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A COMPARATIVE STUDY OF TOXIC AND ESSENTIAL TRACE ELEMENTS IN CAT FISH SPECIES FROM LAKES AND FISH POND LOCATED IN SOUTH EAST, NIGERIA.

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ABSTRACT

The concentration of some toxic elements (mercury, lead, cadmium and arsenic) and some essential trace elements (cobalt, zinc, manganese, selenium, chromium and copper) in fish obtained from Oguta Lake, Agulu lake and fish pond in South East Nigeria were determined using a rapid, highly sensitive method. A stepwise digestion procedure using concentrated nitric acid and hydrogen peroxide was used for complete oxidation of organic tissues. The concentrations of the various elements were measured using Varian AA240 Atomic Absorption Spectrophotometer. A total of 46 fish samples covering three Species of cat fish namely: *Clarias lazera, Clarias anguillaris and Clarias gariepinus* were analyzed for their elemental contents. Results of the study indicate low level of exposure to mercury, cadmium, lead, chromium, zinc, copper, manganese, cobalt selenium and high level of arsenic and mercury in fish species found in Oguta Lake. Higher values of these toxic metals detected may pose a significant health risk to the individuals and to a greater extent the general population buying fish from this source.

Keywords: Toxic Elements; Essential Elements; Clarias species; Oguta Lake, Agulu Lake; Fish Pond.

INTRODUCTION

Trace elements have positive and negative effects on human health and the environment. Many researchers are interested in the analysis of the trace metal contents of the environmental samples and especially foods (Agusa and Sudaryanto, 2007). Accurate and adequate food composition data are invaluable for estimating the adequacy of intakes of essential nutrients and assessing exposure risks from intake of toxic non-essential heavy metals (Canli and Atli, 2003). Toxic elements can be very harmful even at low concentration when ingested over a long time period. The essential metals can also produce toxic effects when the metal intake is excessively elevated (Mendil *et al.,* 2010).

The pollution of the aquatic environment with heavy metals has become a world-wide problem in recent times because they are indestructible and most of them have toxic effects on organisms (Macfarlane Burchett, 2000). Among environment pollutants, metals are of particular concern, due to their potential toxic effect and ability to bio-accumulate in aquatic ecosystems (Censi *et al.,* 2006).

Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are persistent and all have the potential to be toxic to living organisms (Storelli *et al.,* 2005). Studies on heavy metals in rivers lakes, fish and sediments (Ozmen *et al.,* 2004; Begum *et al.,* 2005; Fernandeset *et al.,* 2008) have been a major environmental focus especially during the last decade. Bioaccummulation and magnification is capable of leading to toxic level of these metals in fish, even when the exposure is low. Fishes are notorious for their ability to concentrate heavy metals in their muscles and since they play an important role in human nutrition, they need to be carefully screened to ensure that unnecessary high level of some toxic metals are not being transferred to man through fish consumption (Adeniyi and Yusuf, 2007). Consumption of aquatic food enriched with metal may cause serious health hazards through the food chain magnification (Miretzky *et al.,* 2004).

Recent studies have suggested that nutritional deficiencies in some essential elements may increase the toxicity of lead, and some essential elements may influence the food concentrations of lead and other toxic metals. Hg, Pb and As are known for their toxicity, whereas Mn, Cu, Zn, Se, Cr in the +3 state and Co are essential elements (Bazzi *et al.,* 2008). Mercury (Hg) is noted for its neurotoxicity and at high doses; it can cause serious damage to the central nervous system, brain and /or kidney. Low doses may have some developmental effects on fetuses and infants exposed via the mother's diet (Holmes *et al.,* 2009). Lead (Pb) is noted for intellectual impairments. Zinc (Zn), deficiency is common in developing countries particularly where high phytate diet is consumed. An antioxidant – component of Cu-ZnSOD; healing of wounds and sores, Zinc deficiency weakens the immune functions of the cells, especially those of the helper-T-lymphocytes and killer cells. Selenium (Se) now recognized as micro mineral of great importance is an essential nutrient, modulating a variety of cellular functions, an Inhibitor of tumorigenesis. Selenium maintains biological activity of ascorbate and α–tocopherol (Reduced state). Elemental Se and organo-selenium at comparatively low concentrations inhibit mutagenesis, chromosome breakage (anticlastogen), and cell proliferation from chemical carcinogens; providing anticarcinogenic activity.

Copper (Cu) is an antioxidant (Cu-ZnSOD); biomarker – ceruloplasmin; an indicator to diagnosis and prognosis of cancer. Cu plays very important role in erythropoiesis. Only 80mg of Cu exists in adult human; an immensely important cell-protective mineral. Transport protein is caeruloplasmin.

Chromium (Cr), currently considered a component of glucose tolerance factor, an organic moiety necessary for insulin action on the cell surface. Deficiency causes insulin resistance or DM with associated hyperlipidaemia in otherwise well nourished patients. Manganese (Mn) Mitochondrial SOD – potent antioxidant; UCP -2-GSIS. Prothrombin synthesis Important in pregnancy and Involved in nitric oxide (NO) metabolism.

Cobalt (Co) functions as a co-factor in enzymes catalyzed reaction and are involved in the production of erythroprotein a hormone that stimulates the formation of erythrocytes. However, only scanty data are available on Co and Cr levels in fish worldwide. These findings, coupled with the scarcity of available data on some essential and toxic elements in fish from Oguta, Agulu Lake and fish pond in South East Nigeria; in addition to increase in industrialization, call for the need to monitor the concentration of some toxic and heavy metals as well as essential elements in cat fish (*Clarias spp)*, one of the heavily consumed fish from the lake and fish pond. Oguta Lake is one of the Nigeria's largest natural oligotrophic lakes which serve as a source of water and freshwater fish especially catfish for the people of Nigeria. It is expected that the results of this study will help in generating data for the assessment of metal intake from fish for the general populace.

MATERIALS AND METHOD

Three species of cat fish (12 specimens) each were collected from each of the lakes and an artificial fish pond around the study area. The fishes were harvested from different points of the lakes with fish net and hooks. Samples obtained were therefore reflective of species meant for consumption. A total of 36 fish samples covering three different species of cat fish namely: *Clarias lazera, Clarias anguillaris and Clarias grepineus* were obtained. Samples were sorted by species, placed in clean plastic bags and stored on ice in ice chest. They were then transported to the laboratory at zoology department of Nnamdi

Azikiwe University, Awka and identified. The total length and total weight of each fish were taken. They were then stored on dry-ice at a temperature of 20°C and transported to Research Laboratory for chemical analysis. In the digestion procedure as described by Kwaansa-Ansah *et al.,* (2012), 10.0mg sample of the fish tissues were taken in pre-weighed Teflon tubes placed in heating blocks of a dry-bath incubator set at 25°C. 2.0ml concentrated nitric acid was added to each Teflon tube. The dry-bath incubator was heated stepwise to 60°C, 70°C, 85°C and 95°C. The samples were maintained at 95°C until the samples turned clear. The dry-bath incubator was cooled to 25°C and 2.0ml of hydrogen peroxide added to each tube. The temperature was raised to 60°C and the tubes heated for 10 minutes. The temperature was increased to 70°C, and heating continued for 20 minutes. Finally, the temperature was increased to 95°C and the samples were heated for 30 minutes. The stepwise heating was to ensure that the hydrogen peroxide decomposes, reduce excessive effervescence and subsequent loss of sample. The solutions were cooled to 25°C and diluted to 25ml with water. The concentrations of various elements were determined using Varian AA240 Atomic Absorption Spectrophotometer.

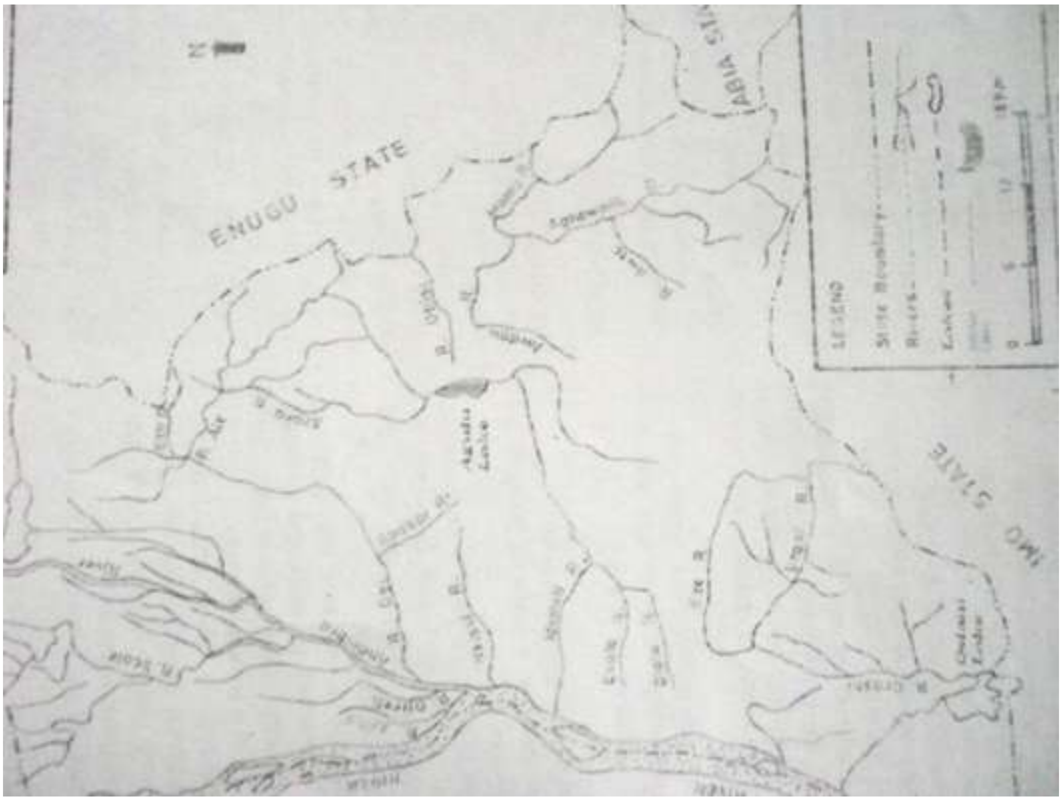


Fig. 1: Map of Anambra State Showing Agulu Lake and Its Major Tributes

Fig. 2: Map of Nigeria Showing the Study Area (Oguta Lake in Imo State)

STATISTICAL ANALYSIS

The results were analyzed using statistical package for the social sciences (SPSS) for windows version 17.0 software. All data were expressed as mean ±SD. Statistical analysis was performed as ANOVA Significant level of $P < 0.05$.

RESULTS AND DISCUSSION

Concentration of arsenic, cadmiun, lead, mercury, chromium, cobalt, copper, manganese, selenium and zinc in the three species of fishes were determined. The validity of the methodology and the determination of its accuracy and precision were obtained from analysis of 10mg sample of a certified reference material NRC TORT-2 Lobster Hepatopancreas reference material for Trace Metals that were brought into solution following the analytical procedure and found to compare favourably with those reported in literature.

Table 1: The Average Weight and Length of *Clarias gariepienus*

Fish	Average Weight (g)	Length (cm)
Oguta	350 – 400	25 -30
Agulu	300 -350	20– 25
Fish pond	360– 400	24 -28

Table 2: The Mean Concentrations of *Clarias species* in Oguta, Agulu Lakes and Fish Pond

Fish species	Concentration of Trace Element on µg/g									
	Toxic Elements				Essential Trace Elements					
	As	Cd	Pb	Hg	Co	Zn	Mn	Se	Cr	Cu
Oguta (n=12)										
Mean	1.76	0.26	0.17	0.88	0.17	1.75	0.50	1.14	0.30	0.40
SD	1.32	0.20	0.14	0.76	0.01	1.02	0.19	1.03	0.25	0.29
Mean± SD	1.76±1.32	0.26±0.20	0.17±0.14	0.88±0.76	0.17±0.01	1.75±1.02	0.50±0.19	1.14±1.10	0.30±0.25	0.40±0.29
Agulu (n=12)	Ψ	Ψ	Ψ			Ψ	Ψ	Ψ		
Mean	0.73	0.00	0.00	0.01	0.03	6.14	1.06	0.03	0.02	1.09
SD	0.05	0.002	0.00	0.01	0.02	1.86	0.61	0.02	0.01	0.52
Mean ± SD	0.073±0.05	0.00±0.00	0.00±0.00	0.01±0.01	0.03±0.02	6.14±1.86	1.06±0.61	0.03±0.02	0.02±0.01	1.09±0.52
Fish pond (n=12)	Ψ	Ψ	Ψ			Ψ	×	Ψ		
Mean	0.02	0.00	0.00	0.00	0.43	4.99	0.05	0.04	0.07	1.39
SD	0.01	0.00	0.00	0.00	0.38	1.48	0.02	0.03	0.04	1.09
Mean ± SD	0.02±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.43±0.38	4.99±1.48	0.05±0.02	0.04±0.03	0.07±0.04	1.39±1.09
p value	0.009	0.001	0.001	0.036	0.071	0.001	0.000	0.000	0.079	0.054

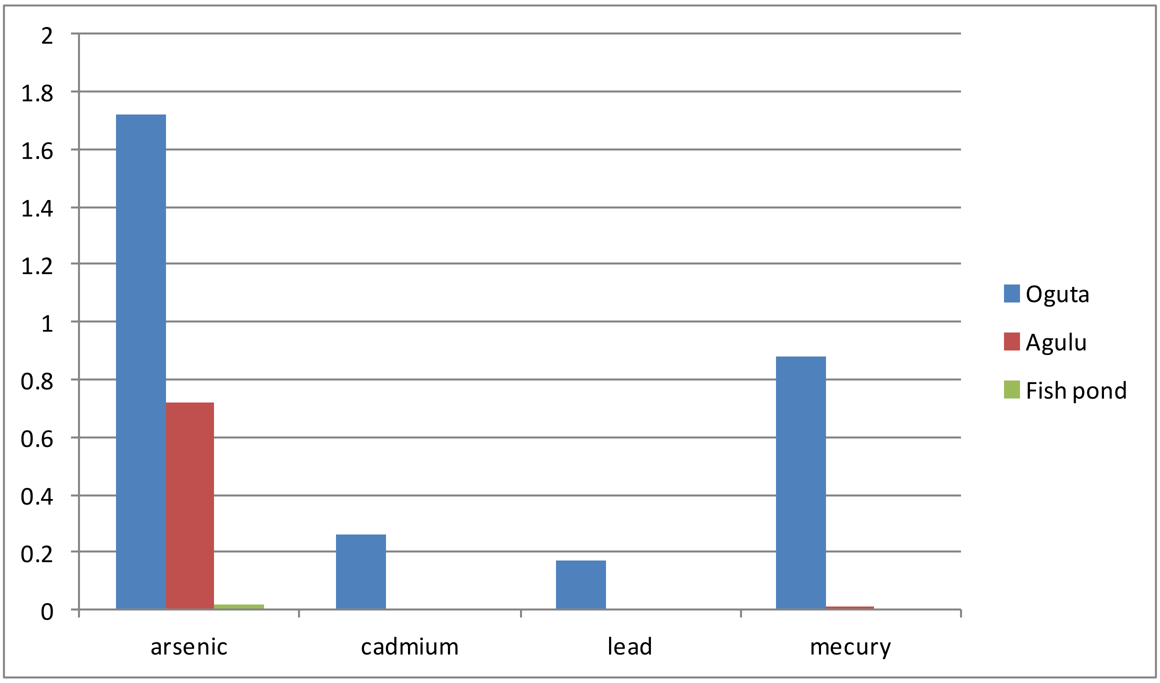
KEY:

Significant at $p<0.05$

Ψ= Significance when compared with *Clarias gariepienus* in Oguta Lake

× = Significance when compared with *Clarias gariepienus* in Fish pond

Mean concentration of toxic metals



Trace Elements

Figure 1: Showing As, Cd, Pb, and Hg Conc. in Cat Fish

Mean Concentration of Essential Elements

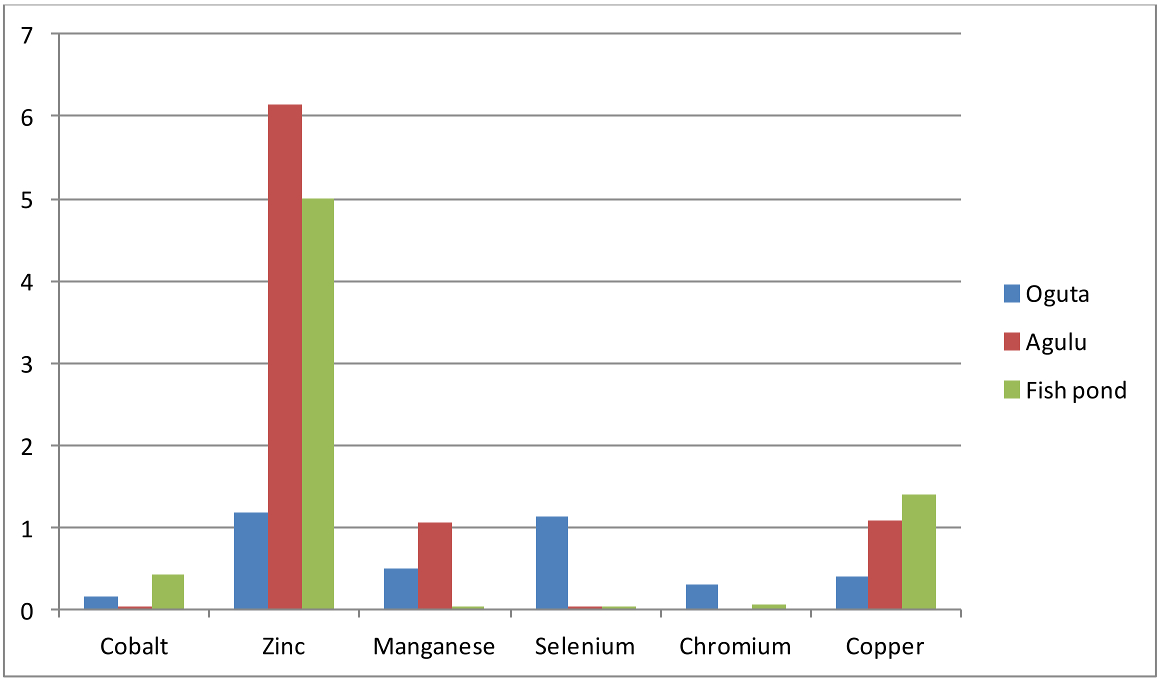


Figure: 2 Showing Co, Zn, Mn, Se, Cr and Cu Concentration Essential Trace Elements in Cat Fish

The results indicate that the metal content in the samples studied depends on the fish subspecies and the concentrations of these metals showed some variations. The variations are generally related to the geographical location. All the three species of fish belong to the same Cichlidae family (all being herbivores). It is pertinent to determine and monitor heavy metal levels in foodstuffs, particularly seafood especially in this era of switch over from animal meat to sea foods, because heavy metal ions can easily accumulate in such food compared to other foodstuffs and cause harmful effects on human health. Discharge of industrial waste water without a pretreatment into lakes, rivers, stream, and sea primarily causes an increase in heavy metal concentration in such water environments.

The mean concentrations of Arsenic in *μg/g* wet weight of the three *Clarias species* of fishes studied were significant ($p<0.05$).Higher values were obtained from fish species in Oguta Lake. Lowest values were obtained in fish species from fish pond. Higher values obtained in Oguta Lake may be attributed to the high crude oil content of the area and oil mining activities of the area.

For most people diet including fish is the largest source of exposure to arsenic. Mean dietary intakes of total arsenic of 50.6ug/g/day and 58.5ug/g/day have been reported for females and males (MacIntosh *et al.,* 1997). In humans inorganic arsenic is methylated to methylarsonic acid and dimethylarsenic acid and these are rapidly excreted. The organic arsenic compounds present in seafood are excreted unchanged.

The mean concentrations of Cadmium in fish species from Oguta ranges from 0.050-0.363 *μg/g*, 0.00- 0.005 *μg/g* in fish species from Agulu lake, and 0.00-0.002 in fish species from Fish pond. Cadmium level in all the sampled fish species from Oguta and Agulu Lakes exceeded the maximum permitted value of Nigerian Industrial Standard, 0.003mg/l. Several studies have reported high levels of Cd in different fish samples from Nigerian rivers. Ishaq *et al.,* (2011) reported 0.927ppm in *Clarias gariepinus* in River Benue. Okoye *et al.,* (1991) reported concentration of 2ppm for Cd in Lagos lagoon sediments. The present study indicated much lower level of exposure to cadmium. Cadmium is selectively accumulated in the kidney and liver, particularly in the renal cortex where it causes liver and kidney damage.

Lead content in the examined cat fish meat were below detection limit in Agulu Lake and fish pond. This may be attributed to the geographical location of the lake which is relatively free from the industrial activities. Fish pond however is free from contaminants and so are free from these toxic metals. Turkmen and Ciminli (2007) found 0.014ug/g in cat fish from lake Golbasi. Some Authors reported values over 0.100 ug/g (Has-Schon *et al.,* 2006). Much higher concentration was reported by Kwaansa-Ansah *et al.,* (2012) in Tilapia species from Volta Lake, Ghana. Pb is noted for intellectual impairments, and can influence neuro behavioural performance in children.

Mercury levels in muscles of fish, depending on the sources varied significantly. The highest mean Hg concentration of 0.880 ug/g was recorded in Oguta Lake. Much lower mercury levels were recorded in West Pomeranian fish on average 0.015-0.030 µg/g reported by Szefer *et al.,* (2003) and by Perkowska and Protasowicki in pike muscle from Swidwie Lake in 1999. There is high level of significance at $p>0.05$ when compared to fish pond and Agulu Lake. The presence of these toxic elements in fish species poses a serious danger to human health. Fish species from fish ponds is free from these toxic elements because their habitats are relatively free from these contaminants. From this study, fish species from Oguta Lake has highest level of toxic elements especially mercury and arsenic, this may be due to the crude oil content of the area and oil producing industries located in this environment. Waste products and effluents from these industries may be discharged into the lake.

Cobalt is an integral component of vitamin B12 and is an essential nutrient for non-ruminant animals and humans. Vitamin B12 is a cofactor for two enzymes, methionine syntheses which methylates homocysteine to form methionine, and methylmalonyl coenzyme A (Co A) mutase which converts L-methylmalonyl Co A, formed by the oxidation of odd-chain fatty acids, to succinyl CoA.The mean concentration of Cobalt ranges from 0.03-0.43 µg/g in *Clarias gariepienus* species from the lakes and fish pond. Cobalt intake in United States has been estimated to be 5-40 µg/day (Jerkins, 1980), with relatively high concentration of Cobalt occurring in fish and vegetables (Barceloux, 1999). In France, the estimated average daily intake is 29 µg/day (Bargio *et al.,* 1988).The mean concentrations of cobalt in *Clarias species* from the three sources fall within recommended limit.

Zinc content in the muscles of the examined fish species *(Clarias gariepienus)* ranged between 1.75- 6.14 µg/g with cat fish from Agulu having the highest concentration. For comparison, slightly higher Zn levels were reported for fish from the Dnieper River (Sapozhnikova, 2005). Much higher concentrations of this metal were observed by other authors (Kuznetsova *et al.,* 2002; Liang et al., 1998; Frakas *et al.,* 2002). The levels were far higher than that obtained in this study. However, Turkmen and Ciminli (2007) reported lower Zn concentration (0.456 mg/kg w.w.) in fish from lake Golbasi. On average, 20 to 40 percent of dietary Zn is absorbed in healthy people (Rao, 1980). According to Kunachowicz *et al.,* (2005), a 100-g edible portion of fish contains from 0.30 to 1.75 mg of Zn.Zinc functions as a component of transcription factors known as zinc fingers that bind to DNA and activate the transcription of a message, and imparts stability to cell membranes.

Manganese (Mn) is present in all tissues and a vital trace metal for functioning of many organic systems. Mn plays a major role in regulation of blood sugar and reproduction, digestion, and bone growth. Mn also acts as a cellular antioxidant. In our study, Manganese content in fish meat ranged from 0.05 to 1.06ug/g. Luczynska *et al.,* (2006) found Mn concentrations in the range of 0.071-0.117 µg/g in *Clarias gariepinus* fishes from north-east Poland. About 5% lower Mn levels were reported by Alibabic and Waheic (2007).Our results were in the same range with the study done elsewhere. However, fish meat is assumed to contain 0.01 – 0.06 mg Mn per 100 g edible portion (Kunachowicz *et al.,* 2005). The variation in the Mn concentrations was not that marked, though there was high level of significance among the fish species from the three sources.

Selenium is a component of enzymes that catalyze redox reaction; these enzymes include various form of glutathione peroxidase, iodothyronine 9<-deiodinase and thioredoxinreductase. In this study, Selenium in the muscle tissue of the fish species ranged from 0.03 – 1.14 ug /g in *Clarias gariepinus* with Oguta lake having the highest concentration; there is high level of significance among each species from the three sources at $p>0.05$. Kwaansa-Ansah *et al.,* (2012) recorded about 10% lower values in some fish species from Volta Lake, Ghana. The differences in Se concentrations found between the different fish species may be a reflection in the intake manner of this trace element. Uptake of selenium by biota can originate from water or from diet. However, dietary exposure of fish to selenium is usually the dominant pathway of uptake, because they are typically at higher trophic levels in the aquatic food webs (Hamilton, 2004). Selenium has the propensity to bio accumulate within the base of food webs; from water and sediment to aquatic plants and invertebrates, and finally, to fish (Hamilton, 2004).

Chromium (Cr) is considered as a basic trace element and found in human body, and it is known to control blood sugar and cholesterol level. Exposure of humans to Cr element is by inhalation, consumption of food containing Cr, and contact with Cr compounds. Mean concentration of chromium in the examined fishes ranged from 0. O2– 0.074ug /g. Sapozhnikowa *at al.,* (2005) and Rashed (2001) reported similar chromium levels in fish. Ikem *et al.,* (2003) observed 0.01 μg Cr per kg w.w. in fish muscle from Tuskegee Lake, USA. Dietary Cr usually satisfies human demand for the element, which varies from 50 to 200 ug per day in adult people. In a study done with tilapia species by Kwaansa-Ansah *et al.,* (2012) in Volta Lake Ghana, they reported the highest mean Cr level of 1.1 ug /g.

Mean concentration of copper in the examined fish ranged from 0.40 -1.39 ug /g. According to the tables of food composition and nutritional value (Kunachowiez *et al.,* 2005), a 100.g edible portion of fish contains from 0.02 to 0.23 mg of Cu. Copper levels in the fish examined in Volta Lake, Ghana, varied from 8.15 to 13.42 *μg/g* edible portion. The recommended daily allowance of Cu for both women and men varies within 1.5 -2.5 mg per person (Ziemlanski, 2001). The lowest mean level of Cu was recorded by fish species from Oguta Lake and by fishes from fish pond. However, there was no significance among the fishes from the different sources.

CONCLUSION

The results of this study indicate relatively low levels of exposure to some toxic and essential elements, lead, cobalt, zinc, manganese, selenium, chromium and zinc through consumption of the three species of fishes analyzed in Agulu and Fish pond. The concentration level of arsenic, cadmium and mercury exceeded the maximum permissible limit of Nigerian Industrial Standard. The levels of these toxic elements pose a significant risk to the individuals and to a greater extent the general population that consumes these fish species for now and the situation may change over time due the ability of the elements to bioaccummulate in the tissues of the fish. However, fishes from Oguta Lake showed some high level of significance of toxic elements when compared to other sources .This may be attributed to the oil mining activities in the area, so the aquatic lives may be contaminated by waste effluents from these industries. The fishes from fish pond has insignificant level of these toxic elements even though the essential trace elements are lower when generally compared with that obtained from the lakes. The fish dietary intake studies in Nigeria are needed particularly among the artisanal oil mining area in order to be able to relate the actual fish consumption patterns among the populations and toxic metals particularly mercury and arsenic. It is recommended that safe disposal of domestic sewage and industrial effluents should be practiced by operators for the protection of the water bodies. Finally, future studies should determine levels of these elements in human especially women and children (using biomarkers such as blood, nail and hair) who are known to be sensitive groups in terms of chemical pollution.

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