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Parameterization of Non – Essential Heavy Metals Concentration in Different Tissues of Inland Commercial Fish *Oreochromis niloticus* from Vea Dam, Bolgatanga, Northern Ghana

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A B S T R A C T

The consumption of inland fish dietetically either for their nutritional or health value in Northern Ghana has been an important tradition since antiquity. This unparalleled natural fatty – free, cosmopolitan and highly – valued animal protein in recent times has been threatened by various factors including the excessive use of hazardous xenobiotic chemicals in fishing. Therefore, the concentration levels of six perilous heavy metals (As, Cd, Cr, Hg, Pb and Sb) were determined in different tissues of *Oreochromis niloticus* found in the water body of Vea Dam, Northern Ghana, at various strata and seasons by NAA and AAS. The results of the heavy metal contents of fish tissues were categorized from the highest concentration metals to the lowest; Cr > Cd > Pb > As > Hg > Sb. Variations of these metals were also observed between the different seasons, strata and tissues. Of all the tissues studied, the maximum concentrations of heavy metals were found in the liver; with the muscles tissues recording the lowest. Thus, notwithstanding the contamination of Vea Dam by heavy metals from diverse sources, the level of the metals in the fish muscles (i.e the edible part) did not exceed the recommended permissible limit. Hence, the fish was considered safe for human consumption.

Introduction

Northern Ghana, which is generally characterized by a sub – Sahelian climate, has many man – made Agricultural dams occasioned principally by a very short raining season.

Despite the recent production of crude petroleum and its related products in commercial quantities (Appenteng *et al*., 2012), Agricultural activities are still the main driving forces behind Ghana's

economy. Hence these Dams play vital roles through employment generation, poverty alleviation, food security and enhancing the standard of living by increasing income levels of the catchment communities during the dry season (Tampoare *et al*., 2012).

One of such Agricultural dams is the Vea Dam that is located in the dry guinea savannah area of Bolgatanga municipality of Northern Ghana. A detailed discussion about geographical location, physical dimensions and climatic conditions at this dam is presented in published literature (Kplig – ba, 2011). Aside the primary aim of providing freshwater for irrigation purposes, the Vea Dam most importantly provides water to Ghana Water Company for treatment into good drinking water that supply the riparian districts in the municipality. Also in recent times, commercial fishing activities in this dam has been on the shrill increase partly caused by the rapid increase in population coupled with the demand for the highly – valued fatty – free animal protein and lipids for nutritional needs. Thus, the stupendous contribution of the Vea Dam for the sustainable development of the Ghanaian economy is very vital.

However, the continuous extensive and often indiscriminate misuse of synthetic hazardous chemicals such as fertilizers, pesticides and weedicides with their associated undesirable heavy metals in farming around the dam ineluctably have devastating effects on the ecological balance of the environment and the diversity of aquatic organisms in the dam especially, the consumable commercial fish, *Oreochromis niloticus*. Besides, the quantum regular damping of pollutants into the dam by storms (especially the harmattan winds), huge inputs of terrigenous discharges and major anthropogenic activities such as domestic uses, the extraction of gold and damping of its tailings in the dam, all result in a significant release of heavy metals into the water body. Furthermore, although enormous works has been carried out on heavy metal levels in various media, coastal belts and marine animal species (Zhang *et al*., 2007; Jezierka and Witeska, 2006; Nwani *et al*., 2010; Eneji *et al*., 2011), there is a paucity of information in the heavy metal concentrations in the food web along the inland water body of Vea Dam. Thus, the effect of various heavy metals entering the microbial food web is still not well understood (Kumar and Achyuthan, 2007).

Most depressingly, the deliberate use of xenobiotic chemical contaminants as an ease, reliable and economic way of fishing may cumulatively result in high levels of heavy metal concentrations which can pose serious health – related problems due to their toxicological effects. Unfortunately, these metals are known to be persistent and non- biodegradable but can easily be bio – accumulated by most edible aquatic organisms (Sen *et al*., 2011; Stanciu *et al*., 2005; Aderinola *et al*., 2009). Hence, they are classified among the most dangerous groups of anthropogenic environmental pollutants (Nyarko *et al*., 2006).

Based on these developments at this important dam, the present study was undertaken to both qualitatively and quantitatively ascertain the heavy metals concentration levels in the predominant consumed fish, *O. niloticus*, from Vea Dam in Bolgatanga, Northern Ghana. As a result, a detailed study on the assessment of heavy metals in this freshwater animal is inevitable as the evaluation of its concentrations along the food chain may throw light on the heavy metal input to the human body from fish food. It is hoped that the parameterization of heavy metals concentration in different tissues of *O. niloticus*, from Vea Dam will

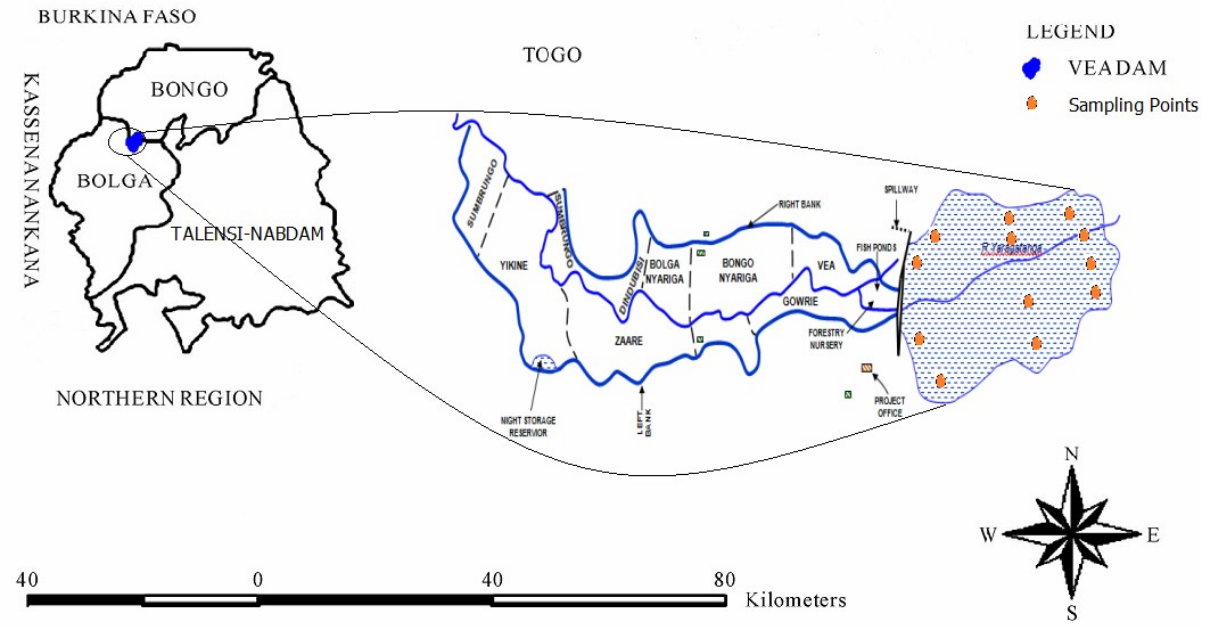
provide baseline data that will help define the permissible levels of chemical contaminants for the inland fishery industry and the country's Food Codex Standard.

Materials and Methods

Sampling

A map of Vea Dam showing the sampling sites is presented in Fig. 1 below. Three series of independent sampling coded named: First Sampling Series I (FSSI), Second Sampling Series II (SSSII) and Third Sampling Series III (TSSIII), were characteristically chosen to purposively represent the major climatic conditions at the Vea Dam. The First Series of sampling was done in December, 2012. This period was generally characterized by very dry and dusty Harmattan winds. The Second Series was in March, 2013 and the Third Series was in August, 2013; which clearly represented the dry and wet climatic seasons around the Dam respectively. During the dry season, temperatures were usually very high (over 370C during the day) coupled with low humidity and intermittent rainfall. The converse is true for the wet season. The mean rainfall during this period ranges from 850 to 1000 mm which occurs in the months of May-October (Pelig-Ba, 2011). The O. niloticus samples were caught by trap anchored using locally made hoop-fyke traps and cast nets at three different locations (i.e. upper strata, middle strata and lower strata) in the water body of Vea Dam from 2012 to 2013. These strata were chosen so as to represent the different degrees of pollution around the Dam. The physicochemical parameters of the water body have recently been done (Pelig-Ba, 2011). The lengths and weights of each samples were measured (Table 1) and recorded before been frozen in polyethylene bags for transportation to the laboratory for chemical analysis.

Figure.1 Location of Vea Dam in the Bolgatanga Municipality of Ghana



Sample Preparation

All samples were thawed and then thoroughly washed with distilled water followed by double distilled deionized water at the Centre. A clean high quality rush – free and corrosion resistant stainless steel knife was used to cut about 2 g of muscle tissue from each sample along the lateral line. Also, the operculum of each fish from each sampling series was carefully opened and the gills extracted. Again, the livers of each fish sample were obtained after appropriate dissections. The parts of liver, muscle and gills of all the *O. niloticus* samples which were carefully categorize per tissues per sampling series per strata were pooled for further preparation and analysis. The samples were then separately pulverized using a laboratory mortar and pestle manually, before been lyophilized at -30 °C and 0.370 mbar to a constant weight using a Christ Gamma 1 - 16 in order to maintain the initial texture and prevent degradation of the sample. The pulverized samples were finally milled in a Retsch RS 100 model vibratory disc mill and stored at -20 °C in closed polyethylene bottles with screw caps until analysis.

Chemical Analysis

Quadruplicates (about 200 mg) of each categorized pulverized sample and a standard reference material (NIST 1566b, Oyster Tissue) were simultaneously accurately weighed into acid – clean polyethylene foils, carefully wrapped with forceps, capped and then heat – sealed. Irradiations were carried out in Ghana's Research Reactor – 1 (GHARR -1), Ghana Atomic Energy Commission, Accra – Kwabenya, Ghana, at a thermal power of 15 kW. It was observed that, As and Sb could be determined in a single irradiation of 30 minutes and Cr at half a day. The distinct energy – signatures of the activated radionuclides of interest were ascertained by a standard procedure described by Nyarko *et al*. (2003). The truthfulness and repeatability of the neutron analytical technique at GHARR – 1, have been well demonstrated by Appenteng *et al*. (2012) and Adazabra *et al*. (2013) for a wide range of different compositional reference materials. Elemental concentration levels were evaluated using the relative comparator method with NIST 1566b, Oyster Tissue as an appropriate compositional comparator standard.

To augment the neuron analytical technique, the analysis of elements such as Hg and Pb as well as Cd was done by the Atomic Absorption Spectrophotometer (AAS) analytical method. For AAS analysis, digestion beakers, test tubes and volumetric glassware were first cleaned by a procedure described by Appiah *et al*. (2012). Four replicates of each category of pulverized samples were again accurately weighed (approximately 0.5 g) and directly dissolved into Teflon vessels containing a (6:1 v/v) mixture of HNO_3 and $HClO_4$. These vessels were then swirled gradually to form a homogeneous mixture, fitted into an ETHOS 900 microwave digester and digested for 25 minutes. After appropriate cooling, each digested solution was transferred into a measuring cylinder and diluted to 20 ml using de-ionised water. These solutions were then analysed using a Flame Atomic Absorption Spectrophotometer model VARIAN AA 240 FS. Calibrations, precision together with accuracy of this system has previously been done (Anim *et al*., 2012).

Results and Discussion

The parameterization of perilous heavy metal concentrations such as Cd, Cr, Hg, Pb,

etc., in different tissues of commercially consumed fishery resources found in inland water bodies is very vital because fish which forms an essential staple part of daily food, may significantly contribute to the transfer of undesirable heavy metals to higher trophic levels especially to humans. Thus, the accumulation of heavy metals in aquatic animals is therefore nowadays not of scientific interest in heavy metal chemistry alone, but of utmost interest to remarkable groups such as Food and Drug Authorities, Nutritionists, Toxicologists, Policy-makers and even Governments at large (Kumar *et al*., 2007).

We have combined two highly sensitive and most extensively used state – of – the – art analytical methods; Neutron Activation Analysis (NAA) and Atomic Absorption Spectrophotometer for the determination of concentration levels of heavy metals of interest in this study.

The reliability, precision and accuracy of these two analytical methods in the determination of elements are well – established in published literature (Emurotu *et al*., 2012; Nyarko *et al*., 2003; Appenteng *et al*., 2012), hence was not considered in this current work. The mean concentration values of six heavy metals determined by NAA and AAS in different tissues of *O. niloticus* at different strata and seasons with the standard deviations as their experimental uncertainties for four replicate measurements are presented in Table 2.

Table 2 shows the concentration of heavy metals per strata per tissues for each sampling series. It is worth noting that, even though, the same fish species were analyzed at the same hydrographic medium each time, varying concentration levels existed among the various tissues. Similar findings were recorded by Bahnasawy *et al*. (2011) and Mastan (2014). One conceivable interpretation of these differences result from different affinity of metals to fish tissues, different uptake, deposition and excretion rates (Jezierka and Witeska, 2006). The difference in metal concentrations in the same fish tissue at different strata confirms that these locations indeed had different degrees of pollution.

An alternative approach in discerning significant trends, easy comparison of data and better representation of the massive number of data acquired in Table 2 is the application of a statistical pictorial technique known as the graphical method. This technique has been successfully applied in many chemical field studies involving biological and environmental samples (Stanciu *et al*., 2005; Zheng *et al*., 2007; Tulonen *et al*., 2006; Begum *et al*., 2009; Ozuni *et al*., 2010). Thus, the concentrations of elements listed in Table 2 are graphically presented per strata in Fig. 1, 2 and 3, for the various sampling series. Even though, the predominant pathways for heavy metal uptake, target organs, and organism sensitivity are highly variable, and are dependent on factors such as metal concentration, age, size, physiological status, habitat preferences, and growth rates of fish (Chapman *et al*., 1996); significant heavy metal accumulation trends were observed in this studied as shown in the figures below.

It is apparently clear in Fig. 2 that, the mean concentrations of all the heavy metals in the muscle tissues were well below 10 µg/100g. The highest metal recorded for these tissues was Cd (9.8 µg/100g) in the lower stratum whereas Hg recorded the lowest. Following the muscle tissues was the gill tissues which recorded similar concentration levels of metals.

Table.1 Food Source, mean weight, mean length and water content of the sampled fish from Vea Dam, Bolgatanga, Northern Ghana

Sampling Series	Food Source	Upper Stratum		Middle Stratum		Lower Stratum		Moisture Content (%)
		Mean Weight (g)	Mean Length (cm)	Mean Weight (g)	Mean Length (cm)	Mean Weight (g)	Mean Length (cm)	
FSSI	Mainly Algae	164 ± 17	15.7 ± 1.9	172 ± 19	15.3 ± 1.8	169 ± 15	18.4 ± 2.1	78.3 ± 2.7
SSSII		180 ± 22	18.0 ± 2.4	188 ± 16	18.5 ± 2.1	197 ± 24	17.9 ± 1.6	81.6 ± 4.4
TSSIII		186 ± 20	21.3 ± 2.7	193 ± 24	22.6 ± 2.5	211 ± 31	26.1 ± 3.2	83.5 ± 3.9

Table.2 Concentrations of heavy metals (µg/100g) compared in different strata in muscles, liver and gills

Elements	**Inhabit Strata**	**First Sample Series I**			**Second Sample Series II**			**Third Sample Series III**		
		Muscles	**Liver**	**Gills**	**Muscles**	**Liver**	**Gills**	**Muscles**	**Liver**	**Gills**
As	*Upper*	4.16±0.32	10.01±1.63	5.78±0.48	3.05±0.14	10.81±1.99	5.74±0.80	<0.1	14.96±1.92	9.43±1.97
	Middle	<0.1	8.30±1.86	5.06±0.52	2.75±0.10	7.09±0.66	3.76±0.41	3.11±0.27	9.65±0.99	6.05±0.73
	Lower	5.71±0.41	9.18±1.89	3.52±0.16	<0.1	6.37±0.52	4.83±0.37	<0.1	8.77±1.00	6.24±0.77
Cd	*Upper*	6.42±0.94	8.17±1.70	2.90±0.11	ND	15.48±2.01	<0.1	13.02±1.00	14.35±2.27	3.60±0.30
	Middle	<0.1	16.19±2.06	6.29±0.77	9.06±1.00	17.58±2.01	3.01±0.29	ND	23.87±3.00	5.27 ±0.70
	Lower	9.82±1.81	23.15±3.91	12.33±1.39	9.63±1.15	25.37±3.88	ND	13.02±1.00	31.56±3.09	11.36±1.30
Cr	*Upper*	6.92±1.00	11.00±1.74	7.03±0.91	2.00±0.01	3.69±0.36	1.32±0.01	16.21±2.00	34.77±4.11	23.85±3.27
	Middle	4.00±0.51	11.39±1.90	ND	7.13±0.39	19.58±2.19	8.90±0.91	18.00±2.11	35.09±4.10	26.01±3.78
	Lower	<0.1	10.47±1.87	5.01±0.81	8.17±0.71	27.15±3.06	23.00±2.72	14.79±1.93	28.64±3.94	21.01±3.29
Hg	*Upper*	ND	1.98±0.07	0.73±0.00	ND	1.01±0.01	ND	<0.1	3.18±0.41	1.80±0.03
	Middle	<0.1	2.05±0.11	0.94±0.01	<0.1	2.33±0.02	1.99±0.02	1.27±0.01	4.00±0.09	1.95±0.03
	Lower	<0.1	1.83±0.10	1.02±0.01	1.02±0.01	3.72±0.19	2.80±0.21	2.00±0.13	3.91±0.47	2.73±0.21
Pb	*Upper*	4.51±0.37	12.79±1.24	5.69±0.30	3.18±0.17	15.68±2.73	7.01±0.80	4.27±0.83	9.85±1.19	5.28±0.90
	Middle	6.12±0.88	11.07±1.82	4.82±0.31	2.95±0.10	15.01±2.86	7.81±0.56	4.13±0.91	8.74±1.27	7.03±0.40
	Lower	5.06±0.73	10.00±1.65	5.19±0.61	3.46±0.20	18.37±0.37	10.27±1.01	5.39±0.89	10.23±2.01	6.10±0.37
Sb	*Upper*	<0.1	2.88±0.14	<0.1	0.49±0.00	2.09±0.01	1.31±0.01	ND	3.11±0.10	2.37±0.16
	Middle	ND	2.16±0.13	<0.1	0.83±0.01	2.16±0.01	<0.1	<0.1	2.79±0.17	2.88±0.22
	Lower	1.33±0.04	3.01±0.18	1.78±0.08	ND	3.91±0.17	1.70±0.02	0.27±0.01	3.59±0.36	<0.1

ND – Not Detected

Table.3 Mean values (µg/100g) of Heavy Metals levels from Dec., 2012 – Aug., 2013

Sampling Series	Tissues	As	Cd	Cr	Hg	Pb	Sb
First Sampling Series I	*Muscles*	3.290	5.413	3.640	0.000	5.230	0.443
	Liver	9.163	15.837	10.953	1.953	11.287	2.683
	Gills	4.787	7.173	4.013	0.897	5.233	0.593
Second Sampling Series II	*Muscles*	2.900	11.345	7.287	1.020	3.197	0.660
	Liver	8.090	19.477	16.807	2.353	16.353	2.72
	Gills	4.777	3.010	11.073	2.395	8.363	1.505
Third Sampling Series III	*Muscles*	3.11	8.680	16.333	1.090	4.597	0.090
	Liver	11.127	23.260	32.833	3.697	9.607	3.163
	Gills	7.240	6.743	23.623	2.160	6.137	1.750
Mean Concentration		**6.054**	**11.215**	**14.062**	**1.729**	**7.778**	**1.512**

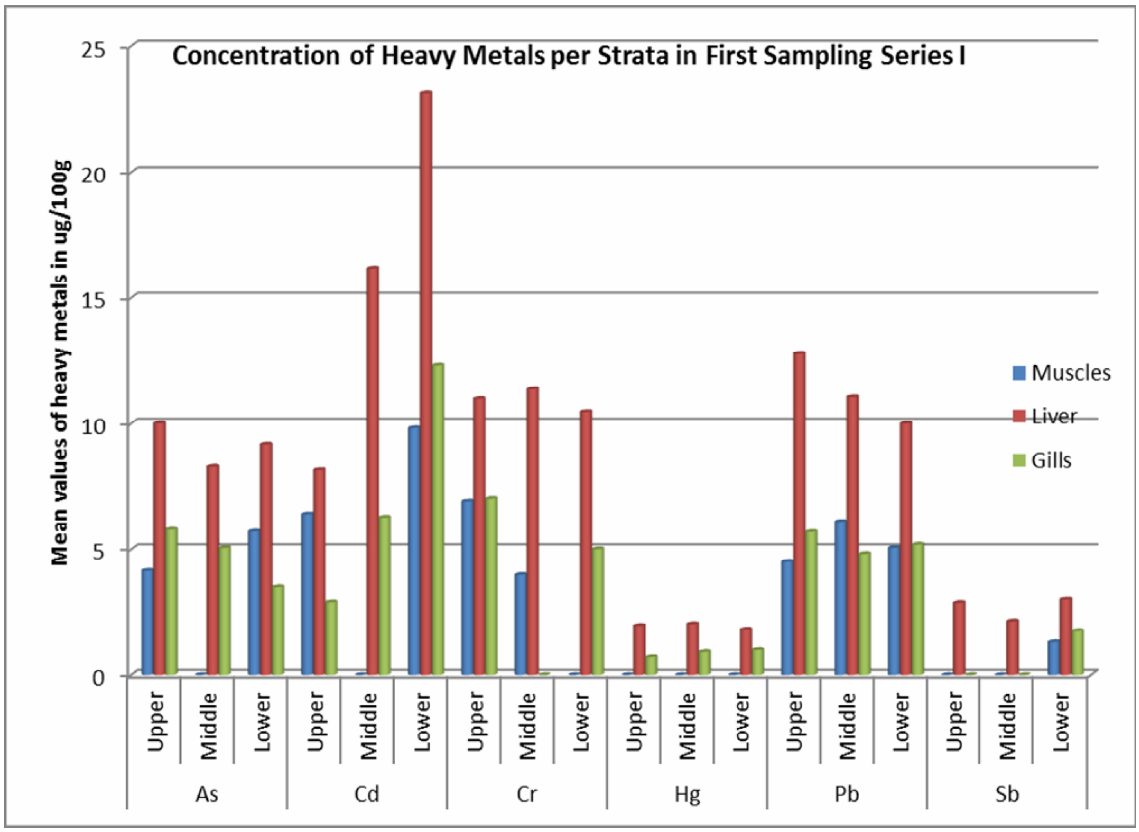


Fig.2 A histogram showing concentration levels of heavy metals at various strata for FSSI

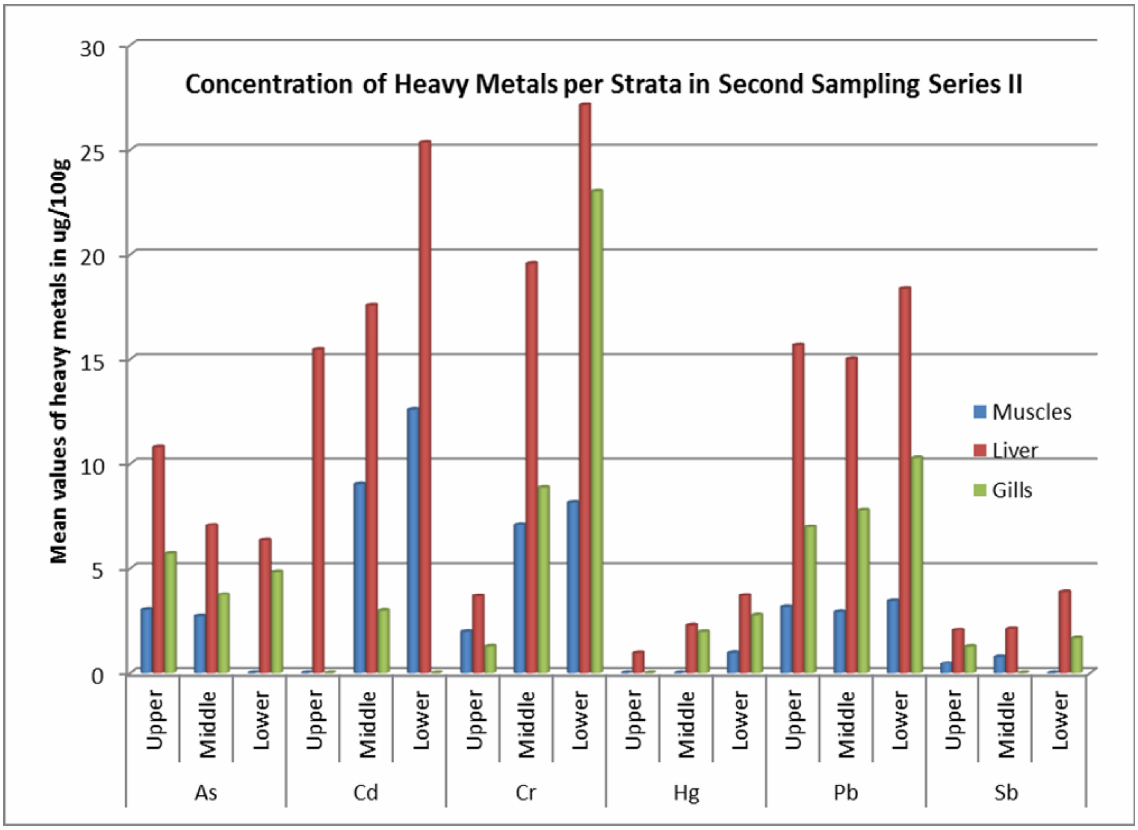


Fig.3 A histogram showing concentration levels of heavy metals at various strata for SSSII

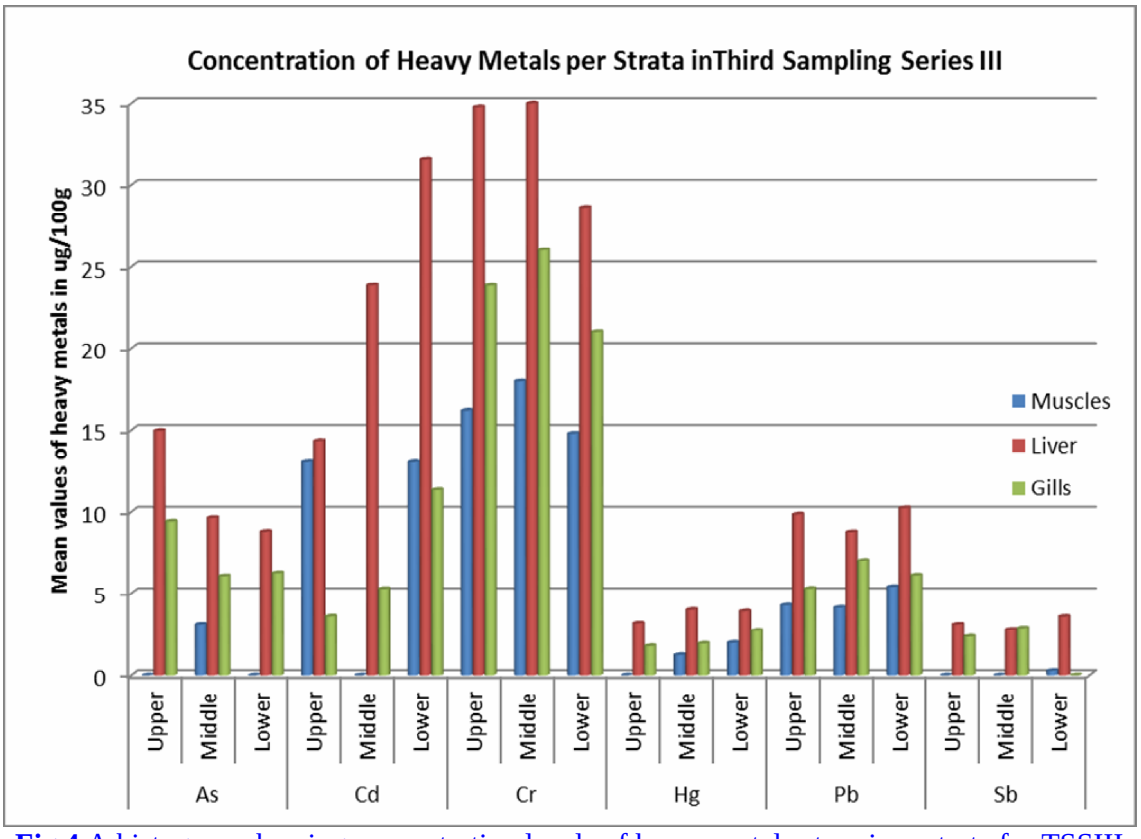


Fig.4 A histogram showing concentration levels of heavy metals at various strata for TSSIII

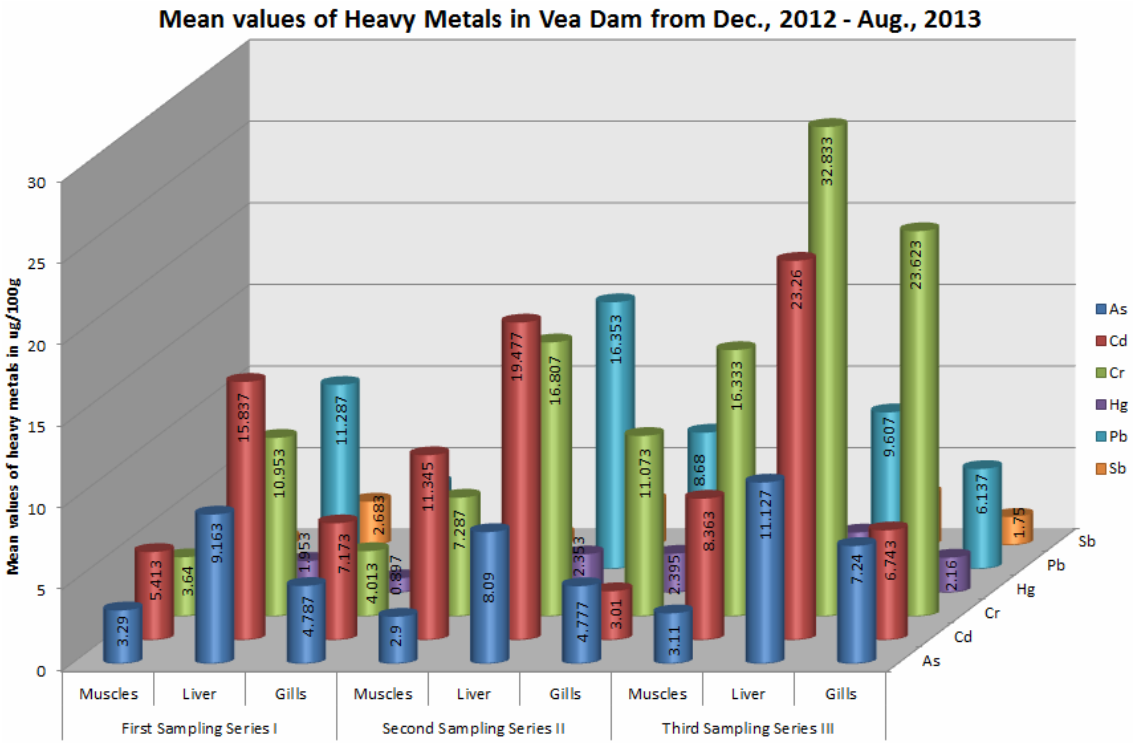


Fig.5 A 3 – D histogram showing concentration levels of heavy metals at from 2012 to 2013

Again except for Cd (lower) all the mean values of heavy metals were within 10 µg/100g. Generally, the concentrations of heavy metals in the gill tissues were found to be higher than those in the muscle tissues except that of As (lower), Cd (upper) and Pb (middle). The high content of metals in gill tissues can tentatively be attributed to the fact that fish gills play a distinct role in metal uptake from the environment (Bahnasawy *et al*., 2011). Thus, the pattern of heavy metal accumulation in the tissues of *O. niloticus* fish species is largely liver > gills > muscles. This is in agreement with the findings of many other works (Shakweer, 1998; Mastan, 2014; Dsikowitzky *et al*., 2012).

In comparison to Fig. 2, it is worth noting that, the SSSII recorded higher concentration of metals (i.e. over 25 µg/100g) than in the FSSI particularly with respect to Cd (25.4 µg/100g) and Cr (27.2 µg/100g) in the liver tissues as shown in Fig. 3. The highest concentration of metals in the muscle tissues was again observed to be Cd (12.6 µg/100g). Thus, it was marginally higher than that recorded in FSSI. The concentrations of all the metals were again well below 10 µg/100g except for Cd in the lower stratum. Hg and Sb were mostly found in traces; thus their concentrations were below 5 µg/100g. The high concentrations of metals in the liver in SSSII than that in FSSI clearly suggest that, there were seasonal variations of heavy metals uptake in the tissues of aquatic organisms. This phenomenon is in accordance with findings by Mathana *et al*. (2012) and Tiina *et al*. (2006).

The highest concentration of metals was observed in TSSIII as shown in Fig. 4 below. The progressively increasing metal concentrations from FSSI to TSSIII certainly confirm the bio – accumulative traits of *O. niloticus* fish species. Even though our data showed similar variations of heavy metal concentrations from one season to the other, it is largely inconclusive as to the predominant sources of metal uptake to the fish. The haphazard patterns of the metal concentrations in the figures can partly be attributed to the fact that, the *O. niloticus* fish species are itinerant and hence may not

be distinctly confined to a particular stratum of habitat.

A critical examination of all these figures confirms the assertion by Jezierska and Witeska (2006) that, considerable amounts of various metals may be deposited in fish tissues without causing mortality. Nevertheless, the cumulative average concentrations of the heavy metals for the study period are typically well below their permissible levels as specified by WHO (2011) as shown in Table 3. Table 3, was derived from Table 2 by applying stereotypical statistical concepts such as additions and averages for each metal based on each tissues. In comparison to other literature reports (Saeed and Shaker, 2008; Dsikowitzky *et al*., 2012; Al-Kahtani *et al*., 2009) on heavy metal content in different tissues of *O. niloticus* fish species, our data (Table 3) may be considered to be in moderate levels of concentrations.

It is fastidiously noting that, irrespective of the season or stratum, the liver tissue had high metal accumulation than the gills and muscles. This was exemplified in Table 3, where all entries in the liver row had the highest values for each metal in all sampling series; thus, the dominant accumulating tissues were the liver. One plausible explanation for the high accumulation potential of the liver is a result of the activity of metallothioneins, metal-binding proteins, which play an important role in metal regulation and detoxification of non-essential metals (Roesijadi, 1992). Thus, the liver is considered a good biomonitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment (Jezierska and Witeska, 2006). The muscle tissues recorded the lowest accumulation for most of the metals studied. We therefore suggest that, the liver tissues should be removed before using the fish in the preparation of meals as a precautionary measure to drastically reduce the level of human exposure to these metals.

A three-dimensional graphical representation of Table 3 is shown Fig. 5. The dominant metal was found to be Cr as shown in Fig. 5. Consequently, the metals concentrations in this studies was in a decreasing order of Cr > Cd > Pb > As > Hg > Sb. The toxicological effects of these heavy metals are established in literature and hence, was not discussed here (Adazabra *et al*., 2013).

Conclusion

The concentrations of six non-essential heavy metals were determined in different tissues of extensively consumed inland fish, *O. niloticus*, found in Vea Dam, at different strata by NAA and AAS. The liver tissues had the highest concentrations of these metals with the muscle tissues recording the lowest. It is therefore recommended that the liver tissues should not be consumed. The order of these metals were Cr > Cd > Pb > As > Hg > Sb. Even though, the concentration levels of these hazardous metals studied in this dam are below permissible levels, their presence in detectable amounts is worrisome. Consequently, regular close monitoring of these metal loads and evaluation of pollutants in the dam is highly recommended in view of the possible risks to health of consumers in future.

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