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Microbial Diversity and Heavy Metals Concentration of Wood Smoked Fish from Edonwhii Fishing Settlement in Akwa Ibom State, Nigeria

U. S. Fred[1], O. D. Akan[2,3*], J. P. Essien[1] and S. I. Umana[2]

[1]Department of Microbiology, University of Uyo, Uyo, Akwa-Ibom State, Nigeria.
[2]Microbiology Unit, Department of Biological Science, Akwa-Ibom State University, Ikot Akpaden, Akwa-Ibom State, Nigeria.
[3]College of Food Engineering, Central South University of Forestry and Technology, Hunan, PR China.

Authors' contributions

This work was carried out with the collaboration of all the authors. Authors USF and JPE designed the study. Authors USF and ODA carried out the experiments, performed the statistical analysis, wrote the protocol. Author SIU wrote the first draft of the manuscript. Authors USF and ODA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The microbial diversity and heavy metal concentrations of wood smoked fishes (*Clarias gariepinu*s and *Pseudotolithus elongatus*) from Edonwhii fishing settlement in Akwa Ibom State, Nigeria were investigated using standard microbiological protocols and analytical procedures, to ascertain the level of fish contamination. The study revealed variations in the densities of the different microbial groups. The fresh fish samples of *Clarias gariepinu*s and *Pseudotolithus elongates* had Total heterotrophic bacterial count of 7.1 x 10^5 and 4.1 x 10^7 cfu/g respectively as compared to the smoke-dried fish which had 4.8 x10^2 and 6.7 x 10^2 cfu/g respectively obtained 16 hours after smoke dying. The study reveals a rich microbial assemblage of the fish samples. *Streptococcus* sp, *Salmonella* sp, *Shigella* sp, *Escherichia coli, Vibrio cholerae* and *Bacillus* sp. were the different bacteria isolates encountered in the study while *Absidia* sp., *Candida* sp., *Penicillium* sp., *Cladosporium* sp., *Aspergillus* sp., *Trichophyton* sp., *Torula* sp., *Saccharomyces* sp., *Verticillium* sp. and *Mucor* sp were the fungal isolates identified. The metal analysis result showed that Zn was found to be the most abundant metal. However, Cd levels in *Clarias gariepinu*s (0.23 mg/kg) was relatively higher than the value recorded for *Pseudotolithus elongatus* (0.16 mg/kg) but all were within the FAO/WHO permissible level of (0.5 mg/kg) of Cd in sea food. Similarly, the Pb levels were within the FAO/WHO permissible level of 0.3 mg/kg, indicating that the smoked fish samples were still suitable for human consumption.

**Corresponding author: Email: otobongakan@aksu.edu.ng;*

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1. INTRODUCTION

Aquaculture development in Nigeria is fast gathering momentum [1]. The need for more fish supplies in the market is seen in the increased importation of about 900,000 metric tons annually which is double the local production/catch, estimated at only 450,000 metric tons. Nigeria is Africa's biggest fish consumer [2,3]. The reason for this high consumption may stem from the fact that fish is adjudged to be nutritionally superior to other sources of animal protein in that they contain most of the essential amino acids particularly lysine, leucine, valine, methionine and tryptophan. Hence fish is regarded as first-class protein producer [4]. Fish is an extremely perishable food item, therefore much interest has also been channelled into its preservation to avoid waste [5] by adopting various preservation techniques like smoking, refrigeration, sun-drying, etc. Rashed [6] opined that fishes are also considered as one of the most significant indicators of metal pollution in aquatic environments, asides also hosting microbial populations. It has been proven that fishes absorb dissolved elements and heavy metals from surrounding waters and ingested food. Although these metals and pollutants are found in traced quantities in the water environment, their ability to bioaccumulate is of interest to researchers as they affect both human and environmental health [7]. Seymore [8] found that these pollutants accumulate in various fish tissues in significant amounts, eliciting toxicological effects at critical targets. Preservation of fishes by Smoking is commonest traditional method of fish preservation in Nigeria. This method combines the effect of the destruction of bacteria by compounds (eg phenols) in the smoke and the high temperature cooking of the fish. This results to a longer shelf life, which has been attributed to the drying, antibacterial and antioxidant and cooking effects of smoke [7]. This study evaluates the effect of smoking on the microbial and heavy metal properties of smoked processed fishes harvested from Edonwhii community.

2. MATERIALS AND METHODS

2.1 Study Area

The Edonwhii fishing settlement is located along the coast of the Bight of Bonny at the mouth of Iko River Estuary. The estuary is a major tributary of Imo River. This area is situated in the Niger Delta fringe between Imo River and Qua Iboe River estuaries and lies between latitudes $4^{o}50`$ and $7^{o}55`$ East.

2.2 Sample Collection

Precisely three samples each of two different fish (*Pseudotolithus elongatus* and *Clarias gariepinus*) giving a total of 6 smoked fish samples were subjectively obtained 16 hours after smoked-drying (low heating). The smoked fish were conditioned in sterile plastic bags and preserved in ice-packed coolers. Representative fresh samples of selected fishes were also purchased to serve as the control (in all twelve fish samples). Samples of red mangrove woods (used as fuel in the smoking process) were also collected from the area.

2.3 Processing of Fish Samples

The samples were carefully and aseptically dismembered with sterile surgical blades to obtain intestine and skin. The organs were homogenized using a sterile pestle and mortar. One gram (1.0 g) of each organ sample was serially diluted and used for microbiological analysis.

2.4 Culture Media Preparation and Sterilization

The media used for the study were: Nutrient Agar (NA), *Staphylococcus* 110 medium, Thiosulphate citrate Bile salt, Eosine Methylene Blue Agar (EMBA) *Salmonella-Shigella* agar (SSA), mineral salt medium, Sabouraud dextrose agar and Dermatophytic medium for the enumeration and isolation of heterotrophic bacteria, *Staphylococcus aureus*, *Vibrio*, faecal coliform (*Escherichia coli*), *Salmonella-Shigella,* hydrocarbon degraders, fungi and dermatophytes species respectively. They were aseptically prepared according to the manufacturer's instructions, sterilized by autoclaving at 121℃ for 15 minutes.

2.5 Estimation of Microbial Contaminants from Fresh and Smoked Fishes

The density of heterotrophic and potential pathogens was determined using standard analytical procedures. *Staphylococcus aureus*,

Vibrio, faecal coliform (*Escherichia coli*), and *Salmonella-Shigella* loads on the samples was determined using the pour plate technique and incubated at 37℃ for 24 hours while the densities of fungi and dermatophytes were determined using spread plate technique and incubated at room temperature for 4 days.

The densities of hydrocarbon utilizing microorganisms were determined using pour plate technique and incubated at (28 ± 2℃) for 7 days. Discrete colonies that appeared on the culture plates were enumerated with the aid of a Quebec colony counter and recorded as Colony Forming Units (CFU) per gram of fish sample.

2.6 Characterization and Identification of Microbial Isolates

The pure colonies obtained from the samples were characterised using standard biochemical procedure as described by *Bergey's Manual of Determinative Bacteriology* [9]. The colonies were subjected to Gram's stain and standard biochemical tests; catalase, coagulase, citrate, indole, MR/VP, motility, spore, sugar fermentation, urease. Fungal isolates were identified according to the method of Barnett and Hunter [10].

2.7 Determination of Heavy Metals in Fresh Fish, Smoked Fish and Fire Wood Samples

The heavy metals in fresh, smoked fishes and wood-fuel samples which were analyzed included Cadmium (Cd), Chromium (Cr), Zinc (Zn), Lead (Pb) and Nickel (Ni). The concentrations of heavy metals in the collected samples were determined (after nitric acid digestion) by means of an atomic absorption spectrophotometer (S series S4 AA system-Thermo Electron Cooperation) [11].

3. RESULTS AND DISCUSSION

3.1 Microbiological Properties of Sampled Fishes

Figures 1 and 2 show that the fresh fish samples of *Clarias gariepinus* and *Pseudotolithus elongates* had total heterotrophic bacterial count of 7.1 x 10^5 and 4.1 x 10^7 cfu/g respectively as compared to the smoke-dried fish which had 4.8 x10^2 and 6.7 x 10^2 cfu/g respectively obtained 16 hours after smoke dying. *Salmonella-Shigella, Escherichia coli, Vibrio* and hydrocarbon degraders were not detected in the smoked-dry fish samples while *Staphylococci* sp were found in all the smoke-dried fish samples, whereas Dermatophytes were found in all the smoked fish samples.

3.2 Microbial Diversity of the Fish Samples

Streptococcus sp, *Salmonella* sp, *Shigella* sp, *Escherichia coli, Vibrio cholerae* and *Bacillus* sp. were the different bacteria isolates encountered in the study while *Absidia, Candida* sp, *Penicillium* sp., *Cladosporium* sp., *Aspergillus* sp., *Trichophyton* sp., *Torula* sp., *Saccharomyces* sp., *Verticillium* sp. and *Mucor* sp. were identified fungal isolates.

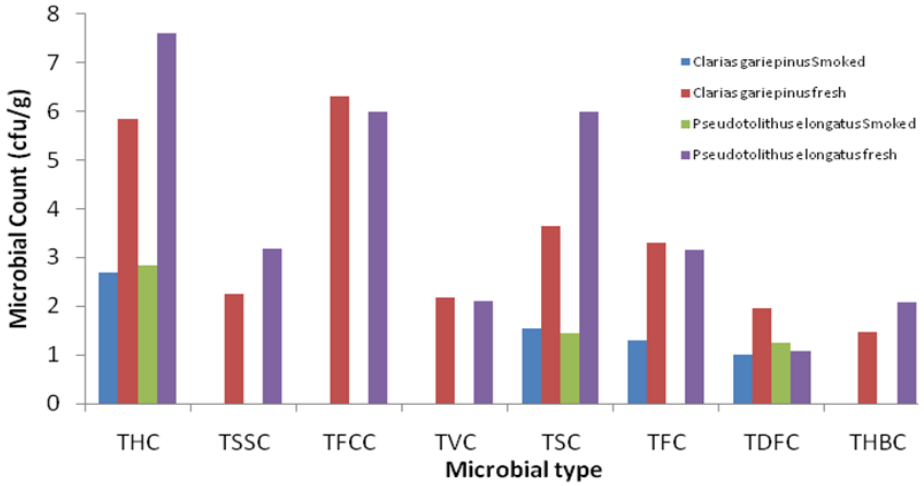

Figure 1: Microbial properties of fish skin samples (Cfu/g)

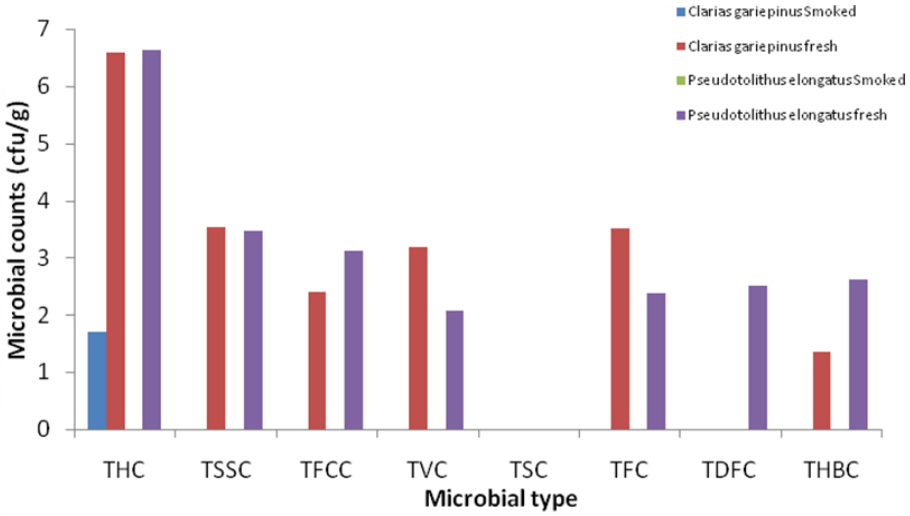

Figure 2: Microbial properties of fish intestinal samples (Cfu/g)

Key:-

Total heterotrophic bacteria count (THC)
Total Salmonella-Shigella count (TSSC)
Total Escherichia coli count (TFCC)
Total Vibrio count (TVC)
Total Dermatophytic fungi count (TDFC)
Total Hydrocarbonoclastic bacteria count (THBC)
Total Staphylococci count (TSC)
Total Fungal count (TFC)

3.3 Heavy Metals Load of the Fish Samples

Heavy metals load of smoked, fresh fishes and the mangrove firewood are presented in Figure 3 and Table 1 respectively. Values recorded for the smoked fish were slightly higher than those of fresh fish samples. Variation in concentrations of metals between fish spices was also noticed and Zn had the highest cumulative amount in all the analyzed samples.

Table 1. Heavy metal levels (mg/kg dw) of mangrove firewood

Heavy metal	Value
Cadmium	0.005
Chromium	2.066
Nickel	4.452
Lead	1.006
Zinc	20.59

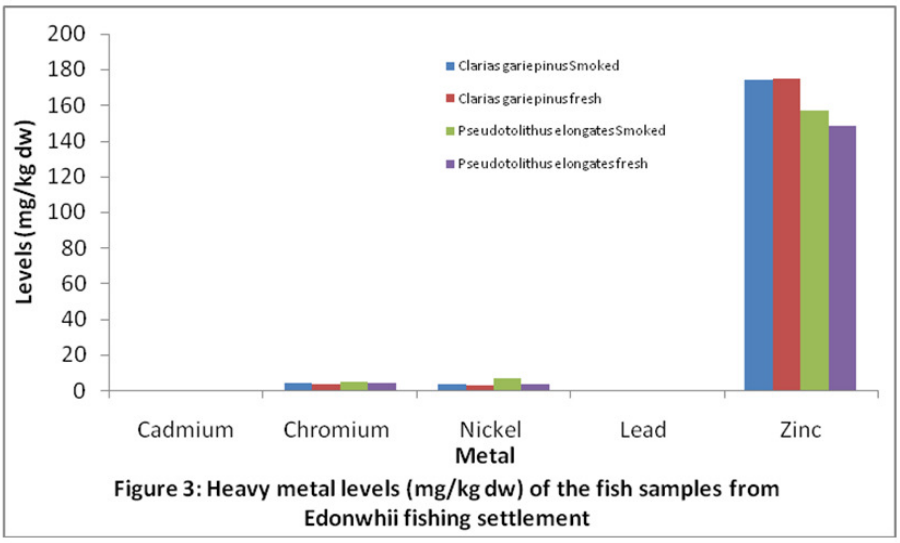

Figure 3: Heavy metal levels (mg/kg dw) of the fish samples from Edonwhii fishing settlement

3.4 Discussion

The microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish live and not of the fish species [12]. The results obtained from this study have shown that fresh fish samples are laden with microbial contaminants including pathogenic groups of microorganisms. Fish, because of their soft tissues, aquatic environment, high water content, neutral pH and high amino acids are extremely susceptible to microbial contamination and spoilage [13]. Run-off water may also introduce pathogenic organisms into the aquatic ecosystem from which the fish are harvested. This may result in faecal coliforms contamination resulting in high numbers of coliforms in harvested fish [14,15]. The high values in the fresh fish may have been due to contamination from the aquatic habitat, poor or unsanitary handling of harvested fish and invasion of the fish flesh by bacteria due to its high moisture content [12,16]. This observation also agrees with the findings of Salan et al. [17] who experimented on smoke as a preservative method. The elimination of pathogenic bacteria such as *Salmonella* sp and *Shigella* sp from smoked fish intestinal samples as compared to fresh fish intestinal samples is indicative of the preservative function of smoke from the wood. Despite the high heterotrophic bacterial loads of the fish samples, some of which were still within that acceptable range limit of 5.0×10^5cfu/g (5.7 log cfu/g) for good quality products [18]. Smoking the fish samples drastically reduced the microbial loads of the fishes sampled.

The bacteria encountered in the smoked fish skin samples included *Streptococcus* sp, *Salmonella* sp, *Shigella* sp, *Escherichia coli, Staphylococcus aureus*, *Vibrio cholerae* and *Bacillus* sp. The occurrence of *Escherichia coli* and *Staphylococcus aureus* in smoked dried fish skin samples have also been previously reported by Martin [19]. *Staphylococcus aureus* constitute the normal flora of the human skin and mucous membrane hence their presence in the fish samples might have been through contamination by handling and poor sanitary condition. These organisms prevalence is of public health concern because they have been implicated in food-borne intoxication and infection cases [20].

Relatively more fungal isolates were encountered on the smoked *Clarias gariepinus* than *Pseudotolithus elongatus* fish samples. The isolates include *Absidia, Candida* sp., *Penicillium* sp., *Cladosporium* sp., *Aspergillus* sp., *Trichophyton* sp., *Torula* sp., *Saccharomyces* sp., *Verticillium* sp. and *Mucor* sp. Some of these fungal isolates especially the dermatophytic species of *Trichophyton* have been reported to be resistant to heat treatment [21]. Previous studies of Essien *et al*. [22] had shown strains or species of *Penicillium* sp, *Aspergillus flavus* and *Cladosporium* sp isolated from Shark fish have the proteolytic and mycotoxigenic potentials. It is important to state that the majority of the fungal isolates obtained from this study are of veterinary and medical importance. *Aspergillus* sp. are probably the notorious of the common isolates because of their high potentials in producing aflatoxin and ochratoxin respectively.

The results revealed that heavy metals were detected in both smoked and fresh fish samples. However, the results show that slightly higher heavy metals loads were recorded for the smoked fish samples. It shows the mangrove wood fuel had contributed little to heavy metals burden of the smoked fish samples despite being harvested from apparently crude oil contaminated ecosystem.

The metal levels accumulated by the smoked and fresh fish samples is more of an indication of man-induced pollution of the water body where the fishes were obtained and less of enrichment through the traditional fish processing method by wood smoking, the former is possible through oil spillage, run off of fertilizer, herbicides, pesticides and discharge of untreated sewage and industrial effluents containing metals into the water bodies, as well as the natural ability of the sediment to act as sink [23]. It has also been reported that mangroves potentially accumulate low metals in contaminated environments [24]. This may be due to the bioavailability in the mangal sediments, exclusion of metals by the mangroves and physiological adaptations that prevent metal accumulation in mangrove plants. It has also been reported that mangrove roots appear to be barriers that prevent metals from reaching the more sensitive part of plants [25]. Oxygen exuded by the underground roots forms iron plaques that adhere to the root surfaces and prevent heavy metals from entering the root cells. Where the metals do enter, there are apparent mechanisms to keep them from circulating freely from the plants [24]. This implies that mangrove wood fuel used in fish smoking may suggestively have contained heavy metals despite being derived from an oil

impacted ecosystem. The reason for the comparatively low metals content of the fish samples dried with mangrove wood fuel.

Of the heavy metals examined in the smoked fish samples Zn was found to be the most abundant metal. However cd levels in *Clarias gariepinu*s (0.23 mg/kg) was relatively higher than the value recorded for *Pseudotolithus elongatus* (0.16 mg/kg) but all were within the FAO/WHO permissible level of (0.5 mg/kg) of Cd in sea food. Similarly, the Pb levels were within the FAO/WHO permissible level of 0.3 mg/kg, indicating that the smoked fish samples are suitable for human consumption [26].

4. CONCLUSION

The research study has revealed the preservative potency of wood-smoking against microbial contaminants although post treatment contamination may readily occur due to pope or unsanitary handling of smoked products. On the other hand, the levels of Cd, Cr, Pb, Ni and Zn in the smoked and fresh fish samples investigated showed that wood-smoking contributed little or nothing to the concentrations of heavy metals as little variation were recorded for some heavy metals between smoked and fresh fish samples analyzed. The levels of all the metals analyzed were within the FAO/WHO recommended guidelines values implying that the biota may have been exposed to small amount of heavy metals pollution during the smoking process. To check the undesirable addition of chemical contaminants on the smoked fish products, it is important to always be mindful of the type and source of wood used for smoke-drying.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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