Chain of Contamination of Seafood with References to Seasonal Distribution of Microbial Loads and Trace Metals in Fishes from Regional Markets, Niger Delta Nigeria

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Abstract

The distribution pattern of microbes and trace metals in the dominant seafood in the major markets in Southern Nigeria was investigated. Three markets were chosen for this study; Swali, Opolo and Otuoke Markets. Swali market is the landing point for the fishes, while the other markets make purchases from the former. 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 microbial isolates include *Actinomycetes* species, *Klebsiella pneumonia*, *Enterococcus* species, *Salmonella* species. *Proteus* species, *Micrococcus* species, *Bacillus cereus*, *Listeria monocytogenes*, *Chromatium* species, *Enterobacter aerogenes*,*Yersinia* species, *Shigella* species, *Pseudomonas* species, *Micrococcus* species, *Staphylococcus albus*, *Actinomycetes* species, *Mucor* species,*Aspergillus flavus*, *Fusarium* species, *Rhizopus stlonifera*, *Trichophyton* species, *Candida tropicalisi* and *Aspergillus* species. Spatial distribution revealed that Otuoke market had the highest microbial load, while Swali had the least. However, no descripancy in the heavy metals loads in the three stations, but certainly above the regulatory limit. The high microbial loads reported in the markets with the exception of the Swali market could be attributed to chain of contamination by the fish's mongers and retailers. Fish is one of the most affordable commodity and so great concern and awareness must be created in order to curb the spread of microorganisms and heavy metals that could cause adverse health effects.

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Introduction

Infectious material like bacteria, yeast, fungi, virus, prions, protozoa or their toxins and trace metals can easily contaminate sea foods either unintentionally or accidentally. The normal flora of both dried and fresh fish depends on the place of capture, fish handlers and processors, the atmospheric exposure and storage methods. The contamination pathway of seafood may also be associated with bad quality of water, farm run-off, feed and conditions of non-sanitary production (Essien *et al*., 2006, Nummelin *et al*., 2007). Seafood living in polluted water may gather higher amount of poisonous heavy metals through their food chain. Fishes reared in artificial fish ponds and lakes often produce heavy metals due to environmental pollution and contamination during processing (Voegborlo *et al.*, 1999).

Many scenarios of microbial and heavy metals contaminations have been reported in Nigeria. For instance, low concentrations of heavy metals and high bacterial contamination found in smoked fish species were recorded in Benin City, Nigeria, due to excessive smoking and unhygienic handling of goods (Daniel *et al.*, 2013)

Abolagba and Iyeru (1988). reported that the absence of proper smoking and unhygienic handling of smoked fish products result in a very high microbial load and open flame smoking of fish has been reported to generate cancer-promoting compounds in the body. *Salmonella typhi* and *Salmonella Entridis* were isolated among other organisms. This is a problem with food safety, because smoked fish could be a potential agent of transfer of these species to unsuspecting clients, the presence of *Staphylococcus aureus* which is a normal floral of the human body may have been through contamination in handling process. Smoked fish are commonly hawked in many developing communities without taking awareness of the microbial environmental pollution. Before reaching customers, smoked fish products may be

infected with microorganisms and heavy metals from processing units and market centers because many processors and fish mongers typically display them freely in a way that could be possible sources of contamination.

Heavy metals such as cobalt, copper, zinc, manganese and iron, and manganese at low concentrations are important for living, when contaminated fish are ingested, they can have possible health consequences. (Chen and Chen, 2001.

The objective of this study is to investigate the pattern in which seafood samples can be contaminated through the transfer chain, with the view to accentuate the hygienic condition of seafood through exchange of hands by retailers or consumers.

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, HNO_3, 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 *C. garipineus, C.lateceps and C.furcatus* were purchased from three major markets (Swali, Opolo and Otuoke) within Yenagoa metropolis Bayelsa State, Nigeria. The markets were chosen based on their proximity to the people and availability of the investigated fishes. Otuoke is a weekly market while Opolo and Swali are the daily markets. Samplings were done on monthly basis and coincided with Otuoke 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^{o}c for one hour. Each fish sample was crushed separately in a mortar in a homogenized using an electric blender, from which 0.1g was homogenized in 9ml of sterile water and allows to stands 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. Enumeration of fungi and bacteria was performed on appropriate media. Total heterotrophic bacteria on nutrient agar, total coliform bacteria on McConkey agar, *salmonella\shigella* on *salmonella\shigella* agar, *vibrio* on thiosulfate citrate bile salt sucrose, *staphylococcus* on mannitol agar, and fungi on sabourand dextrose agar and potato dextrose 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^{o}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^{o}c for 72 hours. After incubation, colonies on each plate were counted using the colony counter. Bacteria isolate was identified by standard biochemical test including gram reaction, catalase, motility, coagulase, methyl-red Voges, Proskauer test, starch hydrolysis, citrate, urease, and sugar fermentation (Hott *et al*., 1994).

The total fungi count of the fish sample was duplicated and inoculated on potato dextrose agar at room temperature for 72 hours. For fungal growth and suppression of bacteria growth, *streptomycin* and *penicillium* solution (50ug/ml and 50 IU/ml respectively) supplemented medium was used for selective enumeration and isolation of fungi. Discrete colonies that appeared on the culture plate were enumerated with the aid of a Quebec colony counter and recorded as a colony-forming unit (cfu/g). Fungi isolates were identified according to the method of (Barnett *et al*.,1974; Domesch *et al*.,1980).

Heavy Metal Analysis

Five (5)g dry weight sample was put into a 50 ml beaker with 5 ml of HNO_3 and 5 ml of H_2SO_4. When the fish tissue stopped reacting with HNO_3 and H_2SO_4, the beaker was then placed on a hot plate and heated at 60°C for 25 minutes. The beaker was allowed to cool, 10 ml of HNO_3 was added and returned to the hot plate to be heated slowly to 120°C. The temperature

was increased to 150°C, and the beaker was removed from the hot plate when the samples turned black. The sample was then allowed to cool before adding H_2O_2 until a clear solution appeared. The content of the beaker was transferred into a 50 ml volumetric flask and diluted to the mark with ultra-pure water then stored with PFTE until analyzed with Atomic Absorption spectrometer Perkin-Elmer model 306.

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

Results

The microbial counts of dominant fish species from three major markets in South-south Nigeria during the dry season (December to March, 2019) and wet season (April to November, 2020), showed that the fishes from this region were highly contaminated (Table 1,2 and 3).

The highest bacteria counts were recorded in Otuoke market for both seasons (56.20 and 94.30) cfu x 10^5, while the lowest bacteria count (14.15, 32.20) cfu x 10^5 was found in Swali market for both seasons, the spatial distribution was Otuoke>Opolo>Swali,

Similarly, the highest fungal count (5.80 and 36.10) cfu x 10^5 was also recorded in Otuoke market for both seasons while the least fungal count (2.10) cfu x 10^5 was recorded in Opolo market during the dry season

Table 1: Seasonal variation of microbial load from smoked fishes from major markets in Niger- Delta Districts Nigeria

Markets		Number of colonies (cfu x 10^5)		
		C.gariepinus	*C.laticeps*	*C.furcatus*
Dry Season				
	TBC	14.15^a	6.03^b	11.10^a
Swali	TFC	4.70^a	4.30^a	2.70^a
	TBC	22.04^b	35.88^c	41.56^c
Opolo	TFC	5.20^a	3.60^a	2.10^b
	TBC	56.20^c	45.10^c	50.20^c
Otuoke	TFC	5.80 ^{a}	4.80 ^{a}	3.20^b
Wet Season				
	TBC	32.20^a	12.50^a	17.78^a
Swali	TFC	12.80 ^{b}	5.20^a	4.20^b
	TBC	72.36^b	56.50^b	63.30^b
Opolo	TFC	33.20^b	18.40^b	13.20^b
	TBC	83.40^c	94.30^c	74.90^c
Otuoke	TFC	36.10^b	21.30^b	17.20^c

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

Seasonally, wet season had the highest bacterial and fungal counts (94.30, 36.10) cfu x 10^5 with Otuoke market having the highest microbial loads. The dry season had the least bacteria and fungi load, with Opolo market having the least fungi load (2.10) cfu x 10^5 and Swali market having the least bacteria load(6.03) cfu x 10^5.

C.gariepinus had the highest bacteria and fungi load (56.20, 5.80) cfu x 10^5 during the dry seasons respectrively, the lowest bacteria load (6.03) cfu x 10^5 was found in *C.laticeps* and that of fungi (2.10) cfu x 10^5 was found in *C.furcatus*.

The microbial isolates for both seasons in the three markets are; *Actinomycetes* species, *Klebsiella pneumonia*, *Enterococcus* species, *Salmonella* species. *Proteus species*, *Micrococcus* species, *Bacillus cereus.*, *Listeria monocytogenes*, *Chromatium* species, *Enterobacter aerogenes*,

Yersinia species, *Shigella* species, *Pseudomonas* species, *Micrococcus* species, *Staphylococcus albus*, *Actinomycetes* species, *Mucor* species,*Aspergillus flavus, Fusarium* species. *Rhizopus stlonifera* species, *Trichophyton* species, *Candida tropicalis*, *Aspergillus* species

In both seasons, the highest microbial isolates were recorded in fishes from Otuoke market,18 isolates for dry season and 26 isolates for wet season. The lowest microbial isolate was observed in samples from Swali market with total number of isolates for dry and season were 13 and 15 respectively (Table 2 and 3)

Table 2: Bacteria isolated from smoked fishes during the dry and wet seasons from major markets in Niger- Delta Districts Nigeria

Markets	*C.gariepinus*	*C.laticeps*	*C.furcatus*
	Dry Season		
Swali	*Actinomycetes* species, *monocytogenes* *Klebsiella pneumonia*, *Enterococcus* species *Salmonella* species	*Enterococcus* species *Proteus species, Micrococcus* species and *Bacillus cereus*	*Listeria* *Chromatium species and Bacillus cereus*
Opolo	*Enterobacter aerogenes*, *Enterococcus* species, *Listeria monocytogenes*, and *Bacillus cereus,*	*Bacillus cereus*, *Yersinia* species, *Chromatium species* *Enterococcus* species	*Actinomycetes* species *Salmonella* species and *Bacillus cereus*
Otuoke	*Chromatium* species, *Listeria monocytogenes*, *Shigella* species, *Yersinia species*, *Proteus species,*	*Proteus species, Pseudomonas* species, and *Yersinia* species	*Enterococcus* species *Chromatium species* and *Yersinia species*
	Wet season		
Swali	*Micrococcus* species, *Shigella Species, Klebsiella pneumonia, Enterococcus* species *Staphylococcus albus*	*Listeria monocytogenes Yersinia species, Micrococcus* species *Enterococcus* species	*Actinomycetes* species *Proteus species* *Shigella* species
Opolo	*Klebsiella pneumonia*, *Shigella* Species, *Enterococcus* species, *Listeria monocytogenes*, *Bacillus cereus, Actinomycetes* species, *Enterobacter aerogenes,*	*Micrococcus* species, *Yersinia* species, *Chromatium species* *Salmonella* species	*Pseudomonas* species *Salmonella* species *Listeria monocytogenes*
Otuoke	*Pseudomonas* species, *Listeria monocytogenes*, *Salmonella* species, *Yersinia species*, *Proteus species, Shigella species, Staphylococcus albus,* and *Chromatium species*	*Actinomycetes* species, *Klebsiella pneumonia*, *Yersinia* species, *Bacillus cereus*	*Micrococcus* species *Chromatium species* *Enterobacter aerogenes* *Salmonella* species

Table 3: Fungal isolated from smoked fishes during the dry and wet seasons from major markets in Niger- Delta Districts Nigeria

Markets	*C.gariepinus*	*C.laticeps*	*C.furcatus*

	Dry Season		
Swali	*Aspergillus flavus* and *Fusarium* species	ND	ND
Opolo	*Mucor* species and *Rhizopus stlonifera*	*Aspergillus flavus* and *Trichophyton* species	ND
Otuoke	*Candida tropicalis*, *Trichophyton* species *Aspergillus flavus* and *Aspergillus niger*	*Rhizopus stlonifera* *Aspergillus niger*	*Aspergillus niger*
	Wet Season		
Swali	*Trichophyton* species and *Candida tropicalis*	*Rhizopus stlonifera*	ND
Opolo	*Fusarium* species, *Mucor* Species and *Rhizopus stlonifera*	*Candida tropicalis*	*Aspergillus flavus*
Otuoke	*Aspergillus flavus* , *Aspergillus Niger, Candida tropicalis*, *Rhizopus stlonifera* and *Mucor* species	*Fusarium* species *Trichophyton* species and *Mucor* species	*Trichophyton* species, *Rhizopus stlonifera* and *Mucor* species

Heavy metals; Cd, Pb, Zn and Cu were detected in various concentrations in the fishes examined from the three major markets in Southern Nigeria. Cd concentration (4 .50 ± 0.20) µg/gdw and (1.60 ± 0.10) µg/gdw were observed in. *C.gariepinus* and C.*furcatus* in the dry season wet season respectively.

The highest Cu concentration (1900 ± 5.20) µg/gdw was observed in *C. gariepinus* during the dry season the lowest(732 ± 0.20) µg/gdw was recorded in *C.furcatus* during the wet season, the highest Zn concentration (5120 ± 3.40) µg/gdw was observed in the dry season in *C. gariepinus* while the least Zn concentrations(2180 ± 3.20) µg/gdw was observed in *C.furcatus* during the wet season. The highest (12.30 ± 1.500) µg/gdw and lowest (3.10 ± 0.20) µg/gdw Pb concentration were observed in *C.furcatus* in both dry and wet season .

The concentration of Zn in *C. garipineus, C.lateceps* and *C.furcatus* was above that of FEPA (3000) for both season, expect *C.furcatus* which is below that of FEPA in the wet season

Similarly, the concentration of Cd in *C. garipineus, C.lateceps and C.furcatus* in the dry season is above the FEPA (03) and WHO(03) recommendation limits . While in the wet season, the concentrations of Cd in *C. garipineus* and *C.furcatusis* were below that of FEPA(03) and WHO(03) with expection of *C.lateceps* which were above the recommendation limit set by the two regulatory bodies

The concentrations of Cu in *C.gariepinus, C.lateceps* and *C.furcatus* for both seasons were above the FEPA limit(1000) but below the WHO limit (2000) excluding *C.furcatus* which was below that of FEPA and WHO limit during the wet season

The concentrations of Pb in *C.gariepinus, C.lateceps* and *C.furcatus* was below that of FEPA and WHO in both season excluding *C.furcatus* which was above the FEPA and WHO limit during the dry season.

Table 3: Heavy metal (µg / gdw) concentrations in smoked fishes during the dry and wet seasons from Swali market, Niger- Delta Districts Nigeria and the recommendation limits

	Cu	Zn	Cd	Pb
Wet season				
C. gariepinus	$1302 \pm 2.80^{a}_{a}$	$4110 \pm 3.40^{a}_{a}$	$2.10 \pm 0.01^{a}_{a}$	$6.10 \pm 0.50^{a}_{a}$
C.laticeps	$1113 \pm 0.50^{b}_{a}$	$3110 \pm 4.20^{a}_{a}$	$3.40 \pm 0.30^{b}_{a}$	$7.30 \pm 1.40^{b}_{a}$
C.furcatus	$732 \pm 0.20^{c}_{a}$	$2180 \pm 3.20^{c}_{a}$	$1.60 \pm 0.10^{c}_{a}$	$3.10 \pm 0.20^{b}_{a}$
Dry season				
C. gariepinus	$1900 \pm 5.20^{a}_{b}$	$5120 \pm 3.40^{a}_{b}$	$4.50 \pm 0.20^{a}_{b}$	$9.20 \pm 1.10^{a}_{b}$
C.laticeps	$1410 \pm 2.10^{b}_{b}$	$3710 \pm 3.20^{b}_{b}$	$3.80 \pm 0.20^{b}_{a}$	$9.80 \pm 0.30^{b}_{a}$
C.furcatus	$1032 \pm 0.20^{c}_{b}$	$3110 \pm 1.80^{c}_{b}$	$3.20 \pm 0.0^{c}_{b}$	$12.30 \pm 1.50^{b}_{b}$
Recommendation Limit				
FEPA	1000	3000	03	10
WHO	2000	-	03	10

Means with the same superscript and subscript within the column are not significantly ($p > 0.01$) different; Superscript = Matrixes; Subscript = Season

Discussion

Microbial Count

Contaminations of the dominant fish species in some major markets in southern Nigeria revealed a chain of contaminations in the investigated markets, which were observed in all the markets.

The presence of the microbes at Swali market, that serves as a distribution centre indicates that the fish products could be contaminated with bacteria and fungi from the field, the processing units and the market centers before reaching the consumers because many processors and fish mongers usually display them openly in a manner that could be potential sources of microbial contamination. The order of contamination of microbial loads was: Otuoke>Opolo>Swali.The high microbial loads recorded in Otuoke and Opolo markets, indicates that processed fish are easily contaminated with microorganisms in nature, through handling and processing. Contamination can occur from the process of transportation of these commodities to their various point of sales. Fish mongers buys fish from the major point of distribution and may decide to sell in the other markets. the products are then placed in locally made baskets or jute sacs that may be contaminated.

Wet season had the highest microbial load. During the wet season the moisture content is usually high and fish has the tendency of absorbing high moisture and when they are also kept in cold rooms they have the tendency of being contaminated. The moisture content of fish plays a significant role in spoilage, thus reducing moisture delays spoilage. Seasonal variation in moisture content of fish may also be as a result of variable drying time, environmental changes and amount and type of salt used However, the moisture content seems to be an exact measure of the tendency of the substance to experience microbial spoilage. Abolagba and Iyeru(1988). also reported that the absence of proper smoking and proper hygienic handling of smoked fish products result in a very high microbial load.

The dry season had low microbial loads this could be as a result of low moisture content, proper drying, packaging and storage. Smoking provides a longer shelf life through its anti-bacterial and oxidative effect, lowering pH, also imparting desirable colorations in addition to giving the product a desirable taste and odours (Kumolu and Johnson *et al*., 2009, Abolagba and Melle,2008).

C.gariepinus had the highest bacteria and fungi load during the dry season. *C.gariepinus* also known as catfish can be reared both naturally and artificially, the presence of microbial load in this sample could be contamination from the ponds, feeds, materials used for capture, processing storage and unhygienic display of the commodity.

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 (Marshall *et al*.,1998).

Isolates

The microbial isolates for both seasons in the three markets are; *Actinomycetes* species, *Klebsiella pneumonia*, *Enterococcus* species, *Salmonella* species. *Proteus* species, *Micrococcus* species, *Bacillus cereus.*, *Listeria monocytogenes*, *Chromatium* species, *Enterobacter aerogenes*, *Yersinia* species, *Shigella* species, *Pseudomonas* species, *Micrococcus* species, *Staphylococcus albus*, *Actinomycetes* species, *Mucor species Aspergillus flavus*, *Fusarium* species, *Rhizopus stlonifera* species,

Trichophyton species, *Candida tropicalis, Aspergillus* species. *Staphylococcus aureus and Eschericia coli* are one of the commonest micro-organisms associated with smoked fish. The presence of *Staphylococcus aureus* in fish could be as a result of contamination through handling, *Staph aureus* is one of the most common causes of human disease and they constitute the normal flora of the human skin and mucous membrane.The presence of *Eschericia coli* and *Staphylococcus aureus* in the smoked-dried fish samples was noted to be the most common species linked with smoked fish.

The presence of *Candida* spp which is a normal floral of the female reproductive tract may be as a result of displaying fish samples close to areas where people urinate, it could also be as a result of improper handling. Some microorganism is present as spores in air and dust and can easily contaminate the fish sample when dust is raised or when exposed to contaminated air. Some *Aspergillus* spp can cause liver problems due to aflatoxins produced by these microorganisms. The presence of *A. flavus* in the studied fish samples is of great health concern because of their mycotoxigenic potentials.*A. flavus* and *A. fumigatus* produce aflatoxins, which destroyed the liver and kidney in man resulting to death. The presence of these organisms in the fish could be as a result of handling processes during smoking and cross contamination during storage, or during sales of smoked fish (Essien *et al*., 2005).

The bacteria group of *Staphylococcus aureus* is one of the most common causes of human disease and they constitute the normal flora of the human skin and mucous membrane without resulting in a diseased condition (Herman *et al*., 2011).

Staphylococcus spp has pathogenic strains which could cause food poisoning due to the heat stable *Staphylococcus enterotoxin* which is resistant to gastrointestinal enzymes. *S. aureus*, is one of the most common causes of boils, impetigo and folliculitis and in some cases, bacteremia and infections of the bones and wounds (Okareh and Erhahon, 2015). *E. coli* and *Salmonella* are fecal borne pathogens and they could occur as a result of contamination from the handlers. Fish harvested from contaminated waters can also habour some of these microorganisms thus making them unfit for human consumption. *E. coli* usually cause diarrhea and kidney damage as well as uncomplicated community acquired urinary tract infections while *Salmonella* caused gastroenteritis and typhoid fever (Adelaja *et al.,* 2013). *Salmonella sp.* May naturally, be present in tropical aquatic environments (Tiamiyu *et al*., 2011). *Klebsiella, Proteus, Bacillus, Streptococcus, Pseudomonas, Staphylococcus* spp. are the microbial flora found in smoked fish.

Heavy Metals

The analysis of the metals in the study review the order of Cd< Pb < Zn < Cu. With the highest concentrations observed in dry season. This could be attributed to changes associated with increased water temperatures during the season. These variations in the metal contents of the fish samples can also be ascribed to the different pollution levels at these locations. Contamination of heavy metals could be from diverse sources; these metals can be carried away or blown by wind from land surface to rivers used by man for various activities or it can be obtained from various surrounding waters. Bioaccumulation of these metal can occur when the rate of uptake far outweighs the rate of ejection.

Zn is an essential element as well as copper and cobalt for both animals and humans. The varying concentration of zinc in the muscles of the fish samples could be due to the presence of large numbers of fishing vessels and trawlers which use galvanized metal coatings to prevent rusting and this ultimately find its way into the ambient media through leaching. Zinc has also been reported to be necessary for embryo development in fish .(carpene *et al.*, 1994).The recommended limit of Zn in *C. garipineus, C.lateceps and C.furcatus* is above that of FEPA (3000) in both season, expect *C.furcatus* which is below that of FEPA in the wet season. This reveals that the fish samples excluding *C.furcatus* during the wet season is not suitable for eating, when these samples are injested it could cause adverse health effects

The recommended limit of Cu in *C.gariepinus, C.lateceps* and *C.furcatus* for both seasons was above the FEPA limit(1000) but below the WHO limit (2000) excluding *C.furcatus* which was below that of FEPA and WHO limit during the wet season. This fish sample is good for consumption according to the WHO limit and not suitable for consumption according to the FEPA limit. Cu is an essential metal and it is necessary for hemoglobin synthesis. Excessive intake can cause kidney damage and death

The presence of Cd in fish sample can be endorsed to the immediate environment of the fish where these metals was taken up by the fish this is could be as a result of the sample gotten from contaminated water bodies where these samples are prevalent.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(Muniz *et al*.,2004). Chronic exposure to Cd in human cause kidney malfunction, hypertension, anemia and liver problem (Erah *et al*.,2002).

The recommended limit of Cd in *C. garipineus, C.lateceps and C.furcatus* is above that of FEPA (03) and WHO (03) in the dry season. While in the wet season, the recommended limit of Cd in *C. garipineusand C.furcatusis* below that of FEPA(03) and WHO(03) with exception of *C.lateceps* which is below that of FEPA and WHO. Intake of fish samples whose limit Are above that of FEPA and WHO may lead to adverse health implications such as damage in kidney, hypertension,tumor and hepatic dysfunction, convulsion, cramps, and still death in human.

The recommended limit of Pb in *C.gariepinus, C.lateceps* and *C.furcatus* was below that of FEPA and WHO in both season excluding *C.furcatus* which was above the FEPA and WHO limit during the dry season. This indicates that all fish samples below the recommended limit are suitable for consumption and will not cause any potential health risk.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 (Celik *et al*.,2006). In addition to this Pb occurs naturally and can also be produced industrially this element is very toxic to humans especially in children where it causes renal dysfunction. It also led to muscle weakness.

Conclusion

The investigation had shown that fishes sold in the major markets in southern Nigeria harbour microbes and traces of metals. It also showed that there were chains of contaminations considering the major collection and distribution centre (Swali market) and the other smaller markets Otuoke market having the highest contamination level. Chain of contamination may also be attributed to poor methods of transportation, poor handling of the samples, contamination through fish mongers who display these samples in inappropriate environments. The presence of these microbes and toxic metals in these sea food put human at risk as these commodities can be transported for consumption both locally and internationally and when ingested can produce adverse health effects therefore there is need for proper monitoring of these fish species produced in this regions in order to reduce health complications accompanied with these commodities.

References

Abolagba, O.J. and Iyeru, O.A. (1998). Study of Insect Pest Infecting Traditionally Processed Fish Sold in Benin City Metropolis, Nigeria. Nig. *J. Applied Sci.* 16: 25-29.

Abolagba, O.J. and Melle, O.O. (2008). Chemical composition and keeping qualities of a scaly fish tilapia, Oreochromis niloticus smoked with two energy sources. Afr. J. *Gen. Agric.* 4(2):113-117.

Adelaja, O.A., O. J., Olaoye, O.J., Ikenweiwe, N.B. and Ashley-Dejo, S.S; (2013). Comparison of microbial load associated with smoked fish (Chrysichthys nigrodigitatus) from Oyan Lake and Ogun Waterside in Ogun State, Nigeria. Global *J. Sci. Frontier Res. Agri. and Vet.,* 13(8): 34-39.

Barnett HC, Hunter BB. Illustrated Genera of Imperfect Fungi 3rd Ed. Mimeapolis Burgers Publishing Company. 1974; pp 180-200

Carpene, E., Gumiero, B., Fedrizzi G. and Serra, R. (1994). Trace elements (Zn, Cu, Cd in fish from rearing ponds of Emilia-Romagna region (Italy). Sci. Total Environ., 141: 39-146

Chakrabarti, R. and Varma, P.R.G. (1997). Fungi in salted and dried fish at Kakinada coast, Fishery Technology 34: 4 – 8.

Celik ES. Baliklarin Kan Parametleri Uzenne Agir Metalle rin Etkisi E.U.S.U Urunlneri Dergisi 2006;23(1/1) 49-55

Chen, Y. and Chen M. (2001). Heavy metal concentrations in nine species of fishes caught in coastal waters of Ann-ping SW Taiwan. *J Food Drug Anal*. 9:107-14

Daniel, E.O., Ugwueze, A.U. and Igbegu, H.E. (2013). Microbiological quality and some heavy metals analysis of smoked fish sold in Benin City, Edo State, Nigeria. World *J. Fish Marine Sci.* 5(3):239-243

Domesch KH, Gams W, Anderson TH. Compendium of Soil Fungi Vol 1-2 London Academic Press 1980; pp 1-860

Essien, J. P., Ekpo, M.A. and Brooks, A.A. (2005). Mycotoxigenic and proteolytic potential of moulds associated with smoked shark fish (Chlamydoselachus anguincus). *J. App. Sci. Enviro. Management*, 9: 53-57.

Essien, J.P., Antai, S.P.and Benson, N.U. (2006). "Microbial population dynamics as a function of sediment salinity gradients in the Qua Iboe Estuary Mangrove Swamp (Nigeria), Research *Journal of Microbiology*, vol. 1, no. 3, pp. 255–265, 2006. View at: Publisher Site | Google Scholar

Erah, P.O., Akujieze, C.N. and Oteze, G.E. (2002). The Quality of Ground Waters in Benin City: A Baseline Study on Inorganic Chemical and Microbial Contamination of Health Importance in Boreholes and Pen Wells *Trop J OF Pharm Res*;1(2):75-82

Herman, H. H. and Lin, W., Petrecca, P.J., Simmons, M.R. and Houghton, J., (2011). Centrifugal

bioreactors and their application in remediations. *Remediation J.*, 11(4): 15-33

Hott, J.G., Krieg, N.R. (1994). Berger Manual of Determinative Bacteriology Williams Wilkins, Baltimore USA. 1994; pp 680

Kumar, H.S., Sunil, R.M.N., Venugopal, I. and Karunasagar, S. (2003). Detection of Salmonella species in tropical seafood by polymerase chain reaction. *International Journal of Food Microbiology* 88: 91-95.

Kumolu-Johnson, C.A., Aladetohun, N.F. and Ndimele, P.E. (2009). The effects of smoking on the Nutritional Qualities and Shelf-Life of Clarias gariepinus (BURCHELL 1822*). Afr. J. Biotechnol.* 9: (1): 73-76

Marshall, T.R. and Divinny, J.S. (1998). Microbial Ecosystem in Petroleum Waste Lands Treatment Water Science Technology,20(11/12):285-291

Muniz, P., Danulat, E., Yannicelli, B., Garcia, A.J., Medina, G., Bicego, M. (2004). Assessment of Contamination by Heavy Metal and Petroleum Hydrocarbon in Sediments of Montevideo Harbour (Uruguay). *Environmental int*., 29:1019-1028

Nummelin, M., Lodenius, M., Tulisalo, E., Hirvonen, H. and Alanko, T. (2007) "Predatory insects as bioindicators of heavy metal pollution," Environmental Pollution, vol. 145, no. 1, pp. 339–347

Okareh O.T. and Erhahon, O.O. (2015). Microbiological Assessment of Food and Hand-wabs Samples of School Food Vendors in Benin City, Nigeria,Food and Public Health, (5)1: 23- 28

Tiamiyu, A. M., Emikpe, B. O. and Adedeji, O. B. (2011). Isolation and Identification of aerobic bacteria flora of the skin and stomach of wild and cultured Clarias gariepinus and *Oreochromis niloticus* from Ibadan, Southwest, *Nigeria. Journal of Applied Sciences* Research, 7 (7): 1047-1051

Voegborlo, R.B., Methnani A.M.E. and Abedin M.Z. (1999). Mercury, cadmium and lead content of canned Tuna fish. Food Chem. 67(4):341-345.7.