

Determination of Toxic and Essential Elements in Tilapia Species from the Volta Lake with Inductively Coupled Plasma – Mass Spectrometry

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Abstract- The concentrations of some toxic elements (mercury, lead and arsenic) and some essential elements (cobalt, copper, chromium, manganese, selenium and zinc) in fish obtained from the Volta Lake in Ghana were determined using a rapid, highly sensitive and accurate method. A stepwise digestion procedure using Optima Fisher concentrated nitric acid and hydrogen peroxide was used for complete oxidation of organic tissue. The concentrations of the various elements were measured using an Agilent 7500c quadrupole Inductively Coupled Plasma Mass Spectrometer (ICPMS; Agilent Technologies, Palo Alto, CA) equipped with a dynamic reaction cell and a Cetac ASX-500 autosampler. A total of 62 fish samples covering four different species of tilapia: *Oreochromis niloticus*, *Tilapia zilli*, *Tilapia dageti* and *Sarotherodon galilaeus* were analyzed for their elemental contents. Results of the study indicate low levels of exposure to arsenic, chromium, cobalt, copper, lead, manganese, mercury, selenium and zinc through consumption of these species of tilapia and do not pose a significant health risk to the individuals and to a greater extent the general population.

Keywords- Toxic Elements; Essential Elements; Tilapia Species; Volta Lake

I. INTRODUCTION

The pollution of the aquatic environment with heavy metals has become a worldwide problem in recent times because they are indestructible and most of them have toxic effects on organisms (Macfarlane and Burchett, 2000). Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate 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; Fernandes et al., 2008; et al., 2008; Pote et al., 2008 and Praveena et al., 2008) have been a major environmental focus especially during the last decade. Bioaccumulation and magnification is capable of leading to toxic level of these metals in fish, even when the exposure is low. The presence of metal pollutant in fresh water is known to disturb the delicate balance of the aquatic systems. Toxic elements accumulation in different species of fish depend on the feeding habits, size and length of the fish (Al-Yousuf et al., 2000) and more particularly their habitat (Canli and Atli, 2003). 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 toxic 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 blood concentrations of lead and other toxic metals. Hg, Pb, 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). 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). Pb is noted for intellectual impairments. Low blood Pb levels can influence neurobehavioral performance in children and may be a contributor to Attention-Deficit/Hyperactivity Disorder (ADHD). Mn is an essential trace element for the development and function of the central nervous system and it is a constituent of many enzymes involved in carbohydrate and protein metabolism. Cu, Zn, Se, Co, and Cr are important elements for human biological activities. Cu has been recognized as an essential element for many years because of its presence in important enzymes and proteins. Zn is also a component of a wide variety of enzymes and hormones such as progesterone and testosterone. Se's importance in human nutrition is well established and it is an essential element with important biological functions. Both Se deficiency and excess can lead to harmful effects. Highly regulated, Se is important because of its incorporation in selenoproteins. Co functions as a co-factor in enzyme catalyzed reactions and is involved in the production of erythropoietin a hormone that stimulates the formation of erythrocytes. Cr is also an essential nutrient required for sugar and fat metabolism. 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 the Volta Lake in Ghana and the introduction of methylcyclopentadienyl manganese tricarbonyl (MMT) to gasoline, call for the need to monitor the concentration of some toxic and heavy metals as well as essential elements in tilapia, one of the heavily consumed fish from the lake. The Volta is one of the world's largest man-made oligotrophic lakes which serves as a source of water and freshwater fish especially tilapia for the people of Ghana. 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. The choice of instrumentation, ICP-

MS is due to the fact that it is very rapid, highly sensitive and accurate for multi-element determination.

II. MATERIALS AND METHODS

The fish species were collected from random commercial catches in villages/towns along the Volta Lake depending on the availability of the species for sale between June 2009 and January 2010. Samples obtained were therefore reflective of species meant for consumption. A total of 62 fish samples covering four different species of Tilapia: *Oreochromis niloticus*, *Tilapia zilli*, *Tilapia dageti* and *Sarotherodon galilaeus* 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 Kwame Nkrumah University of Science and Technology, Kumasi 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 the Environmental Health Sciences laboratory of the University of Michigan, Ann Arbor, USA for chemical analysis. In the laboratory, the samples were washed with deionized water, dried on tissue and a portion of the edible muscle tissue removed from the dorsal part. In the digestion procedure, 10.0 mg 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 . An aliquot of 2.0 ml of Optima Fisher 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 2.0 ml of hydrogen peroxide 30% Supra-pur® added to each tube. The temperature was raised to 60°C and the tubes heated for 10 minutes. The temperature was raised to 65°C and maintained for 10, minutes. The temperature was increased to 70°C , and heating continued for 20 minutes. The temperature was raised to 80°C and maintained for 30 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 25 ml with milliQ water and transferred into clean polyethylene bottles for storage prior to ICP-MS analysis. The concentrations of the various elements were determined using An Agilent 7500c quadrupole Inductively Coupled Plasma Mass Spectrometer (ICPMS; Agilent Technologies, Palo Alto, CA) equipped with a dynamic reaction cell and a Cetac ASX-500 autosampler. The instrumental operating conditions are presented in Fig. 1.



Fig. 1 Map of Africa and Ghana showing the Volta Lake and its major tributaries

III. RESULTS AND DISCUSSION

Concentrations of arsenic, chromium, cobalt, copper, lead, manganese, mercury, selenium and zinc in the four species of tilapia were determined. The validity of the methodology and the determination of its accuracy and precision were obtained from quintuplet analysis of 10 mg 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 analyzed. The results indicate reasonable agreement between the found and claimed values and good coefficient of variation (better or equal to 5%). The Method Detection Limits were computed as three times the standard deviation of the digested blank samples and found to compare favourably with those reported in literature Table 1.

TABLE 1 AGILENT 7500C QUADRUPOLE ICP-MS OPERATING CONDITIONS

RF Power	1600W
Reflected Power	<5 W
Sample Uptake Rate	0.4 mL min ⁻¹
Gas Flow Rate	
Plasma Gas	15 L min ⁻¹
Auxiliary Gas	0 L min ⁻¹
Carrier Gas	0.86 L min ⁻¹
Makeup Gas	0.29 L min ⁻¹
Ion Sampling Depth	8 mm
Torch	Fassel, Quartz 2.4 mm id fitted with Agilent Shield Torch system
Nebulizer	Glass expansion Micro Mist
Sample Cone	Platinum, orifice diameter 1.0 mm
Skimmer Cone	Platinum, orifice diameter 0.4 mm
Spray Chamber	Water cooled Scott type (double pass) (2°C)
Acquisition	Full quantitative mode with no reaction gas (i.e. normal mode), helium reaction gas (i.e. helium mode) or hydrogen reaction gas (i.e. hydrogen mode), depending on the element. Three points/peak with three replicates. Internal standard (~50 µg L ⁻¹ added online using T-connector). Washing to <1% carryover, rinse with 5% nitric acid.

The results indicate that the metal content in the samples studied depends on the fish species and the concentrations showed some variations. The variations are generally related to the fish species, the age or weight of fish, the feeding habit of the fish and the geographical location. All the four different species of tilapia belong to the same *Cichlidae* family (all being herbivores) and numerical trophic level (2.0 – 2.3) but have different habitats. *Oreochromis niloticus* is a benthopelagic; while *Sarotherodon galilaeus*, *Tilapia dageti* and *Tilapia zilli* are all demersals or profundals.

The average concentrations of the elements in µg /g wet weight of fish were: arsenic 1.21, chromium 1.06, cobalt 0.43, copper 958, lead 25.6, manganese 3.52, mercury 0.054, selenium 0.017 and zinc 2350. Generally, concentrations of the elements determined in all the fish species were lower than those found in literature except for cobalt, copper and chromium which were higher than those found in other studies.

In our study, Manganese content in fish meat ranged from 3.05 to 4.12 µg /g w.w. and mean Mn concentration of 1.21 µg /g w.w. (Table 2). Łuczyńska et al. (2006) found Mn concentrations in the range of 0.071 - 0.117 mg/kg in fish from north-east Poland. Higher Mn levels were reported by

Alibabić and Vahčić (0.62 - 0.91 mg/kg w.w.) (2007), yet our results was about 450% higher. However, fish meat is assumed to contain 0.01 - 0.06 mg Mn per 100 g edible portion (Kunachowicz et al., 2005). Species *Sarotherodon galilaeus* had the highest mean Mn concentration of 3.68 µg /g but highest individual concentration was found in a *Tilapia dageti* species. The variation in the Mn concentrations was not that marked. Zinc content in the muscles of the examined fish species ranged within 176.4 – 298.6 µg /g w.w. (Table 2). Zn concentrations observed by Szefer et al. (2003) in the Pomeranian Bay and Szczecin Lagoon and by Protasowicki [1991] are far below those found in this study. 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; Farkas et al., 2002) yet the levels were far lower than that obtained in this study. However, Türkmen and Ciminli (2007) reported lower Zn concentration (0.456 mg/kg w.w.) in fish from Lake Gölbaşı. 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. In our study, Zn levels ranged from 17.64 to 29.85 mg/100 g edible portion. Among the fish species examined, *Oreochromis niloticus* had the highest mean Zn concentration of 270.2 µg/g and the highest individual level of 298.0 µg /g. The lowest mean Zn concentration of 195.6 µg /g was found in *Tilapia zilli*.

TABLE 2 MEAN AND RANGE OF ELEMENT CONCENTRATION FOR ALL FISH

Element	Range of Concentration(µg /g)	Mean Concentration (µg /g)
As	0.59 – 2.45	1.21
Pb	18.6 – 32.14	25.6
Mn	3.05 – 4.12	3.52
Cu	81.5 – 134.2	95.8
Co	0.23 – 0.66	0.43
Cr	0.68 – 1.74	1.06
Se	0.008 – 0.031	0.017
Zn	176.41 – 298.62	235.0
Hg	0.018 – 0.181	0.054

Mean concentration of Copper in the examined fish ranged from 81.5 - 134.2 µg /g w.w. (Table 2). Lower Cu concentrations (0.2-0.4 mg/kg w.w.) were reported by Szefer et al., (2003) and also by Karadede and Ünü (2000) in carp (2.23 mg/kg w.w.). According to the tables of food composition and nutritional value (Kunachowicz 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 our study varied from 8.15 to 13.42 mg/100 g edible portion. The recommended daily allowance of Cu for both women and men varies within 1.5 - 2.5 mg per person (Ziemiański, 2001). *Sarotherodon galilaeus* recorded the highest mean Cu level of 102.1 µg /g with a highest individual level of 134.2 µg /g. The lowest mean level of Cu was recorded by *Tilapia dageti* and highest by *Sarotherodon galilaeus* as indicated by the plot. Mean concentration of Chromium in the examined fish ranged from 0.68 – 1.74 µg /g w.w.(Table 2).Sapozhnikova et al., (2005) and Rashed (2001) reported similar chromium levels in fish. Ikem et al., (2003) observed 0.01 mg Cr per kg w.w. in fish muscle. Much higher Cr concentrations were observed in fish

from the Una River basin, by Alibabić and Vahčić (2007). Dietary Cr usually satisfies human demand for the element, which varies from 50 to 200 µg per day in adult people. In our study, *Sarotherodon galilaeus* recorded the highest mean Cr level of 1.31 µg /g with the highest individual level of 1.74 µg /g. *Tilapia dageti* recorded the lowest mean Cr concentration of 0.87 µg /g.

Lead content in the examined fish meat ranged within 18.6 to 32.14 µg /g w.w. (Table 2).Türkmen and Ciminli (2007) found far lower Pb concentrations in fish from Lake Gölbaşı (*Clarias gariepinus* – 0.014 mg/kg w.w., and *Carasobarbus luteus* – 0.008 mg/kg w.w.). Some other authors reported values over 0.100 mg/kg w.w. (Has-Schön et al., 2006). Much higher concentration was reported by Jurkiewicz-Kamakowska (2001) in fish from Zegrzyński Reservoir (from 8.9 to 12.7 mg/kg w.w.) but these levels were far lower than that found in this study. *Tilapia zilli* recorded the highest mean Pb concentration of 26.3 µg /g w.w. and *Oreochromis niloticus* the lowest mean level of 24.8 µg /g w.w. The highest individual Pb concentration of 32.4 µg /g and lowest of 18.6 µg /g were all recorded by *Tilapia dageti*. Mercury levels in the muscles, depending on the examined fish species, varied between 0.018 and 0.181 µg /g w.w. (Table 2), while the maximum allowable level is 1.0 µg /g w.w. (O.J. L 364, 2006). Similar mercury levels in West Pomeranian fish (on average 0.015-0.030 mg/kg w.w.) were reported by Szefer et al., (2003) and by Perkowska and Protasowicki in pike muscle from Świdwie Lake (1999). In contrast, higher Hg levels were found by Has-Schön et al., (2006) in muscles of Croatian fish and by Dušek et al., (2005) in fish from the Elbe River. The highest mean Hg concentration of 0.131 µg /g w.w. and highest individual level of 0.181 µg /g w.w. were recorded by *Oreochromis niloticus* and the lowest individual level of 0.018 µg /g was recorded by *Tilapia dageti*. In this study, Selenium in the muscle tissue of the fish species ranged from 0.008 - 0.031 µg /g w.w. with a mean value of 0.017 µg /g w.w. The differences in Se concentrations found between the different tilapia 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 bioaccumulate within the base of food webs: from water and sediment to aquatic plants and invertebrates, and finally, to fish (Hamilton, 2004). Mean Arsenic concentration in the examined fish ranged from 0.59 – 2.45 µg /g w.w (Table 2). For most people, diet including fish is the largest source of exposure to arsenic. Mean dietary intakes of total arsenic of 50.6µg/day (range of 1.01–1,081 µg/day) and 58.5µg/day (range of 0.21–1,276 µg/day) have been reported for females and males (MacIntosh et al., 1997). The results obtained in this study indicates low levels As. *Sarotherodon galilaeus* species recorded the highest mean level of 2.03 ug/g w.w. with a range of 1.98 – 2.45 µg /g w.w. (Table 3). The lowest mean As level was recorded by *Oreochromis niloticus* 0.86 µg /g w.w. in the range of 0.59 - 1.63 µg /g w.w. Cobalt as an essential element had mean concentration range of 0.23 – 0.66 µg /g w.w. (Table 2). The Total Diet Study in the United Kingdom in 1994 estimated the population average intake of cobalt to be 0.12 mg/day (MAFF, 1997). Cobalt intake in the United States has been estimated to be 5–40 µg/day (Jenkins, 1980), with relatively high concentrations of cobalt occurring

in fish and vegetables (Barceloux, 1999). In Canada, the estimated average daily intake is 11 µg/day (Dabeka and McKenzie, 1995). In France, the estimated average daily intake is 29 µg/day (Biego et al., 1998). *Sarotherodon galilaeus* species recorded the highest mean Co concentration

of 0.57 µg /g w.w. in a range of 0.38 - 0.66 µg /g w.w and *Tilapia dageti* recorded the lowest mean Co concentration of 0.35 µg /g w.w. in the range of 0.29 - 0.4 µg /g w.w. The levels of Cobalt recorded in this study fall within the ranges set by other countries.

TABLE 3 FISH SPECIES AND THE CORRESPONDING CONCENTRATION OF ELEMENTS IN MG /G W.W

Fish Species	Concentration of Element in ug/g								
	As	Pb	Mn	Cu	Co	Cr	Se	Zn	Hg
Oreochromis niloticus (n=10)	0.86	24.8	3.49	96.2	0.41	0.95	0.021	270.2	0.131
Mean Level	0.05	5.8	0.47	15.6	0.04	0.24	0.003	25.8	0.023
Std Dev.	0.59-	21.4-	3.23	89.7-	0.32-	0.81-	0.013-	254.1-	0.090-
Range	1.63	27.3	3.81	102.8	0.55	1.27	0.029	298.6	0.181
Sarotherodon galilaeus (n=14)	2.03	25.2	3.68	102.1	0.57	1.31	0.021	260.7	0.035
Mean Level	0.41	6.4	0.52	17.8	0.08	0.21	0.001	24.3	0.009
Std Dev.	1.98-	20.3-	3.14-	95.3-	0.38-	1.05-	0.015-	224.5-	0.028-
Range	2.45	28.1	3.92	134.2	0.66	1.74	0.031	292.0	0.041
Tilapia dageti (n=18)	0.97	26.1	3.39	89.7	0.35	0.87	0.012	195.6	0.026
Mean Level	0.22	5.9	0.77	10.5	0.15	0.17	0.003	25.8	0.005
Std Dev.	0.74-	18.6-	3.05-	81.5-	0.29-	0.68-	0.008-	176.4-	0.018-
Range	1.43	32.4	4.12	92.6	0.41	0.99	0.017	201.5	0.039
Tilapia zilli (n=20)	0.98	26.3	3.52	94.8	0.39	1.11	0.015	213.5	0.025
Mean Level	0.14	6.5	0.97	17.8	0.16	0.25	0.003	56.8	0.001
Std Dev.	0.62-	20.5-	3.12-	85.3-	0.23-	0.71-	0.011-	202.1-	0.019-
Range	1.38	29.4	4.09	98.6	0.44	1.56	0.019	221.4	0.031

IV. CONCLUSION

The results of this study indicate low levels of exposure to the toxic and essential elements arsenic, chromium, cobalt, copper, lead, manganese, mercury, selenium and zinc through consumption of the four species of tilapia analyzed. The levels of the elements do not pose a significant risk to the individuals and to a greater extent the general population that consumes these fish species for now but the situation may change over time due the ability of the elements to bioaccumulate in the tissues of the fish. However, fish dietary intake studies in Ghana are needed particularly among the artisanal gold mining areas in order to be able to relate the actual fish consumption patterns among the populations and toxic metals particularly mercury. 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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