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Microbiological Qualities and some Heavy Metals (Mercury and Cadmium) Levels of fresh and Dry Fish Species sold in Benin City, Edo State, Nigeria

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ABSTRACT
The microbiological qualities and heavy metals (mercury and cadmium) levels of fresh and dry fish samples sold in Benin City, Edo State, Nigeria were examined. Five different fish species [Tachurus trachurus (Sese), Scomber scombrus (Mackerel), Clarias gariepinus (Catfish), Protopterus annectens (Mudfish) and Pseudolithus croaker (Meluza)] were purchased from New Benin market, Oba market and Yanga market in Benin City, Edo State, Nigeria. Standard aerobic pour-plate techniques were used for microbial enumeration while heavy metal concentrations in fish were determined using atomic absorption spectrophotometer (AAS). Fresh Clarias gariepinus (Catfish) had the highest mean bacterial (2.23x10^{7} ± 4.1 cfu/g) and fungal counts (5.02x10^{4} ± 7.7 cfu/g). Dry Pseudolithus croaker (Meluza) had the highest bacterial counts (1.70x10^{6} ± 10.1 cfu/g) while dry Clarias gariepinus (Catfish) had the highest fungal counts (4.28x10^{4} ± 10.3 cfu/g). The microbial isolates were Pseudomonas, Proteus, Staphylococcus, Klebsiella, Streptococcus, Cercospora, Trichoderma, Mucor, Penicillum, and Aspergillus. Cadmium levels (0.03 – 0.52 mg/kg) in both fish samples were within the FAO limits for fish samples while mercury levels (0.542 – 1.733 mg/kg) in dry fish except in Scomber scombrus (0.425mg/kg) were found to be higher than FAO recommended limits for fish samples. A considerable high mean fungal load (4.28 x 10^{4} cfu/g) and high mean mercury level (1.73 mg/kg) were observed in dry Clarias gariepinus (Catfish) sample compared to other fish samples.
***Key words**: Heavy metals, Fresh and dry fish, Microbiological qualities.*

INTRODUCTION

Fish is a high-protein, low-fat food that provides a range of health benefits. Fish is lower in fat than any other source of animal protein, and oily fish are high in omega-3 fatty acids. As our body does not make significant amounts of these nutrients, fish are important source. However, most fish nowadays has lost their nutritional values due to environmental pollution. The impact of pollution on fish and the potential health implication of eating contaminated fish are areas of considerable concern for the government bodies and general public. Fish consumes much toxic wastes which are polluted materials discharged into their ambient environment. These toxic wastes are usually industrial wastes, sewage, pesticides and heavy metals such as mercury and cadmium. The pollutants especially mercury could possible end up in human bodies due to the consumption of affected fish [1]. The microbiological flora on fresh and dry fish depends on the environment in which it was caught, the hands of the handlers and processors, exposure to the atmosphere and methods of storage.

Mercury occurs naturally, large amounts enter the aquatic environment from anthropogenic sources [2]. Mercury enters the food chain with the help of certain bacteria. Bacteria that process sulfate in the environment take up mercury in its organic form and convert it to methyl-mercury through metabolic processes. These methyl-mercury containing bacteria may be consumed by the next higher level in the food chain, or the bacteria may excrete the methyl-mercury to the water where it can quickly adsorb to plankton, which are also consumed by the next level in the food chain. Because animals accumulate methylmercury faster than the eliminate it, animals consume higher concentrations of mercury at each successive levels of the food chain, small environmental concentrations of methylmercury can thus readily accumulate to potentially harmful concentrations in fish and people. Methylmercury biomagnifications can result in toxic effects in consumers at the top of these aquatic food chains [3].

Cadmium is a natural element in the earth's crust occurring in combination with zinc. Besides its natural occurrence, elevated cadmium levels in air and dust in urban areas may be associated with emissions from burning fuel, household wastes and motor vehicles [4]. In aquatic environment, cadmium is mostly taken up by organisms directly from water but may also be ingested with substantially contaminated food. Cadmium is toxic at very low levels. In humans, long-term exposure results in renal dysfunction, characterized by tubular proteinuria.

This study examined the microbiological qualities and some heavy metals mainly mercury and cadmium in fresh and dry fish species sold in Benin City, Edo State, Nigeria with a view of determining possible potential risk of large scale consumption of such fresh and dry fish species.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION FOR ANALYSES:

Five different species namely *Tachurus trachurus* (Sese), *Scomber scombrus* (Mackerel), *Clarias gariepinus* (Catfish), *Protopterus annectens* (Mudfish) and *Pseudolithus croaker* (Meluza) were purchased from New Benin market, Oba market and Yanga market in Benin City, Edo State, Nigeria. The fresh samples were wrapped in sterile polyethylene bags, labeled accordingly and transported to laboratory for microbiological and heavy metals analyses.

ENUMERATION, ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF MICROBIAL ISOLATES:

The aerobic colony count of fish samples were done by the pour-plate method as described by Cheesbrough [5]. The homogenized nutrient agar (NA) was cooled to 45^0C and poured into petri-dishes for bacterial culture. The total heterotrophic bacteria counts of fish samples were performed in duplicates by plating out 1.0ml of the 10^{-1}, 10^{-3}, and 10^{-5} dilutions of the fish samples stock dilutions on nutrient agar plates containing 0.5ml of antifungal. The plates were swirled gently for proper mixing before incubation for 24 hours. The mean counts of bacterial colonies were determined and their means and standard error calculated. Characterization and identification of pure cultures of isolates were done using the criteria in Krieg and Holt [6].

The total fungi counts of the fish samples were performed in duplicates by plating out 1.0ml of the 10^{-1}, 10^{-3}, and 10^{-5} dilutions of the fish sample stock mixture and inoculated on potato dextrose agar (PDA) amended with antibiotics. The plates were incubated at room temperatures for 72 hours. Developing colonies were counted and mean and standard error calculated. Fungi isolates identification was done with reference to the manual by Barnett and Hunter [7].

DIGESTION OF FISH SAMPLES FOR MERCURY AND CADMIUM:

1.0g of the fish samples was weighed into digestion glass tube. 10ml of H_2SO_4 was added to the crushed sample. A small quantity (< 0.5g) of Selenium catalyst was introduced into the glass tube. The glass tube was then placed in the aluminum digestion block where it starts to digest. 10ml of Nitric acid was added and digestion continued until the sample changed colour from black to colourless showing that the digestion has completed. The blank was also prepared in the same way as the samples. Filtration of sample was carried out using the method of Atuanya and Okoro (12007). After filtration, the sample was allowed to stand for few minutes for colour development before digest was taken to a spectrophotometer (UV 2100 Spectrophotometer, UNICON) to get the instrument reading for mercury and cadmium using the method described by Willard et al*.* [8].

RESULTS AND DISCUSSIONS

The total microbial counts obtained at different dilutions for different species of fresh and dry/smoked fish samples are shown in Table 1 while bacteria and fungi isolates from fresh and dry fish samples are shown on Table 2. The mean microbial counts for fresh fish samples were highest in fresh *Clarias gariepinus* with bacterial and fungal counts of 2.23 x 10^7 cfu/g and 5.02 x 10^4 cfu/g respectively. However, the mean bacteria counts for dry fish samples was highest (1.70 x 10^6 cfu/g) in dry *Pseudolithus croaker* (Meluza) while the mean fungal counts for dry fish samples was highest (4.28 x 10^4 cfu/g) in dry *Clarias gariepinus*. The microbial isolates obtained from fresh and dry fish samples included bacteria of the genera *Pseudomonas, Proteus, Staphylococcus, Klebsiella,* and *Streptococcus* while the fungi genera were *Aspergillus, Penicillum, Cercospora, Yeast, Mucor, and Trichoderna. Proteus* and *staphylococcus* were more predominant with a frequency of 100% and 70% respectively while *Cercospora* was the most predominant fungi with a frequency of 70%. The considerable high microbial loads and the types of isolates obtained from the fresh fish samples could possibly be due to poor handling by sellers and buyers of different hygienic profile having regular skin contact with the fishes. Another possible explanation could be unhygienic environment of the fish source (river) and the open market. However, the lower microbial counts obtained from the dry fish samples could be as a result of the low moisture content in the fish samples that made it unable for microorganism to grow. Also, the method of drying (smoking) contributed to the low microbial loads in dry fish samples. Smoke is known to contain antioxidants and chemicals that have bacteriostatic and bacteriocidal effects. Most of the microbial load of smoked fish especially those sold in open markets occurs on the surface of the fish. Deep tissues are most often infected in the process of cutting, gutting, skinning and filleting [9]. The presence of *Klebsiella sp.* (Table 2) in the fish samples showed faecal contamination which may be from the aquatic environment where the fish was harvested. The presence of *Pseudomonas sp.* is in accordance with previous study. Gram and Huss [10] and Huss *et al*. [11] found high counts of *Pseudomonas sp.* on fresh fish undergoing spoilage than other bacterial isolates such as *Escherichia coli and Staphylococcus aureus*.

Table 1: Mean microbial heterotrophic counts (cfu/g) of fresh and dry fish samples.

	Fresh		Dry	
Species	**Mean Bacteria ± S.E**	**Mean Fung ± S.E**	**Mean Bacteria ± S.E**	**Mean Fungi ± S.E**
A*	$1.75X10^6$ ± 1.3	$3.37X10^4$ ± 5.9	$1.40X10^5$ ± 10.6	$3.90X10^4$ ± 7.2
B	$1.97X10^6$ ± 6.6	$1.53X10^4$ ± 0.9	$1.56X10^6$ ± 6.3	$1.22X10^4$ ± 1.4
C	$2.23X10^7$ ± 4.1	$5.02X10^4$ ± 7.7	$1.16X10^5$ ± 4.7	$4.28X10^4$ ± 10.3
D	$1.85X10^7$ ± 7.3	$4.98X10^4$ ± 9.7	$1.49X10^6$ ± 4.7	$2.63X10^4$ ± 4.8
E	$1.94X10^5$ ± 3.9	$2.36X10^4$ ± 1.8	$1.70X10^6$ ± 10.1	$2.67X10^4$ ± 4.4

*A = *Tachurus trachurus* (Sese), B = *Scomber scombrus* (Mackerel), C = *Clarias gariepinus* (Catfish), D = *Protopterus annectens* (Mudfish), E = *Pseudolithus croaker* (Meluza)

The mean concentration of mercury and cadmium in the five different species of fresh and dry fish samples from three open markets in Benin City, Edo State, Nigeria presented in Table 3 showed that fresh *Pseudolitus croakar* had the highest mercury concentration (0.549 mg/kg) and fresh *Clarias gariepinus* had the lowest mercury concentration (0.383 mg/kg) while dry *Clarias gariepinus* had the highest mercury concentration (1.733 mg/kg) for dry fish samples. Cadmium concentration was highest in fresh (0.32 mg/kg) and dry (0.52 mg/kg) *Protopterus annectens* fish samples. The mercury levels in fresh *Pseudolithus croaker* (0.549 mg/kg) and dry/smoked fish samples were higher than Food and Agricultural Organization (FAO) limits [12] except for dry *Scomber scombrus* (0.425 mg/kg). However, the cadmium values in both fresh and dry fish samples were within the FAO limits. The high mercury concentration observed in *Trachurus trachurus* (Sese), *Clarias gariepinus* (Catfish), *Protopterus annectens* (Mudfish) and *Pseudolithus croakar* (Meluza) may be due to effect of the drying process that caused weight (moisture and fat) loss thereby increasing the concentration of mercury in the dry fish samples. This is in agreement to the findings of Morgan *et al.* [13] that showed that some cooking practices and increased cooking times increases mercury concentration due to loss of moisture and fat.

Table 2: Frequency distribution of microbial isolates from both fresh and dry fish samples.

Microorganism	Isolates	Percentage frequency
	Proteus sp	100
	Staphylococcus aureus	70
Bacteria	*Pseudomonas sp.*	10
	Streptococcus sp	10
	Klebsiella sp.	20
	Yeast	40
	Penicillium italicum	60
Fungi	*Trichoderma sp.*	60
	Aspergillus niger	20
	Penicillium sp.	40
	Cercospora sp.	70

Table 3: Heavy metals concentration (mg/kg) in various species of fresh and dry fish samples.

Samples	Mean Mercury Conc.		Mean Cadmium Conc.	
	Fresh	Dry	Fresh	Dry
A*	0.438	0.675	0.06	0.03
B	0.458	0.425	0.05	0.27
C	0.383	1.733	0.04	0.34
D	0.433	0.992	0.32	0.52
E	0.549	0.542	0.05	0.03

Note: FAO limit for heavy metals in fish = 0.50mg/kg.
*A = *Tachurus trachurus* (Sese), B = *Scomber scombrus* (Mackerel), C = *Clarias gariepinus* (Catfish), D = *Protopterus annectens* (Mudfish), E = *Pseudolithus croaker* (Meluza)

The active role of some bacteria in mercury bioaccumulation and methylation should not be overlooked. Generally, heavy metals in fish could be due to environmental pollution, type of fish species, microflora of aquatic environment, age of fish, biological and physical processes in aquatic environment, and even handling and storage processes. A considerable high mean fungal load (4.28 x 10^4 cfu/g) and high mean mercury levels (1.73 mg/kg) observed in smoked/dried *Clarias gariepinus* (Catfish) samples could be attributed to biological and physical processes in aquatic environment plus handling and storage processes. Further investigation revealed that all the smoked/dried *Clarias gariepinus* (Catfish) sold and consumed in Benin City are transported from Maiduguri (Chard river basin) unlike the fresh *Clarias gariepinus* (Catfish) with low mercury and cadmium levels that are products of concrete pond cultivations. Based on the results from this study, the consumption of dry/smoked fish especially *Clarias gariepinus* (Catfish) from river by pregnant women, nursing mothers and young children that are not up to six years should be minimized. However, people should be encouraged to rear culturable fish in concrete ponds since study has shown that fish from concrete ponds contain lower levels of heavy metals [14]. In view of the low levels of heavy metals got from the fresh samples analyzed in this study, constant evaluation should be carried out periodically in order to ascertain when the levels of heavy metals is above the acceptable limit for safe consumption.

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