Determination of Mn, Cu, and Na in *Sarotherodon Melanotheron* (Blackchin Tilapia) From the Fosu Lagoon, Cape Coast - Ghana, Using Instrumental Neutron Activation Analysis (INAA)

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Concentrations of Mn, Cu, and Na trace elements in *Sarotherodon melanotheron* (Blackchin tilapia) from the Fosu Lagoon in Cape Coast, Ghana have been determined by employing the technique of Instrumental Neutron Activation Analysis (INAA) at the Ghana Atomic Energy Commission using the Ghana Research Reactor -1, (GHARR-1) operating at 15 KW at a thermal flux of 5×10^{11} n.cm^{-2}.s^{-1}.

Standard reference materials used were International Atomic Energy Agency (IAEA) -350; Trace Elements in Tuna Fish Homogenate and National Institute of Standard and Technology (NIST), USA SRM-1566b; Trace Elements in Oyster Tissues. The *Relative standardization* method was used in the quantification of all the elements. The mean daily intake of the elements determined in the fish were ^{56}Mn (22.54 mg/d), ^{66}Cu (59.22 mg/d), and ^{24}Na (2.24 mg/d). The determined mean daily intake of Cu and Mn exceeded their Maximum Upper Limit (UL) of the Recommended Dietary Allowance (RDA) for all life stage groups, while that of Na was far below the RDA. The mean daily intake of Na with absolute error margin of ±0.0245 was adequate for consumption. However, the mean daily intake of Cu and Mn far exceeded their RDAs and Maximum Upper Limit, (UL), making *Sarotherodon melanotheron* from the Fosu Lagoon unsafe to eat.

1. Introduction

Living organisms require nutrients in right quantities for their proper growth. Some of these nutrients are protein, carbohydrates, fats and oil. In Ghana, fish has been identified as a cheap source of protein [1]. Attention has therefore been given to the protein gained from fish to the neglect of the other micronutrients that are derived from it [1]. However, apart from the protein obtained from fish, it is a good source of both trace and major elements like Chlorine, Copper, Iron, Iodine, Manganese, Selenium, Sodium, and Vanadium. These elements in their right quantities are essential for human growth but can have health implications when taken in excess.

For example, sodium (Na) is an electrolyte/mineral that functions as a major ion of the extra cellular fluid. It also aids nerve impulse transmission. However, high levels of Na contribute to high blood pressure in susceptible people. When taken in excess, it can lead to an increase in calcium loss in urine and increases the amount of water the body holds thus causing the swelling of the legs and hands [2,3].

Copper (Cu), on the other hand, is a very important trace element that aids in iron metabolism. It is needed by enzymes involved in protein metabolism and hormone synthesis. It also helps oestrogen metabolism required for women reproductive system for fertility and maintains pregnancy. Cu stimulates production of the neurotransmitters epinephrine, norepinephrine and dopamine in the nervous system [4]. But high intakes of Cu can cause liver and kidney damage. Vomiting and nervous system disorders have been identified as some of the effect of consuming Cu above 10 mg/d [5,6].

Manganese (Mn) is also an essential trace element that aids in the action of enzymes involved in carbohydrate metabolism [7]. Shortages of manganese in the body can cause health effects like fatness, glucose intolerance, blood clotting, skin problems, skeletal disorders, birth defects and neurological symptoms [7]. The central nervous system is the chief site of damage from high levels of Mn, which may result in permanent disability. A high incidence of pneumonia and other upper respiratory tract infections has been found in workers exposed to dust or fume of Mn [8].

Fish is therefore said to be toxic (poisoned) when it contains some of these elements beyond a desirable threshold or quantity. The concentration

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of these elements in various parts of the fish may vary since the rate of absorbtion by the respective tissues also varies [9].

The rationale behind this study was to use INAA to determine the concentrations of ^{24}Na (15 hours), ^{56}Mn (2.579 hours) and ^{66}Cu (5.120 min), in *S. melanotheron* in the Fosu Lagoon, in Cape Coast, Ghana. The study is to determine if the concentrations of these elements fall within the Recommended Dietary Allowance (RDA), and hence safe for human consumption [10].

2. Study Area

The Fosu Lagoon is located within the Cape Coast metropolis of the Central Region of Ghana, along the Gulf of Guinea, (Fig. 1). The Lagoon is an important source of livelihood for more than 500 fishermen [11]. The main fish caught in this Lagoon is *S. melanotheron*.

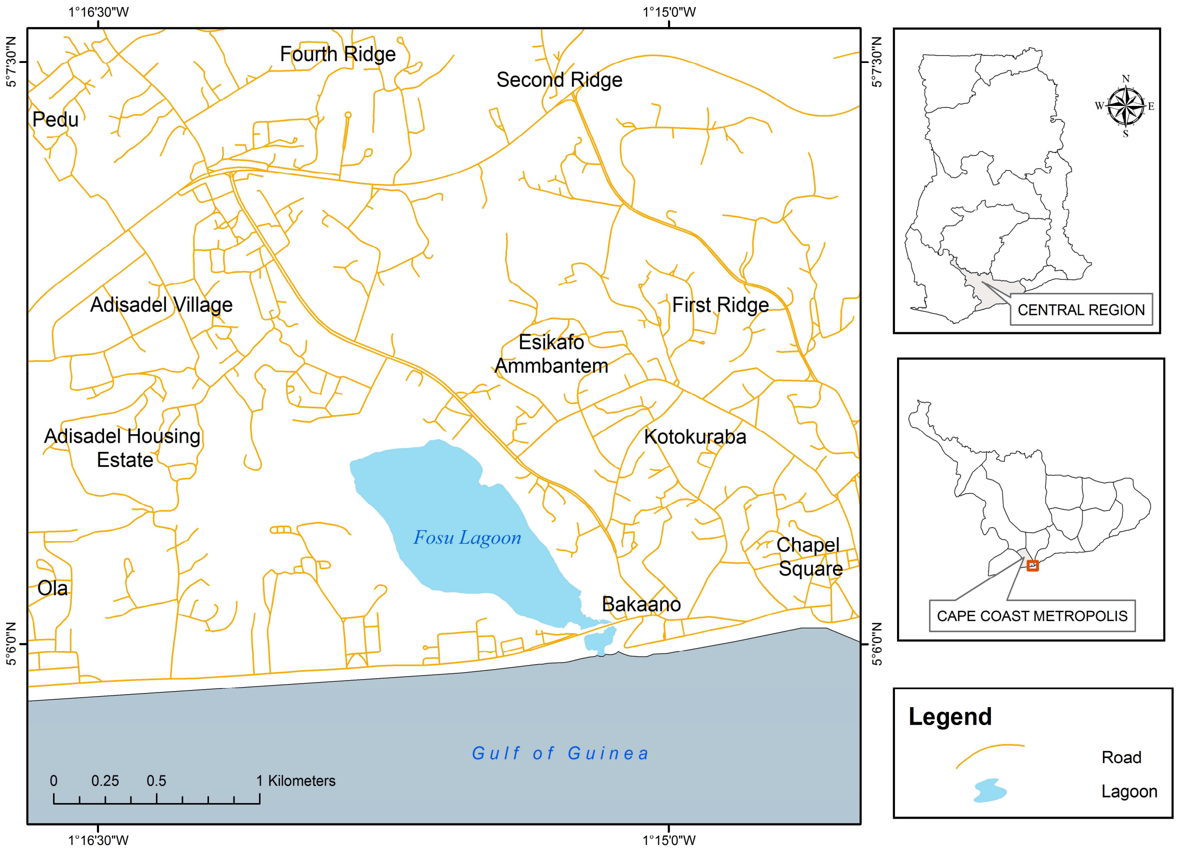

Fig.1: A map of Cape Coast, Ghana, showing the sampling site, Fosu lagoon [12].

3. Pollution of Fosu Lagoon

The Fosu Lagoon has been reported by the Environmental Protection Agency (EPA) of Ghana to be one of the polluted Lagoons in the country. The Lagoon is drying up as a result of heavy pollution [13,14], and this threatens the livelihood of the fishermen. Sources of the pollution have long been identified, but so far no actions have been taken [11]. Residents unfortunately still continue to patronise the fish from the polluted Lagoon.

The Lagoon is polluted with both solid wastes, such as metal scraps and iron filings from the local auto mechanic shops along the bed of the Lagoon, and liquid wastes from domestic activities, commercial food vendors, hairdressers and burning of domestic wastes along the boundaries of the Lagoon, (Fig. 2). The Cape Coast district hospital and two boarding schools, which are also along the bed of the Lagoon, discharge both solid and liquid waste into the Fosu Lagoon [15].

The fish in the Lagoon are reportedly dominated by tilapias, which make up 60 - 80% of all fish caught [16]. The most abundant being the blackchin tilapia, *S. melanotheron*, which is the mainstay of the fisheries of many Lagoons in West Africa including Ghana [17,18]. The Lagoon tilapia is important to the fisheries, but there have been growing concerns about the health implications of fish from this Lagoon. Since the size of *S. melanotheron*, is small, (Fig. 3), the locals in Cape Coast normally eat the tissue, gills and bones together.

Fig.2: Four different sites along the Fosu Lagoon showing some the debris in and around it.

Fig.3: *Sarotherodon melanotheron*, a tilapia species from Fosu Lagoon.

4. Instrumental Neutron Activation Analysis (INAA)

Instrumental Neutron Activation Analysis (INAA) was used to determine the concentration of Manganese, Copper, and Sodium in the Fosu Lagoon.

INAA is a nuclear process used for determining the concentration of elements in a vast amount of materials. INAA allows discrete sampling of elements as it disregards the chemical form of the sample and focuses solely on its nucleus. Compared to many analytical techniques, the instrumentation cost is relatively low [19]. The strengths of INAA are that it:

- can be used to analyze large number of elements simultaneously;
- is non-destructive;
- has very low detection limits for many elements;
- can be used for small sample sizes (1–200 mg);
- needs no chemical preparation.

5. Relative Standardization

In the relative standardization method, a chemical standard (index std) of known mass, W_{std}, of the element is co-irradiated with the sample of unknown mass W_{sam}. When the sample to be irradiated is short-lived radionuclide, both the standard and sample are irradiated separately under the same conditions, usually with a monitor of the same neutron fluence rate and both are counted in the same geometrical arrangements with respect to the gamma-ray energy. It is assumed that the neutron flux, cross section, irradiation times, and all other variables associated with counting are constant for the standard and the sample at a particular sample-to-detector geometry. With this assumption, the neutron activation equation then reduces to

$$\rho_{sam} = \frac{[(P_A/t_c)]_{sam}[\rho CDW]_{std}}{[P_A/t_c)]_{std}[CDW]_{sam}} \qquad (1)$$

Where, $(P_A/t_c)_{std}$ and $(P_A/t_c)_{sam}$ are the counting rates for standard and sample respectively, ρ_{std} and ρ_{sam} are the counting concentrations of the standard and the element of interest, respectively. C_{std} and C_{sam} are the counting factors for standard and sample D_{std} and D_{sam} are decay factors for the standard and sample, respectively.

Eqn. (1) can be reduced to:

$$\rho_{sam} = \frac{[(P_A/t_c)]_{sam}[CDW]_{std}}{\text{SA } [CDW]_{sam}} \qquad (2)$$

Where, SA is defined as $[P_A/t_c]_{std}/\rho_{std}$ and is the sensitivity of the element. Using the number of counts under the photo-peak area from standardized irradiation and counting conditions, the concentration of the element of interest can be determined.

6. Experimental Procedure

The fish samples were obtained from the Fosu Lagoon between the months of March and June 2009. The fishes had their scales well removed using a wooden knife. It was then separated into tissue, bones and gills. The parts were then washed with deionised water and dry cleaned with blotting paper. The samples were placed in separate Petri-dishes, labelled and freeze dried for a period of three days. The three parts of the samples were then milled separately.

Six portions of each part weighing 200 mg were put in a clean polythene film. The 18 portions were then folded with forceps and heat sealed with a hand held dryer. Each sample was in turn put into a rabbit capsule and smoothly heat sealed with a soldering rod.

Standard samples used were Tuna fish from International Atomic Energy Agency (IAEA) in Austria, and oyster from National Institute of Standard and Technology (NIST) in USA.

Samples were transferred into irradiation sites through pneumatic transfer system at a pressure of 60 psi. The irradiation was categorized according to the half-life of the element of interest. Samples and controls were irradiated in the Ghana Research Reactor (GHARR-1) at the Ghana Atomic Energy Commission (GAEC), operating at 15 KW at a thermal flux of 5×10^{11} $ncm^{-2}s^{-1}$.

For the medium lived elements, ^{56}Mn and ^{66}Cu, samples were irradiated for ten minutes, delayed for one minute and counted for ten minutes.

For the long lived radio nuclide ^{24}Na, samples were irradiated for one hour and delayed for 24hrs with ten minutes counting.

The irradiated samples were taken to a modern gamma measuring systems consisting of a gamma detector, i.e., N-type High Purity Germanium Detector (HPGe detector) connected to a multichannel analyzer [20,21].

7. Qualitative and Quantitative Analysis

The qualitative analysis involves the determination of the Mn, Cu, and Na, in the fish samples by the identification of the spectra peaks and assigning corresponding radionuclides and hence the elements present.

The quantitative analysis, involves the calculation of the areas under the peaks of the identified elements and converting them into concentrations using an appropriate software or equation(s) [22]. The qualitative analysis was achieved by means of ORTEC EMCAPLUS Multi-channel Analyzer (MCA) Emulation software. A Microsoft Window–based software, MAESTRO, was used for spectrum analysis [23]. This software identifies the various photo peaks, estimates and works out the areas under them. The other quantitative measurements were done using the concentration equation (Eqn. 1) in an Excel programme for calculating the elemental concentrations in µg/g.

The daily intake, gram per capita of the various elements, was calculated using data from [24].

8. Results

The mean concentrations of Mn, Cu, and Na in the various parts of the fish are presented in Table 1.

Table 1: Elemental concentrations of Mn, Cu and Na in *S. melanotheron.*

Part of Fish	Mean Concentration µg/g		
	Mn	**Cu**	**Na**
Tissue	Below DL	2.59 ± 0.34	3.28 ± 0.01
Gills	116.95 ± 6.68	974.24 ± 22.51	3.75 ± 0.02
Bones	257.02 ± 8.76	5.72 ± 0.61	2.63 ± 0.01
Total	373.97 ± 15.44	982.55 ± 23.46	9.66 ± 0.04

The detection limit (DL) for Mn is 10 ppb.

The average daily intakes (grams per capita) of the elements were calculated. These are presented in Table 2. These were based on [24], which gives the average intake of fish as 22 kg/caput/year and hence the average intake of fish per day as 60.274 µg/d.

Table 2: Mean daily intake of Mn, Cu and Na in the *S. melanotheron.*

Part of Fish	Mn mg/d	Cu µg/g	Na mg/d
Tissue	Below DL	156.11 ± 20.49	0.197 ± 0.000
Gills	7.05 ± 0.400	58721.00 ± 356.80	0.226 ± 0.001
Bones	15.49 ± 0.53	344.77.00 ± 36.8	0.158 ± 0.001
Total	22.54 ± 0.93	59222.22 ± 1414.03	0.582 ± 0.002

Table 3 gives the Recommended Dietary Allowable (RDA)/Adequate Intake (AI) for Mn, Cu and Na for Life Stage Groups. RDA/AI is the dietary requirement of a micronutrient intake level, which meets a specified criterion for adequacy and thereby minimizing risk of nutrient deficit or excess. The Maximum Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to an individual, unless otherwise specified, the UL represents the total intake from food, water, and other supplements [25].

Table 3: Recommended Dietary Allowable (RDA) / Adequate Intake (AI) and Maximum Upper Limit (UL) for Life Stage Groups [20].

Life Stage Group	RDA/AI mg/d Mn	UL mg/d Mn	RDA/AI μ g/d Cu	UL μ g/d Cu	RDA/AI mg/d Na	UL g/d Na
Infants						
0-6months	0.003	ND	200	ND	120	ND
7-12months	0.6	ND	220	ND	370	ND
Children						
1-3y	1.2	2	340	1,000	1000	1.5
4-8y	1.5	3	440	3,000	1200	1.9
Males						
9-13y	1.9	6	700	5,000	1500	2.2
14-18y	2.2	9	890	8,000	1500	2.3
19-30y	2.3	9	900	10,000	1500	2.3
31-50y	2.3	9	900	10,000	1500	2.3
50-70y	2.3	9	900	10,000	1300	2.3
>70y	2.3	9	900	10,000	1200	2.3
Females						
9-13y	1.6	6	700	5,000	1500	2.2
14-18y	1.6	9	890	8,000	1500	2.3
19-30y	1.8	9	900	10,000	1500	2.3
31-50y	1.8	9	900	10,000	1500	2.3
50-70y	1.8	9	900	10,000	1300	2.3
>70y	1.8	9	900	10,000	1200	2.3
Pregnant Women						
≤18y	2.0	9	1000	8,000	1500	2.3
19-30y	2.0	11	1000	10,000	1500	2.3
31-50y	2.0	11	1000	10,000	1500	2.3
Lactation Women						
≤18y	2.6	9	1300	8,000	1500	2.3
19-30y	2.6	11	1300	10,000	1500	2.3
31-50y	2.6	11	1300	10,000	1500	2.3

Note: *The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water. ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake* [25].

The differences between our calculated mean (Table 2) and the UL of the RDA / AI (Table 3) are presented in Table 4.

Table 4: Differences between the UL of the RDA/AI and the calculated means.

Life Stage Group	mg/d Mn	μ g/d Cu	mg/d Na
Infants			
0-6months	ND	ND	ND
7-12months	ND	ND	ND
Children			
1-3y	+ 20.54	+ 58,220	– 1,497.76
4-8y	+ 19.54	+ 56,220	– 1,897.76
Males			
9-13y	+ 16.54	+ 54,220	– 2,197.76
14-18y	+ 13.54	+ 51,220	– 2,297.76
19-30y	+ 13.54	+ 49,220	– 2,297.76
31-50y	+ 13.54	+ 49,220	– 2,297.76
50-70y	+ 13.54	+ 49,220	– 2,297.76
>70y	+ 13.54	+ 49,220	– 2,297.76
Females			
9-13y	+ 16.54	+ 54,220	– 2,197.76
14-18y	+ 13.54	+ 51,220	– 2,297.76
19-30y	+ 13.54	+ 49,220	– 2,297.76
31-50y	+ 13.54	+ 49,220	– 2,297.76
50-70y	+ 13.54	+ 49,220	– 2,297.76
>70y	+ 13.54	+ 49,220	– 2,297.76
Pregnant Women			
$\leq$18y	+ 13.54	+ 51,220	– 2,297.76
19-30y	+ 11.54	+ 49,220	– 2,297.76
31-50y	+ 11.54	+ 49,220	– 2,297.76
Lactation Women			
$\leq$18y	+ 13.54	+ 51,220	– 2,297.76
19-30y	+ 11.54	+ 49,220	– 2,297.76
31-50y	+ 11.54	+ 49,220	– 2,297.76

Note: *The negative sign (–) denotes that the values are below the ULs, whiles the positive sign (+) denotes that the values are above the ULs.*

9. Discussion

The elemental concentration of Cu was found to be very high in the gills of *S.melanotheron* (Table 2). The tissue registered the least concentration of Cu. The ratio of the elemental concentration of Cu in the tissue to gills to bones is 1:376:2. This correlates with [26].

The elemental concentration of Mn was highest (257.02 ± 8.76 µg/g) in the bones whilst that of the tissue was below the detection limit. This agrees with other results from literature where the concentrations of Mn found in tissues of marine and freshwater fish were found to range from <0.2 to 19 µg/g dry weight [27-31], while levels are about 1.6 times higher in fish from acidified lakes [32]. It also confirms the fact that concentrations of Mn at which adverse effects are observed increased with increasing water hardness [33] and the uptake of Mn by fish significantly increases with temperature [34]. The water temperature of tropical waters exceeds 20°C (68°F) and stays relatively constant throughout the year [35].

The total mean daily intake of Na (0.582mg/d) that is the mean daily intake of Na in the gills

(0.226 mg/d), bones (0.158 mg/d) and the tissue (0.197 mg/d) of *S.melanotheron* is far less than the least Recommended Dietary Allowance (RDA) of Na (120 mg/d) for all the life stage groups.

On the other hand the total mean daily intake of Mn (22.54 mg/d) in *S. melanotheron* from the Fosu Lagoon was far higher than both the highest RDA of Mn (2.6 mg/d) and UL of Mn (11 mg/d) for all the life stage groups (see Tables 2 and 3).

Again from Tables 2 and 3, the total mean daily intake of Cu (59,222.22 µg/d), which comprises the mean daily intake of Cu from the tissues (156.11 µg/d), gills (58721.00 µg/d) and bones (344.77 µg/d) in the *S.melanotheron* from the Fosu Lagoon, far exceeded the highest RDA of Cu (1,300 µg/d) and UL of Cu (10,000 µg/d) for all life stage groups.

10. Conclusion

The mean daily intake of Cu, Na, and Mn were determined by employing the technique of Instrumental Neutron Activation Analysis (INAA) with Epithermal neutrons at the Ghana Atomic Energy Commission with standard reference materials: I.A.E.A-350; Trace Elements in Tuna Fish Homogenate and National Institute of Standard and Technology (NIST), USA SRM-1566b; Trace Elements in Oyster Tissues. The mean concentrations of the elements determined are ^{56}Mn – 22.54 mg/d, ^{66}Cu – 59.22 mg/d and ^{24}Na – 2.24 mg/d. The concentrations of Cu and Mn exceeded their RDAs by 58.32 mg/d and 20.24 mg/d, respectively, while that of Na was far below the RDA by 2.33 mg/d.

In conclusion, the *Sarotherodon melanotheron*, from the Fosu Lagoon in Cape Coast has a low level of sodium but high levels of manganese and copper which make them unsafe to eat.

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References

[1] E. Aggrey-Fynn, “The contribution of the fisheries sector to Ghana’s economy”, a paper prepared on behalf of FAO as an input into the Sustainable Fisheries Livelihoods Study. (SFLP), DFID/FAO (2001).

[2] *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate.* The National Academies (2004); www.nap.edu

[3] *Sodium*, Health supplements nutritional guide, Nutritional Health Resource (2010); http://www.healthsupplementsnutritionalguide.com/Sodium.html

[4] *Agency for Toxic Substances and Disease Registry Public Health Statement* (1990); http://www.aeoncp.com.au/copper.htm

[5] L. Wilson, MD *Copper Toxicity Syndrome,* The Center for Development (2011); http://www.drlwilson.com/articles/copper_toxicity_syndrome.htm

[6] P. Eck and L. Wilson, *Toxic Metals in Human Health and Disease* (Eck Institute of Applied Nutrition and Bioenergetics, Ltd., Phoenix, AZ, 1989).

[7] *Manganese* (CASRN 7439-96-5), Integrated Risk Information System, US EPA, (2011); http://www.epa.gov/iris/subst/0373.htm

[8] *Health Effects of Manganese*, Water treatment solutions, Lenntech (2011); http://www.lenntech.com/periodic/elements/mn.htm#ixzz1Yo2PyN00

[9] W. W. Johnson and M. T. Finley, *Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates, U.S. Fish and Wildlife Service*, Columbia National Fisheries Research Laboratory, Columbia, Missouri 65201 (1965-78).

[10] International Food Information Council Foundation (2009); www.ific.org

[11] F. Schmermer, *Silt and dirt chokes Fosu Lagoon* (Central Press, 2011); http://centralpressnewspaper.blogspot.com/

[12] Fosu Lagoon, Cape Coast, on the map of Ghana, by the Cartography Unit, The Department of Geography and Tourism, University of Cape Coast, Cape Coast, Ghana, (2012).

[13] D. A. Painstil and M. Sey, “Cape Coast Fosu Lagoon needs dredging - as it breeds mosquitoes”, Ghanaian Chronicle, p.1. (2008).

[14] D. K. Essumang, G. K. Togoh and L. Chokky, Bulletin Chem. Soc. Ethiopia **23(1)**, 19 (2009).

[15] F. A. Armah, D. O. Yawson, N. M. Alex, N. M. Pappoe and E. K. A. Afrifa, Sustainability **2**, 383 (2010); doi:10.3390/su2010383; www.mdpi.com/journal/sustainability

[16] E. K. Abban, K. A. Asante and T. M. Falk, Environment of the black-chinned tilapia,

Sarotherodon melanotheron, and their potential effects on the genetic structure of stocks in Ghana, p.14. In Biodiversity and sustainable use of fish in the coastal zone. Eds. E. K. Abban, C. M. V. Casal, T. M. Falk and R. S. V. Pullin, ICLARM Conference Proceedings **63**, 71 (2000).

[17] K. N. Eyeson, Aquaculture **31**, 257 (1983).

[18] J. Jr. Blay, J. Ghana Science **38**, 47 (1998).

[19] A. M. Pollard and C. Heron, *Archaeological Chemistry*, (Royal Society of Chemistry, Cambridge, 1996).

[20] A. Chattopadhyay, and K. N. DeSilva, Trans. Am. Nucl. Soc. **32**, 185 (1976).

[21] E. H. K. Akaho, B. T. Maakuu, B. J. B. Nyarko, E. K. Osae, Y. Serfor Armah, and A. Chatt, J. Ghana Science Association **2,** No.1, 45 (2000).

[22] Z. B. Alfassi, *Chemical Analysis by Nuclear Methods* (John Wiley and Sons, Inc., 1994).

[23] D. Adomako, B. J. B. Nyarko, S. B. Dampare, Y. Serfor-Armah, S. Osae, J. R. Fianko, and E. H. K. Akaho, Environ. Monit. Assess. **141,** 165 (2008).

[24] B. S. Owusu, L. Kuwornu and A. Lomo, Annex 5: Integrated Irrigation-Aquaculture Development and Research in Ghana. In FAO Report; http://www.fao.org/docrep/005/y2807e/y2807e0g.htm (13/08/2005)

[25] Food and Nutrition Board, *Intake Applications in Dietary Assessment* (National Academy Press, Washington DC, 2001).

[26] Y. J. Baker, J. Fisheries Research Board of Canada **26**, 2785 (1969).

[27] Y. A. Greichus, A. Greichus, B. A. Amman, D. J. Call, D. C. D. Hamman and R. M. Potts, Archives of Enviromental Contamination and Toxicology, **6**, 371 (1977).

[28] Y. A. Greichus, A. Greichus, B. D. Amman, and J. Hopcraft, Bulletin of Enviromental Contamination and Toxicology **19**, 454 (1978).

[29] R. Capelli, V. Minganti, and M. Bernhard, Sci. Total Environ. **63**, 83 (1987).

[30] E. Sindayigaya, R. V. Cauwenbergh, H. Robberecht and H. Deelstra, Sci. Total Environ. **144**, 103 (1994).

[31] J. S. Heiny and C. M. Tate, Archives of Enviromental Contamination and Toxicology, **32**, 246 (1997).

[32] G. Moreau, C. Barbeau, J. J. Frenette, J. Saintonge, and M. Simoneau, Canadian J. Fisheries and Aquatic Sciences, **40(10)**, 1685 (1983).

[33] P. S. Reimer, "Environmental Effects of Manganese and Proposed Freshwater Guidelines to Protect Aquatic Life in British Columbia", Department of Chemical & Bio-Resource Engineering, University of British Columbia (1999); http://www.env.gov.bc.ca/wat/wq/BCguidelines/manganese/update.html

[34] D. W. Moreau, R. J. Vetter, and G. J. Atchinson, Health Physics **38(2)**, 221 (1980).

[35] Water Encyclopedia, Science and Issues, Oceans, Tropical - seawater, river, sea, temperature, system, plants, marine, oxygen, Pacific; http://www.waterencyclopedia.com/Oc-Po/Oceans-Tropical.html#b#ixzz20QbsQpd4

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