International Journal of
Science and Engineering Investigations vol. 9, issue 99, April 2020
Received on April 5, 2020 ISSN: 2251-8843

Epithermal Instrumental Neutron Activation Analysis Technique in Determining Bromine, Chlorine, and Iodine in Sarotherodon Melanotheron from the Benya Lagoon, Ghana

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Abstract-Epithermal instrumental neutron activation analysis technique was applied in the determination of concentration levels of three halogens - bromine, chlorine, and iodine in Sarotherodon melanotheron (Blackchin tilapia). The species is a delicacy of the people of Komenda Edina Eguafo Abrem Municipality (KEEA) in the Central Region of Ghana, as such this fish is consumed as a whole. The levels of these halogens in bones, muscles, and gills of the fish samples obtained from the Benya lagoon were determined using the Low Power Ghana Research Reactor-1. Three millimetres thick of flexible boron was used to cut off thermal neutrons in order to assess epithermal neutrons. This procedure yielded an accuracy of 94.7%. 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 the halogens. The estimated health risk calculated showed high levels of Cl and I above the Maximum Upper Limit (UL) of the Recommended Dietary Allowance (RDA) for all life stage groups investigated except for children below 8 years. The total mean daily intake of Br was 5342.08 ± 581.64 µg/d, which exceeded the estimated median daily intake of 2000.00 µg/d – 5000.00 µg/d for food and water. The I levels in the fishes were not encouraging so buttresses its known adverse effect on child development in developing countries. This knowledge may be useful for the designing of the best management plans for Benya Lagoon's ecosystems restoration and ensuring the sustainability of the health status of the species in the KEEA.

Keywords- *Benya Lagoon, Epithermal Instrumental Neutron Activation Analysis, EINAA, Hazard Index, Blackchin Tilapia, Sarotherodon Melanotheron*

I. INTRODUCTION

Fish is a high-protein, low-fat food that provides a range of health benefits including the provision of essential micronutrients such as Cu, Se, and Zn. Fishes such as salmon, cod, and tilapia are rich in omega-3 fatty acids which are essential for cognitive development [1]. The consumption of fish in Ghana has a per capita consumption of 22kg/capita/year which is equivalent to 15 % of protein derived from fish [2, 3]. Being a rich source of protein, vitamins, and minerals, fish is also a good source of trace elements like Bromine, Chlorine, and Iodine which are essential to maintain good health [4].

Iodine is a vital trace element that plays a very important role in human physiological actions of thyroid hormones; both insufficient and excessive intake of this micronutrient can lead to thyroid disorder [5]. Iodine deficiency has been identified as one of the factors that have an adverse effect on child development in developing countries [6]. These deficiencies manifest as stunted physical and mental growth as well as infertility, lethargy and cognitive impairment [7]. Thyroid hormones 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 [8]. Therefore any obstruction of thyroid function has adverse effects on the children as well as the adult population. Every cell in the body (including the brain) depends on thyroid hormones for regulation of their metabolism and promotes growth and development in the body.

A fall in metabolic rate, increase in serum cholesterol and enlargement of thyroid glands results from a condition known as goitre. Iodine deficiency is the leading preventable cause of brain damage which leads to significantly lower the intelligence quotients (IQ) of a population. Concerns have been raised about the continued occurrence of Iodine Deficiency Disorders (IDDs) among children. In a related study in KEEA, [9] reported a high (42.5%) prevalence of iodine deficiency in pregnant women. This challenge may hamper the objectives of the educational reform programme and the nation's developmental efforts. Statistics indicated that about 81,200 babies are born annually with mental impairments as a result of such deficiencies, and suffer from stunted growth and low IQs, thereby impeding their learning abilities [10]. Although Bromine (Br) has not been officially designated essential for humans at the moment, it plays a vital role in controlling inflammation of the thyroid gland [11]. There have been reports of reduced growth, fertility, and life expectancy in some animals as a result of hyperthyroidism due to dietary deficiency of bromide. In humans and animals, Bromine - either as Sodium Bromide or Potassium Bromide - has anti-seizure properties, and it is an effective trace mineral in the treatment of hyperthyroid conditions [11]. Organic bromines are widely used as sprays to kill insects and other unwanted pests. The residues of these sprays enter runoffs and get into main water bodies where they easily get bio-accumulated in the organism in the food chain. The most important health effects that can be caused by bromine-containing organic contaminants are malfunctioning of the nervous system and disturbances in genetic materials [12].

Chlorine (Cl), levels in the human closely parallels levels of sodium intake and output since the primary source is the table salt (sodium chloride). Chlorine compounds (chlorides) play an essential role in the electrical neutrality and pressure of extracellular fluids and in the acid-base balance of the body.

Halogens, which include Fluorine, Chlorine, Bromine, and Iodine are Group 7 elements that are known to produce Sodium salts of similar properties, of which Sodium Chloride (table salt) is well known. The halogens show great resemblances to one another in their general chemical behaviour and in the properties of their compounds with other elements [13]. In a displacement reaction, a less reactive Iodine is removed and replaced by the more reactive Bromine. In view of this, it is of considerable interest to accurately measure Bromine, Chlorine and Iodine content of food items. Apart from neutron activation analysis (NAA), other common analytical techniques mostly employed for determining Bromine, Chlorine, and Iodine are colorimetry, ion selective electrode, isotope exchange, and gas chromatography [14, 15, 16]. However, these methods are more expensive than NAA. The strengths of NAA are that it can be used to analyze a 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 [17].

The objective of this study is to determine concentrations of Bromine, Chlorine, and Iodine in Sarotherodon melanotheron (Blackchin tilapia) from the Benya Lagoon in the Central Region of Ghana using Epithermal Instrumental NAA.

II. MATERIALS AND METHODS

A. *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 Blackchin Tilapia. 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, and this threatens the livelihood of more than 500 fishermen. Although the intensity of pollution has long been identified, no action has been taken. In spite of the spate of pollution in this lagoon, residents, unfortunately, continue to patronize the fish from this lagoon [18].

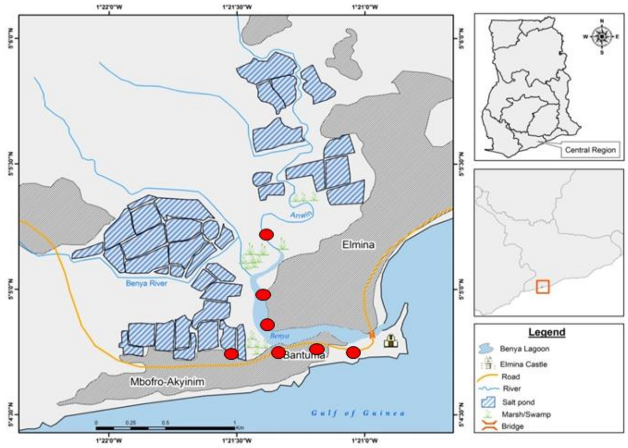

Figure 1. A map of Benya lagoon, Ghana, showing the sampling site [19].

B. *Sample collection*

Figure 2. Sarotherodon melanotheron obtained from the Benya Lagoon.

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 various sampling points. The fish samples obtained from the Benya Lagoon were kept over ice and transported to the laboratory of the Department of Fisheries and Aquatic Sciences, University of Cape Coast. At the laboratory, the length and weight of the specimen were taken after which scales were removed using a stainless steel knife. The specimen were washed with deionized water, dry cleaned with blotting paper and parts separated into tissue, bones, and gills for further analysis. The prepared samples were kept at a temperature of – 10°C and transported to Ghana Atomic Energy Commission Preparation Laboratory.

C. Overview of Neutron Activation Analyses Technique Used (Epithermal Neutron Activation Analyses)

Neutron Activation Analyses (NAA) is a sensitive analytical technique useful for performing both qualitative and quantitative multi-element analyses in samples. NAA offers sensitivities that are sometimes superior to those attainable by other methods. Because NAA is accurate and reliable, it is generally recognized as the "referee method" of choice when new procedures are being developed or when other methods yield results that do not agree [20].

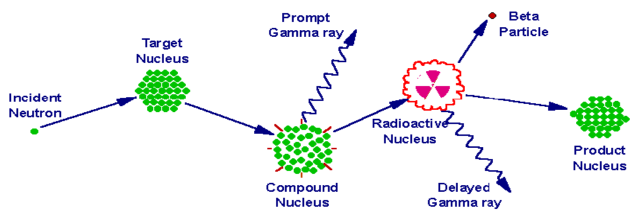

Figure 3. A process of neutron capture followed by the emission of gamma rays [21]

During NAA (Figure 3) a neutron interacts with the target nucleus via a non-elastic collision, a compound nucleus forms in an excited state. The excitation energy of the compound nucleus is due to the binding energy of the neutron with the nucleus. The compound nucleus will almost instantaneously de-excite into a more stable configuration through the emission of one or more characteristic prompt gamma rays. In many cases, this new configuration yields a radioactive nucleus which also decays by emission of one or more characteristic delayed gamma rays, but at a much slower rate according to the unique half-life of the radioactive nucleus. Depending on the particular radioactive species, half-lives can range from a fraction of a second to several years [20].

The basic essentials required to carry out an analysis of samples by NAA are a source of neutrons, instrumentation suitable for detecting gamma rays, and detailed knowledge of the reactions that occur when neutrons interact with target nuclei. The sensitivities for NAA are dependent upon the irradiation parameters (i.e., neutron flux, irradiation and decay times), measurement conditions (i.e., measurement time, and detector efficiency); nuclear parameters of the elements being measured (i.e., isotope abundance, neutron cross-section, half-life, and gamma-ray abundance). Different types of reactors and different positions within a reactor can vary considerably with regard to neutron energy distributions and fluxes due to the materials used to moderate the primary fission neutrons. Most neutron energy distributions are quite broad and consist of three principal components: Thermal, Epi-thermal, and Fast. The thermal neutron component consists of low-energy neutrons (energies below 0.5 eV) in thermal equilibrium with atoms in the reactor's moderator. The fast neutron component of the neutron spectrum (energies above 0.5 MeV) consists of the primary fission yielding neutrons which still have much of their original energy following Fission. The application of purely instrumental procedures is commonly called instrumental neutron activation analysis (INAA) [20]. However, INAA methods using reactor flux neutrons for low-level iodine measurement in biological materials suffer from high background activities from the activation products of major elements like Na, Cl, Mn, K, Br, and Al in the sample [22].

For many elements, the variation of the cross-section is inversely proportional to the neutron velocity (the l/v law) and these are strongly activated by slow (i.e., thermal) neutrons. In contrast, other elements possess resonance cross-sections in the epithermal region, which are usually larger than thermal cross-sections by several orders of magnitude. Therefore, if a sample is irradiated with epithermal neutrons, it is expected that the activation yield of the "resonance" elements will be enhanced relative to those interfering nuclides which are activated mainly by thermal neutrons [23].

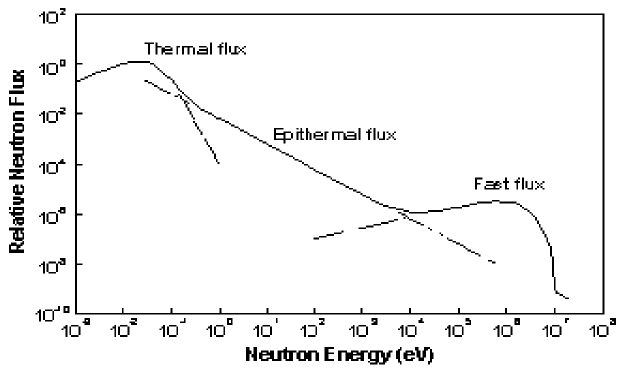

Figure 4. A typical reactor neutron energy spectrum showing the various components used to describe the neutron energy regions [21].

The NAA technique that employs only epi-thermal neutrons to induce (n, γ) reactions by irradiating the samples being analyzed inside either cadmium or boron shields is called epi-thermal neutron activation analysis (ENAA). The epi-thermal neutron component consists of neutrons (energies from 0.5 eV to about 0.5 MeV) which have been only partially moderated. In a typical unshielded reactor irradiation position, the epi-thermal neutron flux represents about 2% of the total neutron flux [24]. In reactor activation, this ENAA technique is performed by enclosing samples in thermal neutron filters such as cadmium or boron, which removes thermal neutrons from the reactor neutron spectrum. It has been applied to a variety of sample matrices including geological and biological materials [23].

Low levels of Iodine in foods make it generally difficult to determine due to its loss during sample preparation by most analytical techniques and cumbersome procedures. With EINAA, the problem of loss of iodine due to sample preparation is avoided. EINAA method is also extensively used for short-lived nuclides like Br and Cl [22].

The EINAA methods are based on the fact that the resonance integral (I0) to thermal neutron (n,γ) cross-section (σ0) ratio (Q0) for 127I (24.8) is much larger than that for some of the interfering elements such as 23Na (0.59), 37Cl (0.69), 27Al (0.71), 41K (0.97), and 55Mn (1.053). The determination of iodine is sometimes hindered by EINAA using a Cd filter due to high dead-time from the neutron-irradiated samples. Additionally, the background in the region of the 443-keV photopeak of 128I is often dominated by Compton background from the γ-rays of 24Na, 56Mn, 38Cl, 42K, 28Al and 82Br. The combination of EINAA using cadmium and/or boron shields and anticoincidence gamma-ray spectrometry (AC) is used to suppress the background, which in turn helps to improve the detection limit of these elements [22].

D. Sample Preparation and Irradiation

Samples were then lyophilized (Christ Gamma 1 – 16) for 72 hrs at – 0°C (corresponding to the vapour pressure of 0.370 mbar). This freeze-drying method was employed to ensure the preservation of the initial sample texture and to facilitate sample milling. Using a commercial blender with stainless steel blades, the freeze-dried samples were then homogenized.

The three parts of the samples were then milled separately. Six portions of each part of the pulverized and homogenized samples weighing 200 mg of the pulverized and homogenized samples were weighed, wrapped and heat-sealed (using soldering rod) in ultra-clean polyethylene films. The 18 portions of each fish were then folded with forceps and heat-sealed with a handheld dryer. Each sample was in turn put into a rabbit capsule and smoothly heat-sealed with a soldering rod.

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 deionized water. The irradiation vials were further soaked in HNO3 for another 24 hrs. They were then rinsed thoroughly with distilled deionized 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 [25 - 28].

Samples were transferred into irradiation sites through a 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 5×1011 ncm-2s-1. Three millimeters (3mm) thick of flexible boron was used to cut off thermal neutrons in order to assess epithermal neutrons. 94.7% accuracy was achieved.

E. Relative Standardization

In the relative standardization method, a chemical standard (index std) of known mass, Wstd, of the element is co-irradiated with the sample of unknown mass Wsam. When the samples to be irradiated are 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:

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

where $(P_A/t_c)_{std}$ and $(P_A/t_c)_{sam}$ are the counting rates for the standard and sample respectively, std and sam are the counting concentrations of the standard and the element of interest respectively, Cstd and Csam are the counting factors for standard and sample, Dstd and Dsam are the decay factors for the standard and sample respectively.

F. Qualitative and Quantitative Analysis

The qualitative analysis involves the determination of the Br, Cl, and I, in the fish samples by the identification of the spectral 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) [29]. 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 60Co. The qualitative analysis was achieved by means of ORTEC EMCAPLUS Multichannel Analyzer (MCA) Emulation software. A Microsoft Windows-based software, MAESTRO, was used for spectrum analysis [30]. This software identifies the various photopeaks, estimates and works out the areas under them. The other quantitative measurements were done using the concentration equation (Equation 1) in a Microsoft Excel programme for calculating the elemental concentrations in μg/g. The detection limit (DL) of the various elements of interest for Neutron Activation Analysis and the nuclear data have been summarized in Table 1.

TABLE I. DETECTION LIMIT AND NUCLEAR DATA OF BR, CL AND I

Element	Radioisotope	Gamma Ray Energy (keV)	Half-life (min)	Irradiation / Counting Time (min)	DL (*μg/g*)
Br	^{80}Br	616.93	17.68	10	0.0001
Cl	^{38}Cl	1642.7	37.24	10	0.001
I	^{128}I	440.9	25	10	0.0001

III. RESULTS AND DISCUSSIONS

A. *Assessment of Concentrations of Br, Cl and I in Various Parts of the Fish*

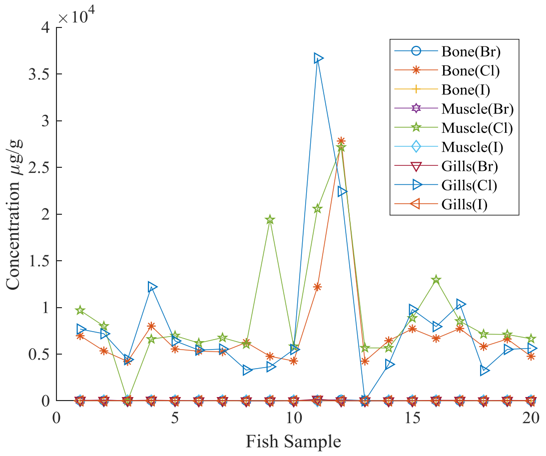

Figure 5. Distribution of Br, Cl and I in the muscles, bones and gills of the sampled Blackchin Tilapia

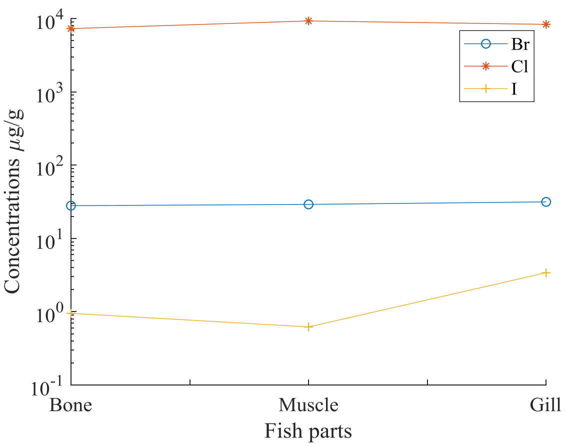

Figure 6. Mean distribution of Br, Cl and I in the sampled Blackchin Tilapia

The concentration of Br, Cl and I fluctuated between 0 - 8000µg/g (Figure 5). Among the three halogens, chlorine recorded the highest concentration whilst iodine as the lowest concentrations in all the fish samples. The high levels of Cl in the fishes could be due to the constant influx of marine water from the Gulf of Guinea which is rich in chlorides into the Benya Lagoon. This lagoon is an open lagoon hence it has constant interaction with the sea throughout the year [31].

The three parts of the fish the muscles recorded the highest concentration while the bones recorded the lowest (muscles > gills > bones). The similar levels of chlorine found in all three tissues analyzed could be due to osmoregulation which is undertaken by the euryhaline (brackish water) species to maintain a constant osmotic balance in the body tissues. Brackish water systems receive a constant inflow of both freshwater and seawater (a major source of NaCl), throughout the year. The uptake of Cl ions from the seawater enable the species to maintain electrical neutrality as well as balancing the K+ and Na+ ions in the body fluids. The low levels of other halogens could be due to low absorption from the brackish water [32].

B. *Correlation Between Br, Cl and I in the S. melanotheron*

The correlation coefficients between the three elements in the bone, muscle, and gills and their respective significance at 95% significance level in the fish are as shown in Table 2. The correlation coefficients between elements give information about their possible same or similar source inputs [29]. There was a strong correlation between chlorine and bromine in all the tissues which were assessed.

It was found out that even though 8 correlations were strong, none was statistically significant. This means that we can exude 95% confidence that the correlations between Br and Cl in the bone are strongly correlated with a coefficient of –0.999 and 0.855.

It can be seen from Table 2 that iodine had no strong correlation with Br or Cl, although there was a strong correlation between Br in the Bone and the Muscle Br, Cl and the Cl in the Gills. It is worth noting that Benya Lagoon has a lot of salt ponds around it hence the Cl. An important source of nutrients, trace elements, and contaminants in many coastal ecosystems come from groundwater discharge [33]. Br is abundant in nature as bromide salts or as organobromine compounds, which are produced by many types of marine organisms. The most recoverable form of bromine is from soluble salts found in seawater, salt lakes, inland seas, and brine wells. Seawater contains bromine in about 65 parts per million [34]. Benya Lagoons' gets water 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 these three halogens. However, the chief source of Cl is from seawater influx from the Gulf of Guinea. Activities along the bed of the Lagoon could also be the source of these three halogens [18].

TABLE II. CORRELATION COEFFICIENTS MATRIX OF THE ELEMENTS IN THE FISH

	Bone (Br)	Bone (Cl)	Bone (I)	Muscle (Br)	Muscle (Cl)	Muscle (I)	Gills (Br)	Gills (Cl)	Gills (I)
Bone (Br)	1								
Bone (Cl)	0.851	1							
Bone (I)	– 0.126	– 0.125	1						
Muscle (Br)	0.641	0.579	0.044	1					
Muscle (Cl)	0.660	0.777	– 0.193	0.489	1				
Muscle (I)	– 0.224	– 0.126	0.261	0.154	– 0.176	1			
Gills (Br)	0.256	0.111	– 0.035	0.477	0.259	0.146	1		
Gills (Cl)	0.668	0.671	– 0.112	0.717	0.690	0.066	0.728	1	
Gills (I)	– 0.031	– 0.067	0.007	0.255	– 0.095	0.073	0.190	0.028	1

C. Factor Analysis

Factor analysis models the interrelationships among observations with the primary focus on the variance and covariance rather than the mean. The underlying idea of factor analysis is that a observed random variables can be written as a linear function of unobserved called the common factors. This can be written as:

$$x_1 = \beta_{11} f_1 + ... + \beta_{1p} f_p + e_1$$

$$....... \qquad (2)$$

$$x_p = \beta_{k1} f_1 + ... + \beta_{kp} f_p + e_k$$

The β_{ij} $(i = 1,...., k \text{ and } j = 1,...., p)$ in equation 1 is called the factor loadings, the error terms e_i are called the specific errors. The error terms are specific to each of the original variables while the f_i are common to all the variables

Figures 7 and 8 are plots for factor 1 and factor 2 shows a strong correlation between chlorine and bromine. However, there was no correlation between iodine and the other elements (chlorine and bromine). It can be seen from the figure that iodine clustered together and there is also some form of the linear relationship between chlorine and bromine. The observation on Figures 7 and 8 buttresses the observation seen in Table 2 that iodine had no strong correlation with Br or Cl and that iodine may be coming from a different source. Although iodine is fairly rare, it is found in both the Earth's crust, in ocean water and in high concentration in some ocean plants such as seaweed. Iodine is also found in underground brines near oil and natural gas reserves [35]. 39.2 km offshore east of Elmina is the Saltpond oil rig and 123.1 km to the west of Elmina are several oil rigs (Jubilee oil fields). These rigs may be the source of iodine towards Benya lagoon from the Gulf of Guinea.

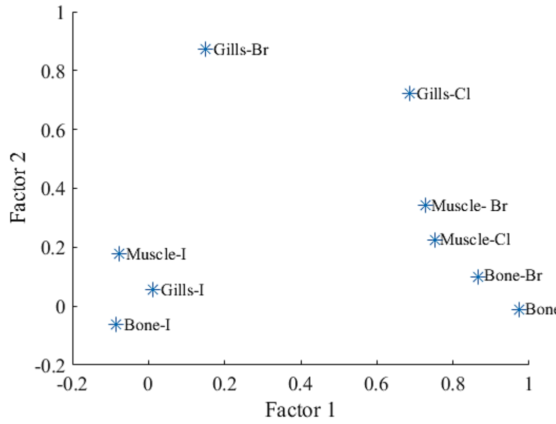

Figure 7. Factor loadings in their unrotated form showing groupings for Iodine and groupings for Chlorine and Bromine.

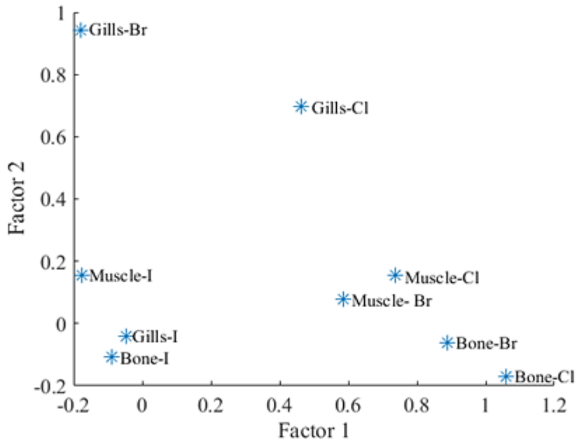

Figure 8. Factor loadings after Promax rotation form showing groupings for Iodine and groupings for Chlorine and Bromine.

D. Assessment According to Dietary Requirements

1) Recommended Dietary Allowable (RDA)

Recommended Dietary Allowable (RDA) is the dietary requirement for a micronutrient. It is an intake level that meets specified criteria for adequacy, thereby minimizing the 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 are 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 [36, 37].

2) 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 [37].

3) 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. 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 the usual intake by an individual [37].

Not Determinable (ND) 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 [37].

TABLE III. RECOMMENDED DIETARY ALLOWABLE (RDA) / ADEQUATE INTAKE (AI) & MAXIMUM UPPER LIMIT (UL) FOR BR, CL AND I [36, 37]

Life Stage Group		Br (μ g/d)		Cl (μ g/d)		I (μ g/d)	
		RDA/AI	*UL*	*RDA/AI*	*UL*	*RDA/AI*	*UL*
Infants	0-6months 7-12months	ND ND	ND ND	125,000 150,000	ND ND	110 130	ND ND
Children	1-3y 4-8y	ND ND	ND ND	200,000 250,000	1,000,000 1,000,000	90 90	200 300
Males	9-13y 14-18y 19-30y 31-50y 50-70y >70y	ND ND ND ND ND ND	ND ND ND ND ND ND	375,000 550,000 550,000 550,000 550,000 550,000	2,000,000 3,000,000 3,500,000 3,500,000 3,500,000 3,500,000	120 150 150 150 150 150	600 900 1,100 1,100 1,100 1,100
Females	9-13y 14-18y 19-30y 31-50y 50-70y >70y	ND ND ND ND ND ND	ND ND ND ND ND ND	375,000 400,000 425,000 425,000 425,000 425,000	2,000,000 3,000,000 3,500,000 3,500,000 3,500,000 3,500,000	120 150 150 150 150 150	600 900 1,100 1,100 1,100 1,100
Pregnant Women	$\leq$18y 19-30y 31-50y	ND ND ND	ND ND ND	450,000 450,000 450,000	3,000,000 3,500,000 3,500,000	220 220 220	900 1,100 1,100
Lactation Women	$\leq$18y 19-30y 31-50y	ND ND ND	ND ND ND	550,000 550,000 550,000	3,000,000 3,500,000 3,500,000	290 290 290	900 1,100 1,100

The mean concentrations of Br, Cl and I in the various parts of the fish are presented in Figure 6. Table 4 also shows the total average mean concentrations of the element in S. melanotheron in μg/g.

TABLE IV. THE TOTAL MEAN CONCENTRATIONS OF THE ELEMENT IN S. MELANOTHERON IN MG/G.

Element	Br	Cl	I
Mean	88.63 ± 9.65	24922.25 ± 3346.78	4.97 ± 1.51

The average daily intake (grams per capita) in Table 5 was calculated from the values in Table 4. 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 [3]. Then the differences between the UL of the RDA/AI and the calculated means were calculated in Table 6.

TABLE V. MEAN DAILY INTAKE OF BR, CL AND I IN THE S. MELANOTHERON IN MG/D.

Element	Br	Cl	I
Daily Intake	5342.08±581.64	1502163.70±01723.82	299.56±91.01

The negative sign (–) denotes that the values are below the ULs, whiles the positive sign (+) denotes that the values are above the ULs. Although Br has a total mean daily intake of 5342.08 ± 581.64 µg/d, it has no Upper Limit for all the life stage groups. This due to the fact that Br has not been officially designated to be essential for humans at this time, however, there have been reports of reduced growth, fertility, and life expectancy in some animals as a result of hyperthyroidism secondary to dietary deficiency of bromide. The estimated median daily intake of Br worldwide from food and water is 2000.00 µg/d – 5000.00 µg/d [11]. The summary of other functions of Br and how it affects the Human Body can be found in Table 7.

For all the life stage groups, the level of Cl and I in the S. melanotheron was far above the recommended daily dose but did not exceed the upper limits. Except for children in the age bracket of 1 to 3 years which did not show the good results of + 99.56 µg/d in I. The effect of having low levels of Cl and I in diet can be found in Table 8.

TABLE VI. DIFFERENCES BETWEEN THE UL OF THE RDA / AI AND THE CALCULATED MEANS

Life Stage Group		Br (mg/d)		Cl (mg/d)		I (µg/d)	
		RDA/AI	*UL*	*RDA/AI*	*UL*	*RDA/AI*	*UL*
Infants	0-6months	ND	ND	+ 1377163.70	ND	+ 189.56	ND
	7-12months	ND	ND	+ 1352163.70	ND	+ 169.56	ND
Children	1-3y	ND	ND	+ 1302163.70	+ 502.16	+ 209.56	+ 99.56
	4-8y	ND	ND	+ 1252163.70	+ 502.16	+ 209.56	– 0.44
Males	9-13y	ND	ND	+ 1127163.70	– 497.84	+ 179.56	– 300.44
	14-18y	ND	ND	+ 952163.70	– 1497.84	+ 149.56	– 600.44
	19-30y	ND	ND	+ 952163.70	– 1997.84	+ 149.56	– 800.44
	31-50y	ND	ND	+ 952163.70	– 1997.84	+ 149.56	– 800.44
	50-70y	ND	ND	+ 952163.70	– 1997.84	+ 149.56	– 800.44
	>70y	ND	ND	+ 952163.70	– 1997.84	+ 149.56	– 800.44
Females	9-13y	ND	ND	+ 1127163.70	– 497.84	+ 179.56	– 300.44
	14-18y	ND	ND	+ 1102163.70	– 1497.84	+ 149.56	– 600.44
	19-30y	ND	ND	+ 1077163.70	– 1997.84	+ 149.56	– 800.44
	31-50y	ND	ND	+ 1077163.70	– 1997.84	+ 149.56	– 800.44
	50-70y	ND	ND	+ 1077163.70	– 1997.84	+ 149.56	– 800.44
	>70y	ND	ND	+ 1077163.70	– 1997.84	+ 149.56	– 800.44
Pregnant Women	≤ 18y	ND	ND	+ 1052163.70	– 1497.84	+ 79.56	– 600.44
	19-30y	ND	ND	+ 1052163.70	– 1997.84	+ 79.56	– 800.44
	31-50y	ND	ND	+ 1052163.70	– 1997.84	+ 79.56	– 800.44
Lactation Women	≤ 18y	ND	ND	+ 952163.70	– 1497.84	+ 9.56	– 600.44
	19-30y	ND	ND	+ 952163.70	– 1997.84	+ 9.56	– 800.44
	31-50y	ND	ND	+ 952163.70	– 1997.84	+ 9.56	– 800.44

E. Assessment According to Health Risk Estimation (HI)

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 [38]:

$$\text{The hazard index (HI)} = \frac{E\,D}{RfD}, \qquad (3)$$

where, ED = Estimated Dose and RfD = Reference Dose

The estimated health risk associated with the consumption of S. melanotheron is presented in Table 7. HI < 1 suggests an unlikely adverse health effect whereas HI > 1 suggests the probability of adverse health effects [38]. 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 Cl is > 1 suggesting a likely adverse health effect for all the life stage groups. Aside Br whose HI values were not presented in Table

7 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. Except for children in the age bracket of 1 to 3 years which again showed a positive result of HI < 1.

Indeed all the three 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.

For example, 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 [39]. Table 8 is a summary of Bromine, Chlorine and Iodine Functions and How They Affect the Human Body.

TABLE VII. HI ASSOCIATED WITH THE EATING OF S. MELANOTHERON FROM THE BENYA LAGOON

Life Stage Group		Hazard Index (HI)					
		Br		Cl		I	
		RDA/AI	*UL*	*RDA/AI*	*UL*	*RDA/AI*	*UL*
Infants	0-6months 7-12months	ND ND	ND ND	0.0832 0.0999	ND ND	0.3672 0.4340	ND ND
Children	1-3y 4-8y	ND ND	ND ND	0.1331 0.1664	0.6666 0.6666	0.3004 0.3004	0.6676 **1.0015**
Males	9-13y 14-18y 19-30y 31-50y 50-70y >70y	ND ND ND ND ND ND	ND ND ND ND ND ND	0.2496 0.2496 0.3661 0.3661 0.3661 0.3661	**1.3314** **1.9971** **2.3300** **2.3300** **2.3300** **2.3300**	0.4006 0.5007 0.5007 0.5007 0.5007 0.5007	**2.0029** **3.0044** **3.6720** **3.6720** **3.6720** **3.6720**
Females	9-13y 14-18y 19-30y 31-50y 50-70y >70y	ND ND ND ND ND ND	ND ND ND ND ND ND	0.2496 0.2663 0.2829 0.2829 0.2829 0.2829	**1.3314** **1.9971** **2.3300** **2.3300** **2.3300** **2.3300**	0.4006 0.5007 0.5007 0.5007 0.5007 0.5007	**2.0029** **3.0044** **3.6720** **3.6720** **3.6720** **3.6720**
Pregnant Women	≤ 18y 19-30y 31-50y	ND ND ND	ND ND ND	0.2996 0.2996 0.2996	**1.9971** **2.3300** **2.3300**	0.7344 0.7344 0.7344	**3.0044** **3.6720** **3.6720**
Lactating Women	≤ 18y 19-30y 31-50y	ND ND ND	ND ND ND	0.3661 0.3661 0.3661	**1.9971** **2.3300** **2.3300**	0.9681 0.9681 0.9681	**3.0044** **3.6720** **3.6720**

IV. CONCLUSION

In this study, the use the three millimetres thick of flexible boron shields was used to cut off thermal neutrons in order to assess epithermal neutrons at 94.7% accuracy enabling the EINAA method to be achieved. The low levels of Br, Cl and I was successful due to the standard reference materials (IAEA)-336; IAEA-407, IAEA-350 and (NIST) USA SRM 1577b.

The analysis of the three elements in the Blackchin Tilapia did not show any good levels that may be good for human consumption due to where these elements may be coming from. The Benya Lagoon needs regular dredging due to the activities around the bed of it.

ACKNOWLEDGMENT

The authors wish to express their sincere appreciation to the technicians and staff of Ghana Atomic Energy Commission at Kwabenya - Accra, for their kind assistance in the analysis of the samples.

TABLE VIII. SUMMARY OF BR, CL AND I FUNCTIONS AND HOW THEY AFFECT THE HUMAN BODY

	Function	Adverse Effect of Deficiency	Adverse Effect of Excessive Consumption	Depleted by	Sources
Br	Has anti-seizure properties, and it is an effective trace mineral in the treatment of hyperthyroid conditions. Helps the body's circulation and water balance.	Seizures, insomnia, agitation, irritability, hyperthyroidism	Poor memory, acne, hyperactivity disorder in children, nausea, vomiting, dizziness, fatigue, drowsiness, coma, blurred vision, skin rash, psychosis, pancreatitis, muscle weakness, increased thirst, hunger and urination, hallucinations hypothyroidism.	Excess alcohol and sugar	Grains, Kelp, seaweed, Fish (marine), nuts, (also some baked goods and fire-retardant compounds).
	[11]				
Cl	Keeps the amount of fluid within and around cells in balance, conserves potassium, critical constituent of hydrochloric acid, a key component of gastric juice secreted by the stomach that is vital for maintaining the normal acidic environment needed by pepsin, and aids digestion and absorption of many nutrients including iron and vitamin B12, helps regulate the pH (acid-alkali / acid-base) balance of body fluids, maintains proper blood volume and pressure.	Burns, heavy sweating, congestive heart failure, or overuse of coffee or laxatives or diuretics, over-hydration certain kidney disorders, Addison's disease, most often seen in infants on chloride-deficient formulae	Coughing, symptoms of asthma such as wheezing and tightness of the chest, blurred vision, redness, and blisters on the skin, burning sensation in the nose, throat, and eyes.	Excess perspiration, prolonged diarrhoea or vomiting	Table Salt, cheese seaweed (such as dulse and kelp), olives, celery, rye, lettuce, tomatoes, bacon, ham, sausages, sausages, yeast extract as potassium chloride found in most foods.
	[40]				
I	Utilized by the thyroid gland for the biosynthesis of the thyroid hormones thyroxin (T4) and tri-iodothyronine (T3). Every cell in the body depends upon thyroid hormones for its metabolism.	Abortions, Goitre, Stillbirths, Infant Mortality, Mental deficiency, obesity, anger, dwarfism, Congenital anomalies, Psychomotor defects, hypothyroidism.	High iodine intakes can also cause thyroid gland inflammation, same symptoms as iodine deficiency, including goiter (an enlarged thyroid gland) and thyroid cancer.	Too much protein	Milk, Fish (fresh water), Fish (marine), Shellfish Meat, Eggs, Legumes, Grains, Fruits, Vegetables
	[41, 42, 43]				

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How to Cite this Article:

Vowotor, M. K., Sackey, S. S., Amoako, G., Sefa-Ntiri, B., Amuah, C. L.Y. & Mireku, K. K. (2020). Epithermal Instrumental Neutron Activation Analysis Technique in Determining Bromine, Chlorine, and Iodine in Sarotherodon Melanotheron from the Benya Lagoon, Ghana. International Journal of Science and Engineering Investigations (IJSEI), 9(99), 5-15. http://www.ijsei.com/papers/ijsei-99920-02.pdf