UNIVERSITY OF CAPE COAST

EPITHERMAL INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS ASSESSMENT OF MICRONUTRIENTS IN FISH, BREAST MILK AND RICE USING GHARR-1: CASE STUDY OF SOUTHERN GHANA

MICHAEL KWAME VOWOTOR

2020

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BY

MICHAEL KWAME VOWOTOR

Thesis Submitted to the Department of Physics of the School of Physical Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in Partial Fulfillment of the Requirements for the Award of Doctor of Philosophy Degree in Physics

JULY 2020

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature ……..……...… Date: ……..…

Name: Michael Kwame Vowotor

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature ……..…… Date: ……

Name: Prof. George Amoako

1st Co-Supervisor's Signature ……..…… Date: ……

Name: Dr. Baah Sefa-Ntiri

2nd Co-Supervisor's Signature ……..…… Date: ……

Name: Dr. Samuel Sonko Sackey

ABSTRACT

The concentrations of ten micronutrients; sodium (Na), magnesium (Mg), chlorine (Cl), potassium (K), calcium (Ca), vanadium (V), manganese (Mn), copper (Cu), bromine (Br), and iodine (I), in polished Jasmine 85 rice, Sarotherodon melanotheron and breast milk was determined using epithermal instrumental neutron activation analysis. The rice samples were collected from farms located in Ashaiman, Afienya, Dawhenya, Aveyime, and Afife, all in Ghana; the breast milk from breastfeeding mothers from the Central Region of Ghana, and the *S. melanotheron* fish was harvested from the Benya Lagoon, Komenda Edina Eguafo Abrem Municipality in the Central Region of Ghana. A-three millimetre thick flexible boron was used to cut-off thermal neutrons and access epithermal neutrons only. 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. Relative standardization method was used in the quantification of the elements. Recoveries of the elemental concentrations ranged from 88% to 111% of the certified values. Rice farms from Aveyime and Afife recorded higher concentrations of the micronutrients. Micronutrients in the breast milk yielded the order K > Na > Ca > Mg > Cu > Mn > I and in the rice will be useful in nutrition planning. The *S. melanotheron* study revealed issues associated with its consumption as it is a delicacy. 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 Lagoon.

KEYWORDS

Benya Lagoon

Breast milk

Epithermal Instrumental Neutron Activation Analysis

Micronutrients

Polished Jasmine 85 Rice

Sarotherodon melanotheron

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For their unparalleled sacrifices, spiritual and moral support, I am grateful to my wife, Vivian, and my daughter, Clare. My sincere thanks go to all other people who were behind my progress throughout my studies. Pardon me if I did not mention your name.

DEDICATION

To my family. I love you so much.

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LIST OF ACRONYMS

A	Mass number
AA	Activation Analysis
ADDI	Average Daily Dietary Intake of Iodine
AI	Adequate Intake
AC	Anticoincidence counting
B2	Calibrating irradiation site
Cd	Epi-cadmium (related to irradiation under cd-cover)
CNAA	Cyclic Neutron Activation Analysis
COMITR	Concentration of micronutrients in the rice
CRM	Certified Reference Standard materials of geological, environmental and biological origin.
DGNAA	Delayed Gamma-Ray Neutron Activation Analysis
DNA	Deoxyribonucleic Acid
ED	Estimated Dose
EINAA	Epithermal instrumental neutron activation analysis
eV	Electron Volt
GAEC	Ghana Atomic Energy Commission
GHARR-1	Ghana Research Reactor-1
GHS-ERC-02/05/15	Sanctioned techniques used by the Ghana Health Service Ethical Review Committee
HI	Hazard Index
HCA	Hierarchical cluster analysis

HCl	Hydrochloric acid
HPGe	n-type high-purity Germanium detectors (model GR2518).
ICPMS	Inductively Coupled Plasma Mass Spectrometry
IDD	Iodine Deficiency Disorder
INAA	Instrumental Neutron Activation Analysis
IQ	Intelligence Quotients
k_0-method	Single Comparator Method
LDA	Linear Discriminant Analysis
MAESTRO	Software used for spectrum investigation
MCA	Multichannel Analyzer
MNSR	Miniature Neutron Source Reactor SLOWPOKE type reactor that was developed and constructed by the China Institute of Atomic Energy (CIAE).
MRIGPD	Mean rice intake (g/person/day)
MW	Megawatts
n	Number of cycles
N	Number of stable target nuclei of interest in the sample
NAA	Neutron Activation Analysis
ND	Lack of data
N_i	Number of nuclei of the parent material
NIST	National Institute of Standards and Technology
ORTEC EMCAPLUS	Multichannel Analyzer Emulation software.
PCA	Principal Component Analysis
PGNAA	Prompt Gamma-Ray Neutron Activation Analysis

RDA	Recommended Dietary Allowable
RfD	Reference Dose
RNAA	Radiochemical Neutron Activation Analysis,
SRM	Standard Reference Materials
UL	Mean and the Maximum Upper Limit
XRF	x-ray fluorescence
Z	Atomic mass number
T3	Triiodothyronine
T4	Thyroid hormones thyroxine

LIST OF SYMBOLS

C_{sam}	Countings for sample
C_{std}	Countings for standard
D	Decay factor ($=e^{-\lambda t_d}$)
D_{sam}	Decay factors for the sample
D_{std}	Decay factors for the standard
E	Neutron energy
E_b	Binding energy of photoelectron in original shell.
E_{e^-}	Energy of the photoelectron produced
E_γ	Gamma ray energy
I_0	Resonance integral for a 1/E epithermal spectrum
Mg/MnO_2	Magnesium/manganese dioxide
P_A	Number of counts in the full-energy peak
$(P_A/t_c)_{sam}$	Counting rates for sample
$(P_A/t_c)_{std}$	Counting rates for standard
Zn/MnO_2	Zinc/manganese dioxide
$t_{1/2}$	Half-life
t_c	Counting time
t_d	Decay time
t_i	Irradiation time
α	Epithermal neutron flux shape factor (parameter describing the $\phi_{epi}(E) \sim 1/E^{1+\alpha}$ neutron flux distribution)
ν_o	Frequency of the incident photon

γ	Absolute gamma ray intensity (gamma ray emission probability)
ρ	Concentration of element
ρ_{sam}	Concentrations of the element of interest
ρ_{std}	Concentrations of the standard element
$\sigma(E)$	(n, γ) neutron cross section at energy E
σ_{eff}	$[G_{th,i}\phi_{th}\sigma_0 + G_e\phi_{epi}I_{0i}]$ is the effective cross section for the (n, γ) reaction
$\sigma(v)$	(n, γ) cross section at neutron velocity v
ϕ_{th}	thermal neutron flux
ϕ_{epi}	Epithermal neutron flux

LIST OF CONSTANTS

E_{cd}	Effective Cd cut-off energy
	(=0.55 eV under standard conditions)
h	Planck's constant
N_A	Avogadro's number
σ_0	$\sigma(v_0)=\sigma(E_0)=2200$ m.s^{-1} (n,γ) cross section
λ	Decay constant

CHAPTER ONE

INTRODUCTION

Background to the Study

Percent composition of a substance is the percentage by mass of each element in the substance. It shows how much the substance consists of one component or the other. Examples include the percentage composition of air, which is 21% weight of oxygen, and water is 80% weight of oxygen and 20% weight of hydrogen. One is bound to get oxygen poisoning by breathing any higher oxygen percentage composition for a prolonged period, and water may be considered unsafe to drink if not in the right composition. In this regard, elemental analysis is a technique where the material is tested for its elementary structure, and often isotopic. It can be either qualitative (determine which elements are present) or quantitative (determine how many of them are present) (Helmenstine, 2019).

Activation Analysis (AA) is a procedure used to determine the trace element concentration of the materials. It is based on nuclear reactions stimulated in the material by irradiating it either with ionizing or non-ionizing radiation and producing radionuclides specific for any given element. AA's high sensitivity and precision, synchronous multi-element diagnostic ability, and grid-free and non-destructive investigation have prompted it being broadly utilized in several of fields including Biology, Geology, Environment, Agriculture, etc. Depending on the type of elements to be examined, one can use neutrons, charged particles, or photons in bombarding the elements in the material. When neutrons are employed, the procedure is known as Neutron

Activation Analysis (NAA) (Glascock, 1996). In NAA, neutrons are used to induce nuclear reaction for an excited isotope formed by neutron capture, known as the intermediate state. The excited isotope intermediately de-excites by emitting prompt γ-ray in 10^{-14} seconds after its formation. The radioactive product then decays via α, β, γ, or delayed gamma-ray emission processes. This γ ray, which is emitted later, is known as a delayed γ-ray. Analytical information, either qualitative or quantitative is obtained by monitoring the emitted α, β, and γ rays. The procedure of neutron capture by an objective nucleus, trailed by the emission of γ-rays is shown in Figure 1.

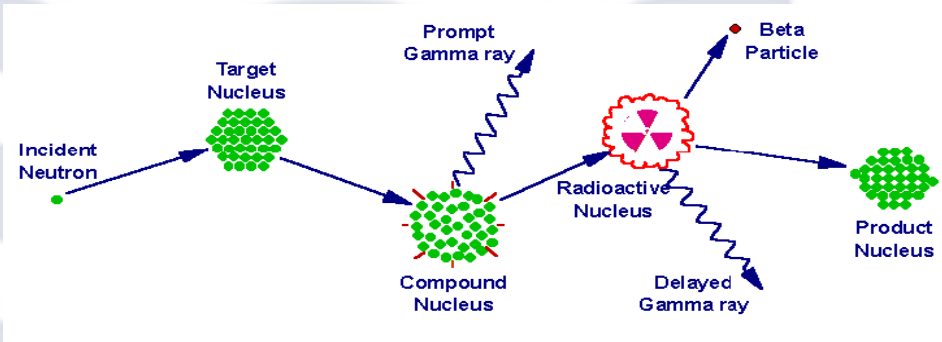

Figure 1: A procedure of neutron capture by a target nucleus followed by emission of gamma rays (Glascock, 1996)

Neutron source, instrumentation appropriate for distinguishing gamma beams, and exhaustive information on the responses that happens when neutrons communicate with the objective cores are the fundamentals expected to lead test examination utilizing NAA. Depending on the species that are radioactive, their half-lives can range from several years to a fraction of a second. Therefore NAA falls into two categories as regards the period of measurement (Ali, 1999):

1. During irradiation, measurements can take place. This process is known as the prompt gamma-ray neutron activation analysis (PGNAA),
2. The measurements are done after irradiation, and the species undergoes radioactive decay. This is also known as the delayed gamma-ray neutron activation analysis (DGNAA).

Mostly used operational mode is the latter. The general assumption is that NAA means the measuring of delayed gamma rays. NAA can be used to estimate about 70% of elements due to their properties (Ali, 1999). DGNAA, oftentimes called the conventional NAA, is helpful because most of the components produce radioactive nuclides. This strategy is flexible since with time the end goal has the affectability of an enduring radionuclide experiencing meddling from radionuclide that is short-lived can be expanded by hanging tight for the rot of the brief radionuclide. DGNAA's principal advantage over other empirical approaches is this selectivity.

In general, the energy of a gamma-ray is characteristic of a nuclide. Gamma-ray energies ranging between 70 and 3100 keV are commonly used for multielement determination using NAA. NAA can be performed in an assortment of ways relying upon the idea of the background matrix. In some cases, the element of interest is concentrated from an interfering matrix before irradiation with neutrons. This technique is termed “preconcentration neutron activation analysis, PNAA”. On the other hand, if the irradiation is done before the separation of the desired elements, the process is well-known as radiochemical neutron activation analysis, RNAA. Appling these techniques

to natural matrices involve several steps such as digestion of the sample, followed by wet chemical separations. This makes PNAA and RNAA time-consuming and not convenient for routine analysis. Furthermore, in RNAA special precautions, such as the addition of carriers, have to be taken into consideration to correct for errors due to loss of the elements of interest. RNAA has an added disadvantage of not being able to make use of short-lived nuclides due to the relatively long experimental times involved. It is therefore not performed frequently due to its high labour cost (Ali, 1999). In any case, both PNAA and RNAA can be very useful for analyzing certain matrices (De Sooete *et al.*, 1972; Ehmann & Vance, 1991).

Problem Statement

The Ghana Research Reactor (GHARR-1) is located on the premises of Ghana Atomic Energy Commission (GAEC), Accra. The need to use k_0-INAA in addition to the relative comparator method for routine NAA had been demonstrated and supported by International Atomic Energy Agency (IAEA) by distributing free k_0-IAEA software to most laboratories of member states including GHARR-1 since the beginning of 2005 (Rossbach *et al*, 2006). However, unlike the relative comparator method, the development of a single comparator method (k_0-method) in analyzing trace elements has been given little attention to in most laboratories, and GHARR-1 is not an exception. This is because the relative comparator method is still simple, sensitive, offers short-turnaround time and it is cheap. This work is intended to implement the relative comparator method at GHARR-1, by characterizing and calibrating irradiation site knowns as B2 and HPGe detector respectively for the

determination routine elemental analysis at GHARR-1 Analytical Laboratory using rice, fish and breast milk as analytes. This entails the analysis of sodium (Na), magnesium (Mg), chlorine (Cl), potassium (K), calcium (Ca), vanadium (V), manganese (Mn), copper (Cu), bromine (Br), and iodine (I) elements present qualitatively and quantitatively. Recommendations to how to minimize iodine deficiency disorder (IDD) would be made.

Objectives of the Study

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 INAA, the problem of loss of iodine due to sample preparation is avoided. Apart from the fact that NAA is a "referee method", it is simple, sensitive, offers simultaneous multielement determination and short-turnaround time.

Due to the high concentrations of elements like Na, K, Ca, Mg, Mn, and Cu in foods, suitable nuclear methods aimed at reducing interferences from these elements are needed. To determine the low levels of iodine in some Ghanaian foods, there is a need to develop methods, which would improve the sensitivity, precision, accuracy, and detection limits of measurements. In this regard, pseudo-cyclic INAA and EINAA methods have been developed and used. EINAA method is extensively used for short-lived nuclides such as Na, V, Cl, K, Ca, Mg, Mn, and Cu (Acharya and Chatt, 2009). These eight elements are in the list of micronutrients in breast milk and other food substances, therefore very necessary to be determine.

Therefore, in this study the following specific objectives have been pursued:

1. To investigate the application of EINAA for fish, rice, and breast milk reactor neutron characterization.
2. To provide validation protocol for EINAA.
3. To help create a national database on elements under observation in fish, rice, and breast milk.
4. To understand and quantify the intake of these nine elements in babies in the Central Region of Ghana.
5. To find out whether the eating habbits of the lactating mothers impact well on their breast milk which will, in turn, affect their infants.
6. Finally, the data will answer the question about why babies in some developing countries does not positively affect the good health of babies after the age of 3 to 4 months than some babies in developed countries.

Significance of the Study

- The EINAA methodology via an EINAA software is a skill-based venture that can be transfered to individuals or similar laboratories. When fully equipped with the understanding of the EINAA method and utilization of EINAA software, individuals could be trained in terms of implementation of the protocols developed for routine use and may be required to entirely set up the method in other EINAA laboratories.

- This research would hopefully develop an in-house spectrum evaluation software that is based on the equipment and analytical requirements. The Software to be developed may be fully applicable to different laboratory setup. Therefore the study would serve as a baseline for developing other methods and to undertake the development of other in-house software (spectrum evaluation software).
- The EINAA methodology has the potential of reducing analytical cost. The design protocol is intended to meet validation criteria such as ease of use, costs per analysis, sample throughput, and turnaround time to obtain maximum information per sample per analysis.
- The validation protocols (in a form of properly prepared document) for EINAA is a special requirement, as the GHARR-1 also undertakes commercial analysis. Hence, it is very necessary for justification, reliability, and consistency in analytical reporting and more importantly for legal purposes.
- The data from this project would probably throw new light on the nutritional requirements of all life stage groups especially the young babies between 0 to 6 months for these elements.
- So far, published recommended dietary allowances (RDAs) by such bodies as the US National Academy of Sciences for 15 elements, and are widely quoted and used in other countries. For some of the elements however, there are differences between the definite intakes observed in the presently used RDAs (Parr, 1983). The results of this study would help create an RDA for Ghana.

- Although, RDA comparisons from the United States National Academy of Sciences is not altogether direct because of changes in bioavailability (e.g. elements like iron are well absorbed in human milk but much less in formula products) there nevertheless seem to be some significant differences between actual intakes and RDAs that need to be explained (Parr, 1983). This is a matter of no little importance since the commercial manufacturers of formula products for babies are starting to supplement their products with trace elements such as copper, zinc, manganese, iron, and, iodine at levels corresponding to the published RDAs. In this respect, therefore, formula products are beginning to have the appearance of being of better nutritional quality than breast milk, which almost certainly cannot be true. It is expected therefore, that this study will help provide definitive new data for establishing the correct nutritional necessities of young infants for essential minor and trace elements (Parr, 1983). The data from this project will help bridge the gap between Physicists, Chemists, Biologists, and the commercial manufacturers of formula products for babies. Ultimately the data from this project may contribute to a better design of infant formula products.

Scope of Work

This project re-characterizes the GHARR-1 facility in Kwabenya, Accra, Ghana, for EINAA by adopting an experimental approach to calibrate and validate the EINAA method by analyzing Certified Reference Standard materials (CRMs) of geological, environmental and biological origin.

As shown in Figure 2, this research will consist of the following steps in the implementation of the EINAA method at GHARR-1:

1. Development of a theoretical background EINAA.
2. Characterizing and calibrating irradiation site B2 and HPGe detector, respectively, for the determination of EINAA-parameter through the validation for routine elemental analysis at the GHARR-1 Analytical Laboratory.

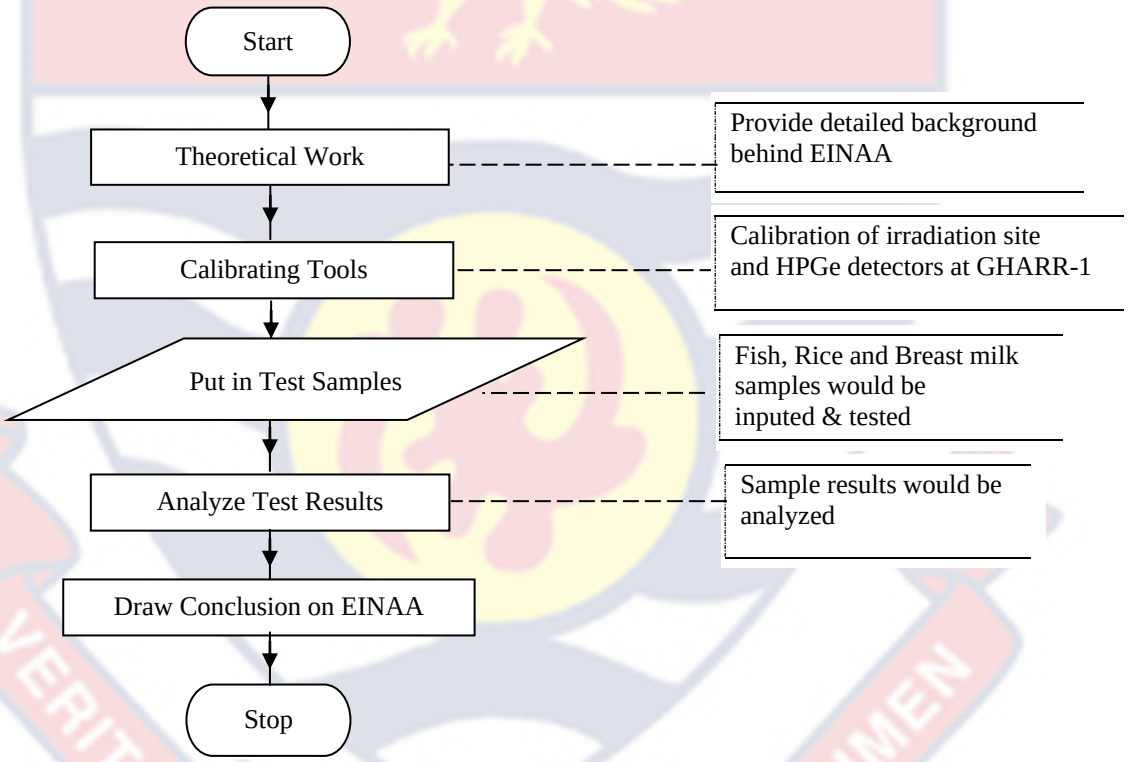

Figure 2: A layout of the scope of work

Limitations of NAA

Although NAA is an accurate (~5%) and precise (<0.1%) multi-element analytical technique, it has several limitations that should be addressed:

- The major limitation is the number of elements that can be analyzed by this technique. For a number of elements the sensitivity is rather poor, and so either their half-lives are very long or very short, the formation cross-sections are low (Lyon, 2020). Several elements of geological interest, such as Nb, Y and some transition metals, are better determined by other analytical methods. For example, more precise Rb, Sr, Y, Nb, and Zr concentrations can be obtained by X-ray fluorescence (XRF). In fact, INAA and XRF are complimentary techniques and rock and mineral chemistries are often determined using both INAA and XRF (Atwood, 2010).
- No direct information on the chemical state of the element is obtained. Also, because there is no chemical pre-separation, the sensitivity of the method is dependent upon the sample matrix. For example, detection limits for all elements are lower in tree ring samples than in rock samples (Atwood, 2010).
- Initial equipment cost for semiconductor spectrometry and irradiation facilities is high (IAEA Nuclear Energy Series, 2014).
- Long counting times may limit the number of samples that can be processed (Kruger, 1971).
- In NAA methods for environmental samples, decay periods of up to one month may be necessary to allow the determination of some long-lived nuclides. Hence, the method would have a time lag for some results (Osei, 2002).
- The average analytical laboratory may not have access to a nuclear reactor (Osei, 2002).

Organization of Work

The research work is presented in five chapters to deal with specific aspects of the study and has been arranged as follows. **Chapter One** deals with the introduction, objectives, and scope of the work. Besides, various methods of analyzing samples and the need to use NAA are discussed. In **Chapter Two**, the literature and theories of NAA and EINAA are treated. The study areas, experimental setup, and results are covered in **Chapter Three**, while in **Chapter Four** details of the Analysis and Discussions of Results are elucidated. In **Chapter Five**, the concluding remarks to the work, future applications, and suggestions for improvements and recommendations to this work are discussed.

Chapter Summary

In summary, an overview of what micronutrients in food are and how they are a key factor that determines the health stutus of a person. Also, how to measure micronutrients are measured using activation analysis was introduced.

CHAPTER TWO

LITERATURE REVIEW

Introduction

There are different sources of neutron (reactors, quickening agents, and radioisotopic neutron producers) often utilized for NAA. Atomic reactors having high transitions of neutrons using uranium parting proposals mostly noteworthy sensitivities available for most components. Several authors have demonstrated that various sorts of reactors and various situations inside a reactor can shift significantly concerning their neutron vitality conveyances and motions because of the tools used to direct (or diminish the energies of) the essential parting neutrons. These they have used to measure elemental content in foods, soils, liquids, etc (Tandoh et al, 2009; Nyarko et al., 2002; Acharya and Chatt, 2009; Tandoh, Bredwa-Mensah, Dampare, Akaho, and Nyarko, 2009; Glascock, 2003).

Instrumental Neutron Activation Analysis (INAA)

In trace analytical work, contamination is an ever-present problem that can be detected using nuclear analytical methods, such as NAA. NAA require much less sample manipulation than other methods like Atomic absorption spectroscopy (AAS). In AAS, there are chemical preparations but NAA are relatively free of reagent and laboratory contamination. NAA also has an automated sample handling used with a solid-state gamma-ray measurement

detector, and data processing which is computerized, and over thirty elements are typically measurable simultaneously without chemical processing in most sample forms. With this technique, no chemical separation is done to treat the sample before or after irradiation. Samples are simply packed in this method, irradiated for the specified period, allowed to decay, then counted, and the results of the elements are checked and recorded. Applying only instrumental procedures are referred to as instrumental neutron activation analysis, INAA. NAA has become one of the preferred methods worthy of notice over other analytical methods. Several elements which are either short-lived or medium-lived radioisotopes are measured using INAA.

Cyclic and Pseudocyclic INAA

Cyclic Neutron Activation Analysis (CNAA) is a technique of NAA where samples are irradiated, delayed, and counted and the process repeated for many cycles to improve the detection sensitivity of short-lived radionuclides. This method increases the sensitivity whilst there is a decreased in statistical error. Rapid counting after a short time irradiation is what is needed for each cycle in analyzing samples. An ideal number of 4 periods of time is important in CNAA. These are irradiation time, decay time counting time, and back transfer time. This method is used to detect trace elements of a short-lived radionuclide with very short half-lives. These include selenium (Se), scandium (Sc) and dysprosium (Dy). These trace elements are found in minute quantities and can only be detected by spectrographic methods or by using radioactive elements (Ehmann & Vance, 1991).

In designing a rapid cyclic form of the CNAA technique, an automated pneumatic system must be installed in the research reactor facility. However, in the absence of such an automated pneumatic system, a manual system known as Pseudo-Cyclic NAA can be used.

A day to several weeks is required for a lot of elements, during analytical period due to the differences in half-lives of nuclides. Examples of such half-lives include ^{181}Hf (45 days), ^{46}Sc (84 days), ^{75}Se (120 days), and ^{110}Ag (250 days). A long irradiation time, delay time, and counting time are the requirements for sensitivity, whereby using elements with short-lived nuclides are preferred in inductively coupled plasma mass spectrometry (ICPMS). When comparing this to other analytical procedures such as their longer-lived activation products as they significantly decrease the analytical period and more samples that are measured each day. That is why activation analysis more economical and profitable. A number of elements, including ^{207m}Pb (0.8 s), ^{19}O (27 s), and ^{20}F (11 s) are determined via only their short-lived nuclides. Therefore, more attention is now focused on NAA using short-lived nuclides (Ehmann & Vance, 1991).

Epithermal Instrumental Neutron Activation Analysis (EINAA)

Radio-isotopic neutron emitters, accelerators, and reactors are the various sources of neutrons used during NAA. However, for most elements, nuclear reactors with high uranium fission neutron fluxes are able to give the utmost sensitivities. For many of the reactors, different locations within a reactor can vary significantly in terms of their distributions of neutron energy

and fluxes owing to the materials used to regulate (or reduce) the primary fission neutrons.

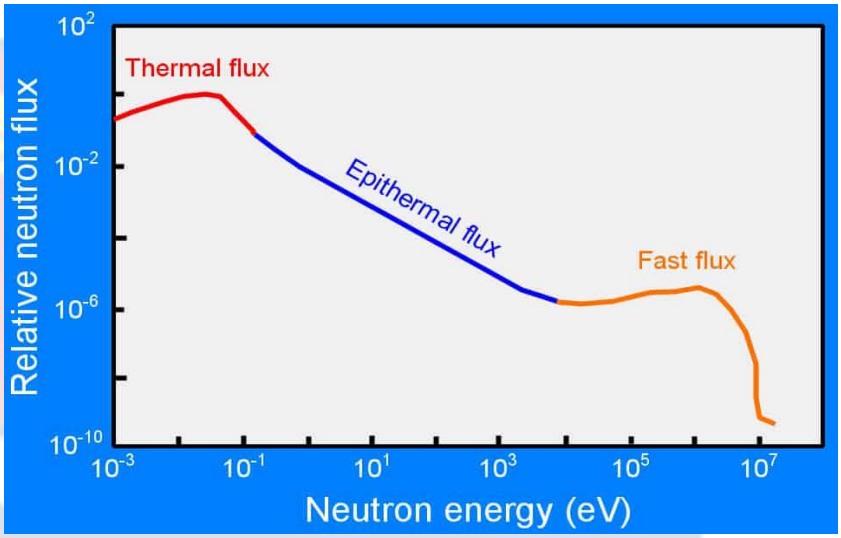

Figure 3: A typical reactor neutron energy spectrum showing the various components used to describe the neutron energy regions (Glascock, 1996)

Figure 3 shows that most distributions of neutron energy are fairly wide and comprise three main components, namely thermal, epithermal, and fast (Glascock, 1996).

The energies below 0.5 eV are the thermal neutron section and they are composed of low-energy neutrons in the reactor moderators and in thermal equilibrium with the atoms. The thermal neutrons' energy spectrum at room temperature is best described by a Maxwell-Boltzmann distribution with a mean energy of 0.025 eV and a most likely velocity of 2200 ms^{-1}. 90-95% of the neutrons that hit a sample are known as thermal neutrons in most reactor irradiation positions. A 1 MW reactor in general has a peak thermal neutron flux of approximately 1.0 $x10^{13}$ $cm^{-2}s^{-1}$. Activation analysis employing this

type of neutrons for sample irradiation is termed thermal neutron activation analysis but generally referred to as NAA.

The component that deals with epithermal neutrons is made up of neutrons of energies between 0.5 eV to about 0.5 MeV and are only partially moderated. 0.025 eV is the most probable energy, with the energy range below epithermal neutron energy (0.1-1eV) (Ehmann & Vance, 1991). All the thermal neutrons are absorbed by a 1 mm thick cadmium foil or boron shield but allows fast and epithermal neutrons above 0.5 eV in energy to go through. The epithermal neutron flux represents approximately two percent of the total neutron flux in a standard unshielded reactor irradiation location. These two neutrons induce (n, γ) reactions on target nuclei. An epithermal neutron activation analysis, ENAA is an NAA procedure that uses only epithermal neutrons to cause (n, γ) reactions by irradiating the samples being studied. The sample is surrounded by either cadmium or boron shield.

In the energy spectrum of above 0.5 MeV (fast neutrons) are composed of the primary fission neutrons, having original energy after fission. Contribution of the fast neutrons are very little to the (n, γ) reaction, instead, they induce nuclear reactions where the ejection of one or more nuclear particles as well as (n, p), (n, n'), and (n, 2n), are prevalent. Around 5% of the total flux is made up of fast neutrons are in a usual reactor irradiation position. The NAA procedure which uses fast neutron-induced nuclear reactions is called fast NAA (Alfassi, 1990).

Reactions that occur with fast neutrons of energies in the MeV range should be considered in two techniques namely; (1) Use of those reactions to

determine certain elements, and (2) Possible interference with certain reactions in deciding those elements by the (n, γ) reaction owing to the formation of the same radionuclide. These can only be resolved by the use of double irradiation, which is, irradiating the sample bare (without Cd cover) and then irradiation within the filters of Cd or B followed by calculation of the contribution from every element. The same treatment is generally used when using (n, ∝) and (n, p) reactions in finding out certain elements. (Glascock, 2003)

The foremost benefit of these reactions is that they create nuclides that are different from those created by (n, γ) reactions. Thus, this leads to faster determinations when producing a short-lived nuclide rather than the long-lived one normally created by the (n, γ) reaction. Other times, these reactions may allow the purpose of elements that cannot be measured via (n, γ) reactions because the created radionuclide is only a β emitter.

Importance of Iodine and Nine Other Elements in Baby Nutrition

Iodine is a vital trace element and a micronutrient that plays a very essential part in the human physiological actions of thyroid hormones (Endocrineweb, 2017). The thyroid gland (Figure 4), is a small gland located in the front of the neck. Its function is to take Iodine (I), found in several foodstuffs, and transform it into the thyroid hormones thyroxine (T4) and triiodothyronine (T3). The thyroid cells are the only cells in the body that can absorb iodine. These cells synthesis iodine and the amino acid tyrosine to make T3 and T4. These T3 and T4 are then released into the bloodstream and conveyed throughout the body where they control metabolism. A normal

thyroid gland produces about 80% T4 and about 20% T3. However, T3 possesses about four times the hormone 'strength' as T4 (Endocrineweb, 2017). The thyroid hormones are depended on by every cell in the body for regulation of their metabolism and promotes growth and development in the body including the brain.

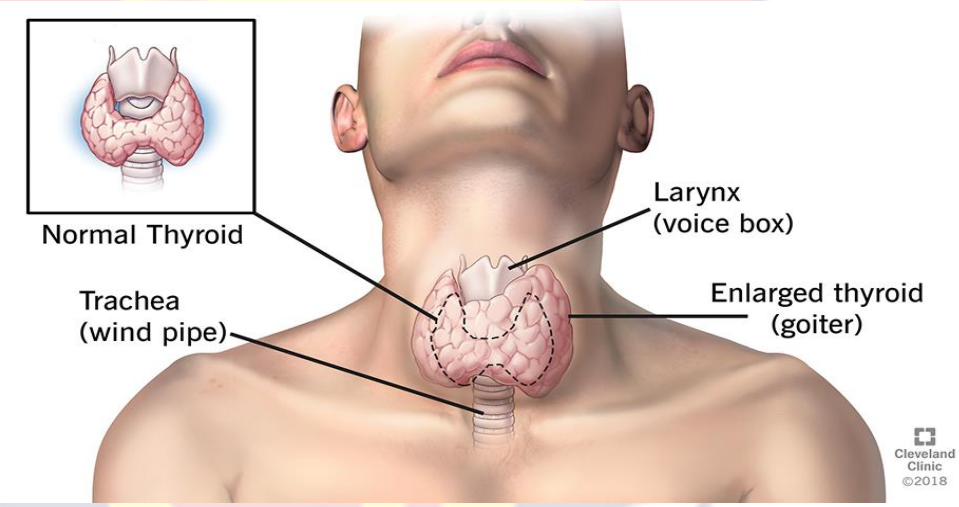

Figure 4: An image of a healthy and damaged thyroid gland (Cleveland Clinic, 2018)

Insufficient and excessive intake of this micronutrient can lead to thyroid disorder (Kapil, 2007). Iodine deficiency has been identified as one of the factors that have an adverse effect on child development in developing countries (Walker et al., 2007). These deficiencies manifest as stunted physical and mental growth as well as infertility, lethargy, and cognitive impairment (Adu & Simpson, 2017). Thyroid hormones assume a significant job in the development and advancement of the brain and central nervous system in humans from the 15th week of gestation to 3 years (Malik & Hodgson, 2002). Therefore, any obstruction in its function has adverse effects on children as

well as adult population. Each cell in the body, including the brain, relies upon thyroid hormones for the control of metabolism and stimulates the growth and development in the entire body.

In Ghana, a major problem affecting a cross-section of the population, especially women and children, is goitre. In the Northern part of Ghana, people are particularly vulnerable to iodine deficiency disorders (IDD). In recent years, there have been national drives to combat the problem of IDD with efforts being made by governmental and non-governmental agencies, and scientists, to estimate the average daily dietary intake (ADDI) of iodine. All these efforts are geared towards the recognition of the population group at risk and the identification of various natural sources of iodine (Nyarko et al., 2002).

A fall in metabolic rate, increase in serum cholesterol and enlargement of thyroid glands results from a goitre. Iodine deficiency is the leading preventable cause of goitre (Chung, 2014), which leads to significantly lowering the intelligence quotients (IQ) of a population. Chung, 2014 raised concerns about the raise in the continued occurrence of IDDs among children. In a related study in Komenda Edina Eguafo Abirem (KEEA), a high (42.5%) prevalence of iodine deficiency was in pregnant women was observed (Simpong et al., 2016). This will hamper the goals of Ghana's educational programme and the country's formative endeavours. Statistics shows that about 81,200 babies are born yearly with mental deficiency as a result of IDD, and these babies suffer from stunted growth and low IQs, thereby impeding their learning abilities (Ghana News Agency, 2007).

Infants up to 6 months need an iodine intake of 90 micrograms per day. Breast milk is the only and best source of iodine for babies breastfeeding and it helps in the development of their brain and nervous system. Breastfeeding mothers might be recommended iodine supplements to ensure that their babies get enough iodine (Mrunal, 2018).

Although bromine (Br) has not been officially designated essential for humans, it plays a vital role in controlling inflammation of the thyroid gland (Acu-Cell Nutrition, 2017). There have been reports of a reduction in growth, fertility, and life expectancy in some animals as a result of hyperthyroidism due to dietary deficiency of bromine. In humans and animals, bromine - either as sodium bromide or potassium bromide - has anti-seizure properties, and is an effective trace mineral in the treatment of hyperthyroid conditions (Acu-Cell Nutrition, 2017). Organic bromines are widely used as sprays to kill insects and other unwanted pests. The residues of these sprays enter runoffs and main water bodies where they get bio-accumulated in the organism in the food chain. The health effects triggered by such bromine-containing organic contaminants are malfunctioning of the nervous system and disturbances in genetic materials (Lenntech, 2017).

Chlorine (Cl) levels in the human body closely parallel that of sodium intake and output as their primary source is 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. Food intake is the main source of vanadium (V) and acts like insulin (US EPA, 2015). Vanadium is used for treating prediabetes and diabetes, low blood sugar, high cholesterol, heart disease, tuberculosis, syphilis, a form of

"tired blood" (anemia), and water retention (edema), improving athletic performance in weight training and preventing cancer (WebMD, 2019). Chlorine is an electrolyte that works with potassium and sodium to regulate the amount of fluids in the body and its pH (Minerals Education Coalition, 2013). Table salt and many vegetables such as celery, tomatoes, lettuce, and seaweeds are good sources of chloride. The recommended daily dose of elemental Chlorine is 3400 mg per day (Lenntech, 2019).

About 80% of vanadium produced is used as a steel additive. vanadium steel alloys are very tough and used for the construction of tools, armour plates, axles, piston-rods, and crankshafts used in cars (Royal Society of Chemistry [RSC], 2015). Manganese in its pure state is too brittle to be of much use and as a result used in alloys such as steel. Steel, which has improved workability and resistance to wear, contains about 1% manganese. Manganese-steel contains about 13% manganese (Royal Society of Chemistry [RSC], 2015). Manganese is an essential nutrient/mineral that is found in several foods including nuts, legumes, seeds, tea, whole grains, and leafy green vegetables (U.S. National Library of Medicine, 2019). Its daily intake is 5 mg (Lenntech, 2019).

Many specialists are hesitant to prescribe oral magnesium preparations due to its known side effect of causing diarrhoea. The recommended daily dose of elemental magnesium is 400 mg per day, with a single dose of 800-1600 mg necessary to produce a laxative effect (Fine, 1991; Gums, 2004; Nishizawa, Morii and Durlach, 2007). Food intake is the main source of vanadium with its dietary intake estimated as 0.34µg/kg (US EPA, 2015) and an Upper Tolerable Limit of 1.8 mg/day (ATSDR, 2012; Institute of Medicine,

2001). Potassium is the third most abundant mineral in the human body and plays an important role in several body processes. A variety of whole foods are excellent sources of potassium, including beet greens, yams, banana, avocado, potatoes, and spinach (Healthline, 2019). Its daily intake is 3500 mg (Lenntech, 2019).

Another important micro-mineral for total health in children and adults is magnesium (Mg). It takes care of approximately 800 enzymatic functions in the human body. To mention just a few of its importance, a requirement of 30 mg per day is needed by babies up to the age of 6 months. It aids children to get better sleep. It is responsible for energy and hormonal health in children. It helps in regulating blood-sugar and therefore in the insulin management in the body. It also ensures good heart health, aids in maintaining blood pressure and DNA formation. It regulates the digestion process, the absorption of various vital nutrients by the body, and bowel movements. The human body depends Mg in building of strong bones and teeth, healthy muscles and nerves. It is beneficial in transporting calcium and potassium to the membranes of the body. It aids in protein synthesis and the respiration process. Mg deficiency in babies may cause sleeplessness, lethargy, and muscle cramps including other various health complications (Mahak, 2018). Studies have shown that both calcium and vitamin D are essential in building bone. Calcium can be found in many foods like Seafood and dairy (Harvard University, 2019). The recommended daily dose of elemental calcium is 1000 mg per day (Lenntech, 2019).

Copper (Cu) helps move broadcast communications flags across telephone wires, permitting correspondence across significant distances. Brain

cells "talk" to one another due to small amounts of Cu within certain enzymes in the brain forming key neurotransmitters. Experts have said that adequate amounts of Cu are needed for the brain to function (US Department of Agriculture, 2007). Babies up to the age of 6 months may require 0.20 mg per day (Food and Nutrition, 2001). The normal-term infant is born with a store of copper in the liver, making copper deficiency a rare event (Widdowson, 1974). Copper deficiency may be responsible for resistant anemia in milk-fed infants because cow's milk is one of the few commonly used foods low in copper (Picciano, 1985). Copper is present in organ-meat foods, seafood, nuts, wheat bran cereals, whole grain, and seeds. The small intestine mainly absorbes Cu, although the stomach also absorbs some. The absorption of copper ranges from more than 50% for intakes below 1 mg/day to less than 20% for intakes above 2 mg/day (Lenntech, 2019). Very high levels of zinc or iron supplements largely affect Cu absorption in the body (Botash, Nasca, Dubowy, Weinberger, and Oliphant, 1992; Morais, Fisberg, Suzuki, Amancio and Machado, 1994; Turnlund, 1999).

Manganese (Mn) helps in bone formation and aids some enzymes, for a example the enzymes involved in carbohydrate metabolism (Food and Nutrition, 2001; Lenntech, 2019). Babies with relatively high or low levels of Mn in their blood may develop a defect in their brain development (Norton, 2010). Obesity, changes in hair colour, abnormal bone, and cartilage function and growth retardation are some of the adverse effects of Mn deficiency (Lenntech, 2019). Babies up to the age of 6 months may require 0.003 mg of Mn per day (Food and Nutrition, 2001).

Natural Mn can be found joined with different components generally circulate the Earth's hull. Manganese is the second bounty component in the earth's covering; it is generally like iron in its physical and substance properties however is more earnestly and increasingly fragile. It happens in various significant stores, of which the most significant minerals (which are for the most part oxides) comprise essentially of manganese dioxide (MnO_2) as pyrolusite, romanechite, and wad. Manganese is basic to plant development and is the cause of the decrease of nitrates in green plants. It is a basic follow component in higher creatures, in which it takes an interest in the activity of numerous proteins. The absence of manganese in the human body causes testicular decay. An abundance of Mg components found in plants and humans can be harmful (Encyclopaedia Britannica. 2014). Magnesium is again used as an anode material in primary batteries due to its high standard potential. Magnesium/manganese dioxide (Mg/MnO_2) battery has twice the service life capacity compared to zinc/manganese dioxide (Zn/MnO_2) battery of the same size, and has a better ability to retain its capacity during storage, even at high temperature. Mg/MnO_2 battery is consequently more durable and better storable as it has a protective cover which is formed on the surface of the magnesium anode (Electrical4u, 2015). A critical screening of the debris along the banks of the Benya lagoon revealed the presence of used, spoilt, and discarded car batteries. This practice can explain the high levels of magnesium and manganese in the mined salt (Vowotor et al, 2011).

Sodium (Na) is an electrolyte/mineral that functions as a major ion for the extracellular fluid and also aids nerve impulse transmission. Babies up to the age of 6 months may need 120.0 mg of Na per day (Health Supplements

Nutritional Guide, 2017). The major source of sodium is salt. A baby gets all the needed sodium from breast milk, and therefore no need to add salt to the food (The National Academies, 2004; Nutritional Health Resource, 2017). Most dietary sodium (over 70%) comes from eating packaged and prepared foods as against salt added to food when cooking or eating. The Daily Value for sodium is less than 2,300 milligrams (mg) per day (US FDA, 2019).

Potassium (K) works with sodium to control the body's water balance, which helps maintain healthy blood pressure. Accordingly, a diet low in potassium but high in sodium is a factor in high blood pressure. Potassium also helps with muscle function and heart rhythm and, in later years, may reduce the risk of kidney stones and osteoporosis. An infant's body keeps a steady amount of potassium in the bloodstream while excreting excess amounts through the urine. The normal amount of potassium for a baby up to 6 months is 400.0 mg daily (Brannagan, 2019; Health Supplements Nutritional Guide, 2017).

Calcium (Ca) is a nutrient that builds strong bones and therefore children who get enough calcium start their adult lives with the strongest bones possible. This helps protect against bone loss later in life. Calcium keeps the nerves and muscles working and plays a role in keeping the heart healthy (KidsHealth, 2017). Young kids and babies need calcium and vitamin D to prevent rickets, a disease that softens the bones causes bow legs and stunted growth, and sometimes sore or weak muscles. The normal amount of potassium a baby up to 6 months may require per day is 200.0 mg (KidsHealth, 2017; Food and Nutrition, 2001).

The consumption of fish in Ghana has a per capita consumption of 22 kg/capita/year which is equivalent to 15 % of protein derived from fish (Mohammed and Uraguchi, 2013; Owusu et al., 2005). 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 (Vowotor et al, 2011).

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 general chemical behaviour of halogens shows great resemblances to one another and in the properties of their compounds with other elements (Encyclopaedia Britannica, 2017). In a displacement reaction, a less reactive Iodine is removed and replaced by the more reactive bromine. As a result, it is of significant interest to measure accurately bromine, chlorine, and iodine content of food items.

The seasonal variation in the chemical composition of plants has been reported by numerous specialists (Cannon, Papp, and Anderson, 1972; Sauchelli, 1969). The uptake of the elements from the soil is determined by many dynamics like the large quantity in the lithosphere, the form of the element, the pH of the soil, the physical condition of the soil, temperature, moisture content, the genetic constitution of the plant species (Teherani, 1987). Sometimes plants undergo a phenomenon known as the Steenbjerg effect, where under severe deficiency conditions, it is possible that the decrease of the concentration of the element results in a small increase in growth (Bista, Heckathorn, Jayawardena, Mishra and Boldt, 2018). The uptake

of elements by plant roots consists of two phases: absorption and accumulation (Teherani, 1987). The relations between ions can result in competition or competitive inhibition or the stimulation of uptake (Hemphill, 1972). Discharges from groundwater are important sources of nutrients, trace elements, and contaminants to many types of ecosystems (Kelly and Moran, 2002). Accordingly, it is important to characterize each distinct source of trace elements and determine their contribution.

Nuclear Reactors

A nuclear reactor is an apparatus used to initiate, maintain, and control a self-sustained nuclear chain reaction. Among other uses, it is utilized at nuclear power plants for the production of energy, artificial elements, and isotopes. Reactors that are used for research are usually made to be in a wide range of civil manner and commercial nuclear reactors which are generally not used for power generation but serve primarily as a neutron source for research and other purposes. Their output (neutron beam) can have different characteristics depending on its use. These research reactors are simpler than power reactors and they are operated at lower temperatures and need far less fuel and far less fission products build up as the fuel is being used. On the other hand, the fuel of research reactors require more highly enriched uranium, typically up to 20% U-235. They also have a very high power density in the core, which requires special design features. Like power reactors, the core needs cooling and a moderator which is required to slow down the neutron and enhance fission. As neutron production is their main function, most research reactors also need a reflector to reduce neutron flow from the core (World Nuclear Association, 2019).

Owing to the high neutron flux, experimental nuclear reactors operating in the maximum thermal power region of 100 kW-10 MW, with a maximum thermal neutron flux of 10^{12} - 10^{14} neutrons cm^{-2} s^{-1}, are the most efficient neutron sources for high sensitivity activation analysis induced by epithermal and thermal neutrons. The reason for the high sensitivity is that the cross-section of neutron activation is high in the thermal region for the majority of the elements (World Nuclear Association, 2019).

Description of Ghana Research Reactor 1 (GHARR-1)

The Ghana Research Reactor-1 (GHARR-1) is a Miniature Neutron Source Reactor (MNSR) SLOWPOKE type reactor that was developed and constructed by the China Institute of Atomic Energy (CIAE). It is a small-in-design, simple, compact, and reliably safe reactor. The Reactor was critical on 17 December 1994 and commissioned on 15th March 1995. It is used mainly for Neutron Activation Analysis, production of short-lived radioisotopes, Education and Training. Figure 5 shows the top view of GHARR-1 whiles Figures 6 and 7 shows the vertical cross-section and core fuel configuration region respectively of GHARR-1.

GHARR-1 adopts the pool tank structure and employs highly enriched uranium as fuel, light-water as moderator and coolant, metal beryllium as reflectors, and is cooled by natural convection. It is rated as a tank-in-pool reactor, thermal power of 30 kW, with a corresponding thermal neutron flux of 1.0×10^{12} n/cm^2s. Since its small excess reactivity cannot compensate for the negative reactivity temperature effect of the moderator and the equilibrium

xenon poising, it cannot operate continuously at the rated neutron flux for a long time.

Figure 5: A photograph of the Ghana Research Reactor-1 (GHARR-1), viewing from the top (Dellaa et al, 2013)

The GHARR-1 consists mainly of the reactor core, metal beryllium reflectors, the reactor vessel, the pool, the control system, the gamma-radiation monitoring system, the thermal-hydraulics monitoring system, and the auxiliary systems. Below are other descriptions:

- Core shape is cylindrical
- Core diameter is 23 cm
- Core height is 23 cm
- Fuel element shape is a thin rod
- Fuel element number in the core is 344
- Total U-235 loading in core is < 1.0kg
- Reactor continues operating (at rate power) is > 2.5 hours

- Refuel period is more than ten years
- Burn up is ~1 %
- Temperature is ~ 0.01 mk/^{o}C (average)
- Control rod is Cd (One at the center of the core)
- Reactor cooling mode is natural convention
- It is 90.2 % enriched U-Al alloy as fuel
- W % of U in the UAl4 dispersed in Al is 27.5 %
- Loading of U-235 is 990.72 g
- It is cooled and moderated with light water
- Light water and beryllium act as reflectors
- Fuel cage consisting of 344 fuel pins, 4 tie and 6 dummy rods concentrically arranged in 10 rings
- Total number of irradiation sites is 10
- Number of inner irradiations sites is 5
- Number of outer irradiation sites is 5 (2 larger, 3 smaller)
- Core has a central guide tube for control rod cladded in Stainless Steel
- Fuel cage is placed on a 50 mm thick and surrounded by 100 mm thick metallic reflector
- The reactor is under moderated with H/U-235 as 197
- On top of the core is placed Al tray which may contain certain thickness of plates for adjustment of core excess reactivity to compensate for fuel depletion and Sm poisoning
- Maximum thermal neutron flux (at rated Power) at inner sites (Φ) is 1.0×10^{12} ncm^{-2} s^{-1}

- Maximum thermal neutron flux (at rated Power) at outer sites (Φ) is 5.0 x 10^{11} ncm^{-2} s^{-1}

Figures 6 and 7 also represent the profile and Hall of GHARR-1 respectively. The main reactor is located in the hall.

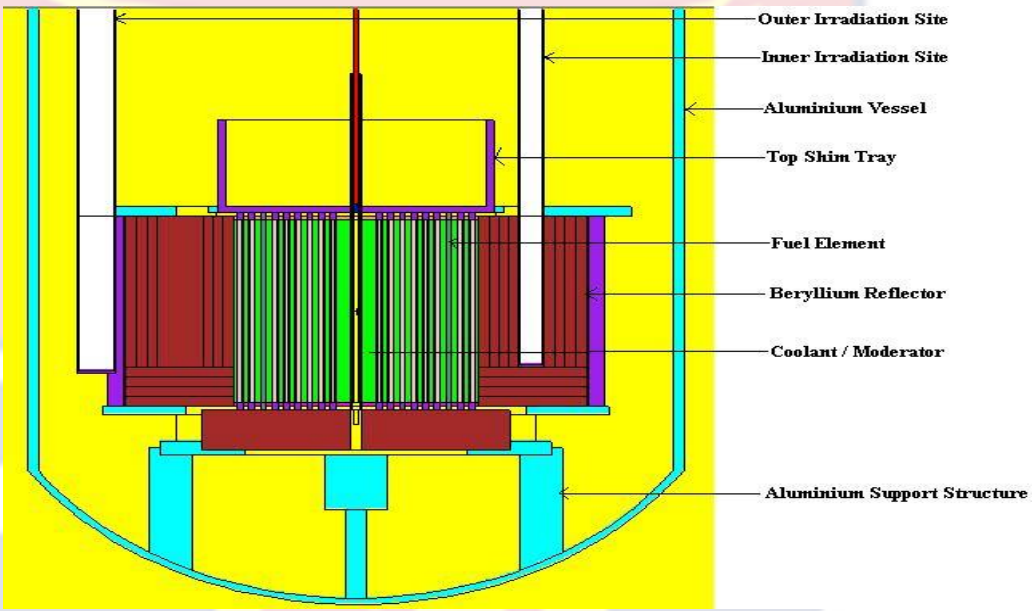

Figure 6: Vertical cross-section of GHARR-1 reactor (Dellaa et al, 2013).

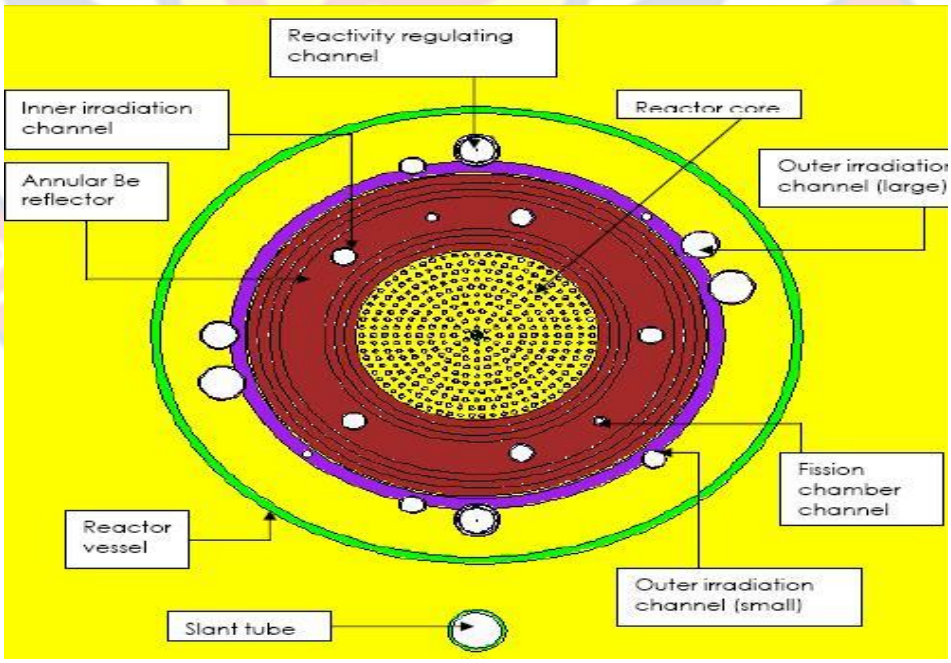

Figure 7: GHARR-1 core configuration showing fuel region: The hall (Dellaa et al, 2013)

Neutron Generated From Nuclear Reactor

A nuclear reactor produces a neutron beam by-product of uranium fission processes occurring within the reactor core. Big reactors do not produce neutrons but electricity, while small reactors, called Research reactors, produce neutrons for research and application in NAA.

Table 1: Common sources of neutrons for NAA

Reaction	Half-life	Average neutron Energy [MeV]	Neutron yield $ns^{-1}Ci^{-1}$ Unless otherwise stated
Photonuclear Source			
^{88}Y with ^{9}Be	106.6 days	0.16	$1.0x10^{5}$
^{124}Sb with ^{9}Be	60.2 days	0.02	$1.9\ x10^{5}$
Alpha emitter			
(α, n) source			
^{239}Pu with ^{9}Be	$2.4\ x\ 10^{4}$ years	3.5	$\sim 10^{7}$
^{226}Ra with ^{9}Be	1600 years	3.6	$1.1x10^{7}$
^{241}Am with ^{9}Be	433 years	3.5	$2.2\ x10^{6}$
Spontaneous			
Fisson source			
^{252}Cf	2.64 years	2.3	$2.3\ x10^{12} ns^{-1}g^{-1}$
Cockroft-Walton			
Accelerators			
$^{1}H(d,n)^{4}He$	-	14.7	10^{8}-$10^{11} ns^{-1}$
Cyclotron			
10 μA of 30 MeV		Broad distribution	$2\ x\ 10^{11}\ ns^{-1}$
deuterons on Be	-		
Nuclear reactor	-	Broad distribution	$10^{12} – 10^{15}\ ncm^{-2}s^{-1}$
Induced fission			

Nuclear reactors produce neutrons with a wide range of energies. The fission process itself results in the emission of fast neutrons

(mean energy ≈ 2.5 MeV) which are moderated to produce thermal neutrons of mean energy ≈ 0.04 eV (most probable energy ≈ 0.025 eV), epithermal neutrons (0.1 – 1 eV) and resonance neutrons (1 eV – 1 keV). Table 1 provides common sources of neutrons for NAA and other information.

Neutron Activation Analysis (NAA) in Multi-element Analysis

NAA was discovered in 1936 when Georg de Hevesy and Hilde Levi discovered that samples containing certain rare earth elements became highly radioactive after exposure to a source of neutrons. From this observation, Georg de Hevesy and Hilde Levi quickly recognized the potential of employing nuclear reactions on samples followed by measurement of the induced radioactivity to facilitate both qualitative and quantitative identification of the elements present in the samples. This is a highly sensitive technique used in trace analysis and can be used to determine about two-thirds of the elements in the periodic table at 10^{-6} g/g or below. This technique has found its importance in both medical and environmental research (Ehmann & Vance, 1991).

Since the discovery of NAA, nuclear analytical techniques have become a versatile analytical tool for multi-element analysis. With the development of high-resolution radiation detectors, automated transfer systems, and computerized multichannel analyzers, activation analysis has become a very sensitive analytical tool, especially for simultaneous multi-element determination.

Activation Equation and Principles of Standardization

If a stable nuclide is exposed to thermal neutron flux, it may capture a neutron to produce a radioactive isotope of that element. This can be represented by a simplified equation as given in equation 1 as:

$$^{A}_{Z}Y(n,\gamma)^{A+1}_{Z}X \quad [1]$$

If n_i is the number of nuclides of a given stable isotope exposed to thermal neutron flux ϕ for a time t_r, σ_i the activation cross section for the (n, γ) reaction and $N_i(t_r)$ is the number of the radionuclide formed, then the rate of reaction is given by:

$$\frac{dN_i(t_r)}{dt} = \text{Rate of production} - \text{Rate of radioactive decay} \quad [2]$$

Production rate $= \phi\sigma_i n_i$

Rate of radioactive decay $= \lambda N_i(t_r)$

$$\text{Therefore, } \frac{dN_i(t_r)}{dt} = \phi\sigma_i n_i - \lambda N_i(t_r) \quad [3]$$

Integrating equation 3 yields

$$N_i(t) = \frac{\phi\sigma_i n_i(1-e^{-\lambda_i t})}{\lambda_i} \quad [4]$$

where $\lambda_i = 0.693/t_{1/2}$. The activity $A_i(t)$ at any time t during the irradiation period according to equation 4 is given by

$$A_i(t) = \lambda_i N_i(t) = \phi\sigma_i n_i(1-e^{-\lambda_i t}) \quad [5]$$

where n_i is expressed as:

$$\mathbf{n_i = \frac{m_i \Theta_i N_A}{M_i}}$$

At the end of the irradiation period, the activity is given by;

$$A_i(t_i) = \frac{\phi\sigma_i\theta_i m_i N_A(1-e^{-\lambda i t_r})}{M_i} \quad [6]$$

If counting is delayed for time t_d then the activity at the end of the delay is:

$$A_i(t_d) = A_i(t_i)e^{-\lambda_i t_d} \quad [7]$$

If after the delay the sample is counted for a time t_c, then the number of disintegration that occurred during the counting period is obtained from equation 7 as:

$$N_i = \int_o^{t_c} A_i(t_d)e^{-\lambda_i t_d}\,dt = \frac{A_i(t_d)(1-e^{-\lambda_i t_c})}{\lambda_i} \quad [8]$$

From equations [6], [7] and [8]

$$N_i = \frac{\phi\sigma_i\theta_i m_i N_A(1-e^{-\lambda_i t_r})(1-e^{-\lambda_i t_c})e^{-\lambda_i t_d}}{\lambda_i M_i} \quad [9]$$

Suppose $\varepsilon(E_i)$ is the photopeak detection efficiency for the gamma-ray energy E_i and total counts recorded by the detector (the photopeak area) is P_A then N_i can be expressed as;

$$N_i = \frac{P_A}{\varepsilon_i(E)\gamma_i} \quad [10]$$

From equation [9] and [10]

$$m_i = \frac{P_A\lambda_i M_i}{\phi\sigma_i\theta_i N_A\varepsilon_i(E)\gamma_i(1-e^{-\lambda_i t_r})(1-e^{-\lambda_i t_c})e^{-\lambda_i t_d}} \quad [11]$$

If W is the weight of the sample used, then the concentration or amount ρ of a nuclide i in the sample is given by:

$$\rho = \frac{m}{W_i} = \frac{P_A \lambda_i M_i}{\phi \sigma_i \theta_i N_A \varepsilon_i(E) \gamma_i G(1-e^{-\lambda_i t_r})(1-e^{-\lambda_i t_c}) e^{-\lambda_i t_d} W} \quad [12]$$

Equation [12] can be written as:

$$\rho = \frac{m_i}{W} = \frac{[P_A / t_c] M_i}{\phi \sigma_i \theta_i \gamma_i \varepsilon(E_i) N_A SCDW} \quad [13]$$

where $\varepsilon(E_i)SD = \varepsilon_i(E)G(1-e^{-\lambda_i t_r})(1-e^{-\lambda_i t_c})e^{-\lambda_i t_d}$ and $P_A \lambda_i = \frac{[P_A / t_c]}{C}$

Principles of Standardization: Methods

Using NAA, the quantification of an element in a sample can be carried out *via* three main methods of standardization, namely; absolute, relative, and single comparator (k_0).

Absolute (Parametric) Standardization

This method of quantification is based on equation 13. By measuring P_A for known timing parameters, *viz.* t_i, t_d, and t_c, the amount of the element present, ρ can be calculated. A reliable determination of ρ requires prior knowledge of accurate values of ϕ, σ, θ, ε and λ. Since these parameters are not usually known with a high degree of accuracy, the absolute measurement does not always provide reliable results; hence, it is not used in many laboratories.

Relative Standardization

In the relative standardization method, a chemical standard (index std) with a known mass *w* of the element is co-irradiated with the sample of known mass *W*. When short-lived radionuclides are employed 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 under the same

geometrical arrangements concerning 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. For low-power research reactors like GHAAR-1, there is no need for a neutron monitor anytime samples are irradiated since neutron flux in the irradiation sites are over a fairly stable period. The neutron activation equation then reduces to:

$$\rho_{sam} = \frac{[(P_A / t_c)CD]_{sam}[\rho W]_{std}}{[P_A / t_c)CD]_{std}.W_{sam}} \quad [14]$$

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 concentrations of the standard and the element of interest, respectively; C_{std} and C_{sam} are the countings for standard and sample; D_{std} and D_{sam} are decay factors for the standard and sample, respectively. Equation 14 can be rewritten as:

$$\rho_{sam} = \frac{[(P_A / t_c)CD]_{sam}}{CD_{std}.W_{sam}SA} \quad [15]$$

where *SA* is defined as $\frac{\left[\frac{P_A}{t_c}\right]_{std}}{[\rho W]_{std}}$, and it is the sensitivity of the element. Using the number of counts under the photopeak area from standardized irradiation and counting conditions, the concentration of the element of interest can be determined (Vowotor et al, 2011).

Single Comparator (k_0-method) standardization

The *k0*-methodology, since 1975 (De Corte, 1987), has not only been a complement to the classical INAA (relative comparator method) but has also ensured complete and convenient multi-elemental analysis. The

k_0-standardisation known as the single comparator method of NAA is based on the fundamental equation for the calculation of the reaction rate R defined in equation 16 as:

$$R = \int_0^\infty \sigma(v)\phi(v)dv \quad [16]$$

Epithermal Neutron Activation Analyses

During NAA (Figure 1) a neutron interacts with the target nucleus through a non-elastic collision, leading to a compound nucleus being formed in an excited state. The excitation energy of the compound nucleus is owing to the binding energy of the neutron with the nucleus. The compound nucleus will almost instantaneously de-excite into a extra 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 (Ali, 1999).

The simple essentials vital to carry out an analysis of samples by NAA are a foundation of neutrons, instrumentation suitable for detecting gamma-rays, and thorough 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) and 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 concerning 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) (Ali, 1999). 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 (Acharya and Chatt, 2009).

For a lot of 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 (Chisela, Gawlik & Bratter, 1986).

The NAA technique that employs only epithermal neutrons to induce (n, γ) reactions by irradiating the samples being analyzed inside either cadmium or boron shields is called epithermal neutron activation analysis (ENAA). The epithermal neutron component consists of neutrons (energies from 0.5 eV to about 0.5 MeV) which have only been partially moderated. In a typical unshielded reactor irradiation position, the epithermal neutron flux represents about 2% of the total neutron flux (Ehmann & Vance, 1991). 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. This has been applied to a variety of sample matrices including geological and biological materials (Chisela, Gawlik & Bratter, 1986).

The EINAA methods are based on the fact that the resonance integral (I_0) to thermal neutron (n, γ) cross-section (σ_0) ratio (Q_0) for ^{127}I (24.8) is much larger than that for some of the interfering elements such as ^{23}Na (0.59), ^{37}Cl (0.69), ^{27}Al (0.71), ^{41}K (0.97), and ^{55}Mn (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 ^{128}I is often dominated by Compton background from the γ-rays of ^{24}Na, ^{56}Mn, ^{38}Cl, ^{42}K, ^{28}Al, and ^{82}Br. 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 (Acharya & Chatt, 2009).

Interaction of Gamma-Ray with Detector Material

Although a large number of possible interaction mechanisms are known for gamma-rays in matter, only three major types play an important role in radiation measurement. They are (i) photoelectric absorption (ii) Compton scattering and (iii) pair production. All of these processes lead to partial or complete transfer of the gamma-ray photon energy to the electron energy in the detector material. The result is sudden and abrupt changes in the gamma-ray photon history, in that the photon either disappears entirely or is scattered through a large average angle (Knoll, 1986).

Photoelectric Absorption

In the photoelectric absorption process, an incoming gamma-ray photon undergoes an interaction with an absorber atom in which the photon completely disappears. When frequency of light is more than threshold frequency, then workfunction energy is consumed into the ejection of electrons and rest energy is consumed into kinetic energy of electron. So, photon completely disappears. In its place, the atom from one of its bound shells ejects an energetic photoelectron (Figure 8).

The interaction is with the atom as a whole, and cannot take place with free electrons. For gamma-rays of sufficient energy, the most probable origin of the photoelectron is the most tightly bound or the K shell of the atom. The photoelectron appears with energy given by;

$$E_{e^-} = h\nu_o - E_b \qquad [17]$$

where E_{e^-} is the energy of the photoelectron produced, h is the Plank's constant, ν_o is the frequency of the incident photon, $h\nu_o$ is the kinetic energy of the incident photon, and E_b is the binding energy of the photoelectron in its original shell.

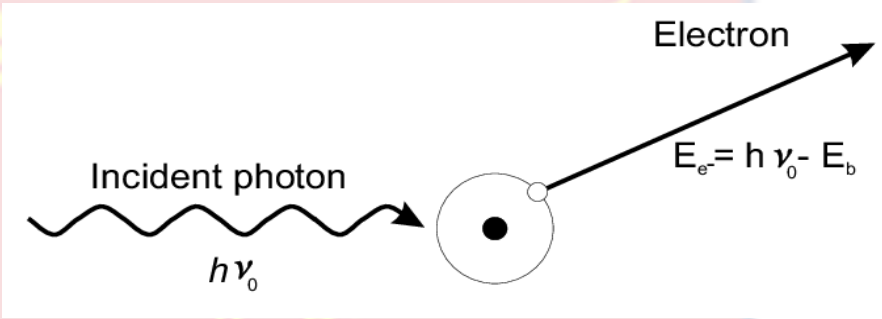

Figure 8: Sketch of the photoelectric absorption process (Lecoq, 2011)

In addition to the photoelectron, the interaction also creates an ionized absorber atom with a vacancy in one of its bound shells. This vacancy is quickly filled through the capture of free electrons from the medium and/or rearrangement of electrons from other shells of the atom. Therefore, one or more characteristic X-ray photons may also be generated. Although in most cases these X-rays are reabsorbed close to the original site through photoelectric absorption involving less tightly bound shells, their migration and possible escape from irradiation detectors can influence their response. In some fraction of the cases, the emission of an Auger electron may substitute for the characteristic X-rays in carrying away the atomic excitation energy.

Compton Scattering

The interaction process of Compton scattering takes place between the incident gamma-ray photon and an electron in the absorbing material. It is

most often the predominant interaction mechanism for gamma-ray energies typical of radioisotope sources. In Compton scattering, the incoming gamma-ray photon is deflected through an angle θ with respect to its original direction (Figure 9). The photon transfers a portion of its energy to the electron (assumed to be initially at rest), which is known as a recoil electron. Because all angles of scattering are possible, the energy transferred to the electron can vary from zero to a large fraction of the gamma-ray energy. The expression, which relates to the energy transfer and the scattering angle for any given interaction can be simply derived by writing simultaneous equations for the conservation of energy and momentum at the speed of light *c*.

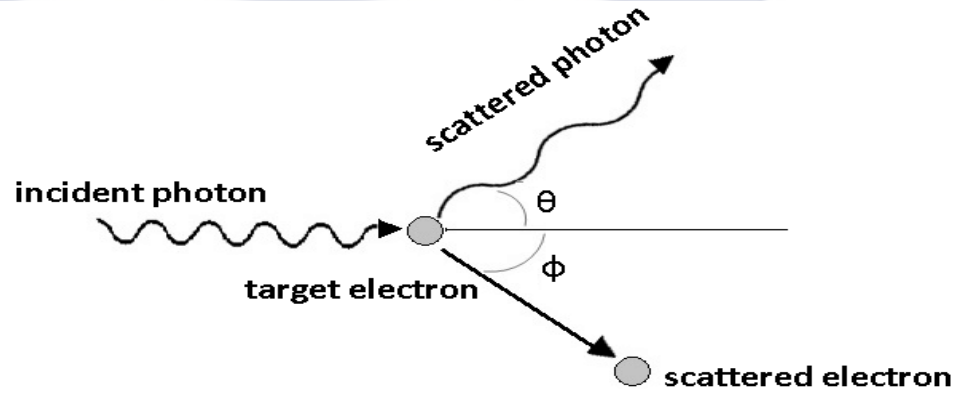

Figure 9: Sketch of Compton scattering process (Lecoq, 2011)

It can be shown that:

$$h\nu' = \frac{h\nu}{1 + \frac{h\nu}{m_0 c^2}(1 - Cos\theta)} \qquad [18]$$

where m_0 is the electron rest mass, ν is the electron frequency, and m_0c^2 is the electron rest mass energy (0.511 MeV).

For small scattering angles θ, very little energy is transferred. Some of the original energy is always retained by the incident photon even in the

extreme case of $\theta = \pi$ (180^0), which is a backscattering scenario. The probability of Compton scattering per atom of the absorber depends on the number of electrons available as scattering targets increase linearly with the atomic number, Z.

Pair Production

If the gamma-ray energy exceeds twice the rest mass energy of an electron (1.02 MeV), the process of pair production is energetically possible (Figure 10). As a practical matter, the probability of the interaction remains very low until the gamma-ray energy approaches twice this value and therefore pair production is predominately confined to high-energy gamma-rays.

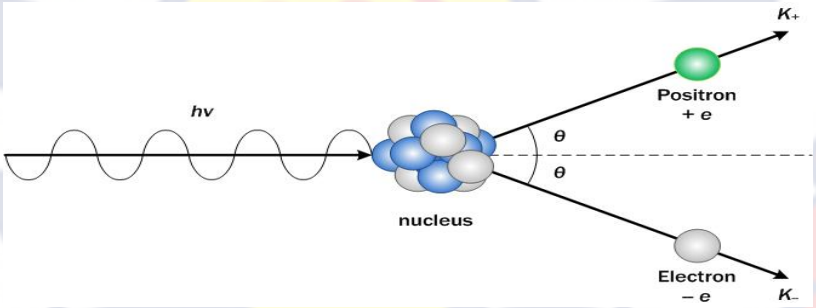

Figure 10: Sketch of the Pair production process (MSE 5317, 2020)

In this interaction (which must take place in the Coulomb field of the nucleus), the gamma-ray photon leaves its' orbit and is replaced by an electron-positron pair. All the excess energy carried in by the photon above the 1.02 MeV required to create the pair goes into kinetic energy shared by the positron and the electron. The positron will be subsequently annihilated after slowing down in the absorbing medium, two annihilation photons are produced as secondary products of the interaction. The subsequent fate of this annihilation

radiation has an important effect on the response of the gamma-ray detectors.

Gamma-Ray Spectrometry

Long before the advent of the first commercially available thallium activated sodium iodide (NaI(Tl)) scintillation detectors in the 1950s, counting of irradiated samples was carried out using proportional counters following chemical separation. In the 1950s when research reactors were made available worldwide, gamma-ray spectroscopy underwent rapid development making multi-elemental analysis possible. However, NaI(Tl) detectors lacked good energy resolution. Around 1960, the first lithium drifted germanium (GeLi) detectors appeared improving in the energy resolution by a factor of about 20 to 30. From that time, gamma-ray spectroscopy has witnessed great improvement leading to the manufacture of high efficiency and high-resolution High-Purity Germanium detectors (HPGe).

Chapter Summary

In summary, a review of the literature on iodine and the other nine micronutrients were done. Also, the literature on the different types of activation analysis and their theories was reviewed. Finally, all the detailed parts of the reactor were used also reviewed.

CHAPTER THREE

RESEARCH METHODS

Introduction

This chapter deals with the experimental work. The study area, types of equipment used, chemicals, and standards used are given. The procedures for sample preparation, irradiation, measurement, counting, and methods of analysis are also described.

Study Areas and Materials

1: Sarotherodon melanotheron (Blackchin Tilapia) obtained from Benya Lagoon

Benya lagoon is located within the KEEA metropolis as illustrated in Figure 11. This metropolis is located along the Gulf of Guinea in the Central Region of Ghana. The main type of fish harvested from this lagoon is the Blackchin Tilapia, shown in Figure 12. The Ghana Environmental Protection Agency (EPA) has reported this lagoon to be polluted some heavy metals like lead (Vowotor et al., 2015). The drying up of this lagoon is as a consequence of this level of pollution and heavy siltation (Vowotor et al., 2015). This threatens the means of support of more than 500 fishermen. Although the intensity of contamination has long been recognized, no move has been made to stop it. Despite the spate of pollution in this lagoon, residents, unfortunately, continue to patronize the fish from this lagoon (Vowotor et al., 2015).

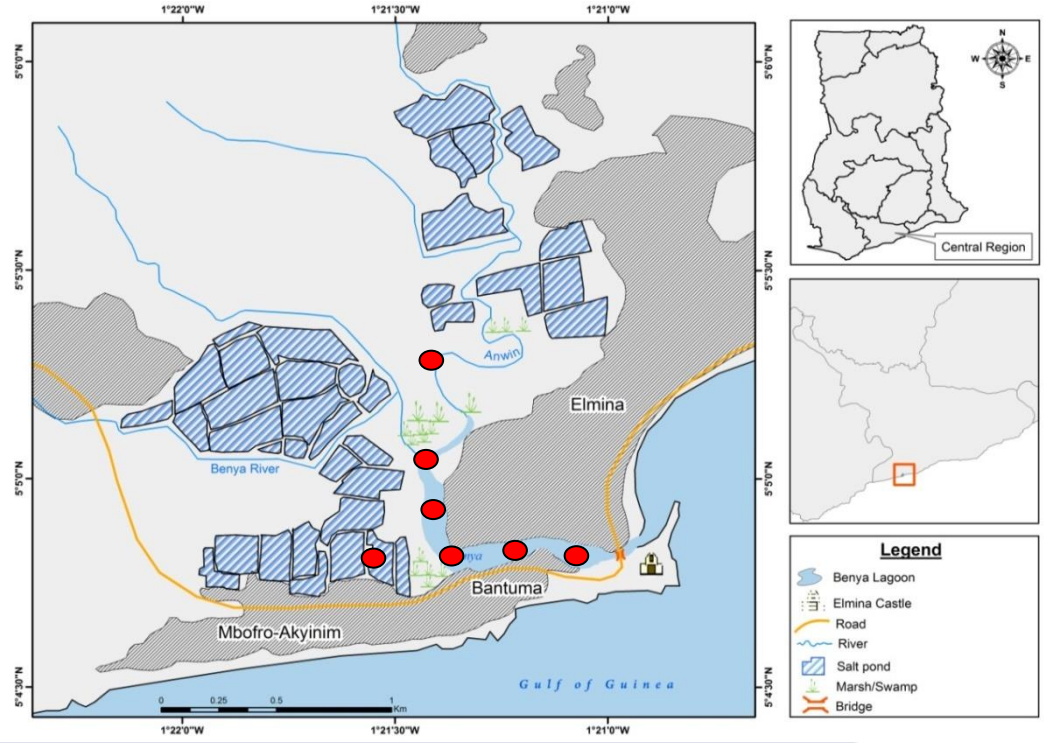

Figure 11: A map of Benya lagoon in Ghana, showing the sampling site (Benya & Co, 2012)

Figure 12: Sarotherodon melanotheron obtained from the Benya lagoon

With the help of some of the fishermen at each location, all through the examining time frame they supported with their vessels and aptitudes in coming to the not-effectively open stations reserved for examination. In total, a hundred fish samples were collected from the fisher folks at the various sampling points. The fish samples obtained were kept in ice before transporting them to the laboratory of the Department of Fisheries and Aquatic Sciences, University of Cape Coast, Cape Coast.

At the laboratory, the length and weight of the each specimen were measured after which the scales were removed using a stainless steel knife. The specimen were washed with deionized water, dry cleaned with blotting paper, and the various parts separated into tissues, bones, and gills for additional analysis. The organized samples were preserved at a temperature of – 10°C before transporting them to GAEC Preparation Laboratory.

At the GAEC preparation laboratory, the samples were arranged, as shown in Figure 13, and for 72 hours samples were lyophilized (Christ Gamma 1–16) at 0°C. This corresponds to a vapour pressure of 0.370 mbar. A stainless steel blade commercial blender was used to mill the samples for homogenization. Before milling the samples, a freeze dryer (Figure 13) was used to ensure the preservation of the texture of the samples.

Figure 13: The fish samples being dried in the freeze dryer

The three parts of the samples were milled separately. Six portions of each part of the pulverized and homogenized samples weighing 200 mg were wrapped. Using a soldering rod, wrapped samples were heat-sealed in ultra-clean polyethylene films. Forceps were used to fold 18 portions of each fish and heat-sealed again with a hand-held dryer. Each sample was in turn put into a rabbit capsule and smoothly heat-sealed with a soldering rod.

The irradiation vials (capsules) used were pre-cleaned by washing them first with distilled water, soaked in an acidic reagent for 24 hours, and rinsed in distilled deionized water. The irradiation vials were further soaked in HNO_3 for another 24 hours. They were 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 also organized and packed similarly as the samples (IAEA, 1999; IAEA, 2003; EVISA1, 2010; EVISA2, 2010).

Samples and controls were sent into the irradiation sites through a pneumatic transfer system at a pressure of 60 psi. With the GHARR-1 operating at 15 kW at a thermal flux of 5×10^{11} $ncm^{-2}s^{-1}$, the irradiation was classified according to the half-life of the species of interest. A three millimeter (3 mm) thick flexible boron element was used to cut-off thermal neutrons to access epithermal neutrons. 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 (Adomako et al., 2008). This software identifies the various photopeaks, estimates and works out the areas under them. The other quantitative measurements were done using the concentration equation (15) in a Microsoft Excel programme for calculating the elemental concentrations in µg/g. A 94.7% accuracy was achieved.

2: Breast Milk of First Six Months Nursing Mothers From Central Region of Ghana

Volunteers Information

Appendix E provides the range of ages and other information on the 27 volunteers used in this research. These volunteers were women who had given birth between 38 to 42 weeks of gestation and were breastfeeding their babies.

They were all practicing breastfeeding exclusively for the first six months of their new borns.

Breast Milk Sample Collection and Preparation

The services of trained midwives and nurses were employed during the sampling period. Before collecting the breast milk sample, 70% ethyl alcohol was used to clean the nipples and areolas of the volunteers breasts. The breast milk expression was done manually. Approximately 10 – 20 mL was delivered directly into a 60 mL vacuum-sealed sterile plastic vials and immediately stored in ice, to prevent the expressed breast milk samples from going bad (BFN, 2009). The samples were sent to the storage point where they were put in a deep freezer at a temperature of below –20 ^{o}C and transported in low-temperature transport containers to GHARR-1 for elemental analysis. Figure 14 shows the samples at the GHARR-1 preparation room being prepared for irradiation.

Figure 14: Breast milk samples being prepared for irradiation at GHARR-1

Ethical Approval and Volunteers Informed Consent

The techniques used were sanctioned by the Ghana Health Service Ethical Review Committee (GHS-ERC-02/05/15). A copy of this approval is shown in Appendix B. An approval was also sought from the administrators of selected health facilities used before the sample collection. The purpose of the study, procedures involved, and possible risks associated were explained in the prospective volunteer's incomprehensible language after which they were given the chance to ask questions. After ensuring that inclusion criteria were met, each volunteer was presented with a written informed consent before the breast milk samples were collected. To ensure the anonymity of the study subjects, the samples were labelled without their names. The study posed no risk to volunteers, and did not bring any direct benefits to them. However, volunteers may benefit indirectly from the results of the study after it has been shared with the Ghana Health Service or published in a scientific journal.

Volunteers Access to Facilities

The volunteers used the selected medical facilities due to easy accessibility. Some of the volunteers were students, unemployed, while others were practicing nurses, teachers, caterers, seamstresses, hairdressers, and petty traders. The facilities are well patronized by breastfeeding mothers from all walks of life and from towns in and around the Cape Coast metropolies and Elmina district. Some of these towns included Pershie, Bantuma, Ayisa, Bakano (Elmina), Essuakyer (Elmina), Iture, Bronyibima, Yesunkwa, Attabadze, Essaman, Ankuanda, Ayensudo, Ampaingyin, Brenu-Akyenu, Sanka, Akotobinsin, Abura, Pedu, Tantri, Adisadel, Nkanfoa, Third Ridge and

Green Hill. The medical facilities used were Adisaldel Health Post, Cape Coast Awim hospital, and Elmina District hospital.

The original inhabitants of the towns and villages (Figure 15), which are in the coastal savannah zones are mostly subsistence farmers and fisherfolks (Vowotor et al., 2011). These coastal savannah zones are characterized by undulating plains with isolated hills and occasional cliffs with sandy beaches and marshy areas. The vegetation around the places can be classified as savannah with grassland and few trees. The forest zone lies between 250 m to 300 m above sea level. It is characterized by dense forest vegetation with palm and cocoa plantations (Opoku-Ansah et al., 2014).

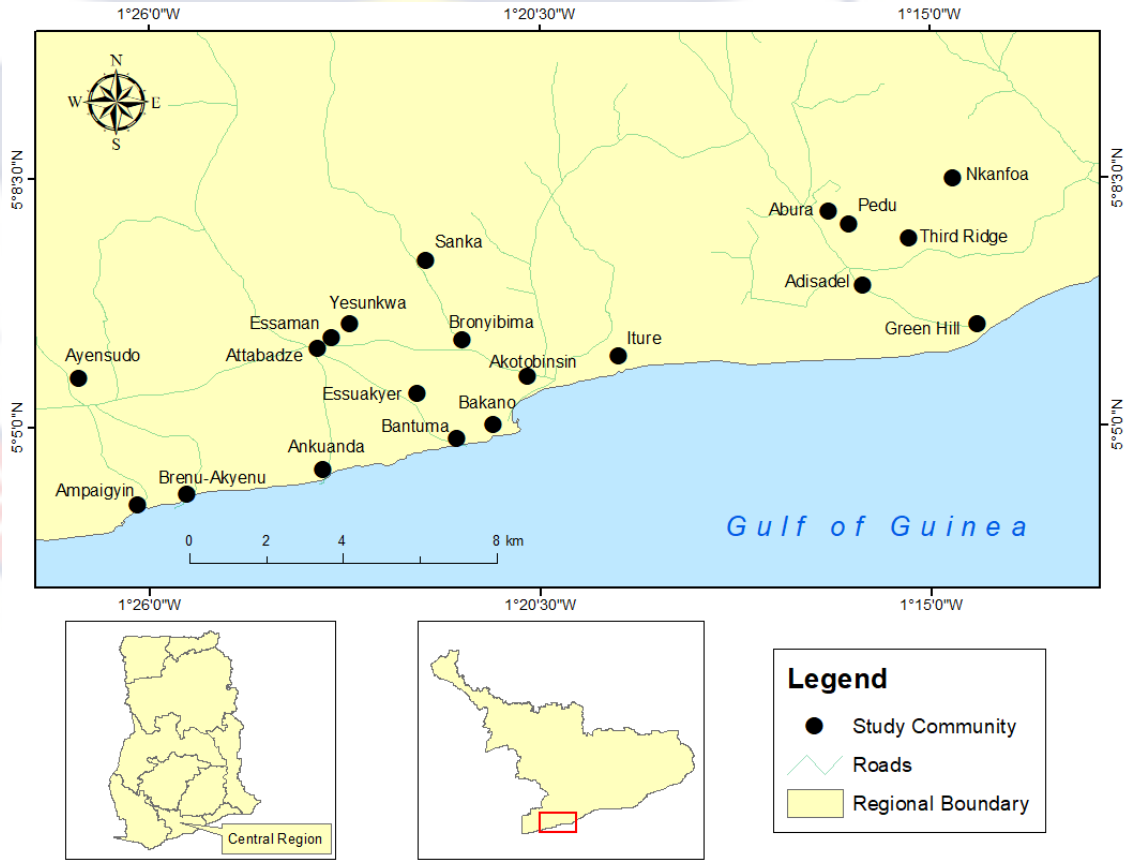

Figure 15: A map showing all towns volunteers are from (Benya & Co, 2012)

3: Polished Jasmine 85 Rice

Rice samples were obtained from five different rice farms in the south-eastern parts of Ghana as shown in Figure 16.

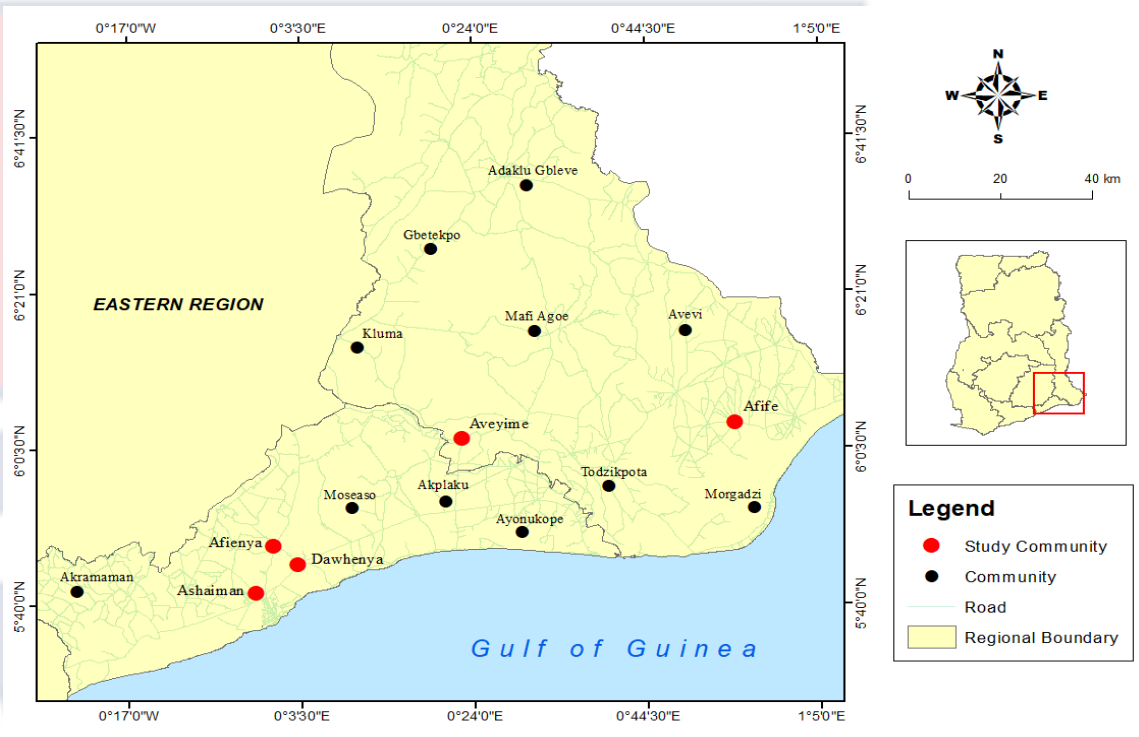

Figure 16: A map of towns in south-eastern Ghana with rice farms (Benya & Co, 2012)

These are the main areas where rice is cultivated in Ghana. Ashaiman is located between coordinates 5° 40’ and 5° 43’ N of latitude and longitudes 0° 05’ and 0° 07’ E. It is at a distance of 26 km North-East of Accra and almost directly north of Tema on the northern boundaries of Tema township in the Greater Accra region of Ghana. Afife is located in the Volta Region of Ghana. It is approximately between latitudes 6° 04’ and 6° 08’ and longitudes 0° 45’ and 0° 55’ East at a distance of 162 km east of Accra. Aveyime is in the Volta region at a distance of 85 km from Accra. Afienya is a town 41.4 km

from Accra in the same longitude with Ashaiman. Finally, Dawhenya is 6 km from Accra and shares the same latitude with Ashaiman. The locations of these sampling areas have been indicated on the map in Figure 16.

All inspecting hardware and sample holders were before and after use, pre-cleaned with heavy metal grade acetone. They were then rinsed with heavy metal grade hydrochloric acid (HCl). Blank determinations were carried out for quality control. Polished Jasmine 85 Rice samples, as shown in Figure 17, were randomly collected in three (3) different areas within each of the farms warehouses. At each farm, samples collected from the three sites were placed in a bowl and mixed to create a composite sample. Coded with an indelible pen, each composite sample was then placed in a plastic container and cross-contamination avoided by putting each composite sample in a labeled Ziploc bag. Samples were kept in a fridge before transporting them to GAEC for analyses. Fifty milligrams (50 mg) in 3 replicates for each sample collected at each farm was wrapped in a see-through polyethylene film.

Figure 17: Jasmine 85 Paddy Rice at the farms

The samples were shaken to guarantee homogeneity before weighing, after which they were further encapsulated into irradiation capsules (Rabbit capsule) of diameter 1.6 cm and height 5.5 cm and heat-sealed for neutron activation (irradiation).

Certificate of analysis standard material 1566b Oyster Tissue (National Institute of Standards and Technology), IAEA-530, and Trace element in tuna fish homogenate was prepared in the same manner as the quality assurance study samples. Recoveries of the elemental concentrations ranged from 88% to 111% of the certified values.

The reference and samples were also irradiated in the GHARR-1 facility using light-water as moderator and coolant and operated at 15 kW at a thermal neutron flux of 5×10^{11} $ncm^{-2}s^{-1}$. (Tandoh et al., 2009) with irradiation times ranging from 10 s to 1 h depending on the half-lives of the elements of interest. For all elements having relatively short half-lives such as Mg, Mn, and V, with half-lives between 2 minutes and 3h, irradiation time was 10 s, and counting time, 10 minutes. The samples were analyzed using irradiation schemes by optimizing irradiation time (t_i), decay time (t_d), and counting time (t_c) based on the half-life of the respective elements. The irradiation scheme was (t_i: t_d: t_c = 0.5-2: 1: 10 minutes).

Qualitative and Quantitative Analysis

The qualitative analysis involves the determination of the Br, Cl, and I, in the fish samples by the identification of spectral peaks and assigning corresponding radionuclides, and hence, the elements present. The quantitative analysis involves the calculation of the areas beneath the peaks of the recognized elements and converting them into concentrations using an

appropriate software or equation(s) (Alfassi, 1994). A PC-based γ-ray spectrometry was used in the counting of the induced radioactivity. It is made of an n-type high purity Germanium (HPGe) detector (model GR2518). This is fixed to a computer-based Multichannel Analyzer through 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 qualitative analysis was achieved employing ORTEC EMCAPLUS Multichannel Analyzer (MCA) Emulation software. The MAESTRO software was used for spectrum investigation (Adomako et al., 2008). This software pinpoints the various photopeaks, estimates, and calculates out the areas below them. The other quantitative measurements were done using the concentration equation (15) in a Microsoft Excel programme for calculating the elemental concentrations in µg/g. Table 2 illustrates the detection limit (DL) of the various species of interest for NAA and the nuclear data that were determined.

Table 2: Nuclear Data of the Ten Elements

Element	Radioisotope	Gamma Ray Energy (keV)	Half-life	Irradiation Time (minutes)	Counting Time (minutes)	DL (µg/g)
Na	^{24}Na	2754.0	15 hr	10	10	0.0010
Mg	^{27}Mg	1014.4	9.46 min	10	10	0.1000
Cl	^{38}Cl	1642.4	37.3 min	10	10	0.0010
K	^{42}K	1524.6	12.4 hr	10	10	0.0100
Ca	^{49}Ca	3084.5	8.72 min	10	10	1.0000
V	^{52}V	1434.1	3.76 min	10	10	0.0010
Mn	^{56}Mn	1810.7	2.58 hr	10	10	0.0001
Cu	^{66}Cu	1039.2	5.1 min	10	10	0.0100
Br	^{80}Br	616.93	17.7 min	10	10	0.0001
I	^{128}I	440.9	25 min	10	10	0.0001

Where hr denotes hours and min denotes minutes

Analysis Methods

Assessment Using the Box and Whisker Plot

A box and whisker plot, as shown in Figure 18, is a chart that presents data from a five-number rundown. It is a useful way of describing the centre, the spread of a distribution, for demonstrating whether a dispersion is skewed and whether there are expected uncommon perceptions (anomalies or outliers) in the informational collection (Statistics Canada, 2017). This will be one of the tools to be used in presenting data collected.

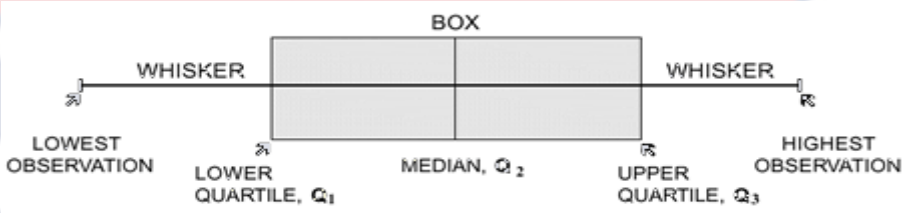

Figure 18: A box and whisker plot indicating five-number summary (Statistics Canada, 2017)

Assessment Using Micronutrient Concentration

This assessment was used to show the total average mean concentrations of the investigated micronutrients and their standard deviations in mg/kg. The elemental composition will reflect their composition in various situations found in another nutrient medium.

Assessment Using Clustering Analysis

A dendrogram is a tree outline used to represent the game plan of groups delivered by various levelled bunching (Spano, 2018). Hierarchical cluster analysis (HCA) is an unsupervised technique that examines the interpoint distances between all of the samples and represents that information

in the form of a two-dimensional plot called a dendrogram (Beebe, 1998). The dendrogram is formed based on the nearness of the sample to each other in a row space. Such clustering analysis was used for analysis.

Multivariate Analysis (Linear Discriminant Analysis)

Linear Discriminant Analysis (LDA) was carried out on the concentration data to determine if any pattern that characterizes or separates the micronutrients at the various stations or farms or mothers can be established.

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 an observed random variable k can be written as a linear function of $p < k$ unobserved called the common factors. This can be written as expressed in equation 19 as:

$$\left.\begin{aligned} x_1 &= \beta_{11} f_1 + \ldots\ldots + \beta_{1p} f_p + e_1 \\ x_p &= \beta_{k1} f_1 + \ldots\ldots + \beta_{kp} f_p + e_k \end{aligned}\right\} \quad [19]$$

The $\beta_{ij} (i = 1, \ldots, k \text{ and } j = 1, \ldots, p)$ in equation 19 is called the factor loadings and 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.

Principal Component Analysis Assessment

Principal Component Analysis (PCA) is a widely used tool for multivariate analysis in which new 'axes' called principal components (PCs)

are constructed by forming linear combinations of original variables (measured parameter). The first PC or PC1 contains the maximum variance from the data; followed by PC2 and so forth (Bro & Smilde, 2014). The contribution of all the other micronutrients can also be inferred using this presentation.

Assessment Using Correlation Coefficient Between Elements

Pearson's correlation coefficient was used to draw parallels between any two of the micronutrients as the matrix between the micronutrients gives information about their possible sources (Currie, 1991; Alfassi, 1994). A correlation coefficient that is significant at a 95% confidence level demonstrate the equivalent or comparative source input, demonstrate the equivalent or comparative source input.

Though 95% confidence level has been used to ascertain the strength of their relationship, there are other strongly correlated elements with high coefficients of determination. Hence, they cannot be ruled out and are also highlighted in blue, green, and black. The focus would be on the strength of the relationship 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. A correlation coefficient exactly ±1.0 depicts a perfect downhill/uphill (negative/positive) linear relationship; ± 0.7 indicates a strong downhill/uphill (negative/positive) linear relationship; ± 0.5 signifies a moderate downhill/uphill (negative/positive) relationship; ± 0.3 signifies a weak

downhill/uphill (negative/positive) linear relationship and 0 indicates the absence of a linear relationship (Rumsey, 2010).

Assessment Using Dietary Reference Intake

Dietary Reference Intakes are rules for the day-by-day admission of supplements (as nutrients, protein, and fats) and other food segments (as fibre). It comprises recommended dietary allowances (RDA), adequate intake (AI), values for nutrients having undetermined recommended daily allowances, and tolerable upper level (UL) values of dietary reference intake (DRI) (Merriam-Webster Medical Dictionary, 2015).

The Recommended Dietary Allowable (RDA) is a consumption level that meets specified criteria for tolerability, thereby, minimizing the risk of nutrient deficiency or extremes. A Maximum Upper Intake Level (UL) is the uppermost level of daily nutrient intake that is likely to pose no risk of adverse health effects to an individual and symbolizes total intake from food, water, and other supplements (Food and Nutrition Board, 2001; Institute of Medicine, 2001).

Adequate Intake (AI) is the endorsed average daily nutrient intake level based on experimentally determined estimates of nutrient intake by groups of apparently healthy people who are assumed to be maintaining an adequate nutritional state. It is expected to meet the needs of most individuals in a specific life-stage and gender group (Institute of Medicine, 2001). The maximum Upper Limit (UL) of RDA and AI for the Life Stage Groups of the elements investigated are presented in Appendix G.

Sodium is an electrolyte/mineral and the most important ion of the extracellular fluid that aids nerve-impulse transmission (The National Academies, 2005; Nutritional Health Resource, 2017). The Daily Value for sodium is less than 2300 milligrams (mg) per day (US FDA, 2019).

Chlorine is an electrolyte that combines with sodium and potassium to control the amount of fluids in the body and its pH (Minerals Education Coalition, 2013). The recommended daily dose of elemental chlorine is 3400 mg per day (Lenntech, 2019).

Many doctors are cautious when recommending oral magnesium preparations owing to its known side effect of causing diarrhea. 400 mg per day is the RDA of elemental magnesium, with a single dose of 800-1600 mg being necessary to produce a laxative effect (Fine, 1991; Gums, 2004; Nishizawa, Morii & Durlach, 2007). Food intake is the main source of vanadium with its dietary intake estimated as 0.34 µg/kg (US EPA, 2015) and an Upper Tolerable Limit of 1.8 mg/day (ATSDR, 2012; Institute of Medicine, 2001). The third most abundant mineral in the body is potassium, and it helps in an important role in numerous body processes. A variety of whole foods are excellent sources of potassium, including beet greens, yams, banana, avocado, potatoes, and spinach (Healthline, 2017). Its daily intake is 3500 mg (Lenntech, 2019).

Studies have shown that both calcium and vitamin D are essential in building bones. Calcium can be found in many foods like seafood and dairy (Harvard University, 2019), with its recommended daily dose being 1000 mg per day (Lenntech, 2019). Manganese is an essential nutrient/mineral that is found in several foods including nuts, legumes, seeds, tea, whole grains, and

leafy green vegetables (U.S. National Library of Medicine, 2019). Its daily intake is 5 mg (Lenntech, 2019).

Iodine is a vital trace element that plays a very important role in the human physiological actions of thyroid hormones. Every cell in the body depends upon thyroid hormones for regulation of their metabolism and promotes growth and development in the body including the brain. (Endocrineweb, 2017). The leading preventable causes of brain damage, which is iodine deficiency can significantly lower the Intelligence Quotients (IQ) of a whole population. The most severe impact occurs during fetal development and in the first few years of life. Its consequence is instability in the development of the brain and the central nervous system which is irreparable with the most serious form being that of imbecility (Chung, 2014). Iodine's daily intake is 150 µg (Lenntech, 2019).

Copper is present in organ-meat foods, seafood, nuts, wheat bran cereals, whole grain, and seeds. The small intestine mainly absorbs it in, although some absorption may also occur in the stomach. Absorption varies with copper intake ranging from more than 50% for intakes below 1 mg/day to less than 20% for intakes above 2 mg/day (Lenntech, 2019). The very high levels of iron or zinc taken as supplements generally affecting Cu absorption in the body (Botash et al., 1992; Morais et al., 1994; Turnlund, 1999).

Assessment Using Health Risk Estimation

Hazard index (HI) is the evaluation of the health effects. This is the projected lifetime average daily dose of each chemical 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 United States of America EPA handbook (Laar et al., 2011). This is expressed in equation 20 as:

$$\text{The hazard index (HI)} = \frac{E\,D}{RfD}, \quad [20]$$

where, ED = Estimated Dose, and RfD = Reference Dose

HI < 1 recommends an improbable adverse health effect, whereas HI > 1 proposes the probability of opposing 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.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

In this chapter, experimental results are analyzed and discussed. For simplicity and easy understanding of the data, the English names and local names of the food items were used instead of their scientific names.

Blackchin Tilapia

The raw data for the concentration of the elements in the sampled Blackchin Tilapia are in Appendix A, and Table 3 gives the summary of the results.

Table 3: Mean Elemental concentrations of Br, Cl, and I in Blackchin Tilapia

Part of Fish	Mean Concentration (µg/g)		
	Br	**Cl**	**I**
Bone	27.98 ± 3.27	7295.85 ± 977.88	0.95 ± 0.070
Muscle	29.13 ± 3.31	9286.70 ± 124.36	0.62 ± 0.093
Gill	31.52 ± 3.07	8339.70 ± 112.54	3.40 ± 0.510
Total	88.63 ± 9.65	24922.25 ± 3346.78	4.97 ± 1.510

Mean value ± SD, where SD is the Standard Deviation

The graphs of the concentrations of Br, Cl and I in the various parts of the Blackchin Tilapia are as shown in Figures 19 and 20. Among the three halogens, chlorine recorded the highest concentration whilst iodine recorded 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 and maintains a constant interaction with the sea throughout the year (Obodai, Yankson & Blay, 1994). Considering the sum of the elemental concentrations in the fish, the muscles recorded the highest concentrations 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.

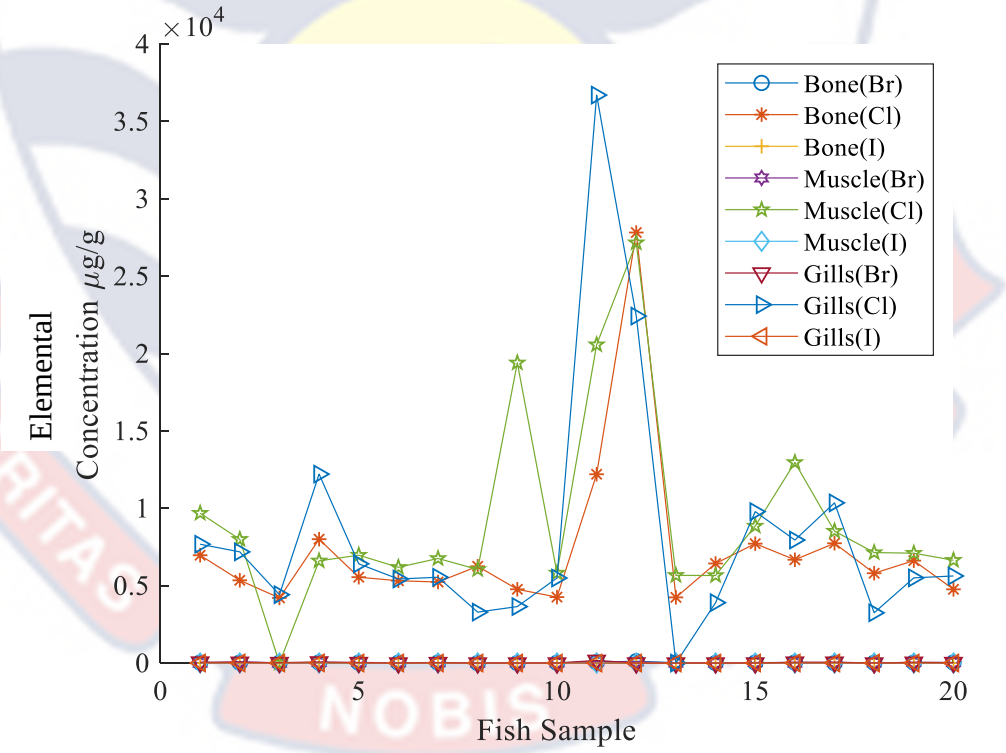

Figure 19: Distribution of Br, Cl and I in the muscles, bones, and gills of the sampled Blackchin Tilapia

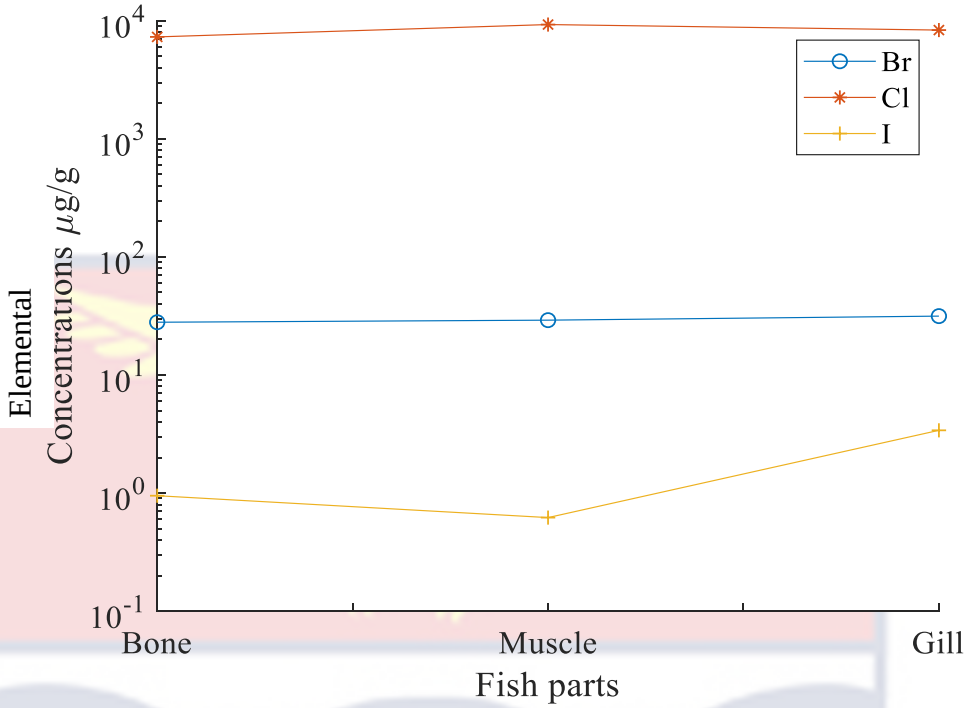

Figure 20: Mean distribution showing the concentration of Br, Cl and I in the sampled Blackchin Tilapia parts

Brackish water systems receive a constant inflow of both freshwater and seawater (the major source of NaCl) throughout the year. The uptake of Cl ions from the seawater enables the species to maintain electrical neutrality as well as balancing the K+ and Na+ ions in the body fluids. The low levels of the other halogens could be due to their low absorption from the brackish water (Fondriest Environmental, 2019).

The correlation coefficients between the three elements in the bone, muscle, and gills and their respective signification at 95% significance level in the fish are shown in Table 4. This is relevant because it gives facts about their likely same or similar source inputs (Alfassi, 1994). There was a strong correlation between chlorine and bromine in all the tissues which were assessed. It was found that even though 9 correlations were strong, none was

statistically significant. This means that we can exude 95% confidence that the correlations between Br's and Cl's in the bone are strongly correlated with a coefficient of 0.777 and 0.851 respectively.

Table 4: Correlation coefficients matrix of the elements in the fish

Part of 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

It can be deduced from Table 4 that iodine (I) had no strong correlation with Br or Cl. However, there was a strong correlation between Br in the Bone and in the muscle; and Cl in the bone and in the gills. It is worth noting that Benya lagoon has over 100 salt ponds around it, hence, the high concentration of Cl. An important source of nutrients, trace elements and contaminants in many coastal ecosystems come from groundwater discharges (Kelly & Moran, 2002). Bromine, 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 (Azo Materials, 2019). Benya lagoon gets water

contributions from more than one groundwater source. These variations in sources, as well as inflows from activities around the bed of the lagoon, could contribute to the concentrations of the halogens within the lagoon. However, the chief source of Cl is the seawater influx from the Gulf of Guinea (Vowotor et al, 2015).

Modelling the interrelationships among the observations with the primary focus on the variance and covariance rather than the mean using Factor analysis, Figures 21 and 22 are plots for factor 1 and factor 2 showing a strong correlation between chlorine and bromine. There was, however, no correlation between iodine and the other elements (eg. chlorine and bromine).

It can be seen from Figures 21 and 22 that iodine clustered at a point, while chlorine and bromine exhibited some form of linear relationship between them and also clustered at another part. This buttresses the information in Table 4 that iodine did not exhibit a strong correlation with Br or Cl, and may be coming from a different source altogether.

Although, iodine is fairly rare, it is found in the Earth's crust, ocean water, and in high concentrations in some ocean plants such as seaweeds. It is also found in underground brines near oil and natural gas reserves (Technological Solutions, 2019). It is worth mentioning that the Saltpond oil rig is 39.2 km offshore east of Elmina, and 123.1 km to the west of Elmina are the Jubilee oil fields which are made up of several rigs. These rigs may be the source of iodine present in the Benya lagoon.

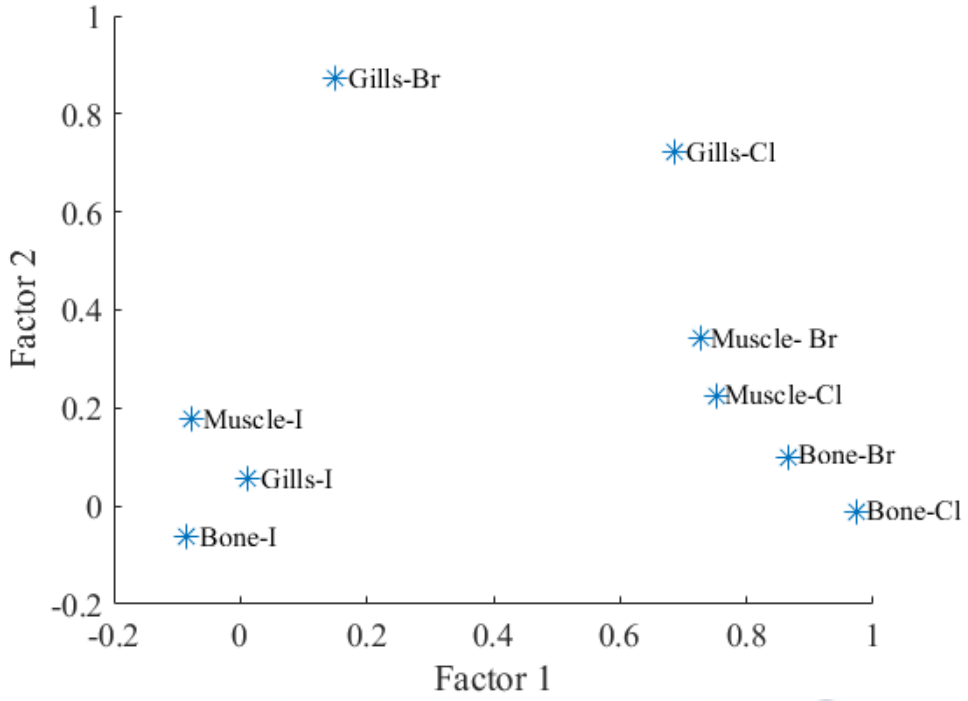

Figure 21: Factor loadings in their unrotated form showing groupings for Iodine and groupings for Chlorine and Bromine

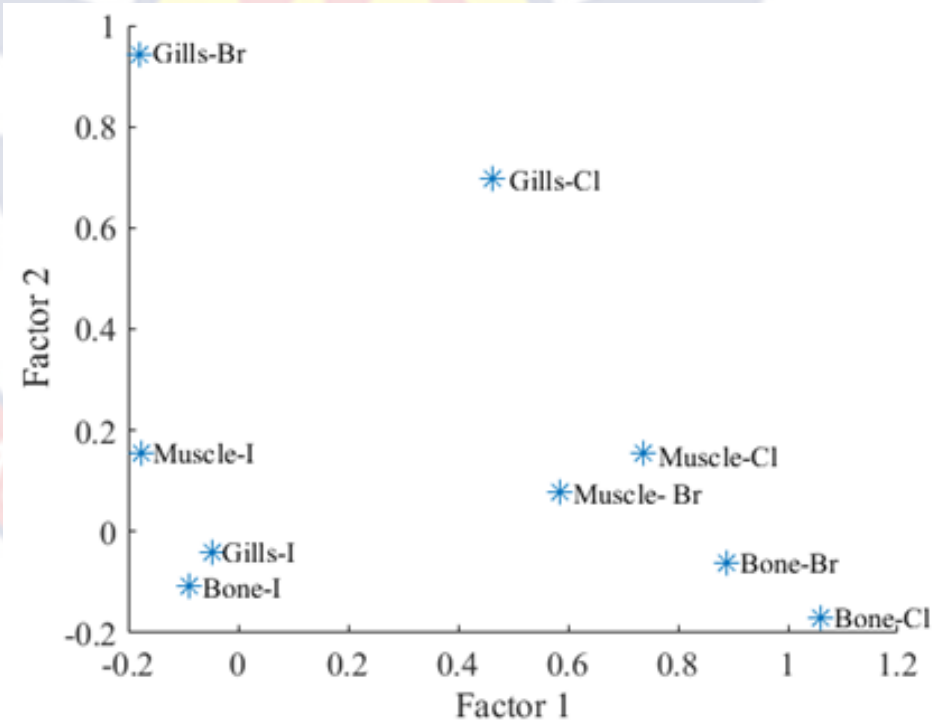

Figure 22: Factor loadings after promax rotation form showing groupings for Iodine and groupings for Chlorine and Bromine

Table 5 shows the total average mean concentrations of the elements in S. melanotheron in µg/g.

Table 5: Mean concentrations of the elements in S. melanotheron in µg/g

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

The mean daily intake (grams per capita) in Table 6 was calculated using the values in Table 5. From a previous study, the average intake of fish was 22 kg/caput/year, and therefore, the average daily intake of fish was calculated to be 60.274 g/d (Owusu et al, 2005). The differences between the UL of the RDA / AI and the calculated means for the various life stage groups were calculated and presented in Table 7.

Table 6: Mean daily intake of Br, Cl, and I in the S. melanotheron in µg/d

Element	Br	Cl	I
Daily Intake	5342.08 ± 581.64	1502163.70 ± 201723.82	299.56 ± 91.01

Table 7: Differences between the UL of RDA/AI and the calculated means for the various life stage groups

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

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 (Dietary Reference Intakes, 2013). The negative sign (–) denotes values below the ULs, whiles the positive sign (+) denotes values above the ULs. Br has a total mean daily intake of 5342.08 ± 581.64 µg/d. Its upper limit for all the life stage groups is yet to be determined, since it has not been officially designated as essential for humans. There have however, been reports of reduced growth, fertility, and life expectancy in some animals as a result of hyperthyroidism,

which is secondary to dietary deficiency of bromide (Minerals Education Coalition, 2013). The estimated median daily intake of Br worldwide, from food and water is between 2000.00 µg/d – 5000.00 µg/d (Acu-Cell Nutrition, 2017). A summary of other functions of Br and how it affects the human body has been presented in Table 27 (in Appendix I).

For all the life stage groups, the levels of Cl and I in the Blackchin Tilapia were far above the recommended daily dose but it did not exceed the upper limit, except for children in the age bracket of 1 to 3 years who registered + 99.56 µg/d for I. The effect of having low levels of Cl and I in diet has been presented in Appendix I.

The projected health risk associated with the consumption of Blackchin Tilapia is presented in Table 8. HI < 1 suggests an unlikely adverse health effect whereas HI > 1 suggests the probability of hostile health effects (Laar et al., 2011). High HI values for the trace elements investigated that registered values > 1 for any life stage group have been highlighted.

All the fish samples collected from the Benya lagoon contained detectable amounts of the elements studied at varying concentrations. Aside Br whose HI values were not presented in Table 8 due to the lack of suitable data on their upper limits, the remaining elements registered HI values > 1 for at least one age group. The only exception was for children in the age bracket 1 to 3 years who recorded HI < 1.

Table 8: 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	0-6 months	ND	ND	0.0832	ND	0.3672	ND
	7-12 months	ND	ND	0.0999	ND	0.4340	ND
Children	1-3 years	ND	ND	0.1331	0.6666	0.3004	0.6676
	4-8 years	ND	ND	0.1664	0.6666	0.3004	**1.0015**
Males	9-13 years	ND	ND	0.2496	**1.3314**	0.4006	**2.0029**
	14-18 years	ND	ND	0.2496	**1.9971**	0.5007	**3.0044**
	19-30 years	ND	ND	0.3661	**2.3300**	0.5007	**3.6720**
	31-50 years	ND	ND	0.3661	**2.3300**	0.5007	**3.6720**
	50-70 years	ND	ND	0.3661	**2.3300**	0.5007	**3.6720**
	>70 years	ND	ND	0.3661	**2.3300**	0.5007	**3.6720**
Females	9-13 years	ND	ND	0.2496	**1.3314**	0.4006	**2.0029**
	14-18 years	ND	ND	0.2663	**1.9971**	0.5007	**3.0044**
	19-30 years	ND	ND	0.2829	**2.3300**	0.5007	**3.6720**
	31-50 years	ND	ND	0.2829	**2.3300**	0.5007	**3.6720**
	50-70 years	ND	ND	0.2829	**2.3300**	0.5007	**3.6720**
	>70 years	ND	ND	0.2829	**2.3300**	0.5007	**3.6720**
Pregnant Women	≤18 years	ND	ND	0.2996	**1.9971**	0.7344	**3.0044**
	19-30 years	ND	ND	0.2996	**2.3300**	0.7344	**3.6720**
	31-50 years	ND	ND	0.2996	**2.3300**	0.7344	**3.6720**
Lactating Women	≤18 years	ND	ND	0.3661	**1.9971**	0.9681	**3.0044**
	19-30 years	ND	ND	0.3661	**2.3300**	0.9681	**3.6720**
	31-50 years	ND	ND	0.3661	**2.3300**	0.9681	**3.6720**

All the three elements investigated in the Blackchin Tilapia from the Benya lagoon are essential as they are needed for the health and growth processes in humans. They, however, can be toxic at concentrations above the recommended levels necessary for their biological functions. Chlorine, for example, is an electrolyte that works with potassium and sodium to regulate the amount and pH of fluids in the body. It enables humans to digest their food and absorb other elements needed to survive. Excessive loss of chlorine in the body can lead to an imbalance of pH which can cause muscle weakness, loss of appetite, dehydration, and can cause coma (Minerals Education Coalition,

2013). Table 27 (Appendix I) shows a summary of bromine, chlorine, and iodine functions, and how they affect the human body.

Breast milk

The concentration of each micronutrient investigated in breast milk is as shown in Appendix E. Meanwhile, Table 9 gives the summary of the results.

Table 9: Summary Concentrations of Micro Nutrients in Breast Milk (mg/kg)

	Na	Mg	K	Ca	Mn	Cu	I
Min	294.400	132.400	1359.000	408.100	0.002	0.224	0.029
Max	2797.000	426.700	5225.000	1497.000	1.115	1.020	0.145
Mean	1098.626	309.248	2438.074	697.404	0.403	0.694	0.061
SD	36.440	23.719	176.349	35.254	0.038	0.008	0.010
CV	1.729	2.784	2.701	3.442	2.098	3.369	0.659

The concentrations of the nutrients ranged within the following intervals: Na: 294.4-2797.0 mg/kg; Mg: 132.4-426.7 mg/kg; K: 1359.0-5225.0 mg/kg; Ca: 408.1-1497.0 mg/kg; Mn: 0.0018-1.115 mg/kg; Cu: 0.2235-1.0199 mg/kg; and I: 0.029-0.1445 mg/kg. Their mean concentrations were: Na: 1098.6259 mg/kg; Mg: 309.2481 mg/kg; K: 2438.0741 mg/kg; Ca: 697.4037 mg/kg; Mn: 0.4028 mg/kg; Cu: 0.6944 mg/kg; and I: 0.0613 mg/kg. Arranging these in terms of abundance in the breast milk yielded the order of K > Na > Ca > Mg > Cu > Mn > I. This has been presented in Figure 23. The low standard deviation (SD) values obtained as presented in Table 9 show that the spatial distribution of the individual micro nutrients in their breast milk was uniform.

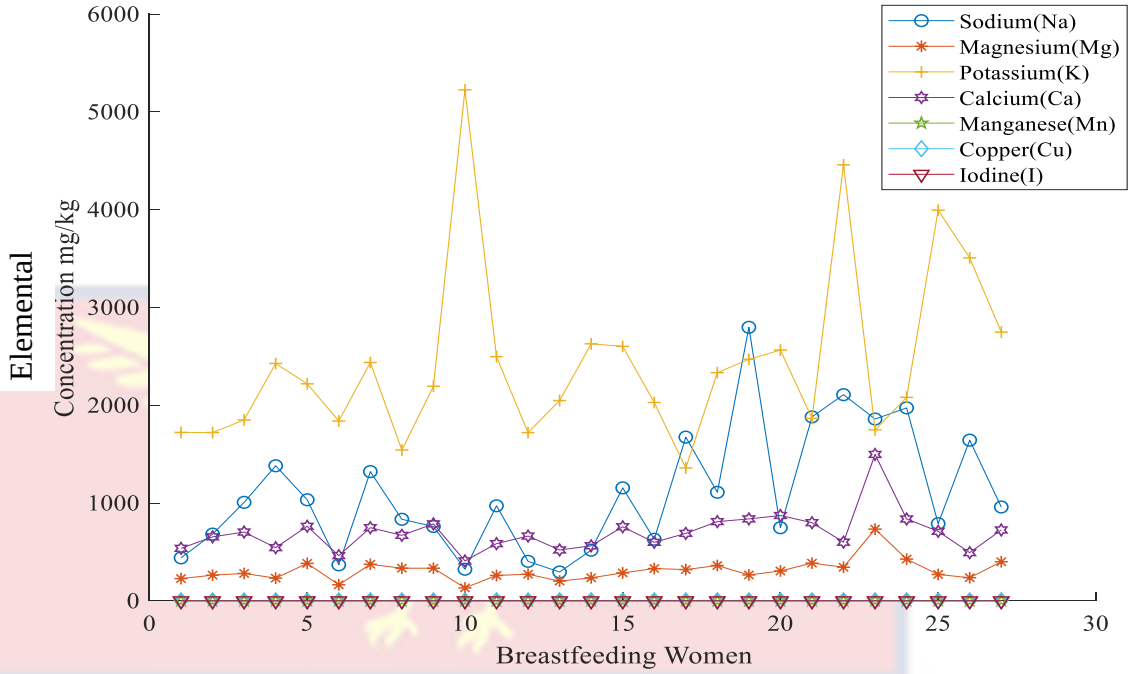

Figure 23: Concentration of the seven elements in the breastfeeding mothers

It is observed in Figure 24 that the notches of the seven boxes do not overlap. This signifies that the median length of the micronutrients is significantly different at the 5 % significance level. The median line in the Na, Mg, Ca, Mn, Cu, and I plots respectively does not appear to be centered inside the box, which indicates that the micronutrient is slightly skewed. Moreover, Mg, Ca, Mn, Cu, and I data contain outlier values with Na not having an outlier. Besides, the median line in K was centred inside the box, and with the micronutrient not being skewed. However, there were outliers within the data.

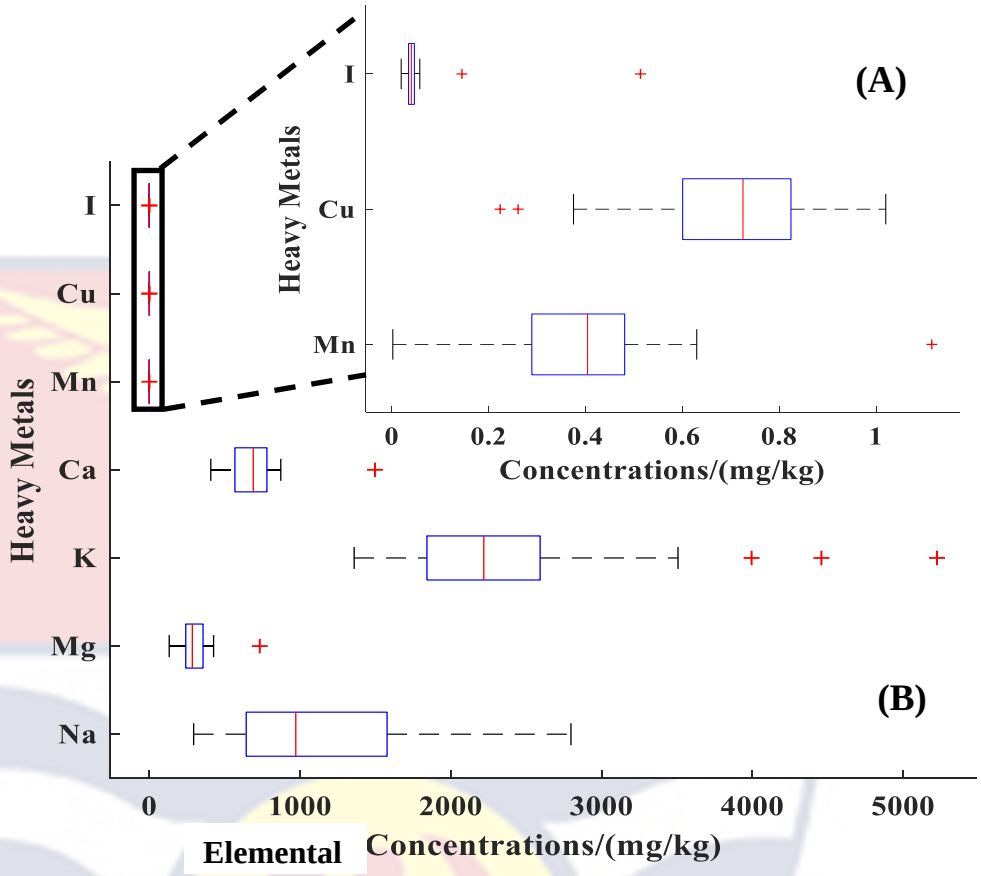

Figure 24: (A) Expanded view from the three macronutrients (Mn, Cu, and I) with low concentrations compared with the other four micro nutrients (Na, Mg, K, and Ca).
(B) Boxplot showing micronutrient concentrations in breast milk obtained for individual lactating mothers.

Clustering Analysis

The elemental concentration data of the breast milk (Appendix E) is presented as a dendrogram in Figure 25. Five (5) clusters were obtained after using a distance threshold of 5. Cluster 1 comprises Cu, I, and Mn. This implies that these three elements show more similarities in their concentrations and may also originate from a common source. As presented in Table 11, Cu, I and Mn have some common sources of dietary intake. With reference to their concentrations, it was observed that these three elements were present in very

low concentrations in all the samples taken. Cluster 2, 3, 4, and 5 are trivial clusters involving Mg, Ca, Na, and K respectively. Thus, at the threshold distance 5, only Cu, I and Mn can be related at that point, but all the other four elements are isolated or they are on their own.

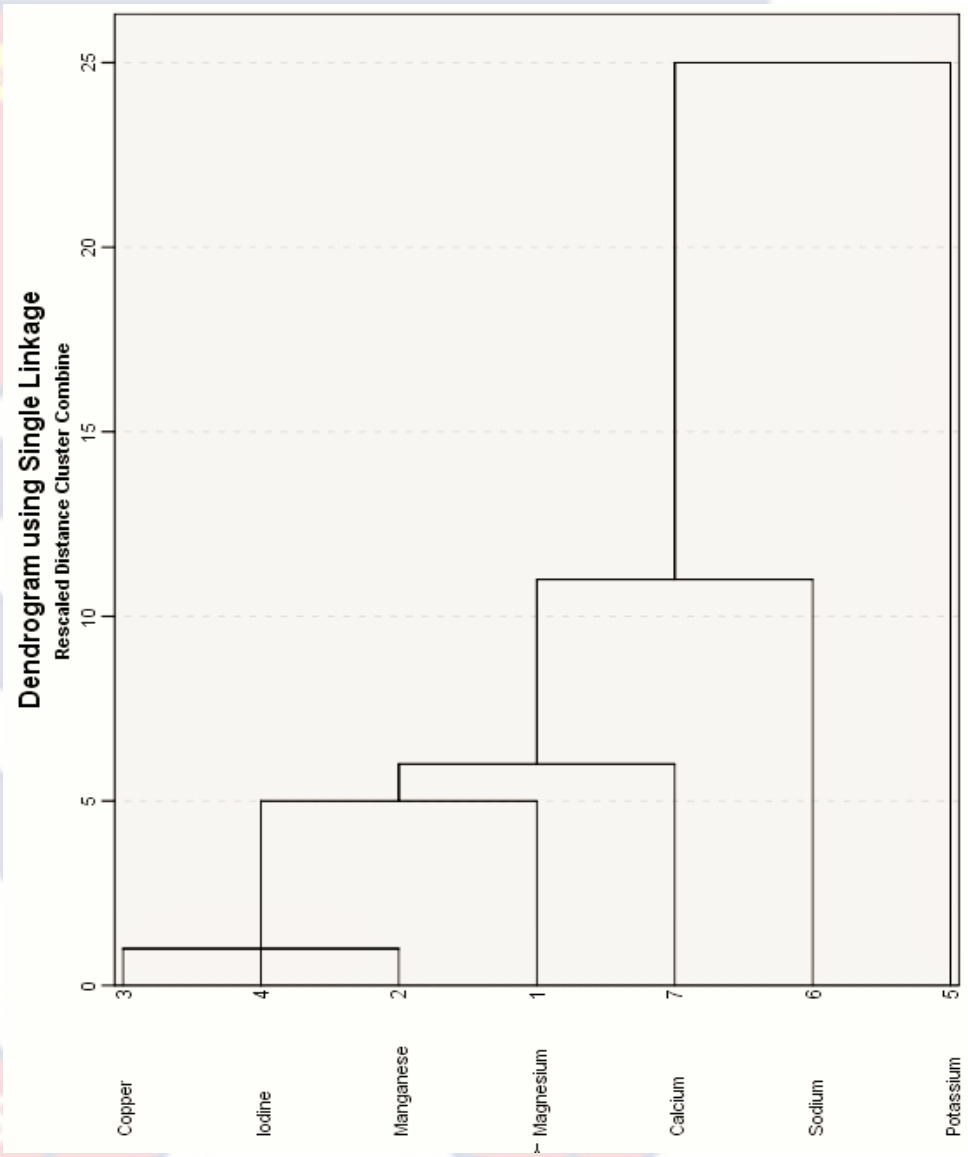

Figure 25: LDA results for the 7 micronutrients on the breast milk

When the threshold distance is increased to a point say 6, Mg merges with cluster 1 to form a new non-trivial cluster. This implies that the four

elements Cu, I, Mn, and Mg may be related in one way or the other. This observation is well explained in the correlation matrix in Table 10.

Multivariate Analysis

The PCA biplot, as presented in Figure 26, combined the scores and loadings to enable easy and quick visualization of the relationships between the mothers (the observations), and how each of the micronutrients (variables) measured from the breast milk contributed to the variations or similarities among them.

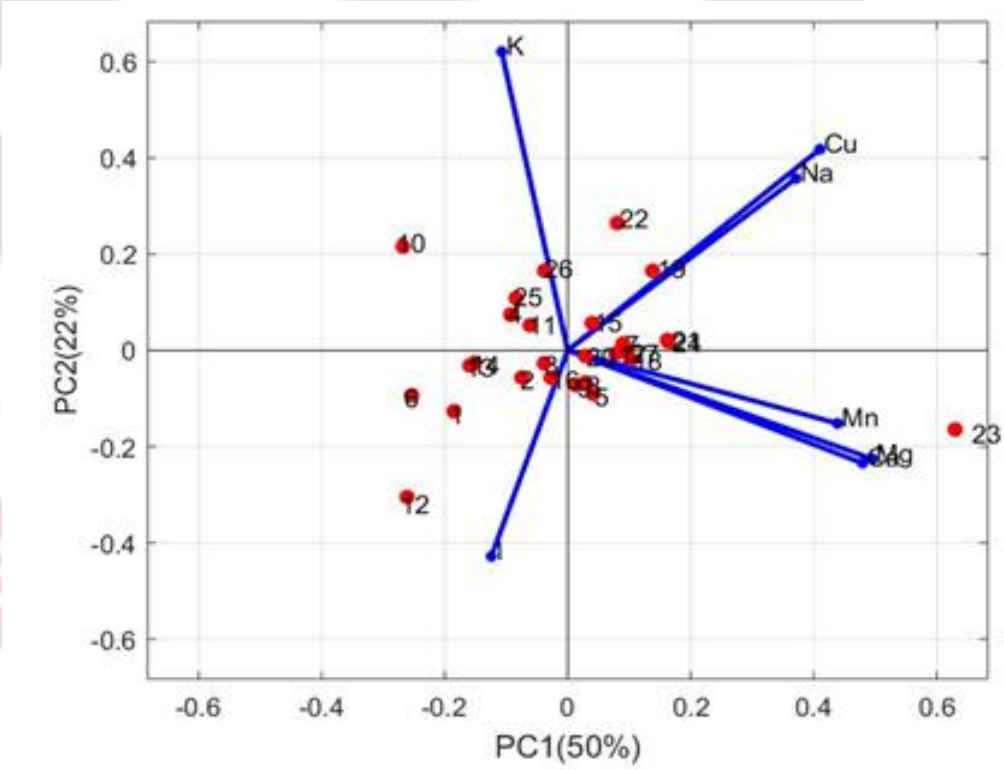

Figure 26: PCA biplot depicting the relationships between the mothers (observations) and how the micronutrients (variables) contributed to the variations or similarities among the mothers

Two groupings can be identified in Figure 26. The first group of observations (breastfeeding mothers 1, 2, 3, 4, 6, 10, 11, 12, 13, 14, 16, 25 and 26) are to the left of the PC1 axis (horizontal-axis), while the second group (breastfeeding mothers 5, 7, 8, 9, 15, 17, 18, 19, 20, 21, 22, 23, 24 and 27) are to the right. It can be inferred that since the second group of breastfeeding mothers are to the right on the PC1 axis, then the major micronutrients causing the differences between these two groups is more in the second group (to the right on the PC1 axis) than the other group of breastfeeding mothers, which is to the left of the PC1 axis. The responsible micronutrient is Mg as it exhibits the maximum positive value along the PC1 axis. This is followed by Ca, which is the second responsible micronutrient exhibiting the second maximum positive value along the PC1 axis. The contribution of all the other micronutrients can be registered as in the following trend line: Mg > Ca > Mn > Cu > Na > K > I.

Also from Figure 26, a general interpretation can be made about the different levels of the concentration of micronutrients present in the breast milk of the breastfeeding mothers. For instance, mothers to the right of PC1 are deficient in iodine and potassium, while those to the left of PC1 are deficient in all the 5 other elements (Na, Mg, Mn, Cu, and Ca). Breastfeeding mothers near the origin (3, 11, 15, and 20) have very low concentrations in all the elements (which is of concern), while breastfeeding mothers at the extremes (6, 10, 12, 22 and 23) have an abundance of some of the elements whiles deficient in others. None of the micronutrients were positioned at the origin of the PCA biplot, which means that all the micronutrients were significant and did not have any neutral effect. The micronutrients in the

negative (K and I) showed an inverse relationship with the other micronutrients at the positive (Mg, Ca, Mn, Cu, and Na).

Two other very useful deductions can be made from Figure 26. The first is that it is possible to trace a particular breastfeeding mother based on the measured levels of concentration of the micronutrients. For instance, an unknown breast milk which has high levels of I, but relatively lower levels in Mg and Cu is most likely to come from breastfeeding mothers 1, 2, 3, 6, 12, 13, 14, and 16. The second deduction is that the type of micronutrients observed can be related to the activity that goes on around the breastfeeding mother. For instance, breastfeeding mothers to the right of the PCA biplot (5, 7, 8, 9, 15, 17, 18, 19, 20, 21, 22, 23, 24 and 27) have Mg as the most dominating heavy metal and is highly correlated with Ca and Mn. These correlations are shown in Table 10.

Correlation Coefficient and Sources of Trace Elements

An important source of nutrients or trace elements is food and water which can be traced to groundwater discharges (Kelly & Moran, 2002). Accordingly, it is important to characterize each distinct source of trace elements and determine its contribution to the breastfeeding mother and baby. The correlation coefficients between elements provide information about their possible same or similar source inputs (Alfassi, 1994). Correlation coefficients between the seven elements and their respective significance at the 90% significance level in the breast milk are as shown in Table 10.

The Pearson correlation coefficients as calculated and presented in Table 10 were considered in the analysis because the concentrations of

elements in the fish were normally distributed, meaning that the mean concentrations are also normally distributed. The variables are continuous and there exists a linear relationship between the variables. The focus was on the strong point of the relationship and the extent of variance shared while reporting statistical significance (Pallant, 2007).

Table 10: Correlation coefficients between concentrations of the seven Elements Concentrations in Breast Milk

	Na	Mg	K	Ca	Mn	Cu	I
Na	1						
Mg	**0.465**	1					
K	0.035	–0.284	1				
Ca	**0.459***	**0.905****	–0.304	1			
Mn	0.250	**0.836****	–0.214	**0.733****	1		
Cu	**0.832****	**0.545****	0.192	**0.531****	**0.389***	1	
I	–0.171	0.002	–0.175	0.020	–0.371	–0.269	1

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

In this study, values between $\pm 0.500 \leq r \leq \pm 0.999$ were considered 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 is 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 six correlations were strong, three were statistically significant. This means that it can exude 90% confidence and that the correlation between Ca and Mg is strongly correlated with a coefficient of 0.905.

There was a moderately strong correlation between Na and Mg, since the main source of Na is the added salt. It is worth noting that salt is one of the cheapest commodities in the communities where these volunteers come from. Salt is mined at Elmina and sold in all the communities in the Central Region. Most of the foods consumed by the volunteering mothers daily are salted. For example, bread eaten is made from wheat which is rich in Mg, but salted before baked. Other salted foods rich in Mg are the soups made from vegetables, sea foods (salmon, mackerel, tuna), and beans. Salted roasted peanuts with banana are a delicacy in Ghana. A strong correlation of 0.905 was established between Na and Ca, and it could be attributed to salted foods rich in Ca. For example, sardines, salmon, beans, and leafy greens are found in most mother's daily diets like; yam and palava sauce, fufu and soup, and gari and beans.

Chocolate, which is rich in Cu, is made from cocoa. Ghanaians consume a lot of cocoa products. Chocolates are salted and can therefore be the source of correlation between Na and Cu. Other Cu-rich foods like lobsters, squid, oysters, and organ meats like liver are used in soups which are also salted before they are consumed.

Mg had a strong correlation of 0.905, 0.836, and 0.545 with Ca, Mn, and Cu, respectively. Mg and Ca-rich foods like the legumes and seafoods may be their sources. The volunteers are known to like gari with beans or soups which are made from sea foods. Most Cu-rich foods like seafoods are also rich in Mg. Table 11 shows the various elements and the foods they are rich in.

Table 11: The seven elements and some foods high in them

Element	Foods
Na	Shrimp, Soup, Ham, Instant Pudding, Cottage Cheese, Vegetable Juice, Salad Dressing, Pizza, Sandwiches, Broths and Stocks, Boxed Potato Casseroles, Pork Rinds, Canned, Vegetables Processed Cheese, Jerky and Other Dried Meats, Tortillas, Cold Cuts and Salami, Pretzels, Pickles, Sauces, Hot Dogs and Bratwurst, Tomato Sauce, Bagels and Other Breads, Canned Meats, Poultry and Seafood, Boxed Meal Helpers, Biscuits, Macaroni and Cheese, Frozen Meals, Baked Beans, Sausage, Bacon and Salt Pork (Healthline, 2019:a)
Mg	Whole Wheat and other grains, Spinach, Quinoa, Nuts and Seeds (Almonds, Cashews, Peanuts…), Dark Chocolate, Legumes (Black Beans, Edamame, Chickpeas, and Kidney Beans), Vegetables (Peas, Broccoli, Cabbage, Green Beans, artichokes, asparagus, brussels sprouts), Fruits (Figs, Avocado, Banana, Raspberries..), Seafood (salmon, mackerel, tuna) (Steen, 2017)
K	White Beans, Potatoes and Sweet Potatoes, Beets, Parsnips, Spinach, Swiss Chard, Tomato Sauce, Oranges and Orange Juice. (Healthline, 2017)
Ca	Seeds (Seeds are tiny nutritional powerhouses), Cheese, Yogurt, Sardines, Salmon, Beans, Lentils, Almonds, Whey Protein, Some Leafy Greens like Broccoli, Parsley, Spinach, Lettuce, Cabbage, “Kontomire”, etc, Milk, Lemon, and Orange. (Healthline, 2019:b)
Mn	Dried Apricots, Pineapple, Spinach, Mussels, Roasted Wheat Germ, Chickpeas, Firm Tofu, Lima Beans, Sweet Potatoes, Brown Rice, Pine Nuts, Soybeans, Quinoa, Corn, Turmeric (Food and Nutrition, 2018)
Cu	Organ meats (liver), Oysters, Lobster, Squid, Spirulina, Shiitake Mushrooms, Nuts and Sesame Seeds, Cashew Nuts, Almonds, Sunflower Seeds, Leafy Greens, Dark Chocolate. (Healthline, 2018:c)
I	Seaweed, Cod, Dairy products (Yogurt, Cheese, etc.), Iodized Salt, Shrimp, Tuna, Eggs, Prunes (Healthline, 2018:d)

It is not surprising to find that Mn also has a strong correlation of about 0.733 and 0.389 with Ca and Cu, respectively. Foods like beans (eat with) and spinach (used for palava sauce) are a rich and good sources of Mn and Ca. Oysters, lobsters and squid which are rich in Cu are usually prepared with

stews. These are normally taken with brown rice which is rich in Mn and are a delicacy in the areas the volunteers came from. The final good correlation of 0.531was established between Ca and Cu. This may probably come from leafy greens like broccoli, parsley, spinach, lettuce, cabbage, "kontomire" (Taro leaves), etc since they are all used in preparing palava sauce.

Jasmine 85 Rice

The concentration of each micronutrient investigated in the polished Jasmine 85 rice samples is shown in Appendix F. Table 12 is a summary of the mean elemental concentrations.

Table 12: Mean Elemental concentrations in the Rice samples in mg/kg

Elements	Na	Mg	Cl	K	Ca	V	Mn	Cu	I
Max	188.10	875.70	718.00	2949.00	2622.00	0.19	14.46	1.68	0.14
Min	142.30	483.20	465.60	514.60	2303.00	0.07	9.96	0.87	0.12
Mean	160.30	730.80	586.10	1611.70	2494.60	0.10	12.70	1.30	0.10
SD	18.01	150.50	105.32	1001.54	122.44	0.05	1.94	0.39	0.01

Appendix F and Table 12 present the total average mean concentrations and their standard deviations in mg/kg. A plot of their mean concentrations at the five farms studied is presented in Figure 27. Elementally, the composition of a plant reflects the composition of the nutrient medium or soil on which it is found. To an extent, plants are known to be selective in their absorption of elements (Teherani, 1987). From Figure 27 (b), rice from Aveyime Farm has the highest quantity of sodium whiles Dawhenya Farm recorded the least.

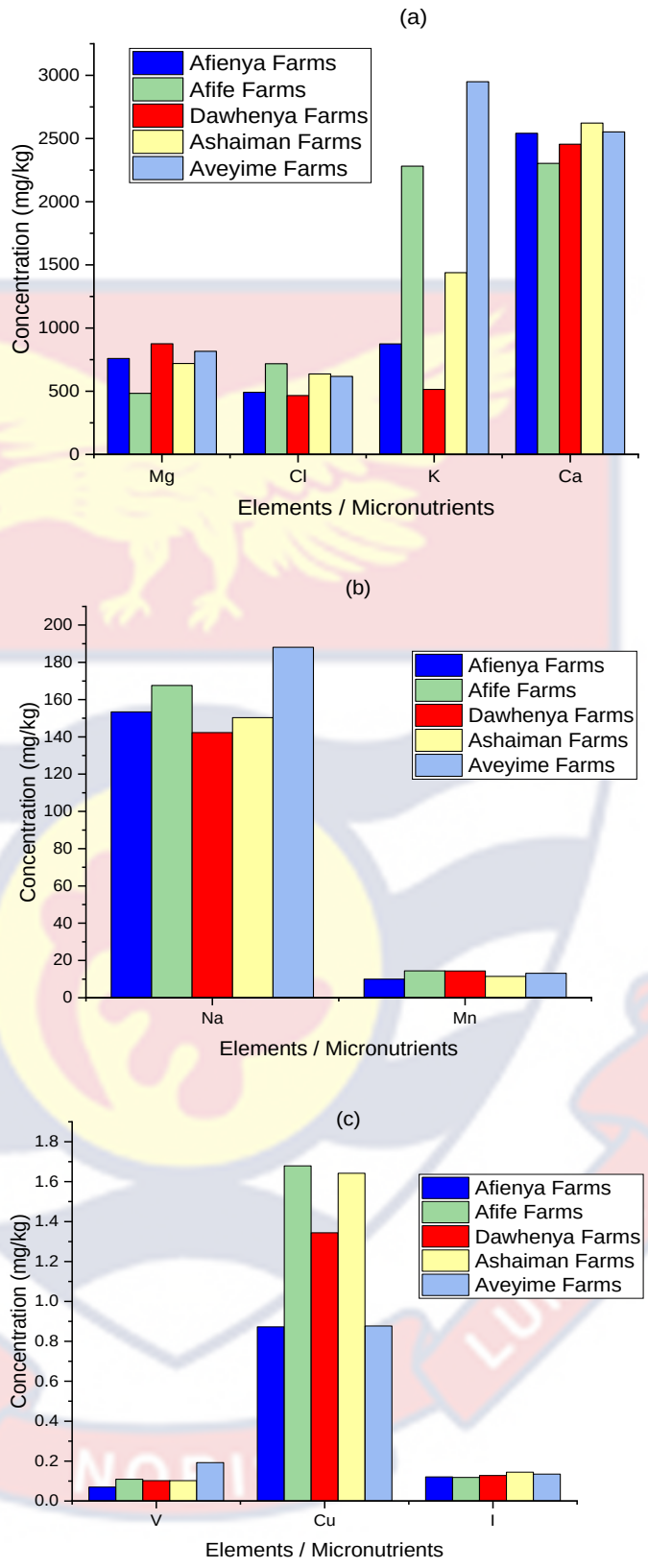

Figure 27: Bar charts showing:

(a) Concentration of the individual elements (Mg, Cl, K and Ca) at the five farms

(b) Abundance of the individual elements (Na and Mn) at the five farms

(c) Abundance of the individual elements (V, Cu and I) at the five farms

On the other hand, Dawhenya Farms had the maximum concentration of magnesium while Afife Farms had the least. Afife Farms recorded the highest concentration of chlorine and the least concentration of calcium. Aveyime Farms and Ashaiman Farms recorded the highest concentrations in potassium and calcium, respectively. The concentration levels of vcanadium, manganese, copper, and iodine were generally very low. Ghanaian foods have been observed to be very low in iodine (Nyarko et al., 2002).

Iodine deficiency has been identified as one of the factors that adversely affect the development of children in developing countries (Walker et al., 2007). This deficiency manifests as stunted physical and mental growth as well as infertility, lethargy, and cognitive impairment (Adu & Simpson, 2017).

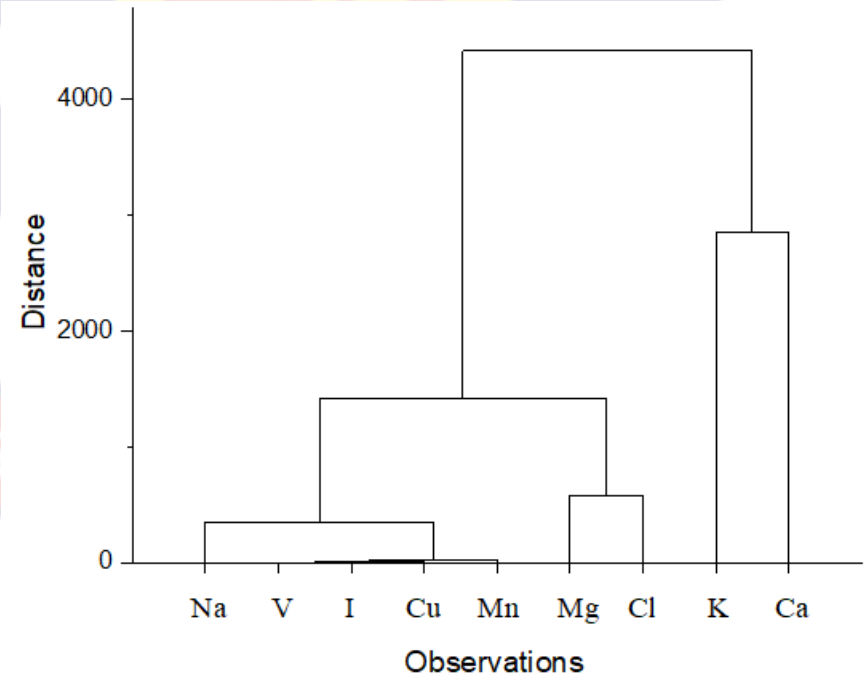

Figure 28: LDA results for the 9 micronutrients with respect to the rice farms

Figure 28 shows the Linear Discriminant Analysis (LDA) carried out on the concentration data to determine if any pattern that characterizes or separates the micronutrients at the various farms could be established. From Figure 28, two major clusters can be identified; Group 1 (K and Ca) and Group 2 (Na, V, I, Cu, Mn, Mg, and Cl). The inference, is that there is a common element that differentiates between these two groups. To know this element, and possibly which of the farms have more or less of it, the PCA biplot as presented in Figure 29 was used.

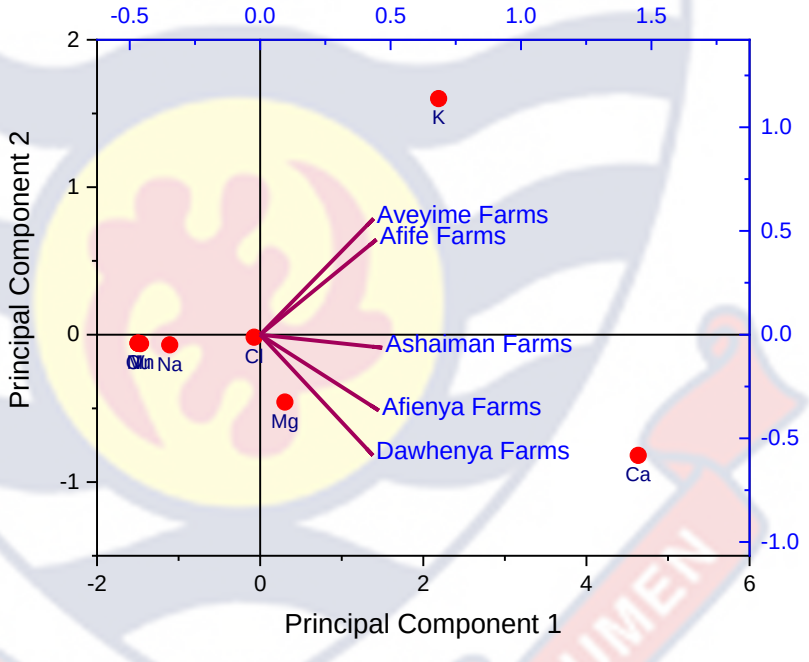

Figure 29: Biplot depicting the relationships between the farms (observations) and how the micronutrients (variables) contributed to the variations or similarities among the farms.

The PCA score plot shows (Figure 29), how the different observations (farms) were related while the loading plot indicated how each of the variables (micronutrients) contributed to the similarities or dissimilarities between the

farms. Figure 29 combines the scores and loadings and was used for an easy and quick visualization of the relationships between the farms and how each of the micronutrients measured from the rice contributed to the variations or similarities among the farms.

From Figure 29, it could be deduced that the levels of micronutrients are not the same in the rice for all the farms. Two groupings can be identified in this respect. The first group of farms (Ashaiman, Afienya, and Dawhenya) is to the low or negative axis of the PC2 axis (vertical-axis) whiles the second group (Aveyime and Afife) is to the high or on the positive axis. It could be inferred that the second group of farms is to the high side on the PC2 axis, then the major micronutrient causing the differences between these two groups is more in the second group (to the positive side on the PC2 axis) than the other group of farms to the negative side of the PC2 axis. The responsible micronutrient is therefore K as it exhibits the maximum positive value along the PC2 axis. This is followed by Ca being the second responsible micronutrient exhibiting maximum positive value along the PC2 axis.

The overall contribution of the micronutrients could be presented as follows; K > Ca > Mg > Cl > Na > Mn > Cu > I > V. The micronutrient positioned at the origin of the PC biplot (Cl) has a neutral effect, whereas those in the negative (Ca, Mg, Na, Mn, Cu, I, and V) show an inverse relationship with the only micronutrient at the positive (K). Two other very useful deductions can be made from Figure 29. The first is that it is possible to trace harvested rice to a particular farm based on the measured concentration level of micronutrients. For instance, rice with high levels of I, but relatively lower levels in Mg and Ca is most likely to come from Afienya or Dawhenya

farms. The second deduction is that the type of micronutrients observed can be related to the activity that goes on around the farming area. For instance, apart from Aveyime Farms and Afife Farms which are high in K but low in the other micronutrients, Ashaiman Farms, Afienya Farms, and Dawhenya Farms are high in all the micronutrients except K.

It could also be inferred that since all the farms are to the right of the PC1 axis, then the major micronutrients running through the five farms are K, Mg, and Ca. However the PC biplot has K as the most dominating micronutrient and is highly correlated with Na, Cl, and V. These correlations between the micronutrients in the rice are as shown in Table 13.

Table 13: Correlation Matrix between the Micronutrients in the Rice Samples

	Na	Mg	Cl	K	Ca	V	Mn	Cu	I
Na	1								
Mg	- 0.195	1							
Cl	**0.549**	**- 0.799**	1						
K	**0.951**	- 0.380	**0.767**	1					
Ca	- 0.078	**0.631**	- 0.321	- 0.131	1				
V	**0.842**	0.186	0.353	**0.812**	0.104	1			
Mn	0.189	- 0.192	0.297	0.280	**- 0.697**	0.399	1		
Cu	-0.361	**- 0.589**	**0.534**	- 0.066	- 0.388	- 0.286	0.427	1	
I	0.014	0.405	0.100	0.125	**0.780**	0.357	- 0.195	0.118	1

Some of the significant correlations as presented in Table 13 could be drawn between the micronutrients Na and K (r = 0.951), Na and V (r = 0.842) and K and V (r = 0.812). These pairs could indicate the same or similar source inputs.

The maximum Upper Limit (UL) of Recommended Dietary Allowable (RDA) and Adequate Intake (AI) for the Life Stage Groups of the elements investigated are presented in the Appendix G.

Calculating the Daily Average Intake based on Dietary Reference

Considering the per capita rice consumption in 2016/17 estimated at about 35 kg/year, with Ghana's population at 28.2 million (Global Agricultural Information Network, 2018), the average daily rice intake was estimated as 95.8904 g/d. Using the Pourgheysari formula presented in equation 21 (Pourgheysari, Moazeni, & Ebrahimi, 2012; Mahan & Escott-Stump, 2008; Ireland, Clifton, & Keogh, 2010), the daily average intake of the micronutrients when one consumes rice from the five farms is presented in Table 14.

$$Daily\ Intake\ of\ Micronutrients = COMITR \times MRIGPD \quad [21]$$

where COMITR is the concentration of micronutrients in the rice, and MRIGPD is the mean rice intake (g/person/day)

Table 14: Calculated mean daily intake of micronutrients in the rice samples

	Mean Concentration of Elements (mg/kg)	Calculated Mean Daily Intake (mg/day)
Na	160.34	15.375
Mg	730.8	70.077
Cl	586.1	0.096
K	1611.702	652,593.484
Ca	2494.6	239.208
V	0.113	0.011
Mn	12.6732	1.215
Cu	1.2830	0.123
I	0.1293	0.012

The calculated mean daily intake of the nine micronutrients values in Table 14 was compared to the Mean and the Maximum Upper Limit (UL) of RDA and AI for all the Life Stage Groups (Tables 21, 22 and 23 respectively in Appendix G) and the difference in values presented in Tables 24 and 25 (in the Appendix H). It could be seen that all the calculated values did not exceed the mean and Upper Tolerable Limit of its RDA, except K, for all Life Stage Groups. The calculated Mg and Ca showed a high value for infants between 0 to 6 months for normal RDAs but did not exceed the UL for RDA. Mg for Children between 1 to 3 years and V for females between 9 to 18 years did also exceed the UL for RDAs. Also, Mn for infants between 0 to 3 years was above the normal RDA values.

To determine the effect on the health of the consumer the Health Risk was estimated by calculating the Hazard Index (HI). Table 26 (in Appendix H) is the calculated HI which estimates the associated health risk associated with the intake of the rice. Apart from the sections that could not be determined due to lack of data (ND), all the calculated values for all the micronutrients were less than 1, suggesting there was no probability of any adverse health effects for all life stage groups. The only HI > 1 was for Mg for children between the ages of 1 and 3. As stated earlier, all these elements investigated are essential to human health but could be toxic at concentrations higher than that necessary for the biological functions of the growth processes. Table 27 (in Appendix I) lists the functions of the elements in the human body and their effects when consumed in deficient or in excessive amounts.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Overview

This chapter presents the summary, conclusions of the research findings, and recommendations.

Summary

In the present research, the EINAA method has been used for the analysis of ^{24}Na(2754.0 keV), ^{27}Mg(1014.4 keV), ^{38}Cl(1642.4 keV), ^{42}K(1524.6 keV), ^{49}Ca(3084.5 keV), ^{52}V(1434.1 keV), ^{56}Mn(1810.7 keV), ^{66}Cu(1039.2 keV), ^{80}Br(616.93 keV), and ^{128}I(440.9 keV) in Polished Jasmine 85 Rice from Afife, Aveyime, Afienya, Ashaiman, and Dawhenya, Breast Milk from zero to six months old nursing mothers from Central Region, and Blackchin Tilapia fish from the Benya Lagoon using GHARR-1, in Accra, Ghana. The use of three millimetres thick of flexible boron shields were used to cut off thermal neutrons to assess epithermal neutrons at 94.7% accuracy enabling the EINAA method to be achieved. Relative standardization method were used in the quantification of the ten elements. The low levels of Br, Cl, and I were successful due to the standard reference materials namely (IAEA)-336; IAEA-407, IAEA-350 and (NIST) USA SRM 1577b. 94.7% accuracy was achieved in the elemental studies of the breastmilk using standard reference materials from the IAEA, and NIST. The reference materials IAEA-530 Tuna fish homogenate and NIST USA 1566b Oyster

Tissue recoveries of the elemental concentrations ranged from 88% to 111% of the certified values in the rice studies.

The I levels in all the samples were below the recommended daily allowance in all the life stage groups in Appendix G. This could cause iodine deficiency disorder espercially among children.

Conclusions

Checks on quality of all the entire protocols used in the research were very important in ensuring precision and accuracy of the good results achieved. The outcomes have shown the usefulness of INAA as a tool for the investigation of fish (Blackchin tilapia), breastmilk, and rice (Jasmine 85) from southern Ghana. The insufficiency or abundance of these elements in the food is known to cause an assortment of a lack of healthy sustenance or well-being issue on the planet. 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. Many health outcomes were recorded to be directly linked to the duration of the breastfeeding, suggesting a potential cumulative effect (Mosca and Gianni, 2017).

This study has provided information on the concentration levels of V, Na, Mn, Mg, K, I, Cu, Cl, Ca, and Br present in these three foods studied. Indeed iodine in all the foods studied were of very low levels making it generally difficult to determine. Elements like Na, K, Ca, Mg, Mn, and Cu also showed very high concentrations. This work has also demonstrated the capabilities of nuclear analytical techniques in determining these ten trace

elements in food at the µg.kg^{-1} levels. All the dietary assessment done hopefully has thrown more light on these ten micronutrients.

This work has shown the low levels of iodine in fish, rice and breastmilk in southern Ghana. Iodine deficiency has been recognised as a factor that has an adverse effect on child development in developing countries (Walker et al., 2007), and this deficiency manifest as stunted physical and mental growth as well as infertility, lethargy, and cognitive impairment (Adu & Simpson, 2017). Therefore the national drive to combat the problem of iodine deficiency by governmental and non-governmental agencies needs to be intensified.

Recommendations

Since there is not enough database for elemental composition of Ghanaian foods and diets (Nyarko et al., 2002), in 2006 the former director of GAEC Prof. B.J.B. Nyarko began working on it. He focused on foods grown in the Northern Regions of Ghana.

1. It is recommended that nutritionists; toxicologists and scientists of different background get together and look at the low intensities of I in the rice, fish, and breastmilk. Irradicating iodine deficiency disorder IDD, would help solve the stunted growth problem and improve the mental capabilities of children in the region since one of the purposes of the education reform program and otherwise the country's development efforts is IDD among children (Caulfield et al, 2006). This would go a long way in helping the country to solve its nutritional and health-related problems.

2. It is also recommended that a national database on elements under observation in fish, rice, and breast milk would be added to earlier work on foods grown in the Northern Regions of Ghana.
3. Nutritionist would be given the data from this research to enable them find out whether lactating mothers and their eating trends are impacting well on their breast milk which will, in turn, affect their infants.
4. The Benya Lagoon needs regular dredging due to the activities around the bed. The results of this study may be useful for designing the best management plans for Benya Lagoon's ecosystem restoration and ensuring the sustainability of the healthy status of the blackchin tilapia species.
5. The information on these nine micronutrients content of the rice from these five farming areas would be useful in research on rice consumption to determine the overall availability of micronutrients for the Ghanaian population and age groups as well as in nutrition design for national rice supply study, particularly in provinces and nations which are known to be vulnerable to deficiencies of these micronutrients.
6. Using this method would enable one to accurately determine the content of these nine micronutrients in rice, fish and breast milk and can be used as traceability and predictability of these micronutrients of the producing area.

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APPENDICES

APPENDIX A

RAW DATA OF THE CONCENTRATION OF ELEMENTS IN FISH

Table 15: Raw Data of the Concentration of Elements in the Twenty Sampled S. Melanotheron

Element	Br		
Fish Part	Bone μg/g	Muscle μg/g	Gill μg/g
1	30.40 ± 3.98	DL	61.55 ± 6.09
2	30.72 ± 3.38	92.05 ± 15.28	48.83 ± 5.03
3	17.28 ± 2.85	DL	28.99 ± 3.42
4	38.43 ± 4.11	39.19 ± 5.05	68.78±7.43
5	24.44 ± 3.13	37.40 ± 3.93	30.00±3.18
6	25.17 ± 3.15	23.03 ± 2.76	DL
7	29.39 ± 3.79	32.61 ± 3.39	28.00±3.44
8	DL	DL	DL
9	DL	DL	DL
10	19.21 ± 2.73	13.61 ± 2.33	DL
11	58.77 ± 5.82	89.18 ± 7.40	171.40 ± 13.19
12	115.60 ± 9.48	86.00 ± 7.31	DL
13	25.04 ± 3.21	DL	
14	DL	DL	DL
15	29.07 ± 4.09	37.32 ± 3.73	DL
16	37.05 ± 3.89	DL	57.06 ± 6.28
17	DL	44.04 ± 4.45	54.81 ± 5.21
18	14.88 ± 2.37	18.43 ± 2.95	DL
19	29.11 ± 3.35	39.17 ± 4.31	49.77 ± 4.58
20	35.04 ± 5.99	30.62 ± 3.28	31.16 ± 3.46
Min	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Max	115.60 ± 9.48	92.05 ± 15.28	171.40 ± 13.19
Mean	27.98 ± 3.27	29.13 ± 3.31	31.52 ± 3.07

The sign (DL) denotes that the value was below the detector's limit of detection

Element	Cl		
Fish Part	Bone μg/g	Muscle μg/g	Gill μg/g
1	6966.00 ± 933.44	9684.00 ± 1297.66	7678.00 ± 1028.85
2	5347.00 ± 716.50	7995.00 ± 1071.33	7184.00 ± 962.66
3	4209.00 ± 564.01	DL	4417.00 ± 596.30
4	8004.00 ± 1072.54	6604.00 ± 884.94	12210.00 ± 1636.14
5	5548.00 ± 743.43	6969.00 ± 932.91	6412.00 ± 859.21
6	5317.00 ± 712.48	6186.00 ± 828.92	5430.00 ± 727.62
7	5237.00 ± 701.76	6758.00 ± 905.57	5545.00 ± 743.03
8	6253.00 ± 837.90	6047.00 ± 810.29	3290.00 ± 440.86
9	4766.00 ± 643.41	19400.00 ± 2599.60	3649.00 ± 488.97
10	4260.00 ± 570.84	5820.00 ±779.88	5495.00 ± 736.33
11	12200.00 ± 1634.80	20570.00 ±2756.38	36700 ± 4917.80
12	27820.00 ± 3727.88	27160.00 ±3639.44	22420.00 ± 3004.28
13	4243.00 ± 568.56	5665.00 ±759.11	DL
14	6433.00 ± 862.02	5657.00 ±758.04	3902.00 ± 526.77
15	7715.00 ± 1033.81	8853.00 ± 1186.00	9785.00 ± 1311.19
16	6675.00 ± 894.45	12960.00 ± 1736.64	7948.00 ± 1065.03
17	7740.00 ± 1037.16	8526.00 ± 1142.48	10350.00 ± 1386.90
18	5804.00 ± 777.74	7139.00 ± 956.63	3247.00 ± 435.09
19	6617.00 ± 886.68	7099.00 ± 951.27	5505.00 ± 869.79
20	4763.00 ± 638.24	6642.00 ± 890.03	5627.00 ± 754.02
Min	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Max	27820.00 ± 3727.88	27160.00 ±3639.44	22420.00 ± 3004.28
Mean	7295.85 ± 977.88	9286.70 ± 1244.36	8339.7 ± 1124.54

The sign (DL) denotes that the value was below the detector's limit of detection

Element		I	
Fish Part	Bone μg/g	Muscle μg/g	Gill μg/g
1	DL	1.10±0.17	3.99±0.60
2	0.64 ± 0.10	0.72 ± 0.11	7.49 ± 1.12
3	1.08 ± 0.16	DL	1.82 ± 0.27
4	0.68 ± 0.10	DL	10.23 ± 1.53
5	10.13 ± 0.17	1.05 ± 0.16	3.14 ± 0.47
6	0.65 ± 0.09	1.30 ± 0.19	2.95 ± 0.44
7	DL	0.78 ± 0.12	2.50 ± 0.38
8	DL	DL	1.86 ± 0.28
9	DL	DL	3.20 ± 0.48
10	DL	0.94 ± 0.14	3.84 ± 0.58
11	DL	0.87 ± 0.13	1.36 ± 0.20
12	DL	DL	2.04 ± 0.31
13	0.74 ± 0.11	DL	DL
14	1.56 ± 0.23	1.56 ± 0.23	2.31 ± 0.35
15	0.54 ± 0.08	1.07 ± 0.16	4.63 ± 0.69
16	DL	DL	4.30 ± 0.65
17	1.52 ± 0.23	1.51 ± 0.23	5.06 ± 0.76
18	0.62 ± 0.09	DL	2.71 ± 0.41
19	0.79 ± 0.12	1.40 ± 0.21	2.78 ± 0.42
20	DL	DL	1.83 ± 0.27
Min	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Max	10.13 ± 0.17	1.56 ± 0.23	10.23 ± 1.53
Mean	0.95 ± 0.07	0.62 ± 0.093	3.40 ± 0.51

The sign (DL) denotes that the value was below the detector's limit of detection

APPENDIX B

COPY OF APPROVED LETTER FROM THE GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. :GHS-ERC: 3
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Tel: +233-302-681109
Fax + 233-302-685424
Email: Hannah. Frimpong@ghsmail.org

19th October, 2015

Michael Kwame Vowotor
University of Cape Coast
School of Physical Sciences
Department of Physics
Cape Coast

ETHICS APPROVAL - ID NO: GHS-ERC: 02/05/15

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"Development of INNA Techniques to Determine Trace Elements in Breast Milk: Case Study Central Region"

This approval requires that you inform the Ethics Review Committee (ERC) when the study begins and provide Mid-term reports of the study to the Ethics Review Committee (ERC) for continuous review. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification without ERC approval is rendered invalid.

You are also required to report all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your sponsor before any publication of the research findings.

Please note that this approval is given for a period of 12 months, beginning October 19th, 2015 to October 18th, 2016.

However, you are required to request for renewal of your study if it lasts for more than 12 months.

Please always quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
DR. CYNTHIA BANNERMAN
(GHS-ERC CHAIRPERSON)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

Appendix B: Copy of approved letter from the Ghana Health Service Ethical Review Committee (GHS-ERC-02/05/15).

APPENDIX C
SAMPLE SIZE CALCULATION

This work is to fine-tune and further improve the use of Relative Standardization INAA in addition to relative single comparator method (k0-method). Nonetheless, proper development and utilization for routine use have become more necessary with the increasing number of research scientist requiring every possible reliable analytical tool for trace element analysis.

A common goal of the research is to collect data representative of a population. The researcher uses information gathered from the survey to generalize findings from a drawn sample back to a population, within the limits of random error (Baarttlletttt et al, 2001). Also, since the data needed for this work is breast milk, it renders it categorical. A categorical data is the statistical data type consisting of categorical variables or of data that has been converted into that form, for example as grouped data. Examples of categorical variables are race, sex, age group, lactating women and educational level. Here it was decided that a small sample size would be needed to test the method.

The appropriate use of Cochran‘s (1977) sample size formula for categorical data was employed for determining the sample size. The t-value is a test statistic for t-tests that measures the difference between an observed sample statistic and its hypothesized population parameter in units of standard error. Here the researcher adjusts the t value used based on the population size, which is required when the population size is 120 or less.

$$\text{Sample size, } S = \frac{(t^2) * (v)}{d^2} \quad [22]$$

For error estimation Cochran's (1977) formula uses two key factors:

(1) the risk the researcher is willing to accept in the study, commonly called the margin of error, or the error the researcher is willing to accept, and

(2) the alpha level, the level of acceptable risk the researcher is willing to accept that the true margin of error exceeds the acceptable margin of error; that is the probability that differences revealed by statistical analyses really do not exist; also known as Type I error.

Another type of error will not be addressed further here, namely, Type II error, also known as beta error. Type II error occurs when statistical procedures result in a judgment of no significant differences when these differences do indeed exist.

Alpha Level, d: The alpha level used in determining sample size in most educational research studies is either 0.05 or 0.01 (Ary, Jacobs, & Razavieh, 1996). In Cochran's formula, the alpha level is incorporated into the formula by utilizing the t-value for the alpha level selected (e.g., t-value for an alpha level of 0.05 is 1.96 for sample sizes above 120). Researchers should ensure they use the correct t-value when their research involves smaller populations. For this work t-value of 2.0 and an alpha level of 0.05 was chosen.

APPENDIX D
CONSENT FORM

The reason behind this research was explained to every volunteer of giving breast milk. Each volunteer was made to sign a consent form. They also answer some few questions on the consent form shown below:

UNIVERSITY OF CAPE COAST
SCHOOL OF PHYSICAL SCIENCES
DEPARTMENT OF PHYSICS

Consent Form

Research Topic - Development of INAA Technique to Determine Trace Elements in Breast Milk: Case Study Central Region

Dear Madam,

This Informed Consent Form is for lactating mothers women who attend weighing at _____ . You are being invited to participate in a research entitled: "Development of INAA Technique to Determine Trace Elements in Breast Milk: Case Study Central Region"

I am Michael Kwame Vowotor, a Ph.D. student from the University of Cape Coast. I am doing research on designing a very good way of detecting nutrients in breast milk.

I am going to give you the information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have

questions later, you can ask them of me, any doctor/nurse or any member of the research staff.

Human breast milk is considered the optimal form of nourishment for infants during the first 6 months of life. It also contains antibodies from the mother's immune system that help the infant fight off infections and diseases. Human milk provides distinct advantages over formula or cow milk for human infants. Not only is breast milk's nutritional composition uniquely designed for the needs of human babies, but if the nutrients are not in the right quantity can affect the growth of the child. So this research is to help us find new wayfinding the type of nutrients in the breast milk. This can help us inform mothers about what would be the best practices in the improvement of their breast milk.

This research will involve you donating about 2 ml of your breast milk once.

Your participation is at your own free will and your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic/centre will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic/centre and you would be told more about it later. You may change your mind later and stop participating even if you agreed earlier.

You would also fill the *questionnaire designed to elicit information on the above topic. The study is purely for academic purposes only. The results from this study we hope would help our nation in the future. We would be very grateful if you could respond to this instrument sincerely and truthfully. Please be assured that your sample and responses will be treated with the utmost privacy and confidentiality. Samples would be destroyed after the research at the Ghana Atomic Energy Commission (GAEC), Accra, according to the standard practice set out by the International Atomic Energy Agency (IAEA), Geneva.*

We truly appreciate your willingness to voluntarily participate in this study. If you have any additional questions please do not hesitate to ask one of the medical staff or researchers. By signing below you are stating that –

I have received a full explanation of the research protocol in which I am electing to participate and have had all of my questions answered to my satisfaction.

Name of participant_____ AND

Thumb print of participant

Signature of participant _____

Date _____

Day/month/year

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____ AND

Thumbprint of witness

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent –

I have explained this research to the potential participant, and to the best of my ability made sure that the participant understood it. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered

correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of Researcher

_____

Signature of Researcher

_____

Date _____

Day/month/year

For any additional information please contact:

Michael Kwame Vowotor, Department of Physics, University of Cape Coast, Cape Coast.

Mobile – 0208157068,
Email – mvowotor@ucc.edu.gh

Ghana Health Service Ethical Review Committee Administrator: Hannah Frimpong

Mobile – 0507041223,
Office – 0320681109,
Email – Hannah.Frimpong@ghsmail.org

Questions to be Asked Nursing Mothers (*QUESTIONNAIRE)*

Topic: *DEVELOPMENT OF INAA TECHNIQUES TO DETERMINE TRACE ELEMENTS IN* BREAST *MILK:* CASE STUDY CENTRAL REGION

Hospital/Health centre:_____

Some Personal Details On Nursing Mother & Baby

Please tick where appropriate

Nursing Mother No. _____

1. Age: 15-19 [ ] 20-24 [ ] 25-29 [ ] 30-34 [ ] 35-49 [ ]
   40-44 [ ] 45-49 [ ]

2. Level of education: Illiterate [ ] Primary [ ] Middle/JHS [ ]
   SHS [ ] Tertiary [ ]
   Others [ ] (specify) _____

3. Marital status: Single [ ] Married [ ] Divorced [ ] Separated [ ] &
   Others(specify) _____

4. Religion: Christian [ ] Islamic [ ] Traditional [ ]
   Others (specify) _____

5. Number of children _____

6. Ethnicity _____

7. Occupation _____

8. Do you use iodized salt in cooking? Yes [ ] No [ ]

9. Which of these diseases does mother have? Asthma [ ] Heart diseases [ ]
   Diabetes [ ] Cancer [ ] Liver complications [ ]
   Any others disease (specify) _____

10. i. Has mother fallen sick in the past 6 months? Yes [ ] No [ ]
    ii. If yes, what sickness?

_____

Baby Background Information

1. Age of infant: 0-1 month[ ] 1-2 month[ ] 2-3 month[ ] 3-4 month[ ]
   4-5 month[ ] 5-6 month[ ]

2. Baby's weight: _____

3. Is the baby on exclusive breastfeeding? Yes [ ] No [ ]

4. What food do you regularly use to feed your infant?
   Breast milk [ ] Animal milk [ ] SMA [ ] Solid foods [ ]
   Others (specify)_____

5. How many times does the infant feed in a day? _____

6. Which of these diseases does the baby have? Asthma [ ] Heart diseases [ ]
   Diabetes [ ] Cancer [ ] Liver complications [ ]
   Others (specify) _____

7. i. Has baby fallen sick in the past 6 months? Yes [ ] No [ ]
   ii. If yes, what sickness?_____

APPENDIX E

BREAST MILK VOLUNTEER INFORMATION

Table 16: Description of the range of ages and other data taken

	Age (Years)	Level of Education	Marital status	Religion	Number of Children	Ethnicity	Occupation
1	20-24	Tertiary	Married	Christian	One	Fante	Students
2	15-19	JHS	Single	Christian	One	Fante	Unemployed
3	20-24	JHS	Married	Christian	Two	Fante	Unemployed
4	15-19	JHS	Married	Christian	One	Fante	Traders
5	30-34	Primary	Single	Christian	Four or +	Ewe	Manual laborer
6	30-34	SHS	Married	Islamic	One	Fante	Teacher
7	25-29	Primary	Married	Christian	Three	Fante	Traders
8	25-29	JHS	Married	Christian	Two	Fante	Traders
9	30-34	JHS	Married	Christian	Four or +	Fante	Traders
10	25-29	Tertiary	Married	Christian	Four or +	Fante	Traders
11	20-24	Primary	Married	Islamic	Two	Fante	Caterer
12	30-34	JHS	Married	Islamic	Four or +	Fante	Traders
13	25-29	SHS	Married	Christian	Two	Fante	Traders
14	30-34	JHS	Married	Christian	Four or +	Fante	Traders
15	35-49	Tertiary	Single	Christian	One	Ewe	Nurse
16	25-29	Illiterate	Married	Christian	Four or +	Fante	Unemployed
17	15-19	JHS	Single	Christian	One	Fante	Students
18	25-29	Tertiary	Married	Islamic	Two	Fante	Seamstress
19	25-29	Illiterate	Married	Christian	Three	Fante	Traders
20	15-19	JHS	Married	Christian	One	Fante	Hairdressers
21	25-29	JHS	Married	Christian	Two	Fante	Seamstress
22	35-49	JHS	Married	Christian	Three	Fante	Seamstress
23	20-24	SHS	Single	Christian	Two	Fante	Unemployed
24	15-19	JHS	Single	Christian	Two	Fante	Unemployed
25	35-49	JHS	Married	Christian	Four or +	Fante	Traders
26	20-24	Illiterate	Married	Christian	One	Fante	Traders
27	15-19	Primary	Single	Christian	One	Fante	Unemployed

Table 17: Description of the foods and other things mothers partake of

	Do You Use Iodated Salt In Cooking?	Do You Have Any Of These Diseases?	Mother's sickness in the last 6 months	Mothers main diet in the morning	Mothers main diet in the afternoon	Mothers main diet in the evening
1	Yes	Skin allergies	Skin allegies	Kooko and bread	Rice and stew	Tea and bread
2	Yes	None	None	Kooko and bread	Rice and stew	Fufu and soup
3	Yes	None	None	Waakye	Banku and stew	Fufu and soup
4	Yes	None	None	Fufu and soup	Gari and beans	Fufu and soup
5	Yes	None	None	Tea and Bread	Yam and palava sauce	Banku and stew
6	Yes	None	None	Tea and Bread	Rice and stew	Gari and beans
7	Yes	None	None	Tea and Bread	Banku and stew	Fufu and soup
8	Yes	None	Malaria	Tea and Bread	Yam and palava sauce	Fufu and soup
9	Yes	None	None	Kooko and bread	Rice and stew	Fufu and soup
10	Yes	None	None	Rice and stew	Fufu and soup	Fufu and soup
11	Yes	None	Aneamia	Rice and stew	Fufu and soup	Fufu and soup
12	Yes	None	None	Rice and stew	Snacks	Fufu and soup
13	Yes	None	Malaria	Tea and Bread	Banku and stew	Fufu and soup
14	Yes	None	None	Tea and Bread	Rice and stew	Fufu and soup
15	Yes	None	None	Tea and Bread	Rice and stew	Banku and stew
16	Yes	None	None	Tea and Bread	Rice and stew	Fufu and soup
17	No	Eye problem	Fever	Tea and Bread	Fufu and soup	Kenkey and stew
18	Yes	None	None	Kooko and bread	Rice and stew	Fufu and soup
19	Yes	None	Headache	Kooko and bread	Kenkey and stew	Fufu and soup
20	No	None	None	Tea and Bread	Kenkey and stew	Fufu and soup
21	Yes	None	None	Tea and Bread	Fufu and soup	Rice and stew
22	Yes	None	Malaria	Kooko and bread	Rice and stew	Fufu and soup
23	Yes	None	None	Rice and stew	Fufu and soup	Tea and bread
24	Yes	Candidiases	Candidiases	Kooko and bread	Kenkey and stew	Fufu and soup
25	No	None	None	Kooko and bread	Rice and stew	Fufu and soup
26	Yes	None	None	Tea and Bread	Rice and stew	Fufu and soup
27	Yes	None	None	Fufu and soup	Fufu and soup	Tea and bread

Table 18: Description of the babies' age and other data are taken

	Age Of Infants	Baby's Weight	Is The Baby On Exclusive Breast Feeding?	What Do You Regularly Use To Feed Your Infant?	How Many Times Does The Infant Feed In A Day?	Have The Baby Fallen Sick In The Past 6 Months?
1	4-5 months	7-10kg	Yes	None	Regular	No
2	2-3 months	4-6kg	Yes	None	Regular	No
3	4-5 months	7-10kg	Yes	None	Regular	No
4	3-4 months	4-6kg	Yes	None	Regular	No
5	1-2 months	1-5kg	Yes	None	Regular	No
6	6 months	7-10kg	Yes	None	Regular	No
7	2-3 months	1-5kg	No	Lactogen	Regular	No
8	4-5 months	4-6kg	Yes	None	Regular	No
9	3-4 months	7-10kg	Yes	None	Regular	No
10	4-5 months	4-6kg	Yes	None	Regular	No
11	1-2 months	1-5kg	Yes	None	Regular	No
12	3-4 months	4-6kg	Yes	None	Regular	No
13	6 months	7-10kg	No	Lactogen	Regular	No
14	3-4 months	4-6kg	Yes	None	Regular	No
15	3-4 months	4-6kg	Yes	None	Regular	No
16	1-2 months	1-5kg	Yes	None	Regular	No
17	1-2 months	1-5kg	Yes	None	Regular	No
18	3-4 months	7-10kg	No	None	Regular	No
19	1-2 months	4-6kg	Yes	None	Regular	No
20	1-2 months	1-5kg	Yes	None	Regular	No
21	1-2 months	1-5kg	Yes	None	Regular	No
22	1-2 months	1-5kg	Yes	None	Regular	No
23	1-2 months	1-5kg	Yes	None	Regular	No
24	1-2 months	1-5kg	Yes	None	Regular	No
25	1-2 months	1-5kg	Yes	None	Regular	No
26	1-2 months	1-5kg	Yes	None	Regular	No
27	1-2 months	1-5kg	Yes	None	Regular	No

Table 19: Breast Milk Concentrations of Baby Mothers (mg/kg)

Women	Sodium (Na)	Magnesium (Mg)	Potassium (K)	Calcium (Ca)	Manganese (Mn)	Copper (Cu)	Iodine (I)
1a	439.4000	227.6000	1722.0000	536.9000	0.4059	0.2235	0.0290
2b	682.4000	262.3000	1721.0000	654.1000	0.3303	0.6048	0.0360
3c	1007.0000	279.7000	1850.0000	703.4000	0.2699	0.6948	0.0575
4d	1381.0000	232.1000	2427.0000	543.6000	0.2145	0.6682	0.0191
5e	1033.0000	382.7000	2219.0000	762.2000	0.4744	0.6490	0.1445
6f	367.9000	164.0000	1839.0000	462.0000	0.2946	0.2606	0.0449
7g	1321.0000	373.7000	2437.0000	748.0000	0.4827	0.7944	0.0455
8h	833.7000	333.6000	1542.0000	669.6000	0.4973	0.7250	0.0396
9i	760.4000	333.4000	2194.0000	786.5000	0.5060	0.5543	0.0343
10j	323.0000	132.4000	5225.0000	408.1000	0.2329	0.5990	0.0320
11k	971.8000	259.5000	2497.0000	584.9000	0.2870	0.7809	0.0440
12l	403.8000	271.9000	1720.0000	662.3000	0.0018	0.3750	0.5133
13m	294.4000	201.9000	2048.0000	520.0000	0.3323	0.5840	0.0315
14n	518.0000	235.2000	2628.0000	562.6000	0.3694	0.4249	0.0302
15o	1155.0000	285.6000	2602.0000	757.3000	0.4040	0.8550	0.0302
16p	629.8000	330.0000	2028.0000	598.0000	0.4967	0.6101	0.0344
17q	1674.0000	319.4000	1359.0000	689.7000	0.4137	0.8310	0.0410
18r	1110.0000	361.1000	2333.0000	809.8000	0.5739	0.7606	0.0393
19s	2797.0000	264.6000	2470.0000	837.8000	0.2546	1.0199	0.0520
20t	747.7000	305.0000	2564.0000	872.3000	0.4092	0.7340	0.0380
21u	1881.0000	385.4000	1866.0000	799.2000	0.4644	0.9081	0.0464
22v	2108.0000	343.4000	4458.0000	599.9000	0.3614	1.0181	0.0452
23w	1859.0000	733.0000	1748.0000	1497.0000	1.1150	1.0113	0.0532
24x	1973.0000	426.7000	2081.0000	837.0000	0.4085	0.8290	0.0460
25y	789.2000	269.6000	3995.0000	710.7000	0.2510	0.6709	0.0398
26z	1643.0000	236.4000	3507.0000	492.5000	0.3940	0.8094	0.0477
27zz	959.4000	399.5000	2748.0000	724.5000	0.6295	0.7517	0.0400

APPENDIX F

MEASURED MEAN CONCENTRATIONS OF THE NINE ELEMENTS AT THE FIVE RICE FARMS

Table 20: **Measured mean concentrations of the nine elements at the five Rice farms in mg/kg**

Elements	Afienya Farms	Afife Farms	Dawhenya Farms	Ashaiman Farms	Aveyime Farms
Na	153.4000	167.6000	142.3000	150.3000	188.1000
Mg	759.3000	483.2000	875.7000	719.5000	816.6000
Cl	492.0000	718.0000	465.6000	636.8000	618.1000
K	875.2000	2281.0000	514.6000	1438.7057	2949.0000
Ca	2541.0000	2303.0000	2455.0000	2622.0000	2552.0000
V	0.0698	0.1091	0.1023	0.1020	0.1925
Mn	9.9560	14.4600	14.3500	11.4700	13.1300
Cu	0.8728	1.6790	1.3440	1.6420	0.8771
I	0.1211	0.1181	0.1277	0.1447	0.1351

APPENDIX G

RECOMMENDED DIETARY INTAKES

Table 21: Recommended Dietary Allowable (RDA) / Adequate Intake (AI) & Maximum Upper Limit (UL) for Br, Cl and I (Food and Nutrition Board, 2001: Dietary Reference Intakes, 2013)

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

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 (Dietary Reference Intakes, 2013).

Table 22: Recommended Dietary Allowable (RDA) and Adequate Intake (AI) for Na, Mg, K, Ca, V, Mn, and Cu for the Life Stage Groups (Food and Nutrition Board, 2001; Institute of Medicine, 2001)

		Na	Mg	K	Ca	V	Mn	Cu
Life Stage Group		*RDA/AI* (mg/d)	*RDA/AI* (mg/d)	*AI* (mg/d)	*RDA/AI* (mg/d)	*AI* (mg/d)	*AI* (mg/d)	*RDA/AI* (mg/d)
Infants	0-6months	120	30	400	200	ND	0.003	0.2
	7-12months	370	75	860	260	ND	0.6	0.22
Children	1-3y	1000	80	2000	700	ND	1.2	0.34
	4-8y	1200	130	2300	1000	ND	1.5	0.44
Males	9-13y	1500	240	2500	1300	ND	1.9	0.7
	14-18y	1500	410	3000	1300	ND	2.2	0.89
	19-30y	1500	400	3400	1000	ND	2.3	0.9
	31-50y	1500	420	3400	1000	ND	2.3	0.9
	50-70y	1300	420	3400	1000	ND	2.3	0.9
	>70y	1200	420	3400	1200	ND	2.3	0.9
Females	9-13y	1500	240	2300	1300	ND	1.6	0.7
	14-18y	1500	410	2300	1300	ND	1.6	0.89
	19-30y	1500	400	2600	1000	ND	1.8	0.9
	31-50y	1500	420	2600	1000	ND	1.8	0.9
	50-70y	1300	420	2600	1200	ND	1.8	0.9
	>70y	1200	420	2600	1200	ND	1.8	0.9
Pregnant	≤ 18y	1500	400	2600	1300	ND	2.0	1
Women	19-30y	1500	350	2900	1000	ND	2.0	1
	31-50y	1500	360	2900	1000	ND	2.0	1
Lactation	≤ 18y	1500	360	2500	1300	ND	2.6	1.3
Women	19-30y	1500	310	2800	1000	ND	2.6	1.3
	31-50y	1500	320	2800	1000	ND	2.6	1.3

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 (Dietary Reference Intakes, 2013).

Table 23: Maximum Upper Limit (UL) of Recommended Dietary Allowable (RDA) and Adequate Intake (AI) for Na, Mg, K, Ca, V, Mn and Cu for the Life Stage Groups (Food and Nutrition Board, 2001; Institute of Medicine, 2001)

Life Stage Group		Na *RDA/AI* (mg/d)	[b]Mg *RDA/AI* (mg/d)	[d]K *AI* (mg/d)	Ca *RDA/AI* (mg/d)	[d]V *AI* (mg/d)	Mn *AI* (mg/d)	Cu *RDA/AI* (mg/d)
Infants	0-6months	ND	ND	400	1000	ND	ND	ND
	7-12months	ND	ND	700	1500	ND	ND	ND
Children	1-3y	1500	65	3000	2500	ND	2	1
	4-8y	1900	110	3800	2500	ND	3	3
Males	9-13y	2200	350	4500	3000	ND	6	5
	14-18y	2300	350	4700	3000	ND	9	8
	19-30y	2300	350	4700	2500	1.8	9	10
	31-50y	2300	350	4700	2500	1.8	9	10
	50-70y	2300	350	4700	2000	1.8	9	10
	>70y	2300	350	4700	2000	1.8	9	10
Females	9-13y	2200	350	4500	3000	ND	6	5
	14-18y	2300	350	4700	3000	ND	9	8
	19-30y	2300	350	4700	2500	1.8	9	10
	31-50y	2300	350	4700	2500	1.8	9	10
	50-70y	2300	350	4700	2000	1.8	9	10
	>70y	2300	350	4700	2000	1.8	9	10
Pregnant	≤18y	2300	350	4700	3000	ND	9	8
Women	19-30y	2300	350	4700	2500	ND	11	10
	31-50y	2300	350	4700	2500	ND	11	10
Lactation	≤18y	2300	350	5100	3000	ND	9	8
Women	19-30y	2300	350	5100	2500	ND	11	10
	31-50y	2300	350	5100	2500	ND	11	10

Note:

[b]The UL for magnesium represents intake from a pharmacological agent only and does not include intake from food and water.

[d]Although vanadium in food has not been shown to cause adverse effects in humans, there is no justification for adding it to food. Its supplements should be used with caution. The UL is based on adverse effects in laboratory animals and this data could be used to set a UL for adults but not children or adolescents.

APPENDIX H

DIFFERENCES BETWEEN THE RECOMMENDED AND CALCULATED DIETARY INTAKES

Table 24: Differences between the RDA / AI and the Calculated Means for the Life Stage Groups

	Na	Mg	Cl	K	Ca	V	Mn	Cu	I
Life Stage Group	*RDA/AI* (mg/d)	*RDA/AI* (mg/d)	*RDA/AI* (mg/d)	*AI* (mg/d)	*RDA/AI* (mg/d)	*AI* (mg/d)	*AI* (mg/d)	*RDA/AI* (mg/d)	*RDA/AI* (mg/d)
Infants 0-6months	-104.625	**+40.077**	-179.904	**+652193.484**	**+39.208**	ND	**+1.212**	-0.077	-0.098
7-12months	-354.625	-4.923	-569.904	**+651733.484**	-20.792	ND	**+0.615**	-0.097	-0.118
Children 1-3y	-984.625	-9.923	-1499.9	**+650593.484**	-460.792	ND	**+0.015**	-0.217	-0.078
4-8y	-1184.63	-59.923	-1899.9	**+650293.484**	-760.792	ND	-0.285	-0.317	-0.078
Males 9-13y	-1484.63	-169.923	-2299.9	**+650093.484**	-1060.79	ND	-0.685	-0.577	-0.108
14-18y	-1484.63	-339.923	-2299.9	**+649593.484**	-1060.79	ND	-0.985	-0.767	-0.138
19-30y	-1484.63	-329.923	-2299.9	**+649193.484**	-760.792	ND	-1.085	-0.777	-0.138
31-50y	-1484.63	-349.923	-2299.9	**+649193.484**	-760.792	ND	-1.085	-0.777	-0.138
50-70y	-1284.63	-349.923	-1999.9	**+649193.484**	-760.792	ND	-1.085	-0.777	-0.138
>70y	-1184.63	-349.923	-1799.9	**+649193.484**	-960.792	ND	-1.085	-0.777	-0.138
Females 9-13y	-1484.63	-169.923	-2299.9	**+650293.484**	-1060.79	ND	-0.385	-0.577	-0.108
14-18y	-1484.63	-339.923	-2299.9	**+650293.484**	-1060.79	ND	-0.385	-0.767	-0.138
19-30y	-1484.63	-329.923	-2299.9	**+649993.484**	-760.792	ND	-0.585	-0.777	-0.138
31-50y	-1484.63	-349.923	-2299.9	**+649993.484**	-760.792	ND	-0.585	-0.777	-0.138
50-70y	-1284.63	-349.923	-1999.9	**+649993.484**	-960.792	ND	-0.585	-0.777	-0.138
>70y	-1184.63	-349.923	-1799.9	**+649993.484**	-960.792	ND	-0.585	-0.777	-0.138
Pregnant ≤ 18y	-1484.63	-329.923	-2299.9	**+649993.484**	-1060.79	ND	-0.785	-0.877	-0.208
Women 19-30y	-1484.63	-279.923	-2299.9	**+649693.484**	-760.792	ND	-0.785	-0.877	-0.208
31-50y	-1484.63	-289.923	-2299.9	**+649693.484**	-760.792	ND	-0.785	-0.877	-0.208
Lactation ≤ 18y	-1484.63	-289.923	-2299.9	**+650093.484**	-1060.79	ND	-1.385	-1.177	-0.278
Women 19-30y	-1484.63	-239.923	-2299.9	**+649793.484**	-760.792	ND	-1.385	-1.177	-0.278
31-50y	-1484.63	-249.923	-2299.9	**+649793.484**	-760.792	ND	-1.385	-1.177	-0.278

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 (Dietary Reference Intakes, 2013).

Table 25: Differences between the UL of the RDA / AI and the Calculated Means for the Life Stage Groups

Life Stage Group	Na *RDA/AI* (mg/d)	[b]Mg *RDA/AI* (mg/d)	Cl *RDA/AI* (mg/d)	[d]K *AI* (mg/d)	Ca *RDA/AI* (mg/d)	[d]V *AI* (mg/d)	Mn *AI* (mg/d)	Cu *RDA/AI* (mg/d)	I *RDA/AI* (mg/d)
Infants 0-6months	ND	ND	ND	ND	-760.79	ND	ND	ND	ND
7-12months	ND	ND	ND	ND	-1260.79	ND	ND	ND	ND
Children 1-3y	-1484.63	**5.077**	-2299.9	ND	-2260.79	ND	-0.785	-0.877	-0.188
4-8y	-1884.63	-39.923	-2899.9	ND	-2260.79	ND	-1.785	-2.877	-0.288
Males 9-13y	-2184.63	-279.923	-3399.9	ND	-2760.79	ND	-4.785	-4.877	-0.588
14-18y	-2284.63	-279.923	-3599.9	ND	-2760.79	ND	-7.785	-7.877	-0.888
19-30y	-2284.63	-279.923	-3599.9	ND	-2260.79	-1.79	-9.785	-9.877	-1.088
31-50y	-2284.63	-279.923	-3599.9	ND	-2260.79	-1.79	-9.785	-9.877	-1.088
50-70y	-2284.63	-279.923	-3599.9	ND	-1760.79	-1.79	-9.785	-9.877	-1.088
>70y	-2284.63	-279.923	-3599.9	ND	-1760.79	-1.79	-9.785	-9.877	-1.088
Females 9-13y	-2184.63	-279.923	-3399.9	ND	-2760.79	**+0.011**	-4.785	-4.877	-0.588
14-18y	-2284.63	-279.923	-3599.9	ND	-2760.79	**+0.011**	-7.785	-7.877	-0.888
19-30y	-2284.63	-279.923	-3599.9	ND	-2260.79	-1.789	-9.785	-9.877	-1.088
31-50y	-2284.63	-279.923	-3599.9	ND	-2260.79	-1.789	-9.785	-9.877	-1.088
50-70y	-2284.63	-279.923	-3599.9	ND	-1760.79	-1.789	-9.785	-9.877	-1.088
>70y	-2284.63	-279.923	-3599.9	ND	-1760.79	-1.789	-9.785	-9.877	-1.088
Pregnant ≤ 18y	-2284.63	-279.923	-3599.9	ND	-2760.79	ND	-7.785	-7.877	-0.888
Women 19-30y	-2284.63	-279.923	-3599.9	ND	-2260.79	ND	-9.785	-9.877	-1.088
31-50y	-2284.63	-279.923	-3599.9	ND	-2260.79	ND	-9.785	-9.877	-1.088
Lactation ≤ 18y	-2284.63	-279.923	-3599.9	ND	-2760.79	ND	-7.785	-7.877	-0.888
Women 19-30y	-2284.63	-279.923	-3599.9	ND	-2260.79	ND	-9.785	-9.877	-1.088
31-50y	-2284.63	-279.923	-3599.9	ND	-2260.79	ND	-9.785	-9.877	-1.088

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

The sign (y) denotes years

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 (Dietary Reference Intakes, 2013).

Table 26: Health Risk Estimates Associated with the Rice collected from the 5 Farms for the Life Stage Groups

		Hazard Index								
Life Stage Group		Na	Mg	Cl	K	Ca	^{d}V	Mn	Cu	I
Infants	0-6 months	ND	ND	ND	ND	0.24	ND	ND	ND	ND
	7-12 months	ND	ND	ND	ND	0.16	ND	ND	ND	ND
Children	1-3 years	0.013	**1.078**	4.17E-05	ND	0.10	ND	0.608	0.123	0.06
	4-8 years	0.008	0.637	3.31E-05	ND	0.10	ND	0.405	0.041	0.04
Males	9-13 years	0.007	0.200	2.82E-05	ND	0.08	ND	0.203	0.025	0.02
	14-18 years	0.007	0.200	2.67E-05	ND	0.08	ND	0.135	0.015	0.01
	19-30 years	0.007	0.200	2.67E-05	ND	0.10	0.006	0.110	0.012	0.01
	31-50 years	0.007	0.200	2.67E-05	ND	0.10	0.006	0.110	0.012	0.01
	50-70 years	0.007	0.200	2.67E-05	ND	0.12	0.006	0.110	0.012	0.01
	>70 years	0.007	0.200	2.67E-05	ND	0.12	0.006	0.110	0.012	0.01
Females	9-13 years	0.007	0.200	2.82E-05	ND	0.08	ND	0.203	0.025	0.02
	14-18 years	0.007	0.200	2.67E-05	ND	0.08	ND	0.135	0.015	0.01
	19-30 years	0.007	0.200	2.67E-05	ND	0.10	0.006	0.110	0.012	0.01
	31-50 years	0.007	0.200	2.67E-05	ND	0.10	0.006	0.110	0.012	0.01
	50-70 years	0.007	0.200	2.67E-05	ND	0.12	0.006	0.110	0.012	0.01
	>70 years	0.007	0.200	2.67E-05	ND	0.12	0.006	0.110	0.012	0.01
Pregnant Women	≤ 18 years	0.007	0.200	2.67E-05	ND	0.08	ND	0.135	0.015	0.01
	19-30 years	0.007	0.200	2.67E-05	ND	0.10	ND	0.110	0.012	0.01
	31-50 years	0.007	0.200	2.67E-05	ND	0.10	ND	0.110	0.012	0.01
Lactation Women	≤ 18 years	0.007	0.200	2.67E-05	ND	0.08	ND	0.135	0.015	0.01
	19-30 years	0.007	0.200	2.67E-05	ND	0.10	ND	0.110	0.012	0.01
	31-50 years	0.007	0.200	2.67E-05	ND	0.10	ND	0.110	0.012	0.01

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 (Dietary Reference Intakes, 2013).

APPENDIX I

FUNCTIONS AND EFFECTS OF MICRONUTRIENTS

Table 27: Functions and Effects of the Ten micronutrients on the Human Body

Element	Function	Adverse Effect of Deficiency	Adverse Effect of Excessive Consumption
Na	Major ion of the extra cellular fluid. Aids nerve impulse transmission.	Hyponatremia; similar symptoms caused by dehydration, and in severe cases the brain may swell and lead to headaches, seizures, coma, and even death	High blood pressure in susceptible people, can lead to an increase in loss of calcium in the urine and increases the amount of water the body, thus causing the swelling of the legs and hands
	(Health supplements nutritional guide, 2017; The National Academies, 2005; Palsdottir, 2016)		
Mg	Maintains normal nerve and muscle function, supports a healthy immune system, keeps heart beat steady, and helps in strong bones, help regulate blood glucose levels and aids in the production of energy and protein.	Anorexia, confusion, delirium, rapid heartbeat, hallucinations, fatigue, insomnia, irritability, muscle twitching, numbness, poor memory, continued muscle contraction	Diarrhoea, abdominal cramping, nausea, stomach upset, vomiting, affects the cardiovascular system, muscle weakness and difficulty in breathing, affects the balance of other minerals in the body, changes in mental status
	(Busse, 2015; National Health and Medical Research Council [NHRMC], 2015)		
Cl	Maintains proper blood volume and pressure, keeps the amount of fluid in and around cells in balance, a critical component of stomach hydrochloric acid, conserves potassium in the body	Heavy sweating, congestive heart failure, over-hydration, Addison's disease, certain kidney disorders, 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.
	(Nutritional Health Resource, 2017)		
K	Help regulate fluid balance, blood pressure, and water retention, nerve signals, and muscle contractions, protect against stroke and prevent osteoporosis and kidney stones.	Muscle weakness	Tiredness or weakness, a feeling of numbness or tingling, nausea or vomiting, trouble breathing, chest pain, palpitations or irregular heartbeats.
	(Raman, 2017)		
Ca	Regulation of heartbeat and blood clotting, building and maintaining bones and teeth, helps body enzymes, help protect against over 10 different types of cancer, the most common being prostate, breast, lung and colorectal, helps in the release of neurotransmitters and contraction of muscles	Fainting, heart failure, brittle nails, chest pains, numbness and tingling sensations around the mouth, toes, and fingers, wheezing, coarse hair, muscle cramps and weakness, bone fractures and tooth decay, fatigue, cataracts, impaired intellectual, dry skin, capacity, seizures, depression, chronic itching, irritability / anxiety	Nausea, vomiting, constipation, muscle weakness, bone pain increased thirst or urination
	(Sharecare, 2019; University Health News, 2019, Davis, 2019)		

Element	Function	Adverse Effect of Deficiency	Adverse Effect of Excessive Consumption
Br	Has anti-seizure properties, effective 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.
	(Acu-Cell Nutrition, 2017)		
V	Controls blood sugar levels, retards tumor growth.	Kidney and heart disease, low blood sugar levels.	Elevation in blood pressure, abdominal cramps, diarrhoea.
	(Murray, 2005; Tracey et al., 2007)		
Mn	Helps bone formation and the action of some enzymes such as those involved in carbohydrate metabolism	Obesity, birth defects, changes of hair colour, abnormal bone function and cartilage, growth retardation.	Neurotoxicity and elevated blood concentration, high incidence of pneumonia and other upper respiratory infections
	(Lenntech, 2019)		
Cu	Fixes calcium in the bones and to build and repair all connective tissue (tendons, ligaments, skin, hair, nails, arteries, veins).	Low libido in women and. Increases the lipid peroxidation in the heart in 2-folds.	Vomiting, nausea, sleep disorder, abdominal pain, homosexual desire, weakness, menstrual cramps, and a metallic taste in the mouth, liver and kidney damage.
	(Nolan, 1983)		
I	Utilized by the thyroid gland for the biosynthesis of the thyroid hormones since every cell in the body depends upon thyroid hormones for its metabolism.	Abortions, Goitre, Stillbirths, Infant Mortality, Mental deficiency, anger, obesity, dwarfism, Congenital anomalies, Psychomotor defects, hypothyroidism.	Thyroid gland inflammation, same symptoms as iodine deficiency, including goitre (an enlarged thyroid gland) and thyroid cancer.
	(Koutras, *et. al.*, 1985; Delange and Burgi, 1999; U.S. Department of Health & Human Services, 2016)		

APPENDIX J

EQUATIONS OF CYCLIC ACTIVATION ANALYSIS

This technique is based on the concept of enhancing the sensitivity of the activation method for the determination of elements with short-lived indication radionuclides. Givens et al in 1969 and Spyrou in 1981 have described the principle of CAA. In this method, the sample is irradiated for a short period of time, and after a delayed period from the end of irradiation the radiation emitted is counted for a short period of time, then the samples is irradiated again and the entire process repeated for a number of cycles (Figure 30).

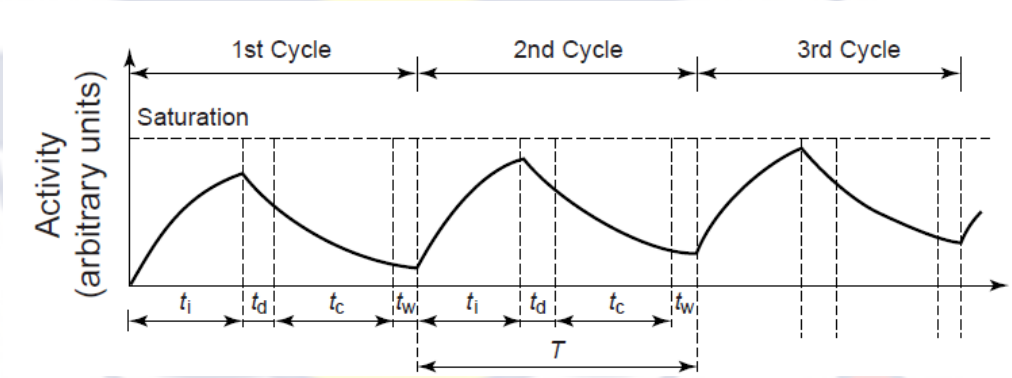

Figure 30: Time parameters of CAA and variation of the activity with time and number of cycles

Frequency, f of detection is given by $1 / T$ [23]

The detected radiation at each counting period is summed and finally a total cumulative detector response is obtained. The cycle period T is given by equation (24)

$$T = t_i + t_d + t_c + t_w \quad [24]$$

Where t_i is the time of irradiation, t_d is the delayed time, i.e. the time between the end of irradiation and the start of counting, which is usually the time required to transfer the sample from the irradiation position to the counting station, t_c is the counting time, and t_w is the waiting time, i.e. the time between the end of counting and the start of irradiation.

The number of count or detector response for the first cycle is given by equation (25)

$$D_1 = \frac{N\sigma\Phi I\varepsilon}{\lambda}\left(1-e^{-\lambda t_i}\right)e^{-\lambda t_d}\left(1-e^{-\lambda t_c}\right) \quad [25]$$

Where

N = number of stable target nuclei of interest in the sample.

Φ = flux density of incident particles (n cm^{-2} s^{-1} in NAA)

λ = the decay constant for the indicator radionuclide (s^{-1})

T = the delay period from the end of a previous delay periods to the end of the next delay period (cycle time, s)

n = the number of cycles.

σ = neutron cross section

I = intensity of the radiation of interest (intensity of γ-ray)

ε = efficiency of the detector

In the second counting period, the detector response or the number of count is the same number of counts due to the second irradiation plus what was left from the first irradiation, and it is given by equation (26)

$$D_2 = D_1 + D_1e^{-\lambda T} = D_1\left(1+e^{-\lambda T}\right) \quad [26]$$

Similarly, for the nth cycle, which can be expressed by equation (27)

$$D_n = D_1\left(1+e^{-\lambda T}+e^{-2\lambda T}+e^{-3\lambda T}+e^{-4\lambda T}+\ldots+e^{-(n-1)\lambda T}\right) \quad [27]$$

The total cumulative detector response or the number of count in all the n cycles is given by equation (28)

$$D_C = \sum_{i=1}^{n} D_i$$

$$= D_1 \frac{n}{\left(1-e^{-\lambda T}\right)} - \frac{e^{-\lambda T}(1-e^{-n\lambda T})}{(1-e^{-\lambda T})^2}$$

$$= \frac{N\sigma\Phi I\varepsilon}{\lambda}(1-e^{-\lambda t_i})e^{-\lambda t_d}(1-e^{-\lambda t_c})\{\frac{n}{(1-e^{-\lambda T})} - \frac{e^{-\lambda T}(1-e^{-n\lambda T})}{(1-e^{-\lambda T})^2}\} \quad [28]$$

Equation (28) is the basic relationship for CAA

Selection of Time Parameters

The detector response may be maximized by the proper selection of the parameters in equation (28). For a given total experiment time, $T(T_t = nT = mt_{1/2})$ the maximum value of D_c occurs when $t_d=t_w=0$ and $t_i=t_c$. However, the fact is that t_d and t_w are not zero and their values ultimately depend on the transfer system used. When the transfer is done manually, the transfer time can be relatively long, in the range of $10s^{-1}$ min. in this case; the method is sometimes referred to as pseudo-CAA (Chattopadhyay and DeSilva, 1979; DeSilva, and Chatt, 1982-1983; and Ismail, *et. al.*, 1998).

In some fast transfer systems, electronically and by computer control, the transfer time is of the order of 0.1s and less (Fanger *et. al*, 1981 and Egan and Spyrou, 1976). The transfer time can be made very short when there is no physical transfer of the sample but a pulsed irradiation is used, which was suggested as "real" CAA (Caldwell et al, 1966 and Givens et al, 1969).

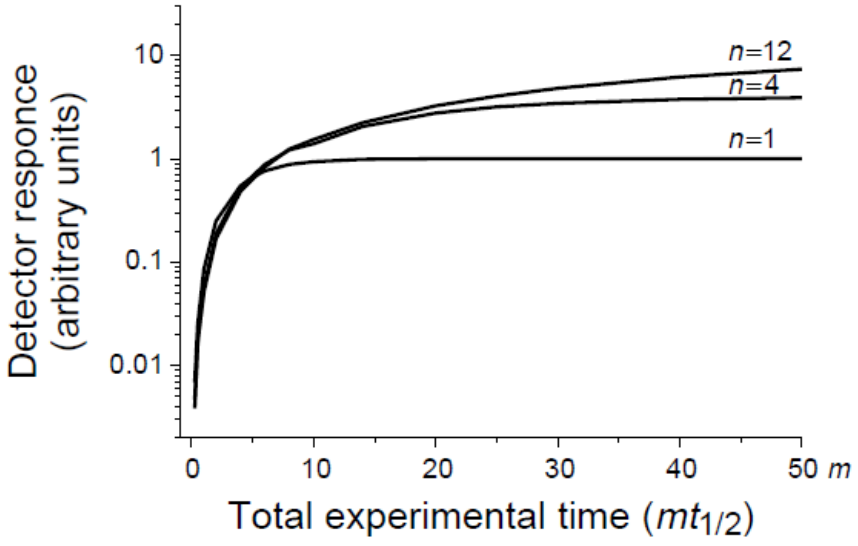

Figure 31: Variation of the conventional and cumulative signals with total with experiment time, $t_d = t_w = 0, t_i = t_c$ and n is the number of cycles.

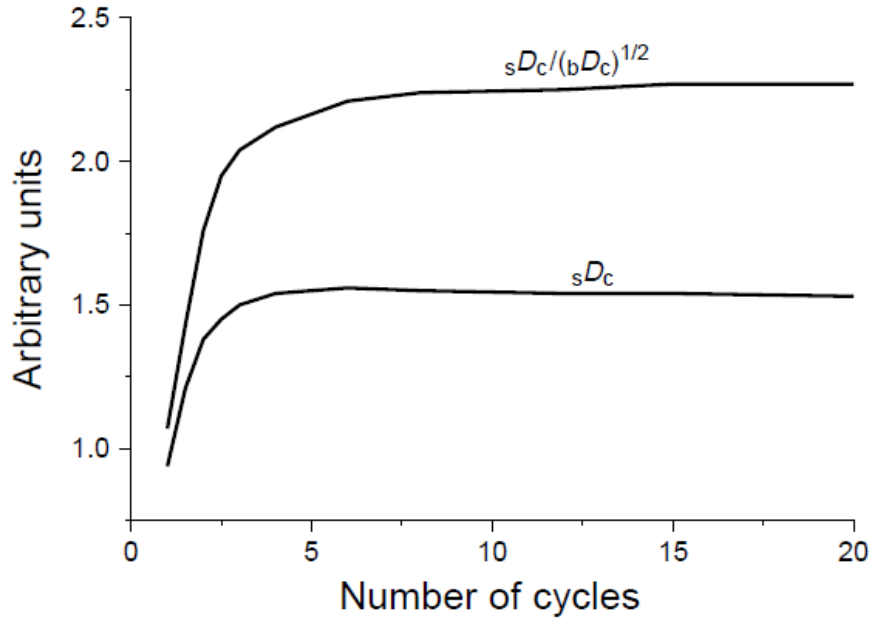

Figure 32: Variation of signal and signal-to-noise ratio $({}_sD_c/\sqrt{{}_bD_c})$ for cyclic and conventional activation with the number of cycles. [Here the underlying background to the signal of interest is provided by the activity of a nuclide with a half-life equal to 10 times the half-life of the nuclide of interest $(T_{1/2} = 10t_{1/2})$]

Figure 32 shows the variation of the detector response for the nuclide of interest with the total experimental time $(T_t = mt_{1/2} = nT)$ in the

conventional and cyclic cases with different cyclic numbers. It is clear that below a certain experimental time, conventional activation is to be preferred to cyclic activation, and the cross-over point of curves between conventional and cyclic activation shifts to a longer experimental time with an increasing of number of cycles. After the cross-over point, cyclic activation has a higher detector response than that of conventional one-shot irradiation.

For a certain total experiment time, such as $T_t = 10t_{1/2}$, the cumulative detector response increases with increasing number of cycles in the beginning, and there is an optimum number of cycles after which the detector response does not increase and slowly decreases (figure 32). In addition, for conventional single-shot activation analysis, the detector response increases quickly with increase in the total experiment time from the start. Saturation activity of 95% is obtained before $5t_{1/2}$, after then a further increase in the total experiment time become longer. For n=4, 95% of saturation activity is obtained after $15t_{1/2}$ and for n=12 after a total experiment time of $50t_{1/2}$ the activity still increases with increasing total experiment time.

However, in CAA, the radionuclides produced from elements in the matrix which have a longer half-life than the nuclide of interest will not decay, or will decay slowly between irradiations, so the underlying background will increase through successive irradiation. Consequently, the detection of an element does not depend solely on the signal from the nuclide of interest and must take into account the background contribution from the matrix elements.

The purpose of CAA is to improve the analytical precision and the detection limit for the element of interest. The analytical precision is usually

expressed by the relative standard deviation of a signal S measured above a background B as shown in equation (29)

$$\delta = \frac{\sqrt{S + 2B}}{S} \quad [29]$$

For the cumulative spectrum of n cycles, it is expressed as equation (30)

$$\delta_n = \frac{\sqrt{{}_sD_c + 2{}_bD_c}}{{}_sD_c} \quad [30]$$

Where δ_n is the cumulative detector response for the nuclide of interest and ${}_bD_c$ is the cumulative detector response for the nuclide making the major background contribution to the signal from the nuclide of interest.

The detection limit l_d is usually given by equation (31)

$$l_d = 3\sqrt{B}\ (\text{counts}) = \frac{3\sqrt{B}}{S/m} = \frac{3m\sqrt{B}}{S}(\mu g) \quad [31]$$

Where S/*m* is the counts per unit mass of the element of interest.

For CAA with n cycles, the detection limit is given by equation (32)

$$L_d = \frac{3\sqrt{{}_bD_c}}{{}_sD_c/m} = \frac{3m\sqrt{{}_bD_c}}{{}_sD_c} \quad [32]$$

Therefore, $\sqrt{{}_bD_c}/{}_sD_c$ or ${}_sD_c/\sqrt{{}_bD_c}$ and $\sqrt{{}_sD_c + 2{}_bD_c}/{}_sD_c$ or ${}_sD_c/\sqrt{{}_sD_c + 2{}_bD_c}$ but not the detector response for the nuclide of interest, must be optimized, when the most suitable cyclic timing parameters are chosen for a nuclide.

In figures 33 and 34 the signal-to noise ratio, ${}_sD_c/\sqrt{{}_bD_c}$, and the ratio of the signal to the square root of the sum of ${}_sD_c$ and

2_bD_c, ${}_sD_c/\sqrt{{}_sD_c+2_bD_c}$ are plotted versus total experiment time ($mt_{1/2}$) for comparison between cyclic and conventional activation analysis. In this case, for illustrative purposes, only one long-lived nuclide with a half-life ($T_{1/2}$) of 10 times the half-life of the nuclide of interest ($T_{1/2}=10t_{1/2}$) is considered to contribute to the underlying background of the signal. It can be seen that the background plays a significant role in emphasizing the difference between the conventional and cyclic case. It is different with the variation of detection response; with increase in the total experiment time, the expressions ${}_sD_c/\sqrt{{}_bD_c}$ and ${}_sD_c/\sqrt{{}_sD_c+2_bD_c}$ has a maximum and this maximum shifts to a longer experiment time with an increasing number of cycles. Compare with figure 32, the cross-over point between conventional and cyclic activation in figure 33 is shifted to a lower value of *m*, that is, cyclic activation becomes advantageous for shorter total experiment times than the case just considering the detector response.

Figure 35 shows the variation of signal-to noise ratios with the total experimental time (number of cycles) under a certain cycle period ($T=2t_{1/2}$). It is clear that in this case, signal-to –noise ratio increases always with increasing total experimental time.

To obtain a better cyclic advantage factor over conventional activation, it is obvious that one must increase the total experiment time ($mt_{1/2}$). The choice of the best total experiment time for cyclic activation is therefore a compromise between the time available for sample analysis and the detection

limit to be achieved. Whenever the total experiment time is chosen, the number of cycle (*n*) or cycle period, $T = T_{t/n}$ has to be decided.

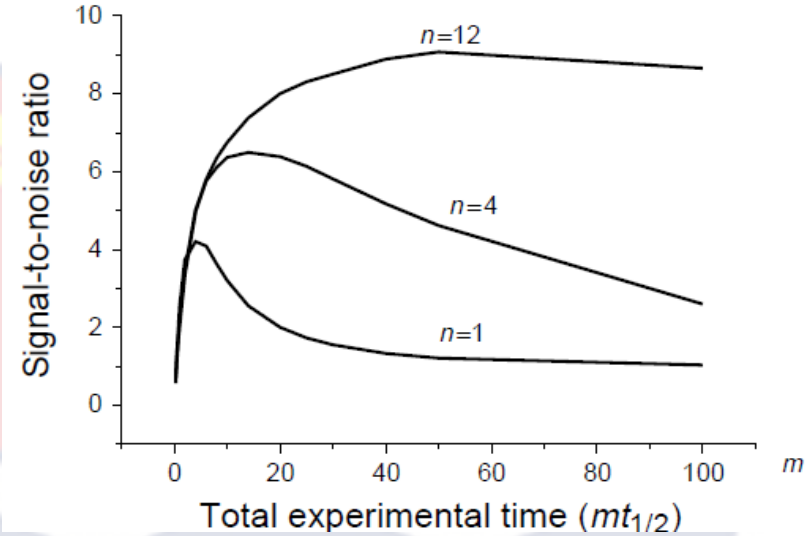

Figure 33: Variation of single-to-noise ratio $({}_sD_c/\sqrt{{}_bD_c})$ for cyclic and conventional activation with a total experimental time $(T_{1/2} = 10t_{1/2})$, m=10, $t_d = t_w = 0, t_i = t_c$.

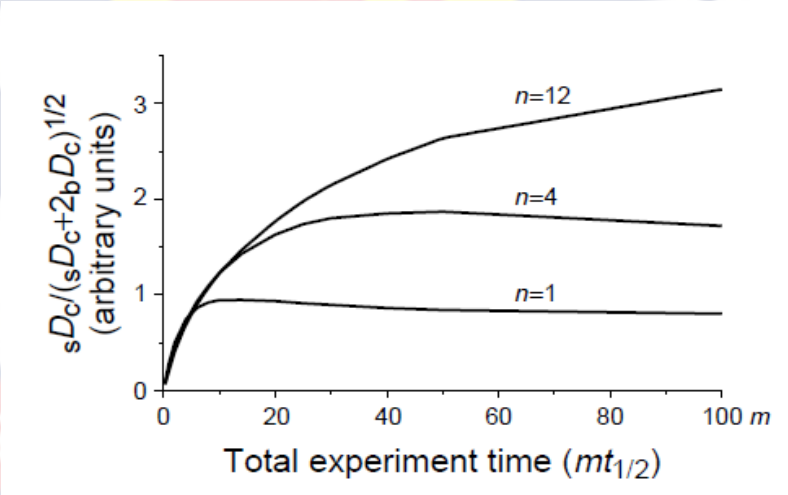

Figure 34: Variation of $({}_sD_c/\sqrt{{}_sD_c + 2{}_bD_c})$ for cyclic and conventional activation with the total experiment time $(T_{1/2} = 10t_{1/2})$.

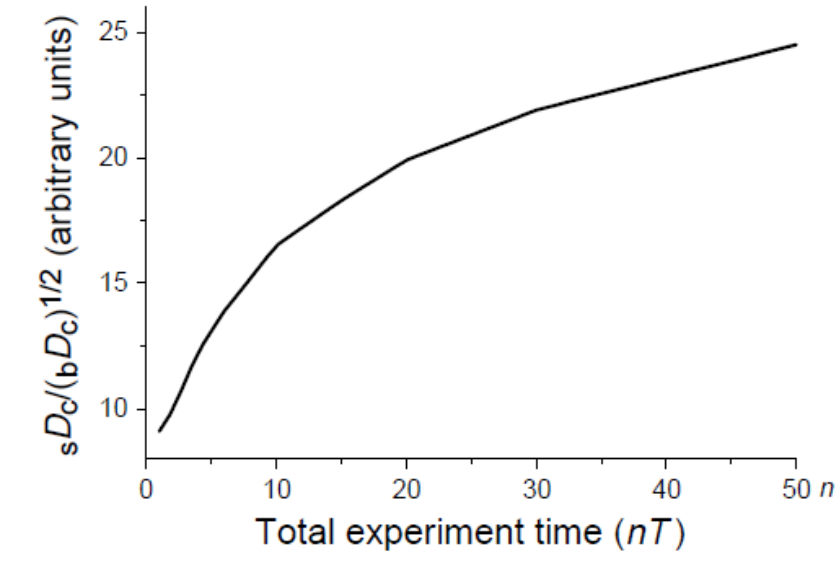

Figure 34: Variation of signal-to–noise $({}_sD_c/\sqrt{{}_bD_c})$ with the total experiment time (nT) for a certain cyclic period $(T = 2t_{1/2})$. $t_i = t_c = t_{1/2}, t_d = t_w = 0$.

In figure 32, the variation of the signal $({}_sD_c)$ and the signal-to-noise ratio ${}_sD_c/\sqrt{{}_bD_c}$ was plotted as a function of the number of cycles $(n = T_t/T)$. it can be seen that after a certain cycle, ${}_sD_c/\sqrt{{}_bD_c}$ and ${}_sD_c$ increase very slowly, and with further increase in the number of cycles become insignificant. After a maximum occurs, ${}_sD_c$ decrease slowly. For example, if $T = 10t_{1/2}$ and $T_{1/2} = 10t_{1/2}$, 95% of the saturation activity of the nuclide can be obtained after four cycles and 95% of the maximum of ${}_sD_c/\sqrt{{}_bD_c}$ after eight cycles. In addition, the optimum number of cycles in order to maximize the signal $({}_sD_c)$ is always smaller than that required to maximize the signal-to-noise ratio $({}_sD_c/\sqrt{{}_bD_c})$, and the optimum number of cycles increase as the half-life of the background increases for the same total

experimental time. However, it becomes less pronounce for background half-lives of more than.

Syrou (1981) and Syprou and Keer (1979) studied the effect of the transfer time (t_d and t_w) on ${}_sD_c$ and ${}_sD_c/\sqrt{{}_bD_c}$. They found that the cross-over point between cyclic and conventional activation was shifted to long experiment time with increasing transfer time. This means that CAA becomes preferable to the conventional method with decreasing transfer time, and the transfer time is an important factor affecting the detection limit and precision.

In real situations, many long-lived nuclides from matrix element not just one, will contribute to the underlying background of the peak of the nuclide of interest after activation. For calculation these contributions and optimization of the parameter of cyclic activation, many computer programs have been developed (Colin et al, 1985), (Tout and Chatt, 1981) and (At-Mugrabi and Spyrou, 1987). In Tout and Chatt(1981) program, by entering the total experiment time, transfer time (t_d, t_w), the mass and relevant nuclear data for matrix elements contributing to the background activity, and the half-life of the nuclide of interest, ${}_sD_c/\sqrt{{}_bD_c}$ can be calculated for individual matrix elements and the total background. The optimum irradiation and counting time (or number of cycles) and its range were finally given for the maximum and 95% of the maximum ${}_sD_c/\sqrt{{}_bD_c}$. Their results indicated that a single value of t_i and t_c can be used to produce results close to the maximum ${}_sD_c/\sqrt{{}_bD_c}$ for nuclide with large range of half-lives, e.g. for a 5-min total experiment time, $t_i = t_c = 6-7s$ will produce values of more than 90% of the maximum ${}_sD_c/\sqrt{{}_bD_c}$ for nuclides with half-lives in the range 3-22s. This

means that a number of short-lived nuclides can be studied at closed to their best detection limits by using only one set of CAA.

In Al-Mugrabi and Spyrou (1987) program, in addition to the contribution to the background of the photo-peaks of nuclides produced by matrix elements, the Compton continuum, single and double escape peaks, and bremsstrahlung were also considered. By entering such information in Tout and Chatt's program, and the flux, mass and nuclear data of the nuclide of interest, and detector parameters, not only the optimized activation conditions were produced but also a simulated γ-spectrum, and the detection limits and precisions for the elements of interest were also presented.

Estimation or Confirmation of the Half-life of Nuclides

Another function of CAA, the estimation and confirmation of the half-life of nuclides by using the data obtained during CAA (Spyrou, 1981), (Spyrou *et. al*., 1982) and (Ortaovali *et. al*.,1981).

For large *n*, $(1-e^{n\lambda T})$ tends to unity and equation (28) can be reduced to equation (33):

$$D_c = D_1\left[\frac{n}{1-e^{-\lambda T}} - \frac{e^{-\lambda T}}{(1-e^{-\lambda T})^2}\right] \qquad [33]$$

$$= \left(\frac{D_1}{1-e^{-\lambda T}}\right)\left(n - \frac{D_1 e^{-\lambda T}}{(1-e^{\lambda T})^2}\right)$$

Where D_c is a linear function of *n* with all other parameters constant. Plotting D_c as a function of *n*, the slope of the line is given by equation (34)

$$a = \frac{D_1}{1-e^{-\lambda T}} \qquad [34]$$

And the intercept by equation (35)

$$b = \frac{D_1 e^{-\lambda T}}{(1-e^{-\lambda T})^2} \quad [35]$$

The half-life of the nuclide measured can be calculated by equation (36)

$$t_{1/2} = \frac{T \ln 2}{\ln(1-a/b)} \quad [36]$$

Dead time and Pile-up Correction

CAA is usually employed for the determination of short-lived nuclides in a matrix of long-lived activation products. The activity of a sample not only changes considerably during a counting period owing to short-lived nuclide decay, but also increases from cycle to cycle as the matrix activity owing to the accumulation of longer lived products. Consequently, a rapidly changing dead time is encountered in a counting period. The basic equation (37) for dead time correction was given by Schonfeld (1966):

$$C = \int_0^{t_c} A_0 e^{-\lambda t}\left[1 - DT(t)\right]dt \quad [37]$$

Where C is the actual acquired net counts in the photopeak of interest, A_0 is the true initial photopeak count rate, and $DT(t)$ is the fractional analyzer dead time at the time t. In order to implement this correctly, the variation of fractional dead time during the counting period must be known. By least-squares fitting of the experimental data, Egan *et. al.*,(1977) found that $DT(t)$ is an exponential function of t, and it can be written as $DT(t) = B - Ce^{-kt}$,where B, C and k are all constant. Hence a correction factor (f) for dead time in a counting period can be obtained using equation and it can be expressed by equation (38):

$$f = \frac{\int_0^{t_c} A_o e^{-\lambda t} dt}{\int_0^{t_c} A_o e^{-\lambda T}(1 - B - Ce^{-kt})dt} \quad [38]$$

For cyclic activation with n cycles, the correction factor (f_n) can be expressed by equation (39):

$$f_n = \frac{\sum_{n=1}^{n} \int_0^{t_c} Ae^{-\lambda t} dt}{\sum_{n=1}^{n} \int_0^{t_c} A_0 e^{-\lambda t}(1 - B - Ce^{-kt})_n dt} \quad [39]$$

DT(t) not only varies with *t* in a counting period but also differs from one cycle to another. f_n is also a function of *n*.

Neutron Interactions

Neutrons, having no charge and with a mass (1.6749 x 10^{-27} kg) slightly higher than that of a proton (1.6726 x 10^{-27} kg), do not interact directly with electrons but are confined to direct nuclear effects (elastic and inelastic scattering) and nuclear reactions (the absorption of a neutron by the nucleus and the emission of electromagnetic radiation or an energetic particle). The nucleus of an atom is 10,000 times smaller than the electron cloud surrounding it. Four types of neutron reactions are possible: elastic scattering, inelastic scattering, neutron capture, fission reaction.

Activation Equation

A nuclear fission reactor produces large number of neutrons. The neutron flux density (Φ) is the number of neutrons incident on a unit area of the target per unit time (neutron $cm^{-2}s^{-1}$). If a sample is placed in a nuclear research reactor, radiation capture reactions will occur.

$$A\ (n, \gamma)\ B \xrightarrow{\lambda_B} C$$

The target atoms (A) capture neutrons to form the radioactive species B, which then decays to the product C. The rate of formation (R) of B is given by

$$R = n\phi\sigma \qquad [40]$$

Where n is the number of atoms of target present, which is assumed to be constant. The flux density is also assumed to remain constant throughout the target. The atoms of i^{th} term are also decaying, at a rate equal to $-\lambda i N_i$, so the net rate of formation of active particles is

$$\frac{dN_i}{dt} = \text{rate of production} - \text{rate of radioactive decay}$$

Where $n\phi\sigma$ is the rate of production

$\lambda_i N_i$ is the rate of radioactive decay

$$\frac{dN_i}{dt} = n\phi\sigma - \lambda_i N_i \qquad [41]$$

$$\int_{N_i=0}^{N_i=N_i} \frac{dN_i}{n\phi\sigma - \lambda_i N_i} = \int_{t=0}^{t=t} dt \qquad [42]$$

A standard form integral for this expression is

$$\int \frac{dx}{a+bx} = \frac{1}{b}\ln(a+bx)$$

Substitution yields

$$\frac{1}{\lambda_i}\ln(n_i\phi\sigma_i - \lambda_i N_i) - \frac{1}{-\lambda_i}\ln(n_i\phi\sigma_i) = t \quad [43]$$

Simplifying equation (43), it becomes

$$n_i\phi\sigma_i - n_i\phi\sigma_i e^{-\lambda_i t} = \lambda_i N_i = A_i \quad [44]$$

This gives

$$A_i = n_i\phi\sigma_i(1 - e^{-\lambda_i t_i}) \quad [44]$$

A_i is the absolute activity of the sample at the end of irradiation and it is the basic equation of activation analysis

The *ni* in equation (45) is expressed as:

$$n_i = \frac{m_i\theta_i N_A}{M_i} \quad [46]$$

Where m_i is mass of the ith nuclide

θ_i = isotopic abundance of the nuclide

M_i = Chemical atomic weight of the nuclide

N_A = Avogadro's number (6.02x10^{23} atoms/mole)

At the end of the irradiation, the activity becomes

$$A_i(t_i) = \frac{\phi\sigma_i\theta_i m_i N_A(1 - e^{-\lambda_i t_r})}{M_i} \quad [47]$$

After delaying for time t_d before counting, the activity of the end of the delay becomes

$$A_i(t_d) = A_i(t_i)e^{-\lambda_i t_d} \quad [48]$$

If after the end of the delay, the sample is counted for a time t_c, then the number of disintegration that occurred during the counting period is obtained from equation 48 as:

$$N_i = \int_o^{t_c} A_i(t_d) e^{-\lambda_i t_d} dt = \frac{A_i(t_d)(1-e^{-\lambda_i t_c})}{\lambda_i} \quad [49]$$

putting equation (47), and (48) into equation (49) becomes:

$$N_i = \frac{\phi \sigma_i \theta_i m_i N_A (1-e^{-\lambda_i t_r})(1-e^{-\lambda_i t_c}) e^{-\lambda_i t_d}}{\lambda_i M_i} \quad [50]$$

Suppose ε(E) is the photo peak detection efficiency for the gamma ray energy E and total counts recorded by the detector (the photo peak area) is P_A then N_i can be expressed as;

$$N_i = \frac{P_A}{\varepsilon_i(E)\gamma_i} \quad [51]$$

Where γ is the gamma ray emission probability

Equating equation 50 and 51 and rearranging, we obtain

$$m_i = \frac{P_A \lambda_i M_i}{\phi \sigma_i \theta_i N_A \varepsilon_i(E) \gamma_i (1-e^{-\lambda_i t_r})(1-e^{-\lambda_i t_c}) e^{-\lambda_i t_d})} \quad [52]$$

If W is the weight of the sample used, then the concentration or the amount ρ of a nuclide i in the sample is given by:

$$\rho = \frac{m}{W_i} = \frac{P_A \lambda_i M_i}{\phi \sigma_i \theta_i N_A \varepsilon_i(E) \gamma_i (1-e^{-\lambda_i t_r})(1-e^{-\lambda_i t_c}) e^{-\lambda_i t_d}) W_i} \quad [53]$$

Equation (53) can be written as:

$$\rho = \frac{m_i}{W} = \frac{[P_A / t_c] M_i}{\phi \sigma_i \theta_i \gamma_i \varepsilon(E_i) N_A SCDW} \quad [54]$$

Reactor Epithermal Neutron Activation Analysis

In the case of nuclear reactions induced by neutrons the radioactivity of the examined isotopes depends on the flux of the neutrons and the cross section of the given nuclear reaction. The cross section and the neutron flux highly depend on the energy of the neutrons, and therefore the usual activation equation is:

$$R = N\int_0^\infty \sigma(E).\varphi(E)dE \qquad [55]$$

N= number of interacting isotopes

$\sigma(E)$= cross section [in cm^2] at neutron energy of E [in eV]

$\varphi(E)$= neutron flux per unite of energy interval [in cm^{-2} s^{-1} eV^{-1}]

R= reaction rate

In nuclear reactors the integral in equation (55) is usually replaced by the sum of the two integrals separating the thermal and epithermal regions, the lower limit of the epithermal component of the reactor neutron spectrum is taken as either μKT (where μ=5 for H_2O and D_2O reactors and 3 for some graphite reactors) or the energy cut-off energy of a filter used to absorb the thermal neutrons. For more common absorber, Cd, this cut-off energy is equal to 0.55eV for a cylindrical cadmium box with a wall thickness of 1 mm. Equation (55) is usually replaced by an equation which involves averages of the cross sections and the flux

$$R = R_{th} + R_{epi} = N\left(\phi_{th}.\sigma_{th} + \phi_{epi}.I_0\right) \qquad [56]$$

σ_{th} = Conventional thermal cross section [in cm^2]

ϕ_{th} = The average thermal flux [cm^2]

ϕ_e = Conventional epithermal neutron flux [in $cm^2\ s^{-1}\ eV^{-1}$]

I_o = Resonance integral cross section (in epithermal region), for 1/E epithermal spectrum [in cm^2]

$$\sigma_{th} = g\sigma_o + \frac{\phi_{epi}}{\phi_{th}} \int_{\mu KT}^{Ecd} \frac{\sigma(E)}{E} dE \qquad [57]$$

or for 1/v nuclides at 293K, σ_{th} (Alfassi, 1990) is written as,

$$\sigma_{th} = g\sigma_0 (1 + 0.45\phi_{epi} / \phi_{th}) \qquad [58]$$

Where σ_0 the cross section for 2200 m/s neutrons and g is the parameter representing the deviation in the thermal region from the 1/v law. ϕ_{epi} is the epithermal flux per unit lnE and I_o is the resonance integral. The common convention is to use for the second term- the I_o term- the cut off energy of 1mm thick Cd filter, 0.55 eV, as the low boundary integral. When reactor systems are composed of constant slowing down density, i.e., when the effects of neutron leakage and absorption can be neglected, the slowing down spectrum follows an almost dE/E distribution. Thus the epithermal neutron activation is given by the equation:

$$R_{epi} = \phi_{epi} \cdot \int_{Ecd}^{1MeV} \frac{\sigma(E)}{E} dE = \phi_{epi} I_o \qquad [59]$$

ϕ_{epi} is not the total epithermal neutron flux which is given in the ideal case by equation

$$\phi_{epi} \int_{Ecd}^{1MeV} \frac{dE}{E} = \phi_{epi} \ln(\frac{1.0x10^6}{0.55}) = \phi_{epi} \ln(1.82x10^6) = 14.41\phi_{epi} \qquad [60]$$

I_o, the epithermal cross section, is only an approximate resonance integral as a precise value of the resonance integral should consider the real

lower energy limit (μkT) and the overlap of the two neutron spectra. The theoretical resonance integral I_t is given by

$$I_t = \int_{\mu kT}^{1MeV} [\sigma(E) - g\sigma_0 \sqrt{\frac{E_0}{E}}] dE \qquad [61]$$

Where σ_o is the cross section for thermal neutrons with most probable velocity of 2200 m/s and g is a parameter which represents the departure of the cross section from the 1/v law in the thermal region (if the 1/v law is obeyed, g=1), and E_o is the thermal energy=0.0253 eV.

However, for activation analysis, the approximate experimental integral I_o is the important one and many resonance integrals are tabulated in Van der Linden *et. al*., (1972), Van der Linden *et. al*., (1974), Gryntakis and Kim (1976), and Sher and Albinson (1974) according to Hogdahl convention in 1962 which set

$$I_o = \int_{0.55eV}^{1MeV} \frac{\sigma(E)}{E} dE \qquad [62]$$

$\sigma(E)$ is taken as the total activation cross section including the 1/v contribution. If the resonance integral is defined with cross section excluding the 1/v contribution, I, the activation rate is given by equation (63)

$$R = \phi_{th}\sigma_{th} + \phi_{epi}(I + 0.45\sigma_0) \qquad [63]$$

The epithermal activation properties of a nuclide can be conveniently expressed by means of the absorber ratio, which gives the ratio of the activity of this nuclide irradiated once with the whole reactor's neutron spectrum and once covered by an absorber of thermal neutrons. Thus the cadmium ratio is given by

$$R_{cd} = \frac{\phi_{th}.\sigma_{th} + \phi_{epi}.\mathrm{I}_0}{\phi_{epi}\mathrm{I}_0} = 1 + \frac{\phi_{th}.\sigma_{th}}{\phi_{epi}\mathrm{I}_0} \qquad [64]$$

The most important fact is not only how the insertion of an absorber of thermal neutrons influences the activity of the specific measured nuclides but also how it influences the interfering nuclides. Thus it would be advantageous to analyze an element by epithermal neutrons irradiation rather than by using the whole spectrum of reactor neutrons for the activation (ENAA vs. RNAA as it is most usually written, epithermal and reactor neutron activation analysis). If the ratio of resonance integral to thermal neutron cross section I_0/σ_{th} is larger than the ratio for the interfering elements.

Several criteria were suggested to determine the advantages of ENAA over RNAA.

Advantages Factors for (n,γ) Reactions

1. Brune and Jirlow's Advantage factor

   Brune and Jirlow (1964) suggested to use as an advantage factor for ENAA activation the ratio between cadmium of the measured nuclide and the interfering nuclide.

$$F_{BJ} = \frac{R_{cd}^{i}}{R_{cd}^{o}} \qquad [65]$$

   Where R_{cd} is the cadmium ratio as defined previously; the superscripts 0 and i stand for the measured element and the interfering nuclides respectively. This is the most advantage factor. In some irradiation facilities where the thermal neutrons absorber is installed permanently, the absorber ratios cannot be determined since the activity without an

absorber cannot be measured. In this case, the advantage factors (called enhancement factor by Gladney *et. al.*, (1980) since they are approximate advantage factors) are measured by comparison of irradiations of the nuclides in the absorber lined position and in a bare irradiation port. Although the spectral distribution in the various places in the reactor might be different, which means that the advantage factor measured in that way will not be the same as measured using only a bare irradiation port with and without an absorbing capsule, the true meaning of those advantage factors are the same. They give the advantage of using the epithermal activation either by using an absorbing capsule or lining the irradiation port with an absorbing material (thermal neutron absorber). Substituting equation (64) to equation (65) gives

$$F_{BJ} = \frac{F_R + S_R^i}{F_R + S_R^0} \qquad [66]$$

Where F_R is the ratio of the fluxes ($F_R = \phi_{ep}/\phi_{th}$) and S_R is the ratio of the cross sections ($S_R = \sigma_{th}/\mathrm{I}_0$). The superscript 0 and i stand as before for the measured and interfering elements, respectively. Equation 66 shows that if $F_R > S_R^i, S_R^0$, then the advantage factor F_{BJ} =1; since the flux is mainly epithermal, the absorber of the thermal neutrons does not change considerably the activity either of the analyzed element or of the interfering element. In the opposite case where $S_R^i, S_R^0 > F_R, F_{BJ} = S_R^i/S_R^0$ which can be looked at as the upper limiting value. For many experimental set ups, F_{BJ} is lower than this

limiting value and consequently it is not expected to obtain the same advantage factors in different studies since S_R^i and S_R^0 are constant but F_R is different for the various reactors. F_R will be higher for less well moderated nuclear reactors (reactors with "hard spectrum" of neutrons).

2. Parry's "Improvement Factor"

In 1980, Parry pointed out that while the advantage factor describes well the increase in the signal-to noise ratio, it does not consider the decrease of the activity of the analyzed element due to the elimination of the activation b y the thermal neutrons and hence does not treat the larger error resulting from the lower counting statistics. Parry suggested that the true criteria should be the improvement in the detection sensitivity. The lower detection limit L_D for a radioactivity measurement, that is, the minimal signal which can be detected above the background at 95% confidence level is given by (Currie, 1968),

$$L_D = 2.33\sqrt{B} \qquad [67]$$

Where B is the background activity. The minimal detected mass in activation analysis (sensitivity) is given by

$$M_D = A/L_D = A/2.33\sqrt{B} \qquad [68]$$

Where A is the specific activity of the analyzed element under the experimental condition for the activation and detection. Since A is proportional to the activity of the analyzed element and since B is due

mainly to the interfering nuclide, Parry suggested that the improvement factor f_p is given by

$$f_p = \sqrt{Ri_{Cd}^{i} / R_{Cd}^{0}} \qquad [69]$$

Methods of Standardization

The two features of neutron induced reaction - high penetrability for neutrons and gamma radiation - ensure that its standardization is potentially easy and accurate. As the signal to concentration ratio is nearly matrix independent, the sample preparation is rather easy; therefore, the risk of systematic or random errors is reduced.

Single Comparator (k_0-method) standardization

The k_0-standardisation also known as the single comparator method of NAA is based on the fundamental equation for the calculation of the reaction rate R defined as:

$$R = \int_0^{\infty} \sigma(v)\phi(v)dv \qquad [70]$$

-Integrating the equation (70) yields

$$R = \phi(v)\sigma(v) = \phi\sigma = nv_0\sigma_{ef}$$

This method involves the simultaneous co-irradiation of the sample and the single nuclide such as ^{197}Au. The activation equation from equation (54) using the k_0 method with Au as comparator standards can be written in the form:

$$\rho = \frac{\left(\frac{P_A/t_c}{SCDW}\right)_i}{\left(\frac{P_A/t_c}{SCDW}\right)_{Au}} \frac{M_i \phi_{Au} \gamma_{Au} \sigma_{effAu} \varepsilon_p(E_{Au})}{M_{Au} \phi_{ii} \gamma_i \sigma_{effi} \varepsilon_p(E_i)} \quad [71]$$

Accurate knowledge of the nuclear data, the detector efficiencies and the specific activities of the nuclides in the sample and the monitor are needed for the determination of the concentration level in the sample. The application of the k_0-method avoids the problem associated with preparation of individual standards for each element to be determined.

Absolute method

This method of quantification is based on equation (54). By measuring P_A for known timing parameters, viz t_i, t_d, and t_c, the amount of the element present, p can be calculated. A reliable determination of ρ requires prior knowledge of accurate ,σ, ε,Φ, θ, and λ Since these parameters are not usually known with high degree of accuracy, the absolute measurement does not always provide reliable results; hence it is not used in many laboratories.

Relative Standardization

In the relative standardization method, a chemical standard (index std) of known mass, w, of the element is co-irradiated with the sample of known mass W. 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. The neutron activation equation then reduces to:

$$\rho_{sam} = \frac{\left[(P_A / t_c)\right]_{sam}\left[\rho WCD\right]_{std}}{\left[P_A / t_c)\right]_{std}.[WCD]_{sam}} \quad [72]$$

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.

Equation (72) can be reduced to:

$$\rho_{sam} = \frac{\left[(P_A / t_c)\, CD\right]_{sam} W_{std}}{CD_{std}.W_{sam} SA} \quad [73]$$

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

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Vowotor, **M. K.,** Sackey S. S., Amoako G., Sefa-Ntiri B., Amoah C. L. C., & 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); Vol. 9, No. 99, pp 5 – 15; http://www.ijsei.com/archive-99920.htm

Michael Kwame Vowotor, George Amoako, Baah Sefa-Ntiri, Samuel Amoah, Samuel Sonko Sackey & Charles Lloyd Yeboah Amuah (2020). *Assessment of Nine Micronutrients in Jasmine 85 Rice Grown in Ghana Using Neutron Activation Analysis*, Environment and Pollution; Vol. 9, No. 2, pp. 29 – 49, https://doi.org/10.5539/ep.v9n2p29 http://www.ccsenet.org/journal/index.php/ep/article/view/0/43828

MANUSCRIPT FROM THIS PROJECT UNDER PREPARATION

Quantitative Epithermal Neutron Activation Analysis of Seven Elements in Breast Milk of First Six Months Nursing Mothers From Central Region In Ghana.