

Seasonal Variations in Microbial Loads and Heavy Metals Concentrations in Fish from Yenagoa Metropolis, Nigeria

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Abstract

In Nigeria and other parts of the world, fishery products have raised serious health issues and are responsible for some of the reported deaths. In this study, the seasonal variation in microbial loads and heavy metal contents in dominant freshwater fishes from major markets in the Yenagoa metropolis was investigated. The microorganisms were isolated by using selected media and identified using Bergey's manual. Heavy metals; Cu, Zn, Pb, and Cd concentrations were analyzed using Atomic Absorption Spectrophotometer. The isolated microorganisms include; *Klebsiella* species, *Bacillus* species, *Shigella* species, *Pseudomonas* species, *Fusarium* species, *Mucor* species, *Aspergillus* species, *Rhizopus* species, *Actinomyces* species, *Staphylococcus* species, *Chromatium* species, *Candida* species, *Trichophyton* species, *Listeria* species, and *Salmonella* species. The microbial loads and isolates varied significantly ($p < 0.05$) within seasons, with the wet season having high microbial load than the dry season. The concentrations ($\mu\text{g/gdw}$) range of the metals in the dry season are; Cu(806 – 4403), Zn (3110 – 5520) , Cd(1.40 – 5.10), Pb(7.40–14.50), while that of wet season is Cu(1280–5120) , Zn(3170 – 6210), Cd(2.50 –6.20) and Pb(11.80 – 16.10). There was no significant difference ($P > 0.01$) in the concentrations of the metals among the markets and the seasons. Zn, Pb, and Cu were above the FEPA and WHO regulatory limits Cd was within the regulatory limit. Fish is one of the most affordable commodities for human for its nutritionally beneficial but can be infected with pathogenic microorganisms and contaminated with heavy metals if not properly maintained, which can pose a serious health hazard to consumers.

Keywords: Microbial loads, Heavy metal, Freshwater fish, Regulatory limit, Health hazard

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1. Introduction

Fish is widely accepted due to its outstanding quality in flavor and taste it is also an excellent source of high-quality protein, Low-fat foods that include all of the essential elements required to complement the diets of both adults and newborns. [1]. Fish consumption is on the increase both in rural and urban areas, as fish lipids provide nutritional vitamins and also helps in reducing some cardiovascular diseases [2]. This aquatic organism is a good source of almost all the minerals found in seawater especially calcium, zinc, iron, phosphorous, selenium, and fluorine. These minerals are easily absorbed by the body [3]. It also has important vitamins with essential health benefits vitamin k in fish is responsible for anti-hemorrhage factors, fish oil and vitamin E reduces inflammatory and joint swelling [4]. The presence of microbes and heavy metals in fish can be attributed to both natural and anthropogenic activities [5]. Though fish is regarded as safe, nutritious, and beneficial aqua-cultural products are usually associated with an increase in microbial loads [6]. Recent studies have shown that many bacteria present in fish are pathogenic under certain conditions. Poor conditions in our local markets, fish smoking facilities, and fishmongers may cause bacteria to emerge in fish, leading to food poisoning, which is a serious concern in low-sanitation countries [7].

Worsening the conditions in our local markets and fish smoking facilities and fish manufacturers could promote the appearance of germs in fish, resulting in food toxicity, which is a worrying issue for countries with low sanitation. [8]. The microbiological flora associated with newly harvested fish mainly depends on the habitat where the fish were collected and hence the indigenous fish microbial population might vary considerably [9]. Fish smoking is one of the traditional processing measures that is aimed at reducing post-harvest loss and microbial spoilage [10]. Some microorganisms associated with fish include *pseudomonas*, *Aspergillus*, *Streptococcus* species, *Acinetobacter*, *Micrococcus*, *Bacillus*, *Salmonella*, *Enterococci*, *molds*, etc The most common in nature are soil hay, cereals, vegetables and food. These bacteria are related with a range of unfavourable health conditions, including diseases/disorders of the haematology, immunology, respiratory and neurological systems. Aflatoxin from these bacteria also has some *Aspergillus* species causing hepatic disease. [11].

Fish contamination can be associated with raw materials, processing equipment, such as lifts, leaks, openings and pesticides in niche processing factories where they can live for a long time, some diseases may even become entrenched. [12]. The water ponds and rivers harbouring fish may, in particular during rainy season, contaminate fish because of the indiscriminate deposition in natural waters of human, animal egested waste and other environmental waste as faecal matter from a number of sources is washed into water bodies from contaminated lands. Animals and birds who travel freely can contribute to water faecal contamination [13]. Likewise, human beings who are the carriers of pathogenic and saprophytic microflora that are known to cause disease among vulnerable people are another substantial source of microbial contamination. Human activities and associated dust contamination can also lead to high bacterial density in fish, since high bacteria are generally found in dust and discharged when soil is elevated [14].

The worldwide concern of environmental pollution is heavy metal pollution as a key pollutant in the aquatic system. They tend to accumulate almost non-bio-degradable form with some toxic effect in water and organisms such as fish [15]. Heavy metals are categorised as a relatively high

atomic weight and hazardous metal component at low concentrations [16]. These metals are found naturally in the environment but can be anthropogenically created to amounts that are sufficiently high to interrupt water species, including malfunctions. At some level of exposure to absorption, all heavy metals are potentially toxic to most organisms, which enter water supplies through direct disposal of trash from industry, waste incinerators, dumping sites or tide flow current [17]. Certain heavy metals, for which low amounts are needed biologically, such as zinc, copper, iron, manganese, etc. [18]. Heavy metal absorption from fish through food and water not only affects their production and reproductive capacity, but ultimately affects the health of man who is a key protein source [19]. Heavy metal content in fish has become an important worldwide threat not only for fish but also for human consumption as a result of health risks associated with the consumption of contaminated fish [20]. Heavy metal accumulates in fish flesh, and some metals of concern to the aquatic system include cadmium, lead, mercury, iron, arsenic, aluminum, nickel etc they find their way into fish mainly via gills [21]. Blade poisoning is related with a significant contamination of heavy metal. The car's plumbing system may be transported as an aerosol, and washed into the aquatic system due to atmospheric repercussions, which can also be put into the market in the form of fish when exposed to air. Lead poisoning in children is more severe than in adults because of their brains and central nervous system [22]. Lead also induces renal tumor, reduces cognitive development, and increases blood pressure in adults, manganese, and iron cause pathological effects such as iron oxide deposition in Parkinson's disease [23]. Excess copper is associated with liver damage and may produce adverse nutritional interaction also zinc suppresses the immune function and reduces the level of high density. Although nickel helps to form enzymes that are needed in the formation of nucleic acid and DNA, it can also be highly toxic at higher concentrations [24].

2. Materials and Methods

Equipment/Reagents

Electric hotplate, Standard flask, Atomic absorption spectrometer (Perkin-Elmer model 306), Electric oven, Beaker, Volumetric flask, Mortar, Electric blender, Petric dish, Quebec colony counter, Sterile cotton wool, HNO₃, Hydrochloric acid, Nitric acid, Streptomycin/penicillium solution, Tetraoxosulphate (iv) acid, Ethanol, Methyl-blue dye, Crystal violet, Mannitol agar, Sabourad detox agar, Thiosulfate citrate basalt sucrose, *Salmonella/Shigella* agar, Nutrient agar, McConkey agar, and peptone water, potato dextrose agar.

Collection of Samples

Three species of dried fish *Tilapia marie*, *Eleotris senegalensis*, *Ctenopoma kingsleyae* were purchased from three major markets (Kpansia, Igbogene, and Zarama) within Yenagoa metropolis Bayelsa State, Nigeria. The markets were chosen based on their proximity to the people and availability of the investigated fishes. Zarama is a weekly market, while Kpansia and Igbogene are the daily markets. Samplings were done on monthly basis and coincided with Zarama market day. Fish samples were collected well labeled, and kept in sterile polythene bags, and transported to Federal University Otuoke, Biological sciences laboratory for microbiological and metal analysis.

Microbiological Analysis

The working area was disinfected with ethanol, all wares were properly washed and sterilized in a hot air oven at 160°C for one hour. In a homogenised mortar using an electric mixer, each fish sample was independently crushed, from which 0.1g was homogenized in 9ml of sterile water and allowed to stand for 5 minutes. A sterile pipette was used to transfer 1ml of the sample into a test tube filled with 9ml of peptone water. 1 ml of the initial suspension was subsequently transferred into a sterilized 9 ml of peptone water to prepare further dilutions to the desirable level of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , etc.

Preparation and Sterilization of Media

All media were prepared and sterilized according to the manufacturer's instructions. Sterility control plates of each media and diluents were made by incubating them overnight at their respective temperatures for the required time. Fungi and bacteria have been listed on suitable mediums. Total heterotrophic nutrient agar bacteria, total coliform McConkey agar bacteria, *salmonella*/*shigella* on *salmonella*/*shigella* agar, *vibrio* on thiosulfate citrate bile salt sucrose, *staphylococcus* Agar mannitol, dextrose dextrose dextrose agar mannitol fungal and agar.

Enumeration of Bacteria and fungi colonies

The pour plate method was used to enumerate the total heterotrophic bacteria. Twenty-five (25) gram of samples was added into peptone water and inoculation was done by adding 15 ml of the mixture into the Plate count agar at 45°C to 1 ml of the sterilized duplicate plate. The inoculums were carefully mixed with the agar medium by rotating the Petri-dishes clockwise and anticlockwise and allowing the medium to solidify, leaving Petri-dishes on a horizontal surface. The inoculated Plate was inverted and placed in the incubator at 35°C for 72 hours. After incubation, colonies on each plate were counted using the colony counter. Standard biochemical tests include gramme, catalase, motility, coagulase, methylred vegele, proskauer test, hydrolysis of starch, citrate, urease and sugar fermentation have been used to identify bacteria isolate [25]. The total fungi count of the fish sample was duplicated and inoculated on potato dextrose agar at room temperature for 72 hours. A fortified medium for selective enumeration and isolation of fungi is employed for fungal growth or suppression of bacterium growth, streptomycin and penicillium solutions (50ug/ml and 50 IU/ml respectively). A colony-forming unit (cfu/g) was documented in a list of discrete colonies which appeared on the cultural plate with the assistance of Quebec colony-counters. The method of identification of fungi isolates [26] and [27].

Heavy Metal Analysis

A 50 ml beaker of 5 ml of HNO₃ and 5 ml of H₂SO₄ was added to the dry weight sample of 5(5) g. The beaker was put on a heat plate and heated at 60°C for about 25 minutes when the fish fabric ceased to react with HNO₃ and H₂SO₄. Beaker cooling was permitted, HNO₃ 10 ml was added and slowly heated to 120° C in the hot plate. The temperature was up to 150°C when the samples went black and the beaker was taken from the hot plate. The sample could then cool until a clear solution was reached before adding H₂O₂. The contents of the beaker were translated into a flask of 50 ml, then diluted with ultra-pure water, stored with PTFE until evaluated by the Perkin-Elmer Model 306 atomic absorption spectrometer.

3. Data Analysis

Data of the seasonal variation in microbial loads in dominant freshwater fishes from major markets in Yenagoa metropolis, Nigeria were subjected to Statistical analysis of the *t*-test and ANOVA Two Factor without replication. Also, data from heavy metals assessment were compared to the acceptable standard of World Health Organization and Nigeria Federal Environmental Protection Agency safety limit.

4. Results

The Seasonal variation of microbial loads from smoked native fishes from major markets in Yenagoa Metropolis, Nigeria is shown in Table 1. The wet season had higher microbial loads than the dry season in all the samples, with *E. senegalensis* having the highest bacteria loads (32.50 CFU x 10⁵), reported in the Kpansia market in the wet season. *T.marie* had the least bacterial loads (3.60cfu x 10⁵), and was noted at the Igbogene market in the dry season. Zarama market had the highest fungal counts (13.10cfu x 10⁵), and was also detected in *E. senegalensis* and occurred in the wet season. However, the least fungal loads (1.20cfu x 10⁵) were observed in the wet season, recorded in *T.marie* in the Zarama market. In *C. kingsleyae*, the TBCs vary significantly ($p < 0.05$) among the markets during the dry and wet seasons. In *E. senegalensis*, TBCs vary significantly between Zarama and Igbogene ($p < 0.05$), and also Zarama and Kpansia ($p < 0.05$) but there was no significant difference in Igbogene and Kpansia markets ($p > 0.05$).

Table 1: Seasonal variation of microbial load from smoked native fishes from major markets in Yenagoa Metropolis, Nigeria.

Markets		Number of colonies (cfu x 10 ⁵)					
		Dry Season			Wet season		
		<i>C. kingsleyae</i>	<i>E. senegalensis</i>	<i>T.marie</i>	<i>C.kingsleyae</i>	<i>E.senegalensis</i>	<i>T.marie</i>
Zarama	TBC	7.20 ^a	12.40 ^b	3.90 ^a	15.20 ^a	23.60 ^b	9.20 ^a
	TFC	2.10 ^a	4.50 ^b	1.50 ^a	3.20 ^a	13.10 ^a	1.20 ^a
Igbogene	TBC	10.60 ^b	13.20 ^c	3.60 ^c	19.40 ^b	21.70 ^c	5.50 ^c
	TFC	3.50 ^b	5.20 ^a	1.70 ^b	4.20 ^b	5.30 ^a	3.80 ^b
Kpansia	TBC	15.20 ^c	23.10 ^c	7.60 ^c	25.40 ^c	32.50 ^c	17.30 ^c
	TFC	3.80 ^b	5.80 ^a	2.50 ^b	5.20 ^b	5.80 ^a	3.80 ^b

Mean with different superscript within the column varies significantly ($p < 0.05$), TBC = Total bacteria counts; TFC = Total fungi counts.

In *T.marie*, TBCs vary significantly ($p < 0.05$) in both seasons in Zarama and Igbogene and also in Zarama and kpanisia markets but there was no significant difference ($p > 0.05$) in Kpansia and Igbogene markets. The TFC of *T.marie* has a considerable change only during the wet season. Zarama markets vary significantly ($p < 0.05$) with Igbogene and Kpansia but there was no significant difference ($p > 0.05$) between Igbogene and Kpansia markets.

Table 2 and 3 shows the Microbials isolated from the smoked native fishes. In Zarama, *Klebsiella* species and *Bacillus* species were abundant in both seasons. *Yersinia* species was present in *E.*

senegalensis during the dry season but absent in *C. kingsleyae*, and *T.marie*. *Yersinia* species was present in all the fishes during the wet season. But during the dry season it was only found in *E. senegalensis* and absent in *T. marie* and *C. kingsleyae*. *Proteus* species was absent in the samples during the dry season, but was detected in the wet season in *E. senegalensis* but absent in *T.marie* and *C.kingsleyae*. *E. senegalensis* had the highest microbial isolate in all seasons but there was moderate bacteria load in *T. marie* and *C. kingsleyae*. In Igbogene *Yersinia* species was present in fish samples in both seasons. *Staphylococcus* species were present in *C. kingsleyae* and *E.senegalensis* but absent in *T.marie* during the dry season. *Chromatium* species were observed only in *C.kingsleyae* and in *T.marie* during the dry season and absent in *E. senegalensis*. *Trichophyton* species was absent in *T.marie* and *C. kingsleyae* but present in *E. senegalensis* during the dry season but was present in all samples during the wet season. In the kpansia market, *Listeria* species were present in all fish samples for both seasons, *Klebsiella* species, *Shigella* species, *Chromatium* species, and *Proteus* species were also present in samples in dry and wet seasons. *Salmonella* species was present in *C.kingsleyae* and *E.senegalensis* but absent in *T.marie* during the dry season but was present in all samples during the rainy season.

Micrococcus species was absent in *C.kingsleyae* but present in *E. senegalensis* and *T.marie* during the dry season, but during the wet season, it was observed in *C.kingsleyae* and *E. senegalensis* but absent in *T.marie*. *Enterobacter* species were present in all samples during the dry season but not during the wet season. During the wet season, it was found in *E.senegalensis* and *T.marie* but absent in *C.kingsleyae*. *Staphylococcus* species were detected in *E.senegalensis* and *C.kingsleyae* during the dry season but not observed in *T.marie* but were present in all samples during the wet season. *Pseudomonas* species was present in *C.kingsleyae* and *E. senegalensis* but not in *T.marie* during the dry season but was present in all samples during the wet season. Fungal isolates were also detected in fish samples from the various markets. In Zarama, *Fusarium* species, *Mucor* species, and *Aspergillus* species were present in samples for both seasons, *Rhizopus* species was present in *C.kingsleyae* and *E.senegalensis* but absent in *T.marie* during the dry and wet seasons. At the Igbogene market, *Actinomycetes* species were observed during the wet and dried seasons. *Candida* species were found in both *E.senegalensis* and *C.kingsleyae* during the dry but not in *T.marie* but it was present in all samples during the wet season. *Aspergillus* species were found in both *C. kingsleyae* and *T.marie* but absent in *E. senegalensis* during the dry season but was present in all samples during the wet season. *Rhizopus* species were present in all samples during the wet season but absent in *E.senegalensis* during the dry season. *Mucor* species was absent in *T.marie* during the wet season, but found in *E.senegalensis* and absent in *C.kingsleyae* and *T.marie* during the dry season. In the Kpansia market, *Actinomycetes* species, *Candida* species and *Rhizopus* species were present in *E.senegalensis*, *C.kingsleyae*, and *T.marie* samples in both seasons, while *Aspergillus* species was present in all the fish samples during the wet season, during the dry season it was present in *E.senegalensis* and *C. kingsleyae* but absent in *T.marie*. *Fusarium* species were detected in *C.kingsleyae* and *T.marie* in the wet season, but not found in *E. senegalensis*, during the dry season. *Mucor* species was observed only in *C.kingsleyae* and *E.senegalensis*. in both seasons.

Table 2: Bacteria isolated from smoked native fishes from major markets in Yenagoa Metropolis, Nigeria

Markets	Isolates	Dry Season			Wet season		
		<i>C.kingsleyae</i>	<i>E.senegalensis</i>	<i>T.marie</i>	<i>C.kingsleyae</i>	<i>E.senegalensis</i>	<i>T.marie</i>

Zarama	<i>Klebsiella</i> spp	+	+	+	+	+	+
	<i>Bacillus</i> spp	+	+	+	+	+	+
	<i>Yersinia</i> spp	-	+	-	+	+	+
	<i>Proteus</i> spp	-	-	-	-	+	-
	<i>Shigella</i> spp	-	-	-	-	+	-
	<i>Pseudomonas</i> spp	-	-	-	+	-	-
Igbogene	<i>Actinomycetes</i> spp	+	+	+	+	+	+
	<i>Yersinia</i> spp,	+	+	+	+	+	+
	<i>Staphylococcus</i> spp	+	+	-	+	+	+
	<i>Shigella</i> spp	+	+	+	+	+	-
	<i>Pseudomonas</i> spp	+	+	+	+	+	+
	<i>Chromatium</i> spp	+	-	-	+	+	+
Kpansia	<i>Listeria</i> spp	+	+	+	+	+	+
	<i>Klebsiella</i> spp	+	+	+	+	+	+
	<i>Proteus</i> spp	+	+	+	+	+	+
	<i>Shigella</i> spp	+	+	+	+	+	+
	<i>Salmonella</i> spp	+	+	-	+	+	+
	<i>Chromatium</i> spp	+	+	+	+	+	+
	<i>Micrococcus</i> spp	-	+	+	+	+	+
	<i>Enterobacter</i> spp	+	+	+	+	+	+
	<i>Actinomycetes</i> spp	+	+	+	+	+	-
	<i>Staphylococcus</i> spp	+	+	-	+	+	+
	<i>Staphylococcus</i> spp	+	-	-	+	-	+
	<i>Pseudomonas</i> spp	+	+	-	+	+	+
	<i>Chromatium</i> spp	+	+	-	+	+	-

Table 3: Fungi isolated from smoked native fishes from major markets in Yenagoa Metropolis, Nigeria

Markets	Isolates	Dry Season			Wet season		
		<i>C.kingsleyae</i>	<i>E.senegalensis</i>	<i>T.marie</i>	<i>C.kingsleyae</i>	<i>E.senegalensis</i>	<i>T.marie</i>

Zarama	<i>Fusarium</i> spp	+	+	+	+	+	+
	<i>Mucor</i> spp	+	+	-	+	+	+
	<i>Aspergillus</i> spp	+	+	+	+	+	+
	<i>Rhizopus</i> spp	+	+	+	+	+	-
Igbogene	<i>Trichophyton</i> spp	-	+	-	+	+	+
	<i>Aspergillus</i> spp	+	-	+	+	+	+
	<i>Rhizopus</i> spp	-	+	-	+	+	+
	<i>Mucor</i> spp	+	-	-	+	-	-
Kpansia	<i>Aspergillus</i> spp	+	+	+	+	+	+
	<i>Fusarium</i> spp	-	+	+	-	+	+
	<i>Rhizopus</i> spp	+	-	+	+	+	+
	<i>Mucor</i> spp	-	+	-	+	+	-
	<i>Candida</i> spp	+	+	-	+	+	-

Heavy Metals

The Seasonal variation in Heavy metal concentrations in smoked fishes in Yenagoa Metropolis, Nigeria, and the recommended limit set by the international regulatory body is shown in Table 4. For copper, *E. senegalensis* has the highest concentrations ($4430 \pm 8.20\mu\text{g} / \text{gdw}$) and it was recorded in the dry season in the Kpansia market. *C.kingsleyae* has the lowest copper concentrations ($806 \pm 1.3020\mu\text{g} / \text{gdw}$) and was recorded in the dry season in Zarama market. *C.kingsleyae* has the highest Zinc concentrations ($6210 \pm 11.80\mu\text{g} / \text{gdw}$) and it was noted in the wet season in Kpansia. It also has the lowest Zn concentrations ($3110 \pm 4.90\mu\text{g} / \text{gdw}$), recorded in the dry season in Zarama market. Means with the same superscript and subscript within the column are not significantly ($p > 0.01$) different; Superscript = Matrixes; Subscript = Season; A₁, A₂ and A₃ represented Zarama Igbogene and Kpansia markets respectively.

The highest cadmium concentrations ($6.20 \pm 0.90\mu\text{g} / \text{gdw}$) were reported in *E.senegalensis* and were observed in the Kpansia market during the wet season. The lowest concentrations ($1.80 \pm 0.0\mu\text{g} / \text{gdw}$) were recorded in *C.kingsleyae* during the dry season and were revealed in the Zarama market. The highest lead concentration ($16.10 \pm 0.30\mu\text{g} / \text{gdw}$) was detected in *T.marie* from the Kpansia market. The lowest lead concentration ($7.40 \pm 0.30\mu\text{g} / \text{gdw}$) was recorded in the dry season in *C.kingsleyae* from the Zarama market. In *C.kingsleyae* there is no significant variation ($p > 0.05$) between the wet and dry seasons in copper, lead, zinc, and cadmium across the various markets. Also, *E.senegalensis* the heavy metal contents do not vary significantly ($p > 0.05$) between both seasons in the different markets. Similarly, there is no significant different ($p > 0.05$) in the heavy metal in *T.marie* across the various markets and seasons. The Cu content ($806 \pm 1.30\mu\text{g} / \text{gdw}$) in *C.kingsleyae* was lower than the WHO (2000) and FEPA (1000) in Zarama but higher in Igbogene and Kpansia during the dry season. During the rainy season, the Cu content in the Zarama market was lower than the FEPA limit but higher than the WHO limit, also the copper content in Igbogene and Kpansia markets was higher than both the WHO and FEPA recommendation.

Table 4: Seasonal variation in Heavy metal ($\mu\text{g} / \text{gdw}$) concentrations in smoked fishes in Yenagoa Metropolis, Nigeria and the recommendation limits

		Cu	Zn	Cd	Pb
Dry season					
<i>C.kingsleyae</i>	A ₁	806 \pm 1.30 ^a _a	3110 \pm 4.90 ^a _a	1.80 \pm 0.01 ^a _a	7.40 \pm 0.30 ^a _a
	A ₂	2120 \pm 12.20 ^b _a	3400 \pm 8.20 ^a _a	4.20 \pm 0.50 ^b _a	11.20 \pm 0.20 ^b _a
	A ₃	3200 \pm 18.50 ^c _a	5120 \pm 3.20 ^c _a	4.60 \pm 0.10 ^c _a	14.50 \pm 1.30 ^b _a
<i>E.senegalensis</i>	A ₁	1006 \pm 7.30 ^a _a	4120 \pm 5.10 ^a _a	3.50 \pm 0.30 ^a _a	10.20 \pm 0.50 ^a _a
	A ₂	4170 \pm 3.70 ^b _a	5440 \pm 6.50 ^a _a	4.20 \pm 0.20 ^b _a	13.50 \pm 0.30 ^b _a
	A ₃	4430 \pm 8.20 ^c _a	5190 \pm 12.80 ^c _a	5.10 \pm 0.90 ^c _a	14.30 \pm 0.70 ^b _a
<i>T.marie</i>	A ₁	910 \pm 2.60 ^a _a	3170 \pm 2.50 ^a _a	1.40 \pm 0.03 ^a _a	11.10 \pm 1.20 ^a _a
	A ₂	2600 \pm 5.70 ^b _a	3800 \pm 4.20 ^a _a	3.10 \pm 0.20 ^b _a	11.80 \pm 1.50 ^b _a
	A ₃	3700 \pm 9.20 ^c _a	5520 \pm 1.80 ^c _a	3.60 \pm 0.10 ^c _a	10.50 \pm 0.20 ^b _a
Wet season					
<i>C.kingsleyae</i>	A ₁	1420 \pm 5.10 ^a _a	3170 \pm 8.20 ^a _a	2.50 \pm 0.20 ^a _a	12.30 \pm 2.10 ^a _a
	A ₂	3200 \pm 7.30 ^b _a	3490 \pm 3.70 ^a _a	4.20 \pm 0.40 ^b _a	14.60 \pm 2.50 ^b _a
	A ₃	4600 \pm 3.30 ^c _a	6210 \pm 11.80 ^c _a	4.90 \pm 0.30 ^c _a	14.80 \pm 1.10 ^b _a
<i>E.senegalensis</i>	A ₁	1280 \pm 3.20 ^a _a	4600 \pm 2.50 ^a _a	3.70 \pm 0.30 ^a _a	11.80 \pm 0.20 ^a _a
	A ₂	4420 \pm 11.20 ^b _a	5960 \pm 12.20 ^a _a	4.50 \pm 0.20 ^b _a	15.30 \pm 0.30 ^b _a
	A ₃	5120 \pm 4.80 ^c _a	5860 \pm 7.50 ^c _a	6.20 \pm 0.90 ^c _a	15.70 \pm 0.20 ^b _a
<i>T.marie</i>	A ₁	1410 \pm 8.10 ^a _a	3450 \pm 6.50 ^a _a	2.60 \pm 0.01 ^a _a	12.80 \pm 3.10 ^a _a
	A ₂	2900 \pm 2.20 ^b _a	4100 \pm 5.10 ^a _a	3.80 \pm 0.30 ^b _a	13.20 \pm 6.50 ^b _a
	A ₃	3900 \pm 4.50 ^c _a	5610 \pm 2.20 ^c _a	4.20 \pm 0.20 ^c _a	16.10 \pm 0.30 ^b _a
Recommendation					
Limit					
	FEPA	1000	3000	03	10
	WHO	2000	-	03	10

In *E.senegalensis*, during the dry season, the Cu content in the Zarama market was higher than the FEPA limit but lower than the WHO limit, in Kpansia and Igbogene the copper contents were higher in both FEPA and WHO limits. During the wet season, the copper content was higher than both WHO and FEPA in all three markets. In the wet season, the Cu content in *T.marie* was higher than FEPA and WHO limit in Igbogene and Kpansia but lower in the Zarama market. In the dry season, the copper content for Zarama was lower than FEPA and WHO recommendations except for Igbogene and Kpansia. The zinc limit for *T.marie* in *C.kingsleyae* and *E.senegalensis* was higher than the FEPA(3000) limit in all three markets and both seasons. The Cadmium limit for *T.marie* in *C.kingsleyae* was lower than the FEPA(03) and WHO(03) limit during the dry and wet season in Zarama but higher in Igbogene and Kpansia, that of *E. senegalensis* was higher in all three markets. The lead content in *C.kingsleyae* was lower than the FEPA(10) and WHO(10) limit in Zarama but higher in the other markets. In *E.senegalensis* and *T.marie*, the Pb contents were higher than recommended limits set by regulatory bodies in all markets during the dry season. Meanwhile, during the wet season, Pb concentrations were higher than the WHO and FEPA limit in all the samples and markets.

5. Discussion

Bacteria Count

The seasonal variation of microbial loads and heavy metals concentration in dominant native fishes in the Yenagoa metropolis showed inconsistencies in their distribution as shown in Table 1-4. Analysis of the microbial loads revealed that the bacteria density was high for both seasons, and maybe attributed to long-term contamination of bacteria through food and water [28]. Moreover, In the dry season months, the occasional plumage of the Niger Delta shore, which enters the river with a few derbies, fecales, and organic waste, produces a high-value total count of bacteria. Previous studies by several authors revealed that precipitation patterns of some areas can affect the interactions of invertebrate bacteria in water environments by change in bacterial concentration in surface water and feeding habits bacteria, and depuration dynamic [29,30]. Seasonally, the wet season had the highest bacteria loads than the dry season with *E.senegalensis* having the highest bacteria load and was observed during the wet season. The seasonal differences could be attributed to the presence of high moisture contents absorbed in the rainy season because of the humid atmosphere as witnessed during the sampling periods. A similar observation was reported from the selected selling point in the city of Muzu Malawi where fresh fish samples were within the maximum limit range during three seasons but the mean total for the wet and cold seasons was higher than that of the dry season [31]. A Buguma investigation also showed that, in the wet season, the tested seafood includes greater bacterial burden than during the dry season. [32], And they stated that an increase in the burden of bacteria can be attributed to an increase in the floral bacteria in adjacent waters since fish can concentrate bacteria in their close vicinity. [33]. *T. marie* had the least bacterial loads distribution with the Igbogene market having the least, and was reported during the dry season. This is because bacterial infection of peaches is also controlled to a degree by seasonal changes with the subsequent change in climate. [34]. This can also be attributed to proper smoking as has been made known by the fishmonger during an oral interview; smoking is known to contain antioxidants and chemicals that have bactericidal and bacteriostatic effects.

Fungi

Contamination of the fungi in these fishes follows a similar trend with the bacteria. All the markets had loads of fungi irrespective of the seasons. This could be a result of the fish handlers, fishmongers, and poor smoking facilities. The hygiene measures that were taken during the capture and processing of fish samples cannot be discounted. Similarly, it could also be as a result of the aquatic habitat in which the samples were caught and equipment used in fish processing that may encourage the growth of fungal species. Zarama market had the highest fungal counts and was also reported in *E.senegalensis* during the wet season. High moisture content that is peculiar to the season is a suspect. The least fungal load was observed in the wet season, recorded in *T.marie* in the Zarama market. This may be attributed to the fact that apart from minimal human – fish contact, Zarama is also a weekly market and so the samples are not too exposed to microbial contaminants such as dust and soils as Igbogene and kpanasia which are daily markets. The response of different microorganisms to change may be due to potentials which may be altered at different seasons, the variation of the climatic condition such as distinct wet and dry season selectively favors the growth

and proliferation of a different physiological type of microorganisms, not limited to tropic environment [35]

Isolates

The microorganisms isolated in this study include *Klebsiella* species, *Bacillus* species, *Yersinia* species, *Proteus* species, *Shigella* species, *Pseudomonas* species, *Fusarium* species, *Mucor* species, *Aspergillus* species, *Rhizopus* species, *Actinomyces* species, *Staphylococcus* species, *Chromatium* species, *Candida* species, *Trichophyton* species, *Listeria* species, *Salmonella* species, *Micrococcus* species, *Enterobacter* species. The presence of these microbes may be due to improper methods of processing of fish samples, contaminated materials used in the capture, unhealthy aquatic environments, the introduction of fecal matters, etc. The presence of *Shigella* species, *Staphylococcus* species, *Salmonella* species, could be a result of contamination. *Staphylococcus* species are pathogenic strains that could cause food poisoning due to heat-stable staphylococcus enterotoxin which is resistant to gastrointestinal enzymes [36]. They are one of the most opportunistic pathogens and a high population in samples determine the degree of spoilage the fish has undergone [37].

The fish feeding practice can also contribute to the presence of these pathogens. *Salmonella* species are fecal borne pathogens that could occur as a result of food handlers, fish harvested from contaminated water that harbor them. *Salmonella* species causes gastroenteritis and typhoid fever, *Shigella* species is causative agents of illnesses like shigellosis in the human who are the only reservoir of these organisms [38]. *Klebsiella* species are found in municipal sea waste. *Listeria* species are ubiquitous in the environment and has been isolated from soil, animal feces, and fresh and marine water, and the contamination of fish by this species can be attributed to contamination of the water bodies by sewage [39] the prevalence and virulence of *Listeria* species is unclear but the presence in water environment serves as a possible point of contamination. *Listeria* species can multiply and survive under low temperatures [40]. *Yersinia* species is common in the environment with pigs and rodents. Birds are also known to be carriers of these human and non-human pathogenic bacteria [41] The presence of *Yersinia* species in fish reflects not only the condition and safety of the aquatic system but also raise a public health concern with regards to fish consumed.

Proteus species are due to fecal contamination and it is also an opportunistic pathogen. *Bacillus* species cause infectious diseases such as abscesses, foodborne infections, ear infections, respiratory and urinary infections [42]. *Bacillus* species are also known to be accountable for food poisoning and their presence as a biological hazard in smoked fish products that are consumed raw have raised many concerns with fish products [43]. The presence of fungal species in dried fish can also be attributed to poor hygiene measures. Fungal species thrive in the soil and are opportunistic pathogens in immune-compromised patients. Retailers expose dry fish in a tray besides refuse heaps and also most samples are exposed to dust which when raised can encourage the growth of fungal species. The occurrence of *Aspergillus* species, *Rhizopus* species, *Actinomyces* species could be due to the fact that during storage the fish samples reabsorbed moist from the environment which then supported growth of microorganisms. *Candida* species can be associated with females it is a normal flora of the female reproductive area, this species can find its way into the fish sample through contaminated hands and places where the fish was

stored, or if the samples were kept where females urinate [40]. Dry fish samples can absorb moisture from the environment which will support the growth of microorganisms although low moisture content suppresses microbial growth some will still survive the dry season for some time before they die especially spore-forming microorganisms.

Heavy Metals

The heavy metals concentrations of *C. kingsleyae*, *E. senegalensis* and *T. marie* from markets in Yenegoa metropolis revealed that; *C. kingsleyae* had the highest and lowest zinc concentrations, recorded in Kpansia market and Zarama in the wet and dry seasons respectively. The Zinc concentrations of the fish samples in all the matrixes were higher than The FEPA limit (3000) during all seasons this shows that the samples are highly contaminated with Zn. It is important to note that Zn is an essential element but at high concentration, it may be toxic to fish, it accumulates on the gills of fish and creates adverse effects on fish by causing structural damage that affect the growth, development, and survival. Studies on the effect of Zn in *Cyprinus carpio* showed that most accumulation took place in the gill tissues, they also suggested that the higher content might have occurred due to mucus secretions and structural alternations in gill tissues caused by contamination [44]. Some aquatic species can store zinc to a certain amount even though they are not harmful when they reach the human chain via consumption, they cause potential health issues. Excessive intake of Zn by man leads to dehydration, vomiting, nausea, and abdominal pains. Sources of Zn include batteries, refuse, pesticides, alloys, fossil fuel; that have easy access to the aquatic environments.

Cadmium in the fish muscle may be associated with the lifestyle of the species at the bottom and/or mud [45]. The highest and lowest cadmium concentrations were reported in *E. senegalensis* and *C. kingsleyae*, and were recorded in Kpansia and Zaram markets during the wet and dry seasons respectively. The highest Cd content observed during the wet season was higher than the WHO and FEPA limits making the sample unfit for human consumption. Cd exhibits high toxicity and has chronic effects on fish and the environment after long exposure. Cd alters the antioxidants defense system and production of free radicals and disrupts calcium metabolism and sublethal effects such as obvious spine malfunction [46]. This heavy metal can be found in foodstuffs including seafood and is a non-essential element that can be sourced from fertilizers, fossil fuel, paints, etc. Municipal waste of industries transport Cd to water, the target organs are kidney and lungs. It has been reported that Cd can be assimilated from anoxic sediment with higher organic matter which generates the potential for bioaccumulation via dietary uptake [47]. Chronic exposure to Cd in human cause kidney malfunction, hypertension, anemia and liver problem [48].

The highest lead concentrations were recorded in *T. marie* from the Kpansia market during the wet season, while the lowest Pb concentrations were recorded in the dry season in *C. kingsleyae* from the Zarama market. The Pb content during the wet season was higher than the FEPA and WHO limit but lower during the dry season. Pb is a dangerous heavy metal and much thought have been given to it due to its considerable health risk in human, its residue could result in gastrointestinal, neurological malfunction prolonged exposure may also cause hypertension and reproductive impairments [49].

E.senegalensis had the highest copper concentrations and it was recorded in the dry season from Kpansia market, while *C.kingsleyae* had the lowest Cu concentrations detected in Zarama. The highest Cu content was greater than the FEPA and WHO limits. The high concentration of this heavy metal could be a result of the disposal of waste containing copper into the water bodies by industries, domestic waste, and automobiles, such as speedboats [50]. The high metal concentration in tissues of fish, observed during the hot season was related to an increase in metabolism during high temperature [51]. Cu has a great tendency to biomagnifies in man when food species are consumed [52]. Aquatic trash enters the ecosystem and the response of aquatic waste systems to the waste input largely depends on the kind of waste, method of waste disposal, exchange rate of water, water volumes, air capacity and biochemical interactions within the aquatic system [53,54].

6. Conclusion

Heavy metal levels were obtained in the three species of fish and were found to be distributed selectively between the various species being considered. The concentration of heavy metals was higher in the weather than in the dry season. Micro-organisms contaminate smoked dried fish sold at Zarama, Kpansia, and Igbogene markets. It was also noted that the change in season has impacts on the growth and development of microorganisms. Caution should be exercised in consuming smoked dried fish shaded openly as such fish could contain microbial cells and reheating may be necessary to deactivate such cells heavy metals are harmful to the environment the effect of consumption of contaminated fish cannot be overemphasized from its effect in fish to adverse health implications in man when it is consumed. The paper also gives essential information and data for aquatic toxicological and microbiological analyses and for the study of the environment, which will improve the safety and sustainability of the aquatic environment.

Conflict of Interest:

The authors declare no conflict of interest

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