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HEAVY METALS DETERMINATION IN SOME SPECIES OF FROZEN FISH SOLD AT ILE-IFE MAIN MARKET, SOUTH WEST NIGERIA

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ABSTRACT

This study determined the concentrations of six heavy metals; cadmium (Cd), mercury (Hg), Chromium (Cr), Nickel (Ni), Arsenic (As) and lead (Pb) in the tissue (flesh and gills) of some frozen fishes sold in Ile-Ife main market using Flame Atomic Absorption Spectrometer. Open acid digestive method was used for sample dissolution. The results obtained showed that, metal concentrations in the muscles of different species were in the range of Ni 10.35-15.40μg/g; Cr, 0.29-0.38 μg/g; Pb, 0.42-0.49 μg/g; Hg, 0.34-0.47 μg/g; As, 0.06-0.07 μg/g; Cd, 0.04-0.07 μg/g with Ni > Pb > Hg> Cr> Cd > As, while in the gills values were: Ni, 9.93-12.67 μg/g; Cr, 0.21-0.33 μg/g; Pb, 0.21-0.43 μg/g;Hg,0.37-0.43 μg/g; As, 0.06-0.07 μg/g and Cd 0.03-0.06 μg/g with Ni > Hg> Pb > Cr > As > Cd. Concentrations of Pb and Ni were above the SON (2007) and WHO (2003) guidelines for fresh water and (EC, 2000) lead permissible value in fish muscles. They are therefore not ideal for consumption as other heavy metals present at varying concentration could bioaccumulate and biomagnify and may result into health related problems in consumer. This study recommends proper scrutiny of the seafood to safe guard the health of the people.

Keywords: Heavy Metal, Market, Flame Atomic Absorption Spectrometer, Environment, Pollution, Anthropogenic Activity.

INTRODUCTION

Heavy metal pollution of water has become a major environmental problem almost since the advent of agricultural and industrial revolution and today most water resources are still being contaminated with heavy metals released from domestic, industrial and other man-made activities (Khare & Singh, 2002; Hayat & Javed, 2008).

The threat of toxic and trace metals in the environment is more serious than those of other pollutants due to their non bio-degradable nature, accumulative properties and long biological half lives. It is difficult to remove them completely from the environment once they enter into it (*Aderinola et al., 2009)*. Heavy metal contamination may have devastating impacts on the ecological balance of natural water bodies including the loss of aquatic diversity (Vosyliene & Jankaite, 2006; Farombi *et al*., 2007; Hayat & Javed, 2008). With increased use of a wide variety of metals in industries and in our daily life, there is now a greater awareness of toxic metal pollution of the environment. Many of these metals tend to remain in the ecosystem and eventually move from one compartment to the other within the food chain (Sadasivan & Tripathi, 2001).

Fish are often at the top of aquatic food chain and when pollutants build up in the food chain, fish are widely used to evaluate the health of aquatic ecosystems. Fish may concentrate large amounts of metals from the water and therefore are responsible for adverse effects and death in the aquatic systems (Mansour & Sidky, 2002; Farkas *et al*., 2002).

The concern about the high levels of trace metals in foods has prompted several statutory bodies such as the WHO to establish maximum allowable concentrations for some of the metals in food (WHO, 1984). Thus the World Health Organization(WHO) as well as the Food and Agriculture Organization (FAO) of the United Nations state that monitoring eight elements in fish Hg, Cd, Pb, As, Cu, Zn, Fe, Sn is obligatory while the monitoring of others though not obligatory may be useful (Staniskiene *et al*., 2006). The assessment of metal burden in fishes and water bodies will create greater environmental awareness regarding the consumption of water and fishes (Adefemi *et al*., 2008). Metals, particularly heavy metals such as lead, mercury, cadmium and arsenic constitute a significant potential threat to human health, both occupational and environmental (Hu, 2000).

Trace elements assimilated from food are transported in the blood, deposited in various tissues and excreted or stored (Kojadinovic *et al*., 2007). The levels of some elements can be related to various pathological conditions in man, hence the determination of trace elements is therefore considered as a useful and important test in surveys of environmental pollution. In the aquatic organisms, it provides information about bio-accumulation and bio-magnification processes and metal bioaccumulation is largely attributed to

different fish species (Tawari-Fufeyin and Kkaye, 2007).

Heavy metal levels and their potential toxic effects on coral *Galaxea fascicularis* from Java sea had been studied (Sabdono, 2009). Many organisms including plankton and fish, indeed, act as bio-monitors and since fish play important role in human nutrition, they need to be carefully screened. This will reveal whether or not unnecessary high levels of some toxic trace metals are being transferred to man through fish consumption. The appreciable levels of heavy metals (HMs) in the fish gives cause for concern when viewed in perspective of public health, as man depend directly on fish as an important source of protein in-take. One of the important ways to control heavy metals pollution in fishes is to monitor some fish as biomonitor of metal pollutants in aquantic environment (Shirvani and Jamili, 2009). This study aimed at determining the concentrations of six selected non-essentials metals: (cadmium (Cd), mercury (Hg), Chromium (Cr), Nickel (Ni), Arsenic (As) and lead (Pb), which are on the U.S. Environmental Protection Agency's (USEPA) list of 126 Priority Pollutants (US Environmental Protection Agency, 1994) in five species of fishes sold in Ile-Ife main market.

MATERIALS AND METHODS

Location of the Study Area

This study was carried out at Ile-Ife in Osun State, South Western Nigeria. Ile-Ife lies approximately between latitudes 07°26'N - 07°33'N of the equator and longitude 004°30'E - 004°35'E of the prime meridian on a general elevation of about 350m above the mean sea level. It is about 35km south of Osogbo, the Osun State Capital and 610 km South West of Abuja, the Nigerian capital. The market is located on latitude 07°28'55"N of the equator and longitude 004°32'56"E of the prime meridian.

Collection and Treatment of Samples

The five fish species collected for investigation were: Atlantic mackerel (*Scomber scobrus),* Atlantic horse mackerel (*Trachurus trahurus),* Atlantic salmon *(Salmo solar),* Herring *(Clupea harengus)* and freshwater sardinella *(Sardinella tawilis*). They were bought at Ile-Ife main market. These species were chosen because they are the commonest species available and sold for human consumption in the market. Atlantic mackerel is a fast swimming pelagic, schooling species distributed in the Northwest Atlantic between Labrador and North Carolina (Anderson, 1980). Maximum observed size in recent years is about 42cm in length and 1.0 kg in weight. Atlantic horse mackerel is a species of mackerel in the family Carangidae. It can be found in the North Eastern Atlantic from Iceland to Senegal, including the Cape Verde islands and also in the Mediterranean and rarely in the Black Sea (Froese and Sampang, 2004). Herring is an oily fish of the genus *Clupea*, found in the shallow, temperate waters of the North Pacific and North Atlantic oceans, including the Baltic Sea (Charles and Leith, 1999). Atlantic salmon is a species of fish in the family Salmonidae, which is found in the northern Atlantic Ocean and in rivers that flow into the North Atlantic and North Pacific (Shearer, 1992). Fresh water sardinella is a fresh water sardine found exclusively in the Philippes. It is unique in that, it is the only member of the family Clupeidae that is known to exist entirely in freshwater, they are epipelagic filter feeders, using their gill rakers to strain plankton from the water while they swim with their mouth open (Castillo *et al.*, 1975).

The fishes were prepared for analysis by cleaning them with deionised water, freeing them of mechanical additives and were allowed to thaw at room temperature. Fish heads, fins, scales and inner organs were removed. Fish flesh was separated from the spinal column and ribs, the gills were separated from the heads and each species was kept in a well tag polyethylene bag and freeze dried before digestion (Staniskiene *et al.*, 2006). The dried fish samples were crushed into a fine powder by porcelain mortal and pestle and stored in amber colored bottles in vacuum desiccators; flesh and gill were analyzed separately.

Sample Preparation and Analysis

All reagents used for this study were of analytical grade (Nitric acid and Hydrogen Peroxide from Riedel-de Haën, Germany). Polyethylene sample bottles and wash bottles together with Teflon beakers were soaked in 10% HNO_3 for 48 hours and later rinsed with distilled de-ionized water prior to use for metals analysis.

About 0.5 g of each blended fish sample (muscle and gills separately) was weighed into a Teflon beaker, 10 mL of 2:1 HNO_3/H_2O_2 was added and the Teflon beaker covered by watch glass. This was placed on thermostatically controlled hot plate maintained at 90°C, 110°C and 120°C respectively at different times for 20 minutes. The best digesting temperature was achieved at 90°C for 20 minutes by comparing the results at these three

different temperatures; as the temperature increases; there is a slight reduction in the concentrations of the metals in the flesh and the gills due to volatilization of the metals hence the use of this temperature (90ºC) for the digestion of the samples was adopted. The beaker and the watch glass were cooled and its content transferred thoroughly into a volumetric flask and made up to 50 mL mark with deionised water. A blank determination was carried out to establish the level of blank metals by digesting the reagents only. The digested samples were analyzed for heavy metal concentration using the Buck Model 205 Atomic Absorption Spectrophotometer (AAS) at the International Institute of Tropical Agriculture, Ibadan. The analytical quality control of determinations was carried out by calibrating the instrument used (AAS) with standard metal solutions prepared by diluting the stock solutions (10mg/L Multi-Element, supplies by Poly Chem, box 17254, Congella, 4013, South Africa) at 8.0, 6.0, 4.0, 2.0 μg/L prior to analysis of samples. Triplicate analysis of the samples was carried out and blanks were run with the Flame Atomic Absorption Spectrophotometry (FAAS) to ascertain the background levels of the analytes of interest in the materials and reagents used for the analysis.

The reliability of the analytical procedures adopted in this study was tested in terms of sensitivity, precision and accuracy. Table 1 shows the values for the calibration curves and wavelengths of the heavy metals respectively. The calibration curves obtained showed high linearity level with r^2 (coefficient of determination) values between 0.993 and 1.000.

Table 1. Calibration Curve and Wavelength Values for Heavy Metals

Heavy metal	Calibration Curve (r^2) Value	Wavelength (nm)
Pb	0.995	279.0
As	0.995	193.7
Cd	0.993	228.9
Cr	0.999	357.9
Ni	1.0	233.0
Hg	0.993	253.7

Statistical Analysis

The statistical analysis of data obtained was based on the Statistical Package for the Social Sciences (SPSS). The actual concentrations of the samples were subjected to descriptive statistical test and the mean values were reported.

RESULTS

The results obtained are summarized in Tables 2 for the six elements investigated, that is, lead (Pb), mercury (Hg), chromium (Cr), arsenic (As), cadmium (Cd) and nickel (Ni).

The concentrations of Pb in the different species of fish flesh ranged between 0.42 μg/g (*Clupea harengus)* and 0.49 μg/g (*Salmo salar)* while the

Table 2: Descriptive Statistics of Levels of Heavy Metals in Fish Species

		Scomber *scombrus*		Trachurus *trachurus*		Salmo *salar*		Sardinella *tawilis*		Clupea *harengus*	
Element	N	Range (µg/g)	Mean±SD (µg/g)	Range (µg/g)	Mean±SD (µg/g)	Range (µg/g)	Mean±SD (µg/g)	Range (µg/g)	Mean±SD (µg/g)	Range (µg/g)	Mean±SD (µg/g)
Hg(Flesh)	3	0.40-0.42	0.41±0.01	0.45-0.48	0.47±0.02	0.43-0.45	0.44±0.01	0.05-0.48	0.34 ±0.25	0.35-0.37	0.36±0.01
Hg(Gill)	3	0.42-0.42	0.42±0.00	0.42-0.44	0.43±0.01	0.39-0.41	0.40±0.01	0.37-0.38	0.37±0.01	0.41-0.45	0.43±0.02
Pb(Flesh)	3	0.42-0.44	0.43±0.01	0.45-0.47	0.46±0.01	0.47-0.50	0.49±0.02	0.43-0.44	0.43±0.01	0.41-0.44	0.42±0.02
Pb(Gill)	3	0.40-0.41	0.40±0.01	0.37-0.39	0.38±0.01	0.42-0.43	0.43±0.01	0.31-0.33	0.32±0.01	0.20-0.22	0.21±0.01
Cr(Flesh)	3	0.31-0.33	0.32±0.01	0.32-0.34	0.33±0.01	0.37-0.39	0.38±0.01	0.36-0.38	0.37±0.01	0.23-0.34	0.29±0.06
Cr(Gill)	3	0.32-0.34	0.33±0.01	0.31-0.32	0.31±0.01	0.33-0.34	0.33±0.01	0.31-0.33	0.32±0.01	0.21-0.22	0.21±0.01
As(Flesh)	3	0.06-0.07	0.06±0.01	0.06-0.07	0.06±0.01	0.06-0.06	0.06±0.00	0.06-0.07	0.06±0.01	0.07-0.07	0.07±0.00
As(Gill)	3	0.06- 0.06	0.06±0.00	0.06-0.06	0.06±0.00	0.06-0.06	0.06±0.00	0.07-0.07	0.07±0.00	0.07-0.07	0.07±0.00
Cd(Flesh)	3	0.07-0.08	0.07±0.01	0.06-0.06	0.06±0.00	0.04-0.04	0.04±0.00	0.05-0.05	0.05±0.00	0.04-0.05	0.04±0.01
Cd(Gill)	3	0.03-0.03	0.03±0.00	0.04-0.05	0.04±0.01	0.04-0.08	0.06±0.02	0.03-0.04	0.03±0.01	0.03-0.03	0.03±0.00
Ni(Flesh)	3	13.00-13.33	13.14±0.17	10.30-10.43	10.37±0.06	10.08-10.88	10.35±0.46	12.98-13.20	13.09±0.11	15.28-15.53	15.40±0.13
Ni(Gill)	3	9.87-10.02	9.93±0.08	10.63-10.92	10.78±0.15	12.55-12.77	12.67±0.11	10.85-11.03	10.95±0.09	10.82-11.00	10.92±0.09

range in gills was between 0.21 µg/g (*Clupea harengus*) to 0.43 µg/g (*Salmo salar and Trachurus trahurus)*. Mercury concentrations ranged between 0.34 µg/g (*Sardinella tawilis*) to 0.47 µg/g (*Trachurus trahurus)* in flesh and 0.37 µg/g (*Sardinella tawilis*) to 0.43 µg/g (*Trachurus trahurus)* in gills. Chromium ranged between 0.29 µg/g (*Clupea harengus)* and 0.38 µg/g *(Salmo salar)* in flesh while it ranged between 0.21 µg/g (*Clupea harengus)* and 0.33 µg/g *(Scomber scombrus* and *Salmo salar*) in the gills.

The other metals did not follow any clear pattern in the fish species either in the flesh or the gills. The concentrations of Arsenic ranged from 0.06 to 0.07 µg/g in all the species flesh and gills except in *Sardinella tawilis* and *Clupea harengus.* Cadmium ranged from 0.04 to *0.05* µg/g in *Clupea harengus* and *Salmo salar flesh* and in *Scomber scobrus* and others ranged between 0.05 to 0.08 µg/g in the flesh. In the gills the mean values ranged from 0.03 µg/g *in Scomber scobrus, Sardinella tawilis and Clupea harengus* to 0.06 µg/g *in Salmo salar.*

In all the samples Nickel concentration was high, it ranged from 10.35 µg/g in *Salmon salar* to 15.40 µg/g in *Clupea harengus,* in the flesh and in the gills it ranged from 9.93 µg/g in *Scomber scobrus to*12.67 µg/g in *Salmon salar* as shown in Table 2.

Nickel showed the highest concentration in the gills of *Salmon salar* and *Clupea harengu*s having highest concentration in the flesh.

DISCUSSION

In food, the allowed amounts of heavy metals (HMs) vary from country to country and are based both on the WHO recommendations and local requirements. According to Lithuanian Standards of Hygiene (LSH, 2001) the maximum tolerable limit (MTL) of Pb in fish meat is 0.4 mg/kg which is same as the value adopted by the European Commission for Pb in marine fish muscle (EC, 2000) while FAO set a limit of 0.5mg/kg (FAO, 1983). Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults (Commission of the European Communities, 2001). FAO of the United Nations and WHO (1994) have established a provisional tolerable weekly intake (PTWI) of lead as 25 mg/kg body weight for humans. In the present study, the means of the lowest and highest levels of lead in fish flesh samples were from 0.42µg/g to 0.49 µg/g.

Lead is a neurotoxin that causes behavioural deficits in vertebrates, decreases in survival, growth rates, learning and metabolism (Weber *et al.,* 1997; Burger and Gochfeld, 2000). Levels of 50 ppm of lead in the diet can cause reproductive effects in some predators and dietary levels as low as 0.10.5 ppm are associated with learning deficits in some vertebrates (Eisler, 1988). In our study, the levels of lead were above the tolerable limits. Among the species of the fishes, *Sardinella tawilis* was found to accumulate less Pb than others while *Salmon salar* and *Trachurus trahurus* had more than other species. Continuous eating of fish of this nature may be dangerous to human health because of bioaccumulation tendency of lead in the body.

Mercury levels in the samples were below the permissible level of 1 ppm as set by Food and Drug Administration (FAO, 1976) for seafood. The most widely established guideline value for

Hg levels in marine predatory fish is 1.0 mg.g-1 w/w (IPCS, 1987; EPA, 1994), which suggests that the fish brought to the market, was relatively less contaminated with mercury. The above result was similar to the result of (Zodape *et al.,* 2011. Mercury is known to be a latent neurotoxin compared to other metals like lead, cadmium, copper and arsenic (Zodape *et al.,* 2011). A high dietary intake of mercury (organic), that is, concentration above 1.0mg/g from consumption of fish has been hypothesized to increase the risk of coronary heart disease (Salonen *et al.,* 1995). When deposited in biota, mercury undergoes biotransformation, in which inorganic mercury may be converted to organic mercury (methyl mercury). Microbes subsequently concentrate mercury through the food chain in the tissue of fish and marine animals (Altindag and Yigit, 2005). Consequently, a large percentage of Hg is present as toxic MeHg in the edible portions of fish consumed by man (Cappon and Smith, 1981; Bloom, 1992; Wagemann *et al.,* 1997).

The speciation of chromium is of great importance for its toxicity. Cr(III), the most stable form of Cr in biological materials, is an essential element for normal glucose metabolism, while Cr(VI) is highly toxic (Costa, 1997). Cr(III) has low toxicity due to low absorption (about 0.5%). The toxic aspects of chromium are related to Cr(VI) (Nordic Council of Ministers, 1995), due to its high absorption, easy penetration of the cell membranes and its genotoxicity and oxidising properties.

WHO (1993) has set a maximum limit of 0.05 mg/l of Cr(VI) in drinking water. Estimates of the daily intake range from 0.025 to 0.2 mg/day (Codex, 1995). Since no standard/ guidelines was set for Cr(III), the stable oxidation state in biological samples, thus Cr found in this study may not be above the recommended daily intake. It was found in this study that Cr is more deposited in *Salmo salar* (gills and flesh) and less accumulated in Clupea *harengus* (gills and flesh). Arsenic has become a highly politicized issue globally particularly in some affected countries due to its carcinogenic characteristics and being a possible natural contaminant of groundwater sources (ARSLAND, 2006). In addition, uptake of arsenic via ingestion is associated with dermal carcinomas and hepatic angiosarcomas in humans (Rossman and Waalkes, 2003).

The statutory limit for arsenic in food is 1 mg/kg for solid food, 0.1 mg/kg for nonalcoholic beverages and 0.2 mg/kg for alcoholic beverages. In flavourings, the limit is 3 mg/kg (EA, 2002). All the fishes analyzed in this study may not be polluted with As since the concentration found were below the statutory limit stated by environmental agency in United Kingdom and of all the fish samples the *Clupea harengus* has the tendency to bioaccumulate As (gills and flesh). This may be due to factors such as size, age and sex of the fish which are not within the scope of the present study.

The accumulation of Cd is proportionally less oriented towards the muscle than Hg, Cadmium is however highly toxic (Jarup, 2003) Cd is associated with nephrotoxic effects particularly at high exposure levels; long-term exposure may cause bone damage. (Friberg *et al.*, 1986).

The threshold concentration of cadmium in fish muscle destined for human consumption set by the European Commission is 0.1 mg/g w.w., thus 10 times lower than that set for Hg. In the present study the concentrations of cadmium in some of the marketed fishes were found to be lower (both in the gills and the flesh) than the consumption safety tolerance in fishes set by countries worldwide. The guideline limit set for Cd by FAO, (1983) is 0.05mg/kg for fish, the concentration range from this study for all the fish species are 0.04-0.07 μg/g in flesh and 0.03-0.08 μg/g in gills. The mean concentration of Cd in the flesh of *Scomber scombrus* is 0.07μg/g and in the gills of *Salmo salar* is 0.06μg/g. These values were above FAO limit of 0.05mg/kg. The Cd concentration in flesh and gills of *Sardinella tawilis* and *Clupea harengus* were within the FAO limit. This is similar to the result of Zodape *et al.* (2011) in their study on marketed seafood of VileParle and Dadar suburban of India in which Cd was within the acceptable limit.

Nickel was found to be outrageously high in all the fish samples (both gills and flesh). The reason(s) for this could be due to marine pollution with crude oil (Osuji and Onojake, 2004). *Salmon salar* had the highest concentration of Ni in the gills with mean value of 12.67 μg/g. Kamaruzzaman *et al.*(2011), reported higher accumulation of heavy metals in commercially important fishes. They concluded that the observation might be due to respiratory mechanism of fishes. The maximum acceptable level of Ni in water as stated by FEPA (1999) is < 1.0 μg/g and WHO, (1984) value of < 0.05 μg/g. The migration of nickel to foodstuffs should be as low as reasonably achievable and not more than: 0.1 mg/kg as a general limit of migration into foodstuffs (Council of Europe, 2001).

Staniskiene *et al.*, (2006) found Ni as high as 10.66

mg/kg in one of the samples in their study. The order of accumulating tendency of Ni in this study is *Clupea harengus> Scombe*r *scombrus> Sardinella tawilis>Salmon salar.*

Non-essential metals do not present any function for the fish's metabolism and are consequently not regulated by the metabolism. The amount of Cd, Hg and Pb in fish organisms can thus serve as an indication of environmental levels of these metals (Kojadinovic *et al*, 2007).

CONCLUSION

This study has shown that, Pb and Ni were high in some frozen fish samples collected from Ile-Ife market, while the levels of As, Hg, Cr and Cd in the fish samples were within permissible limits. These heavy metals may be transferred to man on consumption and may be hazardous to health because of their cumulative effect in the body.

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