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Microbial diversity, heavy metals and hydrocarbons concentration in some fish species from Qua Iboe River Estuary, Akwa Ibom State, Nigeria

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Abstract

The diversity of fish gut microflora, heavy metals and hydrocarbon concentration of two fish species (*Pseudotolithus typus* and *Chrysichthys nigrodigitatus*) from Qua Iboe River Estuary was investigated. The total heterotrophic bacteria and fungi as well as hydrocarbon utilizing bacteria and fungi in the fish guts were abundant and ranged from 6.87 to 7.57logcfu/g, 5.68 to 5.80logcfu/g, 5.42 to 5.48 logcfu/g and 2.76 to 3.40logcfu/g respectively. The microbial counts varied among the fish species. *Proteus*, *Bacillus*, *Staphylococcus*, *Salmonella*, *Vibrio*, *Escherichia*, *Lactobacillus*, *Micrococcus* and *Enterobacter* were the different bacterial genera encountered in the study while *Penicillium* sp., *Aspergillus niger*., *Fusarium* sp. and *Mucor* sp were the fungal species identified. The analysis of the metals showed that Zn, Cu and Fe with concentrations ranging from 0.51 to 4.20mg/kg, 3.51 to 4.11 and 1.19 to 2.04mg/kg respectively were the most abundant and were higher than the maximum permissible limits set by FEPA/WHO. The total mean PAHs and BTEX concentrations were 62.18±4.72 ng/g and 43.67±4.06 ng/g for *C. nigrodigitatus*, 123.52±3.11 ng/g and 211.68±3.53 ng/g for *P. typus* respectively which were higher than acceptable limits. Findings from this study shows poor microbiological quality thus the need for continuous monitoring of our natural waters and proper processing of aquatic foods as the present situation portends a potential concern for ecological risk.

Keywords: Fish gut; Hydrocarbons; Heavy metals; Estuary; Microflora

1. Introduction

Estuaries are body of waters that forms a transition zone between river environments and ocean environments and are subject to both marine influences, such as tides, waves, and the influx of saline water, and riverine impacts, such as flows of fresh water and sediment [1]. Estuaries tend to be naturally eutrophic because land run-off discharges nutrients into estuaries. With human activities, land run-off also now includes the many chemicals used as fertilizers in agriculture as well as waste from livestock and humans. Excess oxygen-depleting chemicals in the water can lead to hypoxia and the creation of dead zones [2]. As an ecosystem, estuaries are under threat due to many anthropogenic activities such as pollution resulting from massive oil exploration, overfishing and other human activities. They are also threatened by sewage, coastal settlement, land clearance and much more. Land run-off, industrial, agricultural, and domestic waste enter rivers and are discharged into estuaries [3].

Aquaculture development in Nigeria is fast gathering momentum [4]. The need for more fish supplies in the market is seen in the increased importation of about 900,000 metric tons annually which is double the local production/catch, estimated at only 450,000 metric tons. Nigeria is Africa's biggest fish consumer [5, 6]. The brackish water zone of the Nigerian Coast which includes creeks, lagoons, rivers and mangrove swamps has tremendous potential for fish farming [7].

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Pseudotolithus typus is a species of croaker or bar, ray-finned fish in the family Sciaenidae [8]. It inhabits coastal waters from shorelines to at least 70 m, but may be more common in deeper waters to 150 m. It is found over muddy and sandy bottoms, uncommon in rocky areas, it feeds on worms, crustaceans and small fishes and spawns off estuaries [9]. Silver Catfish, *Chrysichthys nigrodigitatus*, of the family Claroteidae plays a pivotal role in the ecology and fisheries of Nigeria in particular, and West Africa at large. It is a highly valued food-fish in Akwa Ibom State, Nigeria and other West African countries [10].

The Niger Delta region with its complex ecological form is being subjected to considerable environmental pollutants from agricultural, industrial and domestic activities as well as oil exploration and exploitation. This has resulted in the release of pollutants (hydrocarbons and heavy metals), capable of polluting the terrestrial and aquatic ecosystems [11, 12, 13]. Heavy metals have been reported to have negative effect on metabolic processes in general and may influence the nutritional and biological status of aquatic resources [14, 15]. The contamination of the aquatic system with heavy metals has been on the increase since the last century due to industrial activities [16].

It has been proven that fishes absorb dissolved elements and heavy metals from surrounding waters and ingested food. Although these metals and pollutants are found in traced quantities in the water environment, their ability to bioaccumulate is of interest to researchers as they affect both human and environmental health [17]. Seymore, [18] found that these pollutants accumulate in various fish tissues in significant amounts, eliciting toxicological effects at critical targets. Polycyclic aromatic hydrocarbons (PAHs) and Benzene, Toluene, Ethylene and Xylene (BTEX) are the petroleum hydrocarbons causing adverse effects in aquatic organisms as they are capable of triggering multiple disturbances at varying biological organization levels in the aquatic environment [19, 20]. The entry of BTEX and PAHs into the aquatic food chain via the process of bioaccumulation results in morbidities and mortalities.

Nutritional and environmental factors affects the population level of microbes associated with the guts of hydrobionts. Thus, estimation of the abundance and dynamics of autochthonous and petroleum hydrocarbon-degrading bacteria in the fish gut enables evaluation of participation of such bacteria in biodegradation of pollutants and in the process of self-purification of water [21]. This is because the microflora of the digestive tract of aquatic animals is proved to be the first to be affected by any pollutants appearing in water [21]. Petroleum and its products, apart from being toxic to the majority of bacteria in aquatic ecosystems, are sources of carbon and energy to some bacteria. The result of such an effect is a community of microorganisms with altered species diversity enriched in microorganisms degrading hydrocarbons [22].

Knowledge about the fish gut microbiota, heavy metals as well as hydrocarbon bioaccumulation in the fish gut would help to understand the disturbances and risks of consumption of these species of aquatic resources. Therefore, this study examined the abundance of total heterotrophic and crude oil utilizing bacteria and fungi as well as the heavy metal and hydrocarbon properties of some fishes harvested from Qua Iboe River Estuary.

2. Material and methods

2.1. The study area

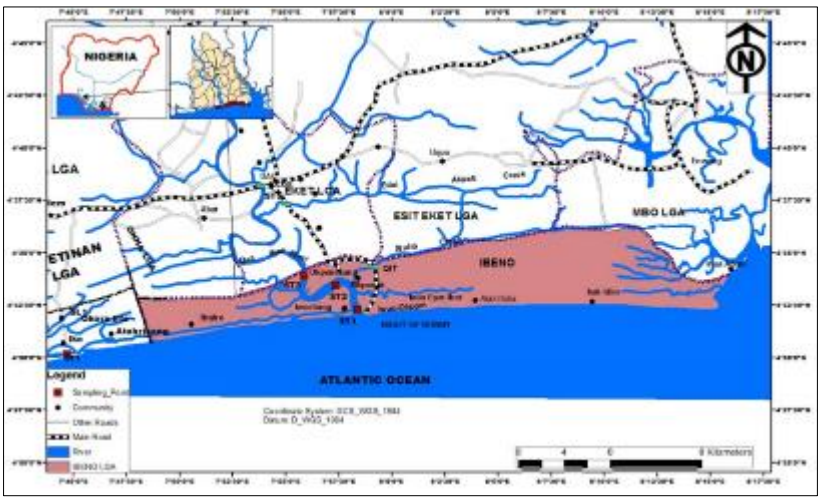


Figure 1 Map of Qua Iboe River Estuary and the sampling points

Qua Iboe River estuary is located in Ibeno Local Government area of Akwa Ibom State South-South flank of Nigeria between latitudes 4°25' and 4°38' North and longitudes 7°48' and 8°05' East (Figure 1). It is one of the most important river systems in the Niger Delta, providing nursery and breeding ground for a large variety of fish. Fishing in the estuary is intense and catch per unit effort is low. Due to the nation's efforts towards speedy industrialization and other human activities, the river is degraded. Fishing is carried out indiscriminately with various traditional and modern fishing gear [23].

2.2. Media preparation and sterilization of consumables

All the media used in this study which included Nutrient Agar, Sabouraud Dextrose Agar and Mineral Salt Medium were all prepared according to manufacturers' instructions. All preparations were done using sterile distilled water and these were adequately sterilized as described below before dispensing into Petri dishes. All the consumables and glass wares such as nutrients, test tubes, beakers, conical flasks, rubber bums, and beaker were autoclaved at 121 ^{o}C and pressure of 15 psi.

2.3. Fish samples collection

20 samples of two different species (*Chyrisicthys nigrodigitatus* and *Pseudotolithus typus*) were purchased from the commercial landings of artisanal and subsistence fishers that land their catches at the water in Qua Iboe River Estuary located in South-Eastern coast of the Niger Delta region of Nigeria. The samples were carefully sorted out, separately contained in sterile polythene bags sealed, labeled and preserved in an ice packed boxes. The samples were immediately within (2- 3 hours of sampling) transported to the laboratory for. representative samples of the fishes were authenticated in the Department of Fisheries and Aquaculture, University of Uyo. The nomenclature of the species conformed to those of Schneider, [24] and Edwards *et al.* [25].

2.4. Microbiological Analysis

2.4.1. Processing of samples

The fishes were processed within 8 hours in order to reduce the change in composition of gut microbial communities over time and with perturbation [26]. Healthy live fish samples were killed by hitting hard object on the head. Before dissection, 70% ethanol was applied to the body surface of the fish samples. The fishes were then dissected with individual-use insect pins or individual-use scalpels and forceps depending on the fish size and their gastrointestinal tracts dissected under sterile condition. Thereafter, 1.0 g of gut contents were homogenized in separate 9 mL of sterile normal saline from which 10-fold serial dilution of each of the sample was carried out using sterile normal saline (0.85% Nacl). Then, 1.0 mL portions of diluted samples were used for isolation of bacteria and fungi.

2.4.2. Determination of microbial densities of fish gut samples

Several methods and media were used for the enumeration of the various microbial groups. The densities of the following microbial groups were determined; Total heterotrophic bacterial counts (THBC), Total heterotrophic fungal counts (THFC), Crude-oil utilizing bacteria (CUB) and Crude-oil utilizing fungi (CUF).

The basic analytical media employed in the course of this research included: Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA) and Mineral Salt Medium (MSM) of Mills *et al*. [27]. The media were prepared according to manufacturer's recommendations (Difco, Biotech). The density of total heterotrophic bacteria was determined by the pour plate techniques using nutrient agar (NA). The NA medium was amended with nystatin (50 μgml^{-1}) to prevent the growth of fungal contaminants. The total fungal count was determined by pour plate technique using Sabouraud dextrose agar (SDA) supplemented with streptomycin (50 μgml^{-1}) to inhibit the growth of bacteria [28, 29]. Inoculated NA plates were incubated at 28 ^{o}C for 24 hours, while the SDA plates were incubated at room temperature for 3 to 5 days before enumeration of microbial colonies.

The density of crude oil-utilizing bacteria and fungi were determined using pour plate methods [27] using vapour phase transfer technique of Amanchukwu *et al*. [30] on mineral salt agar (MSA) and incubated at (28 ± 2 °C) for 7 days. Discrete colonies that appeared on the culture plates were enumerated with the aid of a Quebec colony counter and recorded as Colony Forming Units (CFU) per gram of fish sample.

2.4.3. Characterization and Identification of Microbial Isolates

The pure colonies obtained from the samples were characterized using standard biochemical procedure as described by Bergey's Manual of Determinative Bacteriology, [31]. The colonies were subjected to Gram's stain and standard

biochemical tests; catalase, coagulase, citrate, indole, MR/VP, motility, spore, sugar fermentation, urease. Fungal isolates were identified according to the method of Barnett and Hunter, [29].

2.4.4. Determination of heavy metals in fish gut samples

Dry tissue samples weighing 2.0 g were digested with 6 ml of concentrated nitric acid (HNO_3) and 1 ml of 30% hydrogen peroxide (H_2O_2). The digestion was carried out in a microwave digester using microwave digestion. The completely digested samples were filtered using what-man filter paper and diluted to 25 ml in volumetric flask with distilled water.

The filtrates were analyzed using the atomic absorption spectrophotometer (AAS Model AA320N). The spectrophotometer operational setting was done in compliance with the manufacturer's instructions and was calibrated with analytical grade metal standard stock solutions (1 mg/dm3) in triplicates. The extracted samples were each analyzed for Lead (Pb), Nickel (Ni), Manganese (Mn), Zinc (Zn), Cadmium (Cd), Iron (Fe), Copper (Cu) and Silver (Ag). Stock solutions of 1000 ppm for each metal were prepared from analar grade of the granulated metal salts of high purity (99.9%). Calibration curves were obtained with optimized instrument conditions [32, 33].

2.4.5. PAHs, BTEX and THC Extraction and Analysis

Sample Extraction and Analysis: In the laboratory, the samples were processed by several methods to extract and analyze PAHs. Extraction and cleanup procedures have been described in detail elsewhere [34]. Fish samples were first freeze-dried, then soxhlet extracted with n-hexane. Prior to extraction, each sample and blank was spiked with a range of deuterated PAH compounds (acenaphthene-d10, anthracene-d10, fluorened10, chrysene-d12, and perylene-d12) to monitor analytical recovery. The extracts were reduced in volume on a rotary evaporator, solvent exchanged to hexane, and interfering compounds removed by column chromatography using 10 g silica and 5 g alumina (and 0.5 cm anhydrous Na_2SO_4 at the top of the column to prevent the column from contact with air) and eluting the compounds of interest with 100 mL 1:1 mixture of hexane: DCM. Sulfur compounds in sediment samples, which interfere with later analysis, were removed by soaking the extracts with activated copper powder. The sample extracts were analyzed using an Agilent GC 8860A using splitless injection on a 30m HP5-ms column (0.25mm i.d., 0.25µm film thickness) and helium as carrier gas.

For the oven program of PAH analysis; the inlet temperature was set at 250 ^oC and the detector temperature at 300^oC, oven equilibrium time was 1minute while oven maximum temperature was set at 300 ^oC and ambient temperature at 25 ^oC. Initial temperature was 70 ^oC for 2 mins. Then ramped up at 10 ^oC/min until final temperature of 220 ^oC, the final time was 33 mins and the total run time was 50 mins. This was coupled to an Agilent 5977 GC-Mass Selective Detector (MSD) operated in electron impact (EI) mode using selected ion monitoring (SIM). PAH concentrations in fish gut were calculated by dividing the amounts in extracts by the actual weight of sediment or fish gut extracted after adjusting for moisture (on a dry weight). The oven programs for THC and BTEX are as shown below;

THC: The inlet temperature was 250 ^oC and detector 300 ^oC. The initial temperature was set to 70 ^oC and held for 2 mins, then ramped up at 10 ^oC/min to final temp of 300 ^oC at final time of 20 mins. Total runtime was 47mins. All other oven programs remained the same.

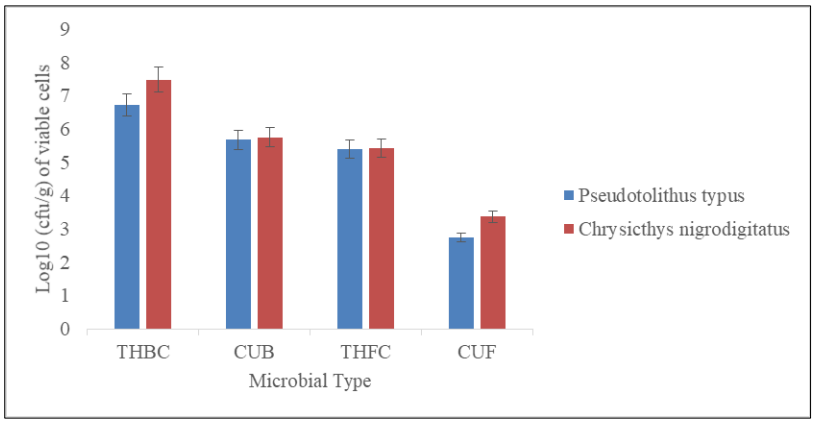
BTEX: The inlet temp was set at 180 ^oC and detector at 180 ^oC. The initial temperature was set to 35^oC for 5mins, then ramped up at 5 ^oC/min to 70 ^oC, then held for 1 min and ramped up again at 15 ^oC/min to final temp of 180 ^oC at final time of 10 mins. The total runtime was 30.33 mins.

3. Results

3.1. Microbiological Properties and diversity of Sampled Fishes

The results presented in figure 2 showed that the ability of the fishes to accumulate microbes in the gut varied between the fish species analyzed. The total heterotrophic bacteria, crude oil utilizing bacteria, total heterotrophic fungi and crude oil utilizing fungi for both *Pseudotolithus typus* and *Chrysichthys nigrodigitatus* were abundant and ranged from 6.87 to 7.57log cfu/g, 5.68 to 5.80log cfu/g, 5.42 to 5.48log cfu/g and 2.76 to 3.40log cfu/g respectively. *Chrysichthys nigrodigitatus* had higher microbial counts in all samples and microbial types examined.

The culturable bacterial genera in the gut of fish as presented in Table 1 were identified as *Proteus*, *Lactobacillus*, *Salmonella*, *Staphylococcus, Micrococcus, Escherichia*, *Vibrio, Bacillus* and *Enterobacter*. Table 2 shows the fungal isolates in the fish gut samples identified as *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., and *Mucor* sp.



THBC = Total Heterotrophic Bacterial Counts, CUB = Crude oil Utilizing Bacteria, THFC = Total Heterotrophic Fungal Counts, CUF = Crude OIL Utilizing Fungi.

Figure 2 Microbial counts in fish gut samples (cfu/g)

Table 1 Morphological, cultural and biochemical characteristics of bacteria isolated from the fish gut samples

Morphology	Gram's reaction	Catalase	Oxidase	Motility	Citrate Spore stain	Indole	Methyl red	Voges proskauer	H_2S production	Starch hydrolysis	Urease	Glucose	Galactose	Sucrose	Fructose	Lactose	Maltose	Mannitol	Probable organism
R	-	-	-	+	+ -	-	+	-	+	+	+	+	-	+	+	-	+	+	*Proteus sp*
R	-	+	-	+	+ -	-	-	+	-	+	-	+	-	+	+	+	+	+	*Enterobacter aerogenes*
R	-	+	-	+	+ -	+	-	+	+	-	-	+	-	+	+	+	+	+	*Vibrio sp*
R	-	+	-	+	- -	-	+	-	+	+	-	+	+	-	+	-	+	+	*Salmonella sp.*
C	+	+	-	+	+ -	+	-	-	-	+	+	+	-	+	-	-	-	-	*Micrococcus sp*
R	+	+	-	+	- +	-	+	+	-	+	-	+	-	-	+	-	-	-	*Bacillus sp*
R	+	-	+	+	+ -	-	+	-	+	-	-	+	-	-	-	-	-	-	*Lactobacillus sp*
R	-	+	-	+	- -	+	+	-	-	+	-	+	+	+	-	+	-	+	*Escherichia coli*
C	+	+	+	-	+ -	-	+	+	-	+	+	+	+	+	+	+	+	+	*Staphylococcus aureus*

Key: R = Rod, C = Cocci

Table 2 Morphological Characteristics of Fungi Isolates from the fish gut samples

Isolates	Cultural characteristics	Morphological features	Microscopy	Probable organism
F1	Yellowish-green mycelium	Conidia in long chains, branched cells	Branched smooth conidiophores, brush-like conidia head	*Penicillin* sp
F2	White to greenish grey with edge	Smooth walled erect conidiophores	Septate, Dome gradually Enlarging	*Aspergillus niger*
F3	White and pale Grey	Sporangiophores	Unseptate, Erect Sporangiophore	*Mucor* sp.
F4	White cottony with felty colony	Macro-conidia with light periphery in chains	Septate hyphae with branched conidiophores	*Fusarium sp*

3.2. Heavy Metals levels of the Fish Samples

Heavy metals load in the fish gut samples is presented in figure 3. The result showed that *P. typus* had higher heavy metal accumulation in their guts compared to *C. nigrodigitatus*. The mean concentration of heavy metals for Zn, Cu and Fe in the two fish species were 2.10 ± 0.02, 4.80 ± 0.06 and 4.60 ± 0.05 for *P. typus* and 1.27±0.07, 0.57±0.00 and 3.81±0.00 for *C. nigrodigitatus* respectively. The mean concentration of total hydrocarbon content (THC), Polycyclic Aromatic Hydrocarbons (PAHs) and Benzene, Toluene, Ethylene and Xylene (BTEX) is as represented in figure 4. The accumulation of hydrocarbons in the fish gut samples showed that *P. typus* recorded higher concentrations in THC (634.43±3.28 ng/g), PAHs (123.52±3.11 ng/g) and BTEX (211.68±3.53 ng/g) while the concentration of THC, PAHs and BTEX in *C. nigrodigitatus* were 627.48±6.27 ng/g, 62.18±4.72 ng/g and 43.67±4.06 ng/g respectively.

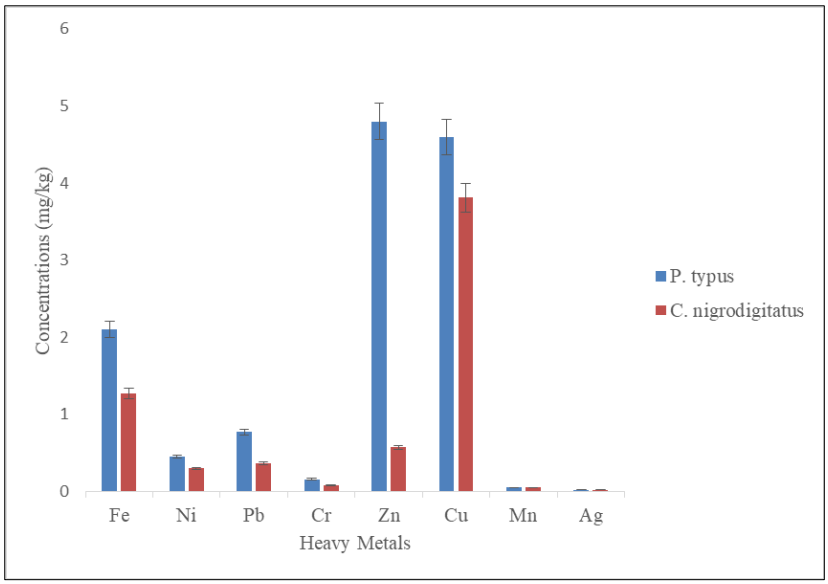


Figure 3 Mean concentration of heavy metals in fish gut samples

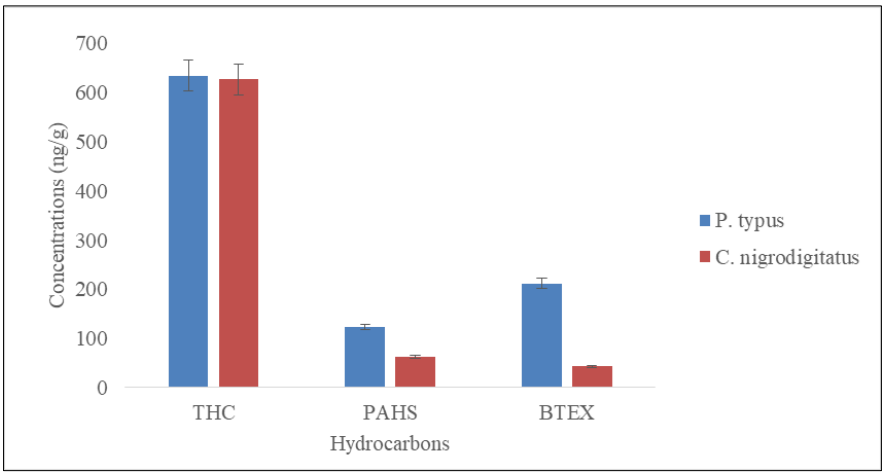


Figure 4 Mean concentration of hydrocarbons in fish gut samples

4. Discussion

According to Shewan [35], the microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish live and not of the fish species. Fish, because of their soft tissues, aquatic environment, high water content, neutral pH and high amino acids are extremely susceptible to microbial contamination and spoilage [36]. The results obtained from this study have shown that the fish gut samples were laden with microbial contaminants including pathogenic groups of microorganisms as well as hydrocarbon degrading microorganism. This is in consonance with the reports of other researchers that reported high number of organisms in the gut of fishes as it provides favourable ecological niches for these organisms [21, 37, 38].

Heterotrophic counts are representative of a small group of active bacteria that react immediately to changes in nutrient supply [39]. The stability of the ecological balance of microflora of the digestive tract of aquatic organisms depends upon the trophism, productivity, toxicity and other chemical and physical parameters of water bodies [40]. This explains why the viable counts of different types of bacteria in the digestive tract varied with fish species. The high bacterial contaminants recorded for the fin-fishes was expected and is in agreement with previous report by Ajayi, [41] and Umana *et al.* [42], who in his study reported a high bacterial population in catfish from fish pond and river respectively.

Microbial Species reported from this work has been isolated from the gut of various fishes [37, 38]. There are several possible sources for the establishment of intestinal gut flora and it is generally believed that the processes of bacterial colonization in fish are complex and depend upon the bacterial flora of live feed and water. *Enterobacter* spp are common in the digestive tract of freshwater and marine fishes and have been found in the gut of rainbow trout and Yellow grouper (*Epinephelus awoara*) [43, 38]. Previous studies of Essien *et al.* [44] and Fred *et al.* [45] had shown strains or species of fungal species isolated in this work e.g *Penicillium* sp, *Aspergillus flavus* and *Cladosporium* sp.

The presence of hydrocarbon-degrading bacteria in the gastrointestinal tract of fish is not surprising since microorganisms have the ability to utilize hydrocarbons [21]. The abundance of hydrocarbon-degrading bacteria in water and in fish digestive tract reflects the degree of contamination of the ecosystem with oil and its products. The ability of intestinal bacteria of aquatic organisms to use petroleum hydrocarbons as a source of carbon indicates that aquatic organisms participate in biodegradation of oil pollutants as well as self-purification of water [21].

The high concentration of heavy metals may be due to agricultural, industrial, domestic as well as other anthropogenic activities such as oil exploration, exploitation and refining which can introduce these metals into the water body and eventually sediment from where fauna feed [11, 46]. Fe, Zn and Cu levels recorded in the fish gut samples in this study were higher when compared to maximum permissible limits and the consumption of these species could cause health hazard to man, hence people are advised to make moderate use of these aquatic fauna to forestall chronic exposure to these pollutants [47, 48]. The mean concentrations of Cr, Pb, Ni and Ag were below the recommended limits, hence these metals may not be an immediate problem in Qua Iboe River estuary but may pose a lot of environmental problems if left unchecked.

The petroleum hydrocarbons causing adverse effects in aquatic organisms are considered to be BTEX and PAH [19]. The presence of these chemicals in fish pose some problems for normal functioning of the fish. The branchial tissue regulates water, ions and the balance of acid-base in fish and this organ has direct contact with water [49]. This may mean that the fish have taken up these chemicals from water. The effect of this bioaccumulation would even be higher in fish eating birds and other animals. Furthermore, some other aquatic animals may have accumulated these chemicals in their bodies from other habitats, and have overtime migrated to the water body sampled in this study. The concentration of PAHs and BTEX recorded in this work is similar to those reported by other researchers [50, 51]. In their study, Akinsanya *et al.* [52] reported a concentration of 49.86±7.30 µg/L and 173.34±4.677 µg/g for BTEX and PAHs respectively as they bioaccumulate in the intestine of *Heterotis niloticus*. The presences of PAH in commercially sold fish was previously reported by Benson *et al.* [53].

5. Conclusion

The result of this study have revealed that the two fish species harbour a high population of diverse microbes including pathogenic strains of bacteria that are commonly associated with human and infant gastroenteritis as well as food poisoning and intoxications. The abundance of total heterotrophic bacteria fluctuated among fish species. The differences in results may be due to the differences in feeding efficiency of fishes. The preponderance of coliform and hydrocarbon degrading bacteria in the guts of the investigated fish species reflects the degree of contamination of the ecosystem with wastewater and petroleum hydrocarbon respectively. Most of the bacteria found in the intestinal content are potential pathogens indicating that fish digestive tract is a reservoir of many opportunistic pathogens. The presence of heavy metals, BTEX and PAHs in fish samples from Qua Iboe River Estuary, Akwa Ibom is established in this study, although not all detected in levels above allowed limits. Anthropogenic activities have been thought to contribute largely to the pollution of the water body. The results of this study call for proper processing of aquatic foods obtained from the apparently contaminated water body.

Compliance with ethical standards

Acknowledgements

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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