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ARTICLE

Analysis of Some Elements and Speciated Compounds in Fish Found in the New Calabar River of the Niger Delta Area, Nigeria

Ndubuisi Kingsley Owhonda*

Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria

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ABSTRACT

The flesh of guinean and blackchin tilapia, and mullet found in Choba river were collected for elemental studies of mercury, cadmium, lead, arsenic, nickel and speciated forms. Analytical method of X-ray fluorescence (XRF) was used for the elemental studies while Gas chromatography-mass spectrometer (GC-MS) was used for the speciated forms. 4.3 mg/kg was the highest concentration of cadmium observed in blackchin tilapia. The three fish species all contained about 0.5 mg/kg of arsenic and 1 mg/kg of mercury. 1.7 mg/kg of lead was detected in mullet while 7.3 mg/kg of nickel was detected in blackchin tilapia. Organometallic compounds found were nickel tetracarbonyl, borane carbonyl in guinean tilapia, nickel tetracarbonyl, borane carbonyl and germanium(iv) pthalocyanine dichloride in blackchin tilapia and [μ-(η6-benzene)] bis (η5-2,4-cyclopentadien-yl) di-μ-hydrodi-vanadium. Borane carbonyl was found in mullet.

1. Introduction

Elements like mercury, arsenic, lead and cadmium are well-known to have major human health problems [1]. Cadmium inhibits membrane-bound enzymes [2]. Cadmium complexes with sulphydryl groups in the active sites of enzymes. Research has shown that these complexes can hinder the formation of enzyme-substrate complex [3]. Research has also shown that using dithiothreitol (DTT) for the treatment of cadmium toxicity is better than other methods since it serves as a chelator and also restores the sulphydryl groups [4]. It is well documented that people ingest arsenic and other toxic metals mainly through food such as poultry, rice, mushrooms and seafood [5,6,7,8].

Mercury through microbe bio-transformation in fishes can form methylmercury (MeHg). Some researchers have reported that the accumulated form in fish are in many cases extremely high compared to the surrounding water [9]. MeHg is known to be toxic and that it can also accumulate in the food chain [10]. MeHg can be consumed through fish consumption. If MeHg is found in animals it is usually in the organs like kidney, liver, nervous system and prenatal

**Corresponding Author:*
Ndubuisi Kingsley Owhonda,
Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria;
Email: kingsleyndubuisiowhonda@gmail.com

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life where it exhibits its toxicity [11]. High lead concentration can reduce aquatic population of mussel [12]. Potato peel is prone to heavy metal accumulation [13]. Thus, it should be removed before consumption. For a similar reason, this is why the amount of leafy vegetables eating in a day should be limited. The presence of high concentrations of nickel in animals can lead to retarded growth and development [14]. Elements exists in various forms. While some these forms are toxic, others are not [15]. The essence of this work was to shed light on the concentrations of these toxic elements since people depend on this river for their source of fish and to also determine the speciated forms in these three fish species.

The exact quantity of these toxic elements present in the fish consumed from rivers around the Niger Delta region is not well documented. This research work sheds light on this.

2. Location, Materials and Methods

2.1 The Study Location

The arrow in Figure 1 shows the sampling site. This river is located between longitude 6°53.95E and latitude 4°53.78N [16]. It is in Choba village. Close to it is an extension of Wilbros Nigeria Limited (WNL), an oil Servicing Company.

Figure 1. Location of Choba river

2.2 (a) Sampling and the Preparation of the Sample for X-ray Fluorescence

Table size fresh samples of the three fish species were taken from the river (Figures 2,3,4). After cleaning, they were wrapped in an aluminum foil and then put into ice. Finally, they were put in a black polyethylene bag and then all in a cooler to the laboratory for analyses.

Figure 2. Guinean Tilapia

Figure 3. Blackchin Tilapia

Figure 4. Mullet

2.3 X-ray Fluorescence Analysis and Quality Control

The concentrations of the elements were done with X-ray fluorescence spectrometer. This was done in accordance with the USEPA 6200. The drying of the fresh fish samples was done in the oven and it was at 110°C and for twenty hours. The edible fleshy part of the fresh fish samples were reduced to lower than 2µm diameter through crushing. Then, pulverized. They were further made into pellets, moved into a prolene foil that was already cleaned and then put into sample vial. They were then labelled

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accordingly before arranging them in the sample tray. Finally, they were taken to the compartment for sample in the X-ray fluorescence equipment (SpectroX-LabPro) for their elemental composition determination. Through previously stored calibration and with a certified reference material the elemental concentrations of the samples were deduced. The source of excitation was X-ray tube (synchrotron). The matrix effect was corrected by the appropriate means. The sample was analyzed several times to check reproducibility.

2.3.1 GC-MS Analysis

This involved 2 stages. Stage one was extraction while stage two was derivatization just before chromatographing.

Scales were removed first from fish using a ceramic knife as specified by IUPAC [17]. Then the cutting of the edible part was done using the ceramic knife. Mortar and pistol were used homogenizing. 2g of homogenized fish sample was collected and 5ml of 20% TMAH was introduced to digest it. It was then taken to a water bath at the temperature of 60^{o}C where it remained for 2 hours. At the end of the 2 hours pH was checked. It is important that the pH remains between 6-8. 3ml of 0.5M NaDDTC was added. 1.5ml of both toluene and benzene were then added. It was then agitated for 2hrs in a mechanical shaker.

For the 2nd stage, a large balloon was filled with pure nitrogen gas. Then, 5ml of the fish sample which was from the initial stage one was collected with a syringe. The air in the sample was then flushed with the nitrogen gas in the balloon and for several minutes. While the flushing was on, 3ml of n-BuMgCl and 2ml THF were introduced. They were then agitated for 10 mins. In case of the presence of any excess Grignard reagent, 10ml of 0.5M sulphuric acid was added to destroy it. Two phases resulted which were then separated. GC-MS analysis was carried out on the organic phase.

3 Results and Discussion

3.1 Results

3.1.1 X-ray Fluorescence

Table 1 shows the concentrations of elements in the three fish species

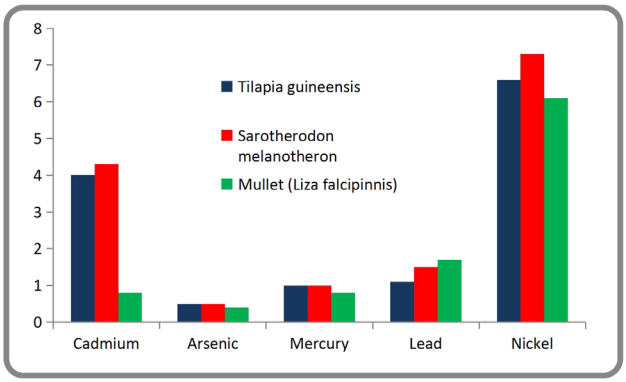

Figure 5. Concentration (mg/kg) of cadmium, arsenic, mercury, lead and nickel in the three fish species

3.1.2 GC-MS Analysis Chromatogram

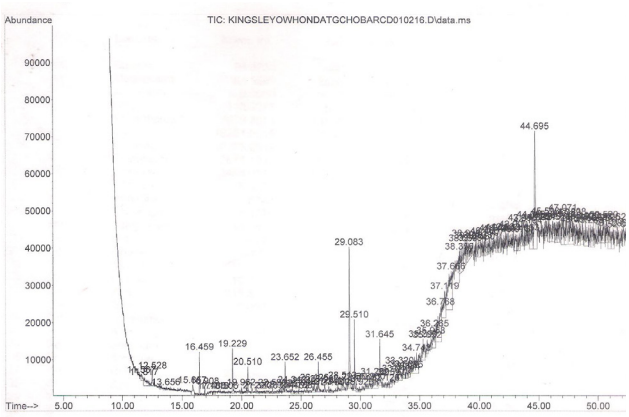

Figure 6. Chromatogram of *Tilapia guineensis* from Choba river

Table 1. X-ray fluorescence analysis result

Fish Specie	Cd(mg/kg)	As(mg/kg)	Hg(mg/kg)	Pb(mg/kg)	Ni(mg/kg)
Guinean Tilapia (Tilapia guineensis)	4.0	0.5	1.0	1.1	6.6
Blackchin Tilapia (Sarotherodon melanotheron)	4.3	0.5	1.0	1.5	7.3
Mullet *(Liza falcipinnis)*	0.8	0.4	0.8	1.7	6.1

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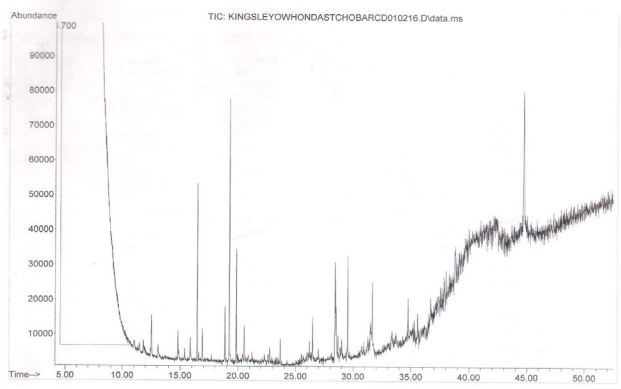

Figure 7. Chromatogram of Sarotherodon melanotheron from Choba river

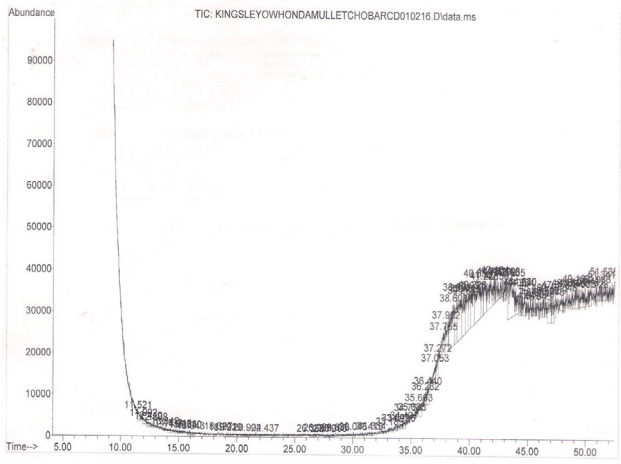

Figure 8. Chromatogram of Mullet (Liza falcipinnis) from Choba river

3.2 Discussion

3.2.1 Cadmium

The cadmium detected was 0.8-4.3mg/kg. Cadmium is known to exerts toxic effect on kidney and body systems. Thus, it has been classified as a carcinogen [18]. It is also known to accumulate in many organisms including crustaceans and molluscs. Vegetables, cereals and starchy roots have shown lower concentrations [19]. Safe amount of cadmium in a month is 25µg/kg body weight [19].

3.2.2 Arsenic

The arsenic detected was 0.5mg/kg or less. The tolerable limit for arsenic in food is yet to be established [19,20]. Thus, the quantity of these fish species consumed per day should be in moderation.

3.2.3 Mercury

For mercury, 1.0mg/kg or less was detected. MeHg was not detected. Research shows that mercury is toxic to humans no matter the form in which it is found [21]. It is even more so to the developing foetus in the uterus and its early life. This is because mercury damages the brain, the lungs and the kidney. 0.5-1mg/kg is recommended as safe limits [22].

3.2.4 Lead

The lead detected was 1.1-1.7mg/kg. There is no known safe level [23].

Table 2. Speciated forms of elements detected in the three fish species

Type of fish	Compound detected	Retention time (minutes)	Amount (%)
Tilapia guineensis	Nickel tetracarbonyl	4.51	8.11x10-3
	Borane carbonyl	5.46	3.36 x10-4
Sarotherodon melanotheron	Nickel tetracarbonyl	4.75	5.41 x10-1
	Borane carbonyl	6.69	3.07 x10-4
	Pthalocyaninedichloridegermanium	44.68	8.20 x10-3
Mullet *(Liza falcipinnis)*	Borane carbonyl	6.42	2.06 x10-4
	[µ-(η6-benzene)]bis(η5-2,4-cyclopentadien-yl)di-µ-hydrodi-vanadium	34.74	1.55 x10-4

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3.2.5 Nickel

The nickel detected was 6.1-7.3mg/kg. 5-50µg of nickel is required per day. While 4.2µg of nickel per kg body weight in a day come from food [24] an upper limit of 1mg of nickel in a day for adults from all sources has been indicated [25].

Nickel tetracarbonyl is an organonickel compound. It is the main carbonyl compound of nickel. It is a pale-yellow liquid with the formula $Ni(CO)_4$ and is extremely poisonous. It is fatal if absorbed through the skin or even when inhaled [26]. The LD_{50} concentration is 3ppm. For humans, the fatal dose remains 30 ppm. This is because the half-life of $Ni(CO)_4$ is just 40s and so, it can decompose very quickly in air [23]. As a result, it will not cause harm when ingested through fish consumption.

4. Conclusions

The results did show that the fish species contain toxic elements. It showed that the quantity of these toxic elements present depend on the type of fish and the river in which it is found. However, the result also suggests that their toxicity may well depend on the quantity of the fish consumed and the totality of all food sources that contain these toxic elements that are consumed daily.

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Cadmium, cadmium chloride, cadmium oxide, cadmium sulphide, cadmium acetate, cadmium sulphate. Geneva, World Health Organization, pp.1075-1318.

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