MULTI ELEMENTAL ANALYSIS OF SELECTED INDIGENOUS SPICES, TUBER CROPS AND MEDICINAL HERBS TAKEN FROM SOUTHERN ETHIOPIA USING INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS TECHNIQUE

By

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This thesis is dedicated to my family

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Abstract

Now days, there are growing interests towards the analytical methods in several fields on the account of their effective and accurate measurement results utilized to improve human life, solve the existing problems, and investigate alternative ways of scientific facts. In order to use them for better future applications in various expertise, constant investigations and improvements have been undergoing.

In this study, nuclear analytical method was utilized in order to get scientific evidences for the existing knowledge gap concerning about contents, status and impacts of nutrients in some indigenous Ethiopian crops and herbs used for various traditional purposes by the local people. The investigation was achieved by the application of the sensitive nuclear analytical technique, instrumental neutron activation analysis (INAA), and gamma-spectrometry method using the IAEA 's quality control and quality assurance procedure. The experiment takes place at centre for energy research and training (CERT) facility using MNSR Nigerian research reactor (NIRR-1) and HPGe detector. The overall INAA method validity was confirmed by using NIST standard reference material (SRM).

The qualitative and quantitative analysis results shows that, various concentrations of constituent essential, trace and other elements of each plant sample were obtained. Most sample's essential elements status such as; Ca, K, Mg, Fe, Zn, and Mn have some significant support to the daily human body nutrient requirements for healthy functions. The estimation of daily intake(EDI) of concentration of constituent elements due to the consumption of these crops and herbs in the traditional purposes are below the tolerable upper intake level set by international organizations.

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Chapter 1

Introduction

1.1 Background

In order to have a quality life, human being needs persistent healthy environment and quality basic needs such as balanced diets and clean environment. The disturbance of one of these life components may results in unprecedented consequence to the human life himself and those living organisms around. Therefore, it needs continuous relevant scientific research to understand the existing status and changes of an ecosystem in order to take appropriate actions. Civilization of human being has its own impact on the environment due to human activity and life style by changing the natural properties of environment. These non-uniform changes depend on various factors as a result the degree of exposition of related impacts also varies[1, 2].

Nowadays, one of the major causes of environmental change is pollution. The dimension of pollution is local and global depending on their properties (physical, chemical, biochemical) and their conditions of released into the environment that can be dispersed and accumulates to a particular region of an environment [3].

The major concern in pollution is the increasing levels of contamination of major

components such as; Soil, water and air. Any environmental change directly affects them and life which depends on them. Environmental pollution changes these major resources either by addition of contaminants or reduction of the essential components. The profound increasing of industries, intensive use of pesticides and fertilizer are among the major human activities which can affect directly or indirectly the essential ingredients of an environment [1, 2, 3].

Environmental pollutants and burdening substances are organic and inorganic chemicals. They are further categorized as degradable and persistent organic compounds, inorganic compounds, and organic or inorganic gases. These environmental chemicals enter into the environment due to human activity and endanger the living organisms and abiotic ecosystems.In addition to their influences in their compound form, they contain either, heavy metals, essential elements, radioactive substances or toxic elements which further affects the life of the ecosystem [1, 3].

Several nations in the world have been facing several problems due to soil, Water and air pollutions effects. Soil and water pollution is most often occurring by local contaminants due to the fast growth of intensive farming and industrial wastages. These contribute the essential minerals for plant growth to decreases gradually, facilitate soil degradation, infertility, contamination and soil acidity [2]. Air pollution categorized under global pollution due to the vast release of greenhouse gases from developed nations of several industries, Vehicles, Machineries, electronics, deforestations, etc. it has the lion share of the causes of the rapidly increasing global warming and its devastating environmental consequences all over the world.

There is a global understanding about the insignificant contribution of developing countries in the current global pollution. Despite the fact that, most often these

nations have been the direct victim of the devastating impacts of pollution. In fact, there are few fragmented local pollutions of an environment due to small agroindustry, small factories, and human activities.However, the degree of their effects to the existing ecosystem problems is insignificant in comparison with the global one. Whatever the degree of either of pollutions, all of them becomes causes of the major issues of developing countries such as; poverty, drought,diseases and malnutrition[3].

Nowadays, With the development of science and technology, some growing demands have been observed in order to assess pollutions (contaminants) of environments and to find alternative solutions for its increasing global impacts using analytical methods. The growth in faster rate of the demands and the advent of several modern instruments used for these analytical methods have a considerable impact for the current achievements[4].

A given analytical sample under study can have physical and chemical properties. Scientists have devised some analytical methods based on these major categories/properties/ of a matter. The choice of any of these methods in each category depends on, the ability to conduct analysis, fundamental characteristics (quality of method and result), personnel concerns(accessibility) and method of status (reachability)[5].

Any technique used for the qualitative and quantitative analysis depends on the matter properties chosen by the researchers and scientists. Some of the physical properties of a matter in given environment includes; mechanical properties (mass-volume-area related, morphometric and structural, rheological, and surface), kinetic properties (quality kinetic constants and microbial growth-decline-death kinetic constants), sensory properties (colour and appearance, odour, sound,tactile, taste, and

texture) and health properties (functional properties, medical properties, nutritional composition, toxicity, and unbalanced intake) [1].

Many analytical methods have been utilized in the world for analyses of geological, biological samples and pollutants. There are some effective methods based on the physical and chemical properties of the fundamental constituent of a matter. Among those, nuclear techniques are based on the radioactive nature of atomic nucleus. It has been accepted as one of the important technique for the evaluation of radioactive substances and the analysis of constituent elements of any geological and biological substances which allows us to get significant evidences about their existing status, future consequences in our environment and to find better solutions[4].

Neutron activation analysis (NAA) technique is one of the best nuclear technique due to its accuracy, sensitivity and speed. In general, nuclear analytical techniques with their broad applicability to almost all matrix types, are indispensable for food, medicinal, and environmental research [5, 6].

Many of the earliest applications of NAA involved geochemistry and Cosmo-chemistry due to its best sensitivity for determining the rare-earth elements (REEs) in terrestrial rocks and meteorites[7, 8]. The information obtained in trace-element fingerprints of Arti-facts helps Archaeologists to reconstruct prehistoric activities such as trade and movement of peoples. Analysis of toxic metals (i.e. Hg, Cd, As, Cu and Sb) in sewage, mining run off and air particulate samples have provided a great deal of information about the pollution caused by the transport and monitoring of these elements in our environment [9].

Some high-purity materials include quartz, metals, plastics and ceramics quality can be determined by using NAA because the Ultra-trace amounts of the transition-group

metals (Le. Cr, Fe, Co, Ni, Cu, Zn, As, Sb and Au) can drastically affect the properties of semiconductors[4, 10].

The other applications of trace-element analysis include biological materials (animal tissues and plants) in which several trace elements with narrow tolerance limits in plants and animals are frequently measured by NAA [6]. Recently, there is an increasing interest in the roles of trace elements in food composition, nutrition and health [10].

1.2 Statement of the Problem

In Ethiopia, there is insignificant pollutions originated from Agroindustry, garment and few chemical industries. However, those impacts caused by global pollutants affecting the nation frequently. Despite the fact that, some measures are under-way in order to minimize the consequences of such pollutions in several regions of the nation . Among those, cultivation of drought resistant and market oriented crops, maintaining soil fertility by reforestation, soil terracing and inter-cropping, encouraging efficient industries, etc. The implementation of these plans becomes effective with the recent scientific knowledge and applications in every sector

There is a tremendous untapped potential for Ethiopia to exploit the rich and diverse plant genetic resources of underutilized crops. These crops are considered as very important in local diets and for local and foreign markets. However, in spite of their potential for food security and for export, very little research has so far been done to utilize, improve the productivity and uses of this important crop category. Some of them are particularly known for their value as an insurance crop in dry seasons, when annual crops fail [11].

The country is also known for its wide range of other crop species that can potentially be used for industrial and medicinal purposes. A wide array of wild plant species has been used as sources of medicine for centuries. About 80% of the rural population in Ethiopia and 95% of their livestock depend on traditional medicine, and more than 95% of the traditional medicines are of plant origin. In order to promote a wider use of indigenous medicinal plants in pharmaceutical industries, it needs significant efforts of the researchers and government organizations.

Some researches have been conducted about the genome and traditional uses of plant species. However, the lack of modern scientific evidences, knowledge and skills to verify their edibility and health significances, several native plant species are known by their limited utility and rarely seen their significances in some part of the nation.

NAA technique in its routine method (INAA) is a common technique adopted for elemental determination in plant and plant products because it is non-destructive, highly sensitive, multi elemental, and requires minimal sample preparation. In addition, instrumental neutron activation analysis is less affected by the matrix of the sample in comparison to other methods because of the high penetrating power of neutrons and gamma rays and is not affected by the chemical and physical states of the elements.

In general, This study focuses on the qualitative and quantitative analysis of multi elements in indigenous plant species (biological materials) using comparative INAA technique. Thus, it can offer; new scientific data relative to the existing body of literature, and provide a meaningful scientific contribution due to the designed objectives. Moreover, the result of the study benefits researchers, governments, related organizations, factories, and customers in particular.

1.3 Objectives of the study

The general objective of the study is qualitative and quantitative multi elemental analysis of each crops and herbal plant samples for better future use.

1.3.1 Specific Objectives

The specific objective of the study is to:

- identify the elemental compositions of each plant samples.
- analyse the concentration and status of identified elements in a given plant sample.
- analyse the levels of each element intake in their traditional purposes.
- evaluate the constituent elements significance in their traditional purposes.
- investigate the relevancy of each plant samples in their traditional values.

1.4 Layout of the thesis

A thorough addresses of the topic under investigation and sufficient justification demonstrated for the work in the context of the existing literature. The sampling plan takes into consideration the natural biological variability within cultivars, species.The number(n) of independent samples analysed and the number of analytical replicates clearly stated in both the materials and methods section and in the data tables (as table footnotes). Appropriate quality control, evidence of method validation, quality of the experimental design and statistical analysis, sampling plan, interpretation of data, and discussion of results in the context of existing literature included.

In chapter two, we will describe the development of the analytical method, the

mathematical principles utilized, recent relevant materials and measurement procedures, the limitations of the method, uncertainties and the scientific optimizations included.

Chapter three discuss all the necessary instrumentations and methodology aspects of the study. It will briefly explain about those materials used in CERT facility for the measurement process. The specification and property of NIRR-1 facility, The specification and calibration process of HPGe spectrometry, sample collection and sample preparation procedures, irradiation and radiation measurement procedures, INAA calibration and data analysis methods will be explained in detail.

Chapter four is the discussion part of the indigenous Ethiopian spices and herbs. we will see review of relevant literatures, sampling and sample preparation methods, irradiation and data analysis procedures, and discussion about the analysis results of these samples. More attention was given in evaluation of their significances in the uses of these plant samples based on their elemental statuses.

Chapter five devoted to discuss the quality of indigenous Ethiopian root and tuber crops used in some parts of the country as food item based on the multi elements measured by comparative INAA technique. In particular, their essential and other elements status as compared to other staple crops and average estimated intake values determined for their best uses.

In chapter six, medicinal herbs, most often utilized for gastrointestinal disease treatments will be assessed based on the existing literatures. special emphasises given on; some essential and trace metals therapeutic property and side effects due to their statuses in these GI organs, their possible bio-availability and involvement the treatment of GI diseases, and the status of these elements in these herbs and possible

impacts.

Finally, the Summary chapter includes;Conclusions, Limitation of the study, and Recommendations based on the results will be discussed respectively.

Chapter 2

Theoretical Background on Activation Analysis

2.1 Radioactivity

An isotope of an atom in a periodic table can be obtained in stable or unstable condition either by artificial means or naturally. The former radionuclide are known as Man-made(induced) nuclide. These nuclide could be destabilized by nuclear interaction of another nucleus, particles or gamma-rays.This interaction is also the principle of many radiation sources from smaller to huge facilities[7, 9].

2.1.1 Naturally Occurring Radionuclide

The origin of Natural radioactivity is from extraterrestrial sources as well as radioactive elements in the earth's crust. The earth's crust is the ultimate sources of primordial radioactive nuclide's in the environment. These radionuclide's are found throughout the environment like rock classes, Various soil types, in the houses, buildings, farms, etc. The level of activity of these radionuclide varies in naturally and the human activity. There are also two main sources of these natural radioactivity.

These includes primordial radionuclide's and cosmic ray particles[9, 12].

(i). Primordial Radionuclides; The primordial radionuclides were obtained from the planetary formation and they are available everywhere up to the present. We can measure them in the environment because these radionuclides have very long half-lives comparable to the age of the earth. Their names come that they are believed to represent a primordial inventory. Based on their number of daughter radioactive nuclei, we can classify them as series radionuclides and non series radionuclides.

Series Radionuclides;- are three main radioactive series known as Uranium, Thorium and Actinium series headed respectively by ^{238}U, ^{232}Th and ^{235}U. The final product in each chain is a stable isotope of lead. In general, naturally occurring radioactive nuclei would undergo radioactive decay in these three chains through a sequence of transformations.

Non-Series Radionuclides; In general, there are seventeen radionuclides in these group but fifteen of them are negligibly in their specific activities and their environmental impact. The known non-series radionuclides are K-40 and Rb-87. K-40 constitutes 0.012% of natural potassium by weight and it is distributed throughout environments and also in the human body. This radionuclide has high activity and emits both beta and high energy gamma radiation. Radioactive rubidium (Rb-87) constitutes 27.8% of rubidium found in the earths crust and it occurs naturally in igneous rocks in varying amount depending on the rock type. Rb-87 is a beta emitter only with high activity and energy [12].

(ii). Cosmic Radiation; Cosmic radiations are elementary particles coming from extraterrestrial activities. Among these particles primary energetic proton and alpha

particles which have a probability to strike the earths atmosphere have a capability to initiates secondary particles or cosmogenic radionuclides which continuously generated by bombardment of stable nuclides in the atmosphere. These cosmogenic radionuclides include H-3, Be-7, C-14 and Na-22. The most significant cosmogenic radionuclide is C-14 which can be taken up by plants and becomes enter the food chains [12].

2.1.2 Induced Radionuclide

There are some radionuclides artificially produced in the nuclear reactors and other similar facilities for various scientific and other purposes. However, in some incidents these radionuclides escaped into the earths surface and atmosphere from these facilities and they have been contaminated the environment. For instance, the Chernobyl reactor accident in the past were the main incidents which give rise to a wide range of radionuclides released in the atmosphere. Such radionuclides may globally have dispersed and the deposited in rain and by dry deposition on terrestrial surfaces. Also, it can be taken up by plants and subsequently be incorporated into foodstuffs. These includes Cs-137 has long half-life to contaminate the environment [12].

(i). Nuclear reactions

Induced nuclear reaction is the collision process between an incident particle and a target nucleus can lead to a rearrangement of the nucleons in the target nucleus, often producing a different product nucleus and one or more particles or radiations.

(ii). Compound nucleus reaction

There is more than one type of nuclear reactions based on nature of particles, energy, etc. However, reactions leading to compound nucleus formation involve a two-step process of formation and evaporation of daughter nuclide. During the formation step, the incident particle merges with the target nucleus so that the total excitation energy (kinetic plus binding) is quickly distributed among all nucleons in the compound nucleus. In the evaporation step, most of the excitation energy carried by evaporated particles or usually by gamma radiations. A typical nuclear reaction can be represented by the expression,

$$a + A \rightarrow Y^* \rightarrow b + B + Q \tag{2.1.1}$$

Where, a is incident particle or ray, A is target nuclide, Y^* is excited compound nucleus, B is daughter nuclide, b is product particle or ray and Q is amount of energy. Most often, neutron capture, $A(n,\gamma)B$, reactions are among the nuclear reactions that involve compound nucleus formation. it involves the low-energy neutrons which result in the compound nucleus has no sufficient energy for particle emission but only gamma-ray emission is possible as shown in Eq.2.1.2. For some higher neutron energies, the compound nucleus has a greater likelihood of emitting charged particles, neutrons and/ or gamma-rays.

$$n +^{75} As \rightarrow (^{76}As) \rightarrow^{76} Se + \beta- + \gamma \tag{2.1.2}$$

Such reaction principle utilized in the irradiation of stable nuclei of a sample by thermalized neutrons of a reactor. During the irradiation process, both production and decay of radioactive nuclide are common events. The mathematical expression for the rate of change in the number of radioactive nuclei during an irradiation is the

difference between the rate of production and the rate of decay, given by[4, 10],

$$\frac{dN}{dt} = RN_0 - \lambda N \tag{2.1.3}$$

In the expression,N_0 is the number of target atom, N, is the number of atoms of the radionuclide present in the sample at a given time t and R is the radioactive nuclide production rate given by,

$$R = \phi\sigma \tag{2.1.4}$$

Where, σ is effective cross-section and ϕ is the average neutron flux. Then substituting for the rate of production from Eq. 2.1.3 leads to an equation

$$\frac{dN}{dt} = N_0\sigma\phi - \lambda N \tag{2.1.5}$$

If Eq. 2.1.5 is integrated, then the number of radioactive nuclei present at the end of irradiation is

$$N(t_i) = \frac{N_0\phi\sigma}{\lambda}.(1 - e^{\lambda t_i}). \tag{2.1.6}$$

The activity of a particular nuclide at the end of the irradiation is given by

$$A(t_i) = \lambda N(t_i) = N_0\phi\sigma(1 - e^{\lambda t_i}) \tag{2.1.7}$$

If $t_i \ll t_{1/2}$, decay during irradiation can be neglected such that the factor $(1-e^{-\lambda t_i}) \sim \lambda t_i$ and Eq. 2.1.6 becomes $N(t_i) = N_0\sigma\phi t_i$. If $t_i \gg t_{1/2}$, then the factor $(1-e^{-\lambda t_i}) \sim 1$. In this case, the rate of production is equal to the rate of decay and irradiation of the sample for times longer than about five times the half-life offers no significant advantage[13]. The maximum number of radioactive nuclei that can be produced is called the saturation value (N_{sat}) gives us

$$N_{sat} = \frac{N_0\phi\sigma}{\lambda} \tag{2.1.8}$$

For a particular reaction, it is possible to increase the saturation value by increasing the flux or the target mass, but not by lengthening the irradiation time[6, 10].

2.1.3 Radiation Detectors

The detection of radiation is generally based on the principles of interactions of ionizing radiations with matter.The radiation detection and measurement systems employed in analytical laboratories have significant improvement realized since the early 1980's.The fast advancements in the areas of material science, electronics, and computer technology have contributed to the development of more sensitive, reliable, and user-friendly laboratory instruments[14].

For the modern radionuclide measurement laboratory, there are four primary radiation measurement systems considered to be necessary. These includes gas-flow proportional counters, liquid scintillation counters, Silicon alpha-particle spectrometer systems, and Ge gamma-ray spectrometer systems. Each of the detection instruments has a specialized field of application. For instance, the gamma-ray spectrometer is the labour-saving device for measuring photon-emitting radionuclide[15].

The choice of a particular radiation detector type for an application depends upon Some operating parameters which can be considered in common for all these detectors, notably upon the energy range of interest, detection efficiency and the radiation background. Additional parameters of detectors with associated spectrometers of concern are the radiation energy peaks that identify and quantify radionuclide, stability of calibrations, energy resolution, peak-to-Compton ratio, and peak shape[12, 14].

1. Gamma-ray Detectors

The most common scintillation crystals used in gamma ray spectroscopy systems were sodium iodide doped with thallium, NaI(Tl) detectors. The advantages of the NaI(Tl) detector for gamma-ray detection are high detection efficiency and the detectors could be manufactured in various dimensions and shapes [14, 15].

The next generation of detectors for gamma-ray spectrometers were Lithium-drifted semiconductor detectors. They can be made based on lithium-drifted germanium and silicon, designated as Ge(Li) and Si(Li), respectively. The Ge(Li) detector was applied to the entire gamma-ray energy range while the Si(Li) detectors were applied for low-energy gamma rays and X- rays. Because of their lower Z value and smaller size, Si(Li) were more good choices for X-ray spectrometers.

(i). Germanium Detectors

The intrinsic HPGe detector now has replaced the Ge(Li) detector. such high pure germanium crystal has a sufficiently low density of free charge carriers to allow it to be used as a gamma detector that does not require lithium drifting for its radiation-sensitive properties. Germanium crystal is chosen as the most suitable material for the construction of gamma radiation detectors because[15]:

(a) Germanium is a semiconductor material which can be refined to an extremely high degree of purity.

(b) More effective at stopping high-energy gamma radiation than silicon because it has a relatively high atomic number.

(c)The mean energy required (about 2.95 eV) for ionization of electrons from valence to conduction bands (about 0.73 ev) is low [16].

The most common type of detector in current use for gamma-ray spectrometry is the

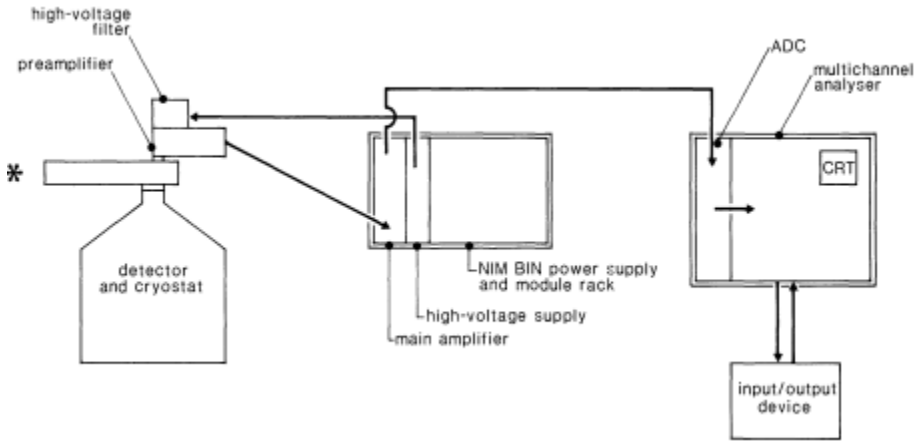

Figure 2.1: Schematic diagram of neutron activation counting system.

coaxial HPGe detector in an aluminum can. These detectors are available with either a p-type or n-type crystal and with their large active volume are most appropriate for the detection of high-energy gamma radiation in the wider gamma-ray energy range. [16]. HpGe detectors, are usually equipped with, radiation shield, Vacuum housing, Liquid nitrogen, and Be cryostat window to take full advantage of their intrinsic energy response as shown in Fig. 2.1

Liquid nitrogen and vacuum housing: -The HPGe detector is not subject to adverse effects if allowed to come to room temperature, but must be cooled to liquid-nitrogen temperature during use to achieve good resolution. In operation, a germanium detector must be cooled to liquid nitrogen temperatures (boiling point 77 ^0K, - 196^0c) in order to minimize electronic noise created by the thermal excitation of electrons from valence to conduction bands [17]. In addition, Germanium detectors must also be mounted in an ultra-high vacuum environment to prevent surface contamination of the germanium crystal in order to prevent a leakage current to flow when the

high-voltage bias is applied to the detector due to tracking across the surface of the crystal. This results in high electronic noise, poor detector resolution and ultimately the inability to maintain an adequate operating bias [16, 17].

Radiation shield: -In order to minimize effects of background radiations in gamma detection and measurement process, effective radiation shield such as, graded lead, are used to enclose gamma detector system. Thickness of a Lead shield should be 100 to 120 mm, the limiting factor being the thickness beyond which no further background reduction is obtained due to increased probability. For this reason, it is normal to line any surface of lead which may be viewed by the detector with a graded shield consisting of cadmium and then copper foil each about 1 mm thick to absorb fluorescence radiation [18].

2. Operating Parameters

Gamma-ray spectrometry is efficiently carried out with NaI(Tl) and HPGe solid-state semiconductor detectors. The key characteristics of a gamma-ray detector system are the detection efficiency, the energy resolution, and the signal-to-background ratio. Among the important operating parameters in the gamma detection and measurement processes are energy and efficiency calibration of the measuring systems [15, 19, 20]. Energy calibration of a germanium detector system is establishing the channel number of the MCA in relation to a gamma-ray energy. it is achieved by measuring mixed standard sources of known radionuclide having well-defined energies within the energy range of interest, usually 60 keV to 2000 keV [18, 20]. In this process, it is recommended that the gain of the system should be adjusted so as to position the 662 keV photo-peak of ^{137}Cs at about one-third full scale and the gain of

the system be adjusted to 0.5 keV/channel [19]. Once these adjustments are made, the gain of the system should remain fixed.

In general, energy calibration represented by a quadratic equation:

$$E = a + bX + cX^2 \tag{2.1.9}$$

It is determined from the six-pair data of peak center channel (X) and the energy (E) by using least square method for determination of the coefficient (a, b and c).

In order to determine the radioactivity of the nuclei present in the sample, it is necessary to know the counting efficiency of the detector for the energies of different peaks identified in the spectrum.

Various kinds of efficiency definitions are in common use for gamma ray detectors[15, 16, 20]:

1. Absolute efficiency: The ratio of the number of counts produced by the detector to the number of gamma rays emitted by the source (in all directions).

*2. Intrinsic efficiency:*The ratio of the number of pulses produced by the detector to the number of gamma rays striking the detector.

3. Relative efficiency: Efficiency of one detector relative to another; commonly that of a germanium detector relative to a 3×3 in. diameter long NaI crystal, each at 25 cm from a point source, and specified at 1.33 MeV only. A standard method to define the relative detection efficiency for a coaxial detector, is described in IEEE standard test procedures[21]. The relative efficiency $\epsilon_R$for a coaxial detector is defined as:

$$\epsilon_R = \frac{\epsilon_{HPGe}}{\epsilon_{NaI(Tl)}} \tag{2.1.10}$$

Where, ϵ_{HPGe}= absolute efficiency at 1332 keV for a coaxial detector measured 25

cm from the source, and $\epsilon_{NaI(Ti)}$ = absolute efficiency at 1332 keV for a 3 in.$\times$ 3 in. NaI(Tl) scintillation detector measured 25 cm from the source.

4. Full-energy peak (photopeak) efficiency: The efficiency for producing full-energy peak pulses only, rather than a pulse of any size for the gamma ray.

Dependable radiation measurement requires a professional understanding of radiation detector calibration, as it applies to each detector and sample. An accurate calibration of the efficiency of the system is necessary to quantify the radionuclide present in a sample. it is not so easy to obtain the absolute peak efficiency,$\epsilon(E, x)$, which is a function of both γ -ray energy (E) and geometry (x) between source and detector.

Taking an approximation that geometry impact on the energy dependency of the peak efficiency is insignificant, one can consider the peak efficiency consists of two components being independent of each other.

$$\epsilon(E, x) = \epsilon(x)\epsilon(E) \tag{2.1.11}$$

where,$\epsilon(E)$ represents the energy dependent efficiency, often called "relative peak efficiency", and $\epsilon(x)$ gives the absolute peak efficiency. In practice, the absolute peak efficiency can be obtained for various geometries using the certified standard gamma sources of specific energy [18]. The relative peak efficiency can be written in the form:

$$\epsilon(E) = e^{[a+b-ln(E)+c.ln(E)^2]} \tag{2.1.12}$$

where a, b, and c are constants.

2.2 Neutron Activation Analysis (NAA)

Neutron activation analysis technique is based on the principle of converting various elements of the sample to radioactive isotopes by irradiating the sample using neutrons of various neutron sources. During irradiation, the naturally occurring stable isotopes of most elements are transformed into radioactive isotopes by neutron capture reaction. The radioactive isotopes so formed decay by emitting the gamma-radiations with specific energies and according to their characteristic half-lives varying from seconds to years[4, 6, 13].

NAA technique is used predominantly for the analysis of specific trace elements with higher sensitivity in the ppm to ppb range. As compared to other compatible analytical techniques, it is especially sensitive for the rare-earth elements such as; La, Ce, Nd, Sm, Eu, Tb, Yb, Lu, Sc, Co, Cr, Cs, Hf, Ta, Th, U and some others [4, 10, 16].

In general, it is a useful method for the simultaneous determination of about 25-30 major, minor, and trace elements present in geological, environmental, and biological samples in ppb-ppm range [6, 10, 13].

2.2.1 Radiation Sources for Activation Analysis

Activating particles in analytical activation analysis method can be neutrons, charged particles (electrons, protons, deuteron, alpha particles), X-rays or gamma rays. Neutrons can be obtained from Neutron sources such as, reactors, neutron generators, photo-neutron sources, radioactive neutron sources and spallation facilities [4, 22].

More than 95% of reports on the analysis of biological materials shows that, the most commonly used activating particles for activation analysis were neutrons.

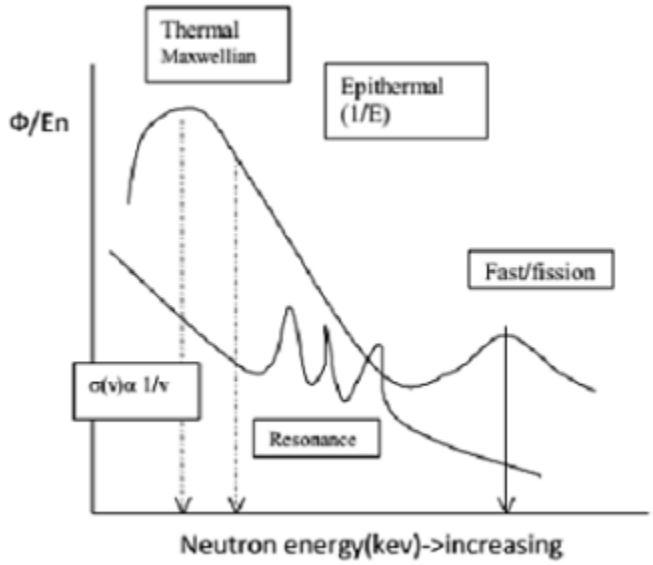

Figure 2.2: Typical distribution of neutron energies in thermal reactor

Samples activated in a nuclear reactor, may be exposed to various fluxes of thermal neutrons, epithermal, and fast neutrons as shown on Fig. 2.2.

Thermal neutrons have very low energies, in the range 0 to 0.5 eV, but with a most probable energy of 0.025 eV at 20^0C (corresponding to a mean neutron velocity of 2200 m /s). This energy is equivalent to that predicted by a Maxwellian distribution function for neutrons that have attained thermal equilibrium with their surroundings[4, 13, 16].

A minimum thermal neutron flux of between 10^9 and $10^{10}n/cm^2s$ is required for thermal activation analysis.Neutron capture (n, γ) reactions is the dominant reaction of thermal neutrons with most periodic elements. It is also more useful for activation analysis than charged-particle- or gamma-ray-induced reactions because they offer greater selectivity and sensitivity for elemental analysis[13, 22, 23].

Epithermal neutrons are obtained among a reactor neutron flux by exposing samples wrapped in cadmium foil to reactor neutrons which has the highest thermal neutron reaction cross-section. Epithermal neutron activation analysis (ENAA)show some advantages when analysing the following elements: Al, Au, Be, Cd, Cl, Cs, Fe, Hf, Mn, Ni, Rb, S, Sb, Sc, Se, Sr, Ta, Th, U, and V[23].

2.2.2 Categories of Neutron Activation Analysis

Neutron activation analysis techniques based on the formation steps and with respect tothe time of measurement are known as prompt gamma neutron activation analysis(PGNAA) and delayed gamma neutron activation analysis(DGNAA), respectively. In the case of PGNAA, the measurements take place during irradiation. The PGNAA technique is most applicable to elements with extremely high neutron capture cross-sections and short half-life such as; B, Cd, Sm, and Gd[4, 16].

DGNAA (sometimes called conventional NAA) is useful for the vast majority of elements that produce radioactive nuclide. In many cases, this new configuration yields a radioactive nucleus which also de-excites or decays by emission of one or more characteristic delayed gamma-rays as shown in Fig.2.3, but at a much slower rate according to the unique half-life of the radioactive nucleus. Such selectivity is a key advantage of DGNAA over other analytical methods because the technique is flexible with respect to time such that the sensitivity for a long-lived radionuclide that suffers from interference by a shorter-lived radionuclide can be improved by waiting for the short-lived radionuclide to decay[4, 13, 23]. DGNAA is one of the few routine techniques available in which the two general procedures employed namely, radiochemical neutron activation analysis (RNAA) and instrumental neutron activation

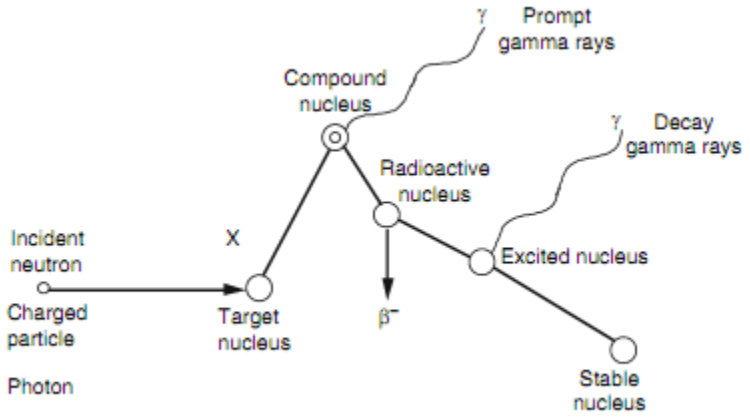

Figure 2.3: Schematic diagram of activation analysis principle

analysis (INAA)[16].

NAA, in principle, is a non-destructive analysis technique. However, when elements having a high neutron absorption cross-section are the major/minor constituents, whose activation products have appreciable half- lives, gamma rays from the matrix complicate the measurements by contributing to the background and dead time of the counting system, may lead to inferior detection limits. In such cases, it is essential to remove the matrix by pre- and post-treatment of a sample using chemical method called RNAA[23].

2.2.3 Basic Equations of Neutron Activation Analysis

1. Reaction rates

A nuclear reaction in NAA depends on the flux and energies of incident neutrons, the number of target nuclei of the particular type available for interaction, and the probability of occurrence for the reaction. Without the loss of generality, a reaction

rate in terms of the kinetic energy, E, of the incident neutron is given by

$$R = \int_0^\infty \sigma(E)\phi(E)\ dE \tag{2.2.1}$$

Which implies that a nuclear reaction is parametrized by the reaction cross section $\sigma(E)$, and the neutron flux spectrum $\phi(E)$. Reaction cross-section defines the property of the material and neutron flux spectrum or it related to the density of the particles travelling through the material[10].

Neutron flux spectrum and neutron capture cross-section can be expressed based on a neutron speed, v, as

$$\sigma = \sigma(v) and, \phi(v) = vn(v) \tag{2.2.2}$$

Where, n(v) is neutron speed distribution [23]. Thus, in terms of neutron speed, the equivalent expression for the reaction rate can be written as

$$R = \int_0^\infty \sigma(v)v\ n(v)\ dv \tag{2.2.3}$$

2. The Hogdahl Convention.

In general, with few exceptions, thermal neutrons have larger cross-sections for radiative capture (n, γ). Other types of nuclear reaction usually have low cross-sections to thermal neutrons. However, some isotopes of elements may exhibits such reaction with epithermal neutrons. Frequently, excitation functions for reactions exhibit peaks when the excitation energy coincides with the energy of an excited state of the compound nucleus. The peaks are known as resonances and are well separated as long as the differences between excited states are large. Based on this fact, the above reaction rate expression has got different parts by Hogdahl in 1965. The extended

Hogdahl convention is based on two principle ingredients: the proportionality of the (n, γ) cross section with $\frac{1}{v}$ up to E_{Cd} and the $\frac{1}{E^{1+\alpha}}$ (α is arbitrary parameter) shape of the epithermal neutron spectrum (E > E_{Cd}) [24].

The contribution of the fission neutrons (fast neutrons) to the total (n, γ) reaction rate is negligible and therefore not considered in the convention. Thus, the integral Eq.2.2.1 can be split into two parts based on a nuclear reactor neutron flux energy spectrum as the thermal part up to energy E_{Cd} (corresponding to neutron speedv_{Cd}) and the epithermal part, which is given by the expression

$$R = \int_0^{E_{Cd}} \sigma(E)\phi(E)\ dE + \int_{E_{Cd}}^{\infty} \sigma(E)\phi(E)\ dE \tag{2.2.4}$$

Due to the strong dependence of the parameters on the reactor neutrons energy, the above expression is neither the cross sections nor the neutron flux spectrum are known accurately. Hence, in order to represent the capture cross section of an isotope to within 0.1% tolerance, several data points are needed.

Without loss of generality, the expression commonly used in NAA for reaction rates representations by the Eq. 2.2.4 is given by

$$R = \phi_{th}\sigma_{th}gG_{th} + \phi_e I G_e = \phi_{th}\sigma_{th}[gG_{th} + \frac{1}{f}Q_0 G_e] \tag{2.2.5}$$

Where, g is generalised g-factor that measured the deviation of the thermal cross section from $\frac{1}{v}$ shape,

G_{th}-thermal flux depression factor,

I -effective resonance integral, $I = \int_{E_{Cd}}^{\infty} \frac{\sigma(E)}{E^{1+\alpha}} dE$

f -ratio of thermal to epithermal flux, ϕ_{th}/ϕ_e,

Q_0 -ratio of the resonance integral and the thermal cross section I/σ_{th},

G_e -resonance self-shielding factor.

The applicability and the accuracy of the above expression in NAA depend on the approximations involved in determining the constants.

(i). Thermal cross section, *(σ_{th})*- it can be calculated if parameters of the neutron resonances including the bound ones are well estimated. In particular, the thermal capture cross section may be approximated by the sum of contributions from separate s-wave resonances at the thermal energy, E_0, which are described by the single-level Breit-Wigner formula [25]. The average reaction cross section of thermal neutrons with several elements of the periodic table varies with the inverse ($\frac{1}{v}$) neutron velocity.

Based on these facts, the contribution of thermal neutrons to the reaction rate, expressed in neutron speed domain is given by

$$R_{th} = \int_0^{v_{Cd}} \sigma(v) v \; n(v) \; dv \tag{2.2.6}$$

For some $1/v$ absorber nuclide, the cross section can be

$$\sigma(v) = \sigma_0 \frac{v_0}{v} \tag{2.2.7}$$

where v_0 thermal neutron speed 2200 m/s by definition, σ_0 cross section at neutron speed v_0 [26]. Substituting into the equation for R_{th},

$$R_{th} = \sigma_0 v_0 \int_0^{v_{Cd}} n(v) \; dv = \sigma_0 v_0 N_t \tag{2.2.8}$$

Where, N_t, is the total thermal neutron density.

The reaction rate proportional to the total thermal neutron density, is independent of the neutron speed distribution [25, 26].In the energy domain, the equivalent

expression for the thermal reaction rate is

$$R_{th} = \int_0^{E_{Cd}} \sigma(E)\phi(E)\ dE \tag{2.2.9}$$

Simplification of the above integral for reaction rate in the energy domain is not possible. The expression for reaction cross-section in the Eq. 2.2.9 for a $1/v$ absorber can be[27]

$$\sigma(E) = \sigma_0\sqrt{\frac{E_0}{E}} \tag{2.2.10}$$

where E_0 is the energy of thermal neutrons corresponding to v_0 and is equal to 0.0253 eV. Assuming that the thermal neutron flux has Maxwellian distribution[26, 27]

$$\phi(E) = E\ e^{\frac{-E}{kT}} \tag{2.2.11}$$

where, k is the Boltzman constant T is the temperature.

Considering the fact that the reaction rate is proportional to the total thermal neutron density, but not to the total thermal neutron flux, then the thermal reaction rate is given by

$$R_{th} = \int_0^{E_{Cd}} \sigma(E)E\ e^{(\frac{-E}{kT})}\ dE \tag{2.2.12}$$

By inserting cross-section expression for a $1/v$ absorber shown in equation (2.2.10)

$$R_{th} = \sigma_0\sqrt{E_0}\int_0^{E_{Cd}} \sqrt{E}e^{\frac{-E}{kT}}\ dE \tag{2.2.13}$$

The average thermal cross section σ_{th} is defined by[26]

$$\sigma_{th} = \frac{\int \sigma(E)\phi(E)\ dE}{\int \phi(E)\ dE} \tag{2.2.14}$$

Substituting Eq. 2.2.10 and Eq.2.2.11 into the above Eq. 2.2.14, we can get

$$\sigma_{th} = \frac{\sigma_0\sqrt{E_0}\int \sqrt{E}e^{\frac{-E}{kT}}\ dE}{\int Ee^{\frac{-E}{kT}}\ dE} \tag{2.2.15}$$

Without loss of generality, we can extend the integration limits from 0 to ∞ and recognize the integral in the numerator as the gamma function $\Gamma(3/2)$ then using the relation between the energy and the temperature $E_0 = kT_0$, then the average cross section gives us

$$\sigma_{th} = \frac{\sqrt{\pi}}{2}\sigma_0\sqrt{\frac{T_0}{T}} \tag{2.2.16}$$

This relation is strictly valid only for a pure $1/v$ absorber nuclide in a Maxwellian neutron spectrum.

(ii). Westcott g-factors: - In practice, the cross sections may deviate from the $1/v$ behaviour and the spectrum may be distorted (depending on the irradiation facility). Westcott, assuming that the spectrum was Maxwellian, attempted to correct for the non-ideal cross section behaviour by introducing the Westcott g-factor.

Despite many of these constrains, it was possible to introduce an alternative definition of the generalized g-factor, used to calculate reaction rates and is applicable to non- absorbers as well as spectra, which deviate from the Maxwellian shape.

Using Eq. 2.2.5 and Eq. 2.2.9, we can get the relation

$$\phi_{th}\sigma_{th}gG_{th} = \int_0^{E_{Cd}} \phi(E)\sigma((E)dE \tag{2.2.17}$$

Arbitrarily we define[26]

$$\phi_{th} = \frac{\sqrt{\pi}}{2}\int_0^{E_{Cd}} \phi(E)dE \tag{2.2.18}$$

Assuming the thermal flux depression factor G_{th} is equal to 1 and inserting Eq. 2.2.18 into Eq. 2.2.17, the definition of the generalized g-factor gives us

$$g = \frac{2}{\sqrt{\pi}}\frac{\sigma_{th}}{\sigma_0} \tag{2.2.19}$$

Substituting the integrals with the expression for σ_{th}, it is easily seen that for a $1/v$ absorber in a Maxwellian spectrum, the above definition gives the well-known Westcott g-factor relation [27].

$$g = \sqrt{\frac{T_0}{T}} \tag{2.2.20}$$

Practically, the values of g-factor for the majority of nuclei are close to unity. Essential deviations of g-factor from unity are observed for the radiative neutron capture at the following nuclei (the values of g are given in brackets): $^{113}Cd(1.34)$, $^{135}Xe(1.16)$, $^{149}Sm(1.71)$, $^{155}Gd(0.84)$, $^{176}Lu(1.75)$, $^{182}Ta(1.64)$, $^{239}Pu(1.13)$. [26, 27].

(iii). The thermal neutron flux depression factor, (G_{th}):- most often, it referred to as the thermal self-shielding factor and it implies primary dependence on the measured nuclide in the sample. This is indeed the case with resonance absorption, but not in the thermal range, where neutron transport effects play a dominant role [26, 27, 28].

2.2.4 Instrumental Neutron Activation Analysis (INAA)

Instrumental neutron activation analysis can be defined as a chemical element analysis made by nuclear activation followed by the measurement of specific induced radio activities without the use of any chemical treatment like RNAA. The main advantages of INAA are economy, speed and non-destruction nature of the sample under study.

Now a days, the application of INAA in any sample provides faster and an analysis at maximum sensitivity due to the rapid stride made in the development of steady- operating reactors, automatic methods to transfer samples to irradiation and measurement systems, efficient and assembled spectrometer systems, and high speed computational gamma-spectrum analysing software [22, 29].

The practical application of INAA is simply based on Eq. 2.1.7 and Eq. 2.2.5. Hence, the expression for the rate of production of radioactive nuclide irradiated for a time interval of ti is

$$A(t_i) = N_0.R(1 - e^{-\lambda t_i}). \tag{2.2.21}$$

In the expression,

$R = (\sigma_{th}\phi_{th}gG_{th} + I\phi_e Ge)$ - nuclear reaction rate,

$A(t_i)$ - the activity of an irradiated sample after time t_i,

$N_0 = (N_A m_x \theta / M_x)$ is the number of the target element, where,

m_x - the mass of an element in a sample(mg),

M_x, - the molar atomic mass(mg/mol.),

N_A - Avogadros number, and

θ - atomic abundance of the isotope of an atom.

Let T, be the end of irradiation period, then activity is given by:

$$A(T) = RN_0(1 - e^{-\lambda T}) \tag{2.2.22}$$

After a delay time, td, the activity becomes

$$A(t_d) = A(T)e^{-\lambda t_d} \tag{2.2.23}$$

The photon emission rate is equal to the product of the decay rate $\lambda N(t_d)$and the gamma-emission probability, p_γ . Then the average characteristic gamma-photon emission rate, R_c, over the time period from t_d to $t_d + t_m$ is obtained by integrating Eq. 2.2.23 as

$$R_c = \int_{t_d}^{t_d+t_m} A(T)(e^{-\lambda t})dt = \frac{A(t_d)p_\gamma(1 - e^{-\lambda t_m})}{\lambda} \tag{2.2.24}$$

Where, t_m is measuring time. Substituting the values for $A(t_d)$ from Eq. 2.2.23, the above equation gives

$$R_c = \frac{A(T)p_\gamma e^{-\lambda t_d}(1 - e^{-\lambda t_m})}{\lambda} \tag{2.2.25}$$

Taking in account of the decay of activated radionuclide during the decay period (t_d) and counting($t_c = t_m$), the total activity of radionuclide is calculated as

$$A(t_c) = RN_0 p_\gamma S.D.C.t_c \tag{2.2.26}$$

where, $S = (1 - e^{-\lambda t_i})$,

$D = e^{-\lambda t_d}$,

$C = (1 - e^{-\lambda t_c})/t_c$

However, in the case of radiation measurements, a detector can record the number of events for a particular radionuclide or only a fraction of the number of gamma-decays calculated from Eq. 2.2.26.

This is because not every decay can emit a characteristic gamma-ray, and once a gamma ray is emitted it may not reach the detector. This event defines the efficiency of a detector for detection and measurement of a particular gamma ray. Thus, $\epsilon(E)$ is the Full-energy peak efficiency of energy, E, and abundance,p_γ, of particular gamma-ray. Therefore, the number of net counts of a given gamma-ray in a neutron activation analysis has the formulation:

$$N_c = \frac{RN_0 \epsilon p_\gamma S.D.C.t_c}{\lambda} \tag{2.2.27}$$

where, N_c is net peak area or net count of the gamma-ray, and ϵ is detection efficiency for this gamma-ray of energy E (including attenuation and true coincidence effects).

Practical INAA technique utilizes the fundamental Eq. 2.2.27 but the measurement procedures may depend on the standardization methods namely; Absolute standardization, relative/comparator/standardization and single-comparator(k_0-INAA) standardization methods.

2.2.5 Standardization methods in INAA

The expression 'standardization' has to be interpreted in the sense of finding the correlation between the concentration of an analyte and the corresponding 'intensity' of the witness signal in the activation spectra. The concentration, C, of an analyte in the sample can be derived from the area of the full energy peak, N_c, corresponding to a characteristic gamma from a decay product. The mass of an analyte in the sample can be calculated from Eq. 2.2.27 considering the dead time correction factors as

$$m_x = \frac{N_c M_x \lambda}{(RN_A\theta)(p_\gamma \epsilon T_L)(S.D.C)} \tag{2.2.28}$$

where, T_l is live measuring time (s).

1. Absolute standardization

In case of absolute standardization, the mass of an element can be calculated using Eq. 2.2.28 by taking the values of some parameters from literatures and corresponding measured parameters. In fact, the values for σ_0, (Q_0), p_γ and θ can be found in literature, but due to their combined uncertainties, they lead to a deterioration of the accuracy and traceability of the analytical results up to 20% [13, 16, 22]. Besides that, the accurate determination of the neutron field characteristics, the detector efficiency, neutron self-shielding factors, require quite some effort from the analyst [30].

2. Comparator standardization (Relative method)

A comparative analysis with a standard reference sample containing a known mass, ms, of the same elements is usually performed. If the standard matches the size and composition of the unknown sample, the irradiation and measurement conditions of the control sample and SRM (Standard Reference Material) are same, the ratio of counts, $(\frac{N_{c,x}}{N_{c,s}})$, can be used for the calculation of the unknown mass. Thus, in the relative method, some parameters will offset each other and not impact the analysis results [26]. Thus, the mass of unknown analyte can be obtained from the formulation,

$$m_x = m_{st}(\frac{N_{c,x}}{N_{c,st}}) \tag{2.2.29}$$

Then, the actual calculation of the concentration of unknown elements, obtained from Eq. 2.2.28 and Eq. 2.2.29, [31];

$$C_x = C_{st}\frac{m_{st}}{wm_x}\frac{A_{c_0,x}}{A_{c_0,st}}R_\theta R_\phi R_\sigma R_\epsilon \tag{2.2.30}$$

Where, w - the dry mass correction factor,

m_x and m_{st} is unknown and standard material samples mass in gram respectively.

A_{c_o} - the decay-corrected counting rate for indicator gamma-ray,

R_θ - the ratio of isotopic abundances for the unknown and standard,

R_ϕ - the ratio of neutron fluences (including fluence drop off, self-shielding, and scattering),

R_σ - the ratio of effective cross sections if the shape of neutron spectrum differs from the unknown sample to standard,

R_ϵ - the ratio of counting efficiencies (differences due to geometry, gamma-ray self-shielding, and dead-time effects) [29, 32].

The decay-corrected counting rate for indicator gamma-ray for a measured nuclide is given by

$$A_{c_0} = \frac{N_c \lambda e^{\lambda t_d}}{1 - e^{-\lambda t_c}} \tag{2.2.31}$$

where N_c is the number of counts in the indicator gamma-ray peak

3. Parametric method (k_0 - Standardisation)

The k_0- standardization method of NAA , a concept can be interpreted as an absolute standardization method [33]. In this method, the so-called k-zero factor, k_0, for each radionuclide is determined experimentally by comparison with the most popular single-element monitor like gold foil. It relies k_0 and Q_0 factors and a few other parameters, which are composite constants that can be derived from the basic nuclear data [34]. The equation to calculate concentrations by the k_0 method is

$$C_x = \frac{A_{sp}}{A^*_{sp}} \frac{1}{k_0} \left(\frac{f + Q^*_0(\alpha)}{f + Q_0(\alpha)}\right) \frac{\epsilon^*}{\epsilon} \tag{2.2.32}$$

where, A_{sp} - specific activity of the comparator element $(*)$ or sample,

k_0 - k-zero factor for this isotope,

f - ratio of thermal to epithermal flux,

Q_0 - ratio of resonance integral to thermal neutron cross-section,

(α) - deviation of the slope of the epithermal neutron flux from $1/E$.

In practice, these parameters usually determined by direct measurements, partly because equivalent constants derived from the basic data are often discrepant. In a parametric method, the values for flux and detection sensitivities are obtained for the irradiation facility and spectrometry equipment through calibration, and tabulated experimental parameters are used for the determination of each individual element [29, 30, 34]

2.2.6 Limitation of Neutron Activation Analysis(NAA)

There are some limitations in NAA as analytical methods, which cannot meet the multitude of the requirements of analytical chemistry. Many literatures have been described limitations like, availability of the required facilities, pollution and health issues, and time needed for measurement. Apart from these issues, one can categorize NAA limitations as compared to the compatible analytical techniques and the existing achievements of NAA results[22, 32]. Thus, we can categorized them based on the resulting errors they influence on the measurement as,

- Chemical limitations: The inability to determine the chemical form of the element in the sample. Chemical errors occur in sampling technique, weighing of samples, chemical yield determination.

- Nuclear limitation; It Occurs during activation part of the technique and affects the overall sensitivity of the required element. Nuclear errors occur by; interfering nuclear reaction and by changing irradiation conditions.

- Gamma-ray spectrometry limitation; It affects the measurement results precision and sensitivity due to the background radiation and stability of measurement system.

- Sensitivity Limitation; This can be due to radionuclide parameters like half-life, nuclide interference, and analysts experiences.

2.3 Measurement Principles in INAA Technique

The major techniques in NAA consists of a series of steps whose parameters in order to optimize the sensitivity and accuracy of the result with maximum effort. Those major techniques include;the optimum nuclear reactor, a suitable irradiation facility, the pre-irradiation treatment, Irradiation condition, post irradiation treatment, optimum radiation measurement system, a desired precision and accuracy.

An important property of instrumental neutron activation analysis is that its strong dependence of the quality of the results on the matrix activities present at the time of radiation measurement. When setting out to solve an analytical problem by means of instrumental neutron activation analysis method, the analyst must select or devise an appropriate procedure. The procedure to be followed must, therefore, be adjusted to suit the type of sample analysed in order to obtain accurate and precise results [30].

2.3.1 Accuracy and Precision

The performance or quality of measurement systems has been traditionally defined in terms of accuracy and precision. Therefore, if sufficient care are made in activation analysis, the result of a given measurement can generally be improved to some level [22, 35].

we can have blithely termed precision is now discussed as repeatability, the variability of a method when applied to measurements on a single sample within a laboratory. The precision of activation analysis is sometimes taken to be identical with the precision of counting, since this is readily calculable from the square root of the total number of counts obtained [36].

The accuracy or absolute sensitivities of NAA of a particular element depend on many factors which includes; the irradiation parameters (i.e., neutron flux, irradiation, and decay times), measurement conditions (i.e., measurement time, detector efficiency), nuclear parameters of the elements being measured (i.e., isotope abundance, neutron cross-section, half-life, and gamma-ray abundance) [22, 23].

The accuracy of an individual NAA of elements determination usually ranges from $10^3 - 10^{10}$grams per gram of a sample. However, the sensitivity of NAA to some of the elements namely B, Be, Bi, C, Li, N, Nb, Ne, O, P, Pb, S, Si, and Tl are very poor or not ordinarily detected by this method [31, 36].

2.3.2 Basic Response Functions

In order to get a quality result from a particular INAA measurement, a variety of response functions have been proposed for use in the optimization of activation analysis. It is important to realize that the function used to compute the response determines what aspect of analytical performance is optimized. One virtue of this response is that the detection limit, the measurement precision, and the analyst's degree of familiarity with the sample type are combined in a single function. As is required, the information content increases as the detection limit and precision are improved and as the number of replicates increases [35].

In the cases of INAA multi element measurement optimization, the strategy employed involves three steps. These optimizations using three response functions proposed includes [31, 35];

1. The average uncertainty in peak area.

2. The average detection limit.

3. The number of elements simultaneously detectable.

1. Gamma-ray spectroscopy

One of the major development of INAA has been the resolution of complex gamma-spectra without the benefit of prior separation by chemical element. It is a technique of determining the number of radionuclide present by identifying gamma-ray photopeak energies and successively subtracting unwanted gamma-ray and background spectrum[35].

The component of a spectrum used to identify and measure a pure radionuclide is full-energy peak (photopeak). The rest components like Compton continuum and pair production events are considered in background spectra because they are not only the sources but it may be a result of detector and geometry factors[15]. However, there may be more complications appear when the number of important radionuclide contributes to the total spectrum is an unknown. For instance, when short-lived radionuclide are present and when the total radioactivity is small. In order to solve all such problems in the complex gamma-ray spectrum, spectrum stripping method was developed using an analytical procedures, electronic means and numerical means by a computer[18, 20].

(i)Peak search and area measurements

A computer program uses data convolution technique to locate peaks in the spectrum, determine the areas, and estimate their energy in complex gamma spectrum analysis.

A gamma spectroscopy software should localize the peaks and calculate their principal characteristics (area, resolution, and energy) without making these parameters

sensitive to the statistics or to the complexity of the spectra [37, 38]. The most important criteria in a peak search program is to detect as few as possible false peaks, but do not neglect the peaks with low intensity.

A gamma-ray spectrum consists of a large number of channels, about 4096-channel spectrum covering an energy range of 2048 keV. Successive channels represent increasing energy and the content of each channel representing the number of counts received within a few keV energy window. Within such a spectrum, a gamma-ray appears as a distribution of counts, approximately Gaussian (Normal distribution), about a central point which we can take to represent the gamma-ray energy [35, 39, 40, 41].

In practice, for instance, the gamma spectroscopy software ($GammaVision^{TM}$), the Gaussian function and experimental peak shape function are the basic mathematical functions to describe the peak shapes in the gamma-ray spectrum [38, 41]. Thus, the Gaussian peak shape may be expressed by a function:

$$Y_i = \frac{N_c}{2.5066\sigma} e^{\frac{-(t-s)^2}{2\sigma^2}} \tag{2.3.1}$$

Where: s- Peak centre, N_c - Peak area (net photo-peak area of the fitted peak), σ - Peak width=0.42466$\times$ FWHM (full width at half maximum).

For a complex gamma-ray spectrum, each has normal distribution function, and the least- squares analysis method are used for analysis in modern computer programs. it has been concluded that an analytical form consisting of a main Gaussian, a tail function riding on a linear background, and a step function are needed to define the photo-peak[37].

The procedure was to locate the highest channel in the peak and then to mark the peak limits or an equal number of channels away from the centroid channel. In this respect, the most useful systems are those which monitor the actual peak uncertainty

continuously during the count and allow acquisition to be terminated when the desired precision is achieved. Precision in peak area measurements is therefore significantly influenced by the magnitude of the background counts [20, 38].

(ii). Peaked-background correction

The background beneath gamma-ray spectrum peaks can arise from many sources. In most cases, the background will represent the Compton continuum from other gamma-ray interactions within the detector, within the sample itself and from general background radiation interaction with the shielding and the detector. Both background radionuclide and other radionuclide in the sample will contribute to this peak background[15, 41].

A background spectrum to the peak is a continuum and its level estimated by using the channel contents at the upper and lower edges of the peak region. The estimation can be made more precise (i.e. less uncertain) by using more channels to estimate the mean count per channel under the peak. Based upon the manufacturer and the situation, most commercial MCA and spectrum analysis programs use up to 5 channels. For instance, GammaVision chooses 5, 3 or 1 channel widths on each side of the peak independently, depending upon whether the channels are deemed to represent a flat portion of the background continuum [18, 35, 38].

The radioactivity of the radioisotopes can be determined from the area of the peaks and the efficiency curve of the detector [31, 39]. Once the net count or measurements of peak areas and peak background correction are done for a given gamma-energy, the variance of the net peak area can be obtained by:

$$Var.N_c = Var.(N_c + B) + Var.B \tag{2.3.2}$$

Thus, the standard deviation sum in quadrature and gives us the expression,

$$\sigma^2 = \sigma^2_{N_c+B} + \sigma^2_B \tag{2.3.3}$$

In certain circumstances, in particular, when small peaks lie on large backgrounds, the uncertainty on the background estimate can dominate the total uncertainty of the peak area measurement. If the sample count rate is higher than background, then the background is proportionately less important and can be counted for a shorter time. Ultimately, of course, as the sample activity becomes very large the background becomes insignificant and we might choose not to measure it at all [35, 41].

2. Measurement decision limits

Liod currie, in his 1968 paper, defined measures of detectability, firmly based on the statistical theory of hypothesis testing. The three specific illustrations of analytical procedures given with worked-out equations for the three quantities were; spectrophotometry, radioactivity, and a complex case of activation analysis. Curries formulation about the concepts of qualitative and quantitative analysis limits have been universally incorporated in many rules of practice governing measurement procedures, international standards regulations, and software [42].

(i). Critical limit (L_C): -is used to test an experimental result or the significance of net count. After a peak area has been measured, it is important to establish its statistical significance. Since a peak becomes non-significant only by being lost in the background, this cannot be done by reference to the peak area alone but must take into account the uncertainties of the background [43].

(ii). Determination limit (L_Q): - refer to the capabilities of the measurement process itself or the measure of statistically maximum reasonable count. The upper

limit is used to assess the statistical validity of a calculated net count. If the net count, N_c, is below or equal to L_Q, then the activity must be declared not detected and determination limit or less-than level quoted [42].

(iii). Detection limit (L_D): - refer to the capabilities of the measurement process itself. it is the minimum number of counts that can be confident of detecting. The detection limit refers in measuring a sample that the count has to be for 95% certainty of detection. However, if the sample activity did happen to be exactly L_C, statistically we would only be able to be 95% sure of detection in 50% of cases because the counts would be distributed symmetrically about Lc [38]. Although for the peak area case the expression for L_D is more complicated, the mathematics for normal distribution of a signal is identical. The final expression is based on Eq. 2.3.6 and Eq. 2.3.7 as

$$L_D = L_C + K\sigma_D = 2.71 + 3.29\sqrt{[B(1 + \frac{n}{2m})]} \tag{2.3.4}$$

Where, B - peak background count, m - the number of step function and n is total number of channels used for the background estimation [35, 42].

Practically, the calculation of L_D or detection limit (DL) would be made once a background or peak spectrum, one which represented the particular situation for which detection limit is needed, had been measured. However, the detection limit relates to a particular confidence of detection in the equations derived above, 95% confidence.

(iv). Minimum Detectable Activity (MDA): - This limit is, an activity rather than a count limit. It is often equated to the activity equivalent of the detection limit, L_D [35, 43]. The MDA defines the performance of the method. all of the commercial spectrum analysis programs give the option of quoting MDA when a peak is not detected .

The procedure should be: Using data from the spectrum, calculate the L_D at the appropriate region of the spectrum and convert to activity. That is the MDA for 95% confidence of detection[38]. Practically, MDA can be obtained using the expression;

$$MDA = \frac{L_D}{p_\gamma \epsilon T_c} \tag{2.3.5}$$

(v). Decision Limits for Physical Quantities: - It is the Limits for qualitative and quantitative analysis value only when expressed in terms of the physical quantity of interest, such as grams or atoms [42]. In order to make such a decision, we needs to know only the net number of counts resulting from a specific experiment, and the critical number of counts, L_C. The connection between decision limits and physical quantities is simply made by means of the relevant calibration factor[38]. For instance, the detection limit, L_D, may be related to the minimum detectable mass, $m_D(g)$, by means of Equation;

$$L_D = \kappa m_D \tag{2.3.6}$$

where κ represents an overall calibration factor in the INAA technique relating the detector response to the mass present.

Based on Eq.2.2.28 and Eq.2.2.30, the estimate of the Lower Limit of Detectable(LLD) element's concentration (in ppm) can be calculated using INAA formula [22, 44].

$$LLD = \frac{M_x L_D e^{\lambda t_d}}{N_A R \theta p_\gamma \epsilon t c (1 - e^{-\lambda t_i})} . \frac{10^6}{m_{sm}} \tag{2.3.7}$$

where Mx - the atomic weight of the element,

L_D- the minimum detectable counts,

N_A- the Avogadros number,

R-reaction rate, and

m_{sm}- the sample mass in gm.

This calculation uses to evaluate the sensitivity of overall analytical procedure for the measurement of the minimum amount of a given element. Thus, optimization of INAA sensitivity takes in account sample size, the properties of an element and matrix, the properties of irradiation facility and condition and the radiation measurement system (specially efficiency of detector).

3. Multi-element irradiation and counting strategies

One of the essence of a multi-element optimization process is that, the separation of the elements to be determined into groups for irradiation and counting regimes. As this grouping entails decisions concerning the active isotopes and gamma-ray energies used for determination, the complexity of the response surface or sensitivity searched in the subsequent optimization stage can be reduced. Most elements require separate irradiation and counting procedures on the basis of half-life of product radionuclide and the neutron spectrum parameters in the irradiation channels of a reactor. These may also be avoided reaction and photo-peak interferences in the standard spectrum by omitting the offending element [31, 44, 45].

An optimum irradiation time of a given sample in a given irradiation facility is to obtain the maximum amount of radioactivity (saturation activity) of a given radionuclide. This can be achieved when a sample irradiated for a sufficient length of time (five times the half-life of a radionuclide) in order to measure 97% of saturation. However, longer irradiation does not give us the required result.

For short-lived radionuclide, irradiation time is not that much difficult but for long lived radionuclide, the choose of irradiation requires balancing of interfering radionuclide with that of obtaining the minimum level of radioactivity[22, 31]. Thus,

it requires a practice to reduce the irradiation time to the minimum value consistent with the desired accuracy and sensitivity.

It is clear from these that any counting strategy must be a compromise to allow the determination of groups of elements with similar half-lives at adequate sensitivity. In a full analysis i.e., the prompt as well as delayed γ -emission analysis, the short-lived nuclide and the most intense gamma- radiations are determined and measured first then observation about their decrease of intensity is made.

2.3.3 Quality Control technique in INAA

Nuclear analytical techniques have been demonstrated many times to be reliable and under complete statistical control. The tools for validating the performance of an INAA procedure have been and still are determinations in an inter-laboratory comparison exercise, comparisons of the procedures results with those obtained from a different analytical method, and determinations in certified reference materials (CRM) [29, 45, 46].

Due to the advantages of being relatively matrix- and amount-independent, it is normally not difficult to demonstrate the accurate performance of nuclear analytical techniques. Based on the discussed advantages, it is also relatively easy for us of INAA to include the concept of traceability in the quality statements on results from the users laboratory [31]. Accuracy of a INAA determination is usually between 0.2% and 10% of the reported value, depending on the element analysed and its concentration in the sample [44].

2.4 INAA Uncertainty Considerations

Analysts can easily obtain estimates for each of the components of uncertainty for each individual sample measurement. This detailed knowledge not only provides realistic estimates of the overall combined uncertainty, but also provides the information needed for a reduction of these uncertainties for a particular measurement problem.

2.4.1 Sources of uncertainty

It was, at one time, conventional to identify uncertainties as random or systematic. According to the ISO terminology, the uncertainties can be divided into Type A (uncertainties evaluated by statistical methods) and Type B (all other). Type A uncertainties are defined as those that have been determined by repeated measurements to assess the magnitude and distribution of the parameter [29].

Modern usage is to treat each source of uncertainty separately and calculate the standard uncertainty, taking into account the type of distribution involved, before combining with other uncertainties. All of the statistical relationships and the equations for combining uncertainties are valid as long as we are consistent in the use of standard uncertainty. It is only necessary to work out the standard uncertainty of the assumed distribution [35, 39].

1. Uncertainty on sample preparation

Ideally, samples presented for gamma spectrometry would be homogeneous. Even though in the real-world samples are often less representative, appropriate sampling technique and homogenization procedure improves samples representativeness. The

uncertainty on the mass is small and can easily be quantified in relation to the significance of the least significant digits on the balance display. In addition, Homogeneity can be properly assessed by a number of measurements on sub-samples[31, 44].

2. Uncertainty in irradiation and Sample Integrity

A common source of error in cases of comparative (relative) INAA arises because sample and standard are not subjected to the same flux of activating particles and the flux in a sample can be significantly affected by the absorption of neutrons. if the absorption of activating particles by the matrices of sample and standards differ, the inner parts of each will be activated in different fluxes, an effect known as self-shielding [31, 36, 44].

This problem is normally overcome by on-line monitoring of the neutron flux, and most research reactors, especially small reactors, have installed such a system. Biological matrices are poor absorbers of both neutrons and gamma rays, but good absorbers of charged particles. In most cases, the uncertainty from this source is low ($< 0.5\%$)

Other uncertainties in the gamma-ray detection yield are matrix effects that can suppress some gamma rays by self-absorption. These effects are gamma ray energy dependent, and can be mathematically or experimentally corrected [45].

3. Geometry Uncertainties in Irradiation and Counting

Irradiation position is a basic requirement for the correct characterization of the irradiation facility. Local and temporal neutron flux density gradients as well as gradients in the neutron energy spectrum of the irradiation position must be well

understood and known for each irradiation geometry [44]. An effective means for quality control of the irradiation are flux monitor foils. It is highly recommended to use these with each irradiation of samples and standards to verify the assumptions about the accurate irradiation geometry [31].

Counting geometry correction is vital usually the case with different energy and activity gamma-rays of samples, this uncertainty can be significant [47]. The uncertainty due to counting geometry can be controlled very reliably by good efficiency calibration of the detector for various source shapes using appropriate software available for the better calculation.

By using an irradiated standard identical to the sample, as is possible in the comparator method, the need for geometry, self-shielding, and self-absorption corrections is eliminated, and thus the potential errors are reduced [44, 47].

4. Timing Uncertainties

An accurate time base is essential for the accurate calculation of decay factors for different counting and decay times during the measurement. More important to consider are uncertainties in the published half-life of the indicator nuclide. The recording of real time and live time of a count with a good precision (with less than 300 s half-life, an order of 0.1 s or even 0.01 s timing resolution) is standard in many modern MCA systems. Other methods of minimizing this type of uncertainty are of course irradiation and counting procedures that are exactly the same throughout a measurement cycle [31].

5. Uncertainties in Signal Detection and Processing

Relevant mechanisms that cause loss of counts from the photo-peak include partial conversions of the incident energy event with part of the energy escaping the detector, true coincidence summing, random summing of pulses (pulse pile-up), and dead time. While the first two effects are part of the detector's efficiency and are the same for all sample and standard counts, random summing and dead time are dependent on a sample's activity. Pile-up losses are corrected by most spectroscopy amplifiers with the employed pile-up detection and rejection circuit. While coincidence losses are not an issue for comparator NAA calibration, a computational correction must be applied [44, 45].

All MCAs exhibit counting losses due to the dead time required to process pulses from any given source of radioactivity. This problem can be solved by the well-proven live-time extension method employed in most nuclear spectroscopy systems [20].

6.Uncertainty in the counting and spectra analysis

Statistical counting uncertainties are always present, of course, but are always taken into account within the spectrum analysis program because these uncertainties vary from sample to sample, from nuclide to nuclide within the sample and even from peak to peak of each nuclide. If these corrections are made by the spectrum analysis program, those uncertainties assigned by the program are reasonable [19, 20].

Spectra analysis uncertainties results mainly from the evaluation of peak area and subtraction of baseline, especially for the analysis of multi-peaks. A major effort has been made to develop effective software to improve the analysis of gamma spectra [18, 48, 49].

7.Uncertainty due to Interferences

There are potential interferences in activation analysis that must be considered and corrected for. These include interfering activation reactions and gamma-ray spectral interferences [40].

In cases of interfering activation reactions, a given radionuclide can often be produced in more than one way. If the indicator nuclide used in the analysis is produced from an element other than the analyte, then a primary interfering reaction occurs. The magnitude of many of the primary interferences in NAA depends on the fast-to-thermal flux ratios in a reactor. This interference can be minimized by irradiation in a well-thermalized neutron flux [49].

Gamma-ray spectral interference arises from the overlap of gamma peaks from two radionuclide emit gamma rays with the same energy, nearly the same energy or due to the energy broadening characteristics of the detection system. For the former two cases nuclide, all practical instances possible are a separation via the half-life or recount the sample after a suitable decay period after which the interfering activity has substantially disappeared [40].

In some instances, a spectral separation is difficult even with the best-resolution detectors when the count rates are high and the peaks get broadened. This interference can be resolved through the use of other gamma-ray lines or select an alternative interference-free gamma photo-peak of the isotope, by utilizing the different half-lives and choosing to use a peak-fitting program rather than a simple total peak area algorithm, so that overlapping peaks may be adequately fitted [47].

8. Calibration uncertainties

Care is usually taken that specifically prepared (multi element) standards minimize interferences and allow approaching the unity condition for comparison of the unknown with the standard. When purchased, the reference material from which the calibration sources are prepared will be accompanied by a calibration certificates. The degree of scatter of the calibration points around the calibration line can be said to represent both the goodness of fit of the calibration data and the uncertainty of estimating the efficiency obtained by calculation from the calibration equation. This interpolation uncertainty is the figure that one would wish to include in the uncertainty budget[49, 50].

The comparator method has been shown to deliver highest accuracy with standards prepared from chemicals of known purity. A high quality INAA measurements achieving uncertainties about 10 % based on the comparator method [49].

2.4.2 Uncertainty Budget

we have a duty to take care that the results are as accurate as possible and the uncertainty that we quote is realistic. It must take into account all of the known sources of uncertainty within the measurement process. Identifying and quantifying those uncertainties provides us with an uncertainty budget.

In setting up the budget, the uncertainty of all parameters which contribute to the final result must be assessed and quantified. It can be pointed out that matters such as errors caused by equipment malfunction or operator error do not form part of an uncertainty budget. A good point at which to start setting up an uncertainty budget is to look at the way in which the result is calculated.

In the case of radioactivity measurement, the equation converting net peak counts, C, to activity per unit mass, Asp, might be [12, 19]:

$$A_{sp} = \frac{(N_{net} - B_p).e^{-\lambda t}.R_{CR}S_{CR}}{mT\epsilon p\gamma} \tag{2.4.1}$$

Where, $e^{-\lambda t}$, the decay correction, B_p, the peaked-background correction, R_{SR}, a possible random summing correction and S_{SR}, a possible self-absorption correction.

An uncertainty budget for INAA of comparator method can be developed on the basis of Eq.2.2.30. All parameters are essentially independent, therefore the combined standard uncertainty in Cx can be obtained from the well-known differential expression for the combination of individual uncertainties [50, 51].

$$\sigma_{c_x}^2 = \sum_i (\frac{C_x}{p_i})^2 u_{p_i}^2 \tag{2.4.2}$$

where pi is the i-th parameter on the right-hand side of Eq.2..2.30 and u is the uncertainty associated with the parameters. Then, the category of uncertainty of these parameters are,

Type A: (m,N_c)

Type B: (C, λ, t_d, t_c, R_θ, R_ϕ (including fluence gradient, neutron self-shielding and scattering), R_σ, R_ϵ (including geometry, gamma-ray self-shielding and counting effects)).

The overall uncertainty on a result depends critically on the counting uncertainty, that, in turn, depending upon the magnitude of the background to the peak [50].

Chapter 3

Instrumentation and Methodology

3.1 Introduction

There are several international organizations and literatures describing about appropriate methodologies, procedures being adopted and materials for the analysis of multi elements or radionuclide of environmental and biological samples [29].

In order to obtain an accurate result form the desired measurement, a standardized neutron activation analysis procedures, needs advanced and appropriate analytical materials. In case of INAA, laboratories should have mandatory materials such as radiation sources/facilities and relevant measurement systems[45, 52]. For an Optimal utilization of INAA, several indispensable and supplementary specific procedures are shown in Fig.3.1.

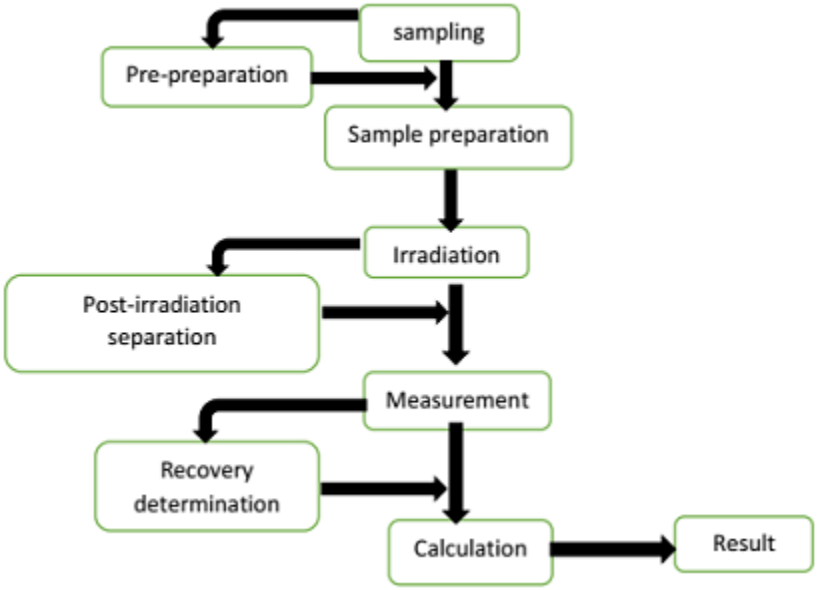

Figure 3.1: Procedures in neutron activation analysis.

3.2 Materials

3.2.1 Description of the NIRR-1 Facility

All research reactor neutrons obtained by the phenomenon of nuclear fission and a flux of neutrons can be obtained in the order of 10^{11} - 10^{17} n/ $cm^{-2}s^{-1}$.

The neutron beam from a reactor consists of thermal, epithermal and fast neutrons. However,the neutron beam has to be thermalized because most often the thermal neutrons are required for NAA due to their maximum absorption cross-sections.

Centre for energy research and training, CERT, is the Nigerian facility, utilizes the research reactor namely, NIRR -1, for INAA purpose since 2004.

It is a miniature neutron source reactor (MNSR) with a tank-in pool structural configuration and working at a nominal thermal power rating of 31 kW. The reactor

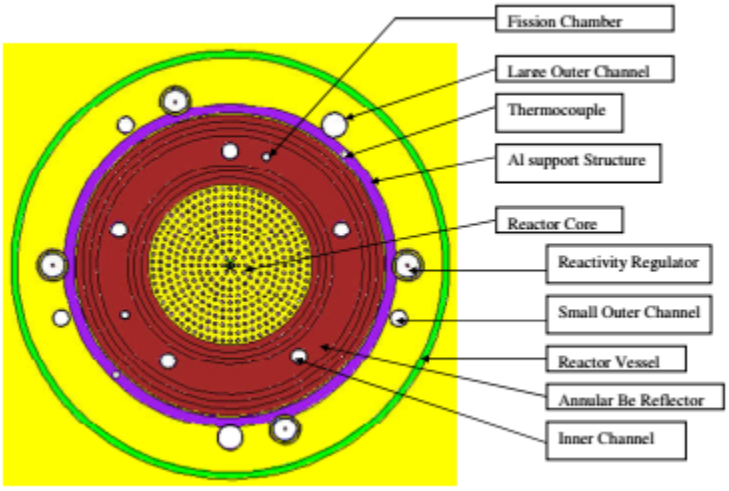

Figure 3.2: Schematic diagram of NIRR-1 reactor core

can operate for a maximum of 4.5 hours at full power (i.e., equivalent to a thermal neutron flux of $1\times10^{12}n/cm^2.s^1$) in the irradiation channels[53]. NIRR-1 have two outer, four inner irradiation channels for thermal neutron activation analysis(TNAA) and one irradiation channel for epithelial neutron activation analysis(ENAA) purposes around the reactor core as shown in Fig.3.2.

For fast and effective sample transfer via the six irradiation channels around its core, automatic pneumatic system (Rabbit Console), was installed in the reactor site. In addition, a control system is connected via each channel for the purpose of neutron flux monitoring during samples irradiations.

The neutron flux at the outer irradiation channels is softer than that of the inner channels allowing for a reduction of uncertainties caused by interference of few radionuclide.

Despite, CERTs facility reactor is a low-power research reactor, it is suitable for

over 30 elements and trace analysis with LOD as low as $10^{-10}\mu$ g for Dy, Eu [54].

3.2.2 Gamma-ray spectrometer

The quality of a germanium detector for measuring gamma-spectra is specified by three parameters such as; FWHM resolution, detector efficiency and signal to noise ratio.

The spread of energies (FWHM) over which mono-energetic gamma rays are detected arises from two sources;

(i),The random nature of the ionization in the detector and,

(ii), the electronic noise generated in the signal amplification and shaping circuits, especially in the coupling between the detector and the first stage of the pre amplifier.

Theoretically, these parameters sum in quadrature for total FWHM value of HPGe detector, however, practical detectors (coaxial detectors) do not always achieve these theoretical limits. Those used in activation analysis normally have FWHM resolutions in the range 1.8-2.2 kev at 1332 kev of ^{60}Co.

HPGe detectors, A good efficiency and high value of signal to noise ratio are highly recommended for the purpose of background reduction and safety issues [20, 37].

In CERT facility, the typical detector is a P-type Coaxial detector system (Ortec-GEM70-S) enclosed with in a graded lead shielding as shown in Fig.3.3. it has coupled to the integrated digital gamma-ray spectrometer hardware (DSPEC jr 2.0), and a computer with the dedicated software's (Maestro-32 and Winspan-2004). GEM70-S detector is specified by; FWHM of 1.85kev, peak to Compton ratio at 1.33 Mev of ^{60}Co is 78:1, the relative efficiency at 1.33 MeV of ^{60}Co is 60%, and shaping time of 12μs.

Figure 3.3: GEM70-S detector mounted on 30-liter liquid nitrogen Dewar and enclosed by lead shield

Figure 3.4: Digital Signal processing module, DSPEC jr 2.0

It is shielded principally with 10 cm of lead and 10 cm of copper, which reduces the laboratory background by approximately two orders of magnitude.

Digital Signal Processing (DSPEC), shown in Fig.3.4, is high performance gamma spectrometer than analogue shaping amplifiers. The shaping functions are then performed in the digital domain rather than with analogue circuitry. DSP filters and processes the signals using high speed digital calculations rather than manipulation of the time varying voltage signals in the analogue domain. The softwares are manufactured by Ortec Company for data accusation and analysis purpose.

3.2.3 Apparatuses for Sample Preparation

The other apparatuses used for samples preparation in the CERT's INAA laboratory includes; Agate Mortar and pestle, Cotton wool, Acetone, Sieve, Air blower, Disposable gloves, Polyethylene bags,Vial, Distilled water, Analytical balance, Marker, and Marking tape.

Disposable glove is used to avoid contamination of sample through sweating. Agate mortal and pestle are to crash samples. Distilled water, cotton wool, brush and Acetone are used for clean up and remove any impurity which can contaminate the samples under study. An analytical balance (Mettlar EA 240), shown in Fig. 3.6, is to record the weight of the samples in four digit level. Each sample wrap up with polyethylene bags and sealed using an air blower. The samples put into a vial, as shown in Fig.3.5, were sealed by cello-tape then marked for irradiation.

Figure 3.5: Vial container used for packing samples

Figure 3.6: Mettlar EA240 weighing machine used in NIRR-1 preparation laboratory

3.3 Instrumentation

All the details of the necessary INAA procedures utilized, i.e, practical considerations and documents in NIRR-1 facility for analysis of geological and biological materials are summarized and the most significant instrumentations for our study will be discussed below.

3.3.1 Interference and Neutron Flux Monitoring

It is very important to determining neutron spectrum parameters of MNSR reactors for INAA experiment to avoid related errors. The neutron spectrum and spectrum parameters determination includes:
(i) absolute, resonance and fast neutron component of the flux,
(ii)flux homogeneity and flux stability determinations.

Reactor neutron flux provides us intense thermal and epithermal neutrons for TNAA and ENAA purposes, however, fast neutrons can be available within the flux at the irradiation sites of samples. Fast neutrons contribute very little to the (n, γ) reaction and in a typical reactor irradiation position, about 5% of the total flux consists of fast neutrons. The usual neutron beams from a research reactor are contaminated by fast neutrons and gamma-rays that originate in the core. Filters, collimators, and shielding can reduce these undesirable components to some extent.Failure to measure and correct such discrepancies can cause analytical errors of the order of 5-50% [22, 23, 24].

In cases of flux homogeneity corrections are necessary, it is essential to know the spatial arrangement of samples and calibration standards relative to neutron flux monitors included in batches of samples prepared for irradiation [55].

Flux stability measurement can be made by irradiation and counting of radioactivity of peace of Al wire doped by 0.1%Au to determine the day-to-day variation of flux at a fixed power level operation. In smaller research reactors, like MNSR, this is not necessarily the case, with samples in slightly different positions receiving different integrated fluxes in the course of irradiation. For most prototype MNSR reactors neutron flux variation in irradiation sites is less than 2% indicating that the reproducibility of flux setting in various periods and this shows the possibility of standardizing of such reactor for multi element INAA measurement [41].

Low-power research reactors, such as, the Canadian Slowpoke and the Chinese MNSR, which run on the same fuel loading for over more than a decade are known to exhibit stable neutron flux characteristics, which make them suitable for INAA via those standardized methods [29, 49].

In order to optimize NIRR-1 for utilization of INAA via relative, absolute and single comparator methods, a careful and complete choose of irradiation positions, decay data and characterization of the neutron flux parameters in the irradiation channels was done. Therefore, the neutron spectrum parameters of NIRR-1 were found to be comparable with those of other reactor facilities, which have similar core configurations as mentioned in Jonah et al. (2006)[54].

3.3.2 HPGe Detector Calibration

In order to accomplish the qualitative and quantitative multi elemental analysis of a given sample, it needs a proper energy and full-energy peak efficiency calibration of HPGe detector which are the essences of neutron activation analysis technique [37].

(1). Energy calibration of GEM70-S detector

The energy calibration of gamma-ray spectrometer relates the pulse height (full-energy peak) to a channel number in the spectrometer. A perfect analyzer would have a constant value of energy increment per channel or gain and constant intercept. However, modern sophisticate gamma- ray spectrometer shows a slight drift in channel number for a given gamma energy in a certain period of time caused by least square computer analysis programs and reference libraries [22]. Therefore, it needs periodic correction or calibration for such shifts as part of the analysis procedure for quality control of spectrometer performance.

The adopted HPGe detector energy calibration was using the three-selected gamma-energies of certified reference radioactive materials(CRM); 661.60 keV of ^{137}Cs, 1173.2keV and 1332 keV of ^{60}Co. The activity of these standard gamma sources was measured by MAESTRO software at a constant geometry along the main axis then the energy and corresponding channel numbers were plotted as shown in Fig.3.7. The theoretical expression for energy calibration of HPGe detector is given by;

$$E = a_1 + a_2.ch + a_3.ch^2 \tag{3.3.1}$$

where, ch is corresponding channel number.

Table 3.1: Energy and corresponding channel numbers of Cs-137 and Co-60 gamma-rays

$ChannelsNo.$	$Energy(kev)$
854	661.62
1486	1173.32
1683	1332.5

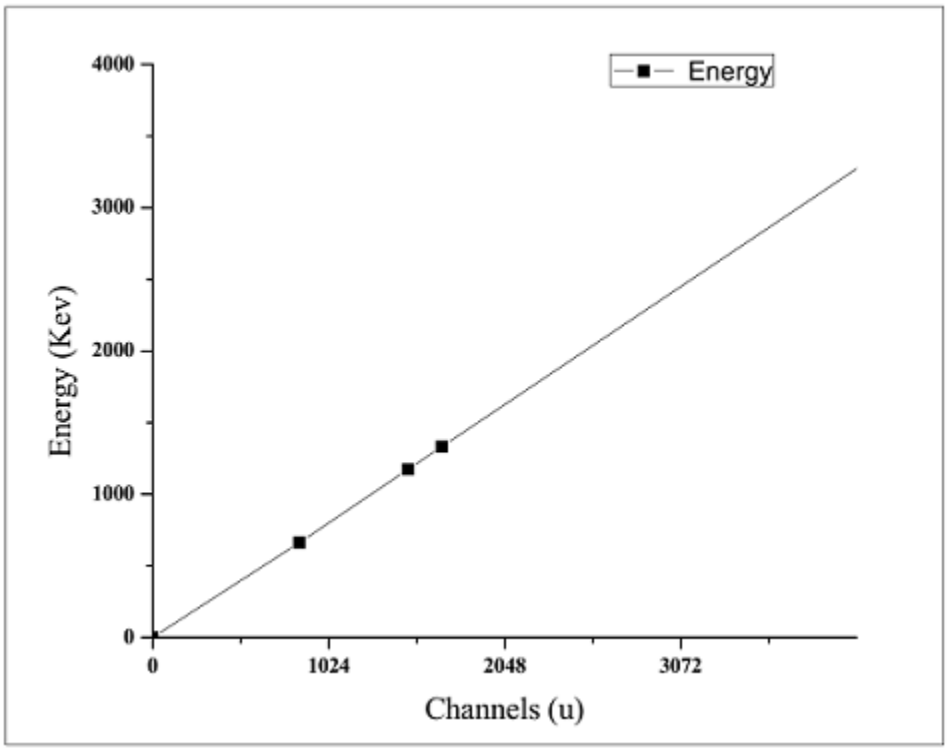

Figure 3.7: Energy Calibration curve of GEM70-S, HPGe detector

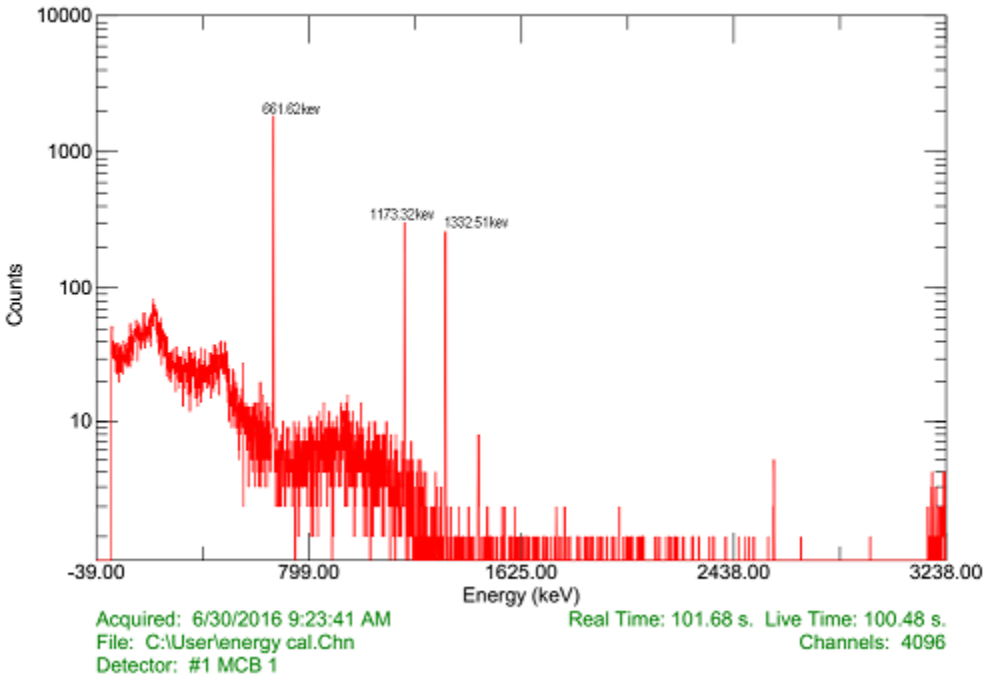

Figure 3.8: Energy Spectrum diagram of standard gamma-ray sources (Cs-137 and Co-60)

The experimental graph in Fig. 3.7 is expressed by the linear equation;

$$E = 0.79.ch \tag{3.3.2}$$

Where, the fitting coefficients are 0, 0.79 and 0.

The stability of the energy calibration is monitored periodically and the calibration is repeated if a shift larger than 0.5 keV is observed. The energy and FWHM (Full Width at Half Maximum) determined for the most intense photo-peaks are well fitted with a first order polynomial function.

(2). Efficiency calibration of GEM70-S(HPGe) detector

The main objectives of efficiency calibration of a detector are to overcome the counting loss (to make dead time <10%) due to the difference in samples geometry and to minimizes the measurement errors in the INAA results.

Since the full-energy peak efficiency of a germanium detector of a given energy is geometry dependent, changes in source-detector distance result in differences in the solid angle of gamma events entering the detector. Therefore, the distance of the sample from the detector (and hence the solid angle) should be adjusted depending on the count rates.

The full-energy peak (photopeak) efficiency calibration of our GEM70-S HPGe detector was done by certified gamma sources; Na-22, Mn-54, Co-57, Co-60, Y-88, Cs-137, Eu-152, Ra-226 and Am-241, in the energy ranges 59-1332 keV.

The MAESTRO emulation software program utilized to obtain the net full peak (background counted for 3600 seconds was subtracted) counts for each photon of interest with gamma-ray emission probability of 13% and above at three different geometries.

Practical calculation of the full-energy peak efficiencies is based on the equation:

$$\epsilon(\%) = \frac{N_c}{T_c p_\gamma A} 100 \tag{3.3.3}$$

Where, N_c - the net peak area of a gamma spectrum, T_c - the counting time(3600s), p_γ- a fraction of gamma-ray emission per disintegration, and A is the present radioactivity (Bq) of certified activity source which is given by

$$A = A_0 e^{-\lambda t_d} \tag{3.3.4}$$

In the formula, A_0 is initial activity(Bq) at the production or certified date, and t_d is

Table 3.2: Full-energy peak efficiency (%) values of HPGe detector at three different geometries

$Radionuclide$	$E(kev)$	$eff\%(2cm)$	$eff\%(5cm)$	$eff\%(15cm)$
$241Am$	59.54	0.292476	0.336199	0.055885
$152EU$	121.78	4.687405	2.04646	0.346436
$152Eu$	244.69	3.47841	1.607749	0.300317
$152Eu$	344.29	2.824388	1.270272	0.247966
$137Cs$	661.66	1.816236	0.80545	0.153186
$152Eu$	778.92	1.421604	0.67356	0.135701
$152Eu$	964.11	1.25557	0.587439	0.120977
$152Eu$	1112.08	1.148735	0.540593	0.111317
$60C0$	1173.2	1.104875	0.51849	0.106737
$22Na$	1274.53	0.95448	0.470791	0.097207
$60C0$	1332.5	1.007504	0.471899	0.097877
$152Eu$	1408	0.966826	0.45031	0.093582

decay time from certified date up to start of counting.

The experimental procedures followed for the determination of full-energy peak efficiency of our HPGe detector were:

I, standard sources were placed at some geometrical positions, Ri, apart from the top face of the HPGe detector using a source holder,

II, gamma-spectrum for a time period (3600s) long were accumulated enough to reduce the counting error,

III, After the measurement has finished, the spectrum is accumulated (integral net count of peak region) for each peak corresponding to the energy, and

IV, Data plot of $\epsilon(\%)$ vs. E(keV) on log-log graph paper was done, then a smooth curve through all points were drawn as shown in Fig.3.9.

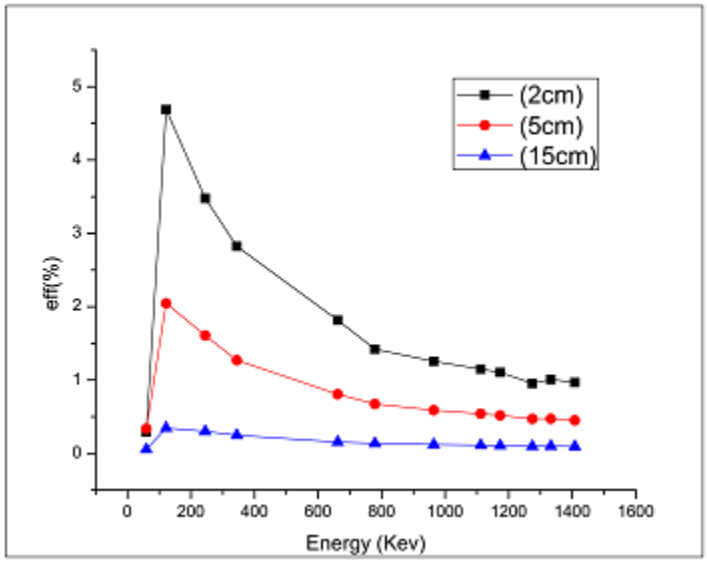

Figure 3.9: Full-energy peak efficiency curves of the HPGe detector at different geometry

At low energies, detector efficiency is a function of cross-sectional area and window thickness while at high energies total active detector volume more or less determines counting efficiency.

The graph of $\epsilon(E)$ fall-off at high energy is to reduce the probability that Compton scattered events derived from the partial detection of high energy gamma photons will contribute to the background spectrum in the 50-200 kev region.

In order to verify optimum geometries for both short lived and long-lived radionuclide analysis,the evaluation of efficiencies for the respective energies mentioned above were plotted against geometries of 2cm, 5cm, and 15 cm from the detector end cap along the main axis. From Fig.3.9, we can observe almost stable efficiency data around the 15 cm regions and as we move toward lower geometries of 2 cm these data deviate significantly. At 2 cm, the separation of the energies was very significant

and was adopted for long-lived radionuclide analysis while 15 cm was adopted for short-lived radionuclide measurements.

Once the detector full-energy peak efficiency calibrated for short, medium and long half- life nuclide based on their half-life and gamma energy, the MAESTO emulated software program utilized to calibrate energy and full-energy peak efficiency at the standardized geometrical position.

3.4 Stages in Neutron Activation Analysis

The overall experimental procedures of INAA can be categorized into four steps [29].

1, Selection and collection of representative samples using appropriate sampling techniques, collection of samples by using clean bags and instruments, Washing and drying with appropriate temperature.

2, After drying of samples; pulverising, homogenising, mass determination, packing, and the preparation of the standards. About 150 to 250 mg of the sample material (depending on the type of material), is packed in high purity polyethylene capsules.

3, After irradiation and appropriate radioactive decay of the sample and standards, these are measured on a coaxial large volume HPGe gamma-ray detector system coupled to a PC-based gamma- spectroscopy system.

4, Using a computer software, the peaks are fitted, their peak areas (and hence the relative intensities of various gamma-transitions) are determined to calculate the abundance of various elements present in the sample.

3.4.1 Sampling and sample preparation

Among the most indispensable steps, Sampling is the first in any meaningful analytical methods.The quality of sampling and sample handling have been given more attention in neutron activation analysis studies [49, 57]. The quality of sampling method and sample influences the result more than the final measurements [56]. With regards to the method, it should be in line with the objective of the study and samples to be collected should be a representative without any contamination until the process of analysis.

A much more comprehensive sampling from different sites have been necessary in order to reliably determine the potential of certain plant samples to deliver nutritionally and pharmacologically relevant elements to humans. Therefore, in this study, total sample size of three or four (n=3 or 4)samples were collected from each plant species in order to yield enough statistical power to allow a reliable estimation of metal loads within certain plant species to represent a good estimation of the population mean. Representative biological(plant) samples with a required size were collected and stored in separate polyethylene bags(films) to avoid any contamination.The details of all the sampling technique, sample size and preparation procedures of each sample in this work will be presented in chapter four to six.

1. Sample preparation

The objective of sample preparation in INAA is to reduce the sample size to allow a representative to be chosen for analysis. Sample preparation procedures and the preparation equipment required may depend on the type of samples.

In each sample preparation, special attention was given about the chemical nature

Figure 3.10: Pre-irradiation sample treatment

of most elements and the potential for contaminations in biological samples because it could be high and may results in elements lost by volatilization. For instance, in case of neutron activation analysis study, some elements can be lost at temperatures less than 100^0c. These include Br, Cl, Hg, I, and Os. while As, Au, Cd, Cr, Se, Pd, Pt, Si, V, Ag and Te may be lost below 500^0c [56, 57].In this study, appropriate materials and adopted procedures were followed in each biological sample preparation which will be discussed in the next chapters.

Standard samples were chosen closely similar to our biological samples both in physical form and in elemental composition. This is because to minimize errors due to self-shielding and self-absorption during counting.

For the purpose of Samples to be encapsulated before irradiation, Low density polyethylene films, as shown in Fig.3.11, which are most extensively used for this

Figure 3.11: Pre-irradiation sample packing by polyethylene films

purpose and have the advantage of low blank levels were utilized.

2. Sample preparation for Irradiation

The polyethylene films and irradiation rabbit capsules /vials/ were cleaned by soaking them into dilute HNO_3 acid more than one day, washed by distilled water and put them in desiccator. Blank concentrations of all the elements of interest were investigated using the adopted procedures and were found to be below the limits of detection set up for the polyethylene films.

All sub- samples brought in CERT were pre-weighed using four-digital electronic balance and put into a dry oven at 65^0c about 1hr and weighed. The process continued until constant mass of each sample were obtained. Similarly, standard CRM powder samples were dried.

Each sub-sample crashed and powdered using a pre-cleaned standard Agate mortar and pestle until the matrix resembles the standard reference samples. In order to avoid cross contamination between different sample matrix, after each sample was crushed the mortar and pestle were first washed by clean tap water, detergent, distilled water and finally washed by acetone.

For long term irradiation of a sample in a reactor, the samples were dried well to avoid any problem of explosion due to the high pressure produced in the container by the radiolysis of water in the samples. In addition, the removal of the water in the sample will improve the determination of the radionuclide of interest because an increased amount of sample can be irradiated and the counting geometry can be improved.

Both for short and long irradiation, biological and CRMs samples weighing about 150- 250mg were prepared and put into pre-cleaned polyethylene bags then sealed by standard heat sealer. All sealed Samples were coded based on their type and customer reference number given by the CERT reference sheet and put them into a pre-cleaned capsules/vials/. Samples in these capsules or vials were carefully covered by clean Wool to avoid any miss-locations at the irradiation process and tightly covered by a sticky cello-tape.

3.4.2 Irradiation and counting procedures

The purpose of sample irradiation is to produce sufficient radioactivity in the sample. This can be achieved, if the analytical conditions such as; neutron flux, irradiation time, decay and counting times have an optimum values.

The choice of the irradiation channel is based on the elimination of errors caused

Table 3.3: Analysis Schemes for Multi-element determination using NIRR-1 Irradiation and Counting Facilities.

Neutron flux and irradiation channels	Irradiation Regime	T_{irr}	T_d	T_c	Activation products
$2 \times 10^{11} n/cm^2.s$ (B_4, A_2)	Short(S)	5min	2-15min	10min	^{28}Al,^{27}Mg,^{38}Cl,^{49}Ca, ^{66}Cu,^{51}Ti,^{52}V
			3-4hr	10min	^{24}Na,^{42}K,^{165}Dy, ^{56}Mn,^{116m}In
$5 \times 10^{11} n/cm^2.s$ (B_1, B_2, B_3, A_1)	Long(L)	6hr	4-5d	30min	^{24}Na,^{42}K,^{76}As,^{82}Br ^{140}La,^{153}Sm,$^{239}Np(U)$
			10-15d	60min	^{46}Sc,^{141}Ce,^{51}Cr,^{134}Cs ^{152}Eu,^{177}Lu,^{131}Ba,^{86}Rb ^{182}Ta,^{160}Tb,^{175}Yb,^{65}Zn $^{233}Pa(Th)$,^{59}Fe,^{181}Hf

by nuclear interferences due to threshold reactions, notably Mg in the presence of Al; Al in the presence of Si; and Na in the presence of P. For instance, due to the proximity of the inner channels of MNS reactors to the core, it can lead to relatively higher ratio of fast-to-thermal neutrons.

The adopted irradiation and counting times were made based on the half-life of product nuclide. The chose of each time was on account of their impacts in minimizing interferences, counting loss and associated errors. Before neutron irradiation, a sample are transferred to an irradiation container for transport to and from the irradiation position. These containers do not contain the element of interest or they add insignificant amount to the total amount of radioactivity produced during activation.

All the prepared biological samples were sent into the reactor through the pneumatic transfer system connected to each irradiation channels.

Table 3.4: Counting Schemes of elements in the adopted experimental conditions

Target sotope	Product isotope by (n; γ) reaction	Half-life	Gamma-energy in (keV)	Counting Regimes
23Na	24Na	14.96h	1368.60	L1
26Mg	27Mg	9.46 min	1014.4	S1
27Al	28Al	2.24 min	1778.99	S1
37Cl	38Cl	37.24	1642.7	S1
41K	42K	12.36h	1524.58	L1
45Sc	46Sc	83.81 d	889.28	L2
48Ca	49Ca	8.72 min	3084.54	S1
50Cr	51Cr	27.7d	320.98	L2
51V	52V	3.75min	1434.08	S1
55Mn	56Mn	2.58h	846.76	S2
58Fe	59Fe	44.5 d	1099.25	L2
59Co	60Co	5.27y	1173.2	L2
64Zn	65Zn	243.9d	1115.55	L2
75As	76As	26.32h	559.10	L1
81Br	82Br	35.3h	776.5	L1
85Rb	86Rb	18.8d	1076.6	L2
121Sb	122Sb	64.8 h	564.24	L1
130Ba	131Ba	11.8d	496.3	L2
139La	140La	40.3h	1596.21	L1
140Ce	141Ce	32.5d	145.44	L2
151Eu	152Eu	13.3y	1408.5	L2
152Sm	153Sm	46.27 h	103.18	L1
159Tb	160Tb	72.3d	879.38	L2
174Yb	175Yb	4.19d	396.33	L1
176Lu	177Lu	6.71 d	208.36	L2
180Hf	181Hf	42.4d	482.2	L2
181Ta	182Ta	115d	1221.4	L2

Both elements leading to short-lived and long-lived products nuclide, irradiated by the thermal neutron flux of $5 \times 10^{11} n/cm^2 s$. After irradiation, the sample with the polyethylene foil is directly measured as it has no significant contribution of elements and radioactivity from the polyethylene film and as there would be difficulties in quantitative removal of the sample from the irradiated film.

The standardized irradiation and counting regimes in CERT facility are shown in Table 3.3 and Table 3.4 respectively. The experimental parameters in this multi-elemental INAA are, mass of the samples, irradiation time (t_{irr}), decay or waiting time (t_d) and the duration of radiation measurement (t_c). The magnitude of this parameters for the two-broad irradiation time has their own predetermined range based on a given reactor type. The relative methods have been used in INAA with MNSR, the calculation results have shown that error from non-contiguous irradiation is less than 0.01%.

3.4.3 Data Analysis Procedures

Peak Searching (Qualitative analysis) is the most important part in the gamma spectrometry of NAA technique in order to provide the most meaningful information about the desired nuclide in the analysed samples. Peak Searching process was accomplished by WINSPAN–2004 software, developed at CIAE, Beijing, China on the basis of the well-known activation equation[41]. Peak search was performed by importing of nuclide database included in the software according to the ray energy of the corresponding nuclide. The major tasks performed in peak searching includes;

- Energy calibration of WINSPAN–2004 software using saved gamma-ray spectrum data of certified gamma-ray sources,

- Finding peaks from the spectra data,
- Machining of the background line under peaks and directly deducting it from the spectra automatically,
- Calculation of the peak centre (Centroid Channel), ray energy, net peak area, statistical error, and FWHM (half peak width),
- By using nuclide database and the efficiency data, calculating the nuclide radioactivity, and
- Finally, the analysis results in a file that can be used in the future or accessed from other applications will be saved.

The instrumental neutron activation analysis or Quantitative analysis calculation was done on the basis of peak searching method. After accepting the required parameters of the INAA calculation using the software, each element content in the sample calculated according to the nuclear database and the peak searching results. The software program generates an activation analysis report based on the use of EOI (Element of Interest) that needs to be analysed. If the specified peak does exist, the element content (ppm) and the analytical error was calculated and listed in the NAA analysis chart. If the specified element does not exist, the detection limit was calculated and listed in the table.

3.4.4 INAA Calibration

The determination of an element concentration is based on the activation equations discussed and the method routinely employed was a multi element comparator (relative INAA) method using Standard Reference Material (SRM). The SRM sample was

irradiated and measured under the same conditions as the unknown samples. It was achieved by first, spectrum reduction which is started by location of peaks and fitted to obtain their energy and net areas. Secondly, standardization and interpretation follow to identify the radioactive nuclei and the stable element amount/concentrations are derived from peak areas [58].

In this study, NIST SRM -1515(Apple leaves), was selected for INAA method validation. It requires that INAA correction factors has to be pre-determined for the analysis of same element in the unknown sample. In the actual calculation of the correction factor of target element can be generated from the Eq.2.2.29,

$$C_m = KC_{cv} \tag{3.4.1}$$

Where, K refers to correction factor and C_m and C_{cv} refers to the measured and certified concentration of an element of the SRM in ppm [39]. Based on Eq. 2.2.29, the expression of correction factors can be,

$$\frac{Nc}{C_{cv}} = K = \frac{Rm\theta N_A}{M_x}.\frac{p_\gamma \epsilon SDC}{\lambda T_L} \tag{3.4.2}$$

In the formula, m is the mass of standard sample in gram and Mx molar mass of an element.

The correction factor of each element obtained by the SRM analysis was saved by the software program. The values can be viewed in the EOI (Element of Interest) edit menu of the program from the corresponding nuclide net peak count [58].

For validity of INAA analytical procedures and quality assurance of the data, double(n=2) control material qualities, NIST-SRM-1515 Apple leaves, were analyzed using optimized irradiation, cooling and counting times. Curries Method was applied

Table 3.5: Elemental concentrations and comparisons of NIST, SRM-1515 (Apple leaves) in ppm (mean ± expanded uncertainty (coverage factor k = 2), n=2)

Element	NIST SRM-1515 Value[a]	NIST SRM-1515 (this work)	RSD (%)	Z
Mg	271 0 ± 120	2704 ± 173	-0.22	-0.03
Al	284.5 ± 5.8	284 ± 6	-0.18	-0.06
Ca	15260 ± 150	15260 ± 442	0.01	0
Cl	582 ± 15	579 ±18	-0.5	-0.126
V	0.254 ± 0.027	0.26 ± 0.06	2.36	0.09
Mn	54.1 ± 1.1	52.7 ± 0.3	-2.6	-1.23
Na	24.4 ± 2.1	24 ± 3	-1.64	-0.12
K	16080 ± 210	16116 ± 60	0.22	0.17
Br	(1.8)	1.80 ± 0.05	0	0
La	(20)	20.00 ± 0.08	0	0
Sm	(3.0)	3.00 ± 0.01	0	0
Sc	(0.03)	0.03 ± 0.008	0	0
Fe	82.7 ± 2.6	83 ± 20	0.36	0.09
Zn	12.45 ± 0.43	12.5 ± 2.6	0.4	0.02
Rb	10.6 ± 1.6	10 ± 2	-5.7	-0.234
Ba	48.8 ± 2.3	49.0 ± 10.3	0.41	0.02
Nd	(17)	17.0 ± 1.7	0	0
Eu	(0.2)	0.20 ± 0.03	0	0
Tb	(0.4)	0.40 ± 0.04	0	0

[a] Certified values are given with uncertainties, non-certified values are given in parenthesis.

for Limits of Detection (LOD) calculation.The analyzed (measured) mean concentration values of elements in the SRM sample and associated INAA method validation parameters are shown in Table 3.5. The contributors to the combined uncertainty during gamma spectrometry could be identified as the counting statistics, mass of samples, counting geometry and dead time. Effects of counting geometry on the measurement uncertainty were brought down to negligible levels, by matching the dimensions of the sample and comparator and maintaining an identical distance from the detector in each set of measurement. The dead time was minimized (<10%) by

digital analyzer, DESPC jr 2.0, using the optimized counting geometries and utilizing an appropriate cooling time of each data.

The major uncertainties contributed to the deviation of the actual concentration of the elements identified were the statistical nature of radioactivity in the gamma ray spectrometric measurements and sample masses. The uncertainties were determined from errors in the peak areas and sample weights and calculated in quadrature.

All uncertainties were calculated at 2σ level to obtain data at 95% confidence interval. The relative errors for most of the major elements in the SRM are less than <10%, while for other elements is more than ten percentage which could be due to their week gamma peak height.

The relative deviation or accuracy of the measurement of the mean concentration of elements in calibrator SRM can also be evaluated through the RSD(%)and standardized difference, Z, computed by the relation;

$$Z = \frac{C_m - C_{cv}}{\sqrt{\sigma_m^2 + \sigma_{cv}^2}} \tag{3.4.3}$$

Where, Cm is measured concentration, Ccv is certified Concentration, σ_m is measured standard uncertainty, and $\sigma_{cv}$is certified uncertainty.

The standardize difference value, Z, and relative standard deviation, (%RSD), values comparison for both certified and information value elements are shown clearly in the Fig.3.12.

The standardized difference, Z, is needed to test the validity of the analysis method because each data values must be corrected by an appropriate weighing factor, which the results of statistics of radioactive decay (elemental concentration) is inversely proportional to the variance of the concentration [38].

As can be seen from Fig. 3.12, except Mn, the standardized difference, Z, values

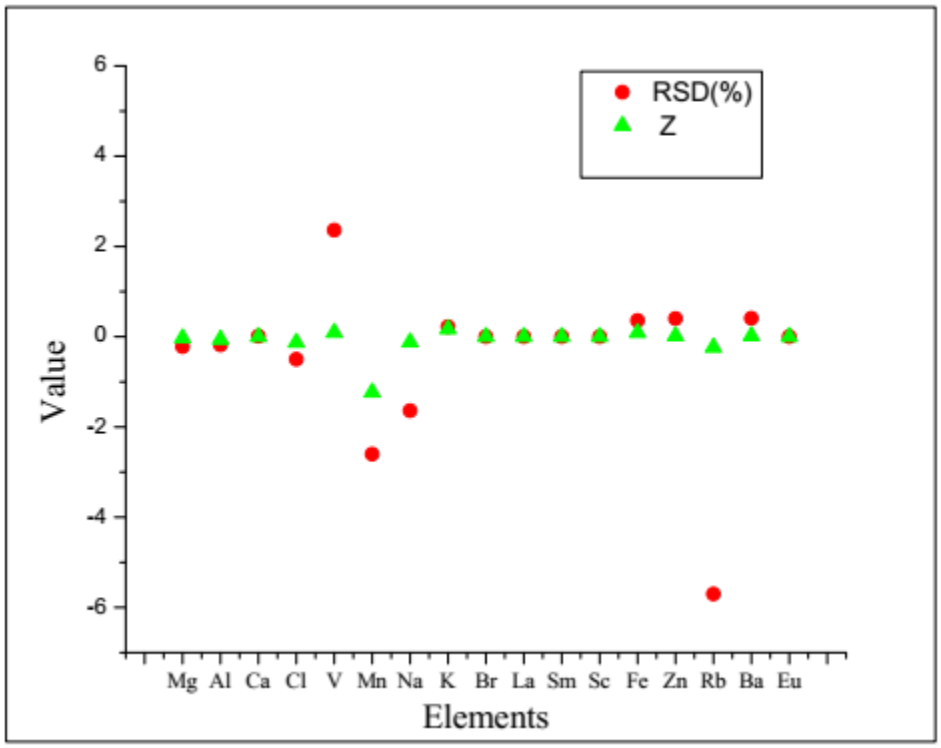

Figure 3.12: Standardized difference(Z) and RSD(%) comparison of NIST,SRM-1515(apple leaves) elemental concentrations

of the most of calibrator elements concentration lie in between -0.1 to 0.1. The sensitivity of standardize difference calculation (Z) signifying the overall precision of the measurement of certified elements as clearly indicated. The percentage difference (%RSD)between the measured concentrations and the certified data obtained shows that, lower values of percentage residuals represent closer match between the certified and measured results for some elements. Both the plots show good agreement between the certified data and observed values since the errors for most elements are <10%, highlighting the reliability of our results.

3.4.5 Analysis of unknown sample spectrum's.

The measurement process for the determination of the concentration of an element by INAA in a blank-free environment can be described by Eq. 2.2.30[29].The ratio factors in this equations are ideally unity, but small deviations and their uncertainties are considered.

For the comparative technique neutron activation analysis, it is essential that spectra from individual samples are compared on the basis of equal live times. The quantitative determinations are made by comparing the areas of these photo peaks with those in the spectrum of a standard sample, NIST ,SRM -1515, irradiated and counted under identical conditions.

Chapter 4

Multi elemental analysis of indigenous food spices in Southern Ethiopia

4.1 Introduction

Spices and herbs are plant parts or plant products, which are mostly used for seasoning, flavouring and thus enhancing the taste of foods, beverages. The International Standards Organization (ISO) defines spices as vegetable products used for flavoring, seasoning, and imparting aroma in foods.[59, 60].

There are several known edible spices and herbs in the world used for hundreds of years in food products. Their primary functions were to flavor food and to provide aroma, texture, and color. However, they also provide secondary effects, such as preservative, nutritional, and health functions in areas where the plants are native[61]. Spices and herbs have their own unique flavor, good aroma and tastes due to their own unique chemical compounds which makes them useful for food preparation and to protect human body against harmful substance and microbes [61].

INAA is a qualitative and quantitative multi- elemental analysis technique useful

Table 4.1: Spices and herbs for culinary purposes [59, 62, 63].

Botanical Name	Vernacular Name	Growing Sites	Parts used	Names of Local spice, C.herb,condiments
Aframomum corrorima	Ethiopian cardamom (*Korarima*)	Natural Vegetation	Seed	Berbere, Shiro, mitmita, Dilih, Awaze, Aferenje, Butter, coffee
Lippa Adonesis Var. koseret sebsibe	Koseret	Home garden	Leaf	Berbere, Butter, Shiro, Mitmita.
Coriandrum sativum	Coriander (Dinbilal)	Home garden	Seed	Berbere, Shiro, mitmita, Aferenj
Trachyspurmem ami Sprague ex Turill	Ajowan (Nechazmud)	Home garden	Seed	Berbere, Shiro, Aferinge, Dilih, butter, 'katikalla'

N. B; (1). spice refers to any dried plant product used primarily for seasoning, be it the seed, fruit, leaves, bark, root, rhizome or flowers.(2).herbs are any plants used for flavoring, food, medicine, or perfume.(3). Culinary herbs (C. herb) are spices derived from green leaves.(4). Condiment is sauce or relish combining vegetable and or spices with other ingredients. (5).Spice mixtures or blends are combinations of dried spices and herbs prepared according to traditional recipes and often associated with particular types of dishes.

to obtain a precise and accurate data of major, minor and trace elements concentration in wide variety of samples. As a result, we can get basic informations about the elemental contribution (their essential and trace element content). Ethiopia has diverse climate and soil types that enable growth of several endemic and indigenous spices, herbs and medicinal plants which are widely adopted to the south western humid and sub humid areas. There are more than 100 known exotic traditional cooking in Ethiopian dishes. The taste and flavor of each dish varies with the combination of an essential indigenous spices and culinary herbs in the several ethnic nations. All these spices are indispensable for the preparation of 'berbre', the pungent pepper powder. In almost all Ethiopian people daily dishes preparation, one cannot do without the known spice blend berbere, shiro and/or spiced butter. The details of these

spices and herb are shown in Table 4.1.

In Ethiopia, there is a limited information about the multi-element composition of indigenous and major spices and herbs. The present study can provide some scientific evidences about the composition and concentration of essential, trace and toxic elements in the selected major Ethiopian spices. In order to do this, a sensitive, non-destructive and versatile INAA technique was employed using the NIRR-1 facility located at the Centre for Energy Research and Training, (CERT), Ahmadu Bello University Zaria, Nigeria[54].

4.2 Experimental

4.2.1 Sampling and sample preparation

Samples of spice analyzed in this study were collected from local marketplaces in Southern Ethiopia. The botanical classification and nomenclature of each is presented in Table 4.1. The sampling was carried out manually using systematic random sampling method. The market places from where the samples were purchased are Hawasa, Dilla and Yergachefe Towns respectively. Each spice s purchased from each market places were weighed and about 1kg was taken. Before purchase of samples, it was made a visual inspection of samples on the level of healthiness and relative purity. All samples were put in a separate strong polyethylene bags and labeled. Each spice sample was turned into four different metal sheets and covered by aluminum sheet which were earlier washed by distilled water.The samples were brought to the laboratory and were washed with distilled de-ionized water to remove any surface contamination and air dried inside dry oven at room temperature.

Figure 4.1: Dry air oven

Each of the sample, was therefore homogenized using quartering technique. The samples were put in cones and the cones divided in to quadrants using pieces of poly wood. The division into quadrant continued manually until a total of 16 homogenized sub-samples for each were obtained. One representative sample among 16 homogenized sub-samples were obtained for each of the four sample types were then put in polyethylene bags and sealed.

All sub-samples were prepared for irradiation in the NAA sample preparation laboratory, Center for Energy Research and Training (CERT), Ahmadu Bello University, Zaria Nigeria. For the determination of moisture contents pre-weighed processed sample of each spice sample was oven dried at 65^0c. After 1hr the samples were reweighed and the moisture content determined through difference in weights.The process continued until constant mass of each sample were obtained. Similarly, two

standard samples of similar matrices were prepared using the standard reference material, NIST, SRM-1515(Apple Leaves).

Each sub-sample was crushed and powdered using a pre-cleaned standard Agate mortar and pestle until the matrix resembles the standard reference samples. In order to avoid any cross contamination, the mortar and pestle were first washed by clean tap water and detergent then washed by distilled water and finally washed by Acetone detergent. The polyethylene bags and irradiation vials were cleaned by soaking them in 60% HNO_3 dilute acid for about 24 hr before the day of sample preparation, rinsed with distilled water and put in desiccator.

Powdered spice samples were then stored in pre-cleaned and labelled polyethylene bottles then screw capped tightly to avoid absorption of moisture and any external contamination. Homogeneity check within bottle for all four sub-samples was determined by randomly taking portion, about 250 mg of each sub-sample after drying and homogenization.

4.2.2 preparation and irradiation

Samples of each spices approximately 250 mg along with NIST, SRM -1515 (apple leaves) as control materials were separately packed, labelled and sealed in pre-cleaned polyethylene capsules for short and long irradiations. Two sets of samples were prepared corresponding to different irradiation schemes. For long irradiation, the spice samples and one standard sample were put together in a single vial and sealed. All polyethylene bags and were properly heat sealed and packed in reactor rabbits, while all vials were sealed and packed for irradiations.

The prepared targets were irradiated in a 31kW swimming pool type research reactor

(NIRR-1) using thermal neutron flux of $5 \times 10^{11} cm^{-2}.s^{-1}$ about 5 minutes for short and 6 hr for longer irradiations in the outer and inner reactor channels respectively.

After irradiations, cooling and counting times for each batch were adjusted in accordance with the half-life of the isotope of the desired element. After appropriate cooling of the active targets the samples and reference materials (RMs) were transferred to pre-weighed clean polyethylene counting vials.

4.2.3 Gamma-ray spectrometry

Gamma-ray spectra for all targets were obtained using DSPEC jr 2.0, multichannel analyzer (MCA) linked to ORTEC coaxial HPGe detector. All data files were subjected to calculations with necessary corrections with background subtraction and results were reported on dry weight basis. For final results error propagation rules were applied at each stage of the calculations to determine overall combined uncertainty for measurements taking into account uncertainties in peak area, background, weighing, balance calibration, and uncertainties in certified values of RMs used for calibration

4.3 Results and Discussion

In order to estimate the accuracy of INAA, the certified reference materials NIST SRM-1515 (Apple Leaves) was analyzed with the samples. The accuracy can be expressed between certified and analyzed value by taking into account the uncertainties as shown in Table 3.5. Based on the available elements in the NIST SRM-1515 (Apple Leaves), a total of 17 major, minor, trace elements and non-essential metals were

analyzed. Using the standardized irradiation and counting schemes [64], the following elements were measured: Mg, Al, Ca Cl, V, Mn, Na, K, Br, La, Sm, Fe, Zn, Rb, Eu, Sc and Ba. The data are presented for the different spices in Table 4.2 below.

Spices and herbs are expected to contribute the exposure of heavy metal contaminants of populations due to the regular consumptions. Despite small proportions in diets, it is recommended that heavy metal contamination should be minimized due to a higher concentration of heavy metals from spices and herbs adds to the burden originating from food. In addition, Spices and herbs belong to a group of condiments, therefore the levels of heavy metals were compared with the suitable safety standards as determined by several international organizations with the Upper Tolerable Limits (UL) applied to other foods and condiment with dry weight content.

The spices under study are commonly used in the preparation of Ethiopian food and are categorized by the amounts such as; one cup (120g) of each Berbere spice blend, shiro and spiced butter preparations needs an average about one teaspoon (2.6g on dry weight basis) of each of these spices [59, 62, 63]. The common cuisines preparation needs at least two of the former spice blends. Traditionally, an adult person can feed on the combination of these spices combinations twice per day. In order to evaluate the average daily intake of the nutrients from the traditional Cuisines from the combination of these spices by taking one teaspoon of each spices (i.e. a total of 10.4g) as the intake of an adult was compared with the UL values in Table 4.3.

Major minerals (i.e. K, Na, Cl) were measured in all spices and other 14 elements were measured in some of the spice samples. The concentrations were determined on

Table 4.2: Concentration of elements in the analysed spices and herb in ppm

Elements	ST0(Korarima)	ST7(Coriander)	ST10(Koseret)	ST18(Ajowan)
Mg	BDL	3897 ± 530 (13.6%)	BDL	4579 ± 421 (9.2%)
Al	37 ± 6 (16.2%)	202 ± 52 (25.7%)	144 ± 25 (17.8%)	253 ± 6 (2.4%)
Ca	BDL	16460 ± 1020 (6.2%)	14400± 878 (6.09%)	47390± 1801 (3.8%)
Cl	1061 ± 40 (3.8%)	839 ± 40 (4.8%)	2109 ± 70 (3.3%)	1739 ± 54 (3.5%)
V	BDL	BDL	BDL	0.20 ± 0.03 (15%)
Mn	349 ±5 (1.4%)	61 ± 2 (3.3%)	212 ± 3 (1.4%)	107 ± 3 (2.8%)
Na	88 ± 2 (2.3%)	244 ± 7 (2.9%)	6945 ± 21 (0.3%)	190 ± 3 (1.6%)
K	8376 ± 235 (2.8%)	90400 ± 2260 (2.5%)	28050 ± 954 (3.4%)	24000 ± 432 (1.8%)
Br	10 ±1 (10%)	13910 ± 83 (0.6%)	43.4 ± 1.6 (3.7%)	2.5 ± 0.7 (28%)
La	0.92 ± 0.08 (8.7%)	3.7 ± 0.3 (8.1%)	BDL	1.4 ± 0.1 (7.1%)
Sm	BDL	0.89 ±0.24 (27%)	BDL	BDL
Sc	0.02 ± 0.009 (45%)	BDL	0.07 ± 0.01‘ (14.3%)	BDL
Fe	106 ± 3 (2.8%)	3053 ± 284 (8.3%)	299 ± 38 (12.7%)	BDL
Zn	36 ± 3 (8.3%)	BDL	36 ± 3 (8.3%)	7 ± 2 (28.6%)
Rb	28 ± 2 (7.1%)	BDL	24 ± 2 (8.3%)	BDL
Ba	BDL	BDL	39 ± 11 (28.2%)	BDL
Eu	BDL	3.1 ± 0.5 (16.1%)	BDL	BDL

BDL refers to below the detection limit’ and values in bracket are relative errors in percentage.

Table 4.3: Comparison of daily intake of elements from spices in traditional dishes with upper tolerable limits of daily intake of elements (UL)[65, 66, 67, 68].

Element	Total (ppm)	Daily intake(mg/day)	UL (per day)
Mg	8476	22	360mg
Al	636	1.7	1mg/kg.BW
Ca	78250	203.5	3000mg
Cl	5748	15	6000mg
Mn	728	1.9	11mg
Na	7467	19	2000mg
K	150826	392	NA
Br	13966	36	1mg/kg.BW
La	5.9	0.016	NA
Sm	0.89	0.0023	NA
Fe	3458	9	40mg
Zn	79	0.2	45mg
Rb	52	0.13	200mg
Ba	39	0.1	NA
Eu	3.1	0.008	NA

NA: Not available

BW: body weight

UL: the maximum level of habitual intake from all sources of a nutrient or related substance judged to be unlikely to lead to adverse health effects in humans.

dry weight basis. The concentration ranges of major elements are as follows, K (8376-90400) ppm, Na (87- 6945) ppm, Ca(14400-47390)ppm and Cl (839-2109) ppm. Mg was found to be a major element in Ajowan (i.e. Bishop weed) and Coriander. The edible parts of both spices are the seeds for spices and medicinal purpose. Mg plays an important role in the growth of human body, formation and function of bones and muscles [64]. The combination of Mg concentration in the spices is below the upper tolerable limit set by WHO (360mg/day).

Coriander has the highest K concentration. It has been recommended that high

intake of K is useful for reduction of Hypertension and diabetes and better cell membrane. The highest Cl and Na concentration were found in Koseret and Coriander respectively. Koseret is an important traditional Ethiopian culinary herb and it is used specifically to add a strong, lemony flavour to butter and local shiro.

According to literatures, Nearly all (99%) of total body calcium is used in human skeleton whereas, the rest parts are used in the teeth, soft tissues, and in the extracellular fluid for various essential body functions[65, 68]. Among the spices, Ajowan has the highest Ca concentration about, 47390 ppm.

Fe was determined in all spices except in Ajowan sample. The highest concentration was found in Coriander to be (3053 $\pm$ 284) ppm and the lowest was obtained in Korerima to be (106 $\pm$ 30) ppm. Edible parts of crops contain Fe have been discussed by several authors. The generally accepted critical value for most crops of Fe = 50 ppm [3, 65]. However, the variation among plants in their ability to absorb Fe is not always consistent and is affected by different changing conditions. In addition, since Fe is easily soluble, plants may take up a very large amount of Fe. It is one of an essential nutrient for several human body systems such as, Oxygen transport and storage and number of vital functions, including growth, reproduction, healing, and immune function [66, 69]. The combination of these spices is below UL of Fe set by WHO (40mg/day) in diets [68].

Mn concentration in Korarima is the highest (349 $\pm$ 5) ppm among the spices and Coriander contains the lowest Mn (61 $\pm$ 2) ppm. Mn is an essential mineral for plants and human. It has been suggested that, the Mn content shows a remarkable variation for plant species, stage of growth, and different organs as well as for different

ecosystems [3, 65, 70]. The physiological function of Mn for human is closely associated with some enzyme activities, involvement in the formation of bone, in amino acid, lipid, and carbohydrate metabolism. The maximum Mn concentration of Mn using the combination of these spices is below the Tolerable Upper Intake Level (11 mg/day) set for adults [68].

Zinc is one of the essential trace mineral in plants and humans. It is a component of a number of various enzymes in the maintenance of the structural integrity of proteins and in the regulation of gene expression [65, 66, 67, 68]. Except in Ajowan, it was measured in all spice samples in trace levels. Once again, the highest concentration was measured in Korarima (36.4 $\pm$ 2.9) ppm. This indigenous Ethiopian spice, is obtained from the plant seeds and it is extensively used in Ethiopian and Eritrean cuisine. The other essential trace elements V was measured in Ajowan spice at trace level.

Aluminum is present in all spices in minor concentration except in Korarima. The concentration of Al varies between 37 $\pm$ 6ppm in Korarima to 253 $\pm$ 6ppm in Ajowan. It has been suggested that, Aluminum availability is defined by its concentration in the soil solution, increasing with decreasing soil PH, increases under anaerobic soil conditions and as a result of physical injury to the root [70, 71]. However, it is known for its toxicity to plants and humans if consumed in high concentration and is stored in body tissue. The concentration in the combination of all spices is below the UL set by WHO (1mg/kg.BW day) [67]. Just like Al, Br is not essential to plants and humans. High Bromine concentration was found in Coriander (13910 $\pm$ 83) ppm but the rest spices are at trace values.

Heavy non-essential metals such as La, Sm, Eu, Sc, Rb, and Ba were determined

in the spices at trace levels. La was determined in all samples except Koseret, Sm and Eu were measured in Coriander only. Furthermore, Sc and Rb were determined in Korarima and Koseret, and Ba was found in Koseret only. Among these metals, Sc and Rb compounds are considered to be toxic in higher intakes. However, the average daily intake of all the metals are less than their toxicity level [3, 70].

As can be seen from the results of daily dietary intake of some essential elements due to the consumption of these four spices, the average intakes are below the recommended upper limit by WHO and other organizations. Therefore, this work can be used as a basis for the determination of database of essential and non-essential elements by INAA in Ethiopian diets in general and spices in particular.

In Ethiopia, there is limited information about the multi-element composition of indigenous and major spices and herbs. The present work attempts to fill that gap through the use of INAA to provide information about the composition and concentration of essential, trace and toxic elements in the selected major Ethiopian spices.

Chapter 5

Evaluation of essential and trace elements status in the indigenous Ethiopian tuber crops using INAA technique

5.1 Introduction

Nowadays, several variety of wild and cultivated root and tuber crops have been known as a staple food items in the world due to their economical and nutritional benefits[72]. Among many species and varieties, the three species, cassava, Irish potato and sweet potato accounts 93% of the root and tuber crops for direct human consumption in the world. Irish potato, cassava, sweet potato, yams and other root and tubers respectively provided 37.5%, 35.3%, 20.2%, 4.3% and 2.7% of the total amount used as human staple food on a dry equivalent basis [73].

Roots and tuber crops are among the important staple foods throughout tropical Africa. They account for well over 50% of the total staple foods produced in Sub-Saharan Africa and thus the backbone of the region's economy. Sub-Saharan African people consumes 27% of total Root and Tuber crops [74]. These crops play a vital

role in the country's food security because they are tolerant of environmental stresses and give reasonable yields under marginal soil conditions.

It has been well understood that tuber based diets can provide one of the cheapest sources of dietary energy (about one-third of that of an equivalent weight of grain), protein, fiber and variety of essential nutrients. The quantities of root and tuber crops used as staple food or of energy available from these crops varies among continents and countries. However, in several African countries where they provide more than a quarter of the available energy which seem root and tubers are unquestionably necessary for the survival of population [75].

The nutritional value of food is determined by adequate concentrations of essential ingredients required by humans and several beneficial substances that must be taken up in balanced proportions and at regular intervals. Human beings healthful diet can be attained through the intake of combinations of a variety of foods. However, for certain groups of people like developing country nations, because of economic restrictions, levels of certain micro-nutrients may not be met from a traditional food alone [76]. Even with sufficient food, deficits of essential food components can cause malnutrition and other related health problems. Unbalanced concentrations of minerals are not only detrimental as such, but also negatively affect the composition of organic food constituents [67]. This fact is verified by medical expertise that about half of all human diseases are caused by inadequate or imbalanced nutrition. Now a day, there are some deficiency diseases observed in several developing countries. In addition, in order to avoid a serious health problem, the minuscule concentrations should not be beyond some tolerable limit of human body system [68].

Recently, interests in implementation of analytical techniques for the analysis of

various food components has increased in order to determine the levels of constituent nutrients and establish limits for human exposure to contaminants from the food [69, 77, 78].

In Ethiopia, several local studies are available about the identification, diversification and subsequence conservation programs which have mainly focused on cereals and pulse crops. However, there are root and tuber plants domesticated in Ethiopia such as, Taro (*Colocasia Esculenta* L.), Yam (*Dioscorea abyssinica* Hochst. ex Kunth), and 'Anchote' (*Coccinia abyssinica* Lam. Cogn.) are among the indigenous crops which needs more attention by researchers. Among various genome types of tubers, 'Anchote' and Yam ('Boye') are native to Ethiopia and there are also exotic indigenous Taro('Boyina') species. Some literatures report shows that, all of them cultivated in specific regions as a traditional co-staple food crop. Local nations consume their tuber parts and consider them as a nutritious, productive, disease resistant, needs less soil fertility [63].

There are limited scientific studies on content of crude fat, utilized carbohydrate. However, the status of multi elements in these native Ethiopian tubers is unknown.Therefore, the current study on the accurate determination of the elemental composition of these tubers are of utmost importance for estimating the populations dietary intake of nutrients and their exposure to toxic elements. As a result, the contribution of dietary consumption of these crops will be estimated. Therefore, the study investigates associated impacts to human health and provides more information about the status of multi-elements content in a dairy food for the benefit consumers.

5.2 Methods

5.2.1 Sampling and Sample preparation

Three healthy matured tuber samples, each weighing about 4 kilograms, were collected from the four market places (Dilla, Yirgalem, Awasa, and Addis Ababa) using simple random sampling technique. The samples were packed in polyethylene bags and transported to Dilla University biology laboratory. Each raw sample was washed thoroughly by tap water and distilled water consequently. The washed sample was sliced to uniform thickness using a pre-cleaned stainless-steel knife and dried using an air-oven at 45^0c for 24 hours. Each same species sample collected from four markets were turned into different pre-washed metal sheet and mixed manually.

The samples, therefore homogenized using quartering technique manually until a total of 16 homogenized sub-samples were obtained. Finally, three sub-samples, one for each tuber crop, were randomly selected among homogenized sub-samples then put in polyethylene bag and sealed.

All the three sub-samples were brought to the NAA sample preparation laboratory of Centre for Energy Research and Training (CERT), facility located at Ahmadu Bello University, Zaria Nigeria. Each sub-sample was pre-weighed using an electronic balance and put into a vacuum-oven at 65^0c about 1hr then weighed again. The processes continued until constant mass of each sample were obtained. A certified reference sample, NIST, SRM -1515(Apple leaves), powder was prepared similarly. Before samples were prepared for irradiation, polyethylene bags and plastic vials were pre-cleaned by socking them into 60% HNO_3dilute acid and dried. The dried sub-samples crushed and powdered using a pre-cleaned standard agate mortar and

Figure 5.1: Anchote (*Coccinea abyssinica*) species

pestle until the matrix resembles the standard reference material. In order to avoid any cross- contamination, in between two consecutive samples, the mortar and pestle were washed by clean tap water, detergent, distilled water and finally washed by Acetone. For both short and long irradiation purposes, the powdered tubers and SRM samples weighing about 250 mg were put into pre-cleaned polyethylene bag and sealed by standard heat sealer.

They were coded based on their type and customer reference number given by the CERT's reference sheet to avoid related errors. Samples were put into a pre-cleaned plastic vials then covered by clean Wool (to avoid any miss-locations in the irradiation process) and tightly covered by a sticky cello-tape. Finally, All packed samples were stored in desiccator, to avoid any moisture absorption from the surrounding environment, till the irradiation processes started

Figure 5.2: Desiccator used for storing prepared samples for irradiation

5.2.2 Instrumentation and gamma-spectrometry

For qualitative and quantitative INAA analysis purpose, the reactor neutron flux parameters, calibration of HPGe detector, irradiation process, data accusation and analysis of all irradiated samples by using Maestro and Winspan-2004 software was done using the adopted procedures mentioned in chapter three.

In the case of INAA analytical method; the sample do not have to undergo any chemical treatment (neither prior, nor after the activation) that makes INAA becomes non-destructive. Among the three known INAA standardizations methods, the multi element comparator standardization (a semi-absolute quantitative INAA) method was selected for its accuracy and speed. it needs both the tuber samples and SRM sample should be irradiated and counted identically. The experimental parameters were, mass of samples, gamma intensity of radionuclide, irradiation time, cooling and

counting time.

5.3 Results and Discussion

Investigation of the nutritional ingredients of these co-stable food crops, the essential and non-essential elements concentration in mg/kg of dry weight basis, were done. In order to approve that the concentration of the nutrients in the tubers are the indications of their own components, contaminations associated with the sampling and sample preparation were minimized by following the quality procedures as mentioned in sampling and preparation paragraph.

In order to validate the quality of instrumental method, the analysis of certified standard reference material, NIST, SRM -1515 (Apple leaves), with known amounts of elements were compared with the certified values as shown in Table 3.5. Thus, the result obtained for the SRM provides a good accuracy in the comparative INAA measurement.

The INAA results of the three tubers along with the uncertainty values are presented in the Table 5.1. A total of 12 elements concentration were measured with the major elements; Ca, K, Cl, Na, Mg, minor minerals; Zn, Fe, Mn and non-essential elements; Al, Br, Rb in all samples. Other non-essential metals; Sm, La, Sc, Ba and Eu are found below the detection limit in all the tubers.

The concentration patterns of an essential elements measured in the tubers were found as;

Coccinia.abyssinica; $K > Ca > Mg > Cl > Na > Zn > Mn$,

Colocasia.esculenta: $K > Mg > Cl > Mn > Na > Zn$, and

Dioscorea.abyssinica: $K > Mg > Na > Zn$.

Table 5.1: Elemental concentration of the three indigenous Ethiopian tubers in ppm

Element	TT0 (*C. abyssinica*)	TT1(*C. esculenta*)	TT2(*D.abyssinica*)
Mg	484.5 ± 99.8 (20%)	1130 ± 264 (23%)	934.8 ± 212.2 (23%)
Al	BDL	BDL	23 ± 4 (17%)
Ca	3073 ± 402 (13%)	BDL	BDL
Cl	122 ± 15 (12%)	270 ± 23 (8.5%)	BDL
V	BDL	BDL	BDL
Mn	13 ± 1 (8%)	89.12 ± 2.50 (8.5%)	BDL
Na	32.2 ± 1.5 (4.7%)	33 ± 3 (9%)	49.8 ± 3.3 (6.6%)
K	9567 ± 411 (4%)	23690 ± 1256 (5.3%)	16530 ± 909 (5.5%)
Br	26.8 ± 1.1 (4%)	5.6 ± 0.7 (12%)	11.6 ± 1.2 (10%)
La	99.1 ± 2.3 (2.3%)	0.51 ± 0.09 (18%)	BDL
Sm	BDL	BDL	BDL
Sc	BDL	0.042 ± 0.006 (14%)	0.013 ± 0.003 (23%)
Fe	BDL	BDL	BDL
Zn	15.8 ± 3.4 (21%)	17.4 ± 2.4 (14%)	5.2 ±1.2 (23%)
Rb	51 ± 3 (6%)	BDL	BDL
Ba	BDL	BDL	BDL
Eu	BDL	BDL	BDL

BDL refers to below the detection limit.values in bracket are relative errors in (%).

The relative errors of concentration of elements in three tuber samples were in most cases <10%. However, in some cases in which the concentrations were very low, in ppb levels and the count rates are extremely low, the relative errors were found to be comparatively high.

In order to assess the adverse effect related to the toxicity of the nutrients intake in a daily consumption of the tubers in traditional diets, we have to make some comparisons of an estimated daily intake (EDI) of elements in mg/day with the elements upper tolerable limit set by international organizations in dry weight basis [68, 77, 78]. Notably, Several root and Tuber crops have very low dry matter Content (70-80% moisture) which goes beyond 30g/100 FW [74].

There is limited scientific data about the consumption of such native root and tuber crops of east African countries. However, there is relevant report by OECD/FAO, (2016), that the average per capita root and tuber crops consumption in Sub-Sahara Africa countries from year 2013-15 was about 2.5kg/cap/year in equivalent dry weight basis[79]. This is equal to 6.85g/cap/day for a person. An estimated daily intake of elements resulting from tubers consumption can be calculated by multiplying the elemental content with the average per capita daily consumption of tubers, in mg/person/day as shown in Table 5.2.

$$EDI = C \times Y \tag{5.3.1}$$

Where, EDI is estimated daily consumption, C is Concentration of element in the sample (in mg/kg) and,Y is average consumption per capita (mg/cap/day. person).

The concentration of these elements in each tubers may vary with the soil type they grow, water, climate, parts of a plant and crop variety and other factors[67]. Calcium and potassium are the most abundant essential minerals in the human body.

Table 5.2: Comparisons of elemental concentration of estimated daily intake(EDI) value with UL.

Element	Total(ppm)	EDI(mg/day)	UL
Mg	2553.3	17.5	350[a]
Al	23	0.16	1[b]
Ca	3073	21.05	3000[a]
Cl	392	2.7	6000[a]
Mn	102.12	0.7	11[a]
Na	115	0.8	2000[a]
K	49787	341	NA
Br	44	0.3	1[b]
La	99.61	0.7	NA
Fe	-	-	40a
Zn	38.4	0.3	45[a]
Rb	51	0.4	200[b]

NB: [a] mg/day, [b] mg/kg BW.Day

According to WHO (1998), the average recommended intake of Ca for adults is 1000 mg/day[68]. In this study, the highest calcium concentration was measured in the 'Anchote' tuber (3073 ± 402) ppm, and the concentrations in the other tubers is below the decision limits. The beneficial effect of a dietary calcium is in the prevention and treatment of a number of chronic disorders or deficiency diseases [80, 81, 82].

Potassium concentration in all tubers have the highest value. According to literatures, among food sources of potassium, the maximum is in beans and peas (Cow peas, pigeon peas, Lima beans, African yam beans) about 13,000 mg/kg [78, 83]. The result of this analysis shows that, the highest content of K was found in Taro ($23{,}690 \pm 1256$) ppm and the lowest is in 'Anchote' (9567 ± 411) ppm. Both Taro and yam are richest in K than beans and peas mentioned in literatures. WHO (2012), recommended that a potassium intake of at least 3510 mg/day for adults. Sodium

concentrations in all analysed samples are in trace level as shown in Table 5.1. However, Yam sample have slightly higher Na concentration. WHO (2012), recommended that, intake of Na less than 2 g/day is more beneficial for disease prevention. Potassium is the major mineral in the tuber crops while sodium tends to be low[84]. This makes the tuber crops particularly valuable in the diet of patients with high blood pressure, who have to restrict their sodium intake. In such cases the high potassium to sodium ratio may be an additional benefit [82, 84, 85].

Magnesium is one of an essential element used in several body functions. It has been documented in literatures that the richest sources of Magnesium include; green vegetables, legume seeds, beans and nuts. however, many highly-refined flours, tubers, fruits, and most oils and fats contribute little dietary magnesium (<100 mg/kg) [68]. In contrary to these data, all tubers provide higher magnesium concentrations. The richest tuber in Mg mineral (1130 $\pm$ 264) ppm and followed by yam tuber (934.8 $\pm$ 212.2) ppm. The concentration of Mg in all tubers is in the range of recommended nutrient intake value [86].

The other nutrient, Zinc was measured in the all tubers in trace levels as shown in Table (5.1). Zn is an essential trace element used in various human body systems [86, 87]. According to literatures, Lean red meat, whole-grain cereals, pulses, and legumes provide the highest concentrations of zinc in the range of 25-50 mg/kg but Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc, having concentrations less than 10 mg/kg [87]. In comparisons with Zn concentration estimation of Tuber crops in the literature, Taro (17.4 $\pm$ 2.4) ppm and 'Anchote' (15.8 $\pm$ 3.4) ppm have better Zn concentration. Thus, the indigenous tubers provide additional source of Zn mineral.

Humans require Cl and uptake it in the form of salt used in nutrition, food and in drinking water. Among food sources, the lowest Cl contents are in cereal grains usually vary 10-20 mg /kg [88]. The amount of Cl in all tuber samples are low in comparison to potato tubers in the literature relative to Ethiopian Yam [85, 86]. Cl concentrations of Taro and 'Anchote' are (270 ± 23) ppm and (122 ± 15) ppm respectively.

Except yam tuber, trace concentration of Mineral Mn were measured in the Taro and 'Anchote' samples. The Manganese concentrations in the Taro (89.12 ± 2.50) ppm and 'Anchote' (13 ± 1) ppm was less than food crops which have been known by their high manganese concentrations like cereal grains, soy beans and sweet potato [88]. Mn is considered as trace essential mineral in several body systems [65, 89, 90, 91]. its concentration in the tubers are relatively below the tolerable upper limit as shown in Table 5.2.

Aluminium is among the most plentiful elements in the earth's crust and it is known as a toxic element to the body. An increased aluminium level in the brain of human may results in Alzheimer dementia and Osteomalacia [65, 67]. The aluminium content in food plants depends upon different factors, however, the soil aluminium content ranges from 10 - 30 ppm [88]. There is no evidence that both Al and Br have no any essential function and efficiency symptoms in animals or humans but most ingested Br is relatively easily excreted from the body. Al is found in Yam only with a higher concentration, (22. ± 4) ppm, and Br concentration in 'Anchote', (26.8 ± 1.1) ppm, was higher than in yam and Taro tubers. Both elements concentrations in the tubers are below the tolerable daily intake of aluminium and Bromine which are approximately 1mg/kg of body weight [65, 67].

Non-essential minerals (Rb, Sc, La) route to plants and human can be through soil and water used by plants through their roots. There is some evidence that Rb is involved in brain functions, but specific roles have not yet been identified. Amounts of Rb in edible plants above 200 mg/ kg might be harmful and its concentrations above 1000 mg/kg in human diet are of a health risk. Most of Rb compounds can irritate the skin, by contact or when breathed [65]. Sc to occur in mammalian tissues and considered as dangerous to human health, but mainly in the working environment. High concentration of Rb is obtained in 'Anchote' tuber (51 ± 3) ppm only and Sc is obtained in Taro and Yam tubers with lower concentrations. La has no any essential function to plants and animals. La concentration in 'Anchote' tuber, 99.1 ± 2.3ppm, and in Taro, 0.51 ± 0.09ppm were measured. The danger of La is mainly from aerial dust inhalation by humans[65, 70].

Iron concentration in all the samples is below the detection level. Iron has several vital functions in the body of humans such as, it serves as a carrier of oxygen to the tissues from the lungs by red blood cell hemoglobin and as an integrated part of important enzyme systems in various tissues [67, 80]. The low Fe concentration may be influenced by soil water PH, Fe-insensitivity, and high levels of P, high rainfall, low temperature, high lime content, compaction, low soil temperature, inhibition of root growth and root activity, low root and shoot ratio[65, 86, 88].

In general, the result shows that the intake of some essential elements due to the dietary consumption of these tuber crops has insignificant contribution of heavy metal intoxication. However, it provides additional contributions for adequate essential nutrients required by human body system and for reduction of the deficiency diseases caused by such nutrients. Therefore, this work can be used as a basis for the

determination of database of essential, trace and non-essential elements in Ethiopian indigenous tuber crops.

Chapter 6

Elemental analysis of traditional medicinal plants used against some gastrointestinal diseases in Southern Ethiopia.

6.1 Introduction

Several systems of medicine have been practiced in the world for the treatment of different diseases caused in human. These medicines involve the available natural resources comprising of herbal medicines which gets Public interest due to their availability and their low prices, along with their therapeutic ability. The purchasing cost of conventional drugs has been affecting the developing nations. This has fostered the use of herbal medicine for the treatment of various ailments alongside with belief that these herbal products are considered as without any adverse side-effects. Another reason for adoption of herbal therapy was lack of general medical facilities mostly in the rural dwellings where the consumers were compelled to the use of herbal or plant medicines [92, 93, 94].

Herbs plants have compatibility to nourish the body and efficacy to provide vitamins, minerals and many trace elements in bioavailable form that is easy to absorb. Minerals and inorganic trace elements are required by living beings for numerous biological and physiological processes that are necessary for the maintenance of good health [95, 96, 97].

There are 17 trace elements (Al, B, Br, Cl, Co, Cu, F, Fe, I, Mn, Mo, Ni, Rb, Si, Ti, V, and Zn) which are known to be essential for all plants [70]. According to Bowen, among those trace elements; Fe, Cu, Mn and Zn are involved in several functions of plants as shown in table(6.1) [70, 98]. Plants readily take up the species of trace heavy metals that are dissolved in the soil solutions in either ionic, chelated and complexed forms. Chemical forms of these trace heavy metals in plant systems exudates differ for each element. For instance, Zn was almost all bound to organic compounds, while Mn was only partly complexed [65, 66, 67, 68, 69, 70]. Most medicinal plants (herbs) have been used for a long time to cure illness. They belong to a kind of herbs known by the accumulation of a greater amount of trace elements than other plants. In addition, it has been stressed that a trace element curative agents are found in their parts [70, 98].

Recent preclinical studies show that, commonly used medicinal plants are the most promising in preventing various gastrointestinal ailments despite detailed investigations are required for pharmaceutical use in the existing knowledge of these plants [99, 100]. WHO reported that more than half percent of world population still uses medicinal plants preparation for gastrointestinal disorder were gets a symptomatic relief and improvement in the physiologic function of the GIT [101].

Table 6.1: Some heavy metals impacts in plants and gastrointestinal tract [99, 102, 103].

Element	Uses in plants	GI disease it affects	GI disease causes
Ca	Catalytic enzymes, parts of organelles	Gastrointestinal Cancer Generic disease	 Deficiency
Mg	Catalytic enzymes parts of organelles	IBD Liver Diseases	Deficiency Deficiency
Al	Activate some enzymes, control some physical properties	NA	NA
Mn	Catalytic enzymes, parts of organelles, storage	Liver diseases GI cancer	Overload Overload
Fe	Antibiotics, porphyrins, Catalytic enzymes, storage, part of organelles	Liver diseases	Overload
Zn	Catalytic enzymes, storage, parts of organelles	GI Cancer IBD Liver diseases	Deficiency Deficiency Deficiency

NA refers to not available and IBD refers to inflammatory bowel disease.

According to several literatures, in order to optimize healthy digestions, diets high in acidic foods should be replaced by food with high alkaline nutrients which contains minerals used for high energy production of cells and to activate essential enzyme catalysts of the body functions like in immune system [104, 105]. These minerals are required supplements for individuals suffering from metabolic acidosis in order to maintain healthy pH homeostasis inside the cell.

Apart from the diet treatment of GI diseases, there are number of conventional and traditional drugs available for the treatment of gastrointestinal diseases. According to several researches, the clinical evaluation of several conventional drugs has shown considerable side effects [99, 102, 103]. Thus, researchers have been actively

investigating drugs which are effective and have less side effects.

The trends of people are shifting towards natural cures to avoid some impacts and side effects of synthetic drugs. It is this revival of interest in these medicines which instigates to study these herbs, especially for the evaluation of their element concentrations. Trace elements, bioactive metals and their compounds are vitally important for various metabolic processes of human body system.Therefore, information about their distribution in medicinal herbs is of significance for consumers and further scientific research.

In Ethiopia, there are several plant species used for medicinal purposes. some medicinal plants widely considered as effective in treatment of some common diseases[59, 63, 98]. Most often, general public used the combinations of various or same herbal medicines leaves, roots, flowers, fruits, barks. it also uses some single herbs for the cure of different ailments in light of specific utility and action of herb or plant product[59, 98, 106].The details of four herbal medicinal plants, used frequently for common disease treatments by the traditional healers, are mentioned in Table 6.2.

There are some researches on medicinal plants about; ethno-botanical, chemo-biotic effects , etc.However, there is no multi elemental analysis of medicinal plants used for gastrointestinal diseases aliments. The current study focuses on the qualitative and quantitative elemental analysis using INAA technique in order to get scientific evidence about constituent elements involved in gastrointestinal diseases treatment. The relative availability of elements from the parts of plants needs to be investigated and documented for their safe and adequate intake. Moreover, this will also help in standardization of the traditional medicines.

Table 6.2: Traditional medicinal plants used for treatment of gastrointestinal diseases[59, 63, 106, 107].

Botanic name	Vernacular Names	Parts used	Therapeutic uses
Taverniera abyssinica	(Dingetegna)	Root	Fever Reduction, stomach ache and headache.
Leonotis ocymifolia (Burm.f.) Iwarsson	(Raskimir)	Leaf	Ulcer of the neck, stomach ailments
Anethum graveolens L.	Dill (Insilal)	Seed	stomach ache, against anorexia, Diure, gonorrhea, heartburn, Diuretic, cough. Colic, Digestive aid, Mild bowel disorders, Flatulence.
Cymbopogon citrates	Lemon Grass (Tejsar)	leaf	chest and stomach complaints

6.2 Experimental

6.2.1 Sampling and sample preparation

Verified and healthy herbal plant species were collected randomly from Sidama traditional healers around Yirgalem, wondogenet and Awasa towns. The samples were packed in polyethylene bags and transported to a laboratory for preparation. The raw sample was washed thoroughly by tap water and distilled water consequently. These washed samples were dried well using an air-oven at 45 ^{0}c for 24 hours. Each same species sample were turned into different pre-washed metal sheet and mixed manually. The samples, therefore homogenized manually using quartering technique until a total of 16 homogenized sub-samples were obtained. Finally, four sub-samples, one for each herbal plant, were randomly selected among homogenized sub-samples and put in polyethylene bag then sealed.

All sub-samples were brought to the NAA sample preparation laboratory of Center for Energy Research and Training (CERT), Ahmadu Bello University, Zaria Nigeria. Samples (including certified reference sample) were pre-weighed using an electronic balance and put them into a vacuum-oven at 65^0c about 1hr then weighed. The processes continued until constant mass of each sample were obtained. Before samples were prepared for irradiation, polyethylene bags and plastic vials were pre-cleaned by socking them into 60% HNO_3 dilute acid and dried.

The dried sub-samples crashed and powdered using a pre-cleaned standard agate mortar and pestle until the matrix resembles the standard reference material. In order to avoid any cross contamination, in between two consecutive samples, the mortar and pastel were washed by clean tap water, detergent, distilled water and finally washed by Acetone reagent. For short and long irradiation purposes, the powdered medicinal samples and SRM samples weighing about 250mg were put into pre-cleaned polyethylene bag and sealed by standard heat sealer. They were coded based on their type and customer reference number given by the CERT reference sheet to avoid related errors. Finally, samples put into a pre-cleaned plastic vials then covered by clean Wool (to avoid any miss-locations in the irradiation process) and tightly covered by a sticky cello-tape.

6.2.2 Irradiation and Measurements

Two sets of samples were prepared corresponding to different irradiation schemes. All polyethylene capsules were properly heat-sealed and packed in reactor rabbits and sent to the corresponding channels using Rabbit console shown in figure 6.1. The prepared targets were irradiated in a 31kW swimming pool type research reactor(NIRR-1) with

Figure 6.1: Rabbit console in NIRR-1 used for automatic sample transfer purpose

thermal neutron flux of $5 \times 10^{11} n.cm^2 s^1$ in short and long irradiation schemes. using the reactor inner and outer reactor channels, 5 minutes irradiation for short and 6hr for longer irradiations of samples were done.Certified gamma radiation sources, Cs-137 and Co-60, were used for the Calibration of the system covering energy range from 121.8keV to 2254keV and were extended to 4000keV by semi empirical method.

After appropriate irradiations, cooling and counting times for each batch of samples (which was adjusted in accordance with the half-life of the isotope of the desired element), first INAA calibration by reference material(RMs) activity of the detector was done using the WINSPAN–2004 software then the content of target elements of the samples was to determined.

As mentioned in chapter four, all data files were subjected to calculations with necessary corrections with background subtraction and error propagation rules were applied in final results at each stage of the calculations to determine overall combined

uncertainty for measurements taking into account uncertainties in peak-area, background, weighing, balance calibration, and uncertainties in certified values of RMs used for calibration.

6.3 Results and Discussion

For the validation purpose of the analytical method, INAA, the certified reference material, NIST SRM-1515(apple leaves) were analyzed and compared with the certified elemental concentration as shown in Table 3.5. A total of 17 elements were measured with a good accuracy. For most of the elements, no significant discrepancy was observed between the measured concentrations and reference values with a good precision signifying the reliability of the analytical methodology employed.

The spectrum of measurements total metal concentrations in plant dry matter were assessed. A total of 16 elements were measured in all four medicinal plants. Among analyzed elements, Ca, Mg, Cl and K were measured in major minerals level whereas Al, Na and Fe were found to be minor minerals and the rest minerals were found in trace levels as shown in Fig. 6.2, Fig.6.3 and Fig. 6.4. As can be seen from the result, the relative errors of the concentration of elements in each herbal plant were less than 10% except few non-essential elements shown in Table 6.3.

According to WHO (2007) report, the use of herbal medicinal products contributes insignificant exposure of heavy metal contaminants to the population due to the lower amount and rarely intake of herbal medicines and their products. However, it is recommended that heavy metal contamination should be minimized due to the fact that the heavy metal content of herbal medicines adds to the burden originating from food[108].

Table 6.3: Elements concentrations in the four traditional medicinal plants in ppm.

Element	MT1(*Anethum graveolens*)	MT5(*Cymbopogon citratus*)	MT6(*Leonotis ocymifolia*)	MT8(*Taverniera abyssinica*)
Mg	2667 ± 427 (16%)	BDL	4080 ± 424 (10%)	1537 ± 223 (14%)
Al	338.1 ± 14.2 (4.2%)	217 ± 29 (13%)	494.1 ± 13.8 (2.8%)	244.6 ±10.5 (4.3%)
Ca	18640 ± 969 (5%)	3438 ± 40.2 (1.2%)	20700 ± 973 (4.7%)	22040 ± 1036 (4.7%)
Cl	2272 ± 64 (2.8%)	4228 ± 93 (2.2%)	6973 ± 118 (2%)	1935 ± 60 (3.1%)
V	BDL	BDL	BDL	BDL
Mn	80 ±2 (2.5%)	80 ± 2 (2.5%)	198 ± 3 (1.5%)	18 ± 1 (5.6%)
Na	228.5 ± 5.5 (2.4%)	138 ± 1 (0.7%)	218 ± 4 (1.8%)	1173 ± 8 (0.7%)
K	17040 ± 920 (5.4%)	21800 ± 262 (1.2%)	50350 ± 1007 (2%)	7097 ± 546 (7.7%)
Br	8.6 ± 1.5 (17.4%)	135 ± 1 (0.7%)	34 ± 2 (5.8%)	32.3 ± 1.5 (4.6%)
La	0.36 ± 0.09 (25%)	.6 ± 0.1 (17%)	0.7 ± 0.1 (14.3%)	0.42 ± 0.12 (28%)
Sm	BDL	0.14 ± 0.04 (28%)	0.16 ± 0.04 (25%)	BDL
Sc	0.07 ± 0.01 (9.8%)	0.05 ± 0.01 (20%)	0.16 ± 0.01 (25%)	BDL
Fe	280 ± 44 (1.4%)	427.0 ± 53.4 (12.5%)	700.8 ± 46.3 (6.6%)	212.1 ± 32.2 (15.2%)
Zn	43 ± 3 (6.9%)	26.3 ± 3.2 (12.2%)	43.16 ± 3.15 (7.3%)	30 ± 3 (10%)
Rb	9.26 ± 1.73 (18.7%)	22.08 ± 2.14 (9.7%)	13.0 ± 2.3 (18%)	13.1 ± 1.6 (12.2%)

In this study, we considered the facts that, the potential of an element to be absorbed within the gastrointestinal tract is depending on its chemical speciation at the absorption site and total amounts do not give enough information to estimate element bioavailability. In addition, without testing these plant materials within biological assays (ideally in controlled animal studies), the discussion of such data in light of potential beneficial or adverse effects on organisms in health and disease is just speculative.

The metabolism of heavy metals is regulated by numerous factors such as the availability of nutrients with the integrity of the gastrointestinal tract and on liver function. It has been documented by literatures that; Mg, Mn, Fe and Zn are the major beneficial minerals in digestive health as shown in Table 6.1, above. In addition, some of the heavy metals such as Al, Ca and Na compounds, in conventional drugs, are used for the treatment of some gastrointestinal diseases [102, 103, 104, 105].

The chemical and biochemical aspects of organic-based metallo-drugs in the biological system describes that, a metallo-drug in the form of metal ions introduced into a body attaches itself to a part of amino acids found in protein and also interact with DNA which are potential targets for metallo-drugs. Thus, metallo-drugs could affect the protein to perform its biological task, which could be enough to kill the cell [109]. In addition, there are also inorganic metal complexes drugs used for treating and diagnosing diseases exposed to different physiological environments by making some changes of chemical composition in the way through a body from the site of administration to target molecules in the cell [109, 110].

Aluminium is one of those metals, present in pharmaceuticals used for the treatment of a number of diseases. Some study shows that Helicobacter pylori associated

gastritis treatments are effective using the combination of metal salts containing aluminium [110, 111, 112, 113]. Aluminium compounds have been primary components of number of vaccines in the form of immune adjuvant and also used as antacids either alone or in synergistic mixtures with other compounds like Magnesium. These antacids are also taken as useful attacks against the gastric mucosa, Heartburn, to protect, restore and heal ulcerations through actions [114]. The concentration of Al metal in medicinal plants measured in the range, 217- 494 ppm. it has been considered as essential for some Al resistant plants but has no function for human body instead it results in adverse effect when ingested beyond upper tolerable limit.

Calcium, magnesium and Sodium are essential minerals obtained from various diets and they have several essential human body functions within a recommended dietary allowance. However, there are adverse effects in gastrointestinal tissues related with their deficiency and interaction with other minerals [99, 102, 103].

Magnesium was not measured in Lemon Grass but higher concentration was measured in *Leonotis ocymifolia*, about 4080ppm. However, higher concentration of Ca and Na were measured in *Taverniera abyssinica*, an endemic herb of Ethiopia, an effective herb and its root is taken orally for the treatment of fever and stomach complaints.

Manganese has beneficial effect in several catalytic enzymes activities of human body systems. In addition, the therapeutic role of manganese compounds is a good reliever from inflammatory pain, gastrointestinal dysfunction associated with alcohol and Helicobacter pylori diseases [114]. Among the medicinal plants, in *Leonotis ocymifolia*, the highest and, in *Taverniera abyssinica*, the lowest Mn concentration were measured. All the medicinal plant sample have trace level of Zn concentrations

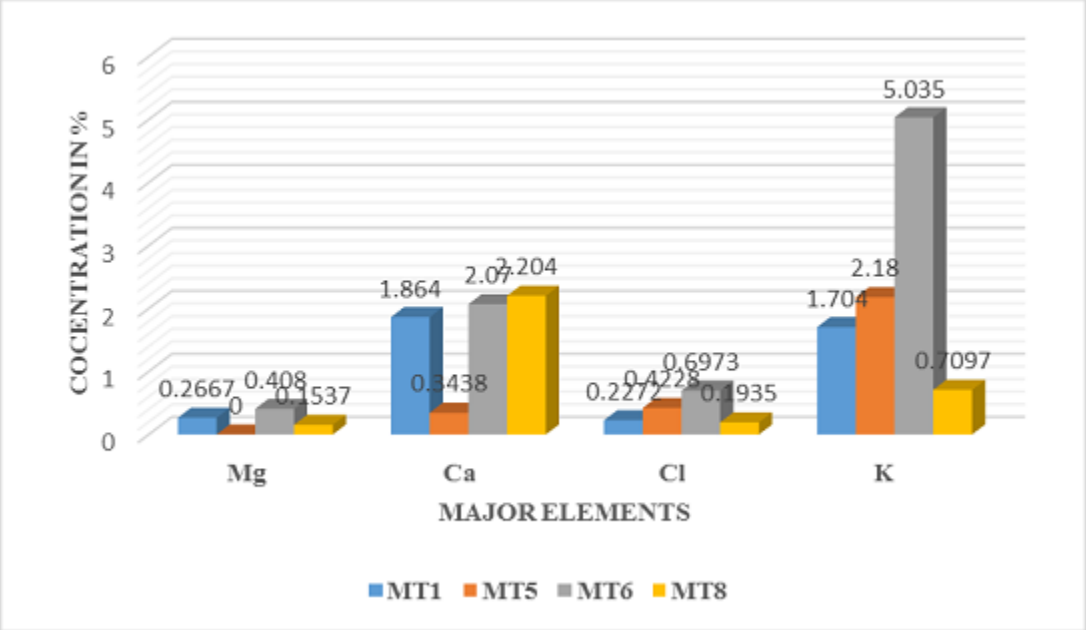

Figure 6.2: Comparison of major elements concentration in medicinal plants

but Dill (*Anethum graveolens*), *Leonotis ocymifolia* and *Taverniera abyssinica* root have considerable zinc concentrations. The seed of Dill is traditionally taken orally for most of gastrointestinal diseases as mentioned above in the Table 6.2. There are some evidences on the Zinc metabolism and gastrointestinal diseases. For instance, Gastric acidity is essential for homeostatic regulation of zinc absorption, some food nutrients result in reduction of zinc bioavailability and others induce higher Zinc absorption [115, 116, 117]. All these and inadequate zinc intake may contribute the deficiency of Zn which causes associated diseases. The reduced Zn levels in the blood correlated with inflammatory bowel disease (IBD) and/or active ulcerative colitis [118]. Therefore, Additional Zn supplements might have some impacts in elevation of Zn bioavailability. As a result, it can suppress the anti-gastrointestinal diseases

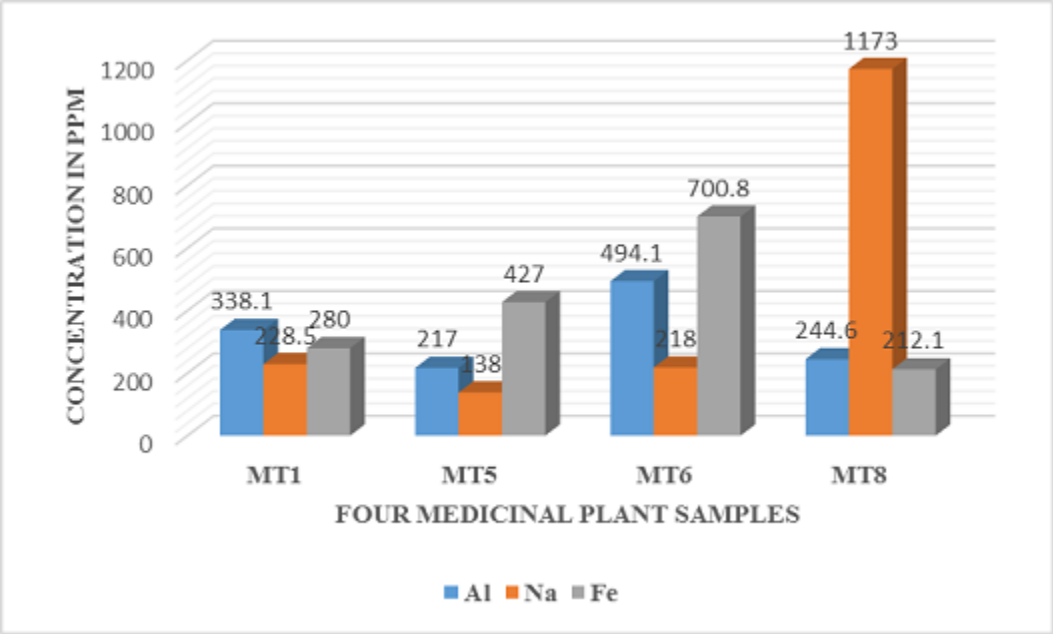

Figure 6.3: Concentration of minor elements in medicinal plants

activities such as; stimulates mucosal regeneration, mucosal resistance to acids, stabilizes plasma membranes, and has antioxidant properties and the others results in Zn absorptive function [102, 103].

Among the medicinal plants *Leonotis ocymifolia*has highest Fe concentrations followed by Lemon grass. It has been documented that, sometimes the metabolism of Fe results in high deposition of iron in gastrointestinal tissues like stomach and liver. This causes several diseases such as hepatocellular carcinoma, cirrhosis and several gastrointestinal inflammations [99, 103, 119]. According to Wu et al, increased concentrations of various trace elements such as Fe and K were independently associated with gastric cancer. It has been suggested that the elevated potassium levels were related to the presence of lymphatic duct metastasis [120, 121]. The concentration

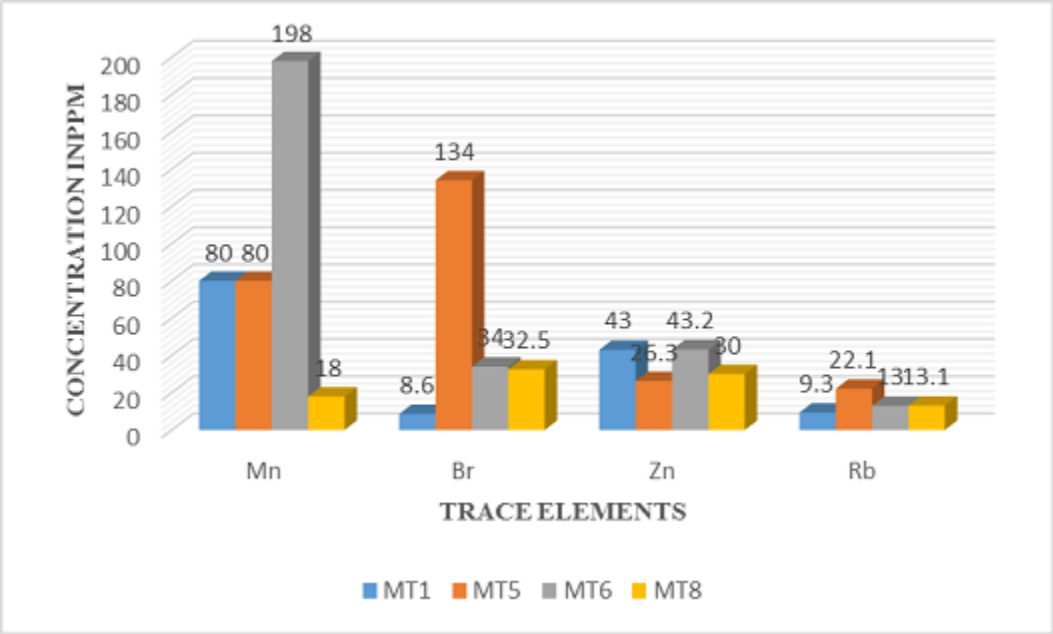

Figure 6.4: Comparison of trace elements concentration in medicinal plants

of potassium in all medicinal plant samples are the highest ranging from 7097ppm in Dingetegna to 50350ppm in Raskimir.

The rest elements such as; Cl, Br, La and Rb measured in all samples, as shown in Fig. 6.3 and Fig. 6.4. whereas Sm and Sc were measured BDL in Dill and Dingetegna respectively. Among these elements only Cl is useful for human body functions. Based on several literatures explanations, care must be taken in concentrations of these elements in the traditional medicinal plants to avoid any significant adverse effect to human health [68, 109, 122].

There is limited information about the multi-element composition of indigenous medicinal plants used for gastrointestinal diseases. Therefore, this work can be used as a basis for the determination of database of essential and non-essential elements

of these medicinal plants in particular. As can be seen from the results, the intake of some essential elements due to the consumption of these medicinal plants, may contributes in the status of concentration /bioavailability/ inside the GI tract affected by the deficiency of such elements.

The present work attempt to fill that gap through the use of INAA to provide scientific evidence about the composition and concentration of elements in traditional medicinal plants used for GI disease treatments .

Chapter 7

Summary

7.1 Conclusions

The qualitative and quantitative analysis of an essential, essential trace minerals (K, Ca, Mg, Na, Fe, Mn, Zn, V, Cl) and non-essential minrals (Al, Br, La, Sc, Sm, Rb, Ba, Eu) were done in the ingenious spices; Korarima, Ajowan, Coriander and Koseret using instrumental neutron activation analysis (INAA) method. The amounts of traditional combination of spices in the nations cuisines were used to compare the daily average elemental intakes with UL values for adults. The result shows that, the indigenous spices and herb, are found as the good source of combination of K, Ca, Na, Mn, Fe and Zn minerals. The traditional average daily intake of all elements in the combination of four spices in the nations cuisines are below the upper tolerable limits. Therefore, in addition to their primary use, the spices can be good sources of essential minerals for human health.

The evaluation of three indigenous Ethiopian tubers using INAA technique shows that the macro-nutrients; K, Mg, Cl, Na and micro-nutrient Zn were found in significant concentrations in all tubers but Ca and Mn minerals were measured in 'Anchote'

(C. abyssinica) and Taro (C. esculenta) only. Those non-essential metals; Al, Br, Rb, Sc and La concentrations were measured in trace levels. The highest K, Mg, Cl, Mn and Zn concentrations were measured in Taro tuber whereas moderate concentration of essential and trace elements such as; Ca, K, Mg, Na, Cl, Mn, and Zn were measured in indigenous 'Anchote' tuber. In addition, based on OECD /FAO estimates, an estimate daily intake(EDI) of elements for the average per capita daily consumption of these tubers are below the upper tolerable limits set for staple food crops. Therefore, the consumption of these tubers as a staple food can provide additional health benefits and becomes an alternative food sources for the nations.

Major, minor and trace elements of four medicinal plants, traditionally used for the treatment of gastrointestinal disease, are identified and their concentration analysed. The result of the analysis shows that the endemic herb root; 'Dingetegna' have high concentrations of Mg, Ca, Zn and low concentration of K, Mn and Fe whereas Dill seed has high Zn and moderate concentrations of Mg, Ca, Mn, K andFe. Several literatures about the bioavilablity of these metals in herbal plants and some of the impacts due to their involvement in human GIT nutrients metabolism. Thus, our result implies that 'Dingetegna' and Dill samples may provides better combinations of the beneficial nutrients for some of gastrointestinal diseases.

7.2 Limitation of the study

The focus of the study was restricted to crops and herbal plants taken from selected parts of the country(some parts of southern and south-western part of Ethiopia) thus, it don not represented all identical plant species multi elemental status. The experimental facility is found outside of the country which limits our interest to take

multiple measurements for a given sample type.Finally, the experimental conditions of the facility namely, lower thermal neutron flux, the use of thermal neutrons for NAA purpose, use of multi element comparator INAA only, and the absence of cyclic INAA limited the sensitivity and number of elements to be measured.

7.3 Recommendations

As can be seen in the results, the significance of these crops and herbal plants will be invaluable for the nations if there is more attention given and curious involvement of government, concerned stack holders, consumers and researchers for better protection, conservation, production and additional scientific assessments. Now a days spices, root and tuber crops, and herbal plants have a considerable influence for several nations for healthy life style and additional income generation. Nations like Ethiopia, should have to use such untapped resources to improve lifestyle in various ways by using a competitive scientific knowledge and technology.

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- Tamene H. Melkegna, A. K. Chaubey, Getaneh A. Beyene, Teshager A. Bitewlign, S. A. Jonah, Y. A. A. Ahmed, N. Abubakar. *Multi elemental analysis of indigenous food spices in Southern Ethiopia using INAA technique.* J Radioanal Nucl Chem (2017) 314:pp 917-921

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Appendix A: Common Abbreviations and Acronyms

BDL - Below Detection Limits

BW - Body weight

CERT - Center for Energy Research and Training

CRM - Certified Reference Material

DGNAA - Delayed Gamma Neutron Activation Analysis

EDI - Estimate Daily Intake

ENAA - Epithermal Neutron Activation Analysis

EOI - Element of Interest

FAO - Food and Agriculture Organization of the United Nations

FW - Fresh Weight

FWHM - Full Width Half Maximum

GIT - Gastrointestinal Tract

HPGe - Hyper Pure Germanium

IAEA - International Atomic Energy Agency

IBD - Inflammatory Bowel Disease

INAA - Instrumental Neutron Activation Analysis

ISO - International Organization for Standardization

LLD - Lower limit of Detectability

MCA - Multi Channel Analyser

MDA - Minimum Detection Activity

MNSR - Miniature Neutron Source Reactor

NAA - Neutron Activation Analysis

NIRR-1- Nigerian Research Reactor-1

NIST - National Institute of Standards and Technology

OECD - Organisation for Economic Co-operation and Development

PGNAA - Prompt Gamma Neutron Activation Analysis

RNAA - Radiochemical Neutron Activation Analysis

RSD - Relative Standard Deviation

SRM - Standard Reference Material

TNAA - Thermal Neutron Activation Analysis

UL - Upper Intake Level

WHO - World Health Organization

Appendix B: Parts of summary of Analytical reports

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* *
* Neutron Activation Analysis Report *
*Nuclear Science and Technology Section *
********** Unit=ppm

2016-11-09 19:58:00

Active Time: 600.0s True Time: 614.7s Geometry No:1 Group No:3

Energy	Concentration	Background	Element	Nuclide	T(1/2)	Average(ppm)	Rate
1014.4keV	9.827e+002 +/-48.9%	5.283e+003	Mg	27Mg	9.46m	9.827e+002+/-48.9%	5.50%
1779.0keV	1.438e+002 +/-17.1%	0.000e+000	Al	28Al	2.24m	1.438e+002+/-17.1%	0.81%
1642.7keV	2.211e+003 +/- 4.7%	0.000e+000	Cl	38Cl			
2167.7keV	2.018e+003 +/- 4.5%	0.000e+000	Cl	38Cl	37.2m	2.109e+003+/- 3.3%	11.81%
3084.5keV	1.440e+004 +/- 6.1%	0.000e+000	Ca	49Ca	8.72m	1.440e+004+/- 6.1%	80.60%
320.1keV	< 479.3413ppm	0.000e+000	Ti	51Ti	5.76m	< 479.34ppm	< 2.684%
1434.1keV	5.859e-001 +/-71.8%	0.000e+000	V	52V	3.75m	5.859e-001+/-71.8%	0.00%
846.8keV	1.695e+002 +/- 1.2%	0.000e+000	Mn	56Mn	2.58h	1.695e+002+/- 1.2%	0.95%
1039.2keV	< 9.0728ppm	0.000e+000	Cu	66Cu	5.1m	< 9.07ppm	< 0.051%
566.1keV	< 0.4691ppm	0.000e+000	Se	81Se	18m	< 0.47ppm	< 0.003%
191.9keV	< 0.0752ppm	0.000e+000	Mo	101Mo	14.6m	< 0.08ppm	< 0.000%

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* *

* Neutron Activation Analysis Report *

*Nuclear Science and Technology Section *

********** Unit=ppm

2016-11-02 00:03:00

Active Time: 1800.0s True Time: 1804.9s Geometry No:7 Group No:14

Energy	Concentration	Background	Element	Nuclide	T(1/2)	Average(ppm) Rate
1368.6keV	2.989e+001 +/-13.2%	0.000e+000	Na	24Na		
2754.0keV	3.537e+001 +/-11.0%	0.000e+000	Na	24Na	15h	3.314e+001+/- 8.5% 0.00%
1524.6keV	2.369e+004 +/- 5.3%	0.000e+000	K	42K	12.4h	2.369e+004+/- 5.3% 0.00%
834.1keV	5.776e+010 +/-63.3%	0.000e+000	Ga	72Ga	14.1h	5.776e+010+/-63.3%100.00%
559.1keV	2.749e-001 +/-10.4%	0.000e+000	As	76As	26.3h	2.749e-001+/-10.4% 0.00%
554.3keV	9.004e+000 +/-10.4%	0.000e+000	Br	82Br		
776.5keV	9.818e+000 +/- 8.0%	0.000e+000	Br	82Br	35.3h	9.515e+000+/- 6.4% 0.00%
336.3keV	5.673e-003 +/-64.8%	0.000e+000	Cd	115Cd		
527.9keV	4.045e-003 +/-82.0%	0.000e+000	Cd	115Cd	53.5h	5.046e-003+/-51.5% 0.00%
487.0keV	< 0.9398ppm	0.000e+000	La	140La		
1596.2keV	5.099e-001 +/-19.0%	0.000e+000	La	140La	40.3h	5.099e-001+/-19.0% 0.00%
103.2keV	4.683e-002 +/-93.9%	0.000e+000	Sm	153Sm	46.3h	4.683e-002+/-93.9% 0.00%
411.8keV	2.547e-003 +/-49.9%	0.000e+000	Au	198Au	64.8h	2.547e-003+/-49.9%

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* *

* Neutron Activation Analysis Report *

*eactor Facility, CERT, ABU, Zaria, Nige *

********** Unit=ppm

2016-08-04 19:19:59

Active Time: 3600.0s True Time: 3787.8s Geometry No:1 Group No:13

Energy	Concentration	Background	Element	Nuclide	T(1/2)	Average(ppm) Rate
889.3keV	2.725e-002 +/-30.7%	0.000e+000	Sc	46Sc	83.8d	2.725e-002+/-30.7% 0.00%
320.1keV	1.777e+000 +/-29.7%	0.000e+000	Cr	51Cr	27.7d	1.777e+000+/-29.7% 0.00%
1099.3keV	2.343e+002 +/-20.2%	0.000e+000	Fe	59Fe		
1291.6keV	1.840e+002 +/-22.7%	0.000e+000	Fe	59Fe	44.5d	2.121e+002+/-15.2% 0.00%
1173.2keV	5.930e-002 +/-68.1%	0.000e+000	Co	60Co		
1332.5keV	7.229e-002 +/-36.7%	0.000e+000	Co	60Co	5.27y	6.937e-002+/-32.4% 0.00%
1115.6keV	2.694e+001 +/- 9.9%	0.000e+000	Zn	65Zn	344d	2.694e+001+/- 9.9% 0.00%
136.0keV	< 0.1056ppm	0.000e+000	Se	75Se		
264.7keV	< 0.1107ppm	0.000e+000	Se	75Se	120d	< 0.11ppm < 0.000%
776.5keV	3.230e+001 +/- 4.6%	0.000e+000	Br	82Br	35.3h	3.230e+001+/- 4.6% 0.00%
1076.6keV	1.308e+001 +/-12.3%	0.000e+000	Rb	86Rb	18.7d	1.308e+001+/-12.3% 0.00%
514.0keV	6.003e+001 +/-15.3%	0.000e+000	Sr	85Sr	64.8d	6.003e+001+/-15.3% 0.00%
140.5keV	2.673e-001 +/-13.2%	0.000e+000	Mo	99Mo	65.9h	2.673e-001+/-13.2% 0.00%

DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other University. All sources of material used for the thesis have been duly acknowledged.

Tamene Hailu ——————————————
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This thesis has been submitted for examination with my approval as University advisor.

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