



Heavy metals, parasitologic and oxidative stress biomarker investigations in *Heterotis niloticus* from Lekki Lagoon, Lagos, Nigeria

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ABSTRACT

Heavy metal toxicity in aquatic life as a result of human activities poses a grave health threat to water quality, aquatic and human life. Parasites may serve as indicators of heavy metal pollution. This research investigated the health status of the fish *Heterotis niloticus* viz-a-viz quality of the water and sediments in Lekki lagoon, parasitic infection, presence of heavy metals and oxidative stress response in the liver and intestine of the fish. Parasites recovered were also analyzed for the extent of bioaccumulation of heavy metals. The metals in water, sediments, parasites, and fish were analyzed using Atomic Absorption Spectrometry. Heavy metal concentrations in the surface water were generally below regulatory limits of World Health Organization. Sediment had high levels of aluminium (124.78 mg/kg) and iron (327.41 mg/kg); other heavy metals were below regulatory limits. *Tenuisentis niloticus*, an acanthocephalan, was the only parasite recovered. Seventy (70) out of 100 fish sampled were infected with the parasite. *T. niloticus* bioaccumulated Cd, Ni, and Pb between 65 to 100 times more than the liver and 12 to 200 times more than the intestine. Other metals bioaccumulated from the host tissues by the parasite had the magnitude between 1 to 12 times as the liver and 1 to 30 times as the intestine. There were significant differences in the activities of antioxidant enzymes between the parasitized and non-parasitized fishes. Fish tissues also showed histological alterations, ranging from mild infiltration of inflammatory cells to moderate inflammation and haemorrhagic lesions. Human activities that introduce stressors into the lagoon should be controlled.

1. Introduction

The need to conserve the aquatic environment and prevent it from contamination cannot be overemphasized. Effluent discharge from various industries, run-off from agricultural lands, and sewage discharge from residential areas have all led to increasing pollution of the aquatic environment, lowering the quality of water while simultaneously negatively impacting the biota in the water body. Heavy metals and pathogens find their ways into water bodies via industrial and sewage discharge [1].

The pursuit of quality livelihoods and the strive for energy have meant that man's activities have led to continuous release of pollutants, including heavy metals into nearly all environmental media [2]. The

threat from heavy metal toxicity is serious because of the bioaccumulation and biomagnification of these toxins in the food chain [3]. Recently, there has been much effort in monitoring the levels of heavy metals in fish and other food products to assess the potential risk to humans [4]. Some of these metals, such as copper, iron, manganese, and zinc are described as essential because they play vital roles in biological systems [5] but can become toxic at high concentrations [6]. Others such as cadmium, chromium, lead and mercury exert adverse effects even at very low concentrations [7].

Fish can easily accumulate metals into their bodies from any of sediment, water, or food, by virtue of their position in the aquatic food chain [8,9]. When metals accumulate in fish, they trigger reduction-oxidation reactions capable of generating reactive oxygen

Abbreviation: APHA, American public health association; CAT, catalase; COD, chemical oxygen demand; FAO, food and agricultural organization; GSH, reduced glutathione; H&E, haematoxyline and eosin; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; WHO, world health organization.

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species (ROS) which can cause oxidative stress, morphological and biochemical changes in their tissues [10,11]. Humans, being higher in the food chain, may also be affected by the toxicity of these metals [12]. Some of the documented health risks posed by heavy metals in humans include liver and kidney damage, cardiovascular diseases, and, in extreme cases, death [13,14].

In the last twenty years, there has been intensive research into the field of Environmental Parasitology, and it has been reported that endoparasites can be useful accumulation bioindicators [15–18]. Environmental Parasitology is the field of science that studies the interaction of parasites with environmental pollutants. In this field, helminthes, particularly intestinal helminthes are used to monitor pollution arising from heavy metals in the environment [19,20].

Parasites infecting fish have been shown to be highly sensitive either by way of their physiological response to contaminants in water or by their ability to accumulate pollutants [21,22]. The acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus lucii* were reported to accumulate cadmium and lead from their fish hosts [23,24]. Two cestode species, *Monobothrium wagneri*, and *Bothriocephalus scorpii* were also reported to accumulate lead and cadmium in their freshwater fish host [25]. *Anguillicola crassus*, a nematode was observed to harbour lead concentrations while parasitizing the fish *Anguilla anguilla* [26]. In all these studies, heavy metal concentrations were reported to be several thousand times higher in the parasites than in the fish hosts. Al-Hasawi [27] reported accumulation of lead and cadmium in *Procamallanus elatensis* (Nematoda) and *Sclerocollum rubrimaris* (Acanthocephala) from the fish *Siganus rivulatus* (Siganidae) collected in the Red Sea, Egypt, in concentrations higher than those of their fish hosts. Similarly, Elsayed et al. [28] also reported significantly higher concentrations of heavy metals in trematodes infecting fish species in the Arabian Gulf in comparison with their fish hosts.

Antioxidant enzymes are a vital component of the body's natural antioxidant defense system [29]. Susceptibility of aquatic organisms to oxidative effects from pollution or pathogens in the environment is high, most importantly when they have the ability to generate or enhance the production of reactive oxygen species (ROS) [30]. Superoxide dismutase

(SOD), catalase (CAT) and glutathione S-transferase (GST) are three prominent enzymes in these antioxidant defense system.

There is a need to constantly study the pollution status of the Lagos lagoon. This aquatic ecosystem offers the opportunity to understand the effects of human activities in the form of oil spillage, industrial and agricultural pollution, heavy metals etc on the natural environment. The Lagoon drains into the Gulf of Guinea through the Lagos harbour. The area is a rural settlement, most of the human population being concentrated along the lagoon bank. Human activities such as agriculture, sand mining, fishing and public transportation occur.

This study was conceived with the aim of investigating parasites in *Heterotis niloticus*, while using the parasites as sentinel organisms to quantify the biological availability of heavy metals in fish, water and sediment. A comparison of the activities of anti-oxidant enzymes between parasitized and non-parasitized fish was further carried out.

2. Materials and method

2.1. Study area

The Epe axis of Lagos Lagoon was the study site for this investigation. It lies between longitude 5°30' - 5° 40'E and latitude 3°50' - 4° 10'N (Fig. 1). It has a depth of 6 m maximum and covers a surface area of about 225 km. It lies between the Lekki and Lagos Lagoons. A mangrove swamp surrounds the vegetation of the lagoon [31]. There is an overwhelming spread of the growth hydrophytes, specifically water hyacinth, on the lagoon, a scenario synonymous with pollution of the lagoon [32].

2.2. Fish collection and sampling

One hundred (100) samples of *Heterotis niloticus*, 90 males and 10 females, were obtained between April and September 2018 from the Lagoon with the assistance of fishermen using appropriate fishing gears. The fish were taken on ice to the Laboratory and examined immediately for parasites. Morphometric measurements of the fish were taken.

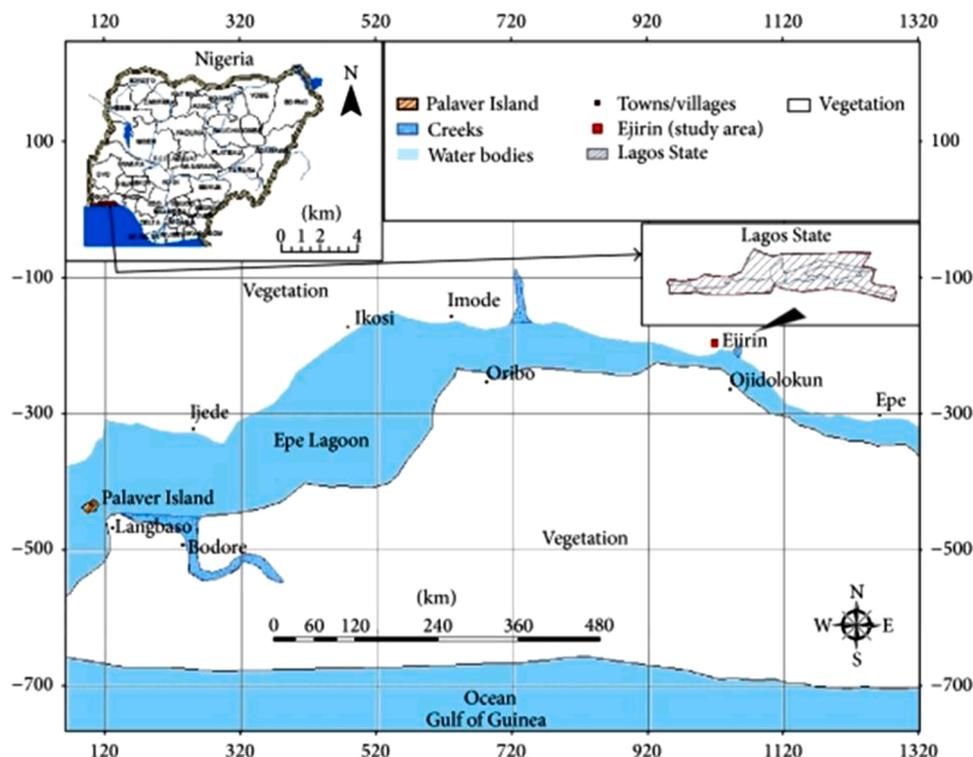


Fig. 1. Map of the Epe Axis of Lekki Lagoon.

2.3. Analysis of water parameters

Water parameters such as pH, conductivity, turbidity, total dissolved solids, total suspended solids, chemical oxygen demand, dissolved oxygen and other parameters were measured. Water samples were taken at three different points in the sampling location in deionized polyethylene bottles and care was taken to avoid any contamination. The pH, conductivity, and turbidity were measured onsite using a pH meter, conductivity meter, and turbidity meter respectively. Other parameters like total dissolved solids, total suspended solids, chemical oxygen demand, dissolved oxygen, salinity, nitrates etc were measured according to APHA [33].

2.4. Examination and identification of parasite

Examination of fish parasites was done following the techniques of Akinsanya et al. [34]. Each fish was dissected and the fish gut was excised and further separated into its constituent parts and placed in petri dishes containing 0.09 % physiological saline. The different parts were further opened up by cutting through longitudinally for easy viewing with a dissecting microscope and to ensure easy recovery of parasites. Worms that emerged were noticed by their wriggling movements in physiological saline. Those that remain attached to the walls of the gut were gently pulled out using forceps. All recovered parasites were counted, recorded and thereafter fixed in 5% formalin. Erlich's Haematoxylin solution was used for the staining of the parasites overnight. The parasites were then passed through graduated alcohol for 45 min each to dehydrate. The parasites were identified at the pathology laboratory of the Department of Veterinary Pathology, University of Ibadan, Nigeria. Guts that had parasitic infection were fixed in Bouin's fluid for 7 h and kept in 10 % phosphate buffer formalin.

2.5. Histopathological examination

Infected guts were later decanted and kept in 10 % buffered formalin for tissue preservation. The preserved tissues were randomly selected based on infection status. The tissues were routinely dehydrated in an ascending series of alcohol at 30 min interval; they were then embedded in molten paraffin wax [35]. 4–5 microns were sectioned from the tissues, processed, and stained with haematoxylin and eosin (H&E) stains. Tap water and 1% alcohol were used to wash off both the stained and over-stained tissues respectively. The tissues were thereafter examined under the microscope.

2.6. Heavy metal analysis

Fish intestine and liver of twenty randomly selected parasitized fish and the parasites were analyzed for heavy metals. Three water samples that were collected from different locations in sampling site. The samples were filtered, thoroughly mixed, and acidified with pure HNO₃. The concentration of metals in the water samples are expressed as mg/l. Samples that could not be analysed within 24 h were insulated in boxes with ice and kept in the dark, and the temperature was maintained at a maximum of 10 °C [33]. Three sediment samples were also taken from the sampling site. They were dried in an oven at 100 °C for about 6 h and grounded. One gram of dried samples was sieved through a 200 µm sieve to normalize for particle size, and digested using a mixture of concentrated acids (HNO₃/HClO₄/HF = 3/2/1) according to Oregoni and Aston [36]. To the residue, 3% HCl was added, made up to 50 ml and analysed for metals. Concentrations of metals in sediment are expressed as mg/kg dry weight. For the biological samples, about 200 mg of fish tissues and 50 mg of parasites were homogenized, digested with HNO₃, and made to cool as described by Jensen et al. [37]. Standards and blanks were prepared accordingly. Determination of heavy metal concentrations in the digested samples was done by comparing absorbance with known standards using an Acetylene Flame Atomic Absorption

Spectrophotometer, AES 2000 series. Accuracy of analytical procedure was ensured by using certified reference material (DORM-3). Metal concentration of fish intestine, liver and parasites are expressed as mg/kg wet weight.

2.7. Redox status biomarkers

To check for the activities of antioxidant enzymes, tissue homogenates of ten (10) samples each from the parasitized and non-parasitized fishes were used. Lipid peroxidation was measured by TBARS assay performed by malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA) formation according to the methods of Niehaus and Samuelsson [38] and Jiang et al. [39]. Tissue homogenate of 0.1 ml (Tris-HCl buffer, pH 7.5) /serum was treated with 2 ml of (1:1:1) TBA-TCA-HCl reagent and placed in water bath for 15 min. It was then cooled and centrifuged for 10 min. at 3000 rpm at 20 °C. Absorbance was measured against blank of clear supernatant at 535 nm.

Activity of reduced glutathione (GSH) was determined according to Elman [40]. A mixture of the homogenate and 10 % TCA was centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 1.0 ml of 0.1 % sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

Catalase (CAT) was assayed calorimetrically at 620 nm and expressed as micromoles of hydrogen peroxide (H₂O₂) consumed/min/mg protein as described by Quinlan et al. [41]. The total volume of reaction mixture was 1.5 ml, made up of 1.0 ml of 0.01 M pH 7.0 phosphate buffer, 0.1 ml of Plasma and 0.4 ml of 2 M H₂O₂. The reaction was discontinued when 2.0 ml of dichromate-acetic acid reagent was added.

Superoxide Dismutase (SOD) activity in tissue homogenates was determined by modifying the method of Marklund and Marklund [42]. The method is based on the inhibition of autoxidation of pyrogallol by SOD. The mixture contained 970 µL of buffer (100 mM Tris-HCl, 1 mM EDTA, pH 8.2), 10 µL of homogenates and 20 µL pyrogallol 13 mM. Cuvettes were used for the assay at 25 °C and a spectrophotometer (Spectronic 20D) at 480 nm was used for recording changes in absorption. One unit of SOD activity was taken as the amount of enzyme inhibiting the auto-oxidation of 50 % the total pyrogallol in the reaction and expressed as units per milligram of protein.

2.8. Statistical analysis

Analysis of Variance (ANOVA) was used to analyze the data and Duncan Multiple Range Test was performed where significant difference was observed in the mean heavy metal concentrations of parasite, liver and intestine while T-test was used to compare mean concentrations of heavy metals between water and sediment, and between activities of enzymes between parasitized and non-parasitized fishes using SPSS 20 software. Results are expressed as arithmetic mean ± SD. P values less or equal to 0.05 were deemed significant.

3. Results

3.1. Physicochemical parameters and heavy metals in surface water and sediment of Lekki lagoon, Lagos

The physicochemical parameters analyzed in water samples from the lagoon included pH, salinity, turbidity, electrical conductivity, dissolved oxygen, chemical oxygen demand, total suspended solids, and total dissolved solids. Also present in water samples were anions such as ammonia, nitrate, nitrite, bicarbonates, total phosphorus, chloride, and sulphate. The pH value of the water was just about neutral. Values for other parameters tested for are presented in Table 1.

Heavy metal characterization of surface water and sediment in Lekki lagoon are shown in Table 2. Of the four toxic heavy metals (Cr, Cd, Pb, and Ni), Pb has the highest value, 0.83 mg/kg, followed by Cr > Cd > Ni

Table 1
Physicochemical Parameter of water measured in the Lekki lagoon and their values.

Parameter	Value
pH	7.54 ± 0.51
Electrical Conductivity (µS/cm)	2305 ± 22.41
Total Dissolved Solids (mg/l)	1125 ± 12.22
Salinity (ppt)	17.33 ± 1.22
Chloride (mg/l)	9571.5 ± 8.54
Sulphate (mg/l)	138.6 ± 5.68
Total Suspended Solids (mg/l)	28.5 ± 3.21
Turbidity (ntu)	35 ± 3.33
Ammonia (mg/l)	8.95 ± 2.65
Nitrate (mg/l)	0.1202 ± 0.01
Nitrite (mg/l)	0.073 ± 0.01
Bicarbonate (mg/l)	95 ± 5.88
Total Phosphorus (mg/l)	0.1426 ± 0.02
Chemical Oxygen Demand (mg/l)	32 ± 3.36
Dissolved Oxygen (mg/l)	8.93 ± 3.22

Values given as mean ± SD.

with respective values of 0.35 mg/kg, 0.13 mg/kg, and 0.05 mg/kg in the sediment samples. Apart from Cr, Cd, and Pb, all other metals in the surface water were generally low and below regulatory limits (Table 2).

3.2. Parasitological investigation

A total of 100 *Heterotis niloticus* were randomly collected and investigated for parasites over a six-month period. Of these, 90 were males while 10 were females. Seventy (70) fishes were infected, giving a prevalence of infection rate of 70 %. Only one parasite species, *Tenuisentis niloticus*, an acanthocephalan was identified in the gastrointestinal tract of fish. Sixty two (62) of the infected fishes were males, with a total parasite load of 279. Eight (8) of the 10 female fish sampled were infected, with a total parasite load of 22. All parasitic infections were restricted to the intestine. A total of 301 parasites were recovered and they all belong to the acanthocephalan group. The prevalence and intensity of infection with respect to fish size, which was taken as a measure of fish length is presented in Table 3. The various length groups (40 cm–49.9 cm, 50 cm–59.9 cm, 60 cm–69.9 cm, and 70 cm–80 cm) show variation in prevalence, intensity, and parasite load. The highest prevalence of infection was between 50 cm–59.9 cm and 60 cm–69.9 cm, while the highest intensity of infection was between 40 cm–49.9 cm for males. Length range 40 cm–49.9 cm showed the highest prevalence of infection in females. The parasite load was highest in the length range 60 cm–69.9 cm in males and 40 cm–49.9 cm in females. Overall, the prevalence of infection was highest in the length range 50 cm–59.9 cm while the intensity of infection and parasite load were highest in the length range 40 cm–49.9 cm.

Table 2
Elemental analysis of water and sediments in the Lagos lagoon.

Heavy Metal	Water	Sediment
Al (ppm)	0.0747 ± 0.02 ^a	124.78 ± 5.14 ^b
Ba (ppm)	0.0153 ± 0.001 ^a	0.190 ± 0.001 ^a
Cd (ppm)	0.065 ± 0.02^a	0.127 ± 0.001 ^a
Co (ppm)	0.0118 ± 0.001 ^a	0.0439 ± 0.01 ^a
Cr (ppm)	0.0647 ± 0.002^a	0.3467 ± 0.03 ^a
Cu (ppm)	0.0483 ± 0.001 ^a	0.0982 ± 0.02 ^a
Fe (ppm)	0.2257 ± 0.01 ^a	327.4 ± 10.25 ^b
Mn (ppm)	0.1424 ± 0.01 ^a	4.0842 ± 1.02 ^b
Ni (ppm)	0.0345 ± 0.02 ^a	0.0539 ± 0.01 ^a
Pb (ppm)	0.0622 ± 0.02^a	0.8276 ± 0.05 ^a
V (ppm)	0.0347 ± 0.02 ^a	0.1488 ± 0.01 ^a
Zn (ppm)	0.0472 ± 0.01 ^a	0.4548 ± 0.04 ^a

Values (mean ± SD) with bold fonts are higher than WHO permissible limits; values with the same superscript on the same row are not significantly different.

Table 3
Abundance of recovered parasites, prevalence and intensity of infection in *Heterotis niloticus* from Lekki Lagoon.

Length (cm)	No of fish Examined	No Infected	Prevalence (%)	Parasite load	Intensity %
Male					
40–49.9	24	14	14	94	13.43
50–59.9	26	22	22	66	6.0
60–69.9	32	20	20	101	10.1
70–80	8	6	6	18	6.0
Female					
40–49.9	6	6	6	16	5.33
50–59.9	0	0	0	0	0
60–69.9	2	0	0	0	0
70–80	2	2	2	6	6.0
Both Sexes					
40–49.9	30	20	20	110	11.0
50–59.9	26	22	22	66	6.0
60–69.9	34	20	20	101	10.1
70–80	10	8	8	24	6.0
Total	100	70	70	301	8.6

3.3. Histopathology

The histological alterations in the intestine of *Heterotis niloticus* in the Lekki lagoon are as presented in Fig. 2. The alterations observed in the intestines of parasitized fish include focal areas of inflammation in the mucosa, mild infiltration of inflammatory cells to the submucosa, and focal areas of inflammation in muscularis (Fig. 2b to d). Other alterations such as moderate inflammation of the submucosa, moderate inflammation of the muscularis and moderate haemorrhagic lesion were also observed. The unparasitized fish examined showed normal arrangement of intestinal tissues (Fig. 2a).

3.4. Heavy metals in major fish organs and parasites

The concentrations of heavy metal in the liver and intestine of *Heterotis niloticus* and the parasite *Tenuisentis niloticus* is shown in Table 4. The differences in the concentrations of Cu, Co and Mn in the parasites, compared with fish tissues were not significant. However, all the other metals have significantly higher values in the parasite in comparison with the host's tissues. Mn was the highest in the liver, followed by Zn and Pb was the lowest. Al was the highest in the intestine, followed by Cd while Pb was still the lowest of all the metals. Cd was also the highest in the parasite, followed by Fe while Co was the lowest.

3.5. Anti-oxidative responses in the tissues of *Heterotis niloticus*

Table 5 shows the mean concentrations of antioxidant parameters in the liver and intestine of parasitized and non-parasitized fish sampled from the Lekki lagoon. Activities of reduced glutathione (GSH) and CAT were significantly higher in both fish tissues of parasitized fish compared with the non-parasitized fish. Although SOD and TBARS activities were higher in parasitized fish in the two fish tissues, these differences were not significant.

4. Discussion

The pH value of the water was within the WHO limits of 6.5–8.5. Electrical conductivity was also high, indicating that there are metals dissolved in water capable of transmitting electrical current. Standard values for electrical conductivity, dissolved oxygen, COD, turbidity, and total dissolved solids in surface waters are 800 µS/cm, 5 mg/l, 10 mg/l, 5 Ntu, and 1000 mg/l respectively [43]. The value recorded for the water parameters measured does not show too much variability from standard ranges. The relatively high dissolved oxygen of 8.93 mg/l O₂ can be attributed to the shallowness of the water body, lack of thermal stratification, and an effective regular mixing due to tidal movement

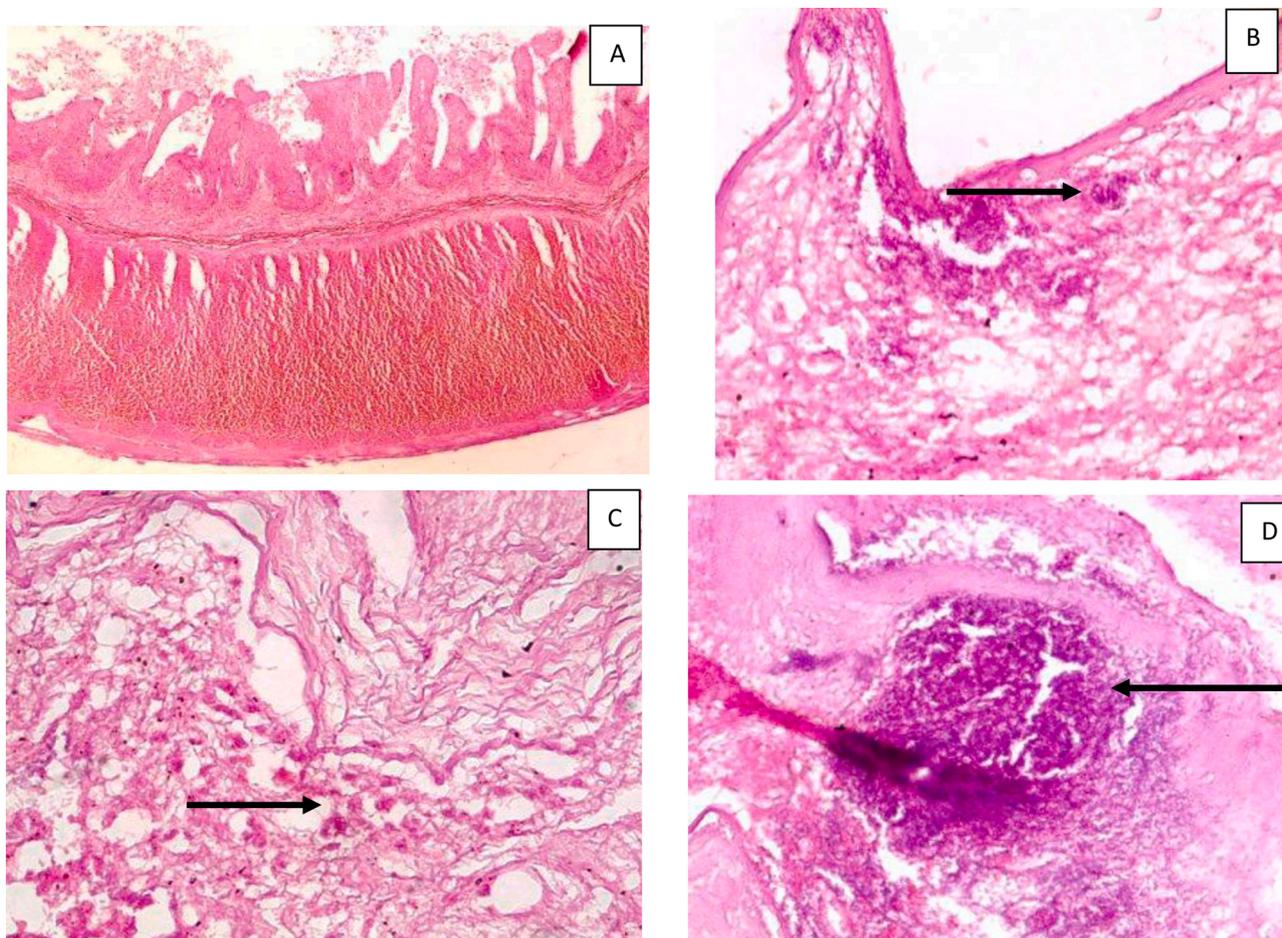


Fig. 2. Histological sections of the fish intestine. (a) Normal section of the fish intestine (b) Focal areas of inflammation in the mucosa; (c) Mild infiltration of inflammatory cells to the submucosa (d) Focal areas of inflammation in muscularis.

Table 4
Mean elemental concentrations (\pm SD) in the organs of *Heterotis niloticus* and in Parasites.

Heavy Metals (mg kg ⁻¹)	Liver	Intestine	Parasite
Zn	1.67 \pm 1.05 ^a	0.58 \pm 0.16 ^a	6.20 \pm 1.02 ^b
Cd	0.28 \pm 0.13 ^a	2.40 \pm 0.37 ^b	12.85 \pm 0.67 ^c
V	0.87 \pm 0.29 ^a	1.04 \pm 0.50 ^a	4.22 \pm 1.51 ^b
Fe	1.51 \pm 0.26 ^a	1.63 \pm 0.33 ^a	9.22 \pm 2.51 ^b
Ba	0.54 \pm 0.22 ^a	0.11 \pm 0.02 ^a	3.22 \pm 0.52 ^b
Ni	0.10 \pm 0.08 ^a	0.45 \pm 0.16 ^a	8.21 \pm 1.26 ^b
Co	0.68 \pm 0.12 ^a	0.16 \pm 0.08 ^a	1.01 \pm 0.89 ^a
Cu	0.12 \pm 0.04 ^a	0.14 \pm 0.06 ^a	1.21 \pm 0.01 ^a
Pb	0.02 \pm 0.02 ^a	0.01 \pm 0.01 ^a	2.00 \pm 1.09 ^b
Mn	1.69 \pm 0.58 ^a	2.22 \pm 0.21 ^a	2.01 \pm 1.00 ^a
Cr	0.61 \pm 0.28 ^a	0.65 \pm 0.15 ^a	3.21 \pm 1.16 ^b
Al	0.34 \pm 0.16 ^a	2.41 \pm 1.26 ^b	4.01 \pm 1.86 ^b

Values with the same superscript on the same row are not significantly different, where n = 20.

which are features of a well aerated water body. The high COD value of 32 mg/l can be attributed to discharges in form of domestic sewage, run-off from agricultural lands, direct dumping of industrial and municipal wastes [44]. Total suspended solids value of 28.5 mg/l infers a reduced visibility as confirmed by the high turbidity of 35 Ntu, total dissolved solids value of 1425 mg/l is probably an indication of high nutrient content. Total organic hydrocarbon content was low, suggesting little presence of organic compound at the time of sample collection in concordance with the low COD. It has been suggested that natural abiotic factors including, oxygen, temperature, concentration of

hydrogen ions and even eutrophication can positively influence the occurrence of parasite populations [45,46].

The overall prevalence of *T. niloticus* in the lagoon was 70 %. Seventy individuals were infected with the parasitic acanthocephalan *T. niloticus* out of which 62 (62 %) were males and 8 (8%) were females. Variation in incidence of helminthic infection in fish may be influenced by the life cycle of the parasites. The overall prevalence of infection of 70 %, observed in the present study, could be attributed to food availability. The high prevalence of the acanthocephalans in water bodies has been attributed to the pollution state of water especially from industrial and municipal effluents [47]. Factors such as improper disposal of waste and management, whereby waste is dumped along the coastal areas of the lagoon, could be adduced as reasons for the prevalence of intestinal parasitic acanthocephalan as reported in this study. Conventional fishing methods with the ability to destroy the plankton community in the lagoon, thereby enhancing food competition and other environmental stressors arising from anthropogenic activities along the shores, are other reasons that may be ascribed to the prevalence of parasitic infection in this study [48]. Furthermore, significantly higher intensity of infection by acanthocephalans in male fish than in female fish have been previously reported [49,50]. The influence of sex on the susceptibility of animals to infections may be due to genetic predisposition and differential susceptibility owing to hormonal control, sampling season and geographical differences [50,51].

In this study, the overall prevalence of infection was higher than that reported by Olofintoye [52], who reported 60.23 % prevalence of intestinal parasite in *Tilapia*. This is suggestive of the interplay of factors such as pH, dissolved oxygen, temperature, and host-parasite

Table 5
Antioxidant enzymes in the liver and intestine of non-parasitized and parasitized *Heterotis niloticus*.

LIVER		Non-Parasitized Fish			Parasitized fish		
		Min	Max	Mean± S.D	Min	Max	Mean± S.D
GSH	μmol/mg protein	0.36	1.88	1.12 ± 0.60 ^a	0.48	2.47	1.50 ± 0.44 ^b
SOD	Unit/mg prot.	0.73	4.11	2.62 ± 1.32 ^a	0.72	4.95	2.86 ± 1.03 ^a
CAT	μmol H ₂ O ₂ /min/mg prot.	3.19	11.18	7.79 ± 3.16 ^a	4.96	13.81	10.54 ± 2.61 ^b
MDA	nmol/MDA/mg prot.	0.07	0.161	0.11 ± 0.03 ^a	0.15	0.341	0.26 ± 0.04 ^a
INTESTINE		Min	Max	Mean± S.D	Min	Max	Mean± S.D
		Min	Max	Mean± S.D	Min	Max	Mean± S.D
GSH	μmol/mg protein	0.92	1.66	1.23 ± 0.31 ^a	1.09	2.19	1.60 ± 0.24 ^b
SOD	Unit/mg prot.	1.91	3.64	2.70 ± 0.69 ^a	1.98	4.06	3.28 ± 0.46 ^a
CAT	μmol H ₂ O ₂ /min/mg prot.	5.11	8.81	7.05 ± 1.51 ^a	5.91	12.14	10.12 ± 1.25 ^b
MDA	nmol/MDA/mg prot.	0.051	0.159	0.10 ± 0.04 ^a	0.068	0.219	0.15 ± 0.02 ^a

Values (mean±SD) with the same superscript on the same row are not significantly different, where n = 10.

relationship being responsible for the variation in the distribution of parasites between habitats. The choice of location of parasites in the different regions of the gut could be attributed to differences in the prevailing conditions such as food content, oxygen and osmotic tension, and pH [53]. Furthermore, the highest prevalence, intensity, and load were within the fish length range 70 cm and 80 cm for males. Fish length ranges of 40 cm–49.9 cm and 70 cm – 80 cm have the highest prevalence in females. The parasite infect fishes with larger length in male and female fish compared to short-length fishes. According to Roberts [54], smaller fishes have smaller surface area for parasites to attach as compared with older fish. This may be a plausible reason for the observation in this study. Furthermore, the observation in the present study is in agreement with the findings of Ayanda [55]. Same author also suggested that fish in this category are able to compete better, thereby having more contact with food items and consequently having higher chances of getting infected with parasites. However, the possibility exists that during competitive foraging, the worst and best competitors are able to take up less and more nutrients respectively, the previously poor competitors becoming competitive while hitherto better competitors gradually become unable to sustain dominant feeding as a result of the weakening effects of infection [56]. The combined effects of reduced ability and increased motivation associated with infection may therefore have the effect of standardizing the competitive ability of hitherto poor and good competitors. It is of current interest, factors determine the growth of parasites while inside their hosts.

In this study, the intestines of *Heterotis niloticus* which harbours *T. niloticus* showed degree of histological alterations. This ranges from mild infiltration of inflammatory cells of the epithelial mucosa, sub-mucosa, muscularis and serosa to moderate inflammation and haemorrhagic lesion. Changes in the histology architecture fish intestines due to acanthocephalan infections may depend on several factors such as parasite species, host nature of the tissues affected, and host-parasite interactions [57]. In this study, significant pathologic conditions were observed as a result of parasitic infection in *H. niloticus*. This is not in agreement with the works of Paperna [58], Khalil [59], Mashego and Saayman [60] and Boomker [61] who all reported no pathological effects in the tissues of clarias species from infections by Camallanids (*Paracamallanus cyathopharynx* and *Procamallanus laevionchus*) even though it was observed that their buccal capsule was firmly attached to stomach mucosa of the fish. However, Santoro et al. [62] reported various pronounced inflammatory responses, necrosis, and fibrosis due to parasitic infection in fish. Pathogenicity, arising from infection by acanthocephalans, is as a result of the adult parasite attaching to the digestive tract and also due to the larval stages encapsulating in the host's tissues. There is a positive correlation between the depth to which the proboscis penetrates and the degree of damage [63]. The attachment of parasite to the fish intestine using its holdfast organs may have brought about the histopathological changes observed in this study. This may further erode the walls of the intestine thereby hampering the

process of food digestion. More studies to understand the community structure of the invertebrate fauna of Lekki Lagoon may be necessary in order to have adequate knowledge of the intermediate hosts of these parasites.

The evidence of heavy metal contamination in Epe axis of Lekki Lagoon is shown by their concentrations in water, sediment, and the biota. The sediment concentration of the metals was higher than in the water medium and below the regulatory limits for most metals. However, three of the toxic heavy metals were higher than regulatory limits (Table 2). Lekki Lagoon for many years has been considered a tropical water body relatively free from heavy metal stressors and perturbations, particularly because of the absence of industries and industrial activities within its immediate vicinity. A previous study, however by Akinsanya and Kuton [64] reported concentrations exceeding WHO [65] approved limits for Ni in the gill and liver of *Synodontis clarias*, Pb in the liver of *Synodontis clarias* and Cr in the gills and liver of *Clarias gariepinus*. This study reported twelve metals detected in the water and sediment media, and bioaccumulated in the biota. The concentration of lead in the organs of *Heterotis niloticus* were low and within WHO permissible limits of 2.0 mg/kg [65]. Cd and Ni concentrations were also within the WHO permissible limits of 2.0 and 0.5–0.6 mg/kg respectively [65]. On the whole, the intestine accumulated higher concentrations of metals than the liver. However, except for Mn, Cu and Co, the parasite accumulated significantly higher concentrations of all the other metals than the tissues of the host. This is similar to the reports by Saliu et al. [66] and Kuton et al. [67] that *T. niloticus* is a good bioaccumulator of heavy metals and potential bioindicator of heavy metal pollution in the Lekki lagoon. Anthropogenic waste in the form of industrial effluent discharge and agricultural runoff, may have been the source of heavy metal pollution in the Lagoon. The accumulation of these metals poses a big risk for fish [68] and other animals (including man) that may consume the fish. The higher concentrations in the intestine may probably be due to the fact that the gut is a major site for food digestion, hence parasites will roam around it to feed [69]. In the process of digestion, the heavy metals present in the different diets of *H. niloticus* would have been released in the intestine. There is a plausible reason for the high concentrations of heavy metals in the parasite in comparison with the fish tissues. The intestine is a predilection site for parasites in the host's body where they can easily absorb digested food [70]. The parasite may in this process have taken up a lot of the heavy metals in the intestine and this is reflected in the very high concentrations of heavy metals in the parasite as compared with the fish. Furthermore, the low levels of the metals in the liver may also be suggestive of the ability of the liver to biotransform the metals. According to El-Moselhy et al. [71], the accumulation pattern of metals in fish tissues differs between species.

Activities of CAT and SOD are two indicators of oxidative stress. There is usually a rise of ROS and reactive metabolites as a result of the interactions between different enzyme systems, including detoxifying enzymes [72]. CAT increased significantly in the parasitized fishes in

comparison with the unparasitized fishes, indicating that the fishes responded to the infection by parasites. This supports the claim by Shin et al. [73] that the host body responds to the presence of parasites by releasing antioxidant enzymes. CAT degrades hydrogen peroxide that SOD produces by the dismutation of superoxide ion in periods of prolonged stress [74]. Increased CAT activity have the capacity to prevent the potential toxicity of free radicals [75] which consequently will protect the fish from oxidative damage. SOD, as a first line of action, primarily neutralizes oxyradicals [76] by quickening the dismutation of superoxide (O_2^-) to H_2O_2 which destroys both biological structures and membranes. Although there was increase in SOD activity in the parasitized fish, the increase was not significant. According to Lin et al. [77], activity of an antioxidant enzyme may reduce when fish has been exposed to a toxic agent for some time. As it is unknown, how long fishes in this study have been infected by the parasite, there is a possibility that the activity of this enzyme has initially increased in response to parasitic infection but has reduced over time as observed in the present study.

In this study, the liver and intestines of fish infected with parasites showed increased level of MDA compared with the unparasitized fish, even though the increases were not significant. This may be attributed to the elevated oxidation of molecular oxygen (O_2) to produce superoxide radicals, an indication of the important roles of the fish tissues, particularly the liver in the detoxification process [78]. Increased MDA levels also be as a result of impairment in antioxidant enzymes due to enhanced ROS formation. This could lead to alterations in cell membrane and cellular dysfunction [79]. Furthermore, increase in antioxidant biomarker observed in the intestine and liver of *H. niloticus* could account for the marked lipid peroxidation observed.

GSH functions in signal transduction, synthesis of DNA and proteins, amino acid transport, maintenance of thioldisulfide status and scavenging of free radicals [80]. When stable, it is important in ensuring a normal physiological cell function [81]. Results in the present study indicate high GSH activities in host tissues of parasitized fish, suggesting toxicity as a result of parasitic infection or possibly due to organic pollution as reported by Wilhelm-Filho et al. [82]. Increased GSH level could be an adaptive mechanism to slight oxidative stress [78]. Furthermore, alterations in the activities of GSH in the tissues of fish has been suggested to be as a result of organ-specific responses [83–85].

In conclusion, the current study is a reflection of the negative impact of man on the environment as observed in the sensitivity to changes in the tissue histology, chemical composition of fish and parasites and the biochemical response from the fish host. Over the years, established sentinel organisms have enjoyed wide patronage in environmental biomonitoring and impact assessment studies. It may become important for researchers to begin to consider the use of other organisms, such as parasites. Results from this study indicate an early warning signal which requires caution so as to prevent escalation of the pollution status of the water body.

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Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

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