



# *Tenuisentis niloticus* (Neoechinorhynchida: Tenuisentidae) as Indicator of BTEX and PAH Pollution in Epe Lagoon, Lagos, Nigeria

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## ABSTRACT

The Lagos lagoon is continually being subjected to all forms of pollution. Human activities like industrialization, agriculture, and oil exploration are constantly threatening the health of this aquatic ecosystem. Polycyclic aromatic hydrocarbons (PAH) and mono-aromatic hydrocarbons-benzene, toluene, ethylbenzene and xylene (BTEX) are chemicals derived from fossil fuels that easily get into the aquatic environment. These chemicals were investigated in the fish parasite *Tenuisentis niloticus*, as a reflection of the extent to which these compounds are present in the lagoon. One hundred (100) samples of *Heterotis niloticus* were investigated for parasitic infection for a duration of six months, while the parasites encountered were used as sentinel organisms to check for the presence of PAH and BTEX. GC-MS was used to analyse for the presence of these chemicals, while parasite identification was done using conventional method. Only one parasite species, *Tenuisentis niloticus*, an acanthocephalan was recovered. There was 72% infection prevalence. The result also revealed that both BTEX and PAHs were accumulated in the body of the parasite. Two components of BTEX, (1,2-dichlorobenzene and 1,3-dichlorobenzene) and five PAH congeners-Acenaphthylene, Phenanthrene, benz(a)anthracene, benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene were not detected in the parasite. Total BTEX and PAH concentrations in the parasite was  $47.45 \pm 7.68$  and  $135.12 \pm 2.547$ , respectively. There are ecotoxic concerns in the surroundings of the lagoon and human activities that aid it should be checked.

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### Authors' Contribution

BA conceived and designed the experiments. IOA analyzed and interpreted the data and wrote the paper. BO performed the experiments. JKS contributed reagents, materials and edited the draft manuscript.

### Key words

PAH, BTEX, *Heterotis niloticus*, *Tenuisentis niloticus*, Parasite.

## INTRODUCTION

There is a need to continuously monitor the natural environment which has been subjected to progressive degradation due to biological and industrial pollution (Dzika and Wyzlic, 2010). To adequately assess and quantify the adverse effects of environmental stressors on the wellbeing of aquatic biota, organisms or communities whose important roles very closely correlate with certain environmental factors, so much that they can be useful indicators in the assessment of an area have enjoyed wide usage by researchers. Such organisms are defined as bioindicators (Markert *et al.*, 2003).

The aquatic environment, being rich in both fauna and flora, having diverse niches and relatively stable, presents conducive conditions for the development, transmission and dispersal of parasites (Popiolek, 2001). In theory, aquatic ecosystems with zero pollution normally are richer in parasite communities (Marcogliese, 2005; Hudson *et al.*, 2006).

Natural and anthropogenic activities and emissions

remain the sources of PAHs in both terrestrial and aquatic environment (Obiakor *et al.*, 2014). BTEX are common pollutants found in water in events of accidental oil spills and oil pipeline leakages, and also when contaminated industrial effluents are disposed of (Mazzeo *et al.*, 2010; Costa *et al.*, 2012).

BTEX and PAH are petroleum hydrocarbons reported to have adverse effects on aquatic biota (Akinsanya *et al.*, 2020). Toxicological studies have reported that BTEX compounds are neuro- and hepato-toxins, with the ability to cause significant cognitive and behavioural effects when exposed to it (Santiago *et al.*, 2014). They have also been known to cause malformation of chromosomal and/or genetic aberrations, making them genotoxic (Ekpenyong and Asuquo, 2017). PAHs on the other hand are carcinogenic (Neff, 2003), induce oxidative stress (Dizdaroglu *et al.*, 2002), affects the immune system (Holladay *et al.*, 1998) and disrupt endocrine regulation and development (Gozgit *et al.*, 2004; Navas and Segner, 2000; Rhodes *et al.*, 2005).

Organisms that are able to concentrate certain substances in the tissues of their body in amounts that are higher than those in their surrounding environment are described as accumulation indicators (Beeby, 2001). They are able to provide important information on the

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volume of chemicals in their habitats and by extension provide knowledge about the biological availability of target substances. Parasites are being used as one of such organisms.

Parasitic infection in aquatic organisms is not an uncommon occurrence. Intensive research into the field of environmental parasitology has been on the rise, and it has been reported that parasites can be useful accumulation bioindicators (Sures, 2003, 2004; Vidal-Martínez *et al.*, 2006; Yen *et al.*, 2014).

There have been recent reports (Akinsanya *et al.*, 2019a, b) of pollution (BTEX, heavy metals and OCPs) from the Lagos lagoon. Furthermore, reports of the use of parasites as sentinel organisms from natural waters exists. *Oncomegas wagneri*, a cestode was used to monitor PAHs in the fish *Cylopsetta chittendeni* in Mexico. *Aspidogastrea africanus* was used to monitor PAH from the Lagos lagoon using the fish *Chrysichthys nigrodigitatus* (Akinsanya *et al.*, 2020). BTEX was also reported to be monitored using the parasite *Wenyonia acuminata* recovered from the fish *Synodontis clarias* (Akinsanya *et al.*, 2019a).

The Lagos lagoon is a coastal water aquatic ecosystems at the highest risk of pollution from human

activities and subsequently, environmental degradation due to their vulnerability. The area is a rural settlement, most human population being concentrated along the lagoon bank. Human activities like agriculture, sand mining, fishing and movement of people and goods occur. Wastes from a number of industries that are located in the state are disposed of in these water bodies (Anetekhai *et al.*, 2007). Hydrophytes, occurring around the edges of the lagoon, shrubs and palm trees scattered around the villages surrounding it are some of the dominant vegetation that characterize the Lagos lagoon. Hence this study was designed with the aim of determining the prevalence of parasitic helminth infections in *Heterotis niloticus*, and analysing the parasites for PAHs and BTEX.

## MATERIALS AND METHODS

### Study area

Epe, Lekki, Lagos and Ologe lagoons are the four major lagoons in the state. The Lagoon's coordinates are between latitudes 6°29' N and 6°38' N; and longitudes 3°30' E and 4°05' E (Agboola and Anetekhai, 2008). A large part of the lagoon has maximum depth of about 6.0 m (Fig. 1).

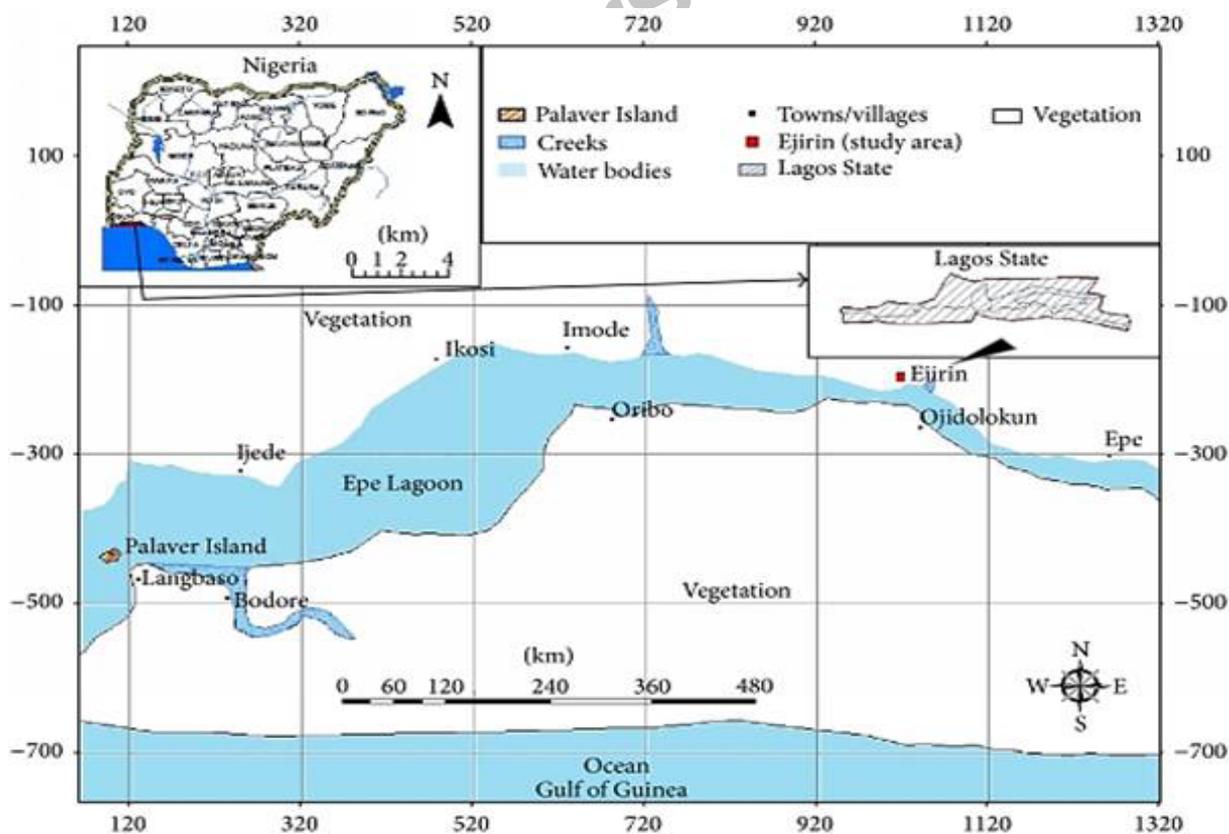


Fig. 1. Map of the Epe Axis of Lekki Lagoon.

#### *Fish collection and examination for parasites*

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.

After dissection, fish gut was excised. To recover the parasites, the alimentary canal was cut into sections and placed in petri dishes containing physiological saline. Furthermore, the intestines were longitudinally cut open to allow the parasites to emerge easily from the gut. The intestines and liver of the fish was also harvested and kept in teflon lined capped vial and refrigerated before further analysis for the presence of hydrocarbon. Thereafter, fixation of the guts that were infected with parasites was done for 7 h in Bouins fluid and were later preserved in 10% phosphate buffered formulation. The number of parasites observed was recorded and fixed in 70% alcohol. For identification, parasites were sent out to the British Natural History Museum, United Kingdom.

#### *Extraction and purification of BTEX and PAHs in parasites*

The method used was the KOH Refluxing/Vortex Extraction EPA Method 3611C where the parasite was macerated, homogenized and the homogenized tissue placed in a 50 mL centrifuge tube. 15 mL of 6N KOH was added to it, and transferred into sealed tubes and incubated for 18 h in a 35°C water bath, while shaking vigorously for 30 second for every ½ hour for the first 4 h. Thereafter, 15 mL of methylene was added to the centrifuge tube and centrifuged at 2000rpm for 5 min to facilitate phase separation. The upper aliquot layer was removed using Pasteur pipettes into a 250 mL round-bottom flask. Sample extracts was concentrated to about 5–10 mL by rotary evaporator prior to fractionation for aliphatic hydrocarbons and PAHs using alumina gel column fractionation and clean up procedure. To prepare a column, 11±0.01g of activated silica/alumina gel was weighed into a beaker, hexane/methylene chloride was added to form a slurry that is poured into the chromatographic column with a based glass wool, rinsing all the silica/alumina gel into the column with the solvent used. Hexane (10 mL) was used to condition the column, and elution was done at a rate of 1 drop/sec. The elution was stopped when the solvent reaches the top of the column after which the eluate was concentrated to 2 ml using rotary evaporator, and about 1 g sodium sulphate was added to remove any remaining water in the extract which was stored in a 2 ml GC vial below 4°C prior to GC analysis.

#### *Chemical analysis*

Agilent 7890B gas chromatograph coupled to flame ionization detector (FID) was used for PAH determination as described by Yao *et al.* (2013). To separate the compounds, HP-5 capillary column coated with 5% phenyl methyl siloxane was used as the stationary phase. Injection was done using 1 µL of the samples, injected at 300°C, 13.74 psi temperature and pressure respectively with a total flow of 21.364 mL min<sup>-1</sup>. To split vent, purge flow was set for 0.75 min at 15 mLmin<sup>-1</sup>. Oven was programmed at 40°C for a min, later at 12°C min<sup>-1</sup> and finally set to 300°C for 10 min. The temperature of the FID was 300°C with hydrogen: Air flow at 30 mL min<sup>-1</sup>: 300 mLmin<sup>-1</sup> while the makeup gas, nitrogen was set at a flow rate of 22 mLmin<sup>-1</sup>. Thereafter, the samples were analyzed and the concentration of the corresponding PAHs was obtained.

BTEX was analysed 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 DB-5 capillary column coated with 95% dimethyl - 5% diphenyl polysiloxane (30m length x 0.32mm diameter x 1.0 µm film thickness) (Agilent Technologies). 1 µL of the samples was injected in split mode with split ratio 100:1 at an injection temperature of 125°C, oven was initially programmed at 35°C (held for 2 min) then ramped at 4°C/min to 50°C and then 10°C/min to 220°C, helium flow rate was set at 1.5 mL min<sup>-1</sup>, while the mass range was set at 35-260 amu and scan time at 0.6\*2 sec/scan.

#### *Statistical analysis*

Result was presented as mean and standard deviation. Comparison of differences in the activities of enzymes between parasitized and non-parasitized fishes was done using Student's t-test and significance was considered at 5% (p<0.05) level.

## RESULTS

#### *Prevalence of intestinal helminths in Heterotis niloticus*

A total of 100 fish specimen of *H. niloticus* were sampled for presence of parasites. Only one parasite species - *T. niloticus* was identified in all the infected fishes. The number of fish infected, prevalence of infection and the number of worms recovered are presented in Table 1. 36 fishes of the total sample are infected with parasite. It can be deduced from the result, an average prevalence parasite infestation of 72%, with, a mean intensity of 55.56, median intensity of 55.0 and a total worm load of 301. 100% infection prevalence was observed in the standard length group 55-59.9 cm and 75-79.9 cm. This may be

attributable to the random selection, while the 45-49.9 cm and 50-54.9 cm had 83% and 62% prevalence rates, respectively. Worm load observed was highest in the 60-64.9 cm length group of 93 worms counted, while the least group with worm load was the 75-79.9 cm length group.

#### BTEX in parasite

The values for the individual component of BTEX in the parasite *T. niloticus*, fish liver and intestine are presented in Figure 2. Total BTEX concentration in parasite was found to be  $47.45 \pm 7.68$   $\mu\text{g/g}$ . Benzene was observed to have the highest value, followed by toluene and m+p xylene. 1,4-dichlorobenzene had the least value while 1,2-dichlorobenzene and 1,3-dichlorobenzene were not detected in the parasite.

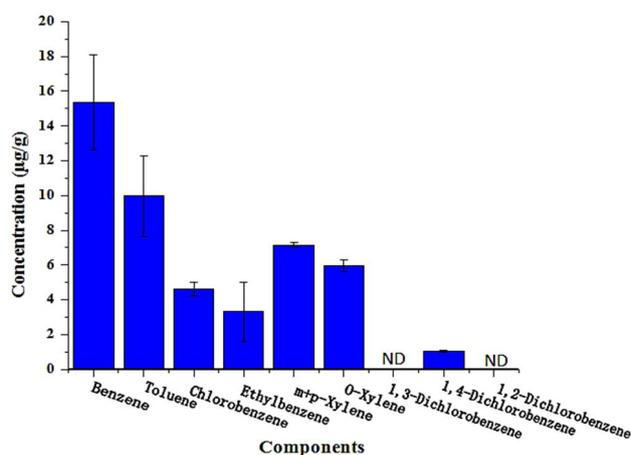


Fig. 2. Mean concentration of BTEX ( $\mu\text{g/g}$ ) in parasite.

**Table I.- Prevalence of helminthic infection in *Heterotis niloticus*.**

Standard length class	No. of fish	No. of infected fish	Prevalence (%)	Worm load
40-49.9cm	30	20	67	110
50-59.9cm	26	22	85	66
60-69.9cm	34	20	59	101
70-80cm	10	8	80	24
Sum	100	70	70	301

#### PAH in parasite

The different PAH congeners and their mean values as measured in the parasite *Teneuiseutis niloticus* are presented in Figure 3. Benzo(e)pyrene has the highest concentration of all the PAHs in the parasite, followed by fluoranthene. The following components of PAH, naphthalene, acenaphthylene, phenanthrene, benz(a)

anthracene, benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene were not detected in the parasite. Benzo(c)phenanthrene had the lowest concentration of all the PAH components. The sum of all the PAH congeners is also presented in Table I.

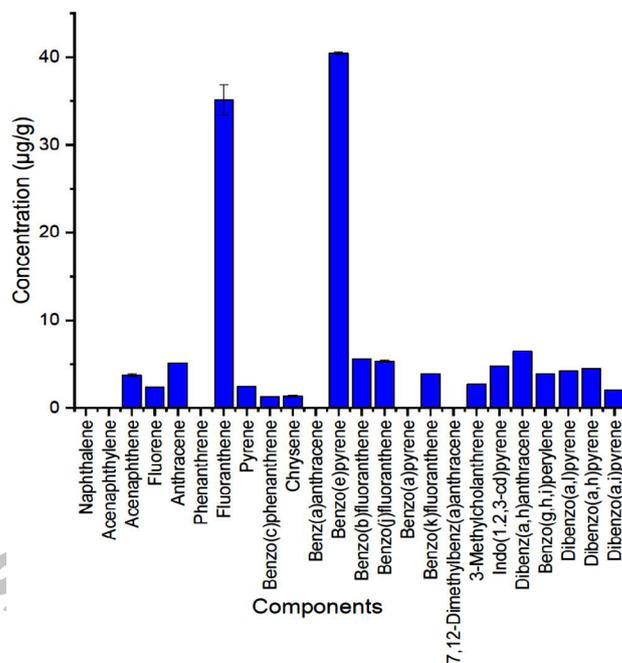


Fig. 3. Mean concentration of PAH ( $\mu\text{g/g}$ ) in parasite.

## DISCUSSION

The presence of parasites in fish may not be unconnected with the pollution state of the particular water body. The threats posed by parasites to fish cannot be overemphasized. Detrimental behavioural responses, reproductive damage, altered growth, mechanical and physiological damage, and inability of parasitized organ to function properly are some health effects of parasitism in fish (Iwanowicz, 2011). Aesthetic value of fish is also greatly reduced. In the present study, the intestinal parasite found in *H. niloticus* was an acanthocephalan- *T. niloticus*, a thorny-headed worm. Previously, researchers have reported the presence of acanthocephalans from freshwater fish (Oniye *et al.*, 2004; Ayanda, 2008). As reported by Akinsanya and Otubanjo (2006), it is possible that differences in geographic factors do not greatly affect the kinds of parasites that infect fish. The length group 30–39.9 cm recorded zero prevalence of infection. It has been reported (Roberts, 1978), that smaller fishes provide less surface area for infection as compared to bigger fishes. Fishes between the length category 50–64.9 cm had the highest parasite load. As reported by Khalil

(1969), infection with parasites is proportional to fish length. Bigger fishes probably provided larger surface area for parasite habitation and feeding. However, the random selection of fishes could also have played a role in this. There is a high overall infection prevalence rate of 72%. This may be a reflection of the pollution status of the Lagos lagoon. Human activities in the form of indiscriminate discharge of wastes and even transport activities may have contaminated the water. This would probably have led to increased contact between parasite and host as fishes desperately search for food. *T. niloticus* was the only parasite species observed to infect *H. niloticus* in this study. It may be a reflection of the abundance of the intermediate host of this parasite in the Lagos lagoon, making it easy for them to easily get into their definitive host.

BTEX and PAH have been reported to be present in parasite tissues in this study. This can be seen as an indication of the prevailing conditions in the body of the fish host, *H. niloticus* and also the habitat of the fish. The sources of introduction of these chemicals into the lagoon are not far-fetched. Lagos is a heavily populated and industrial area. Spillage from oil rig areas, as well as maritime transport are two important processes that have been implicated in water pollution by these chemicals. Additionally, atmospheric deposition into water can also be responsible (Obiakor *et al.*, 2014). As these chemicals are soluble in water, they can spread very rapidly, hence taken up by aquatic organisms.

Some BTEX and PAH congeners were not detected at all in the parasite, they may or may not be present in water and fish host. Of those that were detected, some were high while others were low. Important processes by which BTEX and PAHs in aquatic ecosystems degrade includes chemical oxidation, photooxidation and biological transformation by organisms. This process occurs at a very slow rate in aquatic environments when compared with the time it takes to degrade in the atmosphere. It has been said that the cycling of PAHs in aquatic environments, is poorly understood, as it is for other ecological systems (Neff, 1979).

It has been reported that parasites have ability to accumulate pollutants at levels higher than those found in their immediate surroundings, hence making them effective as sentinel organisms in environmental monitoring studies (Nachev and Sures, 2015). Sures (2001, 2003) reported that parasites show exceptional properties with regards to accumulating substances which have a high affinity for water, hence, making them even better accumulation indicators than conventional bioindicator organisms. Studies on the marine fish-acanthocephalan system (*Nothotenia coriiceps*-*Aspersentis megarhynchus*) have shown that the parasite concentrated higher amount

of pollutants in its body than the levels found in common accumulation indicators like the mussel, *Leternula elliptica* (Sures and Reimann, 2003). Similarly, more detailed studies on the freshwater fish-acanthocephalan systems further shows that acanthocephalans can be regarded as accumulation indicators since they are able to accumulate pollutants better than other parasites and other indicator organisms (Sures *et al.*, 1994, 1999; Sures, 2004). The parasite identified in the present study is also an acanthocephalan, further buttressing these previous reports of them being very good indicator organisms.

Interestingly, Akinsanya *et al.* (2019) in a similar study reported that the parasite *Wenyonia acuminata*, infecting *Synodontis clarias* in the same study area did not bioaccumulate BTEX from fish. This may be due to differences in bioaccumulation factors of chemicals in different parasites species. Fish parasitism is not a desirable condition, be it in fish farms or in the wild; however, that parasites are able to accumulate pollutants from fish when parasitizing them is a good phenomenon. Considering the health implications to man of the buildup of BTEX and PAH in fish, the ability for the parasites to accumulate these chemicals from fish body creates a situation whereby the risk to man when consuming these organisms is greatly reduced.

In conclusion, over the years, established sentinel organisms have enjoyed wide patronage in environmental bio monitoring and impact assessment studies. Further studies investigating the use of parasites as sentinel organisms not only in fish but in other aquatic organisms is encouraged as this will provide more information on pollutant accumulation. This undoubtedly will give a better understanding of the level of pollution in our aquatic ecosystems.

#### Statement of conflict of interest

The authors have declared no conflict of interests.

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