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Quality of the Seafood in Southern Nigeria with References to Microbial Loads and Trace Metals in *Citharidium ansorgii*, An Endemic Fish Species

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ABSTRACT

Background and Objective: Trace metals may contaminate food sources and accumulate the agricultural product and seafood through water, air soil pollution, contamination of soil may further pose risks to human health through direct ingestion with the food chain. Hence, the microbial loads and heavy metals concentrations in *Citharidium ansorgii*, one of the most common fish species in Southern Nigeria were investigated to ascertain its health status. **Materials and Methods:** Sampling was done monthly and a total of 36 fish samples were randomly purchased from fishmongers in six major markets in the region. Bacteriological isolates were analyzed using standard bacteriological procedures, while standard identification keys and atlas were used in identifying fungal isolates. The trace metals were analyzed using the atomic absorption spectrophotometer (G105 UV-VIS). **Results:** The total bacteria population of *C. asorgii* from the markets varied from 8.40-43.10 CFU g^{-1}. The fungi count ranged from 3.80-18.20 CFU g^{-1}. The bacteria isolates were, *Micrococcus* species, *Klebsiella pneumonia*, *Enterococcus* species, *Bacillus cereus*, *Staphylococcus albus, Actinomycetes* species, *Enterobacter aerogenes, Pseudomonas* species, *Salmonella* species, *Listeria monocytogenes*, *Yersinia* species, *Proteus* species, *Shigella* species, *Chromatium* species. *Klebsiella pneumonia*, *Staphylococcus albus*, *Shigella* species and *Salmonella* species had the highest frequency of occurrence. The fungi isolated were *Aspergillus flavus*, *Fusarium* species, *Rhizopus stolonifera*, *Mucor* species and *Trichophyton* species. *A. flavus*, *Fusarium* species and *Trichophyton* species had the highest frequency of occurrence. The concentration ranges of the metals were, Cu, 1120-3540 µg g^{-1} dw, Zn, 2250-7420 µg g^{-1} dw, Cd, 1.60-600 µg g^{-1} dw and Pb, 7.80-7.20 µg g^{-1} dw and were above the standard limits prescribed by regulatory bodies. **Conclusion:** The pathogenic microorganism and heavy metals detected in the endemic fish in this region can pose serious health hazards to consumers as they are not safe for human consumption, an observation that calls for regular monitoring of the kinds of seafood in this region.

KEYWORDS

Trace metals, microbial quality, *Citharidium ansorgii*, endemic fish, Southern Nigeria

INTRODUCTION

Nigeria is a maritime state where nine of the thirty-six federal states have a coastline in the Atlantic Ocean. The coastal federal states of Nigeria are Ogun, Lagos, Ondo, Edo, Delta, Bayelsa, Rivers, Akwa-Ibom and Cross rivers states and are located in the southern part of the country.

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It is well documented that fish is one of the major sources of food and income globally[1] and the importance of the fisheries sector to individuals and the economy of many developing countries cannot be over-emphasized.

It is a major source of animal protein and contains most of the important essential amino acids, particularly lysine, methionine and tryptophan that are lacking in plant proteins[2,3]. Also, important essential amino acids, particularly lysine, methionine and tryptophan that are lacking in plant proteins are abundant in fishes[4]. They have wider acceptability in most parts of Nigeria due to their unique taste, flavor and good texture. Species such as bonga fish (*Ethmalosa fimbriata*) and catfish (*Clarias gariepinus*) are highly accepted and consumed in their smoke-dried form in Nigeria, particularly in Akwa Ibom state due to their affordability and highly delicious flesh[5].

Anthropogenic activities have been a source of contamination in the aquatic environment and aquatic organisms are susceptible to contamination, especially microbial and heavy metals. The microbial organisms, which are either naturally present in the aquatic environment or from terrestrial sources often find the surface or organs of aquatic organisms for colonization[6]. The number and type of microorganisms found in freshly caught seafood are influenced by location, season and rate of environmental pollution.

Aquatic organisms accumulate metals in their different organs and tissues[7]. The accumulation of heavy metals in fish mainly depends on their concentration in food and water. Heavy metal concentration in water, sediment and aquatic animal, such as fish, could indicate the level and tendency of the population. This is important not only for the protection of the environment but for the evaluation of the quality of fish meat either captured from natural water or cultured in fishponds[8]. The mechanism of accumulation and storage of trace metals in aquatic organisms are diverse, varying with the chemical form of metal, mode of uptake and animal species[9]. Many aquatic organisms can excrete the excess proportion of their metal intake under contaminated conditions and thus maintain trace metal concentration in the body at normal levels of the essential elements[10].

Microbial contamination leads to spoilage in fish and makes fishery products to be sources of various foodborne diseases and may affect not only the health of fish but also raise safety concerns with regard to human consumption. Similarly, heavy metals are potentially harmful to most organisms at some levels of exposure and absorption. The ingestion of heavy metals by fish via food and water may affect not only the productivity and reproductive capacities of such fish but also affect the health of man that depends on these organisms as a major source of protein. Heavy metals are natural components of the Earth's crust, they cannot be degraded or destroyed. To a small extent, they enter the body via food, drinking water and air. As trace elements, some heavy metals (e.g., copper, selenium, zinc) are essential to maintain the metabolism of the human body, however, at a higher concentration they can lead to poisoning.

The poor condition in our local markets, fish handlers and fish smoking facilities may contribute to the presence of micro-organisms in smoked fish. These have led to the persistence of food poisoning which is an alarming healthy problem in developing countries where sanitation is low. Trace metals may contaminate food sources and accumulate the agricultural product and seafood through water, air soil pollution, contamination of soil may further pose risks to human health through direct ingestion with the food chain. Hence, the microbial loads and heavy metals concentrations in *C. ansorgii*, one of the most common fish species in Southern Nigeria was investigated to ascertain its health status.

MATERIALS AND METHODS

Study Area: The experiment was carried out in Southern Nigeria, where seafood is prevalent. From January through February 2022, the investigation was conducted.

Equipment/reagents: Sterile stomacher laboratory blender, Durham tube, McCartney bottles, Culture plates, Gallenkamp digital colony counter, Triplicate plates, Microscope, Test tubes, A sterile cotton swab, Oven, Weighing beaker and Mueller-Hinton agar plate, Peptone water, Nutrient Agar (NA), MacConkey Agar (MA), Potato Dextrose Agar (PDA), Eosin Methylene Blue (EMB), Methylene blue dye, lead, copper, cadmium, zinc, nickel, manganese and magnesium.

Collection of samples: A total of 27 samples of smoked *C. ansorgii* were randomly purchased from fishmongers in six major markets in Southern Nigeria (Opolo, Igbogene, Swali, Tombia, Zarama and Kpansia). The samples were collected monthly and transported to the Department of Biological Sciences laboratories of Federal University Otuoke Bayelsa State for analysis.

Microbiological analysis: The fish sample that was initially pulverized in a sterile Stomacher laboratory blender, was homogenized in 225 mL distilled water in the ratio of 1:10 dilution. Further, tenfold dilutions of the sample homogenate to 10-6 were accomplished. Aliquot 0.1 mL of appropriate dilutions were spread plated in triplicate onto Nutrient Agar (NA), for total plate count, MacConkey Agar (MA) for a coliform count and Potato Dextrose Agar (PDA) for the fungal count. One-gram samples were inoculated into Eosin Methylene Blue (EMB) broth with an inverted Durham tube in McCartney bottles and subsequent plating out on Eosin Methylene blue agar after incubation for the coliform test. Cultures on NA, MA and EMB broth were incubated for 24-48 hrs at 37°C. PDA was incubated at 28±2°C laboratory room temperature for 3-7 days.

Enumeration and identification of microbial isolates: Culture plates were examined at the expiration of the incubation period and colonies were enumerated using the Gallenkamp digital colony counter (Gallenkamp, England). Means of the total and specific microbial population from the triplicate plates were expressed as colony-forming units per mL (CFU mL^{-1}). Colonial morphology and other cultural characteristics were observed and recorded and pure cultures of microbial isolates were obtained by repeated sub-culturing on appropriate media. Preliminary identification of bacterial isolates was based on cultural, morphological and basic biochemical characteristics, Gram staining, catalase activity, indole, methyl red, Voges Proskauer test, motility, citrate utilization, urease production, oxidase, starch hydrolysis, gelatin liquefaction, coagulase and fermentation of sugars. Further identification of bacterial isolates was based on standard bacteriological procedures and employing the Biomerieux sa API system. Confirmation for coliform organisms was based on presumptive, confirmatory and completed tests following the description of Speck[11].

Fungal isolates were identified based on cultural and morphological characteristics, pigmentation on media, microscopic characteristics, sporulation, mycelia arrangement and sugar assimilation tests, concerning standard identification key and atlas[12].

Sensitivity test of isolates to commonly used antibiotics and antifungal agents: Disc diffusion technique[13,14] was used to carry out the susceptibility testing of the isolates. Three to five colonies of pure isolates were transferred into test tubes containing 5 mL of peptone water and incubated for 6 hrs. The turbidity of the broth culture was adjusted to that of the 0.5 McFarland standards-approximately $1\text{-}2\times10^8$ CFU mL^{-1} (for bacteria). Turbidity for fungal cultures was adjusted to $1\text{-}5\times10^6$ cells mL^{-1}. A sterile cotton swab was dipped into the adjusted suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The entire dried surface of the Mueller-Hinton agar plate (Oxoid) prepared based on the manufacturer's instruction, was evenly streak inoculated. Mueller-Hinton agar for fungal sensitivity was, however, supplemented with 2% glucose+0.5 µg mL^{-1} Methylene Blue Dye. The plates were allowed to stand for 5 min to allow for any excess inoculum to diffuse before introducing the discs. The following antimicrobial agents were

employed, Cefuroxime (30 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Amoxycillin (25 µg), Co-trimoxazole(25 µg), Nitrofurantoin (50 µg), Gentamycin (10 µg), Nalidixic acid (15 µg), Ofloxacin (5 µg), Tetracycline (10 µg), Streptomycin (10 µg), Chloramphenicol (30 µg) and Amphotericin B (20 µg), Ketoconazole (15 µg), Fluconazole (25 µg), Griseofulvin (10 µg) and Nystatin(100 unit). The plates were incubated at 37°C for 18-24 hrs for bacteria and the fungi culture was kept at room temperature for 5 days after which the zones of inhibition were measured.

Determination of heavy metals: The fish samples collected from the markets were further dried in the laboratory at 60°C to maintain a constant dried weight (1.0 g). Digestion of the samples was done by heating the dried weight in a Teflon beaker with mixed concentrated hydrochloric acid (HCl), hydrogen tetraoxosulphate six acid (H_2SO_4), trioxonitrate acid (HNO_3) and hydrogen peroxide (H_2O_2) in the ration 1:1[15]. Immediately after the digestion process, a hundred times dilution was performed using Mili-Q water and analyzed by the atomic absorption spectrophotometer (G105 UV-VIS, Thermo Fisher Scientific, GeneSys, Madison, USA).

Data analysis: Data of the fish species from the six markets were subjected to Statistical analysis of the t-test and ANOVA two factor without replication. Additionally, data from the heavy metal assessment was compared to the acceptable standard of World Health Organization and Federal Environmental Protection Agency safety levels in regulation and guidance for fish and fisheries products[16].

RESULTS

The analysis of the health status of dried *C. ansorgii* from major markets in Southern Nigeria revealed that the endemic fish harbours microbes and is contaminated with heavy metals in Table 1-3. The level of microbial loads depends on the market's location, with the Igbogene market having the highest TBC 43.10×10^7 CFU g^{-1} and the Zarma market having the least 8.40×10^7 CFU g^{-1}. The magnitude of contamination revealed that Igbogene> Opolo>Tombia>Kpansia>Swali>Zarama markets. The bacteria counts vary significantly among the markets ($p<0.05$) except for, Swali and Tombia markets, Swali and Kpansia and Swali and Zarama markets in Table 1.

The bacteria isolated from the fish shows that the fish was highly contaminated in Table 2. Micrococcus species are found in the fish from Opolo, Igbogene, Swali and Tombia markets but absent in Zarama and Kpansia markets. *K. pneumonia* was present in all six markets. Enterococcus species were present in Opolo and Tombia markets but absent in Igbogene, Swali, Zarama and Kpansia markets. *B. cereus* was present in Igbogene, Kpansia and Tombia but absent in Opolo, Swali and Zarama markets. *S. albus* was present in the six markets. Actinomycetes species were present in Opolo, Igbogene and Kpansia but absent in Swali, Tombia and Zarama markets. *E. aerogenes* was present in Opolo, Igbogene, Swali and Tombia but absent in Zarama and Kpansia markets. *Pseudomonas* species were present in Opolo and Kpansia but absent in Igbogene, Swali, Tombia and Zarama markets. *Salmonella* species were present in all six markets. *L. monocytogenes* were present in Opolo, Swali, Tombia and Kpansia but absent in Igbogene and Zarama markets. Yersinia species was present in Igbogene, Tombia and Kpansia but absent in Opolo, Swali and Zarama. Proteus species were present in Opolo, Igbogene, Tombia and Kpansia markets but absent in Swali and Zarama. Shigella species were present in all the markets. Chromatium species were present in Opolo, Igbogene, Tombia and Kpansia but absent in Swali and Zarama markets.

The total fungal counts of the investigated fish disclosed that Igbogene has the highest TFC (18.20×10^7 CFU g^{-1}), with the least (1.10×10^7 CFU g^{-1}) reported in the Zarma market. The TFCs of the endemic fish in the region shows that Igbogene>Opolo>Tombia>Kpansia>Swali>Zarama market (Table 1). Similarly, the TFCs vary significantly among the markets excepting Opolo and Igbogene markets, Swali and Zarama, Tombia and Kpansa markets ($p>0.05$) (Table 1).

Table 1: Microbial load (CFU g^{-1}) in *C. ansorgii* from major markets in Niger Delta, Nigeria

Markets	TBC ($\times 10^7$)	TFC ($\times 10^7$)
Opolo	34.60^{a}	12.50^{a}
Igbogene	43.10^{b}	18.20^{a}
Swali	11.40^{cd}	1.70^{b}
Tombia	17.80^{c}	3.80^{c}
Zarama	8.40^{d}	1.10^{b}
Kpansia	15.90^{c}	3.30^{c}

Mean with different superscripts within the column varies significantly ($p<0.05$), TBC: Total bacteria count and TFC: Total fungi count

Table 2: Microbials isolated from smoked *C. ansorgii* from major markets in Yenagoa Metropolis, Nigeria

Isolates	Opolo	Igbogene	Swali	Tombia	Zarama	Kpansia
Bacteria						
Micrococcus species	+	+	+	+	-	-
Klebsiella pneumoniae	+	+	+	+	+	+
Enterococcus species	+	-	-	+	-	-
Bacillus cereus	-	+	-	+	-	+
Staphylococcus albus	+	+	+	+	+	+
Actinomycetes species	+	+	-	-	-	+
Enterobacter aerogenes	+	+	+	+	-	-
Pseudomonas species	+	-	-	-	-	+
Salmonella species	+	+	+	+	+	+
Listeria monocytogenes	+	-	+	+	-	+
Yersinia species	-	+	-	+	-	+
Proteus species	+	+	-	+	-	+
Shigella species	+	+	+	+	+	+
Chromatium species	+	+	-	+	-	+
Fungi						
Aspergillus flavus	+	+	+	+	+	+
Fusarium species	+	+	+	+	+	+
Rhizopus stolonifer	+	-	-	+	+	+
Mucor species	-	+	-	-	-	+
Trichophyton species	+	+	+	+	+	+
Aspergillus niger	+	+	-	-	-	+
Candida tropicalis	+	+	+	+	-	+

+: Present and -: Absent

Table 3: Heavy metal concentrations (µg g^{-1} dw) in *C. ansorgii* from major markets in Yenagoa Metropolis, Nigeria

Markets	Cu	Zn	Cd	Pb
Opolo	3540±1.60^{a}	5120±1.50^{a}	2.50±0.10^{a}	15.20±0.60^{a}
Igbogene	1120±5.30^{b}	5960±12.20^{b}	3.80±0.50^{b}	12.10±0.20^{ab}
Swali	3840±12.10^{c}	7420±2.20^{c}	6.00±0.10^{c}	17.20±0.50^{a}
Tombia	3420±1.40^{c}	2250±2.10^{d}	3.20±0.41^{b}	11.30±1.50^{b}
Zarama	1360±2.20^{b}	2730±3.10^{d}	1.60±0.10^{d}	7.80±2.10^{b}
Kpansia	3250±2.80^{c}	3110±5.20^{e}	4.00±0.50^{b}	12.80±1.900^{ab}
Recommendation limit				
FEPA	1000	3000	03	10
WHO	2000	-	03	10

Means with the same superscript and subscript within the column are not significant ($p>0.05$)

Fungal isolated from the fish shows that *A. flavus* and *Fusarium* species were present in all the markets. *R. stolonifera* was found in Opolo, Tombia, Zarama and Kpansia but not found in Igbogene and Swali markets (Table 2).

The heavy metals concentrations in *C. ansorgii* from major markets in southern Nigeria are shown in Table 3. The findings revealed that *C. ansorgii* sampled from the six major markets in the region was contaminated with heavy metals.

Swali had the highest level of copper concentrations (3840±12.10 µg g^{-1} dw) among the six major markets, while Igbogene had the least of copper concentrations (1120±5.30 µg g^{-1} dw). The magnitude of the copper concentration shows that Swali>Opolo>Tombia>Kpansia>Zarama> Igbogene. Statistical analysis revealed that Cu concentrations in Opolo vary significantly ($p<0.05$) with Igbogene and Swali markets and Igbogene varies significantly ($p<0.05$) with Opolo and Swali. Zarama, Kpansia and Opolo vary significantly ($p<0.05$). In contrast, Cu concentration in Swali, Tombia and Kpansia does not vary significantly ($p<0.05$). The Cu concentration in the fish from Opolo, Swali, Kpansia and Tombia markets was higher than the recommended limit set by FEPA and WHO. Also, the metal in the fish from Igbogene and Zarama markets was higher compared to that of the recommended limit of FEPA but lesser than the WHO recommendation limit. The copper concentration in Zarama is higher than the recommended limit of FEPA but lesser than that of the WHO recommendation limit (Table 3).

The Zinc concentration from the six markets shows that Swali had the highest level (7420±2.20 µg g^{-1} dw), while Tombia had the least (2250±2.10 µg g^{-1} dw). The concentration of Zn shows that Sawali>Igbogene>Opolo> Kpansia>Zarama>Tombia. Statistically, the metal concentration in Opolo varies significantly ($p<0.05$) with Igbogene, Swali, Tombia and Zarama, also in Igbogene, it varies significantly ($p<0.05$) with Opolo, Swali, Tombia and Kpansia markest. The Zn concentration in Swali varies significantly ($p>0.05$) with Zarama and Kpansia markets. Similarly, the Zn in Zarama, Kpansia and Opolo vary significantly ($p<0.05$). However, this metal concentration in Tombia does not vary significantly ($p>0.05$) with Zarama). The Zn concentration and their recommendation limit set by regulatory bodies are shown in Table 3.

The fish sampled from all the investigated markets had cadmium with Swali having the highest level of cadmium concentration (6.00±0.10 µg g^{-1} dw), while Zarama had the least (1.60±0.10 µg g^{-1} dw). The magnitude level of Cd concentration revealed that Swali>Kpansia> Igbigene>Tombia>Opolo>Zarama. The Cd concentration in Opolo, Igbogene, Swali and Zarama vary significantly ($p<0.05$). Equally, Cd in Swali varies significantly ($p<0.05$) with Tombia, Zarama and Opolo. Also, the metal in the fish from in Kpansia market varies significantly ($p<0.05$) with Opolo, Swali and Zarama. No significant difference ($p>0.05$) in the metal concentrations in Igbogene with Opolo Swali and Zarama. Similarly, Cd concentration in Tombia does not vary significantly ($p<0.05$) with Kpansia and Igbogene. The cadmium concentration in all the six major markets is greater than the recommended limit of FEPA and WHO.

Lead concentration in the fish sample also showed that Swali had the highest level (17.20±0.50 µg g^{-1} dw), while Zarama had the least (7.80±2.10 µg g^{-1} dw). The magnitude of Pb the fish shows that Swali>Opolo>Kpansia> Igbogene>Tombia>Zarama markets. The Pb concentration in opolo varies significantly ($p>0.05$) with Zarama only, with no significant difference ($p<0.05$) from other markets. Conversely, in the Igbogene market, the Pb concentration in the fish does not vary significantly ($p<0.05$) with Opolo, Swali, Tombia, Zarama and Kpansia. The lead concentration in Opolo, Igbogene, Swali, Tombia and Kpansia is greater than the recommended limit by FEPA and WHO.

DISCUSSION

The microbial loads of *C .ansorgii*, an endemic fish in major markets in Southern Nigeria revealed that all the markets in this region are highly contaminated with microbial load, with the Igbogene market, the most affected. The high level of microbial loads in this region could be attributed to the human activities in the rivers where the smoked fish was harvested. These activities include, bathing, washing of clothes or other materials, disposal of fecal matters and sewage discharges by municipal authorities and independent outfits, another possible explanation could be the unhygienic environment of the fish source and the open markets. This finding corroborated the proposition of another study[17,18] that aquatic organisms, in general, accumulate contaminants from the environment and, therefore, have been extensively used in marine pollution monitoring programs.

The few microbial loads observed in the Zarama market showed that close contact with fish (handlings) enhance the distribution of microbes. Zarama is a weekly market, there is not much contact between the fishmongers and the buyers and that made the microorganism unable to grow and it also reduces the number of bacteria from the fish sample *C. ansorgii* unlike Igbogene, which is a daily market. This is in agreement with the submission that microbes are normally associated with the individual hygiene of the people who handle them in marketplaces[19].

The bacteria isolated from all the six major markets, *Micrococcus* species, *K. pneumonia*, *Enterococcus* species *B. cereus*, *S. albus*, *Actinomycetes* species, *E. aerogenes Pseudomonas* species, *Salmonella* species, *L. monocytogenes*, *Yersinia* species, *Proteus* species, *Shigella* species, *Chromatium* species. *K. pneumonia*, *S. albus*, *Salmonella* species and *Shigella* species had the highest frequency of occurrence, their occurrence was following the assertion of[20] when he stated that these organisms were the commonest microorganisms associated with smoked fish and were also reported by the researcher[21] in his investigation on microbial load of smoke-dried fishes (*Ethmalosa fimbriata* and *pseudotolithus elongatus*) sold in Oba and Koko markets in Edo and Delta States, Nigeria at different seasons. Similarly, this investigation is in agreement with the report[22] that isolated *E. coli*, *P. aeruginosa*, *Salmonella paratyphi* and *B. cereus* from freshly harvested *Trypanosoma* species and *Crassostrea* species from two different creeks in Nigeria.

All the organisms isolated from the smoked *C. asorgii* samples have health implications for man. For instance, *Salmonella* species have been reported to survive and persist in the aquatic environment and have been detected in the gut of tilapia and crabs[23,24] and cause newborn meningitis and infantile diarrhea. It is one of the most important food-borne pathogens and an indication of sewage contamination and it is found to be associated with some non-human hosts, for example, reptiles[25]. *Shigella* species and *Salmonella* species are causative agents of illnesses like *shigellosis* and *salmonellosis* in the human who are the only reservoir of these organisms[26], Some *Bacillus* species are 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[27].

The fungi isolates include *A. flavus*, *Fusarium* species, *R. stolonifera*, *Mucor* species, *Trichophyton* species, *A. niger*, *C. tropicalis*, *Trichophyton* species, *A. flavus, Fusarium* species and *Trichophyton* species had the highest occurrence, the occurrence of *A. flavus*, *Fusarium* species and *Trichophyton* species could be because, during storage, the fish sample reabsorb moisture from the environment which then supported the growth of the microorganisms in addition to the contamination during processing, handling and display in the market stalls[28]. Some of the isolated fungi were identified to be spoilage inducing on smoked fish during storage[29]. *A. niger* and *A. flavus* have also been implicated in causing mycetoma in humans[30]. *A. flavus* is involved in allergic *aspergillosis* (*Pulmonary aspergillosis*) and also produces aflatoxin that is highly carcinogenic[31].

Different metals at high concentrations were detected in the fish sample. The concentration of copper was above the limit[32] of 10 μg g^{-1} dw. Cu is present in most aquatic environments, however, it is also toxic for fish and this is because Copper is an essential part of several enzymes and it is necessary for the synthesis of hemoglobin. Shellfish are the richest sources of Cu especially oysters and Crustaceans[33]. Underwood[33] reported that deficiencies of Cu in infants can lead to anemia and hypoproteinemia and no deficiency of copper in adult have been reported.

The Zinc concentration in the fish from the investigation markets was higher than the recommended limit excepting Tomia and Zarama markets. Zn toxicity is characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite and nausea[28]. However, zinc is an essential element for both animals and humans, the recommended daily allowance is 10 mg/day in growing children and 15 mg/day for

adults[11]. It has a protective effect against the toxicities of both cadmium and lead 8. A deficiency of zinc is marked by retarded growth, loss of taste and hypogonadism, leading to decrease fertility. Zn toxicity is rare, but at concentrations in water up to 40 mg kg^{-1}, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite and nausea[32].

Cadmium was also detected in all the samples, the concentration of Cd in this fish was above the standard limit of FEPA (1000) and WHO (2000). Severe symptoms have been reported to occur with ingestions of 10-326 mg, while ingestions exceeding 350 mg can result in shock and acute renal failure (Calabrese, 1985). Humans are exposed to cadmium through food and the average daily intake for adults has been estimated to be approximately 50 mg[25]. The standard threshold for acute Cd toxicity would appear to be total ingestion of 3-15 mg[33].

Lead was detected in all samples at a very high concentration. The highest was found in Swali and has been implicated in renal failure and liver damage in humans[34].

CONCLUSION

This study has established the microbiological quality and trace metal concentration in *C. asorgii* in southern Nigeria. This revealed that smoked *C. asorgii* sold in southern Nigeria markets are highly contaminated with microorganisms and heavy metals. These revealed that samples of the *C. ansorgii* from the six major markets contain an unacceptable level of microorganisms and the presence of organisms such as *Staphylococcus* species, *Shigella* species and *Salmonella* species implies a poor sanitary condition of the water bodies from where the sample was harvested. Caution should be exercised in consuming smoked fish shaded openly because such fish could contain microbial cells and reheating may be necessary to destroy or inactive such cells. Therefore, there is a need to prevent these fish from contaminants especially the biological hazards from the air and during the processing stages. The seller should wear gloves during production and sales.

SIGNIFICANCE STATEMENT

Heavy metals are important environmental contaminants and their toxicity poses a substantial threat to ecological, evolutionary, nutritional and environmental balances. The presence of heavy metals in *C. ansorgii*, one of the most important seafood in Southern Nigeria, is of great concern because their toxicity can cause a variety of disorders and excessive damage due to oxidative stress caused by free radical formation. Regardless of metal, they all serve important biological activities, in plants and animals. These metals link to protein sites instead of their particular metals by displacing the original metals from their native binding sites, causing cell dysfunction and, eventually, poisoning. Heavy metals that are not digested by the body become harmful and accumulate in soft tissues after entering the human body through diet. Heavy metal exposure might lead to serious issues in the future due to their negative impact on living beings and the environment. However, their chemical coordination and oxidation-reduction characteristics have occasionally provided an added benefit to life.

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