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Aspidogastrea africanus Infections, comparative assessment of BTEX and heavy metals Bioaccumulation, and histopathological alterations as biomarker response in *Chrysichthys nigrodigitatus* (Lacépède, 1803) of Lekki Lagoon, Nigeria

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ABSTRACT

The study was carried out on 120 randomly selected fresh specimens of *Chrysichthys nigrodigitatus* (males and females of different trophic levels) from Lekki lagoon, Lagos, Nigeria. The aim was to investigate the heavy metals and endoparasitic infection in the fish. We compared the impacts of both stressors on the liver and intestine of *C. nigrodigitatus.* Water samples were tested for pH, dissolved oxygen (DO), salinity, electrical conductivity, total dissolved solids using a multiprobe meter. Nitrate, nitrite, phosphorus, ammonia, total suspended solids and colour were measured using VR 5000 spectrophotometer. Alkalinity, acidity, biological oxygen demand (BOD) and chloride were measured via Titrimetry method. Chemical oxygen demand (COD) was measured via a closed reflux titration., and turbidity was measured via a VR 2010. *Aspidogastrea africanus* was the only parasitic infection detected in *C. nigrodigitatus;* though of low intensity. We also observed low heavy metal levels in the environmental media tested. However, the study showed that the liver and intestinal tissues of *C. nigrodigitatus* were slightly impacted by the stressors even at low levels. We therefore suspect synergy between Aspidogasrea infection and heavy metals toxicity. We recommend a further biomonitoring of the aquatic environment in order to ascertain the actual causative factors contributing to tissue injuries in the fish. Findings will help mitigate future aggravation of the tissue toxicity. Low levels of Aspidogastrea infection is an early prognostic information against outbreak of the disease in the aquatic environment.

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Introduction

Heavy metals are chemical elements that have relatively high density. Contamination of the Nigerian aquatic environment with heavy metals has been widely reported [5,19]. Fish is at the top of aquatic food, hence they have high tendency of accumulating heavy metals through biomagnification up the pyramid of aquatic biomass. Of concern is thus the fact that fish are a major source of animal protein requirement in the country [19,32].

Heavy metals such as copper, manganese and zinc, are essential elements required trace amounts. Concentrations of essential elements in organisms are naturally homeostatically regulated according to their respective thresholds of essentiality. Zinc is essential to the animal system, especially if taken orally [2,25]. However, excess amount may cause developmental and reproductive toxicity [26]. Manganese impacts the respiratory tract and central nervous system [3], thereby culminating in compromised cognitive performance and nerve damage. Parkinson disease may ensue from excess manganese intake, lung embolism, bronchitis, schizophrenia, weak muscles, headaches and insomnia may also occur [3].

Furthermore, higher concentration of heavy metals in fish tissues than in aqueous phase is mediated by bioconcentration and bioaccumulation [19,28]. Factors such as season, physical and chemical properties of water may play concerted role in metal accumulation in different fish tissues [17,32]. Ability of fish to accumulate toxicants from ambience depending upon pH, salinity, temperature, and hardness of ambient water; route of uptake, concentration of toxicant, duration, fish age and size, and most importantly the health status of the fish has been widely reported [1,19,22]. These factors however, have been reported to be influenced by parasitic infections [5].

Generally, concentration of heavy metals has been reported to increase from one trophic level to the higher; up the pyramid of biomasses [19,30]. Older and larger fish tend to have higher concentration of heavy metals [4,30] due to availability of storage in the adipose tissue [31,33].

Contaminants such as BTEX and heavy metals may enter the food chain through petroleum related pollution with a significant amount of potential to impair animal and/or human health ([12,34,19,40]). These contaminants may also inhibit various enzyme activities, and induce oxidative stress by altering the activities of enzymes in the antioxidant defense systems of aquatic flora and fauna [11,16,21,23].

Monitoring fish tissue contamination serves as an early warning indicator of water quality problems [9,24]. This fosters appropriate prompt actions to protect public health and the environment.

Chrysichthys nigrodigitatus, also known as Bagrid catfish have numerous endoparasites. However, a predominantly reported of the endoparasites is *Aspidogastrea africanus* [7]. They are trematodes which are widely distributed in fresh and saltwater. They are small group of parasitic flatworms (Neodermata), characterized with ventral holdfast organ with rows of alveoli, a row of rugae or suckers [6]. Aspidogastreans parasitize molluscs as obligate hosts and vertebrates (fishes and turtles) as facultative or obligate final hosts [8]. Depending on the intensity of the parasitic infection, the trematode may inflict alterations on fish membrane selective permeability, thereby influencing the absorption of toxicants by the host fish.

The study was aimed at investigating the impacts of selected heavy metals, BTEX and Aspidogastrea infection on *C. nigrodigitatus*.

Materials and methods

Description of study area

The study was carried in Lekki lagoon (Fig. 1) in Lagos State, Nigeria (longitudes 4º 00′ and 4º 15′ E and latitudes 6º 25′ and 6º 37′ N) between March- August 2017. The lagoon supports a major fishery in Nigeria. Lekki lagoon has a surface area of about 247 km^2 with an avegrage depth of 6.4 m. The Lekki Lagoon is part of an interconnected wetlands which comprise of lagoons and creeks along the coast of South-Western Nigeria. These wetland systems cover a range from the Dahomey border to the Niger Delta region, over a distance of about 200 km. River Oni empties into the North-eastern and Rivers Oshun and Saga empty into North-western parts of the lagoon. The weather conditions of the study area comprise of wet and dry seasons; typical of the southern part of Nigeria. The vegetation around the catchment area is characterized by shrubs and raphia palms, *Raphia sudanica* and oil palms, *Elaeis guneensis* [5].

Sample collection and analysis

Chrysichthys nigrodigitatus

A total of 120 randomly selected fresh specimens of *Chrysichthys nigrodigitatus* (males and females) were purchased at Oluwo market, Epe, Lagos, Nigeria over a period of 6months (March to August 2017). All fish samples were properly identified using standard keys provided by Idodo-Umeh [18] and [41]. The samples were then labelled, and dissected after. The total length was measured using a transparent meter rule and recorded in cm. The weight was assessed using Ohaus electric weight balance (Model number ARC 120). Sex determination of the fish was done by visual examination.

BTEX in fish tissue. BTEX was analyzed in water and fish tissues using the EPA method 8260B Agilent 7890B gas chromatograph coupled to a mass spectrophotometer (GC–MS). The stationary phase of separation of the compounds used was a

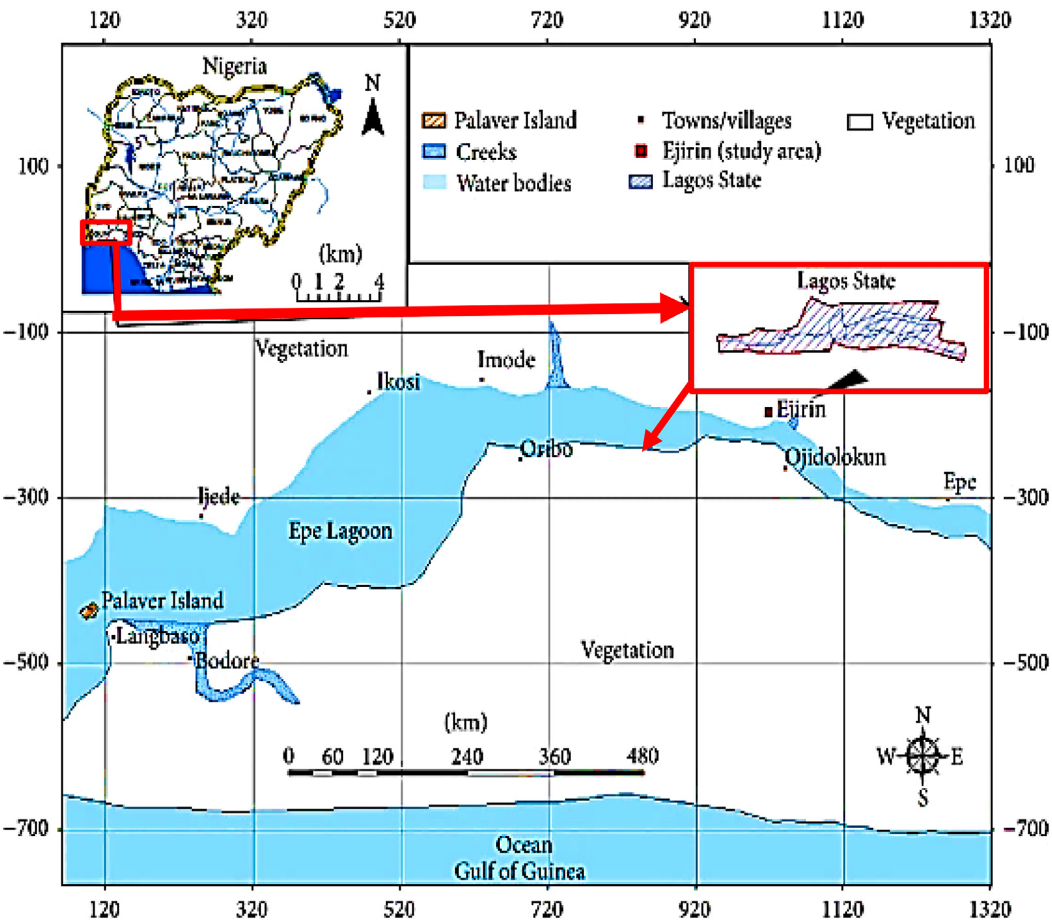

Fig. 1. Map of Lekki lagoon showing sampling sites.
Source: Lagos state surveys office Ikeja (2009).

DB-5 capillary column coated with 95% dimethyl - 5% Diphenyl polysiloxane (30 m length x 0.32 mm diameter x 1.0 µm film thickness). 1µL of the samples were injected in split mode with split ratio 100:1 at an injection temperature of 125 °C.

Heavy metals in fish tissue. Wet weight (10 g) of fish tissue was extracted using 20 mL of HNO3: HClO4 (5:1) at 105 °C for about 24 h [37]. The extract was made up to 25 mL with HNO3 (70%) and diluted with deionized water. Atomic Absorption Spectrophotometer (ASS) was used in reading heavy metals concentration in mg/kg; wet weights. The meter was calibrated for each metal by dissolving 1 g analar grade metal salt in 1 L of distilled water.

Histological assessment. Tissues of intestine and liver were resected for histological preparation after dissection. The intestines were stored in bouins solution in separate universal bottles and decanted after 6 h. The tissue was then preserved with 10% phosphate buffer formalin. The tissues were gradually dehydrated by increasing doubling the alcohol concentrations at 30 min interval. Tissues were then impregnated in molten paraffin three times. The tissues were embedded in molten paraffin wax and allowed to solidify. The solidified tissues were sectioned at 4–5 µm, then soaked into pre-coated slides and allowed to dry. The prepared sections were stained using haematoxylin and eosins stains. The stained tissues were rinsed with distilled water while the overly stained tissues were decolored using 1% acid alcohol. Prepared tissues were mounted using DPX mountant, air-dried and finally examined under the microscope. The photomicrographs were achieved by the team of pathology laboratory of the Department in Veterinary Pathology, University of Ibadan, Nigeria.

Analysis of endoparasites

Examination of intestinal parasites was carried out using the techniques of Akinsanya et al. [6]. Fish specimens were eviscerated using sterile surgical blade. Intestinal parasites were identified morphologically as *Aspidogastrea africanus* using standard identification keys, in conjunction with pictorial guides provided in manuals such as Sinha [36] and Singh [35]. Contents of the intestine were examined using hand lens. All obtained parasites were counted, recorded and fixed in 70% alcohol.

Water physicochemistry

Water samples were collected on monthly basis and preserved for laboratory analysis using standard methods. pH, dissolved oxygen (DO), salinity, electrical conductivity, total dissolved solids were measured using a handheld multi-parameter probe (Horiba Water Checker Model U-10). Nitrate, nitrite, phosphorus, ammonia, and total suspended solids were measured using VR 5000 spectrophotometer. Alkalinity, acidity, biological oxygen demand (BOD) and chloride were measured via titrimetric method. Chemical oxygen demand (COD) was measured via a closed reflux titration., and turbidity was measured via a VR 2010.

Statistical analysis

All indices were statistically computed using SPSS, version 20 ($p<0.05$). Quantitative parasitology software (QP, version 3.0) was used to analyze the prevalence, mean and median intensity of parasite infestation.

Parasitic mean intensity was calculated using the formula according to Ezewanji et al. [13].

$$Percentage\ Prevalence = \frac{\text{Number of infected fish}}{\text{Number of fish examined}}\ X\ 100\ (Saliu\ et\ al.,\ 2014)$$

$$Parasite\ Abundance = \frac{\text{Number of collected parasites}}{\text{Number of fish examined}}(Saliu\ et\ al.,\ 2014)$$

$$Mean\ intensity = \frac{\text{Number of collected parasites}}{\text{Number of infected fish}}\ (Saliu\ et\ al.,\ 2014)$$

Results

The lagoon was slightly alkaline with mean pH of 8.10 ± 0.23, with relatively low turbidity of 1.5 ± 0.71NTU, dissolved oxygen of 4.53 ± 0.14 mg/L and relatively high total hydrocarbon of 8.816 ± 0.02 mg/L (Table 1). The mean chemical oxygen demand (4800.00 ± 2.12 mg/L) was very much significantly higher that the established WHO limit of 80 mg/L ($p<0.01$). The electrical conductivity (16,960 **±** 4.13 µS/cm) and total dissolved solids (8478 ± 8.33 mg/L) were very much higher than the WHO established limits ($p<0.01$). Other physicochemical parameters were below standard regulatory limits i.e. total suspended solids was 4.00 ± 0.15 mg/L, ammonia was 2.92 ± 0.73 mg/L, nitrate was 0.50 ± 0.01 mg/L, nitrite was 0.03 ± 0.00 mg/L, bicarbonates was 293.12 ± 12.23 mg/L, chloride, 5317.50 ± 12.26 mg/L, and sulfate, 110.96 ± 0.33 mg/L.

The range of standard length (SL) examined among *C. nigrodigitatus* was 13- 52.9 cm. The highest parasitic prevalence (50%) was recorded in females of SL of 33.0 – 42.9 cm (Table 2). There was no aspidogastrea infection recorded in the males.

There was no parasitic infection detected in all male weight cohorts (Table 3). In the females, the minimum parasite prevalence was detected in weight cohorts of 40 – 69 g, and 70 – 99. While maximum parasite prevalence of 10% was detected in weight cohorts of 100 – 129 g, and 130 – 159 g. There was no discernible relationship between the parasite prevalence in relative to weight and SL.

Table 1
Physicochemical properties of water in Lekki lagoon, Lagos (March 2017- August 2017).

Parameter	Concentration	[39]	p Value
pH	8.10 ± 0.23	6–8	>0.05
Electrical Conductivity (µS/cm)	**16,960.00 ± 4.13**	400	<0.01
Total Dissolved solids(mg/L)	**8478.00 ± 8.33**	2000	<0.01
Salinity (ppt)	17.33 ± 0.42	–	–
Dissolved Oxygen(mg/L)	4.53 ± 0.14	7.5	>0.05
Chloride(mg/L)	5317.50 ± 12.26	–	–
Sulfate(mg/L)	110.96 ± 0.33	500	>0.05
Total Suspended solids (mg/L)	4.00 ± 0.15	30	>0.05
Turbidity(NTU)	1.5.00 ± 0.71	–	–
Ammonia (mg/L)	2.92 ± 0.73	–	–
Nitrate (mg/L)	0.50 ± 0.01	20	>0.05
Nitrite (mg/L)	0.03 ± 0.00	–	–
Acidity (mg/L)	7.61 ± 0.23	–	–
Alkalinity (mg/L)	78.00 ± 4.22	–	–
Bicarbonate (mg/L)	293.12 ± 12.23	–	–
Phosphorus (mg/L)	0.14 ± 0.00	<5	>0.05
Total hydrocarbons (mg/L)	8.816 ± 0.02	10	>0.05
Chemical oxygen demand (mg/L)	**4800.00 ± 2.12**	80	<0.01

Sample size $N=6$. Emboldened figures are significantly higher than regulatory limits at $p<0.05$ = significant, $p<0.01$ = very much significantly higher, $p>0.05$ = not significant.

Table 2
Prevalence of gastrointestinal Aspidogastrea infections in *C. nigrodigitatus* in relation to standard length (SL).

Sex	Standard Length (cm)	Number Examined	Number Infected	Prevalence (%)	Worm Load	Mean Intensity
	13.0 – 22.9	64	0	0	0	0
Male	23.0 – 32.9	10	0	0	0	0
	33.0 – 42.9	3	0	0	0	0
	43.0 – 52.9	0	0	0	0	0
	13.0 – 22.9	30	2	9	4	15
Female	23.0 – 32.9	7	0	0	0	0
	33.0 – 42.9	6	3	50	26	10
	43.0 – 52.9	0	0	0	0	0
	13.0 – 22.9	94	2	2	4	15
Both	23.0 – 32.9	17	0	0	0	0
	33.0 – 42.9	9	3	33	26	10
	43.0 – 52.9	0	0	0	0	0

Table 3
Prevalence of gastrointestinal Aspidogastrea infections in *C. nigrodigitatus* in relation to weight.

Sex	Weight (g)	Number Examined	No. Infected	Prevalence (%)	Worm Load	Mean Intensity
	40 – 69	22	0	0	0	0
	70 – 99	12	0	0	0	0
Male	100 – 129	5	0	0	0	0
	130 – 159	14	0	0	0	0
	160 – 189	16	0	0	0	0
	190 – 219	9	0	0	0	0
	40 – 69	9	0	0	0	0
	70 – 99	7	0	0	0	0
Female	100 – 129	5	1	20	14	30
	130 – 159	5	2	40	7	15
	160 – 189	9	1	11	2	30
	190 – 219	7	1	14	7	30
	40 – 69	31	0	0	0	0
	70 – 99	19	0	0	0	0
Both	100 – 129	10	1	10	14	30
	130 – 159	19	2	10	7	15
	160 – 189	25	1	4	2	30
	190 – 219	16	1	6	7	30

Table 4
Prevalence of gastrointestinal Aspidogastrea infections relative to sex of *Chrysichthys nigrodigitatus*.

Sex	Number Examined	Number infected	Prevalence (%)	Total number of parasites	Mean Intensity
Male	77 (64.2%)	0	0	0	0
Female	43 (35.8%)	5	12	30	6

Table 5
Summary of parasitic infection in *Chrysichthys nigrodigitatus* of in Lekki lagoon.

Organism	No. Examined	No. Infected	Prevalence (%)	Total parasites	Mean intensity
Chrysichthys Nigrodigitatus	120	5	4	30	6
TOTAL	**120**	**5**	**3.3**	**30**	**6**

In both sexes, minimum parasite prevalence of zero (0) was detected in *C. nigrodigitatus* of weight cohorts of 40- 69 g, and 70- 99 g, while the maximum parasite prevalence was detected in weight cohorts of 100- 129 g, and 130- 159 g.

Of the total fish we examined 77 (64.2%) were males, 43 (35.8%) were females (Table 4). No parasitic infection was detected in the males and all 5 fish infected with 30 Aspidogastrea parasites were females (Table 5). This accounts for significantly higher parasite prevalence and mean intensity in the females than the males ($p<0.05$).

Out of a total of 120 fish specimens examined, only 5 (3.3%) were infected with a total number of 30 Aspidogastrea parasites (Table 5). This accounts for the low percentage parasite prevalence (4%), and mean parasite intensity (6) recorded in *C. nigrodigitatus*

Concentrations of benzene, toluene and chlorobenzene in the intestine were significantly higher than the concentrations in the liver of *C. nigrodigitatus* (Table 6). Furthermore, the concentrations of benzene, ethylbenzene, $m+p$-Xylene, and

Table 6
Comparison of BTEX (mg/L) in intestine and liver of *C. nigrodigitatus*.

BTEX	INTESTINE	LIVER	WHO [42]
Benzene	**1438.00 ± 0.01***	1013.07 ± 0.01*	0.004
Toluene	**10.50 ± 0.01***	9.74 ± 0.01*	8.00
Chlorobenzene	**4.28 ± 0.01**	0	–
Ethylbenzene	7.26 ± 0.01*	6.68 ± 0.01*	0.1
m + *p*-Xylene	7.34 ± 0.01*	7.91 ± 0.01*	0.2
o-Xylene	7.22 ± 0.01*	6.95 ± 0.01*	0.2

Emboldened figures represent significant difference between tissues ($p<0.05$), while asterisked figures represent significant difference between tissues and regulatory standards ($p<0.05$).

Table 7
Comparison of heavy metals (mg/kg) in intestine and liver of *C. nigrodigitatus* with allowable limits.

HEAVY METALS	INTESTINE	LIVER	FEPA [15]
Zn	**0.13 ± 0.02**	0.046 ± 0.01	3.0
V	0.008 ± 0.00	0.006 ± 0.00	–
Cd	BDL	**0.006 ± 0.00**	0.01
Fe	BDL	BDL	0.2
Ba	**0.748 ± 0.16**	BDL	–
Ni	BDL	BDL	0.02
Cu	0.022 ± 0.00	0.038 ± 0.00	–
Pb	BDL	BDL	0.01
Co	0.068 ± 0.01	0.034 ± 0.01	–
Mn	**0.068 ± 0.01**	0.019 ± 0.01	1
Cr	**0.005 ± 0.00**	BDL	–
Al	BDL	BDL	–

Emboldened figures represent significant difference between concentration of heavy metals in intestine and liver ($p< 0.05$). BDL means below detectable limit (<0.001 and <0.002 mg/kg).

o-Xylene in the intestine and the liver were quite higher than the set limit of WHO [42]. The concentration of toluene in the intestine, followed by the liver were also higher than the set limit. Concentration of chlorobenzene was significantly higher in the intestine than the concentration detected in the liver ($p<0.05$).

Comparison of heavy metals in the intestine and liver of *C. nigrodigitatus* with the recommended limits established by the regulatory agency shows that all the metals tested were below the set standards (Table 7). Analysis of variance between the concentrations of heavy metals in the intestine and the liver showed that the former accumulated significantly higher concentrations of Zn, Ba, Mn and Cr ($p< 0.05$). Cadmium was the only heavy metal with detected higher concentration in the liver that the intestine ($p < 0.05$).

Plates 1-3 show histopathological alterations in the liver and intestine of *C. nigrodigitatus.* Results show similar histopathological alterations in all groups of fish irrespective of age or infection status. In the male liver tissues show no observable changes while the intestinal tissue exhibited moderate alterations such as congestion of the submucosa, infiltration of inflammatory cells to the submucosa, and serosa (Plate 1). The observations in both infected and non-infected females also exhibited moderate congestion of the mucosa and submucosa (Plates 2 and 3), which were not different from the observations in the male intestinal tissues. Lower histopathological alterations were also observed in the liver tissues than the intestinal tissues of both infected and non-infected females.

Discussion

Results showed that the essential heavy metals were accumulated in relatively higher concentrations compared to non-essential heavy metals. This is a function of their threshold of essentiality. This observation conforms to findings of Ukwa et al. [38]. The essential elements are homeostatically regulated in fish [10]. On the, other hand, non-essential elements have no biological function or requirement. Current observation of low level of lead in fish is in contrast with the observation of with Ukwa et al. [38], who detected high concentration of lead in *Chrysichthys nigrodigitatus*. The low concentration of non-essential heavy metals could be attributed to the metabolic regulatory functions of the fish species such as excretion of metals which can occur through the gills, bile (via faeces), and kidney and skin. Variability in metal concentrations in the tissues is attributable to biochemical characteristics of the metal [14]. Jobling [20] implicated metallothionein proteins in high accumulation of heavy metals in fish liver. These proteins are however synthesized in liver tissues for detoxification of heavy metals in fish

Accumulate of higher concentrations of nickel in fish tissues than permissible limits has been widely reported in previous literatures ([27,29,38]). On the contrary, nickel accumulation level in the current study was below the recommended

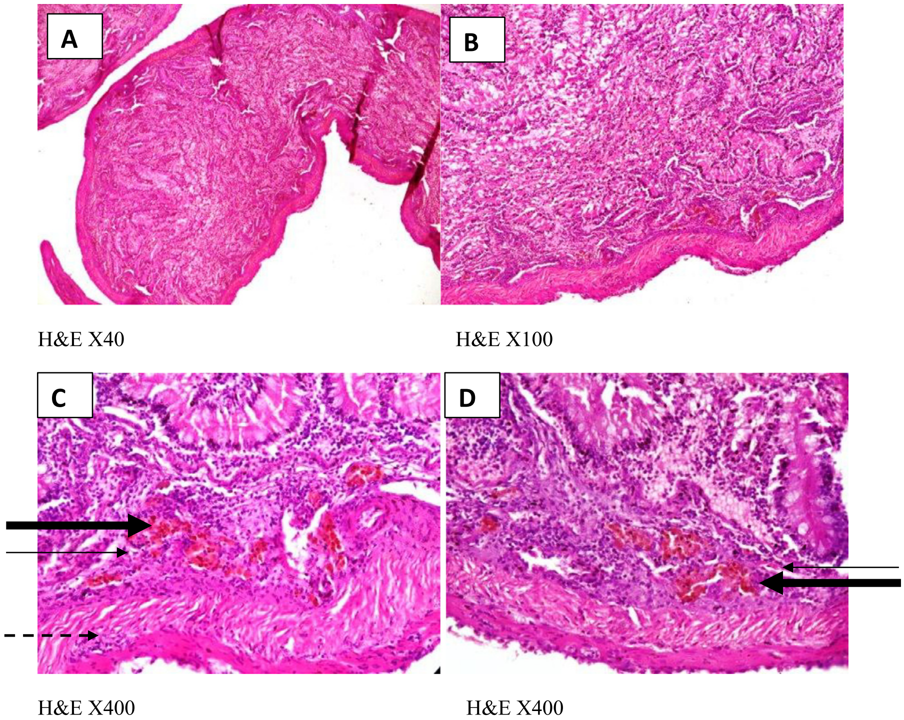

Plate 1. Photomicrographs of male liver tissues (A, B) show no noticeable alterations, while intestinal tissue (C, D) show moderate congestion of the submucosa (black arrow), mild infiltration of inflammatory cells to the submucosa (thin arrow) and serosa (dashed arrow).

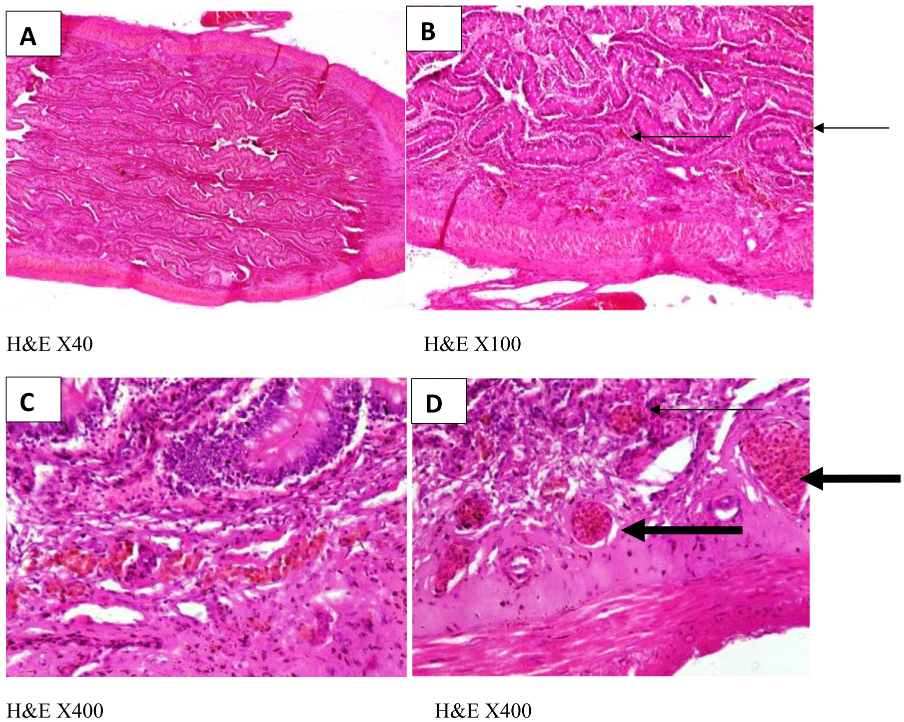

Plate 2. Photomicrographs of non-infected female liver tissues (A, B) show very slight mucosal degeneration (thin arrows), while intestinal tissues (C, D) show moderate congestion of the mucosa (thin arrow) and submucosa (thick arrows).

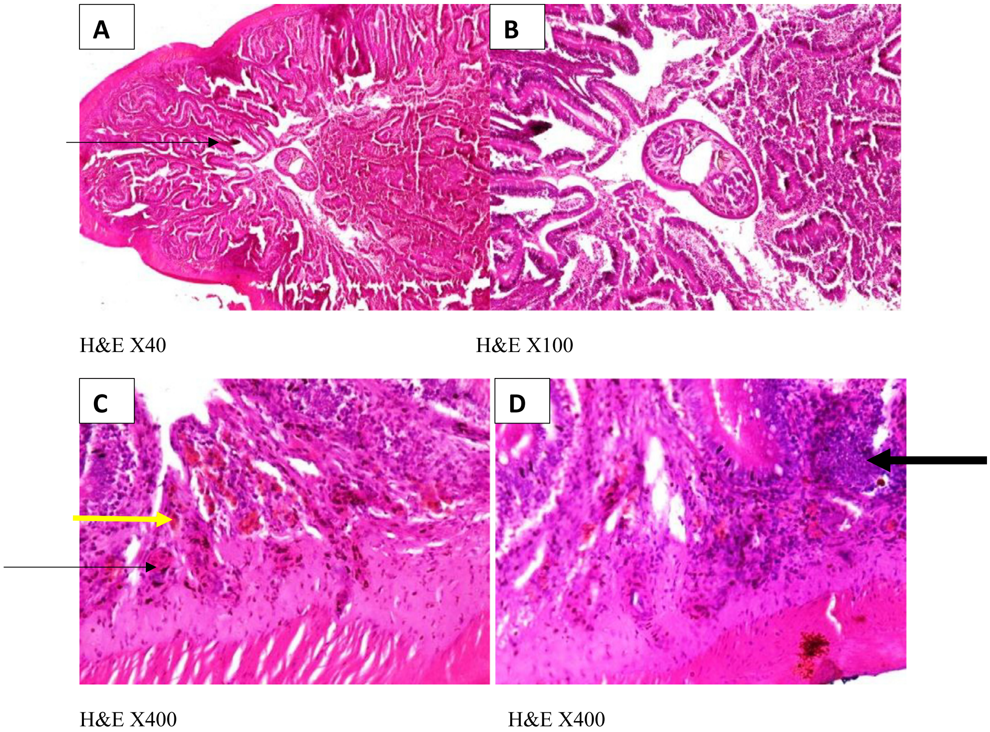

Plate 3. Photomicrographs of infected female liver tissues (A, B) show slight congestion in mucosa (thin arrow), while intestinal tissues (C, D) show presence of parasite in the lumen (thin arrow), mild congestion in the mucosa (yellow arrow), and infiltration of inflammatory cells into the villi (thick arrow).

levels of WHO (0.02 mg/kg). In man, lead toxicity may result in musculo-skeletal, renal, ocular, neurological immunological, reproductive and developmental effects [3]. Levels of lead recorded in this study was below detectable limit compared to the required standard. Although the intestine of the fish accumulated higher concentrations of Zn, Ba, Mn, and Cr than the liver. This can be attributed to possible sorption of the contaminants on settled sediments, coupled with the bottom feeding habit of the fish. However, all the heavy metals in the studied fish species were below the safe limits recommended by FEPA [15]. The study is useful as baseline data for monitoring future anthropogenic activities along the coast. However, there is a need to monitor the Lekki lagoon for heavy metal pollution stability and associated ecological and public health risks.

Higher concentrations of BTEX components in the intestine and liver of the fish than permissible limits indicates a serious prognostic of health and environmental challenges worthy of specified research to ascertain. Though, the intestine of the fish accumulated higher concentrations of the BTEX than the liver, concentrations in both tissues are alarmingly higher than set standards; particularly concentration of benzene which may elicit harmful effects on consumers of the fish. Health effects of accumulated benzene include disruption of the bone marrow, thereby causing decreased red blood cells, hence anemia.

Despite the fact that all male intestinal tissues were not infected with *A. africanus,* their intestinal tissues showed some moderate alterations which were quite similar to the intestinal alterations observed in both infected and non-infected females. This observation implies that Aspidogastrea infection may not be responsible for the alterations observed. This evidence is buttressed by the low parasitic indices observed in the study i.e. overall parasite result shows that only 30 parasites infected a sampled size of 120 *C. nigrodigitatus*, amounting to a low parasite prevalence of 3.3% and mean intensity of 6. However, females constituted 35.8% of the sample size and only 5 of the females were infected among 43 individuals. Furthermore, only 30 parasites accounted for the infections recorded in the 43 females. This amounted to a relatively low parasite prevalence of 12% and mean parasite intensity of 6.

Histopathological evidences of the study quite commensurate with the concentrations of heavy metals, particularly BTEX in the investigated tissues. This indicate that BTEX may be the leading cause of the histopathological injuries observed.

Results imply that *C. nigrodigitatus* of Lekki lagoon has low parasite infection which may aggravate in the absence of monitoring and proper management of the aquatic ecosystem. Results show that *C. nigrodigitatus* of Lekki lagoon are unfit for consumption, hence immediate environmental remediation processes are required in the aquatic ecosystem pending discontinuity of consumption of the fish.

Conclusion

High concentration of toxicants in *C. nigrodigitatus* of Lekki lagoon may compromise the immunity of the fish, thereby increasing the levels of Aspidogastrea infection. Findings ascertained that the water had adequate conditions but *C. nigrodigitatus* had accumulated dangerous levels of contaminants over time. Therefore, regular monitoring of heavy metals and BTEX in the water body as well as in the biota should be undertaken in order to mitigate the environmental pollution and forestall the ensuing debilitating challenges.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2019.e00060.

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