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Daily Intake and Sources of Trace Elements in *Sarotherodon melanotheron* from the Benya Lagoon, KEEA, Ghana, Using Instrumental Neutron Activation Analysis

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Abstract

Consuming fish meal may have health benefits and also contain contaminants. The mean concentrations of ten trace elements (TE) in *Sarotherodon melanotheron* (Blackchin tilapia) obtained from the Benya Lagoon at Komenda Edina Eguafu Abrem Municipality (KEEA) in the Central Region of Ghana were determined. Since this fish is consumed as a whole, the parts of the fish investigated were the bones, muscles and gills. The health index was also determined. The concentrations were determined using Instrumental Neutron Activation Analysis (INAA) and the standard reference materials used were the International Atomic Energy Agency (IAEA)-336; IAEA-407, IAEA-350 and National Institute of Standard and Technology (NIST) USA SRM 1577b. The *Relative standardization* method was used in the quantification of all the elements. The estimated health risk calculated showed that the concentrations of Ca, I, K and Al were high in the order $Ca > I > K > Al$ with all exceeding the Maximum Upper Limit (UL) of the Recommended Dietary Allowance (RDA) for all life stage groups investigated. Cu also exceeded the RDA, for the age group between 1-13 years and Mn for children between 1-3 years. Cl and Na registered concentration values below their RDA. Analysis showed that all the elements except Ca and K which had the highest concentrations in all the parts of the fish, the same amounts of concentrations of the elements is derived irrespective of the date one eats the fish. And anyone who ate some of the fish was likely to derive more Ca and K nutrients. Using Pearson correlation coefficients calculation, the probable sources of the ten trace elements were determined. This knowledge may also be useful for designing the best management plans for the Benya Lagoon's ecosystems restoration and protection efforts.

Keywords: Instrumental Neutron Activation Analysis, (INAA), Blackchin tilapia, *Sarotherodon melanotheron*, Benya Lagoon, Ghana.

Introduction

The essential trace elements (TE) needed by the human body range from metals to non-metals. What makes them essential is their variable oxidation state as they are important parts of oxidation-reduction enzymes in the body that play roles in the transport of proteins and detoxification. TE in general are carried bound to transport proteins in the blood as they are usually toxic when in free form and therefore conveyed bound from entry to exit within the body (Minerals Education Coalition, 2013; Daily-vitamins, 2013; Wilson, 2011; US EPA, 2011). Other TE required by the body are categorized as probably essential (for those that play very limited role in the body chemistry) or non-essential. Such classifications are based on their biological effect, diseases that occur due to their deficiency and toxicity due to overdose.

Our needs for these TE are generally met from our dietary source by eating a variety of foods from the different food-groups, some of which include meat, fish, poultry, milk and eggs, just to mention a few. Although required, such elements must be taken in amounts that are necessary for the maintenance of the vital functions of the body as they may be toxic at concentrations beyond those necessary for their biological functions (Aggrey-Fynn, 2001). Many countries and international organizations like the FAO, WHO and IAEA have shown an increasing interest in the concentrations of TE in foods and have started programs to monitor them as their scrutiny and daily intake has become very important (Smil, 2002).

It is worth mentioning that due to the scare caused by the foot-and-mouth disease, mad-cow disease and bird influenza people these days have resorted to eating more of fish than meat (Smil, 2002). Indeed, the consumption of fish in Ghana is high with an average consumption of 22kg/capita/year, while that of beef is 1.08kg, 0.70kg for small ruminants and poultry meat and 0.49kg for pork (Owusu et al, 2005). Other than taking meat, milk, and egg as their source of protein most Ghanaians now prefer taking fish as they find it relatively affordable, especially so for people staying close to a water body like a lagoon where it is relatively easier for them to get fish at a cheaper price (Aggrey-Fynn, 2001). One of such lagoons is the Benya Lagoon (Figure 1) which is located in the Komenda Edina Eguafo Abrem (KEEA) Municipality in the Central Region of Ghana. The main fish that thrives in this lagoon is the *Sarotherodon melanotheron* (Blackchin tilapia) which is indeed a great delicacy for the locals living particularly in this municipality and the Central Region as a whole. It is enjoyed to the extent that during its preparation for eating it is only the scales and fins that are

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removed as it is consumed with its bones, muscles and gills due to its relatively small size and soft texture when cooked (Vowotor et al, 2012). The issue, however, is that there have been growing concerns about the health implications of consuming *S. melanotheron* from the Benya Lagoon due to the polluted nature of the lagoon.

Fish is therefore said to be toxic (poisoned) when it contains some of these elements beyond a desirable threshold or quantity. The concentration of these elements in various parts of the fish may vary since the rate of absorption by the respective tissues also varies (Johnson, 1980). Contamination of food products through air, soil, and water pollution is becoming an unavoidable problem these days (MacFarlane and Burchett, 2000). Trace elements may come from natural sources, leached from rocks and soils according to their geochemical mobility or come from anthropogenic sources, as the result of human land occupation and industrial pollution. Depending on their solubility, these elements may eventually become associated with suspended particulate matter and/or accumulate in the bottom sediments. The increase of industrial activities has intensified environmental pollution problems and the deterioration of several aquatic ecosystems with the accumulation of these elements in biota and flora (Abolude et al, 2009).

This work investigates the concentrations of ten TE, namely Arsenic (As), Calcium (Ca), Chlorine (Cl), Copper (Cu), Iodine (I), Potassium (K), Manganese (Mn), Sodium (Na), Sulphur (S), (essential TE), and Aluminium (Al), (non-essential TE) in the bones, muscles and gills of *Sarotherodon melanotheron* (Blackchin tilapia) captured from the Benya lagoon using instrumental neutron activation analysis, INAA. The concentrations in the various parts may vary as the rate of absorption by the respective tissues vary (Johnson, 1980). This study determines if the concentrations of these elements fall within the Recommended Dietary Allowance (RDA) and therefore safe for human consumption. The study also determined which activities around the lagoon were the sources of each of the ten elements.

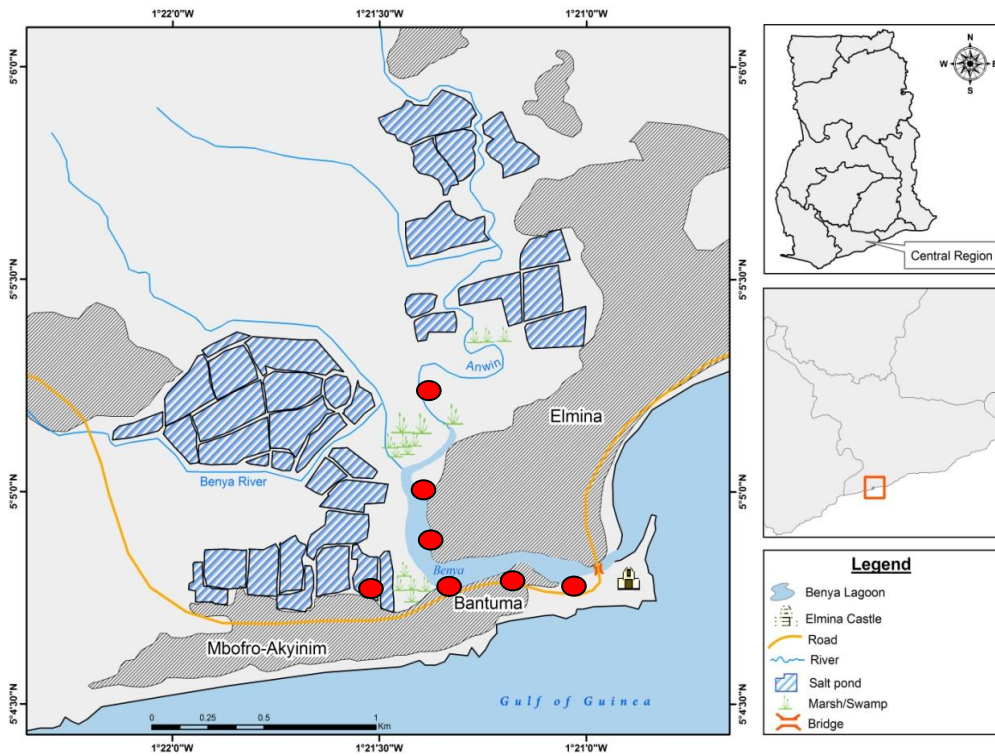


Figure 1: A map of Benya lagoon, Ghana, showing the sampling site (Benya Lagoon, 2012).

Study Area

The Benya Lagoon is located within the KEEA metropolis of the Central Region of Ghana, along the Gulf of Guinea, (Figure 1).

The main fish caught in this Benya Lagoon is *S. melanotheron*. The Benya lagoon has been reported by the Environmental Protection Agency (EPA), Ghana to be one of the polluted lagoons in the country. The lagoon is drying up as a result of siltation and heavy pollution (Essumang, et al, 2009), and this threatens the livelihood of more than 500 fishermen (Schmermer, 2011). Although the intensity of pollution has long been identified, no action has been taken (Environmental Pollution, Central, 2006). In spite of the spate of pollution in this lagoon, residents unfortunately continue to patronise the fish from this lagoon.



Figure 2: Pictorial view of Benya Lagoon and its environs

The Benya Lagoon has become a dumping ground due to poor and inadequate facilities for the disposal of both solid and liquid waste. The lagoon is also being polluted by other activities such as the dumping of 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, hair dressers and burning of domestic wastes along the boundaries of the lagoon, (Figure 2), (Environmental Pollution, Central, 2006). This has almost led to the outbreak of diseases like cholera, yaws, dysentery and malaria.

The fishery in the lagoon is reportedly dominated by tilapias which make up 60 - 80% of all fish caught (Abban, 2000). The most abundant being the blackchin tilapia, *S. melanotheron*, which is the mainstay of the fisheries of many lagoons in West Africa including Ghana (Eyeson, 1983; Blay, 1998). Since most of these fish caught in the Benya Lagoon are stunted (Figure 3).



Figure 3: *Sarotherodon melanotheron*, a tilapia species from the Benya Lagoon

Instrumental Neutron Activation Analysis, (INAA)

Instrumental Neutron Activation Analysis, (INAA), was used to determine the concentration of the ten elements in the *S. melanotheron* from the Benya Lagoon. INAA is a nuclear process used for determining concentrations 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 (Pollard and Heron, 1996). 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) and needs no chemical preparation.

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 samples 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:

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

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.

Experimental Procedure

The services of professional fishermen were employed during the sampling period as they aided with their boats and skills in reaching some of the not-easily-accessible stations earmarked for investigation. Hundred fish samples were bought from the fisher folks at the points marked red in Figure 1 on February 16, 2012 and February 23, 2012. The fish samples obtained from the Benya Lagoon were transported to the laboratory of the Department of Fisheries, University of Cape Coast, and had their scales well removed using a new kitchen stainless steel knife. It was then washed with deionised water, dry cleaned with blotting paper and parts separated into tissue, bones and gills. At a temperature of -10°C samples were transported to Ghana Atomic Energy Commission Preparation Laboratory. Samples were then lyophilized (Christ Gamma 1 – 16) for 72 hrs at -30°C (corresponding to a vapour pressure of 0.370 mbar). This drying method was employed to ensure preservation of initial sample texture and to facilitate sample milling. Using a commercial blender with stainless steel blades, the freeze dried samples were then homogenized.

About 200 mg each of the pulverized and homogenized samples were weighed, wrapped and heat sealed (using soldering rod) in ultra-clean polyethylene films. For short lived radioisotopes, the wrapped films were packaged into a 7.0 ml polyethylene vial (i.e. one wrapped film to one polyethylene vial), which were in turn heat-sealed for irradiation.

The irradiation vials (capsules) that were used were pre-cleaned by washing them first with distilled water and then soaked in an acidic reagent for 24 hrs, and then rinsed in distilled deionised water. The irradiation vials were further soaked in HNO_3 for another 24 hrs. They were then rinsed thoroughly with distilled deionised water and air-dried in a clean fume hood.

Standard reference materials namely IAEA-336 (trace and minor elements in Lichen), IAEA-407 (trace elements and methyl mercury in fish tissue), IAEA-350 (trace elements in tuna fish homogenate) and SRM 1577b (Bovine liver) were prepared and packed similarly as the samples.

However, for the medium lived radioisotopes, the standard reference materials were sandwiched between the wrapped samples and together, packaged into one polyethylene vial for irradiation.

Irradiation of Sample

The prepared samples were all irradiated in the Ghana Research Reactor-1(GHARR-1) facility at the Ghana Atomic Energy Commission, Kwabenya, operating at 15 kW at a thermal neutron flux of $5 \times 10^{11} \text{ ncm}^{-2}\text{s}^{-1}$. Samples were transferred into the irradiation sites via pneumatic transfer system at a pressure of 0.6 Mpa. The categorization of irradiation was done based on the half-life of the elements of interest.

For the medium lived elements, ^{79}As , ^{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 (Chattopadhyay, and DeSilva, 1976: Akaho et al, 2000). The detection limit (DL) of the various elements of interest for Neutron Activation Analysis are shown Table 1.

Table 1: Detection Limit of Elements under consideration in $\mu\text{g/g}$.

Element	Al, Cu and K	Cl and Na	I and Mn	S and Ca	As
DL ($\mu\text{g/g}$)	0.01	0.001	0.0001	1.0	0.00001

Qualitative and Quantitative Analysis

The qualitative analysis involves the determination of the Al, As, Ca, Cl, Cu, I, K, Mn, Na and S in the fish samples by the identification of the spectra peaks and assigning corresponding radionuclides and hence the elements present. The counting of the induced radioactivity was performed by a PC- based γ -ray spectrometry. It consists of an n-type high purity Germanium (HPGe) detector (model GR2518) coupled to a computer-based Multichannel Analyzer via electronic modules and a spectroscopy amplifier (model 2020, Canberra Industries Incorporated). The relative efficiency of the detector is 25% with an energy resolution of 1.8 KeV at γ -ray energy of 1332 KeV of ^{60}Co .

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) (Alfassi, 1994). 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 (Adomako, et al., 2008). 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 (equation 1) in an Excel programme for calculating the elemental concentrations in $\mu\text{g/g}$. Minitab was used in statistical analysis.

Results, Analysis and Discussion

The mean concentrations of ten elements in the various parts of the fish are presented in Table 2 and Table 3. Since the size of *S. Melanotheron*, is small, the locals in KEEA normally eat the tissue, gills and bones together. Only the scales and sometimes the fins are removed. So the concentration of various elements in the tilapia comprise of that of the gills, bones and tissues.

Table 2: Elemental concentrations of the ten elements in *S. melanotheron* on 16/02/2012

Element	Bone $\mu\text{g/g}$	Muscle $\mu\text{g/g}$	Gill $\mu\text{g/g}$	Total $\mu\text{g/g}$
Al	31.49 \pm 4.98	24.01 \pm 3.80	27.83 \pm 4.40	83.33 \pm 13.18
As	27.14 \pm 4.36	36.20 \pm 5.96	36.03 \pm 6.01	99.37 \pm 16.33
Ca	51711.11 \pm 7756.59	1617567.73 \pm 242635.16	31893.00 \pm 4783.95	1701171.84 \pm 255175.70
Cl	5590.70 \pm 749.63	8384.78 \pm 1123.46	6131.00 \pm 822.00	20106.48 \pm 2695.09
Cu	29.98 \pm 4.50	62.8 \pm 6.59	54.84 \pm 6.04	147.62 \pm 17.13
I	0.81 \pm 0.12	0.98 \pm 0.15	4.10 \pm 0.62	208.33 \pm 0.92
K	55257.25 \pm 6459.16	85676.20 \pm 12109.79	125464.40 \pm 14547.01	266397.85 \pm 33115.96
Mn	30.23 \pm 4.90	11.46 \pm 1.92	20.20 \pm 3.28	61.89 \pm 10.10
Na	5639.75 \pm 473.74	4615.67 \pm 387.71	12427.50 \pm 503.51	22682.92 \pm 1364.96
S	4420.33 \pm 663.05	4784.00 \pm 717.60	3781.67 \pm 567.25	12986.00 \pm 1947.90

Table 3: Elemental concentrations of the ten elements in *S. melanotheron* on 23/02/2012.

Element	Bone $\mu\text{g/g}$	Muscle $\mu\text{g/g}$	Gill $\mu\text{g/g}$	Total $\mu\text{g/g}$
Al	15.92 \pm 2.52	22.51 \pm 3.57	26.38 \pm 4.18	64.81 \pm 10.27
As	26.06 \pm 4.41	33.26 \pm 5.56	27.65 \pm 4.58	86.97 \pm 14.55
Ca	16547.90 \pm 2482.19	11331.00 \pm 1699.65	39438.89 \pm 5915.78	67317.79 \pm 10097.62
Cl	9001.00 \pm 1206.13	11027.10 \pm 1477.60	11720.44 \pm 1585.65	31748.54 \pm 4269.38
Cu	27.47 \pm 7.56	30.77 \pm 4.20	40.83 \pm 2.51	99.07 \pm 14.27
I	0.96 \pm 0.14	1.28 \pm 0.19	3.00 \pm 0.45	5.24 \pm 0.78

K	45965.57 ± 5346.86	66864.75 ± 7720.55	31351.40 ± 3690.44	144181.72 ± 16757.85
Mn	8.78 ± 1.39	5.82 ± 0.87	8.67 ± 1.41	23.27 ± 3.67
Na	7687.00 ± 645.71	7422.25 ± 623.47	10995.40 ± 923.46	26104.65 ± 2192.64
S	8737.67 ± 1310.65	9311.50 ± 1396.73	6803.00 ± 1020.45	24852.17 ± 3727.83

The elements were tested for normality using the Kolmogorov-Smirnov Test for normality using a 0.05 level of significance. We usually use Kolmogorov-Smirnov test to check the normality assumption in the Analysis of Variance (Wayne, 1990). The hypothesis was stated as;

H_0 : The element is normally distributed.

H_1 : The element is not normally distributed

Table 4: Results of normality test of elements

Element	P-value	Element	P-value
Al	> 0.150	I	0.044
As	> 0.150	Log I	> 0.150
Ca	< 0.010	K	> 0.150
Log Ca	0.041	Na	> 0.150
Cl	> 0.150	Mn	0.136
Cu	> 0.150	S	> 0.150

All the elements in the exception of Calcium and Iodine were not significant, thus not normally distributed. Hence the above mentioned elements were logarithmically transformed and tested for normality. The p-value for Iodine improved from 0.044 to >0.150, for Calcium from 0.010 to 0.041. Therefore, it can be concluded that, in general, the data was normally distributed.

To make logical inference to the data obtained, hypotheses testing was again applied (statistical inference technique) to test the mean elemental concentration at the two times and the mean concentration in the three parts of the fish at a 95% confidence interval (Weiss, 2008).

The hypothesis for the two-way Analysis of Variance, ANOVA is set as follows;

H_0 : The mean concentrations of elements are equal

H_1 : The mean concentrations of elements are not equal for some two elements.

And;

H_0 : The mean concentrations in the parts of fish are equal

H_1 : The mean concentrations in the parts of fish are not equal.

The ANOVA was then used to analyse the variation in the means. Some of the underlying assumptions considered were: normality of the population, homogeneity of variance and the distribution of the effect of extraneous variables equally over all levels of the independent variables. The p-value is a measure of how likely that no real differences existed. Therefore a p-value less than 0.05 indicates that there are differences in the mean concentration, therefore reject H_0 (Spatz, 2005).

Table 5: A two-way ANOVA table to check for differences in the means of the concentrations of elements and concentrations in the 3 parts of the fish (16/02/2012).

Source	Degree of freedom	Sum-of-squares	Mean sum of squares	p-value
Concentrations of Element	9	41099603583	4566622620	0.000
Parts of the Fish	2	596247469	298123734	0.000
Error	30	808775939	26959198	
Total	59	46948599486		

Table 6: A two-way ANOVA table to check for differences in the means of the concentrations of elements and concentrations in the 3 parts of the fish (23/02/2012).

Source	Degree of freedom	Sum-of-squares	Mean sum of squares	p-value
Concentrations of Element	9	12636688070	1404076452	0.000
Parts of the Fish	2	34039399	17019699	0.216
Error	30	316765248	10558842	
Total	59	15152749449		

The Table 5 and Table 6 show the results from the Two-way ANOVA. In Table 5, the result shows that the mean concentrations of elements differ and the mean concentration of elements in the three different parts of the fish were also significantly different with p-values of 0.000.

The p-value of 0.000 in Table 6 meant the null hypothesis was rejected, which implies that, the mean concentrations of elements were different. And the p-value of 0.216 meant that the null hypothesis was not rejected and the concentrations in the three parts were not statistically significant.

Table 7: Model Summary

	Standard Deviation	R-squared value	Adjusted R-squared value	Predicted R-squared value
16/02/12	5192.22	98.28%	96.61%	93.11%
23/02/12	3249.44	97.91%	95.89%	91.64%

Table 7 summarizes the models in Table 5 and Table 6. The R-square values were 98.28% and 97.91% which mean that more than 50% of variation in the data is adequately explained by the model. Therefore, the ANOVA model is well suited for the data.

Again, to confirm the statistical significance observed in Tables 6 and Figure 5, a Tukey's Honestly Significant Difference Test (Tukey Test for short) was conducted at 95% confidence interval. Tukey Test is a post hoc test for ANOVA. The test pairs each sample mean with every other

mean, a procedure called Pairwise comparisons. For each pair, the Tukey test tells if one sample mean is significantly larger than the other (Spatz, 2005).

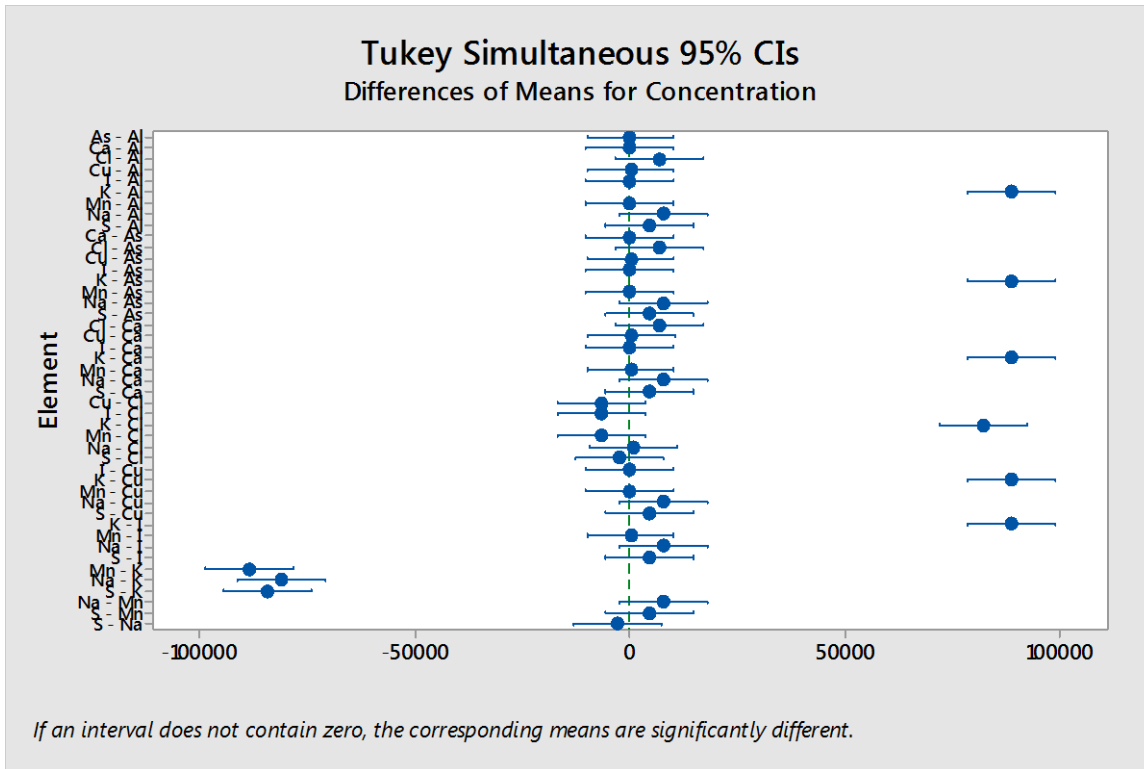


Figure 6: A graph showing pairwise comparison elements for sample 1 (16/02/12).

Table 8: Comparisons for Concentration of elements in Sample 1 (16/02/12)

Element	Mean	Grouping
Al	27.8	B
As	33.1	B
Ca	5.1	B
Cl	6702.2	B
Cu	49.2	B
I	0.2	B
K	88799.3	A
Mn	20.6	B
Na	7561.0	B
S	4328.7	B

*Means that do not share a letter are significantly different

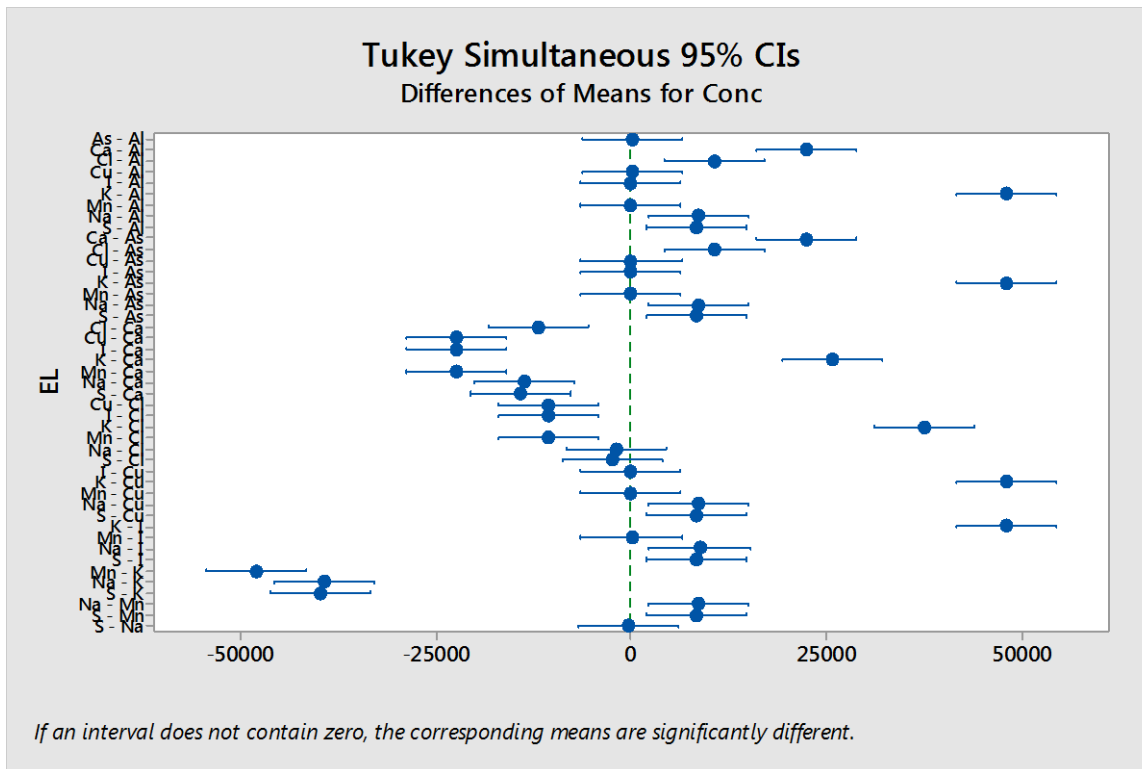


Figure 7: A graph showing pairwise comparison elements for sample 2 (23/02/12).

Table 9: Comparisons for Concentration of elements in Sample 2(23/02/12)

Element	Mean	Grouping
K	48060.6	A
Ca	22439.3	B
Cl	10582.8	C
Na	8701.5	C
S	8384.1	C
Cu	33.0	D
As	29.0	D
Al	21.6	D
Mn	7.8	D
I	1.7	D

*Means that do not share a letter are significantly different.

Figure 6 and Figure 7, are the pairwise comparisons of elements using Tukey's test at 95% confidence interval. It can be observed that, concentration of some element pairs were significantly different. Table 8 indicates that, K is the element causing significant difference in the mean concentrations of elements of the sample collected on 16/02/12. Also, K, Ca, Cl, Na, S also caused statistical significance in the mean concentration of elements of the sample collected on 23/02/12.

However, even though in the ANOVA of the sample 1 the mean concentration of elements in the parts of the fish was significant, a Tukey test showed otherwise. This can be as a result of minute differences in means detected by the model but not the further test; this is shown in Figure 8 and Table 10. Hence can be concluded that, there was no significant difference in the concentration of elements in the three major parts of the fish.

Likewise, the pairwise comparison of fish parts for Sample 2 had the same conclusion as the ANOVA. The mean concentration of elements in the three main parts of the fish was not statistically significant; shown in Figure 9 and Table 11.

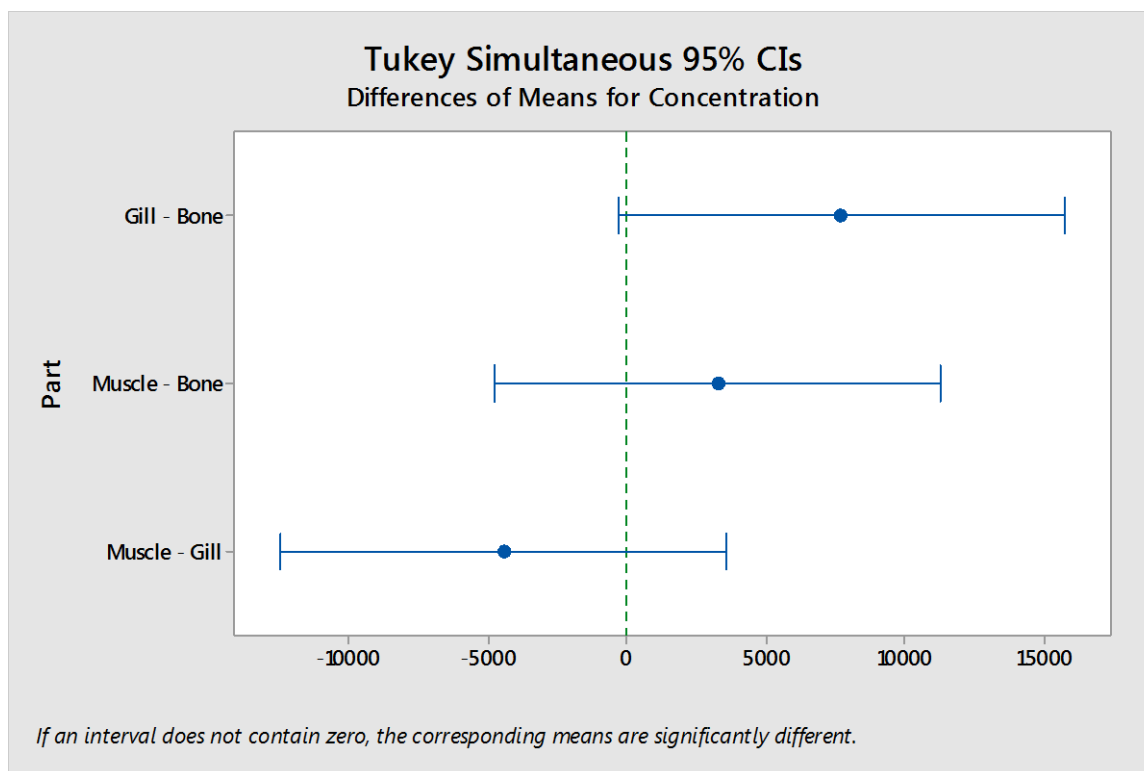


Figure 8: A graph showing pairwise comparison elements for sample 1 (16/02/12).

Table 10: Comparisons for Concentration of elements in Sample 1(16/02/12)

Element	Mean	Grouping
Gill	14794.9	A
Muscle	10360.1	A
Bone	7103.1	A

Tukey Pairwise Comparisons: Response = Conc, Term = FP

Grouping Information Using the Tukey Method and 95% Confidence

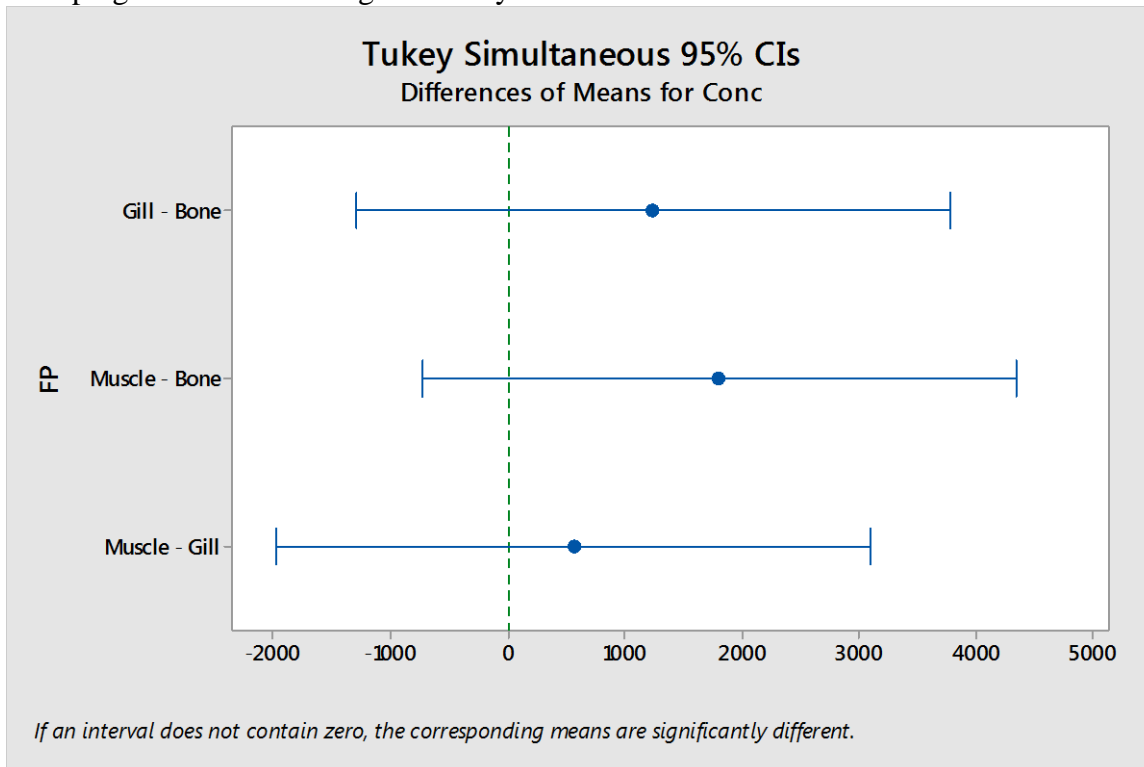


Figure 9: A graph showing pairwise comparison elements for sample 2 (23/02/12).

Table 11: Comparisons for Concentration of elements in Sample 2(23/02/12)

Element	Mean	Grouping
Gill	10605.0	A
Muscle	10041.6	A
Bone	8801.8	A

Means that do not share a letter are significantly different.

The next test was to find out if the concentration of elements in the fish differed statistically as to the time the fish was caught. Sample sizes of at least 30 are needed for methods in hypothesis testing. The reason is that, the central limit theorem enables us to approximate the sampling distribution of the sample mean with the normal probability distribution. The central limit theorem no longer applies for a sample size less than 30. However, the t-distribution can be used to make inferences about the population mean and assume the population is normal and the population standard deviation is unknown (Anderson *et al*, 1987). The F-test precedes the t-test; to test for differences in the sample variances since population standard deviation is unknown and to suggest

which form of t-test to perform (Devore, 2004). Below is the hypothesis for the F-test for mean concentration at the two times 16/02/2012 and 23/02/2012:

$$H_0: \sigma_1^2 = \sigma_2^2$$

$$H_1: \sigma_1^2 \neq \sigma_2^2$$

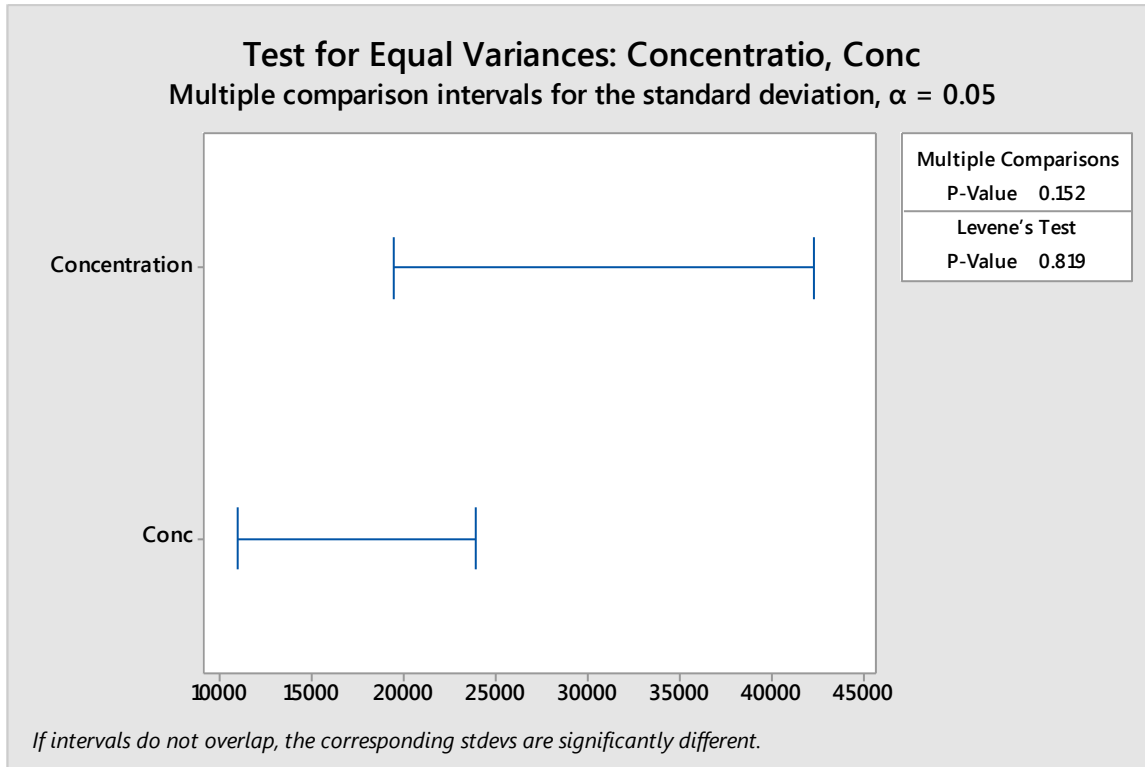


Figure 10: Test for equal variances for mean concentration of Sample 1 and Sample 2

The figures above show results from the F-test, which had p-value of 0.819. There was not enough statistical evidence to reject the null hypothesis. Since the null hypothesis is not rejected, the pooled t-test can be used. However, the procedure without pooled variance was maintained since it was less cumbersome to compute and may not necessarily lead to a different conclusion. The t-test

was then conducted. $t = \frac{\chi}{\Theta}$ (Weiss, 2008), where $\chi = (\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)$ and

$$\Theta = \sqrt{\left(\left(\frac{s_1^2}{n_1} \right) + \left(\frac{s_2^2}{n_2} \right) \right)}$$

with degree of freedom when sample variances are unequal is obtained by the

formula: $\frac{\alpha}{\beta}$, where $\alpha = \left(\left[\frac{s_1^2}{n_1} \right] + \left[\frac{s_2^2}{n_2} \right] \right)^2$ and $\beta = \frac{\left(\frac{s_1^2}{n_1} \right)^2}{n_1 - 1} + \frac{\left(\frac{s_2^2}{n_2} \right)^2}{n_2 - 1}$. The following

were the hypotheses and results:

$H_0: \mu_1 - \mu_2 = \sigma_0$ Versus $H_1: \mu_1 - \mu_2 \neq \sigma_0$. At 95% confidence interval, the mean concentration of elements at the two different times will be between (-7381, 9254). The test statistic value and p-value were; 0.22 and 0.824 respectively. Therefore it can be concluded that the mean concentration of Sample 1 and Sample 2 are not significantly different. Irrespective of the time the fish is harvested, the mean concentration of elements may not be different; however, individual concentration of elements in the fish may differ.

B: Estimation of Health Risk

Recommended Dietary Allowable (RDA)

Recommended Dietary Allowable (RDA) is the dietary requirement for a micronutrient is an intake level which meets a specified criteria for adequacy, thereby minimizing risk of nutrient deficit or excess. A 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 and unless otherwise specified, the UL represents total intake from food, water, and other supplements that is unlikely to pose risk of adverse health effects from excess in almost all (97.5%) apparently healthy individuals in an age- and sex-specific population group (Food and Nutrition Board, 2001; Dietary Reference Intakes, 2013).

Estimated Average Requirement (EAR)

The EAR is the median daily intake value that is estimated to meet the requirement of half the healthy individuals in a life-stage and gender group. At this level of intake, the other half of the individuals in the specified group would not have their needs met. The EAR is based on a specific criterion of adequacy, derived from a careful review of the literature. Reduction of disease risk is considered along with many other health parameters in the selection of that criterion. The EAR is used to calculate the RDA. It is also used to assess the adequacy of nutrient intakes, and can be used to plan the intake of groups. (Dietary Reference Intakes, 2013).

Adequate Intake (AI)

If sufficient scientific evidence is not available to establish an EAR on which to base an RDA, an AI is derived instead. The AI is the recommended average daily nutrient intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people who are assumed to be maintaining an adequate nutritional state. Vowotor, M.K., Sackey, S.S., Hood, C.O., Owusu, A., Tatchie, E., Adukpo, D. C., Mireku, K. K., Agor, S., Letsa, C. B., Nyarko, S. (2015). Daily Intake and Sources of Trace Elements in *Sarotherodon melanotheron* from the Benya Lagoon, KEEA, Ghana, Using Instrumental Neutron Activation Analysis. *Journal of Basic & Applied Sciences*, 1 (3), 1-35

The AI is expected to meet or exceed the needs of most individuals in a specific life-stage and gender group. When an RDA is not available for a nutrient, the AI can be used as the goal for usual intake by an individual. (Dietary Reference Intakes, 2013).

NOTE FOR TABLE 2:

&= The beneficial effects of potassium appear to be mainly from the forms of potassium found naturally in foods such as fruits and vegetables. Supplemental K should only be provided under medical supervision because of the well documented potential for toxicity. So for this work the mean values would be used since K has no ULs.

** = Due to lack of suitable data, ULs could not be established for S, As. This does not mean that there is no potential for adverse effects resulting from high intakes (Dietary Reference Intakes, 2013). Since arsenic has only recently been classified as an essential nutrient it has no official RDA. However, most sources recommend consuming between 0.0125 milligrams (mg) and 0.025mg of this nutrient each day. Organic arsenic has no tolerable upper limit (UL) and consuming high levels is not thought to be dangerous. However, consuming 1mg or more of inorganic arsenic is dangerous and can lead to a number of unpleasant symptoms (Dietary Reference Intakes, 2013).*

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 (Dietary Reference Intakes, 2013).

Average Daily Intake

Using Tables 2 and 3, the total average of the Mean concentrations of element in *S. Melanotheron* was calculated as shown in Table 12. The average daily intake (grams per capita) in Table 13 were then calculated from the values in Table 12. Since the average intake of fish is 22 kg/caput/year. Therefore the average intake of fish per day is therefore 60.274 g/d (Owusu et al, 2005). Then the differences between the UL of the RDA / AI and the calculated means were calculated in Table 15:

Table 12: Total Average of the Mean concentrations of element in *S. Melanotheron* in $\mu\text{g/g}$.

Element	Total Mean concentrations in $\mu\text{g/g}$
Al	74.07 \pm 11.73
As	93.17 \pm 15.44
Ca	884244.82 \pm 132636.66
Cl	25927.51 \pm 3482.24
Cu	123.35 \pm 15.70

Vowotor, M.K., Sackey, S.S., Hood, C.O., Owusu, A., Tatchie, E., Adukpo, D. C., Mireku, K. K., Agor, S., Letsa, C. B., Nyarko, S. (2015). Daily Intake and Sources of Trace Elements in *Sarotherodon melanotheron* from the Benya Lagoon, KEEA, Ghana, Using Instrumental Neutron Activation Analysis. *Journal of Basic & Applied Sciences*, 1 (3), 1-35

I	106.79 ± 0.85
K	205289.79 ± 24936.91
Mn	42.58 ± 6.89
Na	24393.79 ± 1778.80
S	18919.09 ± 2837.87

Table 13: Mean daily intake of Al, As, Ca, Cl, Cu, I, K, Mn, Na and S in the *S. melanotheron*.

Element	Daily Intake in µg/d
Al	4464.50 ± 707.01
As	5615.73 ± 930.63
Ca	53296972.28 ± 7994542.05
Cl	1562754.74 ± 209888.53
Cu	7434.80 ± 946.30
I	6436.66 ± 51.23
K	12373636.80 ± 1503047.31
Mn	2566.47 ± 415.29
Na	1470311.30 ± 107215.39
S	1140329.23 ± 171049.78

Table 14: Maximum Upper Limit (UL) of Recommended Dietary Allowable (RDA)/Adequate Intake (AI) for Life Stage Groups.

(Food and Nutrition Board, 2001: Dietary Reference Intakes, 2013: Dietary Reference Intakes, A Risk..., 2013)

Life Stage Group		Al	* As	Ca	Cl	Cu	I	& K	Mn	Na	* S	
		RDA/AI I (μ g/d)	AI N/A	RDA/AI (μ g/d)	RDA/AI (μ g/d)	RDA/AI (μ g/d)	RDA/AI I (μ g/d)	AI (μ g/d)	AI (μ g/d)	AI (μ g/d)	AI N/A	
Infants	0-6months	ND	ND	1,000,00	ND	ND	ND		ND	ND	ND	
	7-12months	ND	ND	0 1,500,00 0	ND	ND	ND	400,000 700,000	ND	ND	ND	
Children	1-3y	300	ND	2,500,00	2,300,000	1,000	200	3,000,00	2,000	1,500,00	ND	
	4-8y	400	ND	0 2,500,00 0	2,900,000	3,000	300	0 3,800,00 0	3,000	0 1,900,00 0	ND	
Males	9-13y	600	ND	3,000,00	3,400,000	5,000	600	4,500,00	6,000	2,200,00	ND	
	14-18y	800	ND	0	3,600,000	8,000	900	0	9,000	0	ND	
	19-30y	1,000	ND	3,000,00	3,600,000	10,000	1,100	4,700,00	9,000	2,300,00	ND	
	31-50y	1,000	ND	0	3,600,000	10,000	1,100	0	9,000	0	ND	
	50-70y	1,000	ND	2,500,00	3,600,000	10,000	1,100	4,700,00	9,000	2,300,00	ND	
	>70y		1,000	ND	0	3,600,000	10,000	1,100	0	9,000	0	ND
					2,500,00 0 2,000,00 0 2,000,00 0				4,700,00 0 4,700,00 0 4,700,00 0		2,300,00 0 2,300,00 0 2,300,00 0	

Vowotor, M.K., Sackey, S.S., Hood, C.O., Owusu, A., Tatchie, E., Adukpo, D. C., Mireku, K. K., Agor, S., Letsa, C. B., Nyarko, S. (2015). Daily Intake and Sources of Trace Elements in *Sarotherodon melanotheron* from the Benya Lagoon, KEEA, Ghana, Using Instrumental Neutron Activation Analysis. *Journal of Basic & Applied Sciences*, 1 (3), 1-35

Females	9-13y	600	ND	3,000,00	3,400,000	5,000	600	4,500,00	6,000	2,200,00	ND
	14-18y	800	ND	0	3,600,000	8,000	900	0	9,000	0	ND
	19-30y	1,000	ND	3,000,00	3,600,000	10,000	1,100	4,700,00	9,000	2,300,00	ND
	31-50y	1,000	ND	0	3,600,000	10,000	1,100	0	9,000	0	ND
	50-70y	1,000	ND	2,500,00	3,600,000	10,000	1,100	4,700,00	9,000	2,300,00	ND
	>70y	1,000	ND	0	3,600,000	10,000	1,100	0	9,000	0	ND
					2,500,00				4,700,00		2,300,00
				0				0		0	
				2,000,00				4,700,00		2,300,00	
				0				0		0	
				2,000,00				4,700,00		2,300,00	
				0				0		0	
Pregnant Women	≤18y	800	ND	3,000,00	3,600,000	8,000	900	4,700,00	9,000	2,300,00	ND
	19-30y	1,000	ND	0	3,600,000	10,000	1,100	0	11,000	0	ND
	31-50y	1,000	ND	2,500,00	3,600,000	10,000	1,100	4,700,00	11,000	2,300,00	ND
				0				0		0	
				2,500,00				4,700,00		2,300,00	
				0				0		0	
Lactating Women	≤18y	800	ND	3,000,00	3,600,000	8,000	900	5,100,00	9,000	2,300,00	ND
	19-30y	1,000	ND	0	3,600,000	10,000	1,100	0	11,000	0	ND
	31-50y	1,000	ND	2,500,00	3,600,000	10,000	1,100	5,100,00	11,000	2,300,00	ND
				0				0		0	
				2,500,00				5,100,00		2,300,00	
				0				0		0	

Table 15: Differences between the UL of the RDA / AI and the calculated means

Life Stage Group		Al <i>RDA/AI</i> (µg/d)	Ca <i>RDA/AI</i> (mg/d)	Cl <i>AI</i> mg/d	Cu <i>RDA/AI</i> (µg/d)	I <i>RDA/AI</i> µg/d	K <i>AI</i> mg/d	Mn <i>AI</i> (mg/d)	Na <i>AI</i> mg/d	
Infants	0-6months	ND	+5229	ND	ND	ND	+	ND	ND	
	7-12months	ND	7 +1,500	ND	ND	ND	11974 +	ND	ND	
							11674			
Children	1-3y	+ 4165	+5179	- 737	+ 6435	+ 6237	+ 9374	+1	- 30	
	4-8y	+ 4065	7 +5079 7	- 1337	+ 4435	+ 6137	+ 8574	0	- 430	
Males	9-13y	+ 3865	+5029	-	+ 2435	+ 5837	+ 7874	- 3	-	
	14-18y	+ 3665	7	1837	- 565	+ 5537	+ 7674	- 6	730	
	19-30y	+ 3465	+5029	-	- 2565	+ 5337	+ 7674	- 6	-	
	31-50y	+ 3465	7	2037	- 2565	+ 5337	+ 7674	- 6	830	
	50-70y	+ 3465	+5079	-	- 2565	+ 5337	+ 7674	- 6	-	
	>70y		+ 3465	7 +5079 7	2037 - 2037	- 2565	+ 5337	+ 7674	- 6	830 - 830
				+5129 7 +5129 7	- 2037 - 2037					- 830 - 830
Females	9-13y	+ 3865	+5029	-	+ 2435	+ 5837	+ 7874	- 3	-	
	14-18y	+ 3665	7	1837	- 565	+ 5537	+ 7674	- 6	730	
	19-30y	+ 3465	+5029	-	- 2565	+ 5337	+ 7674	- 6	-	
	31-50y	+ 3465	7	2037	- 2565	+ 5337	+ 7674	- 6	830	
	50-70y	+ 3465	+5079	-	- 2565	+ 5337	+ 7674	- 6	-	
	>70y		+ 3465	7 +5079 7	2037 - 2037	- 2565	+ 5337	+ 7674	- 6	830 - 830
				+5129 7 +5129 7	- 2037 - 2037					- 830 - 830
Pregnant Women	≤ 18y	+ 3665	+5029	-	- 565	+ 5537	+ 7674	- 6	-	
	19-30y	+ 3465	7	2037	- 2565	+ 5337	+ 7674	- 8	830	
	31-50y	+ 3465	+5079 7	- 2037	- 2565	+ 5337	+ 7674	- 8	- 830	

			+5079 7	– 2037					– 830
Lactation Women	≤18y	+ 3665	+5029 7	– 2037	– 565 – 2565	+ 5537	+ 7274 + 7274	– 6 – 8	– 830
	19-30y	+ 3465							
	31-50y	+ 3465	+5079 7	– 2037	– 2565	+ 5337 + 5337	+ 7274	– 8	– 830
				+5079 7	– 2037				– 830

The negative sign (–) denotes that the values are below the ULs, while the positive sign (+) denotes that the values are above the ULs

Health Risk Estimation

To estimate the health effects, hazard index (HI), the estimated lifetime average daily dose of each chemical is compared to its Reference Dose (RfD). The reference dose represents an estimate of a daily consumption level that is likely to be without deleterious effects in a lifetime. Based on the equation detailed in the US. EPA handbook (Laar et al, 2011):

$$\text{The hazard index (HI)} = \frac{\text{ED}}{\text{RfD}}, \quad (2)$$

Where, ED = Estimated Dose and RfD = Reference Dose

Table 16: Health risk estimates associated with the eating of *S. melanotheron* from the Benya Lagoon

Life Stage Group		Hazard index							
		Al	Ca	Cl	Cu	I	K	Mn	Na
Infants	0-6months	ND	53.297	ND	ND	ND	30.934	ND	ND
	7-12months	ND	0	ND	ND	ND	1	ND	ND
			35.5313				17.6766		
Children	1-3y	14.8817	21.3188	0.6795	7.4348	32.1833	4.1245	1.2832	0.9802
	4-8y	11.1613	21.3188	0.5389	2.4783	21.4555	3.2562	0.8555	0.7738

Males	9-13y	7.4408	17.765	0.4596	1.4870	10.7278	2.7497	0.4277	0.6683
	14-18y	5.5806	7	0.4341	0.9294	7.1518	2.6327	0.2852	0.6393
	19-30y	4.4645	17.765	0.4341	0.7435	5.8515	2.6327	0.2852	0.6393
	31-50y	4.4645	7	0.4341	0.7435	5.8515	2.6327	0.2852	0.6393
	50-70y	4.4645	21.318	0.4341	0.7435	5.8515	2.6327	0.2852	0.6393
	>70y	4.4645	8	0.4341	0.7435	5.8515	2.6327	0.2852	0.6393
				21.318					
			8						
			21.318						
			8						
			21.318						
			8						
Females	9-13y	7.4408	17.765	0.4596	1.4870	10.7278	2.7497	0.4277	0.6683
	14-18y	5.5806	7	0.4341	0.9294	7.1518	2.6327	0.2852	0.6393
	19-30y	4.4645	17.765	0.4341	0.7435	5.8515	2.6327	0.2852	0.6393
	31-50y	4.4645	7	0.4341	0.7435	5.8515	2.6327	0.2852	0.6393
	50-70y	4.4645	21.318	0.4341	0.7435	5.8515	2.6327	0.2852	0.6393
	>70y	4.4645	8	0.4341	0.7435	5.8515	2.6327	0.2852	0.6393
			21.318						
			8						
			21.318						
			8						
Pregnant Women	≤ 18y	5.5806	17.765	0.4341	0.9294	7.1518	2.6327	0.2852	0.6393
	19-30y	4.4645	7	0.4341	0.7435	5.8515	2.6327	0.2333	0.6393
	31-50y	4.4645	21.318	0.4341	0.7435	5.8515	2.6327	0.2333	0.6393
			8						
			21.318						
			8						
Lactation Women	≤ 18y	5.5806	17.765	0.4341	0.9294	7.1518	2.4262	0.2852	0.6393
	19-30y	4.4645	7	0.4341	0.7435	5.8515	2.4262	0.2333	0.6393
	31-50y	4.4645	21.318	0.4341	0.7435	5.8515	2.4262	0.2333	0.6393
			8						
			21.318						
			8						

The estimated health risk associated with the consumption of *S. melanotheron* is presented in Table 11. $HI < 1$ suggests an unlikely adverse health effects whereas $HI > 1$ suggests the probability of adverse health effects (Laar et al, 2011). High HI values for the

trace element investigated that registered values > 1 for any life stage groups have been highlighted.

All the fish samples collected from the Benya lagoon contained detectable amounts of the elements studied at varying concentrations. The calculated health risks estimates for Na and Cl were < 1 suggesting an unlikely adverse health effects for these trace elements for all the life stage groups. Aside As and S which were not presented in Table 11 due to the lack of suitable data on their upper limits (although their concentrations were determined), all the remaining elements registered HI values > 1 for at least one age group. Ca, I, K and Al were high in the order $Ca > I > K > Al$ where they all exceeded the Maximum Upper Limit (UL) of the RDA for all life stage groups investigated. Other trace elements that also exceeded the RDA were Cu, for the age group between 1-13 years and Mn for children between 1-3 years.

Indeed all the twelve elements investigated in the *S. melanotheron* from the Benya Lagoon are essential components as they are needed for the health and growth processes in humans, but at the same time they can be toxic at concentrations beyond those necessary for their biological functions. Calcium provides for bone and tooth strength, aids in nerve-impulse transmission and required for muscle contraction but its excess intake of over 2 g/d may cause kidney stones in susceptible people and poor mineral absorption in general (Minerals Education Coalition, 2013). Iodine is required for the production of thyroid hormones which play a major role in the growth and development of the brain and central nervous system in humans from the 15th week of gestation to 3 years. Its deficiency manifests in the enlargement of the thyroid, known as goitre (Vance *et al*, 2008).

Potassium functions as a major ion in intracellular fluids and aids in nerve-impulse transmission, but its excess consumption results in slowing of the heartbeat and kidney failure (Minerals Education Coalition, 2013). Aluminum is typically non-essential to the body with its uptake being through food/water, breathing and by skin contact. Long lasting uptakes of significant concentrations can lead to damage to the central nervous system, dementia, memory loss of memory, listlessness and severe trembling (Lenntech BV, 2013; Bernardo, 2013).

Copper aids in iron and protein metabolism, hormone synthesis and oestrogen metabolism required for women's reproductive system but its high intakes above 10 mg/d can cause nervous system disorders, liver and kidney damage (Daily-vitamins, 2013: *Agency for Toxic Substances and Disease Registry Public Health Statement*, 1990). A high level of Mn affects the central nervous system which may result in permanent disability (Lenntech, 2011).

Sodium is an electrolyte/mineral and a major ion of the extra cellular fluid that aids nerve-impulse transmission. Its excess contribute to high blood pressure in susceptible people, and can lead to increase calcium-loss in urine and increases the amount of water the body holds therefore causing the swelling of the legs and hands (The National Academies, 2004; Nutritional Health Resource, 2010). Chlorine is an electrolyte that works with potassium and sodium to regulate the amount of fluids in the body and its pH. It allows us to digest our food and absorb the other elements we need to survive. Excessive loss of chlorine in the body can lead to an imbalance of pH in the body which can cause muscle weakness, loss of appetite, dehydration and coma (Minerals Education Coalition, 2013).

Arsenic is poisonous but up to 20 mg of organic As may be found in the human body to control gene expression, support reproductive health and treat digestive problems (Wilson, 2011). Its overdose causes anaemia, depression, gastrointestinal problems and even death (Parker, 2011; IARC, 1980). Sulfur protects our cells from environmental hazards and slows down the aging process. It helps our liver function properly, digest our food and helps our blood clot when we cut ourselves. The excess S we take is excreted through our urine (Minerals Education Coalition, 2013)

Sources of Trace Elements

An important source of nutrients, trace elements, contaminants, and low salinity water to many types of coastal ecosystems can be from groundwater discharge (Kelly and Moran, 2002). As mentioned in the introduction Benya Lagoons' water gets contributions from more than one groundwater source. These variations in groundwater sources as well as overall discharge rates from activities around the bed of the Lagoon could contribute to the trace elements input. Accordingly, it is important to characterize each distinct source of trace elements and determine its contribution to the Lagoons ecosystem.

Table 17: Correlation coefficients matrix of the elements in the fish

	Al	S	Ca	Mn	Cu	Na	K	As	I	Cl
Al <i>r</i> ²	1									
S <i>r</i> ²	-0.627	1								
Ca <i>r</i> ²	0.662	- 0.999	1							
		0.998								

Mn r^2	-0.181	- 0.653	0.618	1						
Cu r^2	0.908 0.825	- 0.896 0.803	0.915 0.837	0.249	1					
Na r^2	0.739	- 0.988 0.976	0.994 0.988	0.529	0.954 0.910	1				
K r^2	-0.269	0.919 0.845	- 0.900 0.810	- 0.898 0.806	- 0.649	- 0.848 0.719	1			
As r^2	0.353	0.507	- 0.468	- 0.984 0.968	- 0.072	- 0.369	0.806 0.650	1		
I r^2	0.865 0.748	- 0.934 0.872	0.949 0.901	0.338	0.996 0.992	0.977 0.955	- 0.717	- 0.165	1	
Cl r^2	0.992 0.984	- 0.524	0.561	- 0.304	0.847 0.717	0.648	- 0.146	0.468	0.794	1

Correlation coefficients between the ten elements and their respective significance at 95% significance level in the fish are as shown in Table 17. The correlation coefficients between elements give information about their possible same or similar source inputs (Alfassi, 1994). The Pearson correlation was calculated because the concentrations of elements in the fish were normally distributed, so the mean concentrations are also normally distributed. The variables are continuous and there exists linear relationship between the variables. Though 95% confidence level was used to ascertain the strength of their relationship, there are other strongly correlated elements with high coefficients of determination, hence cannot be ruled out. Focus should be on strength of relationship and the amount of variance shared while reporting statistical significance. (Pallant, 2007).

The interpretation of the strength of the correlation coefficients usually depends on the researcher, however there are suggested guidelines. Such as: (Rumsey, 2010)

- Exactly ± 1.0 : A perfect downhill/uphill (negative/positive) linear relationship
- ± 0.7 : A strong downhill/uphill (negative/positive) linear relationship
- ± 0.5 : A moderate downhill/uphill (negative/positive) relationship
- ± 0.3 : A weak downhill/uphill (negative/positive) linear relationship
- 0 : No linear relationship

However, in this research we considered values between $\pm 0.800 \leq r \leq \pm 0.999$ as strong correlations. The level of statistical significance does not indicate how strongly two variables are associated (this is given by rho), but instead it indicates how much confidence we should have in the results obtained. The significance of rho is strongly influenced by the sample size. Smaller sample sizes do not reach statistical significance as compared to larger sample sizes which may even be statistically significant at small (weak) correlations. (Pallant, 2007). It was found out that even though 22 correlations were strong, 2 were statistically significant. This means that we can exude 95% confidence that the correlations between Ca and S and As and V are strongly correlated with coefficient of -0.999 and 0.998 respectively. Significance (2-tailed) was 0.028 and 0.041 respectively. For example for Mn and V, the coefficient of determination calculated by squaring rho is 94.09% This means 94.09% of the variance shared by these elements can be accounted for, which is a good indicator for a strong relationship even though at 0.05 α -level, their correlation of 0.970 is not statistically significant. Thus conclusions can still be drawn using the strength of the correlation coefficients and the r-squared value (coefficient of determination).

As expected there was a strong correlation between Na and Cl since main source of Na and Cl is added salt (NaCl). It is worth noting that Benya Lagoon has a lot of salt ponds around it. Al has a strong correlation with Cu, I, Cl. These may probably come from unwashed vegetables, dust introduced during the cooking and corrosion from aluminium wares used by fishermen and fish mongers. Table 13 contain some perceived bonds from some of the strongly correlated elements. Some of products and processes that these perceived bonds are used for are written below it. It is perceived that these elements might have entered the Benya Lagoon due to the activities around the banks of the Lagoon. Mn combined with other elements is widely distributed in Earth's crust. Manganese is second only to iron among the transition elements in its abundance in Earth's crust; it is roughly similar to iron in its physical and chemical properties but is harder and more brittle. It occurs in a number of substantial deposits, of which the most important ores (which are mainly oxides) consist primarily of manganese dioxide (MnO_2) in the form of pyrolusite, romanachite, and wad. Manganese is essential to plant growth and is involved in the reduction of nitrates in green plants and algae. It is an essential trace element in higher animals, in which it participates in the action of many enzymes. Lack of manganese causes

testicular atrophy. An excess of this element in plants and animals is toxic.

(Encyclopaedia Britannica. 2014).

Table 18: Some perceived bonds from which the twelve trace elements come from

Elements	Perceived Bond	Some Products and Processes From The Perceived Bonds
Ca and I	Calcium iodide	Use in photography
S and K	Potassium sulphide	Use in fireworks
S and Na	Sodium sulphate	Use in the manufacture of detergents
S and Ca	Calcium sulphate	Use as a coagulant in foods like Tofu, Neat Fufu
Na and I	Sodium iodide	Treatment of iodine deficiency
Cu and S	Copper sulfide	Antiseptic and germicide against fungus infections and prevention of malaria,

Conclusion

The concentration of ten TE in the bones, muscles and gills of *S. melanotheron* (Blackchin tilapia) obtained from the Benya Lagoon at Komenda Edina Eguafu Abrem Municipality (KEEA) in the Central Region of Ghana has been investigated by employing the technique of Instrumental Neutron Activation Analysis at the Ghana Atomic Energy. The varying concentrations of the elements in the various parts of the fish indicate that the rates of absorption by these tissues were quite variable. No patterns of distribution, sources and behaviour were noted. These are important factors to be considered for further study.

The two-way ANOVA indicated that, for Sample 1 (16/02/2012), there was statistical evidence to reject the null hypotheses that the mean concentration of elements does not differ, and is the same three main parts of the fish. A further test therefore showed that K had a different mean concentration.

In the Tables 6, the test was to check whether the fish caught at 23/02/2012 had differences in their mean concentration of elements and whether there were differences from part to part.

Ca and K appeared to have very high concentrations and were likely to be the trace elements causing differences. And results from a confirmatory test (Tukey's Test) showed that, Ca and K concentrations dominated the fish. The fishes in general contained more

Calcium and potassium. And the elements were highly concentrated in the gills in both samples.

The F-test result showed that the variances at the concentration for the two different times were equal. The p-values from the t-test were statistically not significant; the null hypotheses were not rejected. This means that, regardless of the time the fishes were caught they mean concentration of the trace elements will be the same. That is, one derives the same amount of nutrient from fishes anytime one eats it, the amount of nutrients one derives from the fish is the same at all times. Though the concentrations of Al, Ca, K, I, As and S are so high in the *Sarotherodon melanotheron*, (Blackchin Tilapia) from the Benya Lagoon in Elmina making it unsafe for human consumption.

The high concentration levels of the trace elements that translated into hazard index values $HI > 1$ for six of the trace elements investigated (Ca, I, K, Al, Cu, Mn) suggest the likelihood of adverse health effects associated with the consumption of the fish. It also gave an indication to the capability of the fish to retain in some specific parts of their bodies some elements they may have ingested from their environment. The consuming of the fish may therefore pose a health risk generally to consumers and particularly to the families of fishermen staying around the lagoon as their meals are more dependent on this fish from the lagoon. Though the *S. melanotheron* from this lagoon may be associated with levels of trace elements above the recommended values and may be unsafe for human consumption, the risk prevention on the consumption this fish should be focused on reducing the amount of dumping of waste into the lagoon so as to reduce the extent of pollution.

It has to be mentioned that our health indeed has an imperative correlation with the balance of trace elements in our body to the extent that imbalanced states will create a series of troubling health problems. Preserving the balance is therefore key to maintaining good health. Excess or lack of these elements can both threaten our health as indeed they are essential components of our biological structures, but at the same time can be toxic at concentrations beyond those necessary for their biological functions.

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