gills > liver >kidney with 34.13±1.41> 32.30±1.40 > 31.04 ±1.08 respectively (Table 1). SOD. There was a significant decrease in antioxidant activity (P<0.05) in SOD activity in the liver and kidney tissue under investigation with 2.88 ±0.17 Unit/mg prot, 2.80.0±0.10 Unit/mg prot and 1.34±0.03Unit/mg prot when compared with control with 2.93.1±0.31, 2.40±0.21 and 4.19±1.01 Unit/mg prot respectively Table 2 illustrates the haematological changes examined in the control group and those exposed to *M. aeruginosa* extract for 28 days. Significant decrease (P<0.05) with increasing concentrations of the extract was observed in PCV, Hb and RBCs values in *O.niloticus* exposed to the extract in a dose dependent manner when compared to control. Based on exposure duration (28 days) and the highest concentration (150.0mg/kg), the PCV, Hb and RBCs values decreased from 5.30±0.90 to 2.01±0.23%, 20.66±1.21 to 13.03±0.31g/dl and 1.80±0.50 to 1.01±0.44 10^{12} cells respectively. The interactions between 28days exposure duration and different concentrations revealed no significant effect on the variables examined (P>0.05).However, there was consistent significant increase (P< 0.05) relative to the control in the values of ESR, WBC, MCH, MCV and MCHC in the fish species when exposed to M. *aeruginosa* extract during the study period (Table 3). ESR increased from 1.66±0.41 to 3.71±1.02mm/hr, WBC (19.41±1.10 to 32.73±0.51 ×10^9 cells), MCH (35.50±1.51to 58.01±0.79 pg), MCV (79.93±1.99to 91.22± 91.22±1.97 fl) and MCHC ranges from 44.03±1.30to 51.39±2.06 %.

DISCUSSION

Fourier-Transform Infrared Spectroscopy of the Cyanobacterial extracts revealed strong and sharp peaks with broad bonds that could be used to characterize algal toxins as described by George (2004). The transmittance ranges identified of 670-710, 750-800, 1200-1400, 1580- 1750, 2110-2165, 2190-2260, 2800-3350 and 3400-3900nw^{-cm} revealed the presence of functional groups O-H, C=O, C-N, N-H, C-H, CYN and-C=C-

Table 1. Oxidative Stress Enzymes Activities in Tissues of Oreochromis niloticus in Thomas Reservoir and Control after 28 days of Exposure

Tissue	Cyano. extr.	Oxidative stress enzymes			
	mg/kg	GSH (nmol/ mg protein)	GST (nmol/min/mg prot.)	CAT (Unit/mg prot.)	SOD (unit/ mg prot.)
Liver	control	62.3±1.12[a]	13.3±0.91[a]	32.3±1.40a	2.93±0.30[b]
	50.00	68.5±2.49[ab]	14.8±1.30[a]	31.3±1.41[a]	1.63±0.10[a]
	100.0	69.3±2.43[ac]	16.6±1.35[b]	43.3±2.01[bc]	1.71±0.14[a]
	150.0	76.0±2.93[a]	14.1±1.20[a]	46.3±1.02[a]	2.88±0.17[a]
Gills	control	46.4±1.94[ac]	10.3±1.10[a]	34.3±1.41[a]	4.19±0.10[a]
	50.00	51.3±1.13[a]	14.9±1.10[a]	18.03±1.22[a]	0.91±0.01[a]
	100.0	52.4±2.30[a]	11.1±1.10[ab]	74.0±5.31[a]	1.34±0.31[a]
	150.0	59.0±2.83[a]	13.5±0.02[a]	81.4±1.61[a]	1.03±0.01[a]
Kidney	control	38.7±2.31[a]	14.1±1.04[b]	31.0±2.81[a]	2.40±0.21[a]
	50.00	40.3±2.33[a]	11.3±1.10[a]	33.4±2.48[ab]	2.21±0.13[a]
	100.0	46.4±1.43[a]	14.3±1.13[a]	41.0±1.79[a]	2.20±0.10[b]
	150.0	46.3±1.08[b]	17.2±1.48[a]	40.1±1.34[a]	2.31±0.13[a]

Values are mean± standard deviation. Superscript with same letters in a column revealed no significant differences (P >0.0 5), F. crit value = 5.14.

Table 2. Effect of Different Concentration of M. aeruginosa on Haematological Parameters of O. niloticus after 28 days of Exposure

Cynobacterial extr. mg/kg	PCV (%)	Hb (g/dl)	RBCs (10 x 12 cells)	WBC (10⁹ cells)	MCH (pg)	MCV (fl)	MCHC (%)	ESR (mm/hr)
7 days								
0.00	21.53±0.31[a]	8.74±0.81[a]	3.13±0.94[b]	14.61±0.43[a]	33.71±1.80[a]	62.45±2.90[a,b]	25.53±5.30[a]	1.12±0.01[ac]
50.00	21.21±0.73[b]	6.43±0.41[b]	1.96±0.81[a]	16.13±1.08[a]	31.20±1.06[a,b]	84.09±1.90[a]	30.95±2.00[a]	1.46±0.03[a]
100.0	20.94±0.91[a]	6.49±0.29[b]	1.80±0.10[a]	16.39±0.81[a]	34.10±2.01[a,c]	80.05±2.39[a]	35.19±2.50[a]	1.67±0.12[a]
150.0	20.66±1.21[a,c]	5.30±0.90[a]	1.80±0.50[a]	19.41±1.10[b]	35.50±1.51[a]	79.93±1.99[a]	44.03±1.30[a]	1.66±0.41[a]
14 days								
0.00	20.93±0.31[c]	6.71±0.87[a]	2.98±0.79[a]	11.05±1.00[a]	29.80±1.36[a]	69.67±2.58[a]	32.36±3.02[a,c]	1.80±0.86[a]
50.00	20.90±0.31[b]	6.03±0.91[a]	1.41±0.90[b]	15.43±0.24[a]	38.63±1.04[a]	77.34±0.98[a]	39.53±1.74[b]	2.37±1.37[a]
100.0	20.51±0.31[a]	5.71±0.29[a]	1.36±0.67[a]	18.13±0.16[a]	42.24±0.87[a]	85.43±1.88[a]	45.13±13.24[a]	2.37±1.37[a]
150.0	20.03±0.31[a]	5.81±0.23[a]	1.30±0.91[a]	19.01±1.09[a]	48.19±0.09[a]	85.18±1.96[a]	50.13±13.24[a]	2.37±1.37[a]
21 days								
0.00	20.90±0.31[a]	6.73±0.23[a]	2.96±0.81[a]	13.93±1.24[a]	32.78±1.94[ab]	67.76±1.91[a]	29.20±1.62[a]	1.41±0.10[a]
50.00	19.73±0.31[a]	5.49±0.23[a]	1.53±0.23[b]	16.11±0.54[a]	47.10±0.49[a]	74.28±1.94[a]	37.90±1.56[a]	1.37±1.37[a]
100.0	18.61±0.31[a]	5.31±0.23[a]	1.33±0.81[b]	17.16±1.70[a]	56.87±0.29[a]	81.13±1.27[a]	36.87±1.90[a,c]	1.29±0.12[a]
150.0	18.13±0.31[a]	5.30±0.23[a]	1.27±0.74[a]	18.10±13.24[a]	60.65±0.94[a]	94.91±1.90[a]	41.35±1.78[a,b]	1.51±0.12[ab]
28 days								
0.00	21.21±0.31[a]	6.61±0.23[a]	2.60±0.91[a]	24.07±1.70[a,c]	30.70±1.37[b]	58.81±6.31[a]	30.16±1.59[a]	1.98±0.47[c]
50.00	16.04±0.31[a]	3.34±0.23[a]	2.36±0.19[a]	28.12±0.27[a]	36.13±0.84[a]	62.41±6.21[a]	37.91±1.68[ab]	2.50±0.92[a]
100.0	15.41±0.31[a]	3.33±0.23[a]	2.01±0.92[b]	32.56±1.14[a]	50.19±0.08[a]	71.89±6.95[a]	43.11±0.66[ac]	2.67±0.16[a]
150.0	13.03±0.31[a]	2.01±0.23[a]	1.01±0.44[a]	32.73±0.51[a]	58.01±0.79[a]	91.22±1.97[a]	51.39±2.06[a]	3.71±1.02[ac]

Means with the same superscript in a column are not significantly different (P>0.05)
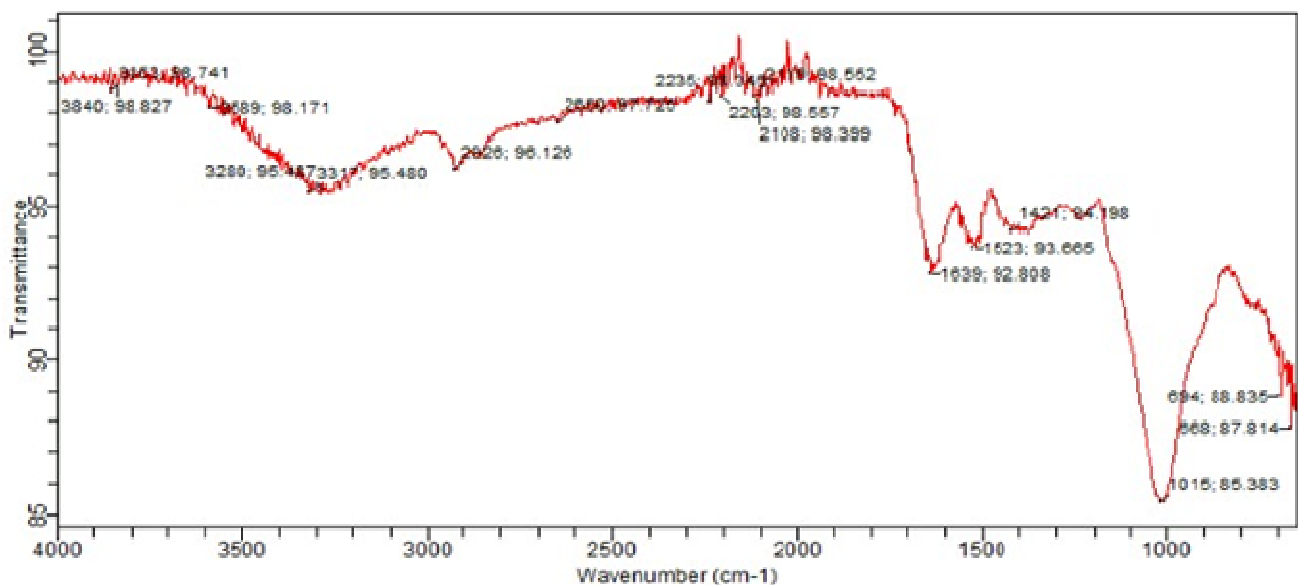

Fig 1. FT-IR Transmittance of Microcystis aeruginosa Extract Obtained from Thomas Reservoir, Kano, Nigeria

belonging to alcohols and phenols, carboxyl, amines, esters and cyanides in the extracts. The peaks of functional groups obtained when compares with the standard evidently proved the presence of Microcystins as reported by Yang (2007). The LC_{50} values for the lethal concentration examined indicated the level of toxicity of the *M. aeruginosa* extracts indicates its toxicity on the fish species under investigation. Camilla *et al.* (2012) reported that the higher the LC_{50} value the lower the toxicity of algal extract. The value recorded in the present study perhaps may be due to the type of response to nutrient levels in the aquatic habitat as reported by Indabawa (2009).

Biochemical Assay: Assessment of oxidative stress biomarkers carried out in liver, kidney and gills of *O.niloticus* was compared with control. The present finding revealed significant higher concentration of GSH in all the tissues examined when compared with control which corroborates with the findings of Prieto *et al.* (2006). GSH has been considered as a cofactor of many enzymes catalysing the detoxification of many toxic compounds. It is an important antioxidant agentwhich covers and secure cell membranes from lipid peroxidation by scavenging oxygen radicals yielding glutathione disulphide (Prieto *et al.*, 2006). The increased in GSH activity recorded in the fish tissues when compared with control might be due to cyanobacterial toxicity as reported by Nahed (2011) and Ogugu *et al.* (2012). It may perhaps be due to the increase in the protective role of the enzymes against lipid peroxidation as reported by Oluwatosin *et al.*(2016). Similarly, the range of GSH values examine are in consistent with other fresh water fishes from the same ecological zone by Abdulkareem and Owolabi (2014) and Akinwande *et al.* (2016). Antioxidant enzymes like GST, SOD and CAT help to neutralize toxic effect of ROS in fish just like in other organisms. GST in conjugation with GSH act as defense against ROS and protect cells against oxidative injuries (Xie *et al.,* 2005). In the present findings, the activity of GST increased in the liver, gills and kidney when compared with control. This might be due to the presence of toxicants in the water body which facilitate GST activity as a result of anthropogenic input along the water course of the reservoir among other factors. This is consistent with the findings of Magalhães *et al.* (2001) and Abdulkareem and Owolabi (2014). Toxic stress has been reported to alter activity of antioxidant enzymes in the vital tissues of *Oreochromis mossambicus* (Jabeen and Chaudhry, 2010).

Therefore, regulating levels of these antioxidant enzymes is essential to prevent oxyradical-mediated lipid peroxidation Arojojoye and Adeosun (2016). Fish among other aquatic biotahas been prone to environmental pollutant and are widely used as bioindicators for aquatic ecosystem assessment (Abdulkareem and Owolabi (2014). Among the widely used bioindicators are Catalase (CAT) and superoxide dismutase (SOD). In the present study CAT activity decrease significantly in the examined tissues (liver, kidney and gills) when compared with control which suggests oxidative stress response due to Reactive Oxygen Species production (ROS) by cyanotoxins as reported by Yunes *et al.* (2005). This observation corroborates with the finding of Farombi *et al.* (2007) in kidney and gills of *Clarias gariepinus*. Catalase is an antioxidant enzyme, found in almost all living organisms exposed to oxygen where it mitigates the toxic effects of hydrogen peroxide by converting it to water and molecular oxygen (Lehman*et al.*, 2005) The observed decrease in catalase activity in the treated fish tissue during the exposure

and the control is an indicator that the fish survive under oxidative stress probably due to induced stress as a results of alterations in the enzyme system among other organic pollutants brought by natural and or anthropogenic activities. Similar observation was reported by John *et al.*, (2014). Pollution induced decrease in the activity of catalase had been reported earlier by Prieto *et al.* (2006) in tilapia fish (*Oreochromis* sp. exposed to Microcsytin LR and RR. Decrease in CAT activity in the present study might be due to frequent superoxide anion radical production as observed by Odjegba and Fasidi (2007). In the present study, higher SOD activity was obtained in the control samples of gills liver and when compared with experimental samples which had lower activity. Decrease in SOD activity significantly in the examined sample tissues indicates oxidative stress response. This could be due to due to SOD's effort to combats oxidative stress or during scavenging reactive superoxide radicals. Similar observation was reported by Oluwatosin *et al.* (2016). Decrease in SOD activity recorded in the fish tissue under investigation could also be attributed to its inability to guard the cells against one of the most dangerous of free radicals (superoxide radicals) or due to denaturation of SOD proteins structure by the superoxide radicals. This is in tandem with findings of Akhiromen and Ogbonne (2018).

Haematological Parameters: The result obtained in the exposure of *Oreochromis niloticus* to different concentrations of cyanobacterial extracts revealed that there was significant decrease ($P<0.05$) with increasing concentrations of the extract in PCV, Hb and RBCs values. The decrease in RBCs, Hb and PCV could be due to with increasing concentration and exposure period of *O. niloticus,* which results to haemolysis and impaired osmoregulation within the delicate tissues of the fish as reported by Papadimitriou *et al.* (2010). It could also be due to the disruption of iron synthesis and inhibitory effect of the toxins on the enzyme metabolism responsible for haemoglobin synthesis as a results of exposure to the cyanobacterial extract (Ferrão-Filho *et al.*, 2007). Decrease in PCV, Hb and RBC count illustrates kidney functions impairment (Gabriel and Marwe, 2013). Toxic cyanobacteria have been known to exert adverse effects on fish, including damage to delicate organs such as liver, gills and kidneys (Ferrão-Filho *et al.,* 2009). Moreover, the white blood cells reflect protection against infectious agent caused by microbial and toxicants (Prieto *et al.*, 2006). In the present study consistent significant increase relative to the control in the values of ESR, WBC, MCH, MCV and MCHC in the fish exposed to the extract might be be related to the immune response to the toxicants. This is in tandem with work of Vasconcelos *et al.* (2001) and Deore and Bansal (2013). The pattern of response of leucocyte (WBC) might be associated with the presence of toxin induced tissue damage and severe disruption of the non-specific immune system leading to increased WBC (Ferrão-Filho *et al.*, 2007). Besides, increase in WBC have been reported in fish species exposed to cyanotoxins (Prieto *et al.*, 2006). Keshavanath *et al.* (1994) observed a time and dose dependent increase in MCV, and MCH, with a decrease in Hb, hematocrits, RBC and WBC count when the toxicity of *Microcystis aeruginosa* was assessed *Oreochromis niloticus.* Camella *et al.* (2012) reported alterations in haematological parameters of fish caused by toxins and their responses differed depending on the fish species, concentration of the toxins and exposure period. These factors might lead to alterations in the haematological parameters of the fish.Krienitz *et al.* (2003) reported

intoxication of icthyfauna and other animals by cyanobacterial toxins in which the blooms of cyanobacteria were enriched by phosphates and nitrates in the water. Many of thesenutrients are generated via human wastes like sewage disposal, detergents applications, industrial pollution, run-off from fertilizers applications among other means (Baker, 2002) and Prieto *et al.* (2006).

Conclusion

From the present finding it revealed that there is relatively moderate damage in the liver, kidney and gills of the fish under investigation due to exposure to the cyanobacterial extracts. Toxicity of a cyanobacterial on *O. niloticus*increased with increasing concentration of the extract. In the aquatic domain this has direct impact on the ecosystem. This perhaps has direct relationship with observed level biomarkers of oxidative stress in the fish tissues. Besides, it can be deduced from the present finding that the fish tissues with cyanobacterial bioaccumulation, has altered oxidative enzymes and haematological parameters significantly when compared with control. It is therefore recommended that uncontrolled discharge of agrochemicals around the reservoir through irrigation and other human activities should be controlled in order to curtail degradation of the aquatic biota over a period of time.

Conflict of interest: none

REFERENCES

Abdulkareemm, S.I. and Owolabi D.O. 2014.Toxicity of Sub-lethal Concentrations of Monocrotophos (MCP) on the Haematological, Biochemical and Growth Responses of hybrid Catfish, *Heteroclarias* and Contaminated *Heteroclarias* fed rats. *International Journal of Current Microbiology and Applied Sciences,* 3(6): 917-931.

Akhiromen, D.I and Ogbonne, F.C. 2018. Biochemical Responses and Heavy Metal Accumulation in *Macrobrachium vollenhovenii* in Epe axis of Lekki Lagoon. Bells *University Journal of Applied Sciences and Environment,* 1(1): 63-72.

Akin-Oriola, G. A., Anetekhal, M. A. and Oriola, A. 2006. Algal Blooms in Nigerian Waters: An Overview. *African Journal*, 28:219-224.

Akinwande, A.A., Abdulkadiri, J.O. and Adesina, B.T. 2016. Oxidative Stress and Antioxidant Response in the Giant African Catfish (*Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809) under Chronic Paraquat Exposure. *Nigerian Journal of Fisheries and Aquaculture*, 4(2): 30 – 37.

Arojojoye, O.A and Adeosun, A.M. 2016. Effect of Environmental Pollution on oxidative stress biomarkers in

African cat fish (*Clarias gariepinus*) from Asejire River in Oyo State, Nigeria. *Journal of Environmental and Occupational Science*, (5)4: 71-76.

Baker, L.C. 2002. Cyanobacterial harmful algal blooms (CyanoHABs): Developing a public health response. *Lake and Reservoir Management,* 18: 20-31.

Burtis, C.A. and Ashwood, E.R. 2001.Tietz fundamentals of clinical chemistry. 5th ed. W.B. Saunders. Philadelphia. pp. 655-657.

Camilla, V., Denise, B. S., Rodolfo, P., Luis, F., Jacqueline, D. T., Stenlo, N.A. and Joao, M.S. 2012. Brine Shrimp Lethality Test as a Biological Model for Preliminary Selection of Pediculicidal Components from Natural Source. *Bioscience Journal, Uberlândia*, 29, p. 255-263.

Dahunsi, S.O and Oranusi, U.S. 2013. Haematological Response of *Clarias gariepinus*. *Ann Rev & Res in Bio.*, 3: 624-635.

Dai, W., Fu, L., Du, H., Jin, C. and Xu, Z. 2009**.** Changes in growth performance, metabolic enzyme activities, and content of Fe, Cu, and Zn in liver and kidney of tilapia (*Oreochromis niloticus*) exposed to dietary Pb. *Biological Trace Element Research,* 128, 176-183

Deore, S.R. and Bansal, G.K. 2013. A study on health hazards caused by microcystins to animal life. *International Journal of Chemical Sciences and Applications*, 4(12): 24-28.

Dutta, H.M. and Areids, D.A., 2003. Effects of endosulfan on brain acetyl- cholinestarate activity in juvenile blue gill sunfish. *Environmental Research,* 91:157-162.

Farombi, E.O, Adelowo, O.A and Ajimoko, Y.R. 2007. Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in African Cat Fish (*Clarias gariepinus)* from Nigeria Ogun River. *International Journal of Environmental Research and Public Health, 4(2)*:158-165.

Ferrão-Filho, A.S., Cunha, R., Magalhães, V.F., Soares, M.C.S. and Baptista, D.F. 2007. Evaluation of sub-lethal toxicity of cyanobacteria on the swimming activity of aquatic organisms by imageanalysis. *J. Braz. Soc. Ecotoxicol.,2*: 93–100.

Ferrão-Filho, A.S., Soares, M.C.S., Magalhães, V.F., Azevedo, S.M.F.O. 2009. Biomonitoring of cyanotoxins in two tropical reservoirs by cladoceran toxicity bioassays. *Ecotoxicol. Environ. Saf.*, 72:479–489.

Fiore, M.F., Genuário, D.B., da Silva, C.S.P., Shishido, T.K., Moraes, L.A.B., Neto, R.C. and Silva-Stenico, M.E. 2009. Microcystin production by a freshwater spring cyanobacterium of the genus Fischerella. *Toxicon.*, 53:754 – 761.

Gabriel, K. and Marwe, D.V. 2013. Classification of Toxic Cyanobacterial Blooms by Fourier-Transform Infrared Technology (FTIR). *Advances in Microbiology,* 3: 1-8.

George, S. 2004. Infrared and Raman Characteristic Group Frequencies: Tables and Charts. John Wiley&Sons. Pp. 18.

Graham, J.L.; Loftin, K.A.; Meyer, M.T.; Ziegler, A.C.(2006). Co-occurrence of toxins and taste-and-odor compounds in cyanobacterial blooms from the midwestern United States. *Environ. Sci. Technol.*, 44:7361–7368.

Habig, W.H; Pabst, M.J. and Jacoby, W.B. 1974. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249(22): 7130-7139.

Haque, R., Bin-Hafeez, B., Parvez, S., Pandey, S,. Sayeed, I., Ali, M. Raisuddin, S. 2003. Aqueous extract of walnut (*Juglans regia* L.) protects mice against cyclophosphamide induced biochemical toxicity. *Hum Exp Toxicol.,* 22(9)473-480.

Ibrahim, J.A. and Raneen, N.M. 2018. Molecular based test for detection of cyanotoxins in the domestic drinking water tanks in Baghdad. *Journal of Pharmacy and Biological Sciences,* (13)1: *76-81*.

Ilavarasi, A., Mubarak, A. D., Praveen, K., Baldev, R. E. and Thajuddin, N. 2011. "Optimization of Various Growth Media to Freshwater Microalgae for Biomass Production" 2011. *Biotechnology,* 10: (6) 360-365.

Indabawa, I.I. 2009. Detection of Variants of *Microcystin* Produced by *Microcystis aeroginosa* in Some Burrow Pits of Kano, Nigeria. *Biological and Environmental Sciences Journal for the Tropics,* 5 (21):141-149.

Jabeen, F and Chaudhry, A.S. 2010. Environmental impacts of anthropogenic activities on the mineral uptake in *Oreochromis mossambicus* from Indus River in Pakistan. *Environ. Monit. Assess.,* 166: 641-651. https://doi.org/10. 1007/s10661-009-1029-z

Jegathambigai, R.N., Rusli, I. and Sreenivasan, S. 2014. Acute Oral Toxicity and Brine Shrimp Lethality of Methanol Extract of *Mentha Spicata* L (Lamiaceae). *Tropical Journal of Pharmaceutical Research,* 13 (1): 101-107.

John, N.K., Babu, M., Racheal, A. and James, G.N. 2014. Haematological and Histopathological Effects of Cyanobacteria (Blue-Green Algae) from lake Victoria Shores of Uganda in Swiss Mice. *International Journal of Applied Science and Technology,* 2(4): 4-16.

Keshavanath, P., Beveridge, M.C.M., Baird, D.J. and Lawton, L.A. 1994. The functional grazing response of a phytoplanktivorous fish *Oreochromis niloticus* to mixtures of toxic and non-toxic strains of the cyanobacterium *Micro cystis aeruginosa. J. Fish Biol.,* 45:123–129.

Krienitz, L., Ballot, A., Kotut, K., Wiegand, C., Putz, S., Metcalf, J.S, Codd, G.A. and Pflugmacher. S. 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser flamingos at Lake Bogoria, Kenya. *FEMS Microbiol. Ecol.,* 43: 141-148.

Kutama, A.S, Umar, S., Fagwalawa, L.D., Ahmed, M.K. and Bilkisu, A.I. 2013. Detection of Fish Saprolegniasis in Thomas Dam and Challawa Commercial Fish Pond: *Global Advanced Research Journal of Agricultural Science,* 2(12): 315-321.

Leena, M. 2012. Haemato-Biochemical Alterations Induced By Chronic Exposure to *Fenthion* In *Cyprinus Carpio. Trendsin Fisheries Research,* 1(3):2319–4758.

Lehman, P.W., Boyer, G., Hall, C., Waller, S. and Gehrts, K. 2005. Distribution and Toxicity of a New Colonial *Microcystis Aeruginosa* Bloom in the San Francisco Bay Estuary, California. *Hydrobiologia,* 541:87-99.

Magalhães, V.F., Soares, R.M., Azevedo, and S.M.F.O. 2001. Microcystin contamination in fish from the Jacarepaguá Lagoon (Rio de Janeiro, Brazil): Ecological implication and humam health risk.*Toxicon,* 39: 1077–1085.

Magwere, T; Naik, Y.S; Hasler, J.A. (1997). Effect of chloroquine treatment on antioxidant enzymes in rat liver and kidney. *Free Radical Biology and Medicine,* 22(1): 321-327.

Molica, R.J.R., Oliveira, E.J.A.; Carvalho, P.V.V.C.; Costa, A.N.S.F.; Cunha, M.C.C., Melo, J.L. and Azevedo, S.M.F.O. 2005. Occurrence of saxitoxins and an anatoxin-a(s)-like anticholinesterase in a Brazilian drinking water supply. *Harmful Algae,* 4, 743–753.

Monad, J. 2012. Growth of Bacterial cultures,.211 pp.Herman and Cie, Paris. P

Montagnolli, W., Zamboni, A., Luvizotto-Santos, R.J. and Yunes, J.S. 2004. Acute effects of *Microcystis aeruginosa* from the Patos Lagoon estuary, Southern Brazil, on the microcrustacean *Kalliapseudesschubartii* (Crustacea: Tanaidacea). *Arch. Environ. Contam. Toxicol.,* 46, 463–469.

Mukund, S., Jegan, G., Palanisamy, M., Muthukumaran, M. and Sivasubramanian, V. 2014. GC MS and FT-IR Analysis of some Bioactive Constituents from *Oscillatoria terebriformisIndian Journal of Pharmaceutical Science & Research,* 4(2):102-107

Mustapha, M.K. 2008. Assessment of the water quality of Oyun Reservoir, Offa, Nigeria, using selected physico-chemical parameters. *Turkish Journal of Fisheries and Aquatic Sciences,* 8: 309-319.

Nafiu, S. A; Rabiu, H. D; Abdullahi, M. T; Halliru, S. N; Kabiru, M.Y; Ishaq, S. A. (2017). Potential Health Impacts of Phytoplankton Composition In Watari Dam, Kano – Nigeria. *International Journal of Advanced Academic Research, Sciences, Technology & Engineering,* 3(5): 51-62.

Nahed, S.G. 2011. Oxidative stress and antioxidant enzymes in *Oreochromis niloticus* as biomarkers of exposure to crude oil pollution. *International Journal of Environmental Science and Engineering, (Ijese)* 1(1): 49-58.

Odjegba, V. J and Fasidi, L.O. 2007. Changes in Antioxidant Enzyme Activities in *Eichhornia crassipes* (Pontederiaceae) and *Pistia strati-otes* (Araceae) under Heavy Metal Stress. *Biology Tropical,* 2007; 55: 3-10.

OECD, 2008. Guidelines for the testing of chemicals. Test guideline 425; Acute Oral Toxicity – Up-and-Down-Procedure.

Ogugu, S.E., Kehinde, A.J., James, B.I. and Paul, D.K. 2012. Assessment of cytotoxic effects of methanol extract of *Calliandra portoricensis* using Brine Shrimp (*Artemia salina*) Lethality Bioassay. *Glob J Bio-Sci Bitech.,* 2, 257- 260.

Olaosebikan, BD. and Raji, A. 1998. *Field Guide to Nigerian Freshwaters Fishes,*Decency Printers and stationary Ltd, Ilorin, Nigeria. 105pp

Oluwatosin, A.A., Olajumoke, O.N., John, A.A. and Jacob, K.A. 2016. Induction of Oxidative Stress in *Clarias gariepinus* from Eleyele River in Nigeria. *Advances in Environmental Research,* 5(3):179-187. DOI: http://dx. doi.org/ 10.12989/aer.2016.5.3.179

Papadimitriou, T., Kagalou, I., Bacopoulos, V. and Leonardos, I.D. 2010. Accumulation of microcystins in water and fish tissues: An estimation of risks associated with microcystins in most of the Greek lakes. *Environ. Toxicol.,* 25: 418–427.

Prieto, A.I., Jos, A., Pichardo, S., Moreno, I. and Cameán, A.M. 2006. Differential oxidative stress responses to microcystins LR and RR in intraperitoneally exposed tilapia fish (*Oreochromis* sp.). *Aquat. Toxicol.,* 77: 314–321.

Sood, R. 2006. Textbook of Medical laboratory Technology. 1st ed Jaypee Brothers Medical Publishers New Delhi, India; 609–672.

Suthers, I.M. and D. Rissik, 2009. Plankton: A Guide to their Ecology and Monitoring for Water Quality. CSIRO Publishing, Collingwood, Vic., pp: 272.

Tarczynska, M., Nalecz-Jawecki, G., Romanowska-Duda, Z., Sawicki, J., Beattie, K., Codd, G.A. and Zalewski, M. 2001.Tests for the toxicity assessment of cyanobacterial bloom samples. *Environ.Toxicol.,*16: 383–390.

Vasconcelos, V., Oliveira, S. and Teles, F.O. 2001. Impact of a toxic and a non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii. Toxicon.,* 39:1461–1470.

Verlencar, X.N. and Desai, S. 2004. Phytoplankton Identification Manual. National Institute of Oceanography New Delhi 1st ed Pp 4.

Xie, L., Xie, P., Guo, L., Li, L., Miyabara, Y. and Park, H. 2005. Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu China. *Environ. Toxicol.,* 20:293–300.

Yang, X. 2007. Occurrence of the Cyanobacterial Neurotoxin, Anatoxin-a, in New York State waters. *ProQuest.,pp* 3-4.

Yunes, J.S., Cunha, N.T., Barros, L.P., Proença, L.A.O. and Monserrat, J.M. 2003. Cyanobacterialneurotoxins from southern Brazilian freshwaters. *Comments Toxicol.,* 9:103–115.

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