World Applied Sciences Journal 34 (2): 268-272, 2016
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2016.34.2.15657

Comparative Assay of Heavy Metals in Gills and Muscles of Common Fishes in Afikpo, Ebonyi State, Nigeria

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Abstract: Proton Induced X-ray Emission (PIXE) was used to determine the concentrations of Iron, chromium, lead, copper, zinc, cadmium and nickel in fish samples from Ndiibe beach, Afikpo. The gills and muscles of *Heterobranchus bidorsalis, Syndontis membrabeceus, Alestes baramose, Chrysichytys nigrodigitatus, Clariias ganepinus and Heterotis niloticus* were samples and assayed in June 2014. The results obtained revealed that heavy metals in the investigated fishes ranged from 42.22±0.3 ppm - 126.33±0.4 ppm for Fe; 2.11±0.2 ppm - 9.18±0.8 ppm for Cr; 0.02±0.1 ppm - 0.84±0.2 ppm for Pb; 11.11±0.1 ppm - 31.12±0.1 ppm for Cu; 10.12±0.3 ppm - 72.12±0.4 ppm for Zn; 1.16±0.2 ppm - 3.46±0.3 ppm for Cd and 0.21±0.1 ppm - 6.62±0.4 ppm for Ni. Cadmium and nickel were not detected in *Clariias ganepinus* and *Heterotis niloticus* respectively. The concentrations of the heavy metals were significantly higher in gills than in the muscles of all investigated fishes (except Cd in *Heterobranchus bidorsalis*). Comparing the results with Maximum Permissible Limits of FAO/WHO, it was observed that in all the fishes, the levels of Cr, Cd and Ni (except Ni in *Clariias ganepinus*) exceeded the legal limits while Cu levels were lower than the Maximum Permissible Limits. Levels of Pb and Fe in *Heterobranchus bidorsalis* and *Alestes baramose* also exceeded the Maximum Permissible Limits. The variation in the levels of heavy metals were found to be statistically significant ($p < 0.05$) as determined by one way analysis of variance. The implications of these high accumulations of metals in the affected fishes were studied with respect to its adverse effect to the health of man who is a member of the food chain. Hence regular monitoring of heavy metal in fishes is expedient for safety of human health.

Key words: PIXE · Fish species · Gills · Muscles · Metals · Ndiibe beach · Afikpo

INTRODUCTION

There is a rapid diversion from consumption of meat to fish and as a result, the rate of fish consumption in Nigeria has increased astronomically. The nation's consumption as at 2013 has risen to 2-6 million tonnes with a per capita consumption of 13.5kg [1]. The reason behind this shift from meat to fish is due to extra nutritional values of fish as high protein supplement especially for heart friendly omega-3 fatty acid in fish which is known to reduce high incidence of heart attack [2]. In Nigeria especially the riverine and coastal areas, the natives depend on fishes as their major staple food. In addition, gills of the fishes are still widely consumed especially by the children in the villages. The major source of these metals is by natural process of weathering or through man's anthropogenic activities [3]. Water bodies such as streams, rivers, lagoons and ponds generally carry large quantity of contaminants especially heavy metals which come from discharge of agricultural, industrial and domestic wastes and other anthropogenic activities. Unlike many organic contaminants which lose toxicity with time as a result of biodegradation, metals are non-biodegradable and their toxic effect can be long lasting. In addition, metals in living tissue can undergo bio-magnification from one trophic level to another. Previous works by [4]; [5] and [6], recorded high concentration of heavy metals such as Cd, Pb, Fe, Cu, Ni,

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Zn, Mn, Mg and Co in some rivers within Nigeria. There are four possible routes through which pollutants such as heavy metals can enter into the fish and they are the skin, the gills, through food and through the water which the fish drinks [7]. Through any of these routes metals may ultimately find their way into the fish. While some of these metals are essential, others are toxic both to the fish and ultimately to man who is a member of the food chain [8]. Proton Induced X-ray Emission (PIXE), used for this study, has many advantages over Atomic Absorption Spectrophotometer (AAS) such as high sensitivity, high speed, multi element and non-destructive analysis in addition to its ability to use very small amount of sample for the analysis. This study aims at determining the levels of some of these heavy metals in fishes from Ndiibe beach and to compare the values obtained with standards such as World Health Organization (WHO), Food and Agriculture Organization (FAO), European Community (EC) and national Federal Environmental Protection Agency so as to establish which metal have exceeded the Maximum Permissible Limits.

MATERIAL AND METHODS

The Study Area: Ndiibe beach is located in Afikpo North Local Government Area of Ebonyi State, Nigeria and is a part of a network of rivers that connect other rivers in Cross River, Ebonyi and Abia States. The Geographic coordinates of Afikpo are Latitude: 5°53'35″ N and Longitude: 7°56'14″ E and the elevation of Afikpo above sea level is 83 m (272 ft). It has a typical hot dry climate and during rainy season there is an ample rainfall within the range of 2250 mm and 2500 mm. The town observes 3 dry months with less than 6 cm rainfall a month. The mean humidity is 71-81% while the temperature is within the range of 27 -34°C. Afikpo being the second largest town in Ebonyi State has few cottage industries. The people are mainly farmers hence industrial waste is not a major source of these metals.

Collection And Pre-treatment Of Fish Sample: The fishes of mean weight 180g±0.5 were bought from local fishermen at Ndiibe beach, Afikpo as soon as the fishermen alighted from their fishing boats at 6.35am in the morning. Fish of similar species and size is a strong index of the age of the fish. The fishes were collected inside a plastic ice box and were labelled appropriately. The fishes were dissected and the gills and dorsal muscle tissues were removed for the purpose of the analysis. The samples were pulverised, sundried and grinded in preparation for PIXE analysis.

PIXE Analysis: Proton Induced X-ray Emission analysis was done following a procedure by [9]. The PIXE process of analysis began with pelletization of the fish samples. A 13mm pellet of the sample was formed using CAVER model manual palletizing machine at a pressure of 6 - 8 torr. The target fish samples were irradiated in PIXE spectrometer for 3 minutes in a vacuum chamber with 3 MeV protons (beam currents of 10-70 nA and beam diameter of 4 mm). The X- rays generated from the target were measured with two Si/Li detectors and the corresponding metal concentration was ascertained by means of incorporated computer device. The samples were analyzed individually in triplicates (n =3).

Fig. 1: The Study Area Afikpo in Ebonyi State Nigeria adapted from the World Atlas

Table 1: Levels of Heavy Metals in Fish from Afikpo

Fish		Fe	Cr	Pb	Cu	Zn	Cd	Ni
Heterobranchus bidorsalis	Gills	117.01±0.6	9.18±0.8	0.84±0.2	11.12±0.1	32.98±0.3	1.24±0.3	6.62±0.4
	Muscles	85.20±0.4	8.01±0.7	0.69±0.1	7.81±0.1	10.12±0.3	2.4±0.4	4.42±0.4
Syndontis membrabeceus	Gills	114.65 ±0.4	2.02±0.2	0.43±0.1	24.12 ±0.1	21.21 ±0.3	2.40±0.3	3.42±0.2
	Muscles	94.11±0.2	2.08±0.2	0.29±0.1	11.12±0.09	11.02±0.3	1.80±0.3	3.00±0.2
Alestes Baramose	Gills	126.33 ±0.4	4.12±0.4	0.82±0.2	21.66±0.1	50.28±0.2	3.12±0.3	1.48±0.1
	Muscles	92.22±0.2	2.00±0.2	0.66±0.2	12.12±0.1	36.66±0.2	3.46±0.3	1.40±0.1
Chrysichytys nigrodigitatus	Gills	101.51 ±0.2	4.12±0.3	0.42±0.1	31.12±0.1	30.2 ±0.1	1.80±0.2	2.52±0.2
	Muscles	80.88±0.2	2.11±0.2	0.40±0.1	10.11±0.1	21.1±0.1	1.78±0.2	2.50±0.2
Clariias ganepinus	Gills	120.97±0.2	8.18±0.8	0.02±0.1	19.18±0.1	66.12±0.2	ND	0.21±0.1
	Muscles	91.00±0.2	3.12±0.2	0.02±0.1	10.12±0.1	25.81±0.2	ND	0.21±0.1
Heterotis niloticus	Gills	64.67±0.3	2.41±0.3	0.12±0.1	14.40±0.1	72.12±0.4	2.00±0.2	ND
	Muscles	42.22±0.3	2.11±0.2	0.12±0.1	11.12±0.1	22.11±0.3	1.16±0.2	ND

Table 2: Maximum Permissible Limit (MPL) of heavy Metals in Fish according to Some International Standards

	Metals							
	Fe	Cr	Pb	Cu	Zn	Cd	Ni	References
FAO (1983)			0.5	30	30	0.05		FAO (1983)
FAO/WHO Limit			0.5	30	40			FAO/WHO (1989)
WHO 1989	100		2	30	100	1.0		Mokhart (2009)
EC			0.2			0.05		EC (2005)
England			2	20	50	0.2		MAFF (2000)
FEPA		0.15- 1.0	0.2	20	50	0.2	0.5-1.0	FEPA (2003)

RESULTS

Tables 1 and 2 present levels of heavy metals in the investigated fishes and the Maximum Permissible Limits of some International Standards.

DISCUSSION

High concentration of heavy metals was observed in gills than in the muscles. Heavy metal accumulation in the gills of *Heterobranchus bidorsalis* and *Alestes baramose* were in the order Fe > Zn > Cu > Cr > Ni > Cd > Pb; *Syndontis membrabeceus* and *Chrysichytys nigrodigitatus* were in the order Fe > Cu > Zn > Ni > Cd > Pb; *Clariias ganepinus* were in the order Fe > Zn > Cu > Cr > Ni > Pb while *Heterotis niloticus* were in the order Zn > Fe > Cu > Cr > Cd > Pb. Similar trend were observed in tissue with few exceptions. The highest concentration of heavy metal was Fe except in *Heterotis niloticus* while the least concentrated metal was Pb.

Iron: Highest concentration of Fe (117.01 ppm) was observed in *Heterobranchus bidorsalis* while the lowest (42.22 ppm) was observed in *Heterotis niloticus*. The concentration of Fe in the gills exceeded the Maximum Permissible Limits of (50 – 100 ppm) [10, 11] and [12], except in *Heterotis niloticus*. The level of Fe obtained from the work was lower than those obtained in the same study area by [13] and this may be as a result of the transient state of metals in the environment. The levels of Fe in these fishes were comparable with the values obtained by [14]. Iron is an essential micronutrient needed for growth and metabolism of fish. However excess of Fe in human body results to hemochromatosis, a genetic abnormality of excessive Fe store which increases the risk of chronic disease such as cancer and heart disease via oxidative mechanism [15].

Chromium: Levels of Cd obtained for this work exceeded maximum recommended limits of 0.15 - 1.0 ppm [16], in fish food. The level of Cr in Syndontis membrabeceus was higher in the gills than in the muscles. Concentrations of Cr in this study were higher than those obtained by [5], from the same study area where Cr in *Chyrsichthys nigrodigitatus* was found to be 1.19 ppm. The difference could be as a result of size and age of the fish which is a function of bioconcentration of metals in the fish body.. The values obtained from this work were lower than those reported respectively by [17], where concentration of Cr in *Parachanna obscura* from Ogba River in Benin ranged from 29.8 - 31.6 ppm and in *C. Gariepinus* ranged from 28.1 - 32.2 ppm. Cadmium toxicity in human includes kidney and liver damage, skin rashes, stomach upset and ulcer, respiratory problems and lung cancer and alteration of genetic materials [17].

Lead: The concentrations of Pb were the least in the study and Pb accumulated more in the gills than in the muscles except in *Clariias ganepinus* and *Heterotis niloticus* where the levels were the same. Highest concentration (0.82 ppm) was observed in the gills of *Heterobranchus bidorsalis*. Lead concentration in *Heterobranchus bidorsalis* and *Alestes baramose* exceeded the Maximum Permissible Limits of (0.22 – 0.5 ppm) [8, 10, 11] but were lower than the Permissible limit of 2.0 ppm prescribed by [19]. The levels of Fe in these fishes were comparable with the values 0.10 - 0.83ppm obtained by [17], in fishes from Ogba River. Levels of Pb in this work were however lower than 0.01-0.06 ppm reported by [20] fishes from bonny estuary and higher than 9 ppm obtained by [4], form fishes in Lagos lagoon. Lead is toxic in human at any concentration. Once in human body, accumulated Pb is sequestered in the bones and teeth. This causes brittle bones and weakness in the wrists and fingers [19. Lead that is stored in bones can re-enter the blood stream during periods of increased bone mineral recycling (i.e., pregnancy, lactation, menopause, advancing age, etc.). Mobilized Pb can be re-deposited in the soft tissues of the body and can cause musculoskeletal, renal, ocular, immunological, neurological, reproductive and developmental effects [15].

Copper: Copper concentrations were found to be more in the gills than in the muscles in all the studied fishes. Except in the gills of *Chrysichytys nigrodigitatus*, the concentrations of Cu were all below the Maximum Permissible Limits of Cu (20 – 30 ppm) [10, 11, 12 and 18]. The concentrations of Cu in fishes reported by [5] in the same study area were lower than those in this present work possibly because of the non-static nature of the environment. Human beings, depending on the age, require Cu between 0.97 mg/day and 3.0 mg/day as RDA (Requirement Dietary Allowance). However, excess copper in human body is known to cause hematemesis (vomiting of blood), hypotension (low blood pressure), melena, coma, jaundice (yellowish pigmentation of the skin) and gastrointestinal distress.

Zinc: Highest concentration of Zn was observed in *Heterotis niloticus* (72.12 ppm) and the lowest in *Syndontis membrabeceus* (11.02 ppm) and these values were within the Maximum Permissible Limit (30-100 ppm) [10-12, 18]. The levels of Zn in this work were comparable with [5 14 and 7]. Zinc as a micronutrient is essential at low-concentrations for normal growth and development of humans, however, at high concentration, zinc toxicity can lead to deficiencies in iron and copper, nausea, vomiting, fever, headache, tiredness and abdominal pain, lassitude, slower tendon reflexes, bloody enteritis, diarrhea, lowered leukocyte count and depression of CNS and paralysis of extremities [21].

Cadmium: Highest concentration of Cd (3.46 ppm) was observed in the gills of *Alestes baramose* even though it was not detected in *Clariias ganepinus*.. Concentration of Cd in both gills and muscles of *Syndontis membrabeceus* and *Chrysichytys nigrodigitatus* and muscle of *Heterobranchus bidorsalis* and they all exceeded the Maximum Permissible Limits of Cd (0.05 – 2.0 ppm). The levels of Cd in these fishes are comparable with the values obtained by [4]. These results are higher than level of cadmium reported by [7], in *Alestes nurse* and *Synodontis nigritisfrom Oguta* Lake which were 1.50 and 1.23 respectively. Lower concentrations of Cd were observed in *C. gariepinus (0.93ppm)* and *T. zillii* (0.99) from River Benue as reported by [17]. High concentration of cadmium is known to cause bone fracture, cancer, diarrhea, stomach pains, severe vomiting, reproductive failure and damage of CNS and DNA when consumed by man through affected fish food [21].

Nickel: Nickel was not detected in *Heterotis niloticus*. Except for *Clariias ganepinus*, all the concentration s of Cd obtained for this work exceeded maximum recommended limits of 0.05 - 2.0 ppm [10-12, 22] in fish food. The concentration of Ni in both gills and muscles of *Clariias ganepinus* were the same. Concentrations of Ni in this study were comparable with the findings of [23], who reported 1.64ppm - 3.58 ppm of Ni in fishes from Olomoro Rivers. Concentrations of Cd obtained from this work were higher than those reported by [14] where Cd content were in *Mormyops deliciosus* and *Mormyrus macrophthalmus* were 0.24 and 0.36 ppm respectively. Deficiency of Nickel have been linked with hyperglycemia, hypertension, depression, sinus congestion, fatigue, reproductive failures and growth problems in humans, while excess intake of Ni leads to hypoglycemia, asthma, nausea, headache and epidemiological symptoms like cancer of nasal cavity and lungs. The prescribed safety limit of Nickel is 3 to 7 mg/day in humans [24].

CONCLUSION

Concentration of many of the investigated heavy metals such as Cr, Cd and Ni, Fe and Pb exceeded the maximum Allowable Limit in some of the studied fishes.

These affected fishes are not fit for human consumption especially the gills which tend to accumulate more toxic metals than the muscles. Regular monitoring of metallic content of edible materials in the environment such as fish is very essential for the purpose of evaluating the safety of consumers.

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