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Bacterial Contaminants and Heavy Metal Accumulating Potentials of Fin-Fishes (*Synodontis obesus* and *Marcusenius senegalensis*) from Humic Freshwater

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The bacterial contaminants and heavy metal accumulating potentials of fin-fishes (*Synodontis obesus* and *Marcusenius senegalensis*) from the humic ecosystem of Eniong River, Akwa Ibom State were investigated. The results obtained revealed that the bacterial loads varied with the type of fin-fish and were much higher in fish intestines, when compared with the skin and gills. The heterotrophic bacterial loads obtained exceeded the 1.2 x 10^5cfu/g limit recommended for fresh fishes. High and unsafe fecal coliform (1.1 ± 0.1 x 10^3cfu/g -2.0 ±0.39x 10^3cfu/g) loads were also obtained. Heavy metal analysis also revealed the presence of Cd, Cr, Cu, Ni and Pb in the humic sediment. Concentrations of Cd (4.71 ± 0.34 to 4.91± 0.39 mg/kg), Cr (18.06 ± 5.78 to 20.22 ± 1.11 mg/kg), Cu (35.33 ± 3.25 to 40.28 ± 2.44 mg/kg), Ni (2.16 ± 0.07 to 2.26 ± 0.18 mg/kg) and Pb (175.85 ± 7.75 to 191. 08 ± 20.11 mg/kg) were found in the order Pb>Cu>Cr>Cd>Ni. Sequential extraction method (SEM) of analysis revealed the poor bioavailability status of heavy metals in sediment. It also showed that the percentage of bio-available and non-bio-available fractions of

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metals in sediment varied with the type of metals. Cu with 62.04% availability rate was the most bio-available element, as against Pb with 25.22% availability rate. These correspond to their 32.4% and 65.2 % residual potency rates. The calculated Biota to Sediment Accumulating Factor values for heavy metals in the fin-fishes revealed varied levels of accumulation in fishes. Cu (3.73±1.39 mg/kg) in *Synodontis obesus* was the most accumulated. However, analysis of the bio-accumulation factors (BCF values) revealed generally low accumulation determined by fish type as well as the metal fractions and bio-available status. The results indicate the poor microbiological quality and poor potential of the fin-fishes as sentinel organism for metals contamination monitoring. These call for proper processing of aquatic foods as well as routine monitoring (but with alternative sentinels) to arrest the growing influence of anthropogenic activities on the level of heavy metals in Eniong River.

Keywords: Heavy metals; freshwater; fin-fishes; bacterial contaminants and humic.

1. INTRODUCTION

In recent years contamination of aquatic environment by metals has risen as a result of increased industrial activities and attendant population surge especially around littoral zones which directly influences the quality of domestic wastes laden with heavy metals. Despite the natural sources of heavy metals in the environment, anthropogenic supply to aquatic ecosystems from industrial effluents/wastes, agricultural and domestic waste waters laden with metal toxicants outweighs the former. Heavy metal pollution is an important environmental problem [1], considering that some are hazardous substances and can bioaccumulate in the environment, plant and animal tissues [2].

Heavy metals enter aquatic environment from natural and human activities [3]. Due to industrialization, the number of factories and pollution has increased rapidly. The contamination of water bodies with a wide range of pollutants has become a matter of concern over the last few years [4]. The natural aquatic ecosystems have extensively been contaminated with heavy metals released from domestic, industrial and other man-made activities [5]. Sediments are an important sink for trace metals especially in river mouth ecosystems. In some cases, sediments may contain 99% of the total amount of trace metals existing in aquatic systems [6]. It is known that metals accumulate on sediment surface, in benthic living things, planktonic organisms and other living matter and is enhanced through food chain. Fish accumulate xenobiotic compounds, especially those with high water solubility because of the very intimate contact with the medium that carries the compounds in solution, suspension and also because fish have to extract oxygen from the medium by passing the enormous volumes of water over gills. For fish, skin and digestive tract are potential sites of absorption of water soluble chemicals. The chemical once absorbed is transported by the blood to either a storage point, such as bone or to the liver for transportation. If transported by the liver it may be stored there, excreted in bile or passed back into the blood for possible excretion by kidney or gills or stored in extra hepatic tissues such as fat [4].

Among the many pollutants, heavy metals show environmental persistence, toxicity at low concentration and ability to incorporate into food chain of aquatic organisms [7]. Due to the deleterious effect of metals on aquatic ecosystem, it is necessary to monitor their accumulation in fishes. The higher the metal concentrations in the environment the more it may be taken up and accumulated by fish [8]. They emphasized that tissue metal level is related to its waterborne concentration only if metal is taken up by the fish from water. The trophic transfer of trace metals from water to aquatic animals of high trophic levels has been reported [9]. Ikemoto et al. [10] reported that significant trophic levels dependence was found in concentrations of Se, Rb and Hg at Hau River in Vietnam. The same researchers also revealed that the bio-magnification profiles of trace metals (Mn, Cu, Zn, Sr, Mo, Ag, Cd, Sb, Cs, Ba, T1 and Pb) were significantly higher in crustaceans, whereas fishes showed higher concentrations of Cr, Pb and Hg). Their findings showed variations in the metal accumulating potentials of diverse fish forms and species in aquatic systems.

Additionally, there are many distinct habitats in the freshwater ecosystem and each is characterized with its communities of microorganisms. For example, the humic freshwater sediment comprises larger amounts of organic deposits in the seafloor, and the source of these humic components or materials may be from the accumulation of dead plants and animals of the lake or stream, which on decomposition settles at the bottom of the water,

thereby forming the river-bed sediment [11]. This river-bed sediment provides a nutrient-rich dwelling ecosystem for bottom animals, as well as other microorganisms. Recent reports by researchers indicate that the freshwater bottom sediment is highly characterized with various bacterial species which may include those of the genera: *Pseudomonas, Bacillus, Azotobacter, Micrococcus, Enterococcus, Acromobacterium, Salmonella, Shigella, Enterobacter, Citrobacter, Flavobacterium* and *Escherichia* species. While the fungal species commonly isolated include those of the genera, *Penicillium, Aspergillus, Candida, Fusarium, Geotricum,* and *Saccharomyces* species respectively [12]. These microorganisms play important roles during the mineralization of complex organic and other toxic chemical pollutants present in the freshwater sediment.

Heavy metals impact both the physiology and ecology of microorganisms [13] and are known to inhibit a broad range of microbial processes including methane metabolism, growth, nitrogen and sulphur concentration. Metals generate many of their deleterious effects through the formation of free radicals, resulting in DNA damage, lipid per oxidation and depletion of protein sulphydryl [14]. In response to toxic concentrations of heavy metals, many organism including microorganisms can develop tolerance [15], resulting in the detoxification of such heavy metals. The development of heavy metal tolerance by microorganisms presents the possibility of utilizing and optimizing microbial mediated reactions as a strategy for removing metal contaminants from the environment. In addition, environmental components may have considerable influence on toxicity and therefore apparent toxicity.

On the other hand, bacteria are known to be ubiquitous in nature and they inhabit most of our food products including fin-fishes. Vibrios of sea-food origin have attracted increasing attention from time to time as it is found to be one of the important causes of food poisoning in man. The majority of outbreaks have also been epidemiologically traced to the consumption of fishes and shellfishes originating from warm coastal waters [16]. Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the exposed individual. They are often bacterial species that are facultative pathogens of both fish and man and may be isolated from fish without apparent symptoms of disease. The infection source may be fish kept either for food or as a hobby [17].

Studies have also been conducted on the heavy metal concentrations in fishes from rivers in Nigeria. The presence of unacceptable levels of Hg and Pb in the tissues of the African catfish, *Clarias gariepinus* from River Niger has been reported [18]. Omoregie et al. [19] also reported enhanced levels of Pb, Cu and Zn in *Oreochromis nilotica* (Nile Tilapia) from River Delimi. However, literatures on elemental burdens in fishes from humic freshwater ecosystem are not available and little or no work has been done on the bioaccumulation of pathogenic bacterial loads and heavy metals in fin-fishes from a humic ecosystem. This is despite the incessant cases of crude oil pollution in the Niger Delta of Nigeria. Therefore this study is focused on bacterial contaminants and heavy metal accumulating potentials of fin-fishes (*Synodontis obesus* and *Marcusenius senegalensis*) from humic freshwater.

2. MATERIALS AND METHODS

2.1 Study Area

The study area is a humic ecosystem of Eniong River, a tributary of the middle course of the Cross River located in South-Eastern coast of the Niger Delta region of Nigeria (Fig. 1). The freshwater ecosystem is unique and the river is characterized by intense colouration due to the presence of humic substances and possibly soluble iron. The ecosystem is home to diverse species of fish resources and supports remarkable populations of fin-fishes including *Synodontis obesus* and *Marcusenius senegalensis* that are widely consumed by the catchment communities in Itu Local Government Area of Akwa Ibom State.

2.2 Sample Collection and Preparation

Twenty samples of four different fish species (*Synodontis obesus*, *Clarias gariepinus*, *Coptodon guineensis* and *Marcusenius* senegalensis) were collected during harvest from fishers from Eniong River. 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 analysis. Representative samples of the fin-fish stocks collected were also taken to the

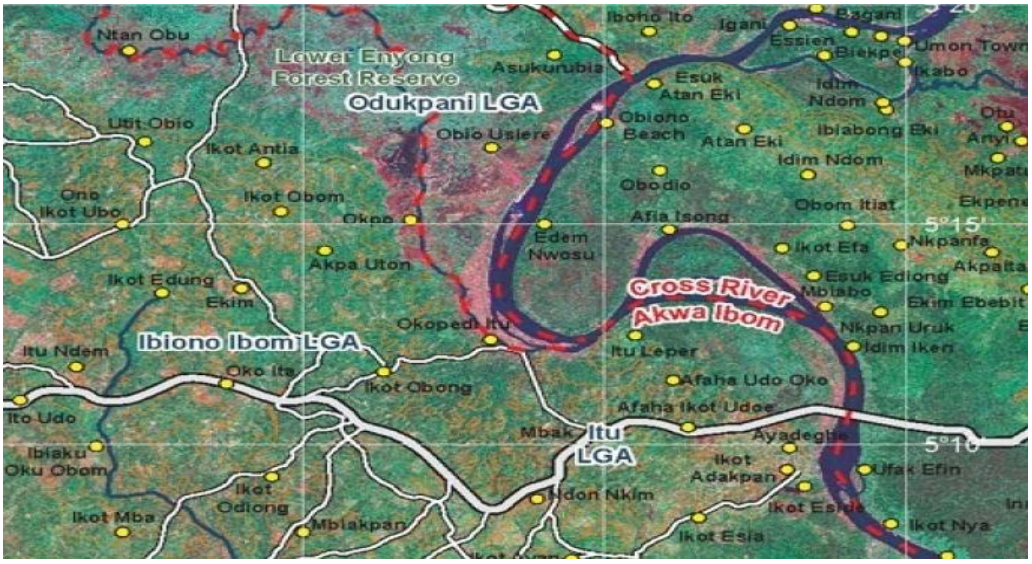

Fig. 1. Location of the humic freshwater Eniong River in Itu Local Government Area where the fishes were harvested

Department of Fisheries, University of Uyo for identification. Also collected were sediment sample with the aid of a metal grab sampler, samples were collected from five different locations, and was stored in clean glass bottles, preserved in iced packed coolers and transported to the laboratory for analysis.

In the Laboratory, the fin fishes were aseptically dissected. The organs (skin and intestine) were removed and macerated using a sterile pestle and mortar. One gram (1.0 g) of each organ sample was serially diluted and used for microbiological analysis. The remaining muscle tissue was placed in a drying oven at 60°C for 4 hours. The dried tissues were then reduced into fine powder in a pestle and mortar, also the sediment samples were digested using ultra pure Nitric acid and heavy metals determined using Atomic Absorption Spectrophotometer (AAS).

2.3 Analysis of Bacterial Contaminants

2.3.1 Serial dilution of fish samples

This procedure was carried out to enhance the enumeration of the bacterial load of the samples. Tenfold serial dilution of 1.0 g of gills, tissue and intestine of each representative fish sample was carried out as described by Cheesbrough (20). Here, 1.0 g of each sample was added to 9 ml sterile water then sequentially diluted to obtain the required dilution.

2.3.2 Culture media preparation and sterilization

The media used for the study were: Nutrient Agar (NA), MacConkey Agar (MCA), Eosine Methylene Blue Agar (EMBA) and Salmonella–Shigella agar (SSA) for the enumeration and isolation of heterotrophic bacteria, total coliform, feacal coliform (*Escherichia coli*) and Salmonella and Shigella species respectively. They were aseptically prepared according to the manufacturer's instructions, sterilized by autoclaving at 121°C for 15 minutes.

2.3.3 Estimation of bacterial contaminant

The density of heterotrophic and potential pathogens was determined using standard analytical procedures. *Staphylococcus aureus, Escherichia coli* (fecal coliform) and Salmonella and Shigella loads on the samples was determined using the pour plate technique. All inoculated plates were incubated at 37°C for 24 hours.

After 24 hours, 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 Characterization and Identification of the Bacterial Isolates

The pure bacterial isolates were grouped into recognizable taxonomic units and

characterized to their generic level using standard procedures. The pure isolates were examined for colonial morphology, cultural and biochemical characteristics according to the methods of Cowan (1985) and Chessbrough, (2006).

2.5 Determination of Heavy Metals Contaminants

2.5.1 Analysis of heavy metals in fin fishes

The analysis of heavy metals was carried out using the method of atomic absorption spectroscopy [21]. Only the fish muscles were used for this analysis. Atomic Absorption Spectrometry (AAS) is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest.

In this study, the samples (fish and sediment) were digested with ultra-pure nitric acid at 100°C until the solution becomes clear. Then the solution were made up to a known volume with deionized distilled water and analyzed for heavy metals (Cadmium, Chromium, Copper, Nickel and Lead) using Atomic Absorption Spectrophotometer (AAS model GPC A932 ver. 1.1). The result obtained was expressed as mg/kg wet weight.

Plate 1. Scientific name: *Marcusenius senegalensis*
English name: Long mouth
Local name: Ono

Plate 2. Scientific name: *Synodontis obesus*
English name: Upside down cat fish
Local name: Ikon Ikon

2.5.2 Determination of heavy metal accumulation using bioaccumulation factors

Bioaccumulation factors (BAFs) are multipliers used to estimate concentrations of chemicals that can accumulate in tissues through any route of exposure. It is referred to as bioconcentration factor (BCF) for aquatic invertebrates. The BCF and biota to sediment accumulation factor (BSAF) of heavy metals from sediment or surface water to animal tissues can be determined in different samples using the following equations:

$$\text{BCF} = \frac{\text{concentration of heavy metal in animal tissue}}{\text{concentration of heavy metal in water sample}} \quad (1)$$

$$\text{BSAF} = \frac{\text{concentration of heavy metal in animal tissue}}{\text{concentration of heavy metal in sediment sample}} \quad (2)$$

2.5.3 Sediment characterization

The major sediments constituents considered in this study included total organic carbon, silt, clay and sand. The preparation, extraction and quantitation of benthic sediment samples for the determination of total organic carbon (TOC) followed the wet chemistry technique as described by Schumacher, [22]. Fine-grained portion of grain-size distributions were determined by sedimentation method [23].

2.5.4 Chemical fractionation of sediment and analysis of heavy metal levels

For the purpose of classifying the biloavailable metallic status in each sample, five sequential chemical extractions were performed with the objective of identifying the metal classifications influenced by various environmental conditions: (a) exchangeable, (b) bound to carbonates, (c) bound to iron and manganese oxides (reducible), (d) bound to organic matter (oxidizable), and (e) residual [24]. The selective extraction of fraction A was performed using 1.0 g of sieved sediment at room temperature for one hour with 8.0 mL of 1 M $MgCl_2$ solution at pH 7.0 with continuous agitation. This fraction sometimes known as acid-soluble fraction provides information on the capacity of the sediment to absorb and desorp heavy metals in relation to changes in the ionic composition of the sediment. Sediment residues from fraction A will be leached at room temperature with 8.0 mL of 1 M sodium acetate at a pH of 5.0 (adjusted using acetic acid) with continuous agitation to obtain metals that are associated with carbonates (fraction B). For the reducible fraction (fraction C) extraction, sediment residues obtained from fraction B will be extracted with 20 mL of 0.04 M hydroxyl ammonium chloride in 25% (v/v) acetic acid for 6 hr at 96°C with occasional agitation of the solution. Fraction C constitutes heavy metals associated with iron and manganese oxides and is sensitive to redox potential variations.

2.6 Statistical Analysis of Data

The data was analyzed using the statistical software Pearson's Correlation Analysis and Factors analysis. Principal Component Analysis (PCA) was employed to explore the interrelationship among heavy metals in sediment and fish samples and identify their probable origin. The analysis was performed with a 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Microbiological properties of fin-fish samples

The results presented in Tables 1 - 2 showed that the ability of the fin-fishes to accumulate bacterial contaminants varied between the genera of fish analyzed as well as in the fish organs as the fish intestine generally accumulated more bacterial contaminants.

3.1.1.1 Bacterial loads of fin-fish skin samples

The bacterial loads of *Synodontis obesus* had the least level of skin contamination with densities of heterotrophic bacteria ($1.5\pm0.87 \times 10^5$ - $3.5 \pm 0.3 \times 10^5$CFU/g of skin scrapings), fecal coliform (0 - $2.3 \pm 0.87 \times 10^3$CFU/g of skin scrapings) and coliform ($2.0 \pm 0.17 \times 10^3$ to $7.7 \pm 0.69 \times 10^4$CFU/g of skin scrapings). The salmonella shigella count recorded ranged from 0 to $1.9 \pm 0.17 \times 10^3$CFU/g of skin scrapings). On the other hand, the bacterial loads of *Marcusenius senegalensis* skin samples were $2.2\pm0.92 \times 10^5$- $3.3 \pm 0.25 \times 10^5$, 0 and $2.0 \pm 0.39 \times 10^3$, $1.0 \pm 0.34 \times 10^3$ and $2.5 \pm 0.26 \times 10^3$ and 0 to $1.9 \pm 0.1 \times 10^2$CFU/g of skin scrapings for heterotrophic bacteria, fecal coliform, coliform and salmonellae shigella respectively.

3.1.1.2 Bacterial loads of fin-fish intestinal samples

The bacteriological loads obtained from the intestine of *Synodotis obesus*is (3) revealed

values that ranged between 2.0 ± 0.26 x 10^5 and 3.5 ± 0.3 x 10^5CFU/g. The total and fecal coliform counts recorded varied between 2.2 ± 0.3 x 10^3 and 2.9 ± 0.53 x 10^3CFU/g and 1.4 ± 0.4 x 10^3 and 2.1 ± 0.3 x 10^3CFU/g respectively, while the Salmonella Shigella count obtained ranged between 1.2 ± 0.1 x 10^2 and 1.9 ± 0.17 x 10^2CFU/g.

For *Marcusenius senegalensis* (Table 4) the values recorded ranged between 2.4 ±0.35 x 10^5 and 4.0 ± 0.26 x 10^5CFU/g, 1.7 ± 0.26 x 10^3 and 2.6 ±0.27 x 10^3 CFU/g, 1.1 ± 0.57 x 10^3 and 2.1 ± 0.3 x 10^3CFU/g and, between 1.0 ± 0.16 x 10^2 and 1.9 ± 0.07 x 10^2 heterotrophic bacteria, coliform, fecal coliform and salmonella - shigella counts respectively.

Table 1. Bacteriological loads of Synodontis obesus skin samples

Sample (Skin)	THBC (x 10^5cfu/g)	Total coliforms (x10^3cfu/g)	Fecal coliforms (x10^3cfu/g)	*Salmonella –Shigella* (x10^2cfu/g)
SO 1	1.5±0.87	2.2 ± 0.92	1.1±0.1	_
SO 2	2.0±0.2	6.3± 3.10	2.3±0.87	1.9 ±0.17
SO 3	3.3 ±0.53	7.7±0.69	1.8 ±0.72	1.5 ±0.44
SO 4	2.8 ± 0.2	2.2 ± 0.2	1.5± 0.44	_
SO 5	2.6 ± 0.4	2.0 ±0.17	_	_
SO 6	3.5 ± 0.3	2.4 ± 0.26	2.0 ±0.17	1.9 ±0.17
SO 7	2.8 ± 0.2	7.7 ± 0.69	1.8± 0.17	_

Values are mean of triplicate determinations ±SD
SD = Standard Deviation

Table 2. Bacteriological loads of *Marcusenius senegalensis* skin samples

Sample	THBC (x10^5cfu/g)	Total coliform (x10^3cfu/g)	Fecal coliform (x10^3cfu/g)	*Salmonella Shigella* (x10^2cfu/g)
MS 1	2.3 ±0.27	1.0 ±0.34	-	-
MS 2	.2 ±0.92	2.0 ±0.17	1.5 ±0.44	-
MS 3	3.3 ±0.25	1.8 ±0.72	1.6 ± 0.32	1.0 ±0.26
MS 4	2.8 ±0.2	2.0 ± 0.24	1.4 ±0.22	-
MS 5	3.1 ±0.26	2.5 ±0.26	2.0 ±0.39	1.9 ±0.1

Values are mean of triplicate determination±SD, SD = Standard Deviation

Table 3. Bacteriological loads of *Synodontis obesus* intestine samples

Sample	THBC (x 10^5cfu/g)	Total coliform (x 10^3cfu/g)	Fecal coliform (x 10^3cfu/g)	Salmonella Shigella (x 10^2cfu/g)
SO 1	2.3 ± 0.1	2.5 ± 0.5	1.4 ± 0.4	1.2 ± 0.1
SO 2	3.2 ± 0.36	2.7 ± 0.26	1.8 ± 0.26	1.3 ± 0.40
SO 3	2.8 ±0.46	2.6 ± 0.26	1.6 ±0.36	1.9 ± 0.17
SO 4	3.0±0.3	2.7 ± 0.26	1.4 ± 0.52	1.5 ± 0.44
SO 5	3.5 ± 0.3	2.7 ± 0.2	1.6 ±0.36	1.7 ±0.17
SO 6	3.0 ± 0.26	2.2 ± 0.3	2.1 ± 0.3	1.4 ± 0.52
SO 7	2.0 ± 0.26	2.9 ± 0.53	2.0 ± 0.26	1.7 ± 0.34

Values are mean of triplicate determination±SD, SD = Standard Deviation

Table 4. Bacteriological loads of *Muarcusenius senegalensis* intestine samples

Sample	THBC (x10^5cfu/g)	Total Coliform (x10^3cfu/g)	Fecal Coliform (x10^3cfu/g)	*Salmonella Shigella* (x10^2cfu/g)
MS 1	4.0 ±0.26	2.0 ±0.36	1.8 ± 0.26	1.0 ±0.45
MS 2	3.0 ±0.1	2.1 ±0.51	1.8 ±0.17	1.2 ±0.27
MS 3	3.2 ±0.44	2.5 ±0.95	2. 1 ± 0.31	1.9 ± 0.07
MS 4	2.4 ±0.35	1.7 ± 0.26	1.1 ±0.57	1.0 ±0.16
MS 5	3.0 ±0.19	2.6 ±0.27	2.1 ±0.3	1.4 ±0.24

Values are mean of triplicate $determination \pm SD$, *SD = Standard Deviation*

3.1.2 Diverse species of bacteria isolated from fish samples

The cultural and biochemical characteristics of the bacterial isolates that the culture-able bacteria associated with the fin-fishes were *Klebsiella* sp, *Bacillus* sp, *Enterobacter*, *Streptococcus* sp, *Micrococcus*sp, *Lactobacillus*, *Serratia* sp, *Proteus* sp, *Salmonella* sp, *Shigella* sp, and *Escherichia coli*.

3.1.3 Occurrence and prevalence of bacterial species on fin-fish samples

Analysis of the occurrence of various bacterial isolates on the fin-fish samples of *Synodontis obesus* and *Marcusenius senegalensis* are shown on Tables 5 - 6 respectively.

3.1.4 Heavy metal loads of the sediment samples

The result of the heavy metals analysis of the sediment samples presented on Tables 7 revealed variation in loads between sample locations and type of element. Comparison of percent total bio-available and non-bioavailable fractions of metals in the humic sediment [8] revealed differences between total metal concentrations and bio-available values. The result of the analysis showed the total percentage of the bio-available and non-bioavailable faction of the metals in the sediment samples varied with type of metals while their distribution in different geochemical phases of the humic sediment is presented in 9.

3.1.5 Heavy metal loads and the biota to sediment accumulation factors (BSAFs) of the fin-fishes from humic ecosystem

The concentration of metals detected in the fish samples varied with the fish species (Table 10). The Biota to Sediment Accumulation Factors (BSAFs) are multipliers used to estimate concentrations of chemicals that can accumulate in tissues through any route of exposure [25]. It is referred to as bio-concentration factor (BCF) for aquatic invertebrates. Analysis of the bio-concentration factor (BCF) of the metals in fin-fishes from humic ecosystem was also determined using standard protocols. The BCF and biota to sediment accumulation factor (BSAF) of heavy metals from sediment to animal tissues was determined in the different samples using the equations given below:

$$\mathrm{BCF}=\frac{\text{concentration of heavy metal in animal tissue}}{\text{concentration of heavy metal in sediment sample}} \quad (3)$$

The results of the BCF for the various metals in the finfish samples are given in Table 11. The results also revealed variation in the metal bio-accumulating potentials of the fin fishes. It also shows that of all the metals analyzed, Chromium (Cr) and lead (Pb) exhibited the least level uptake by fishes and had the least BCF.

3.2 Discussion

Bacteria are widely distributed in nature and are easily accumulated by most of our food products including fishes. According to FFSG (26), fish communities and specific species are excellent indicators of biological and ecological integrity due to their continuous exposure to water conditions. Fishes display an array of biotic responses such as changes in growth, distribution, abundance related to water pollution as well as has a greater potential of bioaccumulating environmental pollutants. Fin-fishes which are among the major class of fish encountered in the freshwater ecosystem of Eniong that constitute an important source of income and aquatic produce for the settlers as well as the nearby community. However, this is not without limitation in microbiological quality.

This study reveals the high load of bacterial contaminants in *Synodontis obesus guineensis* and *Marcusenius senegalensis* harvested from Eniong River. High numbers of coliforms, fecal coliform as well as the *Salmonella* and *Shigella* were found on the harvested fish samples. The level of bacterial contaminants accumulation however varied with the type of fish, and more contaminants were encountered in the fish intestines than the skin and gill. Slight variation was also noticed on the ability of the fishes to accumulate the different groups of bacterial contaminants with the skin of *S. obesus* accumulating more coliforms and fecal coliforms respectively. *Salmonella/Shigella* count was also readily found on the skin of S. *obesus.* On the other hand, *Salmonella* and *Shigella* were predominant in the intestinal samples of *S. obesus.*

Table 5. Occurrence of bacteria on *Synodontis obesus* samples

Isolate	Gills (n = 7)	Skin (n = 7)	Intestine (n = 7)	Frequency of occurrence	% of occurrence
Staphylococcus sp	+(3)	+(5)	-	8	38.1
Bacillus subtilis	+(3)	+(2)	+(4)	9	42.9
Bacillus cereus	+(2)	+(5)	+(2)	9	42.9
Micrococcus sp	-	+(4)	+(2)	6	28.6
Streptococcus sp	+(6)	+(2)	-	8	38.1
Proteus sp	+(3)	+(5)	-	8	38.1
*Serratia*sp	+(3)	-	-	3	14.3
Salmonella sp.	-	+(1)	+(2)	3	14.3
Shigella sp	-	-	+(3)	3	14.3
Escherichia coli	-	+(4)	+(5)	9	42.9
Enterobacter sp	+(5)	+(1)	+(3)	9	42.9
Klebsiella sp	-	+(3)	+(5)	8	38.1
Lactobacillus sp	+(2)	-	+(3)	5	23.8

Table 6. Occurrence of bacteria on *Marcusenius senegalensis* samples

Isolate	Gills (n=5)	Skin (n=5)	Intestine (n=5)	Frequency of occurrence	% of occurrence
Staphylococcus sp	+(5)	+(5)	+(5)	15	100
Bacillus subtilis	+(4)	+(4)	-	8	53.3
Bacillus cereus	-	+(2)	+(2)	4	26.7
Micrococcus sp	+(5)	+(5)	+(5)	15	100
Streptococcus sp	+(5)	+(2)	-	7	46.7
Proteus sp	-	+(4)	-	4	26.7
Serratia sp	+(4)	+(2)	+(2)	8	53.3
Salmonella sp	+(2)	+(1)	+(1)	4	26.7
Shigella sp	-	+(2)	+(3)	5	33.3
Escherichia coli	-	+(2)	+(4)	6	40.0
Enterobacter sp	+(5)	+(1)	+(3)	9	60.0
Klebsiella sp	-	+(2)	+(5)	7	46.7
Lactobacillus sp	+(2)	-	+(3)	5	33.3

Table 7. Comparison of percent total bio-available and non-bioavailable fractions of metals in the humic sediment samples from Eniong River

	Station 1	Station 2.	Station 3	Station 4	Station 5	$\sum_{i=A}^{D} F_i$ %
Cd	31.47(68.52)	29.76(70.24)	31.11(68.89)	30.76(69.23)	29.93(70.08)	30.61±0.66
Cr	59.71(40.26)	58.63(41.38)	59.55(40.44)	59.87(40.12)	65.68(34.32)	60.69±2.53
Cu	59.93(40.06)	63.93(36.07)	58.96(41.03)	69.34(30.67)	58.03(41.96)	62.04±4.16
Ni	51.15(48.85)	48.60(51.40)	49.33(50.67)	45.13(54.87)	46.54(53.46)	48.15±2.11
Pb	26.67(73.33)	25.21(74.79)	23.82(76.17)	24.28(75.90)	26.13(73.87)	25.22±1.07

( ) % total of non-bioavailable fraction

Table 8. Heavy metal distribution in different geochemical phases of the humic sediment

Fraction	Cd (30.61%)	Cr (60.69%)	Cu (62.04%)	Ni (48.15%)	Pb (25.22%)
Residual	62.4	37.3	32.4	46.3	65.2
Oxidizable	17.2	41.7	46.3	21.7	18.2
Reducible	11.3	14.0	15.2	17	9.6
Carbonates	5.1	4.5	6.1	9.5	5
Exchangeable	4.0	2.5	0	5.5	2.0

Table 9. Heavy metals concentrations and sediments characterization variables in the humic freshwater ecosystem of Eniong River

Station	Cd (mg/kg)	Cr(mg/kg)	Cu(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Clay	Sand	Silt	TOC %
Station 1	4.80±0.24	19.35±1.11	38.13±1.70	2.19±0.06	178.36±7.75	21.64±2.26	67.03±1.43	11.33±1.02	7.05±2.00
Station 2	4.79±0.45	20.22±1.11	37.48±5.42	2.16±0.07	177.96±9.60	9.26±1.28	23.12±0.64	68.40±1.74	8.46±2.11
Station 3	4.71±0.34	19.85±0.78	40.28±2.44	2.20±0.04	180.7±4.27	5.08±0.47	24.08±3.25	66.45±4.01	9.45±1.48
Station 4	4.70±0.19	19.85±0.86	35.33±3.25	2.26±0.18	191.08±20.11	19.17±4.75	70.71±5.70	10.12±0.99	5.24±0.71
Station 5	4.91±0.39	18.06±5.78	39.58±2.85	2.18±0.09	175.85±7.74	5.58±0.62	20.69±1.29	69.96±3.97	9.34±3.59

Table 10. Heavy metals concentrations in the fin-fish samples

Fish sample	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)
M. senegalensis	0.245±0.06	0.018±.0.06	3.73±1.39	0.31±0.07	0.05±0.03
S. obesus	0.259±0.06	0.020±0.01	2.273±0.51	0.173±0.03	0.053±0.02

Note: Values are means of each sample number ± SD
SD = Standard Deviation

Table 11. Bio-concentrataion factor (BCF) of the various fish samples

	Cd	Cr	Cu	Ni	Pb
M. senegalensis	0.05±0.19	0.0009±0.03	0.10±0.44	0.14±0.78	0.0003±0.003
S. obesus	0.05±0.19	0.001±0.005	0.06±0.16	0.079±0.3	0.0003±0.002

This findings agrees with previous report by Ajayi [27], who in his study reported a high bacterial population in catfish from fish pond in Akungba-Akoko community, Nigeria. This he attributed to waste materials discharged into water bodies upon which the fishes inhabit/feed. The variations in the bacterial populations reported in this study is indicative of high bacteria accumulation potential of the finfishes and may be attributed to various factors such as body size, feeding pattern, physiology and sediment bioturbation characteristics of the fish samples [26,28].

The culture-able bacteria species associated with the fin-fish samples include *Staphylococcus* sp, *Klebsiella* sp, *Bacillus* sp, *Enterobacter*, *Streptococcus* sp, *Serratia* sp, *Proteus* sp, *Salmonella* sp, *Shigella* sp, and *Escherichia coli*. Similarly, Shewan [29] and Okaeme [30] have reported different bacterial species from the skin of sea-water fish. Sugita *et al*. [31] concluded that the skin of freshwater fishes was the natural habitat of these bacteria. The percentage of occurrence of the isolates in the various fish samples was also found to vary with the fish species. In *S. obesus,* the most prevalent bacterial isolate was *B. subtilis, B. cereus, E. coli* and *Enterobacter* sp while the least prevalent was *Serratia, Shigella* and *Salmonella* sp. In *M. senegalensis, Staphylococcus and Micrococcus* sp had the highest prevalent rate of 100%, while *B. subtilis, Proteus* sp and *Salmonella* sp had the least prevalent (26.4%). This result agrees with Ajayi [27], Shewan [29] and Okaeme [30], who in their various studies reported *S. aureus, Micrococcus* sp., and other Enterobacteriaceae as being the most predominant bacteria in freshwater fishes.

In aquatic systems, benthic sediment act as both sink and carrier for heavy metals and could provide valuable information on the pollution pattern and history of such ecosystems [32]. Heavy metals could be released in both particulate and dissolved forms and known to have high affinities for fine-grained sediment. In this study, detectable concentrations of Cd, Cr, Cu, Ni and Pb were found in the humic sediment of Eniong River. This observation is consistent with the high clay and total organic carbon components of sediment, which are known to be a good accumulator of metallic and organic contaminants [33,34]. Furthermore, sediment has been reported as repositories of heavy metals [35]. Total heavy metal *concentrations* in the aquatic ecosystems reflect varying degree of contamination by different metals. Of all the metals analyzed in the sediments from the humic freshwater ecosystems, Pb was the highest (175.85 – 191.08 mg/kg), followed by Cu (35.33 – 40.28 mg/kg), Cr (18.06 – 20.22 mg/kg), Cd (4.71 – 4.91 mg/kg), and Ni (2.16 – 2.28 mg/kg). The highest levels of Pb in the system may be ascribed to emissions from oil-related industries in the Niger Delta region. The concentrations of each analyzed heavy metal indicated spatial variations between the stations that were characteristically distinctive and correlative with proximity to anthropogenic activities, near-shore area and settlements. Higher concentrations were observed more frequently near the coast (HS-1) of the humic ecosystem resulting in comparable downstream concentration values especially at locations with intense anthropogenic activities. The concentrations of heavy metals generally followed the pattern Pb>Cu>Cr>Cd>Ni. The TOC values ranged from 5.24 ± 0.71 – 9.45 ± 1.45%. The results indicate that the benthic sediments contained comparatively high organic contents, implying high sedimentary metal affinity for humic substances, which might decrease heavy metal bioavailability through complexing [36]. The silt content of sediments ranged from the lowest value of 11.33±1.02 at HS-1 to 69.96 ± 3.77% at HS-5 and may also influence the repository.

Sediment-bound metals are primarily associated with different fractions and they are known to exhibit varied bonding strength, which governs their bioavailability in aquatic ecosystems as well as their attendant ecological risk [37,38]. The sequential extraction method (SEM) employed presents heavy metals in five sediment geochemical fractions (exchangeable + carbonate bound + reducible + oxidizable + residual). The partitioning of heavy metals according to the chemical fractions listed above could have been predominantly influenced by the bonding strengths of the elements, their latent reactivity, and sediment properties [39]. It is generally accepted that the partitioning of heavy metals in environmental matrices provides an indirect assessment of their mobility, bioavailability and the inherent health and environmental risks. Therefore, if the bioavailability of metal is a function of its solubility, then Cu and Cr with low residual rates (32.4 and 37.3% respectively) are expected to have high bioavailability and pose the greatest risks to humans and the environment. Thus, the bioavailability ranking is: exchangeable > carbonate > Fe-Mn oxide > organic > residual.

This order offers only qualitative insights about chemical partitioning of heavy metals viz-à-viz their bioavailability in the labile fractions (exchangeable and carbonate bound). Furthermore, it can be asserted that heavy metals in the mobile or "direct effects" (non-residual) fractions are considered to be more bio-available compared to those found in the residual fraction.

The occurrence of the studied heavy metals (Cd, Cr, Cu, Ni and Pb) in non residual/residual fractions and their bioavailability potentials in benthic sediments of the freshwater ecosystem presented in Tables 7 and 8 have shown that Cu with 62.04% availability rate was the most bio-available element, as against Pb with 25.22% availability rate. These correspond to their 32.4% and 65.2% residual potency rates.

The study reveals that *Synodontis obesus* with the least level of skin contamination with densities of heterotrophic bacteria (1.5±0.87 $x10^5$ - 3.5 ±0.3$x10^5$CFU/g of skin scrapings), fecal coliform (0 - 2.3 ±0.87$x10^3$CFU/g of skin scrapings) and coliform (2.0 ±0.17$x10^3$ to 7.7 ±0.69$x10^4$CFU/g of skin scrapings) recorded a higher level of heavy metal concentration than *Marcusenius senegalensis*. The data of BSAF values for heavy metals in the fin-fishes revealed a significant increase in levels of all the heavy metals in the different fishes. The high bioaccumulation level recorded for these heavy metals in tissue of the fin-fishes may be attributed to their low and high residual rates in the humic sediment which determine the metals bioavailability. It shows that the fin-fish genera cannot serve as good bioindicators for monitoring of these heavy metals in polluted humic ecosystem. Surprisingly, the concentrations of Cd, Cr and Pd accumulated by the fin-fishes studied were very low when compared with that of Cu. This might suggest that despite their comparatively poor availability, the fish species investigated may have low retention of Cr and Pd in humic freshwater ecosystem when compared with other aquatic organisms such as oyster and mussels which had been reported to accumulate Cd in their tissues at levels up to 100,000 times higher than the levels observed in the background environment [40].

The presence of high concentrations of Cu in the fin-fishes indicates anthropogenic input and, hence, the metal accumulation potential of the fishes. The concentration of Cu recorded for the fish samples was below the recommended dietary limit of 4 µg/g in aquatic foods [41,42] and was far lower than the lethal doses Correlation analysis showed a negative value of -0.12549 which indicated that there was no relationship between the heavy metals concentrations in the sediment and fin-fishes.

4. CONCLUSION

The result of this study have revealed that the fin fishes harbour a high population of diverse bacteria including pathogenic strains of *Klebsiella* sp, *Bacillus* sp, *Enterobacter*, *Serratia* sp, *Proteus* sp, *Salmonella* sp, *Staphylococcus aureus*, *Shigella* sp, and *Escherichia coli* which are commonly associated with human and infant gastroentiritis. The spatial distribution, environmental quality and source apportionment of Cd, Cr, Pb, Ni, and Cu in humic sediment of Eniong River and fin-fishes investigated have shown that heavy metals exhibited significant variability between sampling sites and the fin-fish species and values obtained for most of the metals, except Cu were low and did not exceed the FAO/WHO recommended guideline values. Our results indicate that the fin -fishes may serve as sentinel organism for biomonitoring of Cr, Ni and Pb in humic freshwater ecosystem. However, the estimation of BSAF values derived showed that the fin-fishes evidently have the ability to bio-concentrate heavy metals in their edible tissues without apparent ill effect. The high bio-available level of Cu in sediment despite its uptake by the fishes portends danger implying that the aquatic ecosystem and some biota may be exposed to short- and long-term Cu metal pollution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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