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Toxic Metals Assessment in the Tissues and Organs of Three Commonly Consumed Fishes in Igbona Market, Osogbo, Nigeria

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ABSTRACT

A possible route of heavy metal ingestion by human is via heavy metal contaminated food items. Three different commonly eaten species of fresh fish sourced from Igbona market, Osogbo, Nigeria were spectrophtometrically assessed for possible heavy metal contamination. Fifteen (15) fish samples, five each, of Sardine (Scardinella maderensis), Croaker (Johnius bellangeri) and atlantic mackerel (Scomber scrombus) were ashed while digestion of 2.0 g of the ashed sample was done using 5 mL of concentrated nitric acid, filtered and made up to 50 mL with deionized water. Zinc (Zn), iron (Fe), chromium (Cr), copper (Cu), and lead (Pb) contents of the digest were determined using Buck Scientific 210 Variant Giant Pulses (VGP) Atomic Absorption Spectrophotometer (AAS). The three fish samples were very rich in iron with ranges of 24.04 ± 0.42 - 31.01± 0.53, 11.87 ± 0.75 - 22.25 ± 0.57 and 11.98 ± 0.13 -16.03 ± 0.22 µg/g for Scardinella maderensis, Scomber scrombus and Johnius beangerri fishes respectively. Lead, the most toxic of all the studied analytes, was almost not detected in two fish species (Scardinella maderensis and Scomber scrombus), while it was completely not detected in Johnius beangerri. The order of decrease of metals studied in Scardinella maderensis is iron (114.68) > zinc (22.45) > copper (13.43) > chromium (2.34) > lead (0.03) µg/g while that of Scomber scrombus and Johnius beangerri are iron (67.28) > zinc (44.78) > copper (15.36) > chromium (14.05) > lead (0.02) µg/g and iron (55.19) > zinc (22.16) > copper (12.54) > chromium (7.81) > lead (ND) µg/g respectively. The order showed similarity, thus the likelihood of same sources for the heavy metal uptake. It can be concluded that fish samples are rich in essential metals (iron, zinc, copper) and low in xenobiotic metals (lead and chromium). Hence, the fishes found in the study area pose little or no threat to consumption by human.

Keywords: Heavy metals; *Scardinella maderensis; Scomber scrombus; Johnius beangerri*; Igbona market; Essential metals

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INTRODUCTION

In developing countries, a higher percentage of the population has been known to feed on fish rather than buying meat because of the cost, and the protein content present in fish. The muscle of fish is the most part of the fish that is commonly consumed. Fishes are rared in water bodies, and because of this, there is toxic chemicals accumulation such as phthalate esters and toxic metals directly from food and the water they drink [1].

Bioaccumulation is a process by which there is uptake of chemicals by an organism either by consumption of food that contain chemical or by direct intake from contaminated medium [2]. Marine organisms come in contact with toxic metals through food intake, or directly from seawater. There is concentration of metals in some marine organisms more than in the surrounding environment due to accumulation. In general, fauna in marine have been used in monitoring marine pollution programmes because they are affected from the accumulation of contaminants in the environment [3]. Due to the toxicity effect of heavy metals on organism, the heavy metals pollution of aquatic bodies has been a worldwide problem for the past few years [4]. Among the environmental pollutants, metals are of great worry because of their toxicity and bioaccumulation in aquatic systems [5].

Heavy metals are chemical elements which are metallic with high density greater than 5 g/cm^3 which are harmful at low concentrations. They include Arsenic (As), Lead (Pb), Mercury (Hg), Cadmium (Cd), and Chromium (Cr). They can neither be destroyed nor debased. To a minimal extent, they enter our biological system via food, drinking water and air thus accumulating in the body system. Poisoning of heavy metals could be from taking water that is polluted from pipes made of lead, food intake through food chain and also high ambient air concentrations near emission sources. They can also enter water supply from rain with pH of acid (acid rain) by releasing metals into streams, rivers and ground water or from industrial land consumer waste through breaking down of soils [6]. Fish is a vertebrate adapted for a purely aquatic life, which propels and balance itself with the use of fins and thus obtain oxygen from the water for breathing purposes using gills [7]. Fishes like other animals need nutrients to enable them live undisturbed like the common components of foods [8]. Mineral content of fish is the nutrient of interest with respect to this research.

The affluence and possibility of metals in the marine system can be measured indirectly using bioaccumulated heavy metals in marine organism tissues [9], and due to this, contaminated fish tissue being monitored serves as indicator of water quality problems or related sediment contamination [10] and thus help us to take appropriate action in protecting the environment. A lot of factors which includes physical and chemical properties of water can play a significant role in accumulation of metals in different tissues of fish [11]. Studies [12,13] have also shown the ability of fish to retain heavy metals due to accumulation from their environment depending on concentration risk and time and other factors such as hardness, temperature, salinity, and metabolism of animal.

This study focused on investigating the presence and bioaccumulation pattern of heavy metal contaminants (zinc, iron, chromium, lead and copper) in some commonly consumed fish species (Sardine, Croaker and atlantic mackerel) sold in Igbona market (one of the most common markets) in Osogbo, Osun State, to establish their safety for human consumption around the city of Osogbo.

MATERIALS AND METHODS

Description of Site

The fish samples were bought from different fish vendors in Igbona market; Osogbo in Olorunda Local Government whose headquarter is in Igbona, on the outskirts of the state capital Osogbo, Osun State. It has an area of 97 km² and populations of 131,761 during the census conducted in 2006 with a geographical coordinate of 7°52'N4°35'E.

Sample Collection and Preparation

15 fish samples, five each of Sardine (*Scardinella maderensis*), Croaker (*Johnius bellangeri*) and atlantic mackerel (*Scomber scombrus*) were bought from different fish vendors at different time, in the month of August, 2016. These species of fish were put in sterile polythene bags and taken in icebox to the laboratory where they were washed with deionized water. Fish samples identification was done using standard reference sources (www.fishbase.org). All the fish samples were kept separately inside freezer at -10ºC. Prior to digestion samples, the fish samples were put on a dissection plastic tray and allowed to thaw at room temperature for 2 hours. The scales were removed and the fish were dissected using a plastic knife and the fish organs (head, gills, muscles and tail) of different fish samples were removed and the organs for each fish samples were brought together for homogenization after drying them at 105°C for 8 hours. The dried fish samples were each grounded to powder, using a ceramic mortar and pestle in the laboratory, and sieved with 2mm sieve.

Digestion of Sample

Approximately 2.0 g each of the dried organ/tissue of fish samples was accurately weighed into a crucible and ashed in the muffle furnace at 550°C for 5 hours to yield fully ashed samples. The sample-containing crucibles were removed and cooled in a desiccator. The ash was dissolved in 5 mL of concentrated nitric acid and filtered. The filtrate was transferred into a 50 mL standard flask, and made up to mark with deionized water. These were transferred into sterile sample plastic bottles, labeled and kept for determination of heavy metals [12].

Determination of Heavy Metals

Samples were analysed with the use of Buck Scientific 210 Variant Giant Pulses correction(VGP) Atomic Absorption Spectrophotometer (AAS) and the concentration of heavy metals (copper, zinc chromium, iron and lead) were determined at respective wavelength of the metals after proper calibration of the instrument with metal standards . The concentrations of heavy metals were calculated and Microsoft Excel 2010 was used for statistical analysis.

$$\text{Concentration of heavy metals in fish organ sample (µg/g)} = \frac{\left(AAS\ reading - blank\left(\frac{\mu g}{L}\right)\right) \times Vol\ (L)}{Weight\ of\ sample\ (g)}$$

Quality Assurance

Glassware's used were cleaned by soaking overnight in 10% trioxonitrate (v) acid, followed by rinsing with distilled water. The acids used for digestion were of analar grade, while the distilled water was deionized. The samples were analyzed in triplicate, and blank was done.

RESULTS AND DISCUSSION

Results

Concentration of Heavy Metals in Fish Samples

The values are presented in Means ± SD.

Table 1: Mean concentration (µg/g) of heavy metals in the tissues and organs of *Scardinella maderensis* from Igbona market, Osogbo, Osun State, Nigeria

Fish	Organs/Tissues	Zn (µg/g)	Cu (µg/g)	Fe (µg/g)	Cr (µg/g)	Pb (µg/g)
Scardinella maderensis	Head	5.28 ± 0.43	2.99 ± 0.23	27.75 ± 0.08	0.42 ± 0.08	0.02 ± 0.01
	Gills	5.74 ± 0.60	3.90 ± 0.66	31.01 ± 0.53	0.84 ± 0.06	ND
	Muscle	5.96 ± 0.50	3.48 ± 0.07	31.88 ± 0.24	0.78 ± 0.06	ND
	Tail	5.47 ± 0.35	3.06 ± 0.18	24.04 ± 0.42	0.30 ± 0.05	0.01± 0.01
	Total	22.45	13.43	114.68	2.34	0.03

ND: Not detected

Table 2: Mean concentration (µg/g) of heavy metals in the tissues and organs of *Scruber scrubis* from Igbona market, Osogbo, Osun State, Nigeria

Fish	Organs/Tissues	Zn (µg/g)	Cu (µg/g)	Fe (µg/g)	Cr (µg/g)	Pb (µg/g)
Scruber scrubis	Head	8.77 ± 0.15	4.73 ± 0.61	22.25 ± 0.57	3.63 ± 0.18	0.01 ± 0.00
	Gills	12.65 ± 0.50	3.48 ± 0.04	11.87 ± 0.75	3.01 ± 0.15	0.01 ± 0.01
	Muscle	12.58 ± 0.10	3.52 ± 0.31	17.69 ± 0.79	3.69 ± 0.27	ND
	Tail	10.78 ± 0.54	3.63 ± 0.22	15.47 ± 0.23	3.72 ± 0.23	ND
	Total	44.78	15.36	67.28	14.05	0.02

ND: Not detected

Table 3: Mean concentration (µg/g) of heavy metals in the tissues and organs of *Johnius beangerri* from Igbona market, Osogbo, Osun State, Nigeria

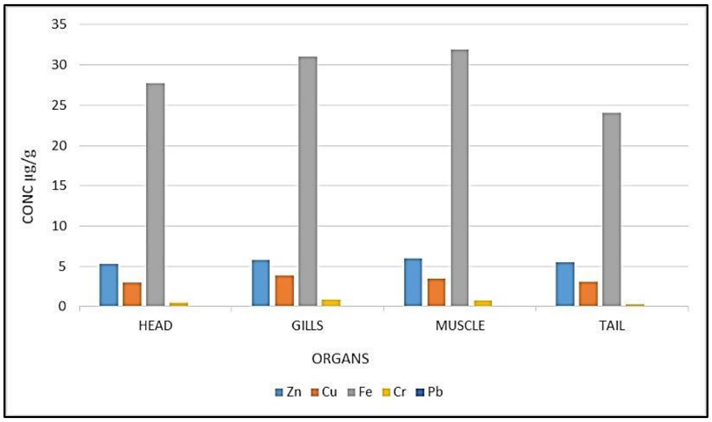
Fish	Organs/Tissues	Zn (µg/g)	Cu (µg/g)	Fe (µg/g)	Cr (µg/g)	Pb (µg/g)
Johnius beangerri	Head	3.69 ± 0.17	3.29 ± 0.73	14.59 ± 0.23	3.01 ± 0.48	ND
	Gills	6.49 ± 0.37	3.42 ± 0.44	12.59 ± 0.39	1.79 ± 0.10	ND
	Muscle	6.84 ± 0.31	3.01 ± 0.12	16.03 ± 0.22	1.80 ± 0.35	ND
	Tail	5.14 ± 0.18	2.82 ± 0.14	11.98 ± 0.13	1.21 ± 0.25	ND
	Total	22.16	12.54	55.19	7.81	ND

ND: Not detected

Discussion

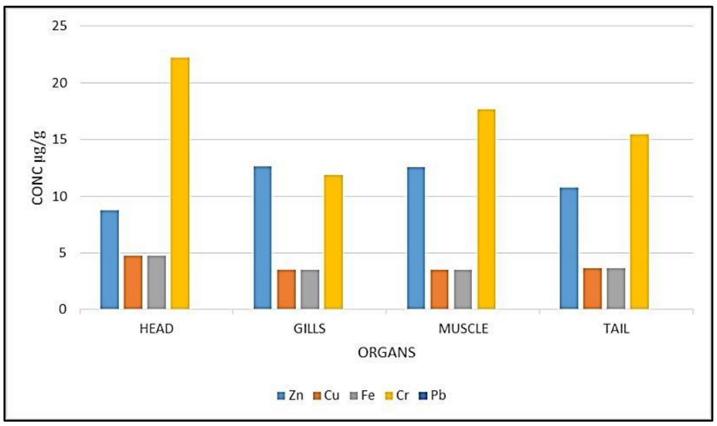
The results of analysed heavy metals in different fish organs/tissues reveal that fish species contained different metal levels in their tissues due to degree of change in nature of feeding, environment and behaviors [14]. The results of the assessment of heavy metals on different fishes with their tissue/organs are shown in Tables 1-3 above. Table 1 reveals the heavy metals level in the tissue/organs (head, gills, muscle and tail) of Sardine (*Scardinella maderensis*). The mean concentration of these metals ranged from 0.42-0.30 µg/g Chromium; 5.96-8.77 µg/g Zinc; 2.99-3.90 µg/g Copper; 24.04-31.88 µg/g Iron; 0.01-0.02 µg/g lead. These results are higher than what Igwemmar et al. [15] discovered in their research (0.31 ppm for Cu, 1.89 ppm for Zn, 3.99 ppm for Fe, and 0.01 ppm for Pb). The levels of their bioaccumulation are in the decreasing order of Fe>Zn>Cu>Cr>Pb. From Figure 1 below, Iron (Fe) has the highest concentration of 27.75 µg/g while lead (Pb) was the least in concentration (0.02 µg/g). The array of bioaccumulation of these heavy metals could be as due to the different accumulation rate of metals in the organs of different fish species (Figures 1-3).

Furthermore, Table 2 clearly shows the contamination level of heavy metals in the organs/tissue (head, gills, muscle and tail) of atlantic mackerel (*Scomber scombrus*). Chromium (Cr) ranged from 3.01-3.72 µg/g; 8.77-12.65 µg/g Zinc (Zn); 3.48-4.73 µg/g copper (Cu); 11.87-22.25 µg/g Iron (Fe); 0.00-0.01 µg/g Lead (Pb). The bioaccumulation order is shown as Fe> Zn> Cu>Cr>Pb. The result reveals that Iron (Fe) has the highest concentration while Lead (Pb) has the least concentration.



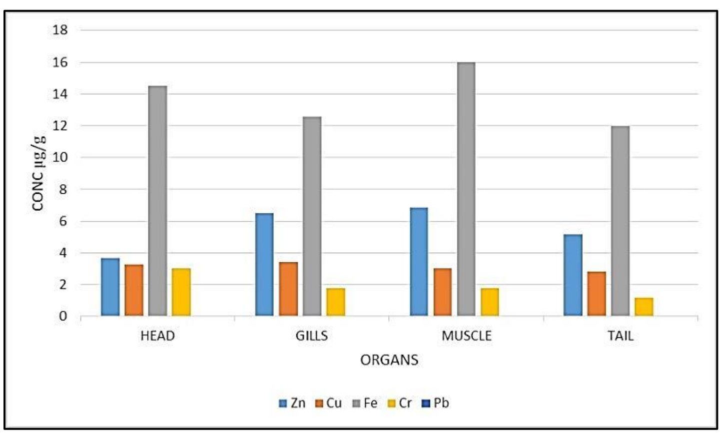
*Values are mean ± SD

Figure 1: Mean concentrations of heavy metals in the head, gills, muscle and tail of *Scardinella maderensis* from Igbona market, Osogbo, Osun State, Nigeria



*Values are mean ± SD

Figure 2: Mean concentrations of heavy metals in the head, gills, muscle and tail of *Scruber scrubis* from Igbona market, Osogbo, Osun State, Nigeria



*Values are mean ± SD

Figure 3: Mean concentrations of heavy metals in the head, gills, muscle and tail of *Johnius belangerri* from Igbona market, Osogbo, Osun State, Nigeria

Also, the level of heavy metals concentrations in different organs/tissue of Croaker (*Johnius bellangeri*) is shown in Table 3. Iron (Fe) levels ranged from 11.98-16.03 µg/g; 3.69-6.84 µg/g Zinc (Zn); 2.82-3.42 µg/g copper (Cu); 1.21

to 3.01 µg/g Chromium (Cr), while lead (Pb) was not detected in croaker. Their level of bioaccumulation is in the order Fe>Zn>Cu>Cr>Pb. In all the organs of the fish species, Iron (Fe) has the highest concentration while Lead (Pb) was not detected, therefore the least concentration.

Nonetheless, the mean concentrations of the various contaminated heavy metals were compared among the various organs/tissue of the fish species. As presented in Tables 1-3, the level of heavy metals for the head in all the fish samples ranged from 14.59-27.75 µg/g Iron (Fe); 0.42-3.63 µg/g Chromium (Cr), 3.69-5.28 µg/g Zinc (Zn); 2.99-3.29 µg/g Copper (Cu) and ND -0.02 µg/g Pb. Iron also has the highest concentration in the head while lead has the least concentration. The levels of heavy metal accumulation in the head of fish samples as reported by [12] is lower in zinc (0.16 µg/g for tilapia and 1.95 µg/g catfish) and iron (0.11 µg/g for tilapia and 0.25 µg/g catfish).

In the gills, Fe ranged from 11.87-31.01 µg/g; 5.74-12.65 µg/g Zn; 3.42-3.90 µg/g Cu; 0.84-3.01 µg/g Cr; ND-0.01 µg/g Pb. The gills surface is the first contact of water-borne metals [16]. The gills have the highest concentration value of Fe while lead has the least concentration. This study revealed that the accumulation of toxic metals in gills is higher in *Scardinella maderensis* compared to other organs or tissues especially muscle. This finding is very common in several researches that have been reported [17,18]. Gills has a contact with the external medium thus act as the major site for entry of different kinds of contaminants such as heavy metals and it has different physiological functions such as gas exchange in respiration, excretion of nitrogen and osmoregulation [19].

The heavy metal levels in the muscles of the fish species ranged from 16.03-31.88 µg/g Fe; 5.96-12.58 µg/g Zn; 3.01-3.52 µg/g Cu; 0.78-3.69 µg/g Cr and Pb was not detected in the muscle of all the fish species. Fe has the highest concentration (31.88 µg/g) in all the metals, and was found to be deposited in the muscle. [20] in their research determined the level of heavy metals in Cu and Pb to range between 1.53-5.61 µg/g and 0.59-3.78 µg/g respectively; this is different from what is reported in this work. Altindag and Yigit [19] and Huang [20] found lower concentration of heavy metals in muscle than gills, which was also confirmed in this research.

The last organ (tail) of all the fish species showed the level of heavy metals to range from 11.98-24.04 µg/g Fe; 5.14-10.78 µg/g Zn; 2.82-3.63 µg/g Cu; 0.30-3.72 µg/g Cr and ND- 0.01 µg/g Pb. In this study, it could be seen that in all the fish species, muscle has the highest concentration of metals (Fe) as seen in Sardine (*Scardinella maderensis*) followed by the gills, head and tail. The tail has the least mean concentration value, and the decreasing order of heavy metals is Muscle>gills>head>tail.

Comparison of Heavy Metals in Fish Species with Standard Values

In this study, it was found that iron dominated all the metals in the samples collected from Igbona market. These observations on this metal are similar and related to the observations of other researchers [21-23]. There is a wide difference in mean concentrations of Fe among different species of fishes with the highest concentration (31.88 µg/g) seen in the muscles of Sardine (*Scardinella maderensis*) while the lowest concentration level (11.87 µg/g) was seen in gills of atlantic mackerel (*Scomber scombrus*). Fe is important in the diet of human, it is a part of hemoglobin which helps in the transport of oxygen from the lungs to the tissues, and hence its deficiency leads to anemia in human. The highest concentration seen in this study was below the high residue of Fe (34-107 ppm) in fish samples on MNW refuge [24]. Therefore, seafoods are high in Fe, and thus suggest a long term contamination in the past few years. Copper plays an important role in the synthesis of haemoglobin and several enzymes. The highest concentration of Cu (3.90 µg/g) was found in Sardine (*Scardinella maderensis*) and its lowest (3.42 µg/g) value in Croaker (*Johnius bellangeri*) fish species. The observed results of this research are higher than what Ishaq et al. [25] (1.56-2.89 µg/g), Kah et al. [26], (0.16 0.27 µg/g) and Abdulali et al. [27] (1.46-1.69 µg/g) for tilapia fish in their research. However, the highest value of 3.90 µg/g was below the FAO guideline of 30 µg/g, hence, the concentration of Cu in the fish samples analyzed were all below FAO recommended guideline [28].

Zinc, a trace element for taste loss and hypogonadin, bringing about reduced fertility. Toxicity of Zn is rare, however, at concentration in water up to 40 mg/kg, may induce toxicity [29]. Zinc is very important in the pathway human metabolism and the deficiency of zinc can result to loss of appetite, changes in skin, and abnormalities in immune and retardation of growth [30]. Due to its significance biologically, zinc is widespread among living organisms. According to Food Codex, the maximum permissible level of Zinc for fishes is 50 mg/kg [31], while the recommended daily intakes of zinc are 15 mg for adult males and 12 mg for adult females. The highest concentration of zinc (12.65 µg/g) was confirmed in the gill of atlantic mackerel (*Scomber scombrus*), while the lowest value of 5.14 µg/g was found in the tail of Croaker (*Johnius bellangeri*) fish species within the FAO [28] guidelines of 30 µg/g. Lead is one of the most toxic heavy metals. Its effect due to high concentration could lead to delayed embryological development, suppressed reproduction, and neurological problems [32]. Lead increases the pressure of blood and heart related adult diseases and also brings about reduced development cognitively and intellectual performance in children. The maximum permissible level of lead for fishes in can is 0.2 ppm [33]. Lead causes behavioral inadequacy, and decreases the survival, growth rates, learning, and metabolism of vertebrates

[34]. Lead concentration of 50 ppm in diets can lead to reproductive effects in some predators. In this study, the highest concentration of Pb (0.02 µg/g) was found in the head of Sardine (*Scardinella maderensis*) and atlantic mackerel (*Scomber scombrus*) while the lead was not detected in most of the organs/tissues of the fish samples. This is however still within the guideline of 0.6 µg/g of FAO [28], thus the concentration levels of lead pose no hazardous effect in this study. Chromium deficiency leads to damaged growth, and disorder in glucose, protein and lipids metabolism [35]. The lowest concentration of Cr (0.30 µg/g) was found at the tail of Sardine (*Scardinella maderensis*) fish species while the highest concentration of 3.72 µg/g also at the tail atlantic mackerel (*Scomber scombrus*) fish species. Chromium has been known to be essential in human health; Chromium (III) is an important nutrient which has helped the body in the proper use of fats, sugars, and proteins, whereas Cr (VI) is known to be carcinogenic [36]. According to Tarley et al. [37], the sufficient diet intake in adults' ranges from 0.50 to 2.00 µg/g. Different researches confirmed that chromium (VI) compounds can increase in the risk of having cancer of the lung and also World Health Organization (WHO) has proposed that chromium (VI) is carcinogenic to human [38]. According to other works, the levels of Cr in fish have been reported to be in the range of 0.10-1.60 µg/g for fish muscles from the Turkish sea [39], 0.06-0.84 µg/g for muscles of fish from the black sea coasts[40], 0.31-0.73 µg/g for muscles of fish from the western coast of the United Arab Emirates [41], which were all lower than the highest level of chromium (3.01 µg/g) in this research but despite that, our findings on Chromium contents were well within the limits prescribed by the FDA. The guideline of 12-13 µg/g stipulated by (USFDA, 1993) was higher than the concentration of Cr determined in all samples.

Table 4: Metal-metal correlation result for sardine (*Scardinella maderensis*)

	Zn	*Cu*	*Fe*	*Cr*	*Pb*
Zn	1				
Cu	-0.925	1			
Fe	-0.779	0.852	1		
Cr	-0.462	0.307	0.683	1	
Pb	-0.305	0.513	0.064	-0.659	1

Table 5: Metal-metal correlation result for croaker (*Johnius bellangeri*)

	Zn	*Cu*	*Fe*	*Cr*	*Pb*
Zn	1				
Cu	0.731	1			
Fe	0.745	0.75	1		
Cr	0.822	0.916	0.949	1	
Pb	-0.941	-0.853	-0.632	-0.806	1

Table 6: Metal-metal correlation result for atlantic mackerel (*Scomber scrombus)*

	Zn	*Cu*	*Fe*	*Cr*
Zn	1			
Cu	-0.045	1		
Fe	0.127	0.048	1	
Cr	-0.618	0.602	0.484	1

Metal-Metal Correlation Study

A correlation coefficient indicates the strength of relationship between two variables. A Microsoft Excel 2007 was used to determine multiple correlation coefficients for metals. According to Oladoye et al. [42] there's no relationship if it's 0.0; a value of 1.0 indicates absolute dependency and if negative they're said to oppose one another. Correlation coefficients <0.50 are less significant than those >0.5. In Sardine *(Scardinella maderensis),* Cu -Zn, Fe-Zn, Cr-Zn and Pb-Cr have negative correlation coefficients indicating that they oppose each other and the fish took them up from different sources. Significantly correlated of all these metals are Fe-Cu, Cr-Fe and Pb-Cu with correlation above 0.50 (Table 4). All the metals have strong relationship (correlation) except Pb-Zn, Pb-Cu, Pb-Fe and Pb-Cr for croaker (*Johnius bellangeri*) as shown in Tables 5 and 6 indicated extreme poor correlation between the metals except for that of Cr-Cu that is significant (>0.50). It can be concluded that *Scardinella maderensis* uptook zinc from source different from other metal sources; *Johnius bellangeri* obtained Pb from sources different from the sources of Zn, Cu, Fe and Cr while Cr-Cu (>0.50) is of similar source for Atlantic mackerel's (*Scomber scrombus*) uptake.

CONCLUSION

In this research work, the highest level of all the metals in this study were detected in the muscle, head and gills of the three fish samples while tail shows the lowest value. It was also discovered that Sardine has the highest concentration of heavy metals and this is attributed to Fe, followed by atlantic mackerel and croaker. The level of heavy metals in the fish species are in the decreasing order of sardine > atlantic mackerel > croaker. Following the results of the analysis in this study, the levels of metals bioaccumulated in organs/tissue of Sardine, atlantic mackerel and croaker are still within the limits that are permissible for heavy metals by FAO, FEPA and WHO. Thus, these fishes in the area of study pose little or no threat to consumption by human.

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