A DATABASE DEVELOPMENT FOR MULTI-ELEMENT CONCENTRATIONS OF MEDICINAL PLANTS USING INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS (INAA) AND INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS).

By

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DECLARATION

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ABSTRACT:

Medicinal plants are used by an estimated 80% of the population in developing countries for a wide range of health applications. The objective of this study is to develop a database for multi-element characterization of leaves and stem bark of medicinal plants using Instrumental Neutron Activation Analysis (INAA) and ethanoic extracts of the plants using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Furthermore, information on South African medicinal plants and their uses; essential or toxic elements and their dietary intake; trace element concentration and their healing capabilities as well as possible linking between traditional and modern medicine based on element concentrations is compiled to fulfill the overall objective. Thirty dry medicinal plant samples were analyzed using INAA and about 11 medicinal plants extracts using ICP-MS. The essential and toxic trace elements that have been found in most medicinal plants include As, Ca, Cd, Co, Cr, Fe, Hg, K, Mo, Mn, Na, Ni, P, Se, Th, U and Zn with in some cases major differences in concentrations providing a possible link between therapeutic actions of medicinal plants and trace elements. It has been demonstrated that INAA and ICP-MS techniques were ideal and relatively cumbersome respectively for this study.

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1 INTRODUCTION, OBJECTIVES AND SIGNIFICANCE OF THE STUDY

1.1 Introduction

Medicinal plants are used by an estimated 80% of South Africans for a wide range of health related applications. The enormous richness of medicinal plants can contributes to traditional cultures, modern medicine and pharmacology worldwide promoting an important therapeutic aid for alleviating ailments of humankind. Traditional healers prescribe medicinal plants for diseases like common colds, malaria, ulcers and infections across the world since ancient time [1,2,3].

Most of the active ingredients in chemically manufactured drugs are originally from medicinal plants extracts or constituents such as sugars, gums, *amino acids*, lecithin, *glycosides*, *tannins, quinines*, *flavonoids*, vitamins, minerals and trace elements. The availability of mixtures of medicinal plants, extracts and capsule forms contribute to the easy use and quick effects. Medicinal plants are the primary source of most of the medicines in the world providing many well known examples of plant-derived drugs including aspirin, *cocaine, quinine, morphine, codeine*, atropine and *reserpine* [2,3].

Trace (essential or toxic) elemental composition and concentration of medicinal plants play an important role in treatment of various diseases and ailments in medicinal science [4]. The analysis of multi-elemental concentrations in this study will be accomplished by using Instrumental Neutron Activation Analysis (INAA) for dried plants and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for liquid extracts.

Medicinal plants are potential renewable resources, therefore the conservation and sustainable utilization must involve a long term, integrated and scientifically oriented action programme to protect these valuable plants. The conservation strategy must define the management of human use of the medicinal plants so that it may yield the greatest sustainable benefit to the present generation while maintaining its potential to meet the needs and aspirations of future generations [2,3,4,5].

1.2 Objectives

The main objective of the study is to develop a database for multi-element analysis parts (leaves, roots, stems) of medicinal plants using Instrumental Neutron Activation Analysis (INAA) and in ethanol extracts using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The following information will be compiled to fulfill the objectives.

- Information on commonly used South African medicinal plants and their medicinal uses.
- Literature on the essential and/or toxic inorganic elements and their daily reference intake.
- Information on trace elements concentrations and their healing capacities.
- Determination of element concentrations by INAA and ICP-MS.
- Possible linking of traditional versus modern medicine based on element concentrations.

1.3 Significance of the study

South Africa offers many medicinal plants of which little is known so far about their essential or/and toxic element content. Knowledge of element concentrations and healing capacities of South African medicinal plants will provide clarity, quality and security to guide the usage of these plants for medicinal treatment of a specific ailment or a number of various disorders. A suite of data collected using INAA and ICP-MS will include the concentrations of essential and/or toxic elements of some of the South African medicinal plants, which can be reviewed against the background daily dietary reference intake of these elements. The information of the quantitative inorganic profile of these medicinal plants could be used to identify effectiveness of these plants for therapeutic use. The choice of the suite of plants is based on their medicinal effect and their widespread use by the populations in South Africa.

CHAPTER 2

2 THEORETICAL BACKGROUND OF MEDICINAL PLANTS

Medicinal plants and plant-derived drugs are widely utilized in traditional cultures all over the world for their medicinal or aromatic properties. South Africa has more than 30,000 plant species representing 10% of the world species. Approximately 3,000 species are used by an estimated 200,000 indigenous traditional healers. During the last century, the use of medicinal plants declined with the development of synthetic drugs especially in developed countries. Recently the use of easily accessible and low cost medicinal plants continues co-existing with modern medicine. It is unfortunate that medicinal plant usage has depleted in wild populations resulting in many plants being destroyed, vulnerable and/or being lost from their natural habitats. The conservation of medicinal plants can be accomplished by protecting these plants in their natural habitats or by cultivating and maintaining plants in botanic garden parks. Plant materials are widely used throughout developed and developing countries as home remedies, nutritional supplements and as raw materials for the pharmaceutical industry, representing a substantial proportion of the global drug market. The two fields where knowledge of metal species in medicinal plants is of great interest are the traditional and modern herbal medicine. A list of 37 commercial medicinal plants giving the correct scientific name, common name, family, trace elements known to be present, main medicinal uses and the dosages (if known) is provided in Appendix I [2,3,6,7,8,9,10,11,12,13].

2.1 Literature survey on plant material.

Plants consist of two main structural components: the root system which anchor the plant in the soil and absorb minerals from and the shoot system consisting of the stem and leaves. The main function of the stem is to support the plant above the ground and is the part through which minerals and water absorbed by the roots are conveyed to leaves. Plants control both the oxidation state and coordination environment of specific elements to maximize their detoxification by directly coordinating the element using the most chemical appropriate ligand to form stable non-toxic complexes. Roots are required by plants to acquire essential minerals such as iron, copper, nickel, zinc and selenium from soil. Though these elements are essential they are also potentially toxic to the plants themselves, so plants posses complex biochemical pathways to control them. The xylem transports elements taken up by the roots and distribute them to the leaves. When the element concentration increases in a plant, the cell membrane controls metal efflux and the metals bind to negative charges in various macromolecules that are either soluble or part of cellular structures in the cell. Other elements may be accumulated in the cytoplasm, the amount depending on the plant species and metals. Soluble molecules in the cytoplasm, such as organic acids or sulfur-rich polypeptides (phytochelatins) form complexes with metals and may also function as carriers to facilitate metal transport in cell vacuole. Phytochelatins (PCs) is probably the most widely studied metal binder in plants and can detoxify metals by forming a metal-PC complex in which the metal is bound to thiol groups of the cysteine units. Other mechanisms of plant resistance to toxic elements include high turnover of organic acids such as oxalate, malate, citrate, phytate, succinate and induction or activation of anti-oxidant enzymes (e.g. superoxide dismutase). The visible symptoms of manganese toxicity for example in plants include brown spots, marginal chlorosis, slow development in growing, necrosis of leaves and crinkled leaves which also varies from species to species [14].

2.2 Medicinal plant parts used and methods of administration.

The whole medicinal plant is rarely used for medicinal purposes because different parts of a plant contain different active ingredients, which may be essential or toxic. The plant parts normally used includes the stem bark, leaves, flowers, roots, tubers, bulbs and stem rhizomes. Some of the medicinal plants parts with interesting exceptions include seeds, fruits, gums, wood, nectar, resins and exudates. Plant medicines are prepared and administered in many ways. External treatment involves the uses of salves, dry powder (snuffs), pastes, bathing,

infusions and applying directly to the skin (topically). Internal treatments ranges from drinking teas, to chewing various plant parts, inhaling the smoke or vapor from burning plants and to the use of enemas [3,6,7,15].

The easiest and the frequent way of herbal administration among most indigenous traditional healers is to chew on the medicinal plant; e.g. the roots of Echinacea and the resins of Myrrh have been ingested directly [3,6,7,15]. Methods used for herbal administration include the following:

- **Herbal infusions and decoctions**

There are two ways of making herbal teas, infusion and decoction. Infusion is prepared by steeping lighter parts of the plant (leaves, flowers, light stems) in boiled water for 5 to 10 minutes before it is filtered. The preparation of infusions is similar to that of tea, certainly making it is the most common and cheap method of extracting the medicinal compounds of herbs. Decoction is prepared by adding cold water to thicker and less permeable plant parts (roots, stem bark) then heated to boiling for 5 to 10 minutes. Both infusions and decoction can be drunk hot or cold. Herbal compresses can also made by soaking a cloth in a herbal infusion or decoction taped with moisture barrier to draw out infection from the affected area.

- **Herbal tinctures**

Herbal tinctures are prepared by steeping a medicinal plant in alcohol extracts forming a liquid mixture that can be stored for long periods taking advantage of alcohol-soluble principles. Ideally tinctures should be made using pure ethyl alcohol, however since the product is not available to the public good Vodka with 35-45% alcohol can be used instead. Most of herbal tinctures are prepared using ethanol and the solution is then diluted with clean water to a predetermined herb-to-extract ratio. For example, in the United States a 1:5 tincture is common, while in UK a stronger 1:2 tincture is mostly used. Alcohol increases absorption by about 10% compared to water extraction.

- **Fluid and solid extracts**

Fluid extract refers to glycerites that are prepared using glycerol as a solvent instead of alcohol. The disadvantage of using glycerol as a solvent is that it inhibits absorption by 30% so higher doses are required. Solid extracts are made by distilling off the alcohol from the medicinal plant extracts leaving a soft paste-like or a dry solid that is stronger than a tincture.

- **Powdered herbs and capsules**

Herbs are dried and milled into a fine powder form. Powdered medicinal plant material can then be compressed into a tablet or filled in capsules. Coated tablets and capsules are used because they have longer shelf life and are easier to swallow while the smooth coating masks the usual unpleasant taste of medicinal plants.

- **Herbal ointments and essential oils**

An ointment is prepared by simmering herbs in waxes or fats containing no water. The resulting product, after separating the simmered herbs by squeezing and cooling, is a solid mixture of the wax or fat with medicinal constituents of that particular plant. Petroleum jelly, soft paraffin wax and bee wax are some common bases used. Essential oils are the volatile oily components of aromatic plants found in tiny glands located in the flowers, leaves, roots, wood and resins. The four main methods that are used to extract essential oils are distillation, in which the oil is extracted from hot-water dissolution followed by selectively condensation, pressure or centrifugation extraction, solvent extraction and lastly effleurage, which is a longer process involving the dissolution of the oils in animal fat and its separation using alcohol. Essential oils are mainly used in cosmetics, perfumery and also for therapeutic purposes.

Essential element content of medicinal plants play an important role in treatment of diseases for example diabetes. Fatima, N. et al [16] studied the amount of these elements in acid and water medicinal plants extracts. The results of this study are given in Table 1 showing the differences in element concentrations between the water and acid extracts.

Table 1: Essential elements obtained in water and acid extracts of selected anti-diabetic medicinal plants [16].

Medicinal plants		*Adiantum capillas*	*Allium sativum*	*Eugena jambolana*	*Gymnema sylvestre*	*Momordica charantia*	*Trigonella foenum graecum*
Chromium (mg/g±SD)	**Acid extracts**	0.009± 0.002(4)	0.006± 0.003(2)	0.006 ±0.001(3)	0.020± 0.0016(2)	0.007 ±0.001(3)	0.023± 0.0016(3)
	Water extracts	0.068x10^3 ±0.09(2)	0.042± 0.05(4)	0.111± 0.27(2)	0.211± 0.02(2)	0.209± 0.39(5)	0.094± 0.46(6)
Iron (mg/g±SD)	**Acid extracts**	0.738± 0.036(2)	0.132± 0.009(2)	0.235± 0.000(2)	1.407± 0.040(2)	0.172± 0.040(2)	0.7023± 0.030(2)
	Water extracts	0.0014 x10^3 ± 0.001(5)	0.0125± 0.009(2)	0.0035± 0.004(2)	0.0110± 0.003(3)	0.0147± 0.001(2)	0.0018± 0.001(6)
Manganese (mg/g±SD)	**Acid extracts**	0.057± 0.004(4)	0.018± 0.018(3)	0.010± 0.001(2)	0.612± 0.003(3)	0.036± 0.004(3)	0.028± 0.013(2)
	Water extracts	0.0075 x10^3 ± 0.007(3)	0.0067± 0.002(3)	0.0023± 0.004(4)	0.0559± 0.02(4)	0.0061± 0.004(2)	0.0016± 0.000(2)
Zinc (mg/g±SD)	**Acid extracts**	0.062± 0.004(3)	0.057± 0.002(2)	0.028± 0.900(4)	0.107± 0.003(2)	0.072± 0.007(2)	0.111± 0.013(3)
	Water extracts	0.0049 x10^3 ± 0.0064(3)	0.0135± 0.002(4)	0.0035± 0.001(2)	0.0092± 0.008(5)	0.0149± 0.015(2)	0.0024± 0.001(3)

SD = Standard deviation
The number of determinations is shown in brackets

2.3 Dosage and dosage forms in herbal medicine

Information based on dosage regimes used in clinical trials with successful outcomes is obtained from reliable traditional sources such as various pharmaceutical companies and expert committees such as Commission E as well as ESCOP (European Scientific Cooperative on Phytotherapy). Many different dosage approaches are found within countries among different herbal practitioners. One extreme assumption in medicinal plants usage is that their therapeutic effect relies on a specific dose of the active chemicals contained in each plant. An appropriate dosage system for modern phytotherapy should be consistent with the dosages ranges used in other important traditions, for example in China, and the dosages currently recommended and established from pharmacological as well as clinical research. Dosages of medicinal plants commonly used in South Africa are also given in Appendix I. One of the biggest concerns for humans is being able to differentiate between toxic and edible medicinal plants according to the dose because potentially poisonous plants can also be medicinal depending on the dosage and mode of preparation. Fatima N et al [16] studied six medicinal plants recommended for the treatment of diabetes mellitus. Giving also the parts of the plant used, dose per day of these selected herbs [3] and the dosage forms used are shown in Table 2.

Table 2: Dosage of medicinal plants recommended for the treatment of diabetes mellitus [16].

Medicinal plant	Parts used	Dose/day (g)	Dosage form
Adiantum capillas veneris	Bark	0.7-1.75g	Water extract
Allium sativum	Cloves	2-4g / 0.008g	Fresh / essential oil
Eugena jambolana	Seeds	2-5g	Powder
Gymnema sylvestre	Leaves	0.4-0.6g	Water extract
Momordica charantia	Fruit	3-15g	Powder
Trigonella foenum graecum	Seeds	15g /10-100g	Powdered solid / water extract

2.4 The traditional medical system

Medicinal plants are an important element in the medicinal system as part of a culture's traditional knowledge. A wide range of health related applications of medicinal plants includes headaches, memory improvement, snake bites, wounds and enhancement of the body's general immunity. Millions of people including spiritual healers, prophets, traditional village practitioners and herbal healers use medicinal plants to ensure healthcare and security.

Traditional medicine consists of health practices, knowledge and beliefs using herbal based medicines and spiritual therapies to treat, diagnose and prevent diseases maintaining health in the broadest sense of physical, spiritual, social and psychological well-being. Heinrich C. et al examined the use of medicinal plants in four indigenous groups of Mexican Indians, Maya, Nahua as well as Zapotec for comparative purposes, and discovered that the widespread use of plant species is due to multiple transfer of species within Mexico, which are usually known in one culture and are part of their traditional knowledge [6,16].

Most people using traditional medicine may not know the scientific rationale behind their medicines, but they understand from personal experience that medicinal plants are highly effective at correct doses. Mathabe M.C et al conducted the ethno-botanical survey on antibacterial activities of 21 medicinal plant species belonging to 14 different families that are used in traditional medical practice for treatment of diarrhea in the Limpopo Province, South Africa [6,18].

The advantages of using traditional medicine includes more accessibility, less costs, few if no side effects when taken wisely, and basically medicinal plants are more natural. The disadvantages of the traditional medical system includes: (a) the effectiveness of some of the medicinal plants remain unproved, (b) the dosages or indications for medicinal use are not clearly stated on most packaging information, (c) traditional medicine may have serious interactions with prescribed drugs, (d) poor hygienic conditions, (e) lack of precision of the somatic diagnosis and the wide range of therapeutic indications for a given medicine and (f) the immaterial aspects of its practice, which open the way to presumed witchcraft and quackery.

Herbal medicines are not all natural because some are synthetic and can be just as dangerous because of incomplete toxicity information for most of these medicinal plants. For example, Whiting D.A [20] did a study on the genuine poison ivy dermatitis in South Africa, which reported four South African patients with allergies from being exposed to *Smodingium argutum*, *Toxicodendron radicans* and *Toxicodendron succedanea*, which are all members of the family *Anacardiaceae*.

Medicinal plants are used to derive plant drugs or medicines (Table 3) that restore health and prevent disorders; e.g. castor oil extracted from seeds of *Ricinus communis* and rooibos tea as a dry product of *Aspalathus linearis* [13,20,21,22].

Table 3: Various herbal drugs from medicinal plants of the *Lamiacae* family [21].

Commercial herbal drug	Medicinal plant
1.Salviae folium	*Salvia officinalis L.*
2.Menthae pip.folium	*Menthe piperita L.*
3.Melissa folium	*Mellisa officinalis L.*
4.Lavandule flos	*Lavandula angustifolia Mill*
5.Basilici herba	*Ocimum basilicum L.*
6.Marubii herba	*Marrubium vulgare L.*
7.Origani herba	*Origanum vulgare L.*

2.5 The modern medical system

The modern medical system is the scientific or classical system of medicine comprising the codified and organized medical wisdom with sophisticated theoretical foundations and explanation. The Minister of Health manages the modern medical system, which provides medical care, health laboratory services, mental, dental, nutritional and maternal child health services. The modern medical system is a system that is government financed with medical needs provided at hospitals, health care centers, clinics and maternity homes. Modern medicine is always updated and advanced through scientific research and it has been based on a materialistic and objective vision of man, natural phenomena and the universe. This system originated by mastering the human anatomy that is gathering knowledge of the physical and organic components of the human being, thus studying cells, tissues and body parts alterations caused by pathogenic agents.

The use of modern medicine is a preferred choice of healing modality because of laboratory and radiology results identifying the cause of diseases. Western medicine may diagnose an illness in terms of bacterial or viral infections, and then effectively treat that infection with antibiotics. When applying this system good hygienic conditions are strictly required. Modern medicine is not accessible to most of the communities in rural areas because poverty and high costs of treatment make it unaffordable [18,20,21,22].

2.6 Classification of trace elements

The human body does not synthesize minerals and trace elements and therefore it must obtain them from external sources such as food, air, plants and beverages. Safe water may be an important source of such essential elements like iron, iodine, fluoride, calcium and copper. Trace elements are elements, which are required in amounts smaller than 0.01% of the mass of the organism. These elements are essential for life or optimum health of an organism and mostly function as catalysts for enzymatic activity of human bodies. Trace elements are classified as either beneficial (essential) or harmful (toxic) in human health [4,5,23] as shown in Table 4.

Table 4: Classification of essential and toxic elements.

Essential trace elements	Ca, Cl, Co, Cr, Cu, Fe, I, K, Mg, Mo, Na, P, S, Se, Zn
Trace elements that are probably essential	B, Mn, Ni, Si, V
Toxic elements	Al, As, Cd, F, Hg, Li, Pb, Sn
Radiotoxic elements	^{232}Th + progeny, ^{238}U + progeny, ^{235}U + progeny

2.7 Essential and toxic elements

Trace metals have a complex as well as fascinating effects on human health. Therefore there has been a growing interest in trace-element research in clinical medicine, biology, toxicology and nutrition. Medicinal plants contain both organic and inorganic constituents, but little research work has been carried out on the role of inorganic elements in medicinal use while more attention has been paid to the organic constituents of medicinal plants. Trace elements co-exist with numerous organic compounds in medicinal plants, therefore concentrations of free trace elements are usually low. Most of medicinal plants contain trace elements that play an important role in the formation of active chemical constituents giving rise to medicinal as well as toxic properties, thereby providing a possible link to the therapeutic action of the medicine. A direct correlation between elemental content of medicinal plant and their healing ability is not yet understood in terms of modern pharmacological concept. The quantitative estimation of a range of trace element concentrations is a key for determining the effectiveness of medicinal plants in treating a range of diseases and also to understand their pharmacological action [23]. Trace elements can be highly toxic to various life forms, others essential elements, but even essential can be toxic in higher doses. The influence and effects of trace elements are largely determined by the nature of ligands around it and by their chemical form; e.g. Cr(VI) ions are far more toxic than Cr(III) [15].

Essential elements are elements required in small amounts for survival by plants and animals. The depletion of these elements may cause various metabolic instabilities due to enzyme dysfunction. An essential element can also be defined as an element meeting the following criteria:

- It is present in all healthy tissues of all living things.
- Its concentration from one species to next is fairly constant.
- Its withdrawal from the body induces reproducibility of the same structural and physiological abnormalities regardless of the species of the species studied.
- Its addition either prevents or reverses these abnormalities.
- The abnormalities induced by deficiency are always accompanied by biochemical changes.
- The biochemical changes can be prevented or cured when the deficiency is prevented.

Berger M.M discovered that enhancing trace element status and antioxidant defense by selenium (0.315 to 0.380mg/d), zinc (26.6 to 31.4 mg/d) and copper (2.5 to 3.1mg/d) supplementation is associated with the decrease of nosocomial pneumonia in critically severely burned patients. High concentrations of chemical elements that are not required by the human body are referred as toxic elements, which may be contained in the diet, drinking water or air. Toxic elements can result in chronic or acute poisoning and nutrient deficiencies, therefore these elements should be eliminated from the environment or lowered by supplying nutrients that protect against their toxic levels [24,25,26,27,28] as given in Table 5.

Table 5: Body organs and tissues affected by some of abundant (toxic) element levels and their protective nutrients [28].

Toxic elements	Body organs and tissues affected by toxic elements	Nutrient protective against the effects of toxic elements
Aluminum (Al)	Stomach, brain, bones	Magnesium (Mg)
Arsenic (As)	Cells (cellular metabolism)	Selenium (Se), Iodine (I), Calcium (Ca), Vitamin C, Sulfur (S), Amino acids
Cadmium (Cd)	Renal cortex of the kidney, heart, blood vessels of the brain, appetite and smell centers of the brain	Zinc (Zn), Calcium (Ca), Vitamin C, Sulfur (S), Amino acids
Lead (Pd)	Bone, liver, kidney, pancreas, heart, brain, nervous system	Zinc (Zn), Iron (Fe), Calcium (Ca), Vitamin C, Vitamin E Sulfur (S), Amino acids
Mercury (Hg)	Nervous system, immune system, cell membranes, appetite and pain centers of the brain	Selenium (Se), Vitamin C, Sulfur (S), Amino acids

2.8 Healing capacities of trace elements

The nutritional importance of trace elements has grown rapidly during the last three decades mainly due to a better understanding of their biological functions. Trace elements serve as building blocks of lives. Human bodies consume these elements through daily intake and therefore they should be incorporated to stay healthy and strong. For example, calcium is used to build strong bones and fluorine makes teeth healthier. Food and plants are amongst the greatest sources of trace elements, and sometimes dietary supplements such as vitamins are taken to maintain the proper chemical balance in human bodies. Abnormal trace element concentrations may be attributed to metabolic disorders, excessive or inadequate uptake. Excessive concentrations of trace elements in plants, food or drinking water may induce toxic manifestations and deprivation of essential trace elements can produces a deficiency syndrome. There is no simple correlation between trace element content and clinical symptoms and for most essential elements no recommended daily allowances have been established, but only ranges of recommended intakes. Inadequate dietary intake of essential elements can results in a deficiency syndrome, which is preventable or reversible by supplementation with this element [5,29,30,31]. As an example of the enormous importance of trace elements the health benefits and negative effects caused by abundance or deficiency of these elements are shown in Table 6.

For example selenium is found in medicinal plants like garlic, fennel seed, ginseng, alfalfa, burdock root, radish, onion and yarrow. The trace element content of selenium in these medicinal plants enables the fighting of infections since it stimulates antibody response and promotes more energy in the body [5,29,30,31]. Manganese is an essential trace element to all living systems and can be found everywhere on earth. It is a major constituent of enzymes (metalloenzymes, hormones and proteins) of human beings. Low manganese dietary levels in blood or tissue have been associated with osteoporosis, whereas high levels are associated with greater risk of several cancers. Other elements, also analyzed in this thesis, will be addressed because of their important function to health; e.g. Calcium (formation and maintenance of bones), Potassium (normal blood pressure) and Zinc (participates in the metabolism of nucleic acids and the synthesis of proteins) [15].

2.9 Dietary recommendations of essential and toxic elements

The *Recommended Dietary Allowance* (RDA) represents the establishment of nutrition for planning and assessing dietary intake levels of essential elements considered to be adequate to meet the needs of essentially all healthy human beings. The minimum dosage required per day to ward off serious deficiency of a particular nutrient is referred to as the Recommended Dietary Allowance (RDA), or in other words the daily dietary intake of essential and/or toxic elements are given in the RDA. The so-called *Tolerable Upper Intake* (ULs) as well as *Adequate Intake* (AI) are recommended intake levels affected by sex, age as well as special physiological states such as lactation and pregnancy as indicated in Table 7. Elements like Al, As, Cd, Hg, Li, Pb, S, Si and Sn daily intake recommendations are not given in Table 7 as insufficient data could be obtained, e.g. due to lack of data of adverse effects in age groups and concern with regard to lack of ability to handle excess amounts.

Factors that influence trace element nutrition in developing countries include environmental pollution, respiration and diarrhea infections. The reference values are important for the monitoring changes in the environment that could influence the trace element supply. Growing industrialization, rising composition of fossil fuels, etc. also increase the environmental exposure to some of more toxic trace elements which is a matter of public health concern. Research projects dealing with dietary intake of trace elements in several countries have been carried out by international bodies such as the World Health Organization (WHO), the Food and Agricultural Organization (FAO) and the International Atomic Energy Agency (IAEA). The intake levels of some of the essential and toxic elements given in global data along with

results from the IAEA study for comparison in Provisional Tolerable Intake levels (PTA)[5,24,32,33,34,35] are shown in Table 8.

Nutritional policies and programmes should be based upon diet and health relationships of a particular relevance to the individual country. Priorities in establishing dietary guidelines must address the relevant public health concerns whether they are related to dietary insufficiency or excess. Dietary guidelines have specific goals such as to promote overall health, control nutritional diseases whether they are induced by deficiency or excess of nutrient intake and to reduce diet-related diseases. Currently more people are at risk of micronutrient deficiencies, actually ill or disabled by dietary intakes causing for example nutritional anemia, mental retardation, learning disabilities, low risk capacity and blindness. Therefore there is an urgent need to establish reliable reference values for all elements essential as well as toxic, with this information it will be possible to know whether an observed trace element concentration may have clinical importance or not [24,32,33].

2.9.1 Dietary goals for vulnerable groups

Infants and children of up to two years are the most vulnerable group in almost every country because special attention is needed to ensure achieving full growth and development potential. Nutritional requirements for 4-6 months old infants are normally fully covered from breast milk depending on the good health of the mother, but the dietary guidelines must include RDA of essential elements in case herbal medicines are used for health applications. Dietary intakes especially in food-deficit, low-income populations are not increased much or at all during pregnancy and thus must be more recommended. Iron deficiency anemia for example is a common disease during pregnancy therefore sources rich in iron should particularly be encouraged [5,35]. Lactating women are also at risk of nutritional depletion of energy and other nutrient intakes that may not cover their increased requirements therefore nutrients supplements must be encouraged also in this case. In nutrient recommendations for older age group beyond 70 years, it is important to maintain adequate nutrient intake which must compensate for changes affecting appetite strength and energy needs [5,35].

The IAEA has supported research on human dietary intakes of trace elements by taking advantage of analytical capabilities offered by neutron activation analysis and other nuclear-related techniques. The Total Diet Study (TDS) was carried out by the IAEA from 1986 to 1993 with the aim of obtaining reliable comparative data on average daily dietary intakes of trace elements in fifteen countries (Australia, Brazil, Canada, China, Iran, Italy, Japan, Norway, Portugal, Spain, Sudan, Sweden, Thailand, Turkey and USA) [36]. A nutritional study of population groups living in areas close to the site of the 1986 Chernobyl nuclear power plant accident was carried out applying the same methodologies in 1990-1991. The daily dietary intakes of some of the essential elements for the recent 'Reference Asian Man (RAM)' project was also studied in eight Asian countries (Bangladesh, China, India, Japan, Republic of Korea, Pakistan, Philippines and Vietnam) in 1995-2003. The results of the above three projects are summarized in Table 9, for adult females and males [36].

The main of quality assurance in this IAEA study comprised of some of the following: written protocols covering all the various steps in the process from definition of the study group up to obtaining the analytical results, homogenization of the samples at the IAEA's laboratory under clean conditions using a Teflon blender, analysis of the samples by experienced analysts, and validation of the analytical techniques before the start of routine analysis with the help of certified reference materials. The greatest achievement of the project was to allow meaningful comparisons of dietary intakes of trace element made between different countries using common methods for sample collection, preparation, analysis and quality assurance [36]. IAEA projects have over 30 years being gratefully appreciated supporting and strengthening nutritional and environmental awareness in its member states particularly. The interest for trace elements has now reached a wider audience and is no longer limited to academic

spheres. The industry and governments are getting involved especially with the advert of new and more stringent regulations [36].

Table 6: Health benefits and negative effects of trace elements [29,30,31].

Trace element	Uses and health benefits	Negative effects or symptoms caused by trace element deficient.	Negative effects or symptoms caused by trace element abundance.
Aluminum	Bone formation.	Depressed growth, decreased life expectancy and an increased number of spontaneous abortions.	Reduce bone formation and *osteomalacia*.
Arsenic	Proper growth, development and reproduction.	Carcinogenic.	Death.
Boron	Promotes healthy bone metabolism, proper function of the endocrine system (ovaries, testes and adrenals).	Decreased bone density, greater risk for prostate cancer, decreased mental alertness in men and women past age of 45.	Skeletal abnormalities, diarrhea, nausea, vomiting, anemia, *edema*, *seizures*, gastrointestinal disturbances, fatigue and cold-like symptoms.
Cadmium	Maximize growth.	Gastrointestinal irritation, vomiting, abdominal pain and diarrhea.	Kidney, skeletal and *pulmonary* damage.
Calcium	Formation and maintenance of bones, muscular growth and normal *blood clotting*.	*Hypertension, osteoporosis*, prostate cancer, kidney stones, miscarriage, menstrual problems, bone diseases, menstrual problems, sleep disturbances, cardiovascular and depressive disorders.	Joint degeneration, stroke, gastrointestinal disturbances, chronic *fatigue* and it inhibit the Vitamin D's cancer protective effect.
Chlorine	Constituent of stomach acid and as an electrolyte for controlled regulation of acid-alkaline balance.	*Metabolic alkalosis*, apathy, slowed growth and delayed speech.	Edema, high blood pressure, greater risk of some cancers, choking, chest pains, nausea and vomiting.
Chromium	*Anti-inflammatory* properties, normal blood sugar and fat metabolism.	Glucose intolerance, elevated total *cholesterol*, inflammatory joint disease and nerve degeneration.	Spinal/ joint degeneration, depressed immune system and lymphatic swelling.
Cobalt	Myelin formation to support the manufacture of red blood cells, metabolism of fats, carbohydrates and synthesis of proteins.	*Demyelination* of large nerve trunk and the spinal cord, reduced white blood cells, shortness of breath, headaches and dizziness.	Asthma, anxiety and cardiac symptoms.
Copper	Wound healing, formation of oxygen-carrying molecule *hemoglobin*, formation red blood cells and bones.	*Anemia*, increased susceptibility for infections, *osteoporosis*, weakened immune system, irregular heart beat and nerve degeneration.	*Anaemia*, nausea, vomiting, abdominal pain, *arthritis*, joint/spinal degeneration, higher risk of some cancers, heart disease and stroke.
Fluorine	Bones and teeth, preventing dental caries.	Weakened bone and dental caries.	Increased bone fractures, fluorosis, osteosclerosis, hearing loss, stomach ulcers and greater risk of some cancers.

Iodine	Production of hormones (thyroxin) by the thyroid gland.	*Goiter*, fatigue, depression, low cardiac output, edema, hair loss, *hypothyroid*, asthma, infertility and weight gain.	Palpitations, *insomnia*, skin rash, sweating, goiter, weight loss and *hyperthyroid*.
Iron	Production of hemoglobin and *myoglobin*, energy production, oxygenation of red blood cells and healthy immune system.	Fatigue, anemia, depression, dizziness, asthma, gastrointestinal disorders, migraine-headaches and weak immune system.	Arthritis, high blood pressure, heart disease, liver disease, nausea, higher risk for several cancers and constipation.
Lead	Functioning of the nervous system and the kidneys.	Liver and kidney damage, mental retardation as well as abnormalities in fertility and pregnancy.	Neurological, neurobehavioral and development effects in children, hematological effects, renal effects and increased blood pressure.
Lithium	Mood stabilizing agent, produce positive effects regarding mental health and immune function.	Gastrointestinal disorders, low stomach acid and heartburn.	Nausea, vomiting, weight gain, *goiter*, liver disease, kidney disease, frequent urination, diarrhea, edema and death.
Magnesium	Formation of bones and teeth, relax muscles, synthesis of protein, important co-factor in most enzymatic reactions, production of energy and for cardiovascular functions.	Kidney stones, high blood pressure, heart problems, insomnia, anxieties, chronic constipation and menstrual cramps.	Neuromuscular problems, gastrointestinal problems, low blood pressure, depression, dizziness, cardiovascular disease, osteoporosis, dry skin and management of premature labor.
Manganese	Protein metabolism, bone formation, carbohydrate (glucose) metabolism, blood clotting, activates enzyme responsible for formation of urea, breast milk in females, DNA and *RNA* synthesis.	*Fatigue*, depression, low blood sugar, joint dislocations, high cholesterol, asthma, migraine-headaches, infrequent menstrual cycles, gastrointestinal disorders, osteoporosis, edema and nausea.	Migraine-headaches, frequent menstrual cycles, dizziness, depression, mental illness, higher risk for several cancers, insomnia, edema and nausea.
Mercury	Nervous and renal system functioning.	*Hypothyroidism* and *anemia*.	Neurological effects, renal disturbances and *hyperthyroidism*.
Molybdenum	Normal *uric acid* levels, anti-cancer properties anti-oxidative properties and protect cells from free radicals.	Spinal degeneration, higher risk for several cancers, insufficient uric acid.	Skin eruptions, itchy skin, inflammatory spinal/ joint disease and decreased growth.
Nickel	Interacts with nucleic acids (*RDA and DNA*), activation of enzymes, constituent of circulating proteins *nickeloplasmin* and *albumin*.	Liver and kidney diseases, thyroid disease, gastrointestinal irritation.	Asthma, cardiac symptoms, potential to cause cancer of the sinuses, throat and lungs.
Phosphorus	Bone and teeth formation, kidney functioning, cell growth and the contraction of the heart.	Osteoporosis, joint/bone pain, arthritis, weakness, weight loss, higher risk for several cancers, dehydration and *kidney stones*.	Arthritis, bone loss, gout, dental problems, skin eruptions, higher risk for several cancers and kidney stones.

Potassium	Normal blood pressure, building muscles, transmission of nerve impulses and heart activity.	Irregular heartbeat, *hypertension*, stroke, asthma, muscle spasms, muscle weakness, frequent menstrual cycles, liver disease, kidney disease, and weight gain.	Low blood pressure, kidney disease, bladder infections, higher risk of several types of cancer, infrequent menstrual cycles, ovarian cysts, joint/back pains, weakened immune system, anxiety and *insomnia*.
Selenium	Anti-aging properties, prevent cancer, stimulates anti-body response to infections, fight cold sores and shingles.	Cardiomyopathy, cardiovascular disease, stroke, nerve degeneration, higher risk for some cancers, arthritis and anemia.	Nerve degeneration, osteoporosis, shingles, loss of hair, abnormal nails, tooth decay, garlic breath and death.
Silicon	Promotes the union of bone after fracture and keeping blood vessel walls healthy.	Cartilage/ joint degeneration, osteoporosis, headaches, cardiovascular disease, vascular degeneration, dry/ brittle nails and hair.	Bruising, stomach irritations, skin rashes and irritations.
Sodium	Acts as an electrolyte, required in the manufacture of hydrochloric acid in the stomach and for normal kidney functioning.	Fatigue, depression, low blood pressure, headaches, dehydration, dizziness, arthritis, kidney stones and seizures.	Edema, hypertension, stroke, dizziness, gout, headaches, kidney damage, stomach problems, nausea and vomiting.
Sulfur	Detoxify the body, cartilage regeneration, assist in immune system and fights the effect of aging.	*Alzheimer's disease*, nerve degeneration, memory loss, arthritis/ cartilage degeneration, reduced insulin production, collagen diseases affecting hair, skin and nails.	Inflammatory disease of the gut, nerve degeneration, asthma, inflammatory vascular/ joint degeneration.
Thorium	No data available.	Lung disease, cancer of the bone, lung or pancreas, liver disease,	Death.
Tin	Supporting adrenals and cardiac functions.	*Fatigue*, depression, low cardiac output, shortness of breath, asthma, headaches and *insomnia*.	Skin rash, stomach problems, palpitations, vomiting, diarrhea, abdominal pains, nausea and headaches.
Uranium	No data available.	Kidney and lung problems.	Reproductive, developmental, mutagenic and carcinogenic consequences.
Vanadium	Stabilize blood sugar levels, formation of bones and teeth.	Spinal degeneration, reduced growth and reproductive and elevated *cholesterol*.	Arthritis, aching bones, weakened immune system chronic colds and gastrointestinal problems.
Zinc	Immune system, synthesis of the nucleic acids RNA and DNA, protect cells from free radicals, normal growth and reproductive development.	Decreased growth, loss of taste and smell, sterility, decreased wound healing, skin rash, heart disease, kidney disease, muscle weakness, several types of cancer, depression and high blood pressure.	Nausea, vomiting, dehydration, gastrointestinal problems, stomach ulcers, higher risk of several types of cancer, *anemia*, hair loss, muscles cramps, weakened immune system and *insomnia*.

Table 7: Dietary recommendations of trace elements [33,34].

Life Stage Group	B	Ca	Cl	Co	Cr	Cu	F	Fe	I	K	Mg	Mn	Mo	Na	Ni	P	Se	Th	U	V	Zn
	UL	AI	AI	RDA	AI	RDA /AI*	AI	RDA /AI*	RDA /AI*	RDA	RDA /AI*	AI	RDA /AI*	RDA	UL	RDA /AI*	RDA /AI*	PTI	PTI	UL	RDA /AI*
m/y	mg/d	mg/d	mg/d	µg/d	µg/d	µg/d	mg/d	mg/d	µg/d	mg/d	mg/d	Mg/d	µg/d	mg/d	mg/d	mg/d	µg/d	µg/d	µg/d	mg/d	mg/d
Infants																					
0-6m		210	0.18		0.2	200*	0.01	0.27*	110*	580	30*	0.003	2*	280		100*	15*	36.2	24.7		2*
7-12m		270	0.57		5.5	220*	0.5	11	130*	1370	75*	0.6	3*	580		275*	20*	36.2	24.7		3
Children																					
1-3y	3	500	1.5		11	340	0.7	7	90	2730	80	1.2	17	1150	0.2	460	20	370	35.6		3
4-8y	6	800	1.9		15	440	1.0	10	90	3900	130	1.5	22	1730	0.3	500	30	476	41.1		5
Males																					
9-13y	11	1300	2.3		25	700	2	8	120	5460	240	1.9	34	2300	0.6	1250	40	653	65.1		8
14-18y	17	1300	2.3	3	35	890	3	11	150	5460	410	2.2	43	2300	1.0	1250	55	653	65.1		11
19-30y	20	1000	2.3	3	3	900	3	8	150	5460	400	2.3	45	2300	1.0	700	55	724	100	1.8	11
31-50y	20	1000	2.3	3	35	900	4	8	150	5460	420	2.3	45	2300	1.0	700	55	724	100	1.8	11
51-70y	20	1200	2.0	3	30	900	4	8	150	5460	420	2.3	45	2300	1.0	700	55	724	100	1.8	11
>70y	20	1200	1.8		30	900	4	8	150	5460	420	2.3	45	2300	1.0	700	55			1.8	11
Females																					
9-13y	11	1300	2.3		21	700	2	8	120	5460	240	1.6	34	2300	0.6	1250	40	653	65.1		8
14-18y	17	1300	2.3	3	24	890	3	15	150	5460	360	1.6	43	2300	1.0	1250	55	653	65.1		9
19-30y	20	1000	2.3	3	25	900	3	18	150	5460	310	1.8	45	2300	1.0	700	55	724	100	1.8	8
31-50y	20	1000	2.3	3	25	900	3	18	150	5460	320	1.8	45	2300	1.0	700	55	724	100	1.8	8
51-70y	20	1200	2.0	3	20	900	3	8	150	5460	320	1.8	45	2300	1.0	700	55	724	100	1.8	8
>70y	20	1200	1.8		20	900	3	8	150	5460	320	1.8	45	2300	1.0	700	55			1.8	8
Pregnancy																					
≤18y	17	1300	2.3		29	1000	3	27	220	5460	400	2.0	50	2300	1.0	1250	60	724	100		12
19-30y	20	1000	2.3		30	1000	3	27	220	5460	350	2.0	50	2300	1.0	700	60	724	100		11
31-50y	20	1000	2.3		30	1000	3	27	220		360	2.0	50		1.0	700	60				11
Lactation																					
≤18y	17	1300	2.3		44	1300	3	10	290	5460	360	2.6	50	2300	1.0	1250	70	724	100		13
19-30y	20	1000	2.3		45	1300	3	9	290	5460	310	2.6	50	2300	1.0	700	70	724	100		12
31-50y	20	1000	2.3		45	1300	3	9	290	5460	320	2.6	50	2300	1.0	700	70	724	100		12

RDA: Recommended Dietary Allowance,
UL: Tolerable Upper Intake Level,
AI: Adequate Intake and
PTI: Provisional Tolerable Intake

Table 8: Intake values of essential and/or toxic elements per day [36].

Essential element	Global data median (range for minimum and maximum intake)	IAEA (PTA)	Unit
Aluminum	4.4 (2.2-17)	3-17	mg/day
Arsenic	41 (3-330)	3-160	µg/day
Cadmium	14 (8-200)	8-25	µg/day
Chromium	50 (20-285)	59-106	µg/day
Copper	15 (0.6-5.8)	1.1-2.0	mg/day
Iodine	190 (50-1050)	50-260	µg/day
Iron	13 (5.1-47)	8.1-30.0	mg/day
Lead	51 (7-515)	21-160	µg/day
Mercury	4.1 (0.7-76)	3-76	µg/day
Selenium	61 (8-1340)	34-133	µg/day
Zinc	10 (4.2-19)	8.3-14.0	mg/day

Table 9: Statistical summary of daily dietary intakes for elements of interest compared with the USA RDAs [36].

Study	Country	No.[a]	Ca mg/d	Cu mg/d	Fe mg/d	I mg/d	Mg mg/d	Mn mg/d	Se mg/d	Zn mg/d
TDS	**Australia**	6	780	1.82	15.2		350	6.5	62	13.6
	Brazil	20	585	1.24	17.6	240	238	3.2	45	9.2
	Canada	14	890	1.06	20.4		290	3.4	114	9.0
	China	63	580	1.56	19.2	90	340	5.7	28	8.6
	Iran	30	690	1.95	18.7	53	360	8.5	66	11.2
	Italy	48	810	1.46	11.2	77	260	2.6	50	9.7
	Japan	5	600	1.25	9.4	260	285	5.4	142	10.4
	Norway	19	980	1.98	13.9	170	355	5.8	85	10.8
	Portugal	15	910	1.25	16.1		300	3.2	71	10.8
	Spain	40	980	1.19	11.4	125	300	2.1	62	10.7
	Sudan	40	580	1.89	31.4	53	410	6.3	59	9.1
	Sweden	12	1550	1.87	23.7		330	3.0	53	12.7
	Thailand	68	435	1.32	10.4	41	220	6.0	73	11.2
	Turkey	16	650	1.94	17.9	80	310	6.9	37	10.5
	USA	12	870	1.27	14.6	230	285	2.5	114	13.8
Chernobyl	**BY, RU, UA[b]**	34	860	0.82	15.4	81	270	3.3	63	9.9
RAM	**Bangladesh**	9	310	1.95	10.8		320	3.4		9.1
	China	48	720	1.39	30.1	330	360	6.7	53	12
	India	20	360		14.9	85			57	7.9
	Japan	27	440	1.06	4.9	245	210	3.5		8.3
	Korea	5	430			87				9.5
	Pakistan	31	480		30	43	520	11.8	109	14.3
	Philippines	31	220	0.95	4.2	40	130	2.3		5.2
	Vietnam	18	640	0.68	7.4	1450	130	2.8		5.2
Median			645	1.32	15	87	300	3.5	62	10.2
Range			200-1550	0.68-1.98	4.2-31.4	40-1450	130-520	2.1-11.8	28-142	5.2-13.8
No.			24	21	23	19	22	22	19	24
RDA			1200	0.9	8	150	320-420	1.8-2.3	55	8-11

[a]Number of total diet samples collected and analyzed for the elements of interest
[b]ISO country codes for Belarus (BY), Russia (RU) and Ukraine (UA)

2.10 Specific studies on potential analytical techniques

2.10.1 Atomic Absorption Spectroscopy (AAS)

Rajurkar N.S et al [39] studied elemental composition (Cu, Cr, Co, Cd, Zn, Ni, Na, Fe, Hg, K, Ca, Cl) of some medicinal plants used for healing urinary tract disorders by atomic absorption spectroscopy (AAS). Arce S. et al [37] found that the element content of *Valeriana officialis* roots were Fe, Al, Ca and V within the range 100-1000 mg/kg; Pb, and Zn within range 10-100mg/kg and Cd with concentration of 0.0125mg/kg when using flame and electrothermal AAS. Elemental composition in the leaves of four traditional medicinal plants (*Murraya koenigii, Mentha piperitae, Ocimum sanctum and Aegle marmelos*), widely used in the treatment of diabetes-related metabolic disorders were studied using AAS by Narendhirakannan R.T et al [38]. The levels of Cu, Ni, Zn, K and Na were obtained at substantial trace level while Fe, Cr, and V levels were marginal in those medicinal plants.

Dogo S. et al [40] determined trace elements in herbal drugs using flame and electrothermal AAS. The elemental concentration data collected is shown in Table 10. Kalny P. et al [41] determined Ba, Cd, Cr, Cu, Fe, Ni, Pb and Zn in birch leaves (*Folium betulae*), dandelion roots (*Radix taraxacae*), hawthorn blossom (*Inflorescentia crataegi*) and their infusions, using graphite furnace atomic absorption spectrometry (GFAAS) after microwave digestion of the plant samples. The results showed high content of Cd in the medicinal plants analyzed and for infusions high levels were observed for Ni and Zn and low levels for Cd and Pb.

Table 10: Element content of herbal drugs (mg/kg), expressed on a dry weight basis [40].

Herbal drug	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Ni	Pb	Zn
Salviae folium	12922	0.12	1.64	13.75	134.0	5450.0	7253.2	49.0	1.5	4.6	20.3
Menthae pip.folium	15134	0.26	0.75	11.37	118.0	7342.2	4935.9	64.0	4.7	3.5	20.0
Melissa folium	1444	0.26	2.46	10.25	236.0	1825.0	7434.4	49.0	3.9	4.3	23.6
Lavandule flos	7270	0.13	1.40	12.50	61.0	4968.6	4370.3	27.0	1.9	2.4	35.1
Basilici herba	13028	0.50	0.58	10.12	141.0	7568.8	7651.6	79.0	2.6	1.6	54.9
Marubii herba	7787	0.17	0.63	11.50	69.0	5751.6	3148.4	32.0	2.9	2.2	30.4
Origani herba	9481	0.10	0.49	7.88	89.0	4367.2	2803.2	32.0	7.5	1.6	35.3

2.10.2 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Queralt M. et al [42] determined the macro- and microelement content of five medicinal plants (*Taraxanum officinale* Weber, *Eucalypus globules* Labill, *Plantago lanceolata* L, *Matricaria chamomilla* L and *Mentha piperita* L) and their infusions were evaluated by the combined use of XRF and inductively coupled plasma (ICP-MS) techniques. The use of XRF techniques offered a good multi-elemental approach for the rapid quality control of raw plant materials whereas ICP techniques are well suited for the analytical control of infusions in order to ascertain the nutritional role of medicinal plants and the daily dietary intake. Table 11 lists the results for infusions determined using ICP-MS in $\mu g.l^{-1}$.

Table 11: Concentrations of elements ($\mu g\ l^{-1}$) in infusions determined by ICP-MS [42].

Herbal infusions	As	Cu	Mn	Pb	Rb	Sr	Ti	Zn
Taraxacum offinale Weber	7	182	566	11	206	2652	58	262
Eucalyptus globules Labill	4	68	19085	49	66	396	32	123
Plantago lanceolata L.	11	105	498	7	188	24315	175	494
Mentha piperita L.	8	112	1206	25	298	777	78	181
Matricaria chamomilla L.	5	207	319	36	111	778	62	380

2.10.3 Proton Induced X-ray Emission (PIXE) and Proton Induced Gamma-ray Emission (PIGE).

Olabanji S.O et al [45] evaluated thirteen medicinal plants, commonly used in South-western Nigeria, for various biological activities such as anti-cancer and antimicrobial. All plants contained many of the major elements of the body such as K, Ca, Ti, Cr, Mn, Fe, Cu and Zn as shown in Table 12. Naga Raju G.J et al [24] carried out trace element analysis in various parts of some anti-diabetic medicinal plants using PIXE, providing justification for the usage of medicinal plants in the treatment of diabetes mellitus since they were found to contain appreciable amounts of K, Ca, Cr, Mn, Cu and Zn, which are responsible for insulin action. The results of trace element analysis of those anti-diabetic medicinal plants are given in Table 13 [24]. Buoso M.C et al [44] studied medicinal plants that are implicated in some herbal recipes for treatment of different severe diseases in south-western Nigeria evaluating elemental composition using PIXE and PIGE. The results of both techniques are given in Table 14 and Table 15 respectively [43,44,45,46].

2.10.4 Instrumental Neutron Activation Analysis (INAA)

Yamashita C.I et al [51] studied trace elements in leaves of *Casearia sylvestris*, *Casearia decandra*, *Casearia obliqua* medicinal plant species which were collected at the Atlantic Forest in Brazil using INAA. The results obtained are shown in Table 16. Serfor-Armah Y. et al determined essential elements in six traditional Ghanaian plant medicines namely Ninga powder, Lippia tea, Ritchiea powder, Momordica powder, Kenken powder and Fefe powder using INAA. In that study a total of seventeen elements haven been determined, of these Sb and Sc were found present at the trace level, Br, Co, Cu, Cr, Mn, Rb, Ta, V and Zn at the minor level, while Al, Ca, Cl, K, and Mg were generally at major levels [52]. Choudhary R.P et al employed INAA for the determination of at least 31 trace elements of Trikatu, which is a formulation of powder spices such as ginger, black pepper and pipali used to promote digestion of food [53]. Diuretic action of medicinal plants attributed by element content was also studied by Kanias G.D et al using INAA [54].

2.10.5 X-ray Fluorescence (XRF) spectroscopy

Ekinci N. et al investigated the elemental composition and concentration of medicinal plants by using energy dispersive X-ray Fluorescence (EDXRF) and found that P, K, Cl, Ca, S, Al, Ti, V, Rb, Sr, Zr, Nb, Mo, In, Sn, I and Ce were present in those medicinal plants. The results of concentrations of inorganic elements in these medicinal plants [48] are shown in Table 17.

Table 12: Element concentrations (ppm) of some medicinal plants in South-western Nigeria [45].

Plant species	Plant's part	Al	Ca	Cl	Cr	Cu	Fe	K	Mg	Mn	Ni	P	S	V	Zn
Acalypha wilkesiana	Leaves	1511 ±103	33699 ±70	5582 ±39	6.9 ±1.59		195 ±4	19520 ±39	5835 ±105	45.2 ±2.3	10.3 ±2.9	4698 ±70	3558 ±42		21.6 ±3.1
Annona senegalensis	Roots	1512 ±58	13485 ±40	2421 ±22		3.2 ±1.28	301 ±3	12413 ±23	3860 ±73	60.2 ±1.8	6.79 ±1.47	1493 ±34	2093 ±25		10.4 ±1.7
Bryophyllum pinnatum	Leaves	8856 ±198	65824 ±151	8011 ±60	21.6 ±3.8	19.3 ±4.4	1562 ±12	54990 ±76	9502 ±185	130 ±5		5414 ±113	4017 ±62	15.5 ±5	79.1 ±5.6
Cassia alata	Leaves	2466 ±113	49004 ±98	856 ±37	7.2 ±2.26		241 ±4	25777 ±54	5258 ±130	26.6 ±2.5	14.6 ±4.2	4270 ±83	2067 ±49		13.1 ±3.5
Chenopodium ambrosioides	Whole herb	4688 ±177	20270 ±154	15403 ±61	7.29 ±2.9	10 ±3.3	1884 ±11	78167 ±62	14765 ±169	147 ±4	13 ±3.9	8315 ±80	4023 ±64	11.4 ±3.3	201 ±6
Chrysophyllum albidium	Root wood	2532 ±52	1186 ±14	57.3 ±15.5	2.79 ±0.92		61.5 ±2.4	2966 ±14	1481 ±79	4.4 ±1.03		1575 ±29	2264 ±26		
Chrysophyllum albidium	Root bark	3780 ±90	63781 ±76	737 ±38		23 ±3.4	1089 ±8	13334 ±42	2372 ±109	41.7 ±3.4	65.7 ±6.2	1615 ±87	11717 ±58	11 ±2.5	22.5 ±3.6
Erythrophyllum ivorensis	Stem bark	745 ±44	8657 ±24	294 ±13		2.89 ±1.17	110 ±2	3204 ±15	1625 ±62	24.1 ±1.3		847 ±27	568 ±17		6.59 ±1.65
Erythrophyllum ivorensis	Roots	842 ±55	3233 ±36	201 ±16		6.29 ±1.37	92 ±2.5	14745 ±28	2252 ±72	16.6 ±1.3		1862 ±30	954 ±21		12.8 ±1.8
Eugenia uniflora	Leaves	2005 ±102	36872 ±81	1838 ±30		27.5 ±2.9	385 ±5	28420 ±42	7597 ±110	79.2 ±2.8	43.8 ±3.7	5582 ±68	2260 ±40		36.5 ±3.5
Hymenocardia acida	Roots	936 ±45	5378 ±19	345 ±13		5.2 ±1.26	199 ±3	3985 ±13	1848 ±62	60.8 ±1.7	4.4 ±0.97	1657 ±28	1053 ±18		11 ±1.6
Hymenocardia acida	Leaves	2962 ±66	20359 ±36	499 ±16		11.4 ±1.6	355 ±5	9856 ±19	9180 ±78	15.4 ±2.3	15.4 ±2.3	2896 ±40	3062 ±23	7.9 ±1.63	32.8 ±2
Hymenocardia acida	Stem	989 ±37	7098 ±19	137 ±8		4.5 ±0.88	97.9 ±1.6	5460 ±10	2606 ±44	3.39 ±0.82	3.39 ±0.82	1123 ±21	562 ±11	2 ±0.71	7.2 ±1.11
Larpotea aestuens	Whole herb	3786 ±179	78429 ±141	15495 ±68		14.9 ±4.9	439 ±7	49719 ±74	11732 ±170	166 ±9	166 ±9	8414 ±124	6306 ±83		39.1 ±5.4
Morinda lucida	Roots	2328 ±42	3202 ±14			11.1 ±1.3	853 ±5	2594 ±10	2203 ±59	5.4 ±1.13	5.4 ±1.13	894 ±22	1577 ±17	3.2 ±1.11	23.6 ±1.9
Rhus longipes	Roots	1193 ±97	12887 ±59	6490 ±38		5 ±1.78	269 ±4	21973 ±35	4285 ±98			4347 ±54	1965 ±34		24.1 ±2.4

Table 13: Mean concentrations and standard deviation (µg/g) of elements present in anti-diabetic medicinal plants, where n is the number of samples [24].

Element	*Cassia auriculata* n=21	*Eugenia* n=15	*Gymnema sylvestris* n=12	*Adhathoba vasica* n=24	*Ocimum sanctum* n=13	*Azadirachta indica* n=15	*Withania somnifera* n=18	*Tinospora cordifolia* n=15
Br		7 ± 3	7.2 ± 2.3	8.8 ± 2.7	26.8 ±6.5	10.4 ± 2.8	5.4 ± 2.7	11.7 ± 1.9
Ca	3200±40	9200 ±100	9200±100	1600±100	11700±700	1400±47	9500±300	1600±30
Cl	16300±800	24900±900	30500±900	28400±1200	48400±3000	8700±500	32300±1400	7200±300
Cr	15.1±1.3	37.4±3.4	68.4±2.7	48.0±6.7	38.8±8.2	48±2	63.2±3.4	48.6±1.6
Cu	8±6	15.8±2.1	18.3±1.8	8.2±1.7	19.4±3.7	6.4±1.5	5.1±1.7	3.5±1.0
Fe	224.5±3.5	206±12	599.7±6.8	462.2±62.2	799.5±60.5	333.6±5.1	451.4±18.1	471.2±5.2
K	8200±200	21300±200	22100±200	16500±600	9500±600	8800±100	14300±600	4100±200
Mn	76.3±6.7	28±4	52.5±4.4	83.2±5.6	40.6±6.2	25.1±2.3	53.4±3.7	67.0±2.3
Ni	5.5±2.1	58±3	16.3±2.4	45.9±3.7	24.1±4.7	21.7±2.2	19.3±2.5	23.6±1.5
Rb	9.9±4.5	19±4	15±4	44.4±6.5		8.1±4.4	8.8±4.4	11±3
Sr	12.3±2.5	22.8±5.6	41.2±5.9	26.6±6.3	31.7±11.2	11±5	29.3±7.7	8.2±3.1
Ti	47.4±4.4	45.2±5.5	67.3±4.8	45.6±3.7	68.9±8.6	18.7±2.6	39.8±4.2	28.4±1.9
Zn	24.5±1.9	43.2±2.6	28.9±2.1	36.3±2.9	58.1±6.8	20.6±2.1	32.9±2.9	24.3±1.4

Table 14: Elemental concentration (ppm) in some medicinal plants obtained using PIXE [44].

Medicinal Plant	Plant part	Ca	Cr	Cu	Fe	K	Mn	Ti	Zn
Annona senegalesis	Root	8917 ±22	13.5 ±6.4		692 ±10	7672 ±16	85.4 ±7.5	71.2 ±5.6	101 ±16
Larpotea aestuens	Whole herb	36951 ±59	1.5 ±1.3	10.5 ±2.0	370 ±5	20673 ±37	122 ±3	30.9 ±2.2	63.9 ±3.5
Chenopodium ambrosoides	Whole herb	19300 ±120	7.9 ±2.4	17.0 ±3.7	3838 ±19	76067 ±84	283 ±5	224 ±4	427 ±10
Morinda lucida	Roots	2387 ±7		27.2 ±7.6	3731 ±9	1351 ±6	92.8 ±4.6	122 ±3	60.0 ±8.7
Hymenocardia acida	Roots	1722 ±4	2.4 ±0.2	1.7 ±0.5	129 ±1	1085 ±3	19.2 ±0.4	8.2 ±0.3	15.5 ±0.8
Hymenocardia acida	Stem	1570 ±4	2.3 ±0.2	2.4 ±0.4	106 ±1	1287 ±3	31.7 ±0.5	5.2 ±0.4	11.0 ±0.6
Cassia occidentalis	Roots bark	12030 ±43		7.9 ±1.4	362 ±14	21734 ±33	105 ±12	28.4 ±1.5	63.5 ±2.8
Cassia alata	Leaves	14231 ±26		4.6 ±1.2	149 ±2	6419 ±17	16.6 ±11.0	15.9 ±1.1	15.2 ±1.8
Garcinia kola	Roots	10004 ±18		27.0 ±12.0	4526 ±15	2478 ±12	282 ±8	272 ±6	56.6 ±13.2
Chrysophyllum albidium	Root wood	693 ±5	1.7 ±0.4	3.7 ±0.6	98.9 ±1.4	1324 ±5	4.8 ±0.4	7.6 ±0.6	10.0 ±0.9
Chrysophyllum albidium	Root bark	15718 ±19	1.6 ±0.5	8.8 ±1.1	377 ±3	3441 ±10	20.4 ±0.8	37.0 ±0.9	19.8 ±1.4
Eugenia uniflora	Leaves	22076 ±57		39.3 ±18.4	759 ±16	17641 ±42	98.4 ±12.5	44.9 ±9.4	91.1 ±19.9
Bryophyllum pinnatum	Leaves	30682 ±61		6.3 ±2.3	1413 ±9	24819 ±40	119 ±3	175 ±3	63.9 ±3.4
Erythrophyllum ivorensis	Stem bark leaves	2938 ±7	0.6 ±0.3	2.8 ±0.7	128 ±1	1504 ±5	15.1 ±0.5	12.1 ±0.5	9.5 ±1.1
Erythrophyllum ivorensis	Roots	1617 ±16	2.3 ±0.5	5.0 ±1.2	142 ±2	8574 ±11	8.7 ±0.7	15.7 ±0.8	20.0 ±1.8
Rhus longipes	Roots	79637 ±263	22.0 ±7.4	56.6 ±11.0	4660 ±37	121758 ±195	257 ±11	105 ±13	264 ±19
Acalypha wilkesiana	Leaves	11003 ±19	2.7 ±0.5	6.4 ±1.1	140 ±2	6502 ±12	22.3 ±0.8	16.8 ±0.7	24.8 ±1.6

Table 15: Elemental concentration (ppm) in some medicinal plants obtained using PIGE [44].

Medicinal plant	Plant part	Al	Cl	Mg	P
Annona senegalesis	Root	112±6	434±68	4600±397	1100±68
Larpotea aestuens	Whole herb	324±18	901±105	9750±766	2200±125
Chenopodium ambrosoides	Whole herb	140±7	434±70	4250±330	1003±74
Morinda lucida	Roots	108±5	258±54	3325±296	838±104
Hymenocardia acida	Roots	135±7	244±46	4138±330	930±38
Hymenocardia acida	Leaves	108±5	282±47	3440±327	834±54
Hymenocardia acida	Stem	112±6	282±43	3418±330	764±40
Cassia occidentalis	Leaves, roots bark	97±5	297±45	2947±279	736±37
Cassia alata	Leaves	213±10	344±25	5556±630	1631±46
Garcinia kola	Roots	114±35	306±47	3655±319	845±36
Chrysophyllum albidium	Root wood	286±12	523±56	7613±957	2223±67
Chrysophyllum albidium	Roots bark	379±24	640±64	9127±1092	2669±74
Eugenia uniflora	Leaves	233±107	401±34	6143±700	1837±65
Bryophyllum pinnatum	Leaves	161±7	312±47	4223±450	1249±44
Erythrophyllum ivorensis	Stem bark	138±7	329±49	4369±613	1122±36
Erythrophyllum ivorensis	Leaves	152±7	325±27	4609±620	1203±44
Erythrophyllum ivorensis	Roots	144±7	291±17	4353±594	1127±52
Rhus longipes	Roots	243±10	564±40	8136±1057	1899±51
Acalypha wilkesiana	Leaves	118±5	364±46	3907±452	924±26

Table 16: Mean values of element concentrations determined in three different *Casearia* Species [51].

Elements	*C. obligua*	*C. decandra*	*C. sylvestris*
Br (μg g^{-1})	5.04±0.01 (2)	3.773±0.008 (2)	3.60±0.01 (2)
Ca (%)	0.22±0.01 (2)	0.56±0.01 (3)	0.48±0.02 (2)
Cl (μg g^{-1})	425±22 (3)	2067±76 (3)	1450±43 (2)
Co (μg kg^{-1})	66±2 (3)	187±4 (3)	65±2 (3)
Cr (μg kg^{-1})	43±10 (2)	64±1 (3)	114±1 (3)
Cs (μg kg^{-1})	142±4 (3)	107±4 (2)	83±3(3)
Fe (μg kg^{-1})	58.0±1.7 (3)	72.7±1.3 (3)	88.8±1.5 (3)
K (%)	1.061±0.003 (3)	1.466±0.003 (3)	1.104±0.004 (3)
La (μg kg^{-1})	36.6±1.6 (2)	80.5±1.7 (3)	59.4±1.3 (3)
Mg (%)	0.23±0.02 (3)	0.23±0.02 (2)	0.17±0.03 (2)
Mn (μg g^{-1})	193±4 (3)	508±15 (3)	357±9 (2)
Na (μg g^{-1})	12.6±0.1 (3)	183.0±0.3 (3)	32.9± 3.0 (3)
Rb (μg g^{-1})	149± 2(3)	98.4±0.8 (3)	79.1±3.0 (3)
Sc (μg kg^{-1})	5.1± 0.1 (3)	5.79± 0.12 (3)	10.2±0.1(3)
Zn (μg g^{-1})	29.9±0.2 (3)	28.8±0.2 (3)	17.4±0.2 (3)

Values in parenthesis indicate the number of determinations.

Table 17: Concentrations of inorganic elements in medicinal plants analyzed by EDXRF technique [48].

Medicinal plant	Plant part	Ca	Cl	Cu	Fe	K	Mg	Mn	Na	Ni	P	Rb	S	Sr	Zn
Anadenathera macrocarpa	Bark	4488 ±750	300 ±91	1.7 ±0.8	55.9 ±4.5	2442 ±238	860 ±294	37.3 ±7.5	2638 ±810	<1	552 ±115	3.9 ±1.4	693 ±87	77.8 ±2.5	8.7 ±2.5
Anadenathera macrocarpa	Ethanol extract	29.4 ±4.5	689 ±52	1.9 ±0.7	4.9 ±0.5	1163 ±28	890 ±60	7.7 ±1.1	4029 ±1700	<1	692 ±39	2.5 ±0.4	183 ±17	3.0 ±1.0	8.3 ±0.7
Schinus molle	Leaves	2837 ±43	1982 ±42	1.6 ±0.6	21.0 ±2.2	6105 ±28	3200 ±500	28.5 ±3.8	<50	<1	1000 ±50	4.4 ±0.9	1954 ±38	6.7 ±0.3	11.8 ±1.2
Schinus molle	Bark	5851 ±49	389 ±66	2.6 ±0.8	65.7 ±5.1	3885 ±112	1776 ±172	35.9 ±5.6	<50	<1	417 ±51	3.2 ±1.1	533 ±85	220 ±71	7.1 ±1.8
Schinus molle	Seed	1729 ±19	1241 ±17	2.1 ±1.2	81.9 ±3.4	12151 ±35	1335 ±217	24.3 ±2.5	<50	<1	1803 ±44	12.4 ±0.6	989 ±12	17.8 ±1.1	18.2 ±2.0
Schinus molle	Ethanol extract	291 ±15	801 ±36	2.2 ±0.8	3.3 ±1.7	2827 ±12	2062 ±250	3.9 ±1.3	2296 ±757	<1	172 ±28	4.5 ±0.6	155 ±28	3.9 ±1.2	6.8 ±1.1
Hymenaea courbaril	Bark	3779 ±41	297 ±98	2.3 ±0.4	49.2± 6.5	5022 ±235	1160 ±536	121 ±15	<50	<1	466 ±125	2.1 ±0.5	500 ±66	12.7 ±7.2	23.1 ±2.2
Hymenaea courbaril	Ethanol extract	75.4 ±7.3	268 ±35	1.9 ±0.8	8.7 ±0.6	2237 ±41	533 ±156	17.3 ±2.3	3244 ±1042	<1	90 ±12	3.4 ±0.7	117 ±17	3.2 ±0.9	6.6 ±1.6
Solidago microglossa	Leaves	6498 ±230	4019 ±94	4.7 ±1.6	237 ±7	26011 ±924	3332 ±888	287 ±9	<50	<1	2138 ±117	8.3 ±1.3	1399± 45	17.7 ±1.6	72.1 ±3.4
Solidago microglossa	Bark	4200 ±520	2453 ±38	2.2 ±0.5	601 ±10	5302 ±1142	2540 ±337	176 ±29	<50	12.2 ±3.5	741 ±222	6.9 ±1.1	390 ±22	17.6 ±3.5	8.8 ±1.7
Solidago microglossa	Flower	701 ±13	907 ±25	1.2 ±0.3	28.9 ±1.6	3559 ±51	593 ±81	7.6 ±0.4	1223 ±75	<1	553 ±56	1.1 ±0.1	357 ±16	<1	3.8 ±0.3
Solidago microglossa	Ethanol extract	136 ±11	641 ±49	2.4 ±1.2	180 ±6	6377 ±107	1370 ±132	58.3 ±3.7	2790 ±1132	1.3 ±0.2	473 ±56	3.7 ±0.7	386 ±10	3.3 ±1.1	17.9 ±0.9
Stryphnodendron barbatiman	Bark	1670 ±44	1555 ±57	2.6 ±1.4	527 ±10	2368 ±30	1187 ±138	54.7 ±2.5	<50	10.7 ±0.4	243 ±50	5.8 ±1.0	444 ±49	5.4 ±0.5	5.5 ±1.6
Stryphnodendron barbatiman	Ethanol extract	41.7 ±7.5	1877 ±3	3.3 ±1.6	3.1 ±0.9	2424 ±2	644 ±55	7.8 ±2.8	3210 ±70	<1	141 ±14	3.3 ±0.7	136 ±16	2.9 ±1.1	6.8 ±1.2
Carinlana leralis	Bark	6967 ±41	787 ±15	2.3 ±0.7	1991± 140	5816 ±16	2612 ±382	700 ±55	<50	90.0 ±1.2	516 ±35	4.5 ±1.4	927 ±21	23.5 ±4.1	10.9 ±2.8
Carinlana leralis	Ethanol extract	26.4 ±3.2	1358 ±4	2.4 ±0.9	4.9 ±0.3	933 ±2	613 ±36	6.6 ±1.8	3094 ±31	<1	587±15	2.3 ±0.5	230 ±8	2.2 ±0.7	9.7 ±1.0

CHAPTER 3

3 TECHNIQUES FOR MEDICINAL PLANT ANALYSIS

Multi-elemental analysis of plants has become important in environmental science particularly for the identification of chemical elements present and the relative levels of toxicity. Medicinal plant analysis is the chemical evaluation of essential element concentrations in plant tissue required to complete the life cycle of a plant [7]. There are many sensitive methods to determine the total concentration of trace elements present, but these do not differentiate between free and bound elements. In order to determine the concentrations of the respective species, preliminary separation is required. The separation techniques available for species-selective analysis in biological materials include High-performance liquid chromatography (e.g. size-exclusion, reversed-phase and ion-exchange chromatography) and Electrochromatography (e.g. capillary zone electrophoresis, flatbed gel electrophoresis, isoelectric focusing and immunoelectrophoresis)[15]. Most of the medicinal plants are analyzed by using analytical techniques including:

3.1 Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectroscopy (AAS) shown in Figure 1 uses the absorption of light to measure the concentration of gas phase atoms evaporated from solid or liquid samples. Each trace element has a characteristic wavelength and the concentrations are determined from a working curve after calibrating the instrument with standards of known element concentration or from the Beer-Lambert's Law, defined as

$$A = \varepsilon bc \qquad \ldots (1)$$

Where:

A	the absorbance
ε	molar absorptivity in $M^{-1}cm^{-1}$
b	the path length expressed in centimeters (cm)
c	concentration of the element in moles (M)

A typical atomic absorption spectrometer consists of an appropriate light source (usually a hollow cathode lamp containing the element to be measured), an absorption path (usually a flame but occasionally an absorption cell), a monochromator (to isolate the light of appropriate wavelength) and a detector. Limitations of AAS [36,37,38,39,40] include:

- All measurements are made following chemical dissolution.
- AAS is a sequential analytical technique (analyzes one element at a time).
- Interferences from other elements or chemical species can reduce atomization and depress absorbance thereby reducing sensitivity.

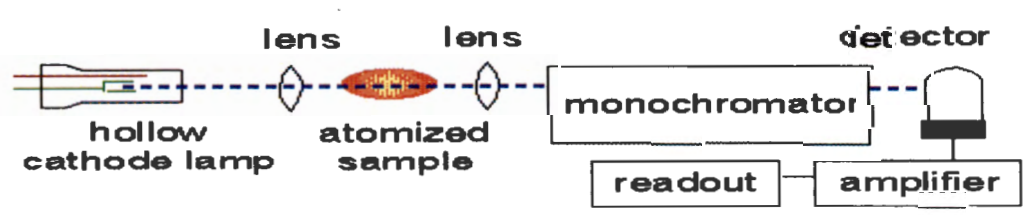


Figure 1: Schematic diagram of atomic absorption spectroscopy.

3.2 Proton Induced X-ray Emission (PIXE) and Proton Induced Gamma-ray Emission (PIGE).

PIXE is a powerful and relatively simple analytical technique used to identify and quantify trace elements. X-rays are produced following the excitation of target atoms induced by an energetic incident beam of protons produced by an accelerator. The energies of X-rays are characteristics of the elements and therefore used to identify elemental composition. By measuring intensities of characteristic X-ray lines, concentrations of almost all elements can be determined. The schematic diagram of an external beam PIXE end-station is shown in Figure 2. The 2.4 MeV (for specific PIXE set-up) proton beam passes through a 7.4 μm Kapton film to the atmosphere. In order to reduce the X-ray absorption in the air and to avoid its possible fluctuation due to temperature change, helium gas is continuously supplied to the cavity around the film, detector and the sample surface. X-ray detection can be accomplished by a Si(Li) detector but currently thin-window HPGe detectors are employed for this purpose [23,43,44].

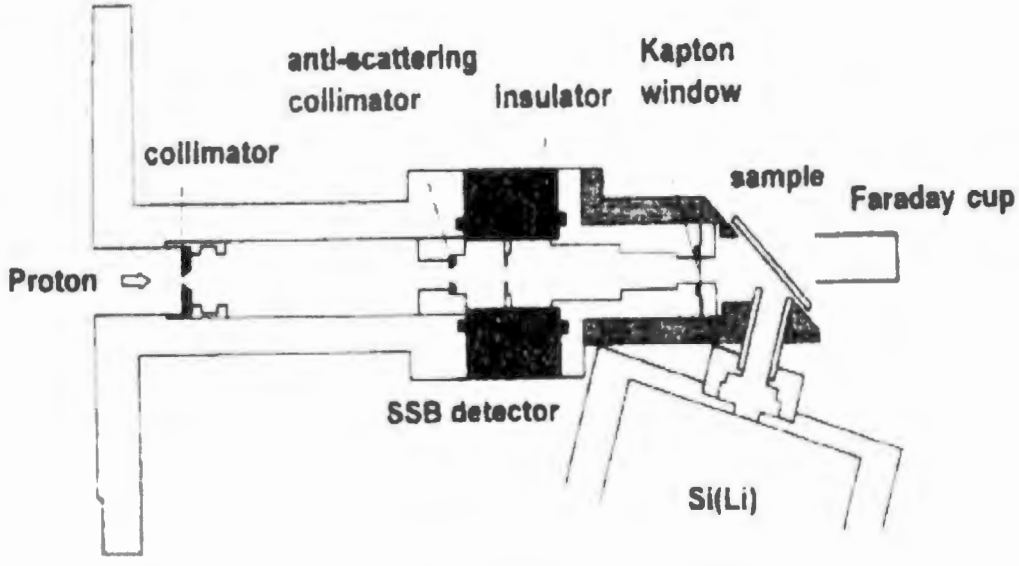


Figure 2: Proton Induced X-ray Emission system.

In PIGE, interactions of protons of a few MeV (kinetic) energy with matter permit microanalysis of sample constituents using gamma rays from the induced nuclear reactions. The samples are bombarded with protons from the accelerator (e.g. a Van de Graaff accelerator) and as MeV protons can penetrate the Coulomb barrier, especially on light elements, nuclear reactions can be induced. The schematic diagram of both internal and external beam of a PIGE set-up is shown in Figure 3. To obtain a finely collimated beam, two tantalum collimators are used each of 2mm diameter and a 4mm diameter cleanup aperture.
In internal beam PIGE, sample excitation and gamma-ray emission are performed within a vacuum chamber. In external beam PIGE samples are irradiated in air by the proton beam through a Be window sufficiently strong to hold vacuum in the accelerator tube. The advantage of the external beam technique over the internal is the reductions of charge build-up on the sample during irradiation as well as that samples can be changed more easily. The external beam is also most useful for irradiation of samples of different shape and size, while limitations in geometry make internal beam irradiation more restrictive. A high purity germanium detector is used for gamma-ray analysis [43].

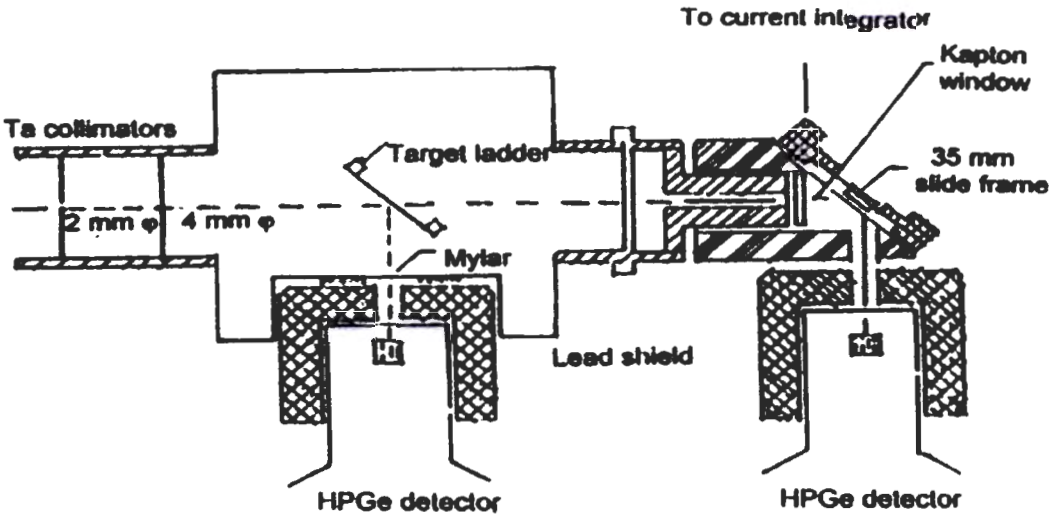


Figure 3: Schematic diagram of the external/internal beam of the PIGE experimental system.

3.3 X-ray Fluorescence (XRF) spectroscopy

In XRF (Figure 4), a beam of electrons produced from a tube consisting of a cathode strikes a target to release a source of continuum X-rays. These primary X-rays are then used to irradiate the sample causing the sample to emit fluorescent (secondary) X-rays which are characteristic of constituent elements. Each element has a unique set of energy levels producing X-rays at a unique set of energies allowing non-destructive measure of the elemental composition of a sample. The fluorescent X-rays emitted by the sample are collimated and diffracted using different analyzing crystals and their intensities are measured with a detector mounted on a goniometer. Components of XRF are put in a vacuum to prevent oxidization and to minimize absorption of X-rays by air. Disadvantage of using XRF is that lighter elements are not easily determined since they intrinsically have less sensitivity and accordingly higher detection limits [47,48,49].

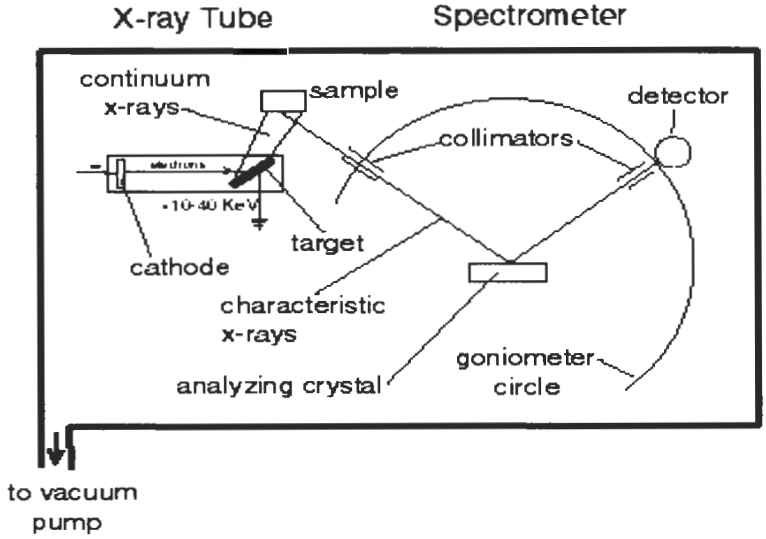


Figure 4: Schematic diagram of a typical wavelength-dispersive X-ray fluorescence spectrometer.

3.4 Instrumental Neutron Activation Analysis (INAA) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as methods of choice.

INAA depends on nuclear rather than on atomic properties of the element of interest so that any sources of uncertainty are independent of those associated with most other techniques of chemical analysis. INAA is generally recognized as the 'reference method' of choice when new procedures are being developed or when other methods yield results that do not agree, as it is almost free from matrix interference. Apart from all above techniques neutron activation analysis requires minimum sample preparation. Theoretical information on INAA will be discussed in detail in Chapter 4 as this technique is used in this study.

Dry medicinal plants will be analyzed using INAA while ICP-MS is chosen to analyze medicinal plant extracts in this study since the samples are already in a liquid form, which is suitable for direct use with this technique. However, it is recognized that the high alcohol content may pose a problem and that there can be a need for solidification of samples by evaporation and dissolution in a more appropriate medium, although in this process volatile organo-metallic compounds may be lost. The principles of ICP-MS will be discussed in detail in Chapter 5.

CHAPTER 4

4 PRINCIPLES OF INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS (INAA)

4.1 Introduction

Neutron activation analysis (NAA) was discovered in 1936 by Hevesy and Levi as an important technique for both qualitative and quantitative multi-element analysis of major, minor, trace and rare elements in samples from almost every field of scientific or technical interest. INAA is a non-destructive method in which neutron induced radionuclides in a sample are basically determined by taking advantage of nuclide specific gamma-ray emission and differences in the decay rates. The concept of INAA (Figure 5) is to produce a compound nucleus by bombarding the target nucleus with neutrons. The compound nucleus will immediately emit prompt gamma-rays or a prompt particle (normally alpha, neutron or proton) to form a product nucleus. In case the product nucleus is a radioactive nucleus it will also de-excite into a more stable configuration by emission of a delayed particle (normally alpha, beta or neutron) followed by delayed gamma rays, which are in most cases species specific. The half-lives of the radioactive nuclei can range from fractions of a second to several years depending upon the particular radioactive species.

On striking a suitable detector, delayed gamma rays are then converted into an electrical signal that is processed as counts in an energy spectrum. The accumulation of gamma counts at a particular energy generates a peak in its area is proportional to the radioactivity of the species, facilitating both qualitative and quantitative identification of the elements present in samples. Signals in INAA are related to the atomic nucleus and therefore results are not affected by the chemical and physical state of the elements. The basic essentials required to carry out INAA are a source of neutrons, instrumentation suitable for detecting gamma rays and accumulating them in a spectra and a detailed knowledge of the reactions that occur when neutrons interact with target nuclei [53,55,56,57,58].

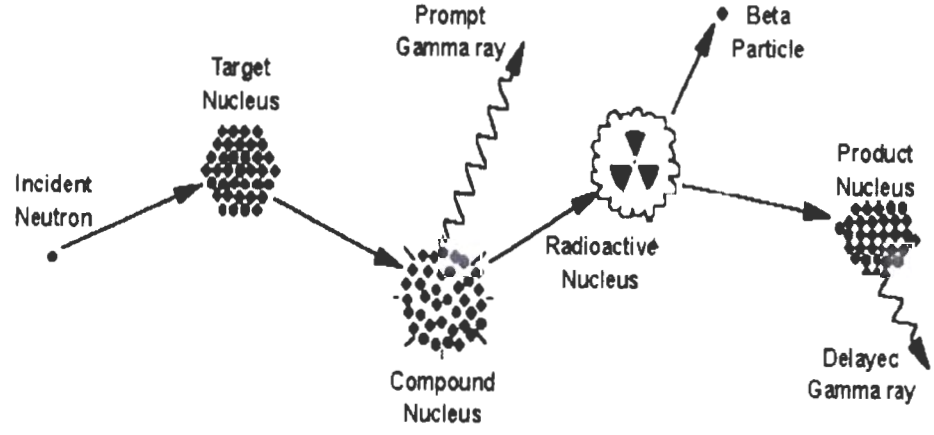


Figure 5: INAA concept.

4.1.1 Applications of Neutron Activation Analysis (NAA)

NAA is an established analytical technique for determining trace elements in wide variety of materials in solid, liquid or gaseous state. World application of INAA is widespread, it is estimated that approximately 100,000 samples undergo INAA each year and about 70% of the elements have properties suitable for measurement by INAA. Among potential applications in which NAA has been utilized are [57,59]:

- **Archaeology**

The use of NAA to characterize archaeological specimens (e.g. pottery, obsidian, ceramic utensils, teeth, bones) and identifying the chemical elements present is a well established application. Over the past decade, large databases of chemical fingerprints for clays, obsidian, chert and basalt have been accumulated through analysis of approximately 30 elements in each of more than 42,000 specimens [57,59,60].

- **Biochemistry**

Neutron activation producing high specific activity radiotracers has been used with great success to study biochemical processes. Trace element and mineral nutrition are important aspects of human health, therefore NAA is one of the methods used to study nutritional bioavailabity and absorption of essential trace elements in human beings using enriched stable isotopes [57,59].

- **Forensic investigations**

NAA has been used as a non-destructive method to analyze evidence as an aid in the investigation and prosecution of criminal cases. Extremely small evidence samples (e.g. gunshot residues, bullet lead, glass, paint, hair, etc.) found at crime scenes can be analyzed due to the sensitivity of NAA to detect elements [57,59].

- **Geological science**

NAA assist geochemists in studying rare earth elements and other trace elements in different samples such as rocks, meteorites, moon samples, gems and minerals. About 30 elements can be measured routinely in almost any geological sample using the NAA method [57,59].

- **Semiconductor materials**

Semiconductor devices are strongly influenced by the presence of impurity elements either added intentionally (doping with B, P, As, etc.) or contaminants remaining due to incomplete purification of the semiconductor material during device manufacture. Small quantities of impurities present at concentrations below 1ppb can have a significant effect on the quality of semiconductors and NAA is widely used for analysis of impurity levels of interest [57,59].

- **Soil science**

NAA has extensive use in agricultural processes such as fertilization, herbicidal and pesticidal control. Stable activatable tracers (e.g. Br) analyzed by NAA have allowed the soil scientists to quantify the distribution of agricultural chemicals under a wide variety of environmental and land use influences [57,59].

4.1.2 Advantages and limitations of INAA

Several chemical methods that have been employed for medicinal plant analysis includes AAS, XRF, PIXE, PIGE and ICP-MS but INAA is the standard and dominated analytical method used for performing multi-elemental analyses with minimum detection limits in the parts per billion range or better. The main advantages of INAA for medicinal plant analysis [52,59] are:

- Multi-element capability. Thirty (30) elements or more can be measured simultaneously without chemical separation.
- Minimal sample preparation. No sample digestion, extraction, volume reduction or dissolution is required. Limited handling reduces sample contamination.
- High precision, accuracy and sensitivity.
- Nuclear technique. Chemical composition and crystal structure do not affect elemental analysis. Isotopic identifications are possible.
- Gamma rays produced are much more energetic (than X-rays) and therefore are less readily absorbed and matrix corrections are not usually important.
- Enables the observation of elements at detection limits in $mg.kg^{-1}$ to $\mu g.kg^{-1}$ range.

The main limitations of INAA are:

- Even though the INAA is essentially non-destructive the irradiated sample may remain radioactive for many years after the initial analysis, requiring handling and disposal protocols for low-level to medium-level radioactive material.
- Number of suitable nuclear research reactors is declining. With a lack of irradiation facilities INAA will decline in popularity and become more expensive.
- Another common limitation of INAA is caused by bulk matrix elements, which can produce a large background that masks the signal of the element of interest reducing the detection limit extensively [52].
- Some elements do not generate suitable radioactive nuclei for gamma spectrometric measurements (e.g. B, P, Tl, Pb).

4.1.3 Sensitivity of INAA

Sensitivity is the smallest amount of a particular element which can be detected in a specific sample of interest. INAA can detect over 30 elements depending upon the experimental procedure with detection limits ranging from 10^{-3} to 10^{-10} mg/g of sample depending on the element under investigation. The sensitivities for INAA depend upon the concentration of the element, the irradiation parameters (i.e., neutron flux, irradiation and decay times), measurement conditions (measurement time, and detector efficiency) and nuclear or radionuclide parameters (isotope abundance, neutron cross-section, half life and gamma ray abundance). The accuracy of INAA usually ranges from 2-10 % of the reported value depending on the element analyzed and its concentration in the sample. Heavier elements have larger nuclei, therefore have larger neutron capture cross- section and are more likely to be activated. Some nuclei can capture a certain number neutrons and remain relatively stable, not undergoing transmutation or decay for many months or even years. Estimate detection limits for INAA using decay gamma rays assuming irradiation in a reactor neutron flux of $1x10^{13}n.cm^{-2}s^{-1}$ is shown in Table 18 [52,59].

Table 18: Lists the approximate sensitivities for determination of elements assuming interferences free spectra [59].

Sensitivity (grams)	Elements
10^{-12}	Dy, Eu
$10^{-11} - 10^{-12}$	In, Lu, Mn
$10^{-10} - 10^{-11}$	Au, Ho, Ir, Re, Sm, W
$10^{-9} - 10^{-10}$	Ag, Ar, As, Br, Cl, Co, Cs, Cu, Er, Ga, Hf, I, La, Sb, Sc, Se, Ta, Tb, Th, Tm, U, V, Yb
$10^{-8} - 10^{-9}$	Al, Ba, Cd, Ce, Cr, Hg, Kr, Gd, Ge, Mo, Na, Nd, Ni, Os, Pd, Rb, Rh, Ru, Sr, Te, Zn, Zr
$10^{-7} - 10^{-8}$	Ca, K, Mg, Pt, Si, Sn, Ti, Tl, Xe, Y
$10^{-6} - 10^{-7}$	F, Fe, Nb, Ne
10^{-5}	S

See also chapter 4.2.6, which refers to minimal detectable activity based on element characteristics and irradiation, counting and reactor parameters

4.1.4 Accuracy of INAA

Accuracy is defined as the closeness of the agreement between the result of a measurement and a true value. The qualitative (identification of elements) accuracy may be affected by

factors such as: peaks being assigned to erroneous radionuclides due to instrumental drift of the spectrometer from calibration conditions, under-estimations of interfering nuclear reactions and gamma ray spectrum interferences (e.g. narrowly spaced peak, and sum peaks). Corrections can be made for many of these effects because for a number of nuclides, in which such situations occur, differences in half-life are taken into consideration. Three types of errors contributing to the uncertainty of quantitative analysis include [55]:

- *Random errors* which are due to the differences in neutron flux between sample and standard caused by badly defined positions during irradiation, sample inhomogeneities and counting statistics.
- *Systematic errors* are mainly due to background subtraction. Examples of sources of systematic error are contamination, moisture content, erroneous standardization, dead-time and pile-up, errors in photopeak efficiency of the detector, blank and natural background corrections, neutron self-shielding, neutron self-moderation, half-lives as well as gamma ray self-absorption.
- *Additional errors* which may either have systematic or random character. These errors may include the choice of the wrong method for drying with consequent loss due to volatilization, erroneously entered sample weights and contamination. An additional source of error for some elemental determinations is due to unresolved interferences for example when identical radionuclides are produced from different nuclear reactions.

4.2 Theory of Neutron Activation Analysis

4.2.1 Introduction

The INAA experimental procedure is characterized a number of steps which include:

- Sample preparation which in most cases involves pulverizing, homogenizing, mass determination, packing as well as the preparation of standards if any.
- Activation via irradiation with (reactor) neutrons,
- Measurement of the gamma radiation after one or decay intervals, and
- Interpretation of the resulting gamma ray spectra in terms of the elements present and their concentrations, and subsequent element concentration calculations [55].

4.2.2 Sample preparation

INAA is capable of producing a comprehensive determination of the sample's total elemental content regardless of the oxidation state, chemical form or physical state of the desired element. Sample preparation is important before neutron irradiation occurs. Placement of the sample for irradiation in the core of the nuclear reactor is accomplished as follows:
(a) For analysis of short lived radionuclides requiring shorter irradiation times (<20minutes), samples are packaged in polyethylene vials and placed into a larger transport system known as a rabbit.
(b) Samples requiring longer irradiation times (hours or days) with moderate high densities are packaged in high-purity quartz vials. The rabbit travels from the laboratory into the reactor by an air drive pneumatic transport system into one of the irradiation positions of the reactor where the sample will reside for a predetermined time. SAFARI No.1 also uses HDPE (high density polyethylene) vials for sample packaging [55]. A number of factors affecting sample preparation are as follows:

- **Impurities of irradiation containers**

Impurities of irradiation containers affect the accuracy of the analysis because of problems associated with blanks. It is therefore recommended that a test analysis should be performed after receiving a new production batch and keeping containers clean through washing with appropriate reagents e.g. using diluted HNO_3 (nitric acid) [55].

- **Encapsulation of liquids**

Leakage testing of containers used for liquid samples is important, but also this test will be of use if powdered material is being irradiated, since it could easily result in dust-like contamination if the capsule leaked. Leakage tests include submerging the capsule in hot water. If the container has alkaline solution, a wipe test with pH paper will indicate leakage. Heat- sealing procedures are used to avoid leakage [55].

- **Moisture content and evaporation losses during drying.**

The moisture content of a sample material must be determined by weighing before and after drying in accordance with a prescribed procedure, also taking into account that certain elements (e.g., As, Hg, Sb, Se) are volatile and may be lost during drying [55] .

- **Quality of homogeneity, standards and the balance used.**

To ensure quality of homogeneity about 6-10 replicate samples should be obtained to determine the uncertainty of the entire analysis. The purity and stoichiometry of the standards provided by the supplier must be verified. The balance should be routinely inspected and calibrated to obtain an accurate indication of the weighed masses [55].

4.2.3 Neutrons

A neutron is a neutral subatomic particle that is part of every atomic nucleus except hydrogen. A neutron has no electrical charge and a rest mass equal to 1.67495×10^{-27} kg, which is slightly greater than of a proton but nearly 1840 times greater than that of an electron. Research reactors are one of the most important neutron sources (besides accelerators, neutron generators and radioisotopic neutron emitters), which produce high fluxes of neutrons essential for sensitive INAA. General information, nuclide data and decay energy of neutrons should be known for a better understanding of the nuclear reactions. The most important reason for using neutrons for irradiation in INAA is that the neutrality of uncharged neutrons allows them to penetrate deeply inside the samples without destroying the samples. Neutrons are classified depending on the energy as: (a) Thermal neutrons with an energy of about 0.025 eV, (b) Epithermal neutrons with energy about 0.2 eV, (c) Resonance neutrons with an energy range of about 1 to 1000 eV, (d) Intermediate neutrons with an energy range of about 1 to 500 keV and (e) Fast neutrons with an energy > 0 5 MeV [57].

4.2.3.1 Neutron reactions

When target nuclei are bombarded with neutrons, many possible reactions can take place producing radioactive nuclides whose radiations can be measured. Since neutrons are uncharged particles, they can approach a target nucleus unaffected by the coulomb forces of electrostatic interactions. The four major neutron reactions [58,59,60,61] are:

- **Neutron capture**-The most common activation reaction in which the target nucleus captures a low-energy neutron, resulting in a product isotope where the mass number is incremented by one. If the product nucleus is unstable, it usually de-excites by emission of gamma-rays. An example of (n, γ) reaction is:

$$^{27}Al + {}^{1}n \longrightarrow [^{28}Al^{*}] \longrightarrow \gamma + {}^{28}Al$$

- **Transmutation-** The second common activation reaction is when a target nucleus absorbs a neutron emitting charged or non-charged particles like alpha, proton, neutron or deuteron. The unstable product nucleus generally de-excites through β^- emission back to the target nucleus. Transmutation neutron reactions are caused by neutrons of high energies (fast or intermediate neutrons). An example of (n,p) reaction is:

$$^{27}Al + {}^{1}n \longrightarrow [^{28}Al^{*}] \longrightarrow {}^{1}H + {}^{27}Mg$$

- **Fission reaction-** Fissionable target nucleus (usually Z > 90) absorbs a neutron triggering the splitting into two large fragments and simultaneously releasing 2 to 3 neutrons. The fission process can become a chain reaction producing a large amount of neutrons, which become source to the nuclear reactor. U, and Pu are examples of fissionable materials with radioisotopes ^{233}U, ^{235}U, ^{239}Pu and ^{241}Pu.

- **Inelastic scattering-** This process differs from the three preceding absorption processes in that the neutron transfers only part of its kinetic energy to the target nucleus, escaping with only a degradation in its energy. The neutron collides with the target nucleus but the rebounding target nucleus is left in excited energy state. The neutron must have a kinetic energy exceeding the excitation energy for this reaction to be possible. The two reactions of inelastic scattering of interest for the production of radioactive isotopes are the (n,n') and the (n,2n). The (n,n') reaction takes place when the scattered neutron imparts sufficient energy to the target nucleus to raise its energy to a metastable state (an isomer of the nuclides). The isomer will then decay back to the stable state with the emission of a gamma ray and the prime on the emerging neutron denotes that the neutron is degraded. The (n,2n) reaction may occur when the scattered neutron imparts enough energy to exceed the binding energy of the least bound neutron in the target nucleus. Examples of (n,n') and (n,2n) reactions are:

$$^{27}Al + {}^{1}n \longrightarrow [^{28}Al^{*}] \begin{cases} \nearrow\ {}^{1}n + {}^{27}Al \\ \searrow\ 2{}^{1}n + {}^{26}Al \end{cases}$$

4.2.4 INAA detectors

The instrumentation used to measure gamma rays from radioactive samples is called a detector, with which the final procedure known as counting is performed. Detectors are used as specialized devices to detect the resultant gamma rays emitted from the irradiation process. The most important performance characteristics to be considered when employing a detector are resolution, detection efficiency, peak shape, peak-to-Compton ratio, shape and price. The detector's resolution is defined as the measure of its ability to separate closely spaced peaks in a spectrum, which is specified in terms of the full width at half maximum (FWHM). The efficiency of detectors depends on the energy of the measured radiation, the solid angle between sample and detector and the volume of the crystal. Gamma radiation interacts with matter via three main processes [59]:

- **Photoelectric effect**

This process describes the interaction between the incident gamma-ray with the orbital electron of the atom of the detector crystal, ejecting that electron from its inner shell as shown in Figure 6. The vacancy created by the ionized electron gets filled by an electron falling from the next higher shell and simultaneously emitting the characteristic X-rays of the detector. Photoelectric interaction predominantly takes place with orbital shells close to the nucleus (K-shell) and is dominant for gamma-ray with energies below 50keV [64].

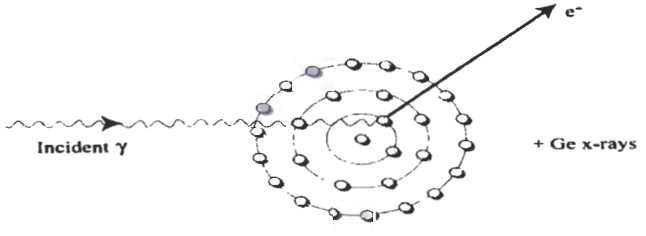


Figure 6: Photoelectric effect.

➢ **Compton scattering**

This is the interaction between the incident gamma-ray and the outer orbital electron in which part of the gamma energy is transferred to the electron to cause its ejection as shown in Figure 7. The remainder of the original gamma-ray energy is emitted with an emission direction different from that of the incident gamma-ray. Compton scattering is a principal absorption mechanism for gamma-rays in the energy range of 100 keV to 10 MeV [64].

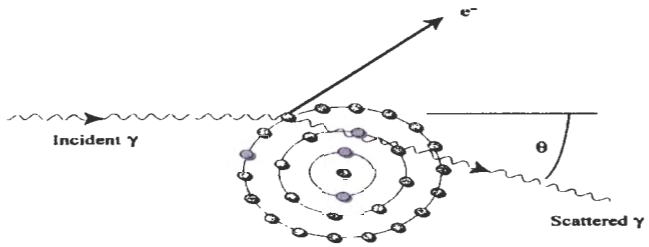


Figure 7: Compton scattering.

➢ **Pair production**

Pair production (Figure 8) is the process whereby a photon with energy in excess of 1022 keV is converted into a pair of a negative electron (negatron) and a positive electron (positron) with short lives of about 10^{-8} seconds. By further collision of the positrons with other electrons they will lose energy and at rest will impulsively annihilate to generate two 511 keV gamma-rays emitted at 180° to one another [64].

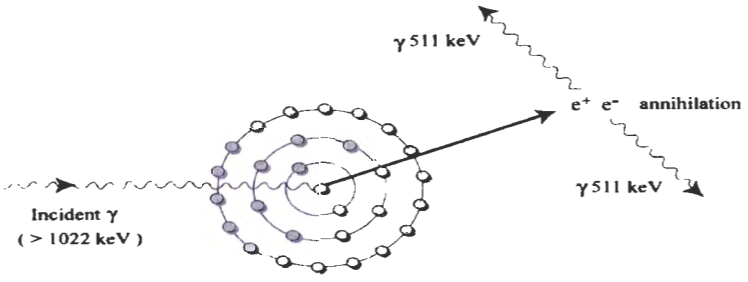


Figure 8: Pair production.

The most common type of gamma detectors include:

4.2.4.1 Scintillation detectors

Scintillation type detectors involve the use of radiation sensitive crystals that emits low energy light when struck by high energy gamma photons. Scintillation detector consists of a phosphor, photocathode, photomultiplier and a charge collector. The kinetic energy of the gamma rays causes ionization and the phosphor converts the ionization into light pulses. These light photons strike the photocathode, which subsequently emits photoelectrons. The photomultiplier tube accelerates and multiplies these photoelectrons. The negative charge is collected as an electrical signal proportional to the energy of the incident gamma rays. The most common scintillators made of inorganic material used in gamma ray detectors are NaI(Tl) (Thallium doped Sodium Iodide) shown in Figure 9 and CsI(Tl) (Thallium doped Cesium Iodide) crystals. These detectors have been accepted as the standard scintillation materials for gamma measurements because of the excellent efficiency and reasonable resolution [60].

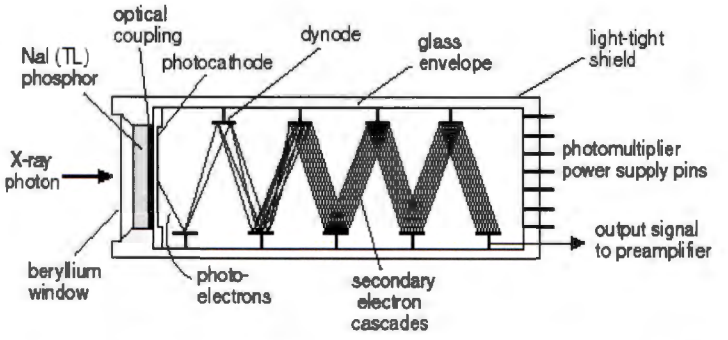


Figure 9: Schematic diagram of a NaI(Tl) scintillation detector and photomultiplier assembly.

4.2.4.2 Semiconductor detectors

A semiconductor detector is a device that uses a semiconductor, usually silicon or germanium (Figure 10), to detect photons. The most commonly used semiconductor material is High Purity Germanium (HPGe), which is nowadays commonly used. The lithium drifted germanium detectors are still around, but this is old technology that was used 20-30 years ago.

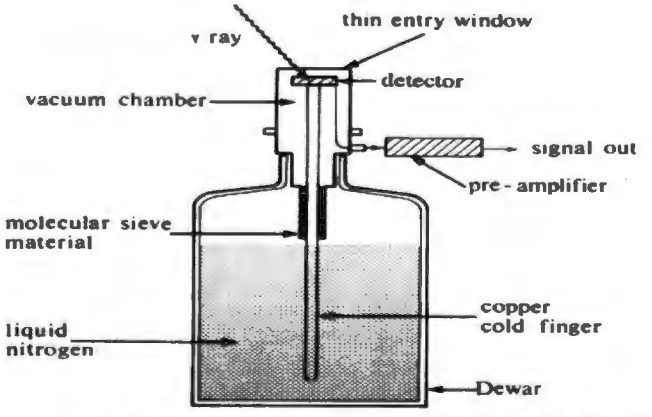
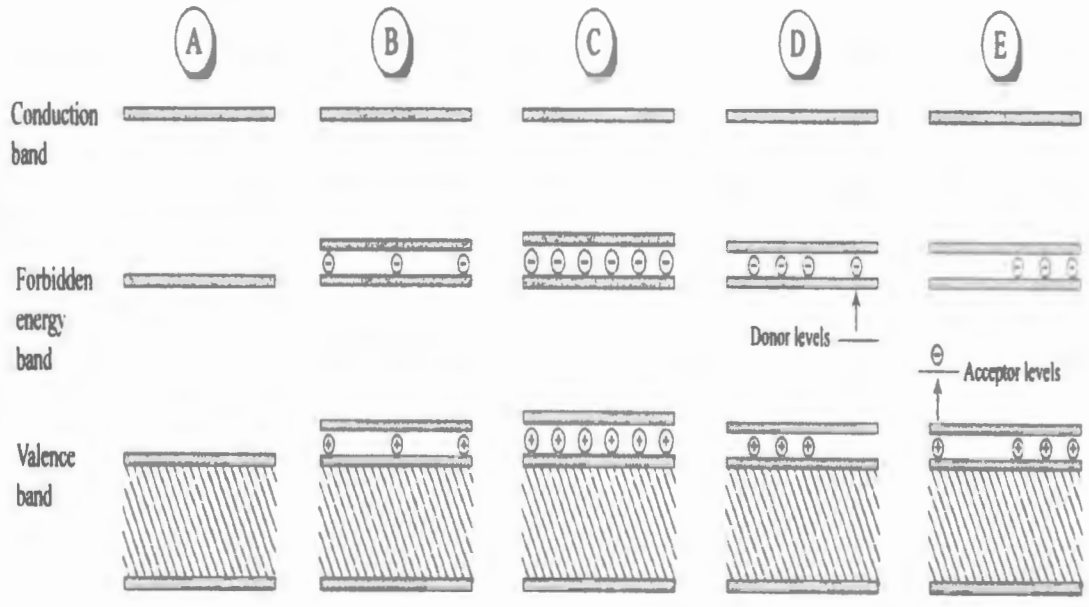


Figure 10: Schematic cross-sectional view of a Germanium detector.

The semiconductor detector is a large reverse proton-neutron diode in which gamma rays are measured by means of charge carriers set free in the detector [60,65,66]. The semi-conducting element Silicon may be used, but Germanium is preferred as its atomic number makes it more efficient in stopping and detecting high energy gamma rays. Both Ge(Li) and HPGe have excellent sensitivity and resolution, but Ge(Li) detectors are unstable at room temperature, with lithium drifting into the intrinsic region, which is not reversible though cooling and in effect destroying the detector. The HPGe crystal is not affected by temperature therefore allowing storage without cooling, though in order to reduce thermal noise the detector should be operated at liquid nitrogen temperature (77 ^{0}K) [2,16]. The detector material itself rests on one end of a thick copper rod, also known as a cold finger, which is immerged in liquid nitrogen to ensure that the detector is always cooled. In this study an HPGe detector has been used; therefore it will be discussed in more detail.

4.2.4.2.1 High Purity Germanium (HPGe) detector

The properties of a typical semiconductor detector used for Neutron Activation Analysis (NAA) are shown in Figure 11 [64,65,67].



KEY:- Shading indicates valence band fully occupied by electrons. Arrows indicate direction of ionization of electrons to or from impurity atoms.

Figure 11: Properties of semiconductor materials for Neutron Activation Analysis (NAA).

Schematic behavior of a semiconductor crystal

A: Perfect (intrinsic) semi-conductor at 0 ^{0}K, the valence band is fully occupied by electrons and the conduction and forbidden energy bands are empty, in this state the semiconductor cannot conduct.
B: Semiconductor at 77 ^{0}K, vast reduction in thermal ionization to conduction band.
C: Semiconductor at room temperature, significant thermal excitation of electrons from valence, forbidden energy bands to conduction band, in this state the semiconductor will conduct.
D: Effect of 'donor' atom impurities in n-type semiconductor material.
E: Effect of 'acceptor' atom impurities in p-type semiconductor material.

Gamma-ray spectrometry with high purity germanium detectors offers the advantages of excellent energy resolution, high detection efficiency and potential spatial resolution. HPGe has impurity concentrations of around one part in 10^{12}, which does not allow the semi-conducting properties of the material to decrease. Typical detector characteristics for an n- and p-type detector are shown in Table 19 for a specific HPGe detector. The physical dimensions of a detector suitable for gamma-ray measurement is normally a cylinder with a diameter of about 50-80 mm and height of 30-100 mm, but the costs of detectors increase rapidly with size [64,65,66].

In order to reconstruct the trajectory of a gamma-ray inside the array, it is important to determine the 3D positions of the gamma-ray interaction points inside the HPGe detectors with a detailed understanding of the following:

- The mechanism through which gamma-rays interact in HPGe,
- The consequent HPGe pulse line-shape and
- How it depends on the position of the interaction points.

Table 19: Characteristics of HPGe detectors [64].

Detector parameters	N-detector	P-detector
Detector diameter	70.4mm ± 0.1mm	54.9mm ± 0.1mm
Detector length	86.4mm ± 0.1mm	54.2mm ± 0.05mm
End cap window material	Aluminum	Beryllium
Window thickness	1mm ± 0.4mm	0.5mm ± 0.05mm
Crystal-window distance	4mm ± 0.4mm	3mm ± 0.5mm
Crystal top dead zone thickness	0.3µm ± 0.03µm	0.3µm ± 0.03µm
Crystal material	Germanium	Germanium
Crystal hole depth	79.3mm ± 0.5mm	47.2mm ± 0.5mm
Crystal hole diameter	11.6mm ± 0.5mm	12mm ± 0.5mm
Detector side cap thickness	1mm ± 0.1mm	1.3mm ± 0.1mm
Detector side cap diameter	94.8mm ± 0.5mm	63.5mm ± 0.5mm
Detector side cap material	Aluminum	Magnesium
Detector type	n-type	p-type
Calibration geometries	Point source, ampoule	Point source, ampoule
Shielding	5 cm thick lead + 0.05 cm thick cadmium + 1.57 cm thick copper	10 cm thick lead + 0.3175 cm thick cadmium + 0.05 cm thick copper
FWHM	1.90 keV at 1.33 MeV 0.97keVat 122 keV	1.8 keV at 1.33 MeV 1.0 keV at 122 keV

A gamma-ray, once inside an HPGe detector, will interact mainly through the photoelectric effect, Compton scattering and pair production. The photon energy produced as a result is transferred to one or more electrons of the material whereby electrons will slow down, which in turn produce tracks of particle-hole excitations. The particle-hole excited by the electrons drifts to the associated electrode and generates an electrical signal that has a distinct line-shape depending on the energies deposited and the positions of the gamma-ray interaction points because of the electric field inside the HPGe detector [64,65,66].

The energy difference between the valency and the conduction bands for germanium is 0.66eV; therefore even at room temperature a fraction of the electrons in the filled valency band can become excited, by thermal agitation, to the conduction band. The accurate analysis of the gamma-ray spectra obtained by HPGe detectors is necessary for accurate measurements of radioactive isotopes. Activity measurements using HPGe detectors are also performed for sample containing radionuclides for which no suitable primary standard calibration method is available for direct measurements. Typical geometrical parameters and materials of the HPGe detector are shown in Figure 12 [64,65,66,67].

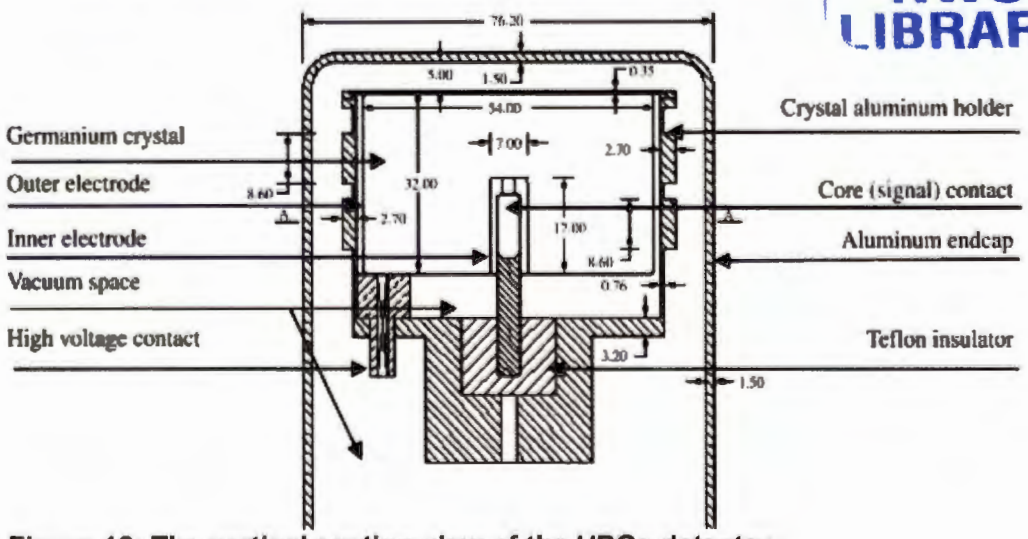


Figure 12: The vertical section view of the HPGe detector.

4.2.5 Quantification of concentration via radioactivity

Radioactive decay is a process in which unstable nuclides lose energy by emitting radiation in the form of particles or electromagnetic waves whereby a parent nuclide transforms to an atom of a different type called the daughter nuclide. A radionuclide can undergo a number of different reactions giving rise to different modes of decay given in Table 20 where atomic number and atomic weight of a nucleus is represented by A and Z respectively [66,67].

Table 20: Modes of decay [66,67].

Mode of decay	Participating particles	Daughter nucleus
Alpha decay	Alpha particle (A=4, Z=2) emitted from the nucleus	(A= -4, Z= -2)
Beta-Negative decay	A nucleus emits an electron and a antineutrino	(A, Z+1)
Beta-Positive decay	A nucleus emits an positron and a neutrino	(A, Z-1)
Electron capture	A nucleus captures an orbiting electron and emits a neutrino. The daughter nucleus is left in an excited and unstable state.	(A, Z-1)
Gamma decay	Excited nucleus releases a high-energy photon (gamma ray)	(A, Z)

The rate at which the number of radioactive nuclides is formed after irradiating a stable nuclide with neutrons is determined by the activation equation [58] given by:

$$\Delta N_p = \Phi \sigma N_t \Delta t - \lambda N_p \Delta t \qquad \ldots (2)$$

Where:

Np number of product atoms that are present in the target
N_T number of target atoms that are present in the same material
σ cross section for the reaction being used in this process (1 barn =10^{-24} cm^2)
Φ flux density of the incident particle ($cm^{-2}.s^{-1}$)
λ decay constant (s^{-1}) for the radionuclide that is produced in the reaction given by ln2 / $t_{½}$ (half-life)
Δt small time interval during which the reaction is being observed (s)

This differential equation (2) can be solved for the number of radioactive product nuclei present after a radiation period of time (t in seconds) resulting in [60]:

$$N_p = \frac{\Phi \sigma N_T}{\lambda}(1 - e^{-\lambda t}) \qquad \ldots (3)$$

or the activity of product atoms in Becquerel (Bq).

$$A_p = \lambda N_p = \Phi \sigma N_t (1 - e^{-\lambda t}) \qquad \ldots (4)$$

In case where $t << t_{½}$

$$A_p = \Phi \sigma N \lambda t \qquad \ldots (5)$$

The number of atoms and activity of a sample material that contains the element to be analyzed at the mass-concentration with the isotopic abundance of the target isotope is given by [60]:

$$N = \frac{mCIN_A}{M10^{-6}} atoms \qquad \ldots (6)$$

Hence,

$$A = \frac{\Phi \sigma m C I N_A \lambda t}{M 10^{-6}} \quad \ldots (7)$$

Where:

N_A Avogadro's number, 6.022×10^{23} and
M molecular weight of this isotope
I isotopic abundance of the target isotope
m mass of the sample material (g)
σ cross section for the reaction being used in this process (1 barn $=10^{-24}$ cm^2)
Φ flux density of the incident particle ($cm^{-2}.s^{-1}$)
λ decay constant (s^{-1}) for the radionuclide that is produced in the reaction given by ln2 / $t_{½}$ (half-life)
t time in seconds
C concentration ($\mu g.g^{-1}$)

4.2.6 Theoretical limits of detection

A limit of detection (LOD) is the lowest quantity of concentration substance that can be distinguished from the absence of that substance (a blank value) within a confidence limit. The different detection limits that are commonly used include the instrument detection limit, the lower level of detection, the method detection limit and the level of quantification. Elemental detection limits of INAA are variable because the production of radioactive nuclides depends on the cross-section of a specific element and the gamma-ray that are emitted. In general, elements that have large cross-sections will have low detection limits. Detection limits are sample matrix dependent therefore it is important to calculate these limits for matrix free elements without disturbing activities from sample after irradiations in the research reactor. The contribution of the natural background to the observed counting rate cannot be eliminated completely therefore detection limit (DL) is important for background correction [67,68] given by:

$$DL = 2.71 + 4.65 B^{½} \quad \ldots (8)$$

Where, *B* is the background reading obtained for the same measuring period.

The minimum detectable activity (MDA) represents an equivalent amount of activity based on the background reading and is an estimate of the capability of the instrument or the method given by:

$$MDA(Bq) = \frac{DL}{Y \varepsilon t} \quad \ldots (9)$$

Where,

DL detection limit (counts) as defined in equation (8)
Y yield during decay of the type of radiation being measured (fraction)
ε counting efficiency of detector- sample configuration (fraction)
t counting periods (seconds)

The approximate detection limits of essential and toxic elements range of INAA given in grams [69] are shown in Table 21.

Table 21: Approximate detection limits of essential and trace elements by INAA [69].

Elements	Detection limits in grams	Elements	Detection limits in grams
Ag	$1x10^{-10}$	Na	$4x10^{-12}$
Al	$3x10^{-8}$	Ni	$6x10^{-9}$
As	$1x10^{-12}$	Os	$1x10^{-11}$
Au	$6x10^{-14}$	P	$5x10^{-5}$
Ba	$2x10^{-10}$	Pt	$1x10^{-11}$
Br	$2x10^{-12}$	Rb	$8x10^{-10}$
Ca	$2x10^{-7}$	Re	$5x10^{-13}$
Cd	$3x10^{-11}$	Ru	$9x10^{-11}$
Cl	$2x10^{-9}$	S	$1x10^{-5}$
Co	$6x10^{-11}$	Sb	$1x10^{-12}$
Cr	$1x10^{-10}$	Sc	$2x10^{-12}$
Cs	$1x10^{-11}$	Se	$1x10^{-10}$
Cu	$4x10^{-10}$	Si	$4x10^{-8}$
Ga	$2x10^{-12}$	Sn	$9x10^{-8}$
Ge	$4x10^{-10}$	Sr	$4x10^{-9}$
Fe	$2x10^{-8}$	Ta	$1x10^{-11}$
Hf	$2x10^{-11}$	Te	$9x10^{-11}$
Hg	$4x10^{-11}$	Th	$1x10^{-11}$
I	$8x10^{-11}$	Ti	$1x10^{-8}$
In	$3x10^{-9}$	U	$5x10^{-12}$
Ir	$3x10^{-13}$	V	$9x10^{-10}$
K	$2x10^{-10}$	W	$1x10^{-12}$
La	$1x10^{-12}$	Y	$4x10^{-9}$
Mg	$2x10^{-8}$	Zn	$1x10^{-10}$
Mn	$8x10^{-13}$	Zr	$1x10^{-3}$
Mo	$3x10^{-11}$		

CHAPTER 5

5 PRINCIPLES OF INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY (ICP-MS)

5.1 Introduction

ICP-MS is relatively new technique developed in the late 1980's for the determination of trace elements with high accuracy and low detection limits. ICP-MS uses the ability of the argon ICP to effectively generate singly charged ions from elemental species within a sample. These ions are then directed into the mass spectrometer, which separates ions introduced from the ICP according to their mass-charge ratio, other than ICP-OES (Optical Emission Spectroscopy), which separates light according to its wavelength. Finally the ions are then measured and detected by the electron multiplier. Interferences of ICP-MS results from signals of oxides (MO^+), doubly charged ions (M^{2+}), hydroxides (MOH^+), argides, isobaric overlaps and molecules with the same ratio of mass and charge as the element of interest. ICP-MS instruments are not stable over a long period of time, which makes it necessary to use an internal reference or apply periodical external standardization [41,42,43].

5.1.1 Applications of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

A wide variety of sample materials can be analyzed for most elements in the Periodic Table. Some important trace-element analysis applications of ICP-MS include [41,42,43]:

- Pharmaceutical (drugs)
- Food and beverage
- Rocks and mineral separates
- Fresh, saline and sediment pore waters
- Sediments, soils and soil leachates
- Archeological materials (ceramics)
- Human teeth and bones, fish bones, shells etc.

5.1.2 Advantages and Disadvantages of ICP-MS

The advantages of ICP-MS are [41,42,43]: (a) multi-element technique, more than 70 elements can be determined, (b) both solid and liquid samples can be analyzed, (c) typically about 3-5 ml sample quantity required, (c) ICP-MS is suitable for the excitation of elements which are difficult to be excited; due to its high temperature complete atomization can be achieved, (d) because of the inert conditions of the ICP-MS chemical interference is very small and the intensities of the spectrum are high, and (e) an extensive analytical range between ppt and ppm can be obtained.

Disadvantages of ICP-MS include [41,42,43]: (a) ICP-MS suffers from severe matrix effects requiring the addition of matrix modifiers and the use of the method of standard additions to quantify some elements, (b) isobaric, molecular and doubly-charged ion interferences are common in ICP-MS, whereby spectral interferences arise from ions having the same mass-to-charge ratio. Molecular species which are produced in the plasma are usually a bigger problem than elemental interferences. These can be controlled in three ways: (1) keeping the solution concentration of the problem species low, (2) properly adjusting the instrument to minimize the creation of the molecular species in the plasma and (3) using collision and reaction cell technology to minimize interferences, (c) destructive technique, (d) strong dependence of signal on plasma parameters for example detection capabilities, temperature etc, (e) cannot analyze very small sample volume less than 3ml and, (f) operating cost of the method is quite high.

5.1.3 Instrument description of ICP-MS

ICP-MS (Figure 13) has four main processes, including [41,42,43].:

- Sample introduction and aerosol generation.
  This system facilitates the transport of sample material. Liquid samples are transported using a peristaltic pump ensuring a constant sample flow rate and allow the sample uptake rate to be adjusted and the autosampler may be used. Various nebulizers (for example cross-flow, Meinhard, Ultrasonic) are used to introduce liquid samples into the plasma in a form of an aerosol.
- Sample ionization by an argon plasma source.
  The excitation source in ICP-MS is called and argon plasma. The argon gas is commonly used because it is comparatively easily ionized at high temperature of about 6000 to 8000 ^{0}K and has a large mass which means it has good impulse transfer properties. The sample gets completely volatilized, atomized and ionized under atmospheric pressure.
- ICP-MS interface.
  Since the ionization occurs at atmospheric pressure, the sample ions are transferred into a mass filter under a vacuum environment.
- Quadropole mass spectrometer.
  The separation of the ions according to mass-to-charge (m/z) is performed by a quadrupole mass analyzer. Finally the ions are then detected by a secondary multiplier.

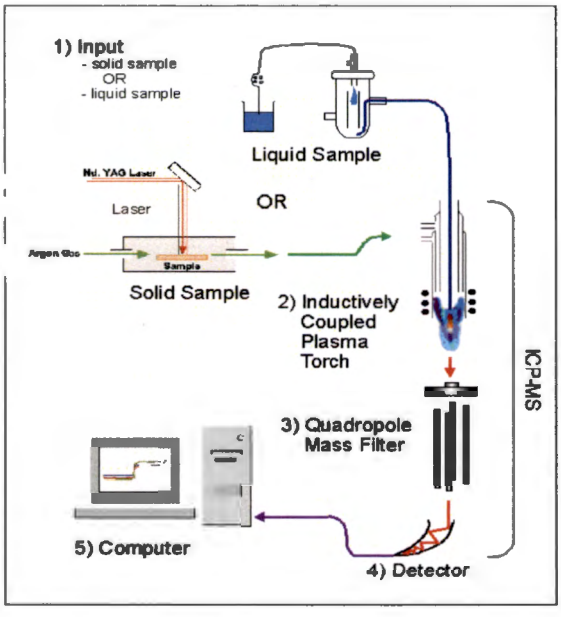


Figure 13: Schematic diagram of Inductively Coupled Plasma-Mass spectrometry.

5.1.4 Sample preparation

For trace element quantification by ICP-MS necessitates medicinal plant samples are to be digested. Various digestion methods may involve a range of different clean acids such as HF, HNO_3 or HCl. In this investigation HNO_3 was used. The sample preparation procedure involved adding 5 ml HNO_3 to the medicinal plant sample in a Teflon beaker and evaporation on a steam bath. The residue was then dissolved in a 1 ml HNO_3 and diluted in a 25 ml volumetric flask. Before analysis of elements can take place the operating conditions need to be optimized, e.g. the nebulizer gas flow rate and the ion lens voltage must be adjusted to obtain the highest signal intensities while still maintaining low levels of oxides and doubly-charged ion production.

CHAPTER 6

6 EXPERIMENTAL PROCEDURE FOR INAA.

The first step in assuring quality, safety and effectiveness of medicinal plants is the correct identification of these plant species. Botanical verification is important and the information required include the currently accepted Latin botanical name, common name, the parts (stem bark, roots, leaves) of the plant used for each preparation together with an identification of whether fresh plant material, dried plant material or tinctures were used.

6.1 The SAFARI No.1 Research Reactor

The South African Fundamental Atomic Reactor Installation (SAFARI) No.1 is an Oak Ridge type material test reactor located at the Pelindaba site of the South African Nuclear Energy Corporation (Necsa) near Pretoria. The reactor became critical on 8 March 1965. SAFARI-1, which initially was a 6.67MW tank-in-pool type light water reactor purchased from the United State of America (USA), but was soon modified to enable operation at 20MW thermal power level. The reactor is housed inside an aluminum vessel with fuel in the form of plates that are about 50 mm wide and 500 mm long whereby each plate consists of an Uranium/Aluminum alloy that is sandwiched between two pure aluminum plates. The reactor was designed for 90% enriched ^{235}U, but has been operated for years at about 40% enrichment due to strategic reasons. A number of plates are mounted inside a long square tube of pure aluminum and couplings are attached to each end of this tube to form a fuel element. At the bottom of the reactor vessel is a rectangular metal grid into which the fuel elements are fitted to form the reactor core. Three edges of the core are covered by aluminum and beryllium reflector plates whereas one edge is close to the cut-away side of the vessel to keep the row of fuel elements close to the poolside face [69].

Some of the grid positions are used for the five control rods and a number of hollow elements (empty fuel cells) into which capsules can be loaded for long-term irradiations. The control rods consist of two parts; the top part is made from cadmium to absorb neutrons and stop the chain reaction and the bottom part is similar to the fuel elements to add extra reactivity when the reactor is operating. The control rods are pushed up vertically to start the reactor and will drop back to quench the chain reaction should anything go wrong. The reactor is located inside a pool with clean water that is about 10 m deep used as a radiation shield for the operational personnel. The pool walls are built from high density concrete that contains large chunks of iron which acts as a biological shield to absorb the intense gamma radiation from the fission products in the core. Currently, the reactor operates on a cyclic programme of 5 weeks full power operation at 20MW and shuts down for necessary maintenance and fuel reload or reallocation over a period of 3 to 4 days [69]. The SAFARI reactor facility is shown in Figure 14 and 15 respectively. SAFARI-1 consists of two pneumatic transfer systems namely the RINGAS and PRS, which were used for the irradiation of the medicinal plant samples with neutrons as discussed in paragraph 6.2..

6.1.1 Restrictions on the material to be irradiated in a reactor building

Personnel are not allowed to [69]:

- bring mercury metal or compounds into the reactor building because mercury may react with the aluminum of the reactor core and pool vessel.
- irradiate water or other material that will produce a gas under gamma radiation because this may lead to pressure build-up and explosions that can damage the reactor.
- Irradiate fissile material because this will affect the reactivity of the core and complicate the control.
- Irradiate large amounts of material that is easily activated, it will basically increase the radiation at the receiving station beyond the level that it can be handled.
- bring in radioactive material which may pose contamination and/or a radioactive hazard.

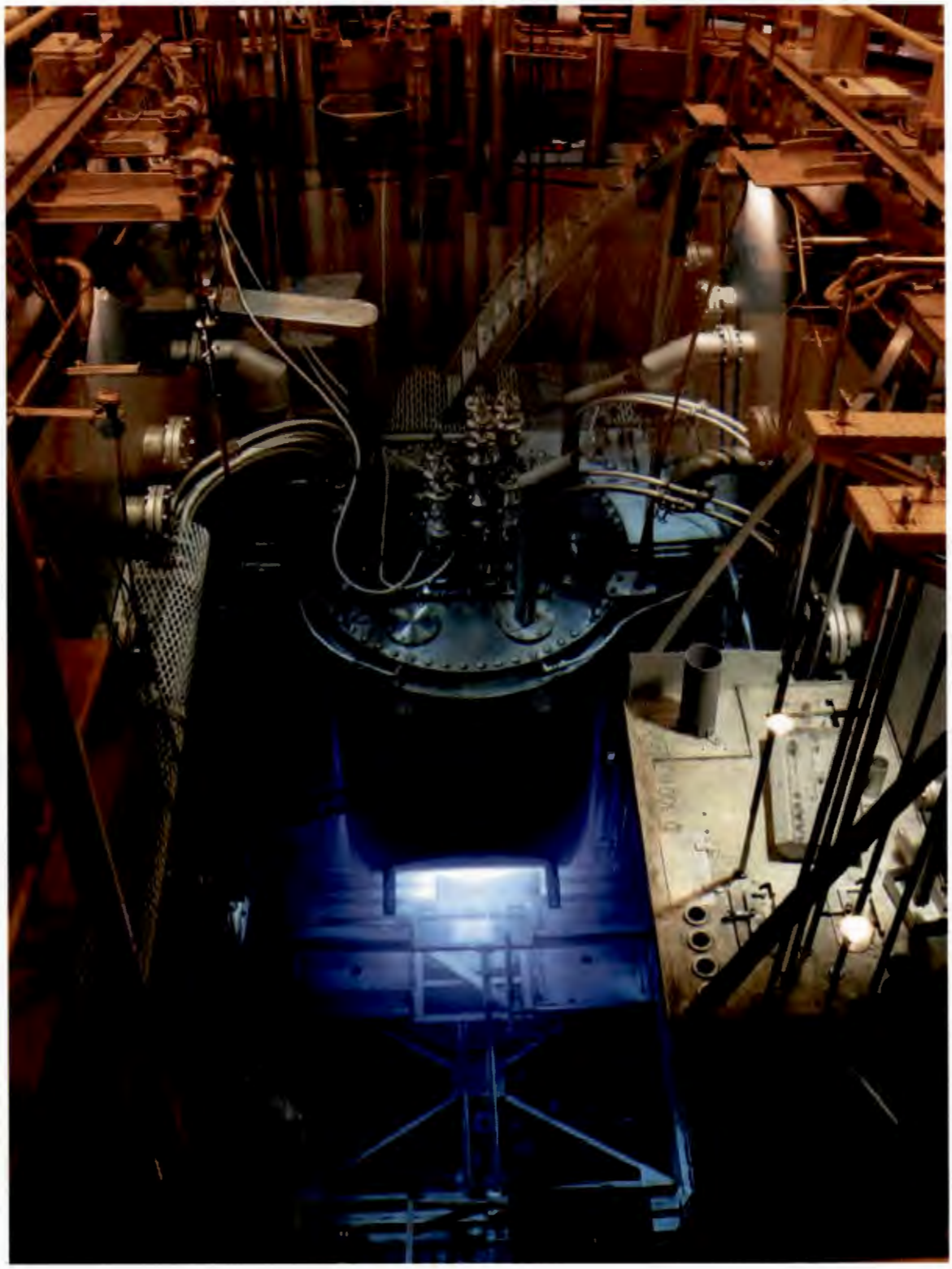


Figure 14: SAFARI No.1 reactor facility.

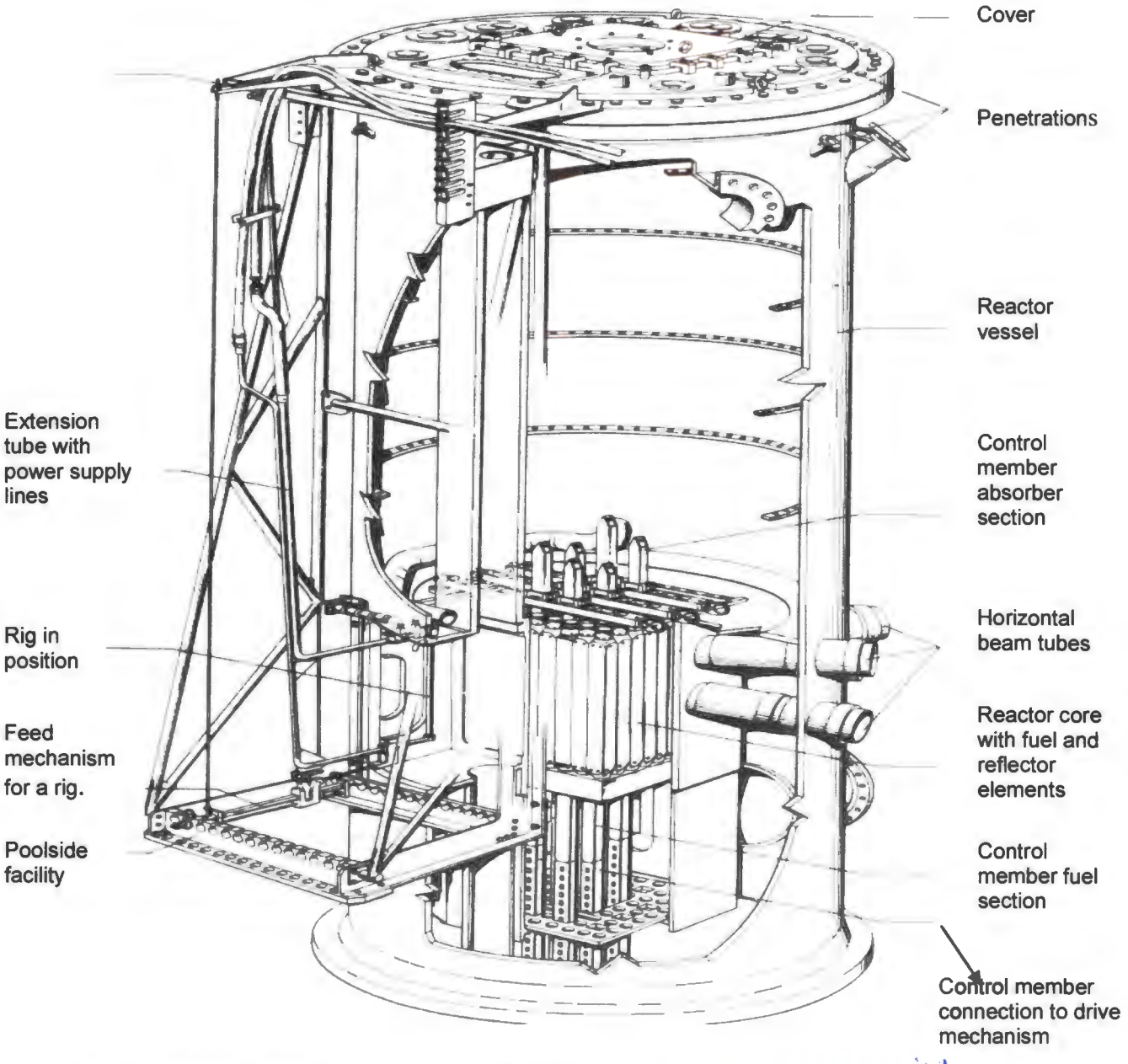


Figure 15: Schematic view of SAFARI-1 reactor.

6.1.2 The role of SAFARI-1 at Necsa

SAFARI-1 is utilized mainly for client service to perform irradiations for Nuclear Technology Products (NTP) Radioisotopes (Pty) Ltd. for the production of radioisotopes for medical applications and Neutron Transmutation Doped (NTD) silicon. Other important roles of this reactor include the utilization of beam-ports for Neutron Diffraction and Neutron Radiography as well as Neutron Activation Analysis for commercial and institutional purposes. In support of the safe operation and commercial needs of NTP, SAFARI-1 applies an integrated ISO 2001 accredited management system incorporating quality, health and environment [70,71,72].

6.2 Irradiation Facilities

6.2.1 RINGAS (Routine Instrumental Neutron and Gamma Analysis System)

The main purpose of RINGAS pneumatic transfer system is to transport rabbit assemblies from the rabbit feed station to the desired in-core position in the reactor, one at time, to be irradiated with neutrons for a predetermined time. The rabbit assemblies are then extracted from the reactor and counted at a delay station to determine their activity; if this exceeds a predetermined level the rabbit will be sent to the waste station to avoid possible over-exposure of personnel at the counting station. If the activity is acceptable, the rabbit assemblies are transported to a separator gate where the samples are separated from the rabbit body to lower background gamma-ray interferences. The rabbit and sample are then counted and thereafter transported to the waste station. The rabbit bodies can be re-used approximately five times before they become brittle due to the high gamma-dose at the irradiation positions..

RINGAS has been utilized to analyze medicinal plant samples for a wide range of elements producing short-lived nuclides with half-lives ranging from 15 seconds to 30 hours. This pneumatic transfer system uses compressed air outside the reactor core and vacuum inside the core (for safety reasons) for high speed transport of the rabbits and samples. A "Dummy" position is available outside the reactor vessel to test the pneumatic transfer system without exposure to neutrons.

RINGAS is operated through a computer controlled system, which is able to detect failures and/or errors during the sample analysis. This RINGAS computer control system indicates the position of the rabbits and samples at various positions in the pneumatic transfer system. The types of failures which can occur include amongst others the following:

- Rabbit stuck in reactor; possible causes of this failure may be that the rabbit did not leave the reactor in-core position after the radiation time elapsed.
- Separator gate failure; the gate did not switch to the position required by the control system.
- The rabbit was not detected at the Cadmium or non-Cadmium Gate; probably due to a vacuum failure in the pneumatic transfer system.
- Feed gate and In/Out valve failure; whereby the gate or valve did not switch to the position as required by control system.

A simplified layout of the RINGAS rabbit and sample travel path is shown Figure 16.

6.2.2 PRS (Pneumatic Rabbit System)

The pneumatic rabbit system is located inside the reactor vessel but outside the core and is primarily used for the analysis of radionuclides with long half-lives from two days to years. The system enables batches of up to ten samples to be irradiated at a time. This facility can be operated manually or automatically. Irradiation times of up to 60 minutes are used. The thermal neutron flux is about $3.9 \times 10^{13} n.cm^{-2}s^{-1}$. The pneumatic rabbit system consists of a 38mm rabbit tube leading from the send/receive station in the pneumatic rabbit room to the irradiation position within the reactor vessel, a send/receive station control panel with automatic/manual control as well as a vacuum system to transport the rabbits. The send/receive station is located within a special lead-shielded fume hood in the laboratory. The pneumatic rabbits (Figure 17) are inserted through a spring-loaded gate and discharged through a coil-type device to reduce the speed when returning from the reactor [70]. Solenoid valves are used to direct the air flow from the station to the reactor, maintaining a vacuum in the system at all times. For the automatic control of the irradiation time, the automatic timer is used.

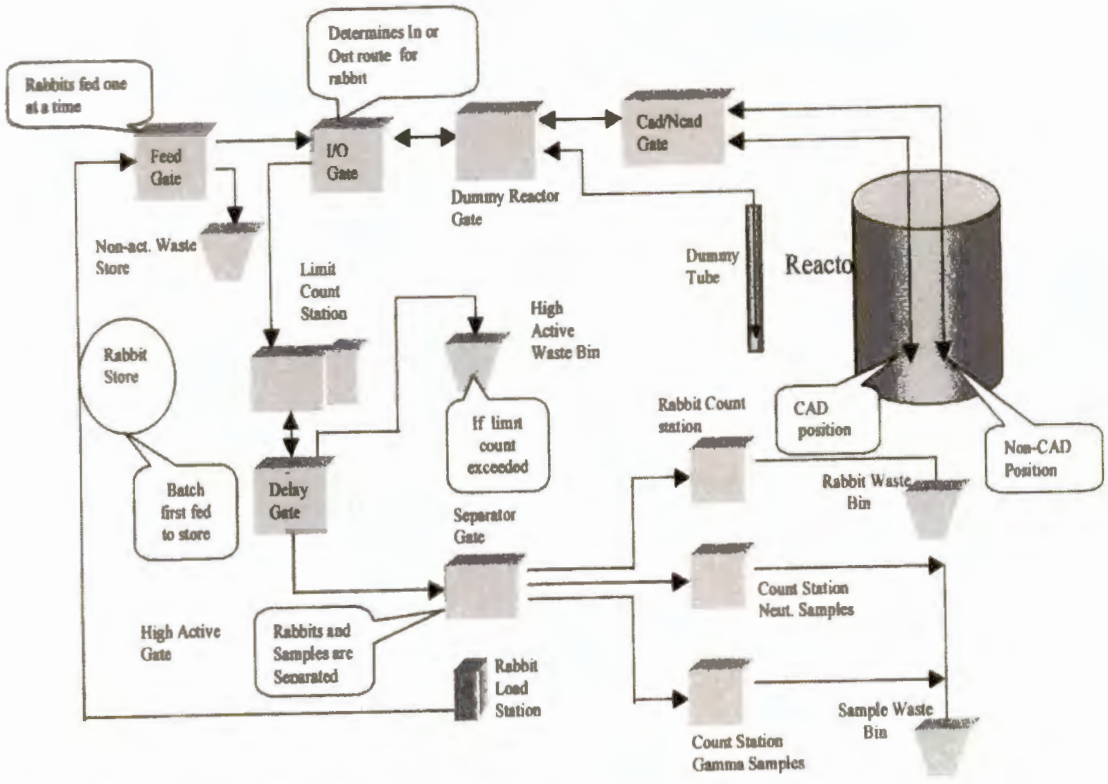


Figure 16: RINGAS rabbit and sample travel path simplified layout.

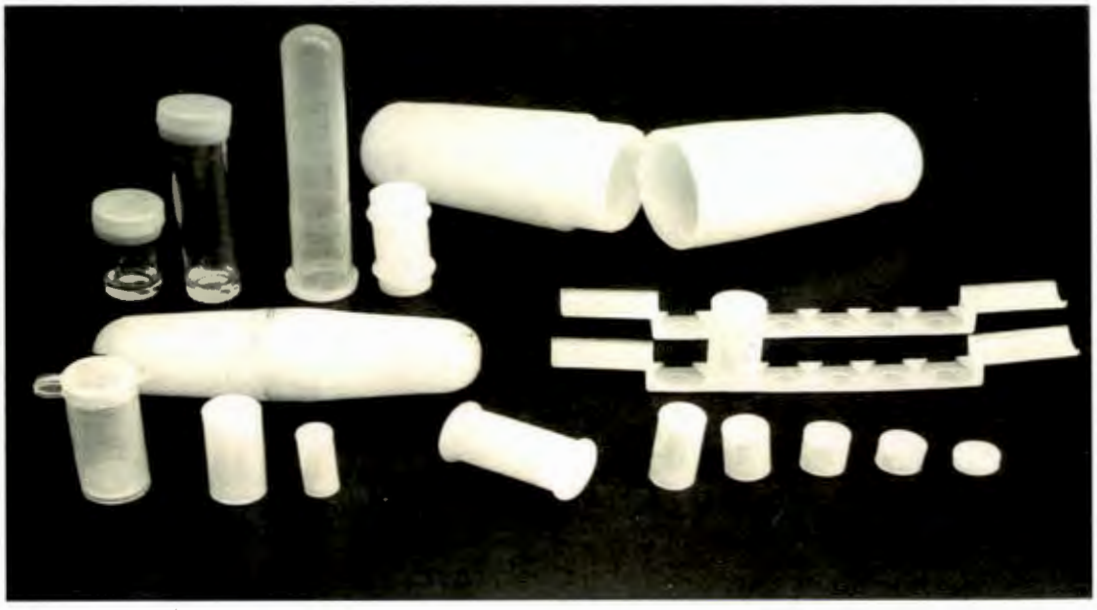


Figure 17: The modular of irradiation capsules and shuttles used in PRS.

Off-gas vacuum is employed to convey the pneumatic rabbits into and out of the reactor. A turbo blower provides the necessary air sweep to cool the sample when it is in the reactor. Air from the blower is discharged into the off-gas system which maintains a slight vacuum and air sweep on the system even when the blower is switched off. A vacuum switch connected to the air supply tube activates an alarm in the control room if the tube vacuum falls below a set value while a rabbit is in the reactor. The schematic diagram of pneumatic rabbit system is shown in Figure 18 and 19. Should the system not function normally after the rabbit has been inserted for irradiation, there is an "Emergency Rule" button on the control box which can be pressed to return the rabbit to the receiving station. This button cancels all other control settings, reverses the direction of air flow and returns the rabbit to the station.

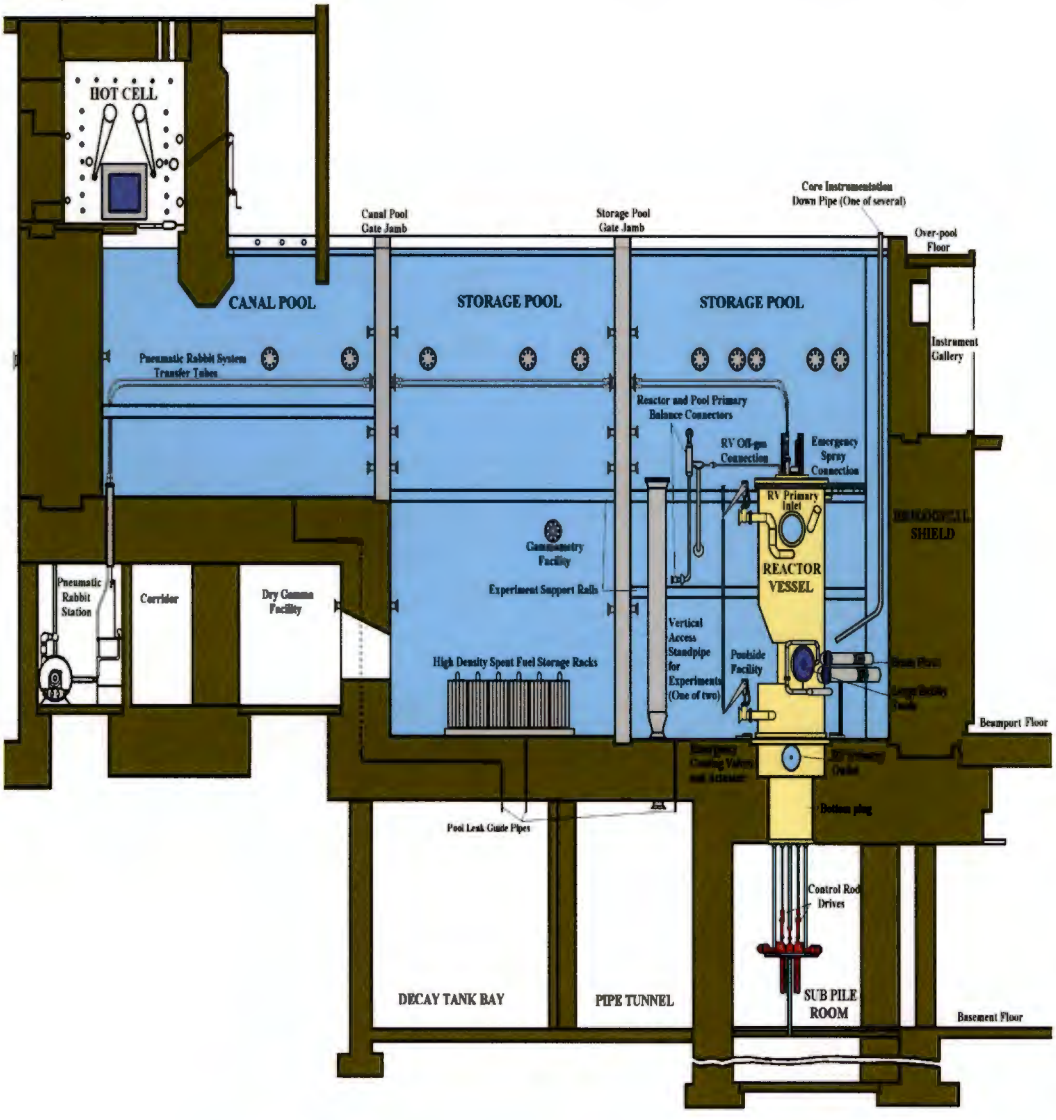


Figure 18: Longitudinal section of pool structure showing reactor and other facilities.

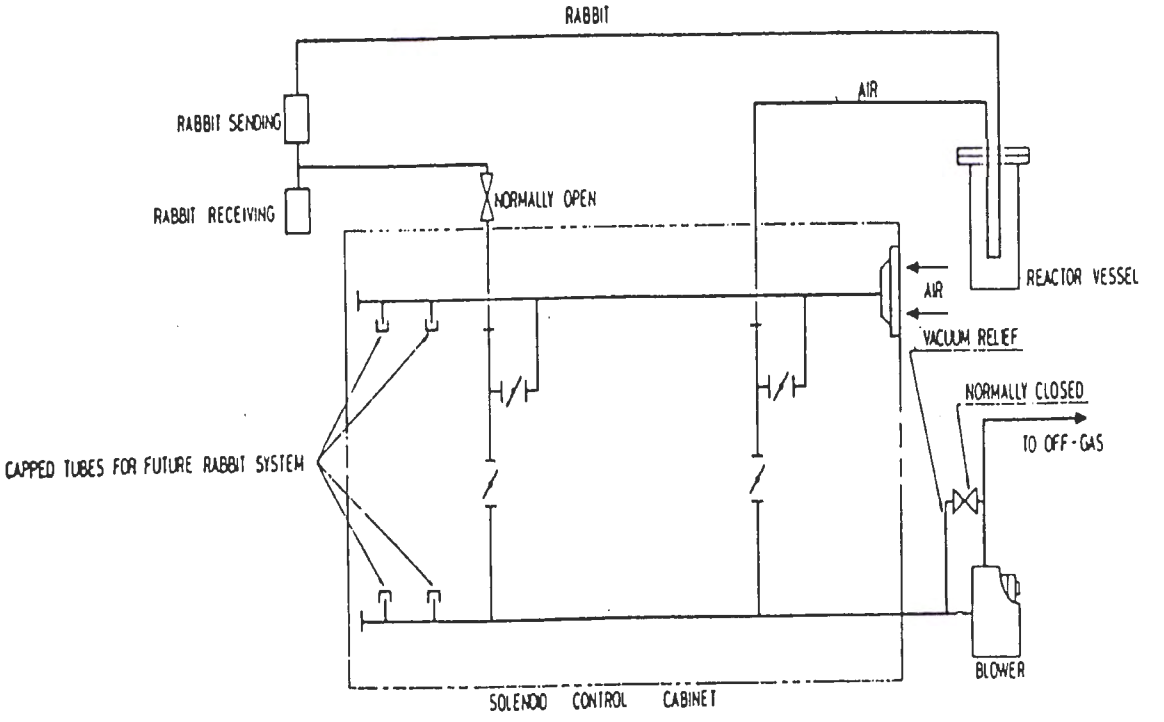


Figure 19: Schematic diagram of pneumatic rabbit system.

The regulations governing irradiations in the pneumatic rabbit system of SAFARI-1are as follows:

- For all the irradiations, there is an overall mass limit of sample material per capsule according to the formula,
  Maximum mass (grams) = 60/P, where P is the reactor power in megawatts.
- The lead shield of 5cm thick is constructed in such a way that exposure of the whole body, blood-forming organs and gonads need not be considered relative to the exposure of the hands when the irradiated rabbit is opened.
- The maximum dose equivalent rate to the hands when the rabbits are opened may not exceed 1.5 rem/h.
- The irradiation time is limited to 80/P hours, to limit radiation damage to the polyethylene containers.

6.3 Sample preparation

Medicinal plant samples (dry plant material and tinctures) were provided by Phytosun Green Laboratories, which is a herbal medicine company, and were registered making sure all the samples are logged, sorted and allocated a unique laboratory sample code. Empty drying trays were weighed and their mass was recorded. Medicinal plant samples were then transferred into those drying trays and wet mass of the sample plus drying trays were determined. For plant material to be dried, each specific drying tray was marked with a specific sample number. All plant material were spread evenly on the drying trays and then dried in an oven at 65°C for about 12 hours. All the plant samples were then removed from the oven to cool. For the determination of dry mass, samples were transferred in the in different containers on the balance and the weights of dry plant materials were recorded. The plant samples were milled in a SiEBTECHNiK agate-vessel swing-mill machine for one minute until fine powdered samples were obtained. The fine powdered samples were placed into polyethylene bottles and capped. The polyethylene sample bottles were weighed and their masses were recorded and marked. Contrad soap and ethanol was used for the cleaning of the all drying dishes prior and after being used. The schematic view of INAA procedure followed for all the medicinal plants analyzed is specified in Figure 20.

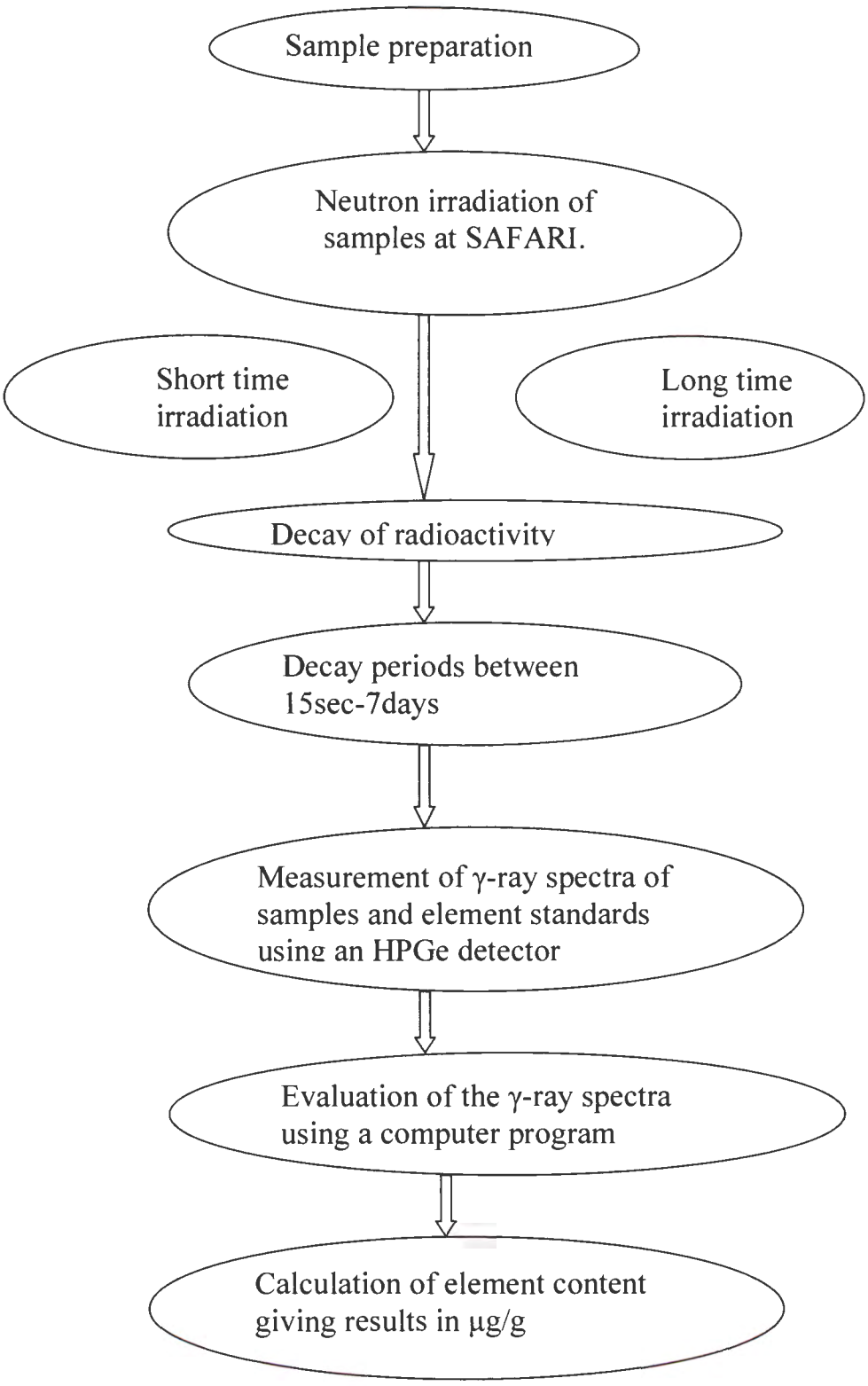


Figure 20: INAA Procedure.

6.4 Irradiation and Counting

The typical irradiation, decay and counting time-regimes used in routine analysis are given in Table 22.

Table 22: The irradiation and counting conditions for analyzed nuclides.

Irradiation facility	Irradiation time	Decay time	Counting time	Nuclides analyzed
RINGAS Short-Lived	8 seconds	30 seconds	120 seconds	^{66}Cu, ^{52}V, ^{28}Al
RINGAS Intermediate-Lived	8 seconds	3-30 hours	15-30 minutes	^{42}K, ^{24}Na, ^{56}Mn, ^{99}Mo, ^{115}Cd, ^{76}As
PRS Long-Lived	30 minutes	5 to 7 days	60 minutes	^{47}Ca, ^{59}Fe, ^{65}Zn, ^{60}Co, ^{51}Cr, ^{58}Ni, ^{75}Se, ^{203}Hg, ^{232}Th, ^{238}U

6.5 Quality Assurance

A histographic comparison of essential and toxic elements contents of various medicinal plants are shown in Figure 21 and 22. The uncertainty of each element concentration is computed as the square root of the sum of variances of different parameters used namely the peak areas, target thickness, weighing and systematic errors.

The seven standard certified reference materials given in Table 23 were used for quality assurance and quality control (QAQC). The reference materials Lake sediment IAEA-SL-1, Seaweed Fucus species IAEA-140TM, Lichen IAEA-336, ACS/A37/02 IAEA H-8, IAEA-Soil-7, Spinach ACS/F19/01 and UREM-4 were analyzed under the same experimental conditions used for the sample in order to evaluate the precision and accuracy of the results.

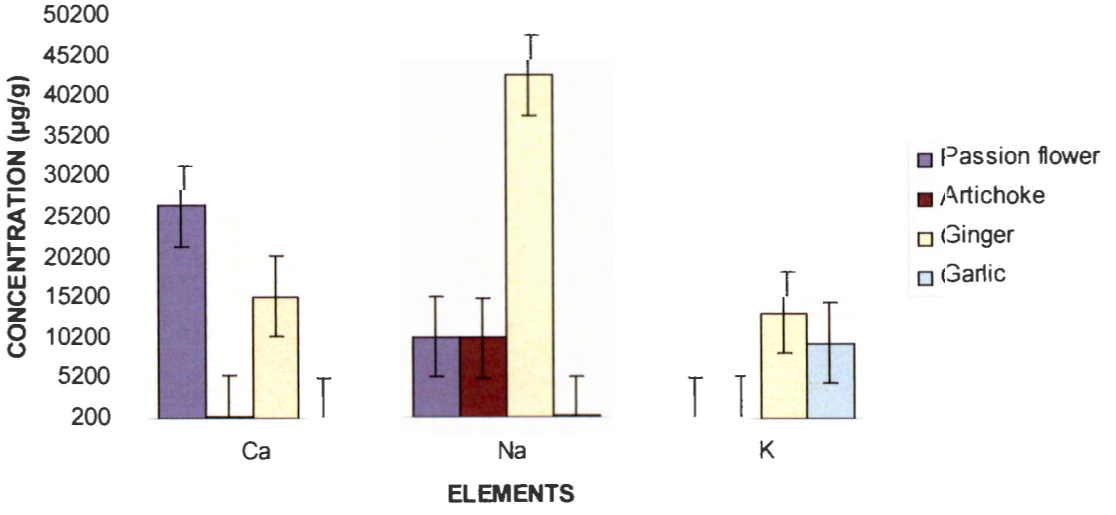


Figure 21: Concentration of essential elements in various medicinal plants using INAA.

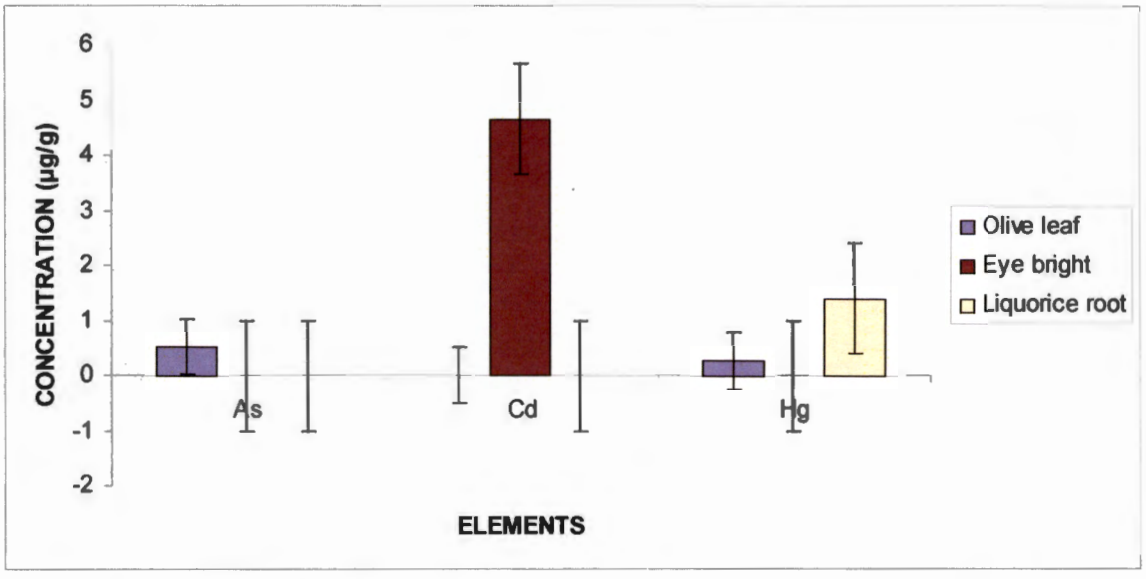


Figure 22: Concentration of toxic elements in various medicinal plants using INAA.

Table 23: The reference materials for analytical quality control.

REFERENCE MATERIAL			Lake sediment IAEA-SL-1			Seaweed Fucus species IAEA-140TM			Lichen IAEA-336		
Element	Nuclide	Energy	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
CA	CA-47	1297.09	2.16E+03	5.45E+02	1.89E+03	1.18E+04	9.34E+02	5.84E+03	1.91E+03	1.52E+02	7.60E+02
CD	CD-115	527.90	2.90E+01	1.62E+00	1.02E+01			1.71E+01			2.64E+00
CO	CO-60	1173.22	1.97E+01	5.20E-01	5.46E-01	6.57E+01	1.72E+00	1.24E+00	1.37E+00	6.28E-02	1.45E-01
CR	CR-51	320.08	1.17E+02	3.01E+00	1.31E+00	1.07E+01	6.22E-01	1.78E+00	1.06E+01	3.23E-01	3.39E-01
FE	FE-59	1099.22	6.61E+04	1.69E+03	2.46E+02	1.06E+03	1.13E+02	3.57E+02	4.02E+02	2.06E+01	5.30E+01
HG	HG-203	279.19	7.27E+00	2.41E-01	7.89E-01	2.16E+00	1.60E-01	1.08E+00	4.09E-01	6.45E-02	1.94E-01
MO	MO-99	140.51			2.03E+00			1.30E+00			3.46E-01
NI	NI-58	810.76			3.43E+02			3.14E+02			5.11E+01
SE	SE-75	136.00			3.50E+00	1.92E+01	8.11E-01	2.08E+00			7.10E-01
AS	AS-76	559.10	3.09E+01	4.40E-01	1.67E+00	3.90E+01	7.42E-01	1.75E+00	6.32E-01	7.75E-02	2.83E-01
K	K-42	1524.67	1.25E+04	3.23E+02	9.16E+02	3.04E+04	4.70E+02	1.20E+03	1.60E+03	6.93E+01	1.59E+02
MN	MN-56	846.75	3.92E+03	4.72E+00	7.70E-01	5.88E+01	1.55E+00	4.75E+00	6.44E+01	5.89E-01	6.98E-01
TH	PA-233	312.17	1.27E+01	3.24E-01	1.12E-01	2.29E-01	3.64E-02	1.18E-01	1.18E-01	8.62E-03	2.50E-02
U	NP-239	277.60	2.51E+00	9.79E-02	3.29E-01	4.17E-01	7.29E-02	4.55E-01			8.88E-02
ZN	ZN-65	1115.55	2.16E+02	5.97E+00	1.13E+01	4.63E+01	3.28E+00	1.89E+01	2.82E+01	9.83E-01	2.42E+00
NA	NA-24	1368.53	1.99E+03	1.55E+01	2.73E+01	3.07E+04	1.33E+02	4.36E+01	2.63E+02	3.98E+00	5.61E+00
Al	Al-28	-	-	-	-	2.93E+03	3.29E+02	2.10E+01	9.57E+02	1.08E+02	8.91E+00
Cu	Cu-66	-	-	-	-			2.41E+02			1.00E+02
V	V-52	-	-	-	-	3.97E+00	8.78E-01	2.73E+00			1.96E+00

REFERENCE MATERIAL			Spinach ACS/F19/01			UREM-4		
Element	Nuclide	Energy	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
CA	CA-47	1297.09	-	-	-	2.87E+03	4.00E+02	2.59E+03
CD	CD-115	527.90	-	-	-			1.05E+01
CO	CO-60	1173.22	-	-	-	2.02E+01	5.29E-01	3.71E-01
CR	CR-51	320.08	-	-	-	1.77E+02	4.55E+00	9.56E-01
FE	FE-59	1099.22	-	-	-	1.64E+04	4.23E+02	1.59E+02
HG	HG-203	279.19	-	-	-	1.87E+02	5.01E+00	6.81E-01
MO	MO-99	140.51	-	-	-	1.67E+02	4.24E+00	1.01E+00
NI	NI-58	810.76	-	-	-			2.41E+02
SE	SE-75	136.00	-	-	-	1.00E+01	4.08E-01	2.35E+00
AS	AS-76	559.10			2.18E+00	-	-	-
K	K-42	1524.67	3.16E+04	4.95E+02	1.06E+03	-	-	-
MN	MN-56	846.75	1.72E+02	2.24E+00	5.53E+00	-	-	-
TH	PA-233	312.17	-	-	-	1.25E+01	3.20E-01	8.96E-02
U	NP-239	277.60	-	-	-	8.16E+01	2.09E+00	3.02E-01
ZN	ZN-65	1115.55	-	-	-	2.76E+01	1.47E+00	7.03E+00
NA	NA-24	1368.53	1.30E+04	6.56E+01	3.69E+01	-	-	-

REFERENCE MATERIAL			ACS/A37 / 02 IAEA H-8			IAEA-Soil-7		
Element	Nuclide	Energy	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
CA	CA-47	1297.09			3.39E+03	1.57E+05	4.16E+03	2.61E+03
CD	CD-115	527.90	2.88E+02	7.74E+00	7.17E+00	8.26E+00	1.03E+00	7.03E+00
CO	CO-60	1173.22	1.02E+01	3.12E-01	5.13E-01	9.76E+00	2.68E-01	4.08E-01
CR	CR-51	320.08	1.94E-01	2.86E-01	9.24E-01	7.30E+01	1.90E+00	9.30E-01
FE	FE-59	1099.22	1.73E+02	3.92E+01	1.27E+02	2.52E+04	6.46E+02	1.79E+02
HG	HG-203	279.19	1.67E+00	9.61E-02	5.07E-01	4.51E+00	1.59E-01	5.89E-01
MO	MO-99	140.51	1.75E+00	1.18E-01	8.20E-01			1.50E+00
NI	NI-58	810.76			1.28E+02			2.41E+02
SE	SE-75	136.00	4.86E+00	2.94E-01	1.66E+00			2.64E+00
AS	AS-76	559.10			1.39E+00	-	-	-
K	K-42	1524.67	1.10E+04	2.35E+02	5.94E+02	-	-	-
MN	MN-56	846.75	4.72E+00	7.70E-01	2.49E+00	-	-	-
TH	PA-233	312.17			9.53E-02	7.33E+00	1.88E-01	7.97E-02
U	NP-239	277.60	2.12E-01	5.19E-02	2.20E-01	1.48E+00	7.10E-02	2.58E-01
ZN	ZN-65	1115.55	1.73E+02	5.50E+00	9.98E+00	9.80E+01	2.97E+00	8.34E+00
NA	NA-24	1368.53	8.43E+03	4.08E+01	2.07E+01	-	-	-

CHAPTER 7

7.1 RESULTS AND DISCUSSIONS

7.1.1 Results and discussions for dry medicinal plants using INAA.

Table 24 shows the results of 16 trace elements concentrations of 30 medicinal plants analyzed by INAA. For each element determined for a particular medicinal plant, detail information on the name of the element, nuclide energy, the radionuclide, the concentration in µg/g, the uncertainty and the minimum detectable concentration (MDC) in µg/g is given. The results obtained below showed that the elements concentrations can vary substantially between the medicinal plant species, e.g. Cr (0.17 – 220 µg/g), Ca (1490 – 26500 µg/g), K (3660 – 42700 µg/g), Fe (110 – 7200 µg/g), Al (185 – 47900 µg/g) and Na (40 – 10100 µg/g).

Some specific observations were made whereby a correlation was observed between a high element concentration in the medicinal plant and their therapeutic application as given in Appendix 1, against the element health benefits described in Table 6:

- Potassium (K) is medically used to detoxify and protect the liver and the highest concentration of this element was found in Artichoke, which is used as a liver-protectant.
- The largest concentration of sodium (Na) was found in Gotu Kola, which both is used in the treatment of stomach and duodenal ulcers.
- Passion flower is traditionally used for nervous gastrointestinal disorders especially in children, which can be attributed to the high calcium (Ca) concentration found in this medicinal plant.
- Artichoke also had the maximum concentration of Co, which is used for lipid lowering.
- Both chromium (Cr) and Boswellia medicinal plant stops or inhibits infection because of their anti-inflammatory properties and the highest concentration of this element was observed in this plant.
- Alfalfa is traditionally used as a tonic to maintain and restores health. The highest concentration of selenium (Se) was obtained in this medicinal plant showing a correlation since Se is known to have anti-aging properties.
- Gotu Kola, with high concentrations of elements such as Ca, Co, Hg, Se, K, Mn, Zn and Na, is mainly use is for treating wounds, burns and ulcers to accelerate healing and to prevent the formation of scar tissue following surgery indicates that this plant also acts as a multi-functional general tonic improving the resistance to physical and mental stress.
- Calcium (Ca) was found to be high in 9 medicinal plants out of the 30 studied, all used to improve general body health.
- Aluminium (Al) and Yarrow are both good for bone formation.
- Copper (Cu) and Burdock are used for treatment of skin disorders such as sores.
- Vanadium (V) and Thyme are applied for improved immune system reducing the occurrence of common colds.

Furthermore, the dosage of each element received from a particular medicinal plant usage can be compared to the daily intake recommendations to verify the safety of these plants for human utilization. Medicinal plants are taken preferably one hour before or after each meal in a dose of 1 teaspoon (up to 1g) three times a day. Considering a daily dose of 3 teaspoons (3g), the highest daily intake of essential and toxic elements from different medicinal plants is found to be Ca (80 mg/d), Cd (15 µg/d), Co (100 µg/d), Cr (660 µg/d), Hg (4 µg/d), Mo (15 µg/d), Se (4.5 µg/d), K (130 mg/d), Zn (0.5 mg/d), As (1,5 µg/d), Mn (1,5 mg/d), Na (30 mg/d) and Fe (20 mg/d). From this and the information in Table 7 and Table 8 it is observed that the recommended daily intake will be exceeded for all age and gender groups for Co and Cr, while Fe, Mo and Mn may be exceeded for infants less than one year old. The daily intake dosages estimated are given in Table 25. All the other trace elements have acceptable intake dosages. However, high dosages of some trace elements are justifiable since the medicinal plants are

only recommended to be taken for less than two weeks; not for a life-time. Therefore some elements may be taken in abundance when really needed.

Table 26 and 27 were compiled so as to understand whether the healing capacity of those relatively rich elements matches with the traditional medicinal uses of the herbs. Confirmation of Gotu Kola as an adaptogenic tonic was obtained from this tables. Cr was obtained in Boswellia, Wormwood, Thyme and Peppermint herbs which all have anti-inflammatory properties. High Ca content was obtained in Alfalfa and Goldenrod used for bone formation. Alfalfa, Yarrow and Artichoke have been used to normalize blood pressure because of high concentration content of K. Co which is used in fat metabolism was found in Yarrow and Artichoke.

7.1.2 Results and discussions for vodka medicinal plant extracts using ICP-MS.

About 11 medicinal plant extracts were provided by Phytosun Green Laboratories, which is Pretoria based medicinal plant vendor. The samples were then taken to Pelindaba Analytical Laboratory (PAL) for analysis using ICP-MS. Twenty-six (26) trace element concentrations were determined and the results are shown in Table 28. Out of the 26 elements only half provided meaningful results as 13 elements were below the lower limit of determination.

The results of these samples showed the presence of essential elements that could enhance the healing process as well as the presence of potentially toxic elements, which, however, should not make administration of plants extracts risky as the daily intake through a 10 ml portion is well below the recommended allowed limits. Interestingly, trace elements such as B, Li, Mg, Pb, Si and Sn were able to be determined in medicinal plant extracts by ICP-MS and none of them were obtained using INAA as the latter technique is not suitable for most of them. Accordingly, the two techniques can be regarded to complement each other to provide an overview of a wide variety of elements.

The knowledge of correlation between the healing capacity of a particular medicinal plant and elements present are important in this study. Also with the plant extract some correlations could be found:

- Feverfew and manganese (Mn) have the same medicinal use, which is to treat gynecological disorders and Feverfew appeared to have a relatively high concentration of Mn.
- The essential element calcium (Ca) showed high concentrations in the Yarrow plant extract, and Yarrow and Ca are both important for treatment of bone disorders such as arthritis.
- The linkage between copper (Cu) and Comfrey was also clear because of the wound healing potential of this medicinal plant showing a relatively high Cu concentration.
- Comfrey also contained the highest concentration of potassium (K) used in treating lung disorders.
- Melissa became popular as a calming and soothing herb and magnesium (Mg) is medicinally used to relax muscles, and Melissa contained the highest concentration of Mg.
- The connection between sodium (Na) and Calendula for improved digestion and stomach problems can also be observed since a high concentration of Na was found.
- Looking at the second and third highest concentrations of trace element found in the medicinal plants, a few correlations were also obtained:
    - Aluminium (Al) is high in Yarrow and Feverfew, which are both used for bone formation.
    - Calendula and copper (Cu) can be correlated for wound healing.
    - Iron (Fe), which is found high in Melissa both act against bacteria, viruses and fungi to improve the immune system.
    - Sodium (Na) is also high in Horehound, which both are applied to help the digestive system.

7.2 CONCLUSIONS

Medicinal plants are the oldest known healthcare products and the information on the levels of elements present is important in understanding their pharmacological and toxicological actions in general. INAA with multi-elemental characterization over a wide concentration range and minimal sample preparation demonstrated to be an ideal technique for analyzing dry medicinal plant species. The use of ICP-MS to determine additional elements in medicinal plant extracts added more significance to this study.

The analysis has shown that there is a correlation between the elemental concentration and medicinal plants and their pharmacological effects, which helps in the identification of the plant use in medicine.

Trace elements play an important role in biological activities whereby the deficiency or excess in human beings can lead to a number of disorders. The difference in concentrations of elements between medicinal plants can be attributed to factors such as the preferential uptake of a particular plant for a certain element, which might be influenced by the age of the plant. Weather conditions and mineral composition of the soil in which the plant grows are other factors that may cause the difference. The effectiveness of medicinal plants is not only due to the trace elements but the organic compounds they contain will also have specific healing capacities, which are not dealt with in this study.

7.3 RECCOMENDATIONS FOR FURTHER INVETIGATIONS

- There are about 30,000 medicinal species in South Africa, so there is still a lot of work to be done if one wants to characterize them all.
- It has been demonstrated that INAA with multi-elemental characterization over a wide range of concentration is ideal for this study and therefore would be the preferred technique to continue.
- Because the extract samples contained alcohol, it was not easy to perform the ICP-MS analysis, and accordingly this technique is not recommended for the determination of large numbers of medicinal plant extracts.
- Background analyses of vodka by ICP-MS still are outstanding and must be done as they contribute to the medicinal plant extracts results.

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9 TABLES AND FIGURES

9.1 List of Figures

9.2 List of Tables

Table 24: The results of medicinal plants trace elements in µg/g using INAA.

	Element1			Element2			Element3		
Element	CA			CD			CO		
Nuclide	CA-47			CD-115			CO-60		
Energy	1297.09			527.9			1173.22		
Medicinal plant	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
1. Alfalfa	2.33E+04	7.30E+02	1.85E+03			4.51E+00	9.84E+00	3.00E-01	4.77E-01
2. Sutherlandia	1.82E+04	6.01E+02	1.81E+03			4.53E+00	1.14E+01	3.31E-01	4.60E-01
3. Olive Leaf	1.48E+04	4.58E+02	7.16E+02			1.94E+00	8.51E-01	6.02E-02	1.71E-01
4.Stinging Nettle	7.93E+03	4.47E+02	2.42E+03			6.27E+00	1.08E+01	3.55E-01	6.93E-01
5. Thyme	1.16E+04	4.17E+02	1.40E+03			3.86E+00	7.70E+00	2.30E-01	3.28E-01
6. Ginger Root			1.36E+03			2.24E+00	7.52E-01	6.30E-02	1.86E-01
7. Eye Bright	9.67E+03	3.34E+02	8.34E+02	4.66E+00	5.46E-01	1.70E+00	7.72E-01	5.70E-02	1.64E-01
8. Yarrow	6.44E+03	3.58E+02	1.90E+03			5.17E+00	1.41E+01	3.90E-01	4.17E-01
9. Astragulus	1.69E+03	1.62E+02	9.51E+02			2.50E+00	9.55E-01	7.54E-02	2.24E-01
10. Fennel	9.80E+03	3.86E+02	1.30E+03			3.65E+00	2.00E+00	1.09E-01	2.95E-01
11. Artichoke	1.02E+04	7.60E+02	4.11E+03			1.12E+01	3.27E+01	9.11E-01	1.11E+00
12. Clove	4.51E+03	3.22E+02	1.62E+03			4.45E+00	2.59E+00	1.53E-01	4.34E-01
13. Comfrey Root	4.56E+03	3.22E+02	1.83E+03			4.95E+00	8.73E+00	2.69E-01	4.38E-01
14. Burdock Root	2.94E+03	2.70E+02	1.65E+03			4.51E+00	8.50E+00	2.51E-01	3.46E-01
15. Hydrangea	5.65E+03	2.25E+02	6.13E+02	3.29E+00	2.54E-01	1.29E+00	3.10E-01	3.63E-02	1.09E-01
16. Echinacea Root	6.09E+03	2.74E+02	1.09E+03			3.30E+00	2.94E+00	1.10E-01	2.30E-01
17. Garlic			1.00E+03			2.14E+00	2.38E-01	4.31E-02	1.36E-01
18. Valerian	3.41E+03	2.38E+02	1.25E+03			3.91E+00	3.14E+00	1.28E-01	2.94E-01
19. Wormwood	1.40E+04	4.94E+02	1.57E+03			4.59E+00	7.79E+00	2.30E-01	3.05E-01
20. Liquorice Root	9.80E+03	3.56E+02	9.91E+02			2.87E+00	8.69E-01	8.25E-02	2.52E-01
21. St.John's Wort	8.28E+03	3.57E+02	1.50E+03			4.86E+00	9.84E+00	2.72E-01	3.38E-01
22. Korean Ginseng	8.68E+03	3.08E+02	6.98E+02	1.50E+00	5.60E-01	1.83E+00	4.72E-01	4.66E-02	1.39E-01
23. Goldenrod	1.46E+04	5.08E+02	1.55E+03			4.84E+00	8.21E+00	2.47E-01	3.66E-01
24. Gotu kola	1.95E+04	7.46E+02	2.93E+03			8.48E+00	1.56E+01	4.61E-01	6.98E-01
25. Horsetail	2.11E+04	6.37E+02	9.49E+02			2.99E+00	1.82E+00	8.03E-02	1.87E-01
26. Rosemary	9.95E+03	3.38E+02	7.36E+02			2.41E+00	1.31E+00	6.24E-02	1.51E-01
27. Boswellia			1.78E+03			1.43E+00	1.04E+00	4.18E-02	6.90E-02
28. Bilberry	1.49E+03	1.02E+02	3.90E+02			1.59E+00	2.77E-01	2.56E-02	7.15E-02
29. Passion flower	2.65E+04	7.82E+02	1.38E+03			4.57E+00	6.98E+00	2.08E-01	2.91E-01
30. Peppermint	1.46E+04	5.43E+02	1.89E+03			6.10E+00	1.08E+01	3.15E-01	4.33E-01

	Element4			Element5			Element6		
Element	CR			FE			HG		
Nuclide	CR-51			FE-59			HG-203		
Energy	320.08			1099.22			279.19		
Medicinal plant	**Concentration(μg/g)**	**Uncertainty**	**MDC(μg/g)**	**Concentration(μg/g)**	**Uncertainty**	**MDC(μg/g)**	**Concentration(μg/g)**	**Uncertainty**	**MDC(μg/g)**
1. Alfalfa	1.37E+01	4.54E-01	7.99E-01	7.46E+02	4.48E+01	1.29E+02			5.59E-01
2. Sutherlandia	3.41E+01	9.33E-01	7.98E-01	8.82E+02	4.95E+01	1.40E+02			5.54E-01
3. Olive Leaf	6.51E+00	2.33E-01	3.76E-01	3.89E+02	1.88E+01	4.59E+01			2.53E-01
4.Stinging Nettle	1.21E+01	4.60E-01	1.01E+00	7.20E+03	1.96E+02	1.96E+02	2.91E-02	7.58E-02	5.02E-01
5. Thyme	5.60E+01	1.47E+00	6.74E-01	2.00E+03	6.34E+01	1.10E+02			4.80E-01
6. Ginger Root	3.18E+00	1.77E-01	4.17E-01	1.62E+02	2.16E+01	6.81E+01			2.97E-01
7. Eye Bright	2.86E+00	1.74E-01	4.34E-01	1.85E+02	1.85E+01	5.67E+01			3.17E-01
8. Yarrow	2.48E+01	7.11E-01	8.42E-01	2.05E+03	6.63E+01	1.22E+02			5.92E-01
9. Astragulus	3.54E+00	1.80E-01	4.11E-01	3.16E+02	2.37E+01	7.00E+01			2.37E-01
10. Fennel	1.73E-01	1.73E-01	5.40E-01	1.79E+02	2.97E+01	9.52E+01			4.56E-01
11. Artichoke	1.58E+00	5.21E-01	1.69E+00	3.39E+02	8.15E+01	2.66E+02			1.17E+00
12. Clove	5.89E+00	2.63E-01	5.97E-01	2.06E+02	3.63E+01	1.17E+02			4.70E-01
13. Comfrey Root	7.60E+00	3.21E-01	7.33E-01	3.37E+03	9.92E+01	1.45E+02	2.69E-01	1.52E-01	4.95E-01
14. Burdock Root	8.78E+00	3.25E-01	6.35E-01	8.42E+02	3.96E+01	1.03E+02	1.84E-01	5.65E-02	3.24E-01
15. Hydrangea	4.34E+00	1.82E-01	3.42E-01	4.80E+02	1.86E+01	3.60E+01	1.63E-01	3.60E-02	1.95E-01
16. Echinacea Root	7.55E+00	2.65E-01	4.49E-01	1.01E+03	3.48E+01	6.59E+01			1.97E-01
17. Garlic	1.96E+00	1.53E-01	4.00E-01	1.10E+02	1.38E+01	4.27E+01			2.67E-01
18. Valerian	1.37E+01	4.28E-01	5.96E-01	1.67E+03	5.57E+01	1.03E+02	5.35E-02	1.45E-01	4.75E-01
19. Wormwood	5.07E+01	1.34E+00	7.17E-01	8.25E+02	3.73E+01	9.43E+01			5.05E-01
20. Liquorice Root	2.54E+00	1.22E-01	3.27E-01	4.59E+02	2.71E+01	7.54E+01	1.39E+00	8.90E-02	2.37E-01
21. St.John's Wort	1.13E+01	3.98E-01	7.58E-01	1.10E+03	4.57E+01	1.11E+02			5.35E-01
22. Korean Ginseng	2.42E+00	1.59E-01	3.27E-01	5.69E+02	2.32E+01	5.09E+01			2.56E-01
23. Goldenrod	1.52E+01	4.68E-01	7.58E-01	1.18E+03	4.72E+01	1.10E+02			3.35E-01
24. Gotu kola	3.94E+01	1.09E+00	3.97E-01	6.66E+02	7.16E+01	2.27E+02	6.67E-01	8.62E-02	5.41E-01
25. Horsetail	7.55E+00	2.60E-01	6.67E-01	2.99E+02	2.00E+01	5.66E+01	8.23E-02	7.01E-02	2.22E-01
26. Rosemary	2.03E+01	5.60E-01	3.61E-01	6.34E+02	2.29E+01	4.36E+01			2.58E-01
27. Boswellia	2.20E+02	5.63E+00	2.94E-01	2.24E+03	6.07E+01	2.71E+01	3.90E-02	6.01E-02	1.87E-01
28. Bilberry	9.90E+00	2.97E+01	2.60E-01	3.87E+02	1.48E+01	2.62E+01			1.68E-01
29. Passion flower	1.77E+01	5.15E-01	5.72E-01	1.07E+03	3.96E+01	8.55E+01			4.34E-01
30. Peppermint	7.16E+01	1.87E+00	8.04E-01	6.36E+02	4.16E+01	1.22E+02			5.61E-01

	Element7			Element8			Element9		
Element	MO			NI			SE		
Nuclide	MO-99			NI-58			SE-75		
Energy	140.51			810.76			136		
Medicinal plant	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
1. Alfalfa	1.42E+00	6.83E-02	4.21E-01			1.19E+02	1.47E+00	1.87E-01	1.07E+00
2. Sutherlandia			4.95E-01			1.17E+02	8.81E-01	3.37E-01	1.11E+00
3. Olive Leaf	3.79E-01	3.60E-02	2.73E-01			5.27E+01	9.32E-01	1.15E-01	6.57E-01
4.Stinging Nettle			6.76E-01			1.63E+02			2.10E+00
5. Thyme	5.59E-01	5.88E-02	3.43E-01			9.45E+01			1.53E+00
6. Ginger Root			3.54E-01			5.36E+01			1.09E+00
7. Eye Bright	4.66E-01	4.68E-02	2.66E-01			5.66E+01			1.22E+00
8. Yarrow			5.80E-01			1.27E+02			1.71E+00
9. Astragulus	4.73E+00	1.31E-01	2.34E-01			6.17E+01			1.10E+00
10. Fennel	3.51E-01	6.29E-02	3.66E-01			8.27E+01			1.67E+00
11. Artichoke			9.66E-01			2.66E+02			2.87E+00
12. Clove			4.28E-01			1.06E+02			1.25E+00
13. Comfrey Root			5.68E-01			1.15E+02			1.61E+00
14. Burdock Root			5.79E-01			9.67E+01			1.67E+00
15. Hydrangea			3.70E-01			5.60E+01			9.18E-01
16. Echinacea Root	1.23E+00	6.02E-02	2.88E-01			8.04E+01			1.23E+00
17. Garlic	2.95E-01	4.92E-02	2.83E-01			3.69E+01	3.88E-01	1.29E-01	7.39E-01
18. Valerian	5.21E-01	6.99E-02	4.39E-01			7.93E+01			1.63E+00
19. Wormwood	1.14E+00	8.20E-02	4.49E-01			9.20E+01			1.77E+00
20. Liquorice Root	2.21E+00	7.19E-02	2.47E-01			6.40E+01			9.08E-01
21. St.John's Wort	6.06E-01	8.21E-02	5.18E-01			9.26E+01			1.92E+00
22. Korean Ginseng			4.22E-01			4.99E+01			1.10E+00
23. Goldenrod			5.83E-01			9.37E+01			1.53E+00
24. Gotu kola			8.26E-01			1.70E+02	1.07E+00	4.51E-01	1.48E+00
25. Horsetail	3.70E-01	4.53E-02	3.18E-01			5.92E+01			1.17E+00
26. Rosemary	4.27E-01	4.33E-02	2.75E-01			4.50E+01			9.39E-01
27. Boswellia	3.13E+00	9.18E-02	2.12E-01			2.93E+01			7.76E-01
28. Bilberry	3.89E-01	4.06E-02	2.45E-01			2.68E+01			8.39E-01
29. Passion flower	1.19E+00	7.19E-02	4.80E-01			8.20E+01	1.24E+00	1.61E-01	9.59E-01
30. Peppermint	8.81E-01	8.01E-02	4.81E-01			1.11E+02			1.72E+00

	Element10			Element11			Element12		
Element	AS			K			MN		
Nuclide	AS-76			K-42			MN-56		
Energy	559.1			1524.67			846.75		
Medicinal plant	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
1. Alfalfa			9.19E-01	2.49E+04	2.49E+02	3.62E+02	7.38E+01	4.11E-01	5.69E-01
2. Sutherlandia			9.42E-01	1.30E+04	2.30E+02	5.79E+02	9.12E+01	4.68E-01	5.64E-01
3. Olive Leaf	5.22E-01	5.77E-02	3.12E-01	8.32E+03	1.31E+02	2.15E+02	4.90E+01	3.31E-01	3.82E-01
4.Stinging Nettle			1.12E+00	1.60E+04	2.21E+02	4.55E+02	1.25E+02	6.31E-01	9.09E-01
5. Thyme			8.12E-01	1.60E+04	1.96E+02	3.05E+02	7.61E+01	4.64E-01	5.82E-01
6. Ginger Root			1.12E+00	1.32E+04	2.01E+02	4.27E+02	4.70E+02	1.47E+00	8.50E-01
7. Eye Bright			8.48E-01	1.91E+04	2.23E+02	3.58E+02	2.23E+02	9.04E-01	7.56E-01
8. Yarrow			1.08E+00	3.17E+04	2.96E+02	3.89E+02	1.20E+02	6.62E-01	8.99E-01
9. Astragulus	2.10E-01	4.65E-02	2.95E-01	6.29E+03	1.21E+02	2.15E+02	1.95E+01	2.85E-01	5.53E-01
10. Fennel			7.30E-01	2.43E+04	2.55E+02	3.56E+02	4.22E+01	4.66E-01	9.69E-01
11. Artichoke			1.83E+00	4.27E+04	4.12E+02	7.41E+02	5.33E+01	7.79E-01	2.14E+00
12. Clove			9.77E-01	1.30E+04	2.05E+02	4.34E+02	2.49E+02	1.13E+00	1.33E+00
13. Comfrey Root			8.97E-01	2.66E+04	2.86E+02	4.48E+02	7.66E+01	6.90E-01	1.35E+00
14. Burdock Root			8.48E-01	2.38E+04	2.53E+02	3.34E+02	2.63E+01	4.62E-01	1.11E+00
15. Hydrangea			3.73E-01	3.66E+03	9.12E+01	1.52E+02	5.89E+01	6.30E-01	1.24E+00
16. Echinacea Root			7.92E-01	1.21E+04	1.54E+02	2.69E+02	5.63E+01	2.26E-01	1.91E-01
17. Garlic			4.16E-01	9.51E+03	1.25E+02	1.78E+02	6.87E+00	8.02E-02	1.48E-01
18. Valerian			8.28E-01	1.47E+04	1.79E+02	3.20E+02	5.88E+01	2.52E-01	2.64E-01
19. Wormwood	3.86E-01	9.89E-02	6.37E-01	3.34E+04	2.93E+02	4.01E+02	7.60E+01	3.10E-01	3.35E-01
20. Liquorice Root	2.70E-01	5.74E-02	3.43E-01	7.33E+03	1.19E+02	2.15E+02	1.87E+01	1.46E-01	2.28E-01
21. St.John's Wort			9.63E-01	1.18E+04	1.62E+02	3.02E+02	7.18E+01	3.09E-01	2.99E-01
22. Korean Ginseng			7.28E-01	1.03E+04	1.50E+02	2.81E+02	7.60E+01	3.26E-01	2.91E-01
23. Goldenrod			1.11E+00	1.46E+04	1.89E+02	3.56E+02	1.37E+02	4.95E-01	4.02E-01
24. Gotu kola			1.77E+00	3.37E+04	3.42E+02	6.60E+02	2.15E+02	7.32E-01	8.39E-01
25. Horsetail			8.57E-01	2.84E+04	2.65E+02	3.62E+02	8.97E+01	3.99E-01	4.27E-01
26. Rosemary			5.84E-01	1.59E+04	1.83E+02	2.57E+02	2.82E+01	2.22E-01	3.36E-01
27. Boswellia	4.08E-01	3.79E-02	1.67E-01			1.04E+02	1.42E+01	1.44E-01	1.24E-01
28. Bilberry			7.68E-01	5.71E+03	1.25E+02	2.82E+02	1.59E+02	6.03E-01	3.94E-01
29. Passion flower			8.88E-01	1.53E+04	1.87E+02	2.90E+02	6.00E+01	3.64E-01	4.83E-01
30. Peppermint			1.13E+00	3.30E+04	3.48E+02	7.12E+02	5.60E+01	3.94E-01	7.23E-01

	Element13			Element14			Element15		
Element	TH			U			ZN		
Nuclide	PA-233			NP-239			ZN-65		
Energy	312.17			277.6			1115.55		
Medicinal plant	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
1. Alfalfa	1.67E-01	2.01E-02	6.39E-02			1.70E-01	4.32E+01	1.71E+00	6.73E+00
2. Sutherlandia	1.25E-01	1.89E-02	6.08E-02			1.71E-01	3.66E+01	1.54E+00	6.43E+00
3. Olive Leaf	1.32E-01	1.07E-02	3.20E-02			8.21E-02	2.08E+01	8.23E-01	2.56E+00
4.Stinging Nettle	1.96E-01	2.29E-02	7.28E-02			1.56E-01	2.33E+01	1.75E+00	9.51E+00
5. Thyme	1.76E-01	1.66E-02	5.17E-02			1.53E-01	7.71E+01	2.30E+00	5.14E+00
6. Ginger Root	9.31E-02	1.09E-02	3.42E-02			9.89E-02	1.91E+00	8.48E-01	3.22E+00
7. Eye Bright	1.58E-01	1.21E-02	3.61E-02			1.05E-01	6.00E+01	1.77E+00	2.72E+00
8. Yarrow	3.84E-01	2.57E-02	7.66E-02			1.95E-01	3.40E+01	1.45E+00	6.17E+00
9. Astragulus	1.14E-01	1.14E-02	3.54E-02			8.25E-02	1.60E+01	8.06E-01	3.62E+00
10. Fennel	1.43E-01	1.80E-02	5.72E-02			1.53E-01	3.70E+01	1.42E+00	5.04E+00
11. Artichoke	2.21E-01	4.18E-02	1.36E-01			3.94E-01	1.86E+01	2.58E+00	1.60E+01
12. Clove	1.40E-01	1.70E-02	5.37E-02			1.62E-01	1.37E+01	1.26E+00	7.50E+00
13. Comfrey Root	1.55E-01	1.90E-02	6.04E-02			1.70E-01	5.29E+01	1.91E+00	7.60E+00
14. Burdock Root	6.57E-02	1.38E-02	4.46E-02			1.16E-01	1.54E+01	1.01E+00	5.70E+00
15. Hydrangea	1.56E-01	8.93E-03	2.41E-02			7.44E-02	2.98E+01	1.00E+00	1.93E+00
16. Echinacea Root	3.55E-01	1.61E-02	4.11E-02			7.52E-02	1.30E+01	7.67E-01	3.52E+00
17. Garlic			3.87E-02			9.98E-02	1.38E+01	9.49E-01	2.62E+00
18. Valerian	1.04E-01	1.47E-02	4.69E-02			1.74E-02	7.06E+01	2.16E+00	4.83E+00
19. Wormwood	1.01E-01	1.86E-02	6.01E-02			1.88E-01	7.34E+01	2.22E+00	4.94E+00
20. Liquorice Root	1.37E-01	6.02E-03	2.65E-02			9.29E-02	1.42E+01	8.03E-01	3.94E+00
21. St.John's Wort	3.45E-02	1.67E-02	5.49E-02			2.03E-01	4.62E+01	1.58E+00	4.99E+00
22. Korean Ginseng	1.50E-01	1.13E-02	3.34E-02			1.01E-01	3.79E+01	1.23E+00	2.54E+00
23. Goldenrod	6.61E-02	1.47E-02	4.77E-02			1.31E-01	3.96E+01	1.45E+00	5.12E+00
24. Gotu kola	1.22E-01	2.76E+02	9.00E-02			2.10E-01	1.26E+02	3.85E+00	1.06E+01
25. Horsetail	9.77E-02	1.25E-02	3.97E-02			9.24E-02	2.98E+01	1.06E+00	2.90E+00
26. Rosemary	7.89E-02	9.94E-03	3.13E-02			1.06E-01	4.02E+01	1.25E+00	2.25E+00
27. Boswellia	1.59E-02	5.81E-03	1.89E-02			8.18E-02	4.23E+00	3.21E-01	1.25E+00
28. Bilberry	4.10E-02	5.92E-03	1.86E-02			7.39E-02	1.05E+01	4.74E-01	1.27E+00
29. Passion flower	3.23E-02	9.65E-03	3.14E-02			1.81E-01	1.79E+02	4.78E+00	4.25E+00
30. Peppermint	6.02E-02	1.71E-02	5.57E-02			2.35E-01	3.08E+01	1.38E+00	6.33E+00

	Element16			Element17		
Element	NA			Al		
Nuclide	NA-24			Al-28		
Energy	1368.53			1778.8		
Medicinal plant	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
1. Alfalfa	8.22E+02	7.58E+00	1.22E+01			5.79E+01
2. Sutherlandia	8.80E+02	7.72E+00	1.12E+01	7.92E+02	8.93E+01	8.79E+00
3. Olive Leaf	1.69E+02	3.40E+00	6.96E+00	8.87E+02	1.00E+02	8.01E+00
4.Stinging Nettle	3.95E+03	2.13E+00	1.55E+01	3.85E+03	4.32E+02	1.81E+01
5. Thyme	2.30E+02	4.37E+00	9.73E+00	2.02E+03	2.27E+02	1.25E+01
6. Ginger Root	3.06E+02	5.55E+00	1.34E+01	4.90E+02	5.55E+01	7.02E+00
7. Eye Bright	1.22E+02	4.02E+00	1.09E+01	8.35E+02	9.43E+01	8.38E+00
8. Yarrow	1.86E+02	5.08E+00	1.38E+01	4.22E+03	4.73E+02	1.95E+01
9. Astragulus	7.49E+02	6.58E+00	7.35E+00	1.22E+03	1.38E+02	9.62E+00
10. Fennel	1.24E+03	9.76E+00	1.23E+01	4.51E+02	5.11E+01	6.24E+00
11. Artichoke	1.01E+04	4.72E+01	2.49E+01	8.73E+02	9.85E+01	1.04E+01
12. Clove	2.90E+03	1.70E+01	1.40E+01	2.75E+02	3.14E+01	5.99E+00
13. Comfrey Root	2.66E+03	1.63E+01	1.49E+03	1.84E+03	2.06E+02	1.15E+01
14. Burdock Root	4.83E+02	6.18E+00	1.16E+01	6.34E+02	7.17E+01	8.58E+00
15. Hydrangea	9.62E+01	2.68E+00	5.33E+00	1.44E+03	1.62E+02	9.78E+00
16. Echinacea Root	2.53E+02	4.14E+00	9.19E+00	2.50E+03	2.81E+02	1.45E+01
17. Garlic	3.90E+02	4.38E+00	6.36E+00	1.85E+02	2.13E+01	4.54E+00
18. Valerian	1.28E+03	9.11E+00	1.08E+01	1.72E+03	1.94E+02	1.29E+01
19. Wormwood	2.35E+02	4.77E+00	1.21E+01	1.21E+03	1.37E+02	1.06E+01
20. Liquorice Root	1.16E+03	8.34E+00	7.97E+00	1.19E+03	1.34E+02	8.25E+00
21. St.John's Wort	4.10E+02	5.20E+00	1.01E+01	1.14E+03	1.29E+02	1.15E+01
22. Korean Ginseng	4.71E+02	5.41E+00	9.48E+00	1.33E+03	1.50E+02	1.01E+01
23. Golden Rod	5.28E+02	6.14E+00	1.19E+01	2.91E+03	3.27E+02	1.57E+01
24. Gotu kola	8.55E+03	4.03E+01	2.35E+01	8.58E+02	9.68E+01	1.22E+01
25. Horsetail	1.49E+02	4.43E+00	1.22E+01	2.54E+03	2.85E+02	1.60E+01
26. Rosemary	1.92E+02	3.85E+00	8.62E+00	7.32E+02	8.26E+01	6.70E+00
27. Boswellia	4.01E+01	1.43E+00	2.44E+00	4.79E+04	5.37E+03	1.03E+02
28. Bilberry	5.48E+01	3.01E+00	8.71E+00	5.04E+02	5.71E+01	5.64E+00
29. Passion flower	4.72E+02	5.67E+00	1.01E+01	7.12E+02	8.06E+01	8.17E+00
30. Peppermint	1.66E+03	1.15E+01	1.45E+01	9.57E+02	1.08E+02	1.09E+01

	Element18			Element19		
Element	Cu			V		
Nuclide	Cu-66			V-52		
Energy	1039.2			1434.06		
Medicinal plant	Concentration(µg/g)	Uncertainty	MDC(µg/g)	Concentration(µg/g)	Uncertainty	MDC(µg/g)
1. Alfalfa			1.26E+02	7.25E+00	7.73E-01	1.95E+00
2. Sutherlandia			1.04E+02	7.17E+00	6.94E-01	1.59E+00
3. Olive Leaf	1.07E+02	4.26E+01	6.38E+01	1.17E+00	3.70E-01	1.16E+00
4.Stinging Nettle			2.15E+02	2.22E+01	1.70E+00	3.31E+00
5. Thyme			1.51E+02	7.40E+00	7.67E-01	1.86E+00
6. Ginger Root			8.44E+01			1.52E+00
7. Eye Bright			1.05E+02			1.82E+00
8. Yarrow			2.24E+02	6.82E+00	1.19E+00	3.60E+00
9. Astragulus			1.15E+02	1.28E+00	3.93E-01	1.23E+00
10. Fennel			8.65E+01	6.27E-01	2.73E-01	8.76E-01
11. Artichoke			1.31E+02			2.09E+00
12. Clove			8.37E+01	9.42E-01	3.20E-01	1.01E+00
13. Comfrey Root			1.54E+02	1.68E+01	1.29E+00	2.43E+00
14. Burdock Root	4.00E+01	2.69E+01	7.58E+01	1.87E+00	3.85E-01	1.13E+00
15. Hydrangea			1.19E+02	1.25E+00	4.55E-01	1.45E+00
16. Echinacea Root			1.58E+02	2.93E+00	6.62E-01	2.04E+00
17. Garlic			5.11E+01			8.90E-01
18. Valerian			1.38E+02	6.40E+00	6.94E-01	1.73E+00
19. Wormwood			1.28E+02	4.07E+00	5.60E-01	1.52E+00
20. Liquorice Root			1.14E+02	1.33E+00	4.39E-01	1.39E+00
21. St.John's Wort			1.24E+02	4.69E+00	6.62E-01	1.85E+00
22. Korean Ginseng			1.21E+02			2.27E+00
23. Golden Rod			1.84E+02	1.25E+01	1.06E+00	2.25E+00
24. Gotu kola			1.45E+02	4.97E+00	7.07E-01	1.99E+00
25. Horsetail				3.39E+00	4.47E-01	2.97E+00
26. Rosemary			9.67E+01	3.39E+00	4.47E-01	1.16E+00
27. Boswellia			9.80E+02			1.78E+01
28. Bilberry			7.94E+01	1.10E+00	2.81E-01	8.42E-01
29. Passion flower			1.06E+02	6.78E+00	6.43E-01	1.40E+00
30. Peppermint			1.21E+02	3.83E+00	5.77E-01	1.63E+00

Table 25: Estimated daily dosage intake of trace elements.

	Estimated daily dosage	Ca	Co	Cr	Fe	Mo	Ni	Se	K	Mn	Th	U	Zn	Na	Cu	V
	g/day	mg/day	ug/day	ug/day	mg/day	ug/day	mg/day	ug/day	mg/day	mg/day	ug/day	ug/day	mg/day	mg/day	ug/day	mg/day
Medicinal plant																
1. Alfalfa	22.5	524.25	221.40	308.25	16.79	31.95	2.68	24.08	560.25	1.66	3.76	3.83	0.97	18.50	2835.00	0.16
2. Sutherlandia	1.5	27.30	17.10	51.15	1.32	0.00	0.18	1.67	19.50	0.14	0.19	0.26	0.05	1.32	156.00	0.01
3. Olive Leaf	1.5	22.20	1.28	9.77	0.58	0.57	0.08	0.99	12.48	0.07	0.20	0.12	0.03	0.25	95.70	0.00
4.Stinging Nettle	10	79.30	108.00	121.00	72.00	0.00	1.63	21.00	160.00	1.25	1.96	1.56	0.23	39.50	2150.00	0.22
5. Thyme	5	58.00	38.50	280.00	10.00	2.80	0.47	7.65	80.00	0.38	0.88	0.77	0.39	1.15	755.00	0.04
6. Ginger Root	1.5	0.00	1.13	4.77	0.24	0.00	0.08	1.64	19.80	0.71	0.14	0.15	0.00	0.46	126.60	0.00
7. Eye Bright	6	58.02	4.63	17.16	1.11	2.80	0.34	7.32	114.60	1.34	0.95	0.63	0.36	0.73	630.00	0.00
8. Yarrow	4.5	28.98	63.45	111.60	9.23	0.00	0.57	7.70	142.65	0.54	1.73	0.88	0.15	0.84	1008.00	0.03
9. Astragulus	20	33.80	19.10	70.80	6.32	94.60	1.23	22.00	125.80	0.39	2.28	1.65	0.32	14.98	2300.00	0.03
10. Fennel	1.45	14.21	2.90	0.25	0.26	0.51	0.12	2.42	35.24	0.06	0.21	0.22	0.05	1.80	125.43	0.00
11. Artichoke	6	61.20	196.20	9.48	2.03	0.00	1.60	17.22	256.20	0.32	1.33	2.36	0.11	60.60	786.00	0.00
12. Clove	4.5	20.30	11.66	26.51	0.93	0.00	0.48	5.63	58.50	1.12	0.63	0.73	0.06	13.05	376.65	0.00
13. Comfrey Root	7.5	34.20	65.48	57.00	25.28	0.00	0.86	12.08	199.50	0.57	1.16	1.28	0.40	19.95	1155.00	0.13
14. Burdock Root	0	21.02	60.76	62.76	6.02	62.76	6.02	62.76	6.02	6.02	62.76	62.76	6.02	6.02	62.76	6.02
15. Hydrangea	0	40.39	2.22	31.02	3.43	31.02	3.43	31.02	3.43	3.43	31.02	31.02	3.43	3.43	31.02	3.43
16. Echinacea Root	4.25	25.88	12.50	32.09	4.29	5.23	0.34	5.23	51.43	0.24	1.51	0.32	0.06	1.08	671.50	0.01
17. Garlic	3	0.00	0.71	5.88	0.33	0.89	0.11	2.22	28.53	0.02	0.00	0.30	0.04	1.17	153.30	0.00
18. Valerian	6	20.46	18.84	82.20	10.02	3.13	0.48	9.78	88.20	0.35	0.62	0.10	0.42	7.68	828.00	0.04
19. Wormwood	3	42.00	23.37	152.10	2.48	3.42	0.28	5.31	100.20	0.23	0.30	0.56	0.22	0.71	384.00	0.01
20. Liquorice Root	5	49.00	4.35	12.70	2.30	11.05	0.32	4.54	36.65	0.09	0.69	0.46	0.07	5.80	570.00	0.01
21. St.John's Wort	3.5	28.98	34.44	39.55	3.85	2.12	0.32	6.72	41.30	0.25	0.12	0.71	0.16	1.44	434.00	0.02
22. Korean Ginseng	3	26.04	1.42	7.26	1.71	0.00	0.15	3.30	30.90	0.23	0.45	0.30	0.11	1.41	363.00	0.00
23. Goldenrod	9	131.40	73.89	136.80	10.62	0.00	0.84	13.77	131.40	1.23	0.59	1.18	0.36	4.75	1656.00	0.11
24. Gotu kola	1.8	35.10	28.08	70.92	1.20	0.00	0.31	2.66	60.66	0.39	0.22	0.38	0.23	15.39	261.00	0.01
25. Horsetail	3	63.30	5.46	22.65	0.90	1.11	0.18	3.51	85.20	0.27	0.29	0.28	0.09	0.45	0.00	0.01
26. Rosemary	6	59.70	7.86	121.80	3.80	2.56	0.27	5.63	95.40	0.17	0.47	0.64	0.24	1.15	580.20	0.02
27. Boswellia	0	0.00	7.43	1572.59	16.01	1572.59	16.01	1572.59	16.01	16.01	1572.59	1572.59	16.01	16.01	1572.59	16.01
28. Bilberry	40	59.60	11.08	396.00	15.48	15.56	1.07	33.56	228.40	6.36	1.64	2.96	0.42	2.19	3176.00	0.04
29. Passion flower	6	159.00	41.88	106.20	6.42	7.14	0.49	5.75	91.80	0.36	0.19	1.09	1.07	2.83	636.00	0.04
30. Peppermint	7.5	109.50	81.00	537.00	4.77	6.61	0.83	12.90	247.50	0.42	0.45	1.76	0.23	12.45	907.50	0.03

Table 26: Therapeutic medicinal plants categories containing relatively rich trace elements.

Medicinal plant		Relatively rich in							Traditional application of the plant			
1. Alfalfa	Ca	K	Se						General tonic	Lowering cholesterol levels	Disorders of bones and joints	
2. Sutherlandia	Ca	Se							Adaptogenic tonic			
3. Olive Leaf	Ca	Se	As						Lower blood pressure	Renal function		
4.Stinging Nettle	Fe	Th							Antirheumatic	Urological problems		
5. Thyme	Cr								Expectorant	Antibiotic	Spasmolytic	
6. Ginger Root	Mn								Colds and influenza	Stomachic	Reduces muscular spasms and tension	
7. Eye Bright	Cd	Mn							Eye complaints	Anti-inflammatory		
8. Yarrow	Co	K	Th						Anti-inflammatory	Diuretic	Anti-arthritic	Fever
9. Astragulus	As	Mo							Adaptogenic tonic	Immune system stimulant		
10. Fennel	K								Carminative	Expectorant	Aromatic	
11. Artichoke	K	Co	Th	Na					Choleretic	Lipid lowering	Liver protectant	
12. Clove	Mn								Carminative	Stomachic	Anaesthetic	
13. Comfrey Root	Fe	K							Wound healing	Anti-inflammatory	Lung disorders	Stomach ulcers
14. Burdock Root	K								Diuretic	Skin disorders		
15. Hydrangea	Cd								Diuretic			
16. Echinacea Root	Th								Immune system stimulant	Skin disorders	Colds and infections	
17. Garlic									Lipid lowering (cholesterol)	Antiviral	Antibacterial	
18. Valerian									Sedative	Restlessness and sleeplessness		
19. Wormwood	Ca	As	K	Cr					Bitter tonic (appetite and digestion)	Indigestion	Bile tract disorders	
20. Liquorice Root	As	Mo	Hg						Expectorant	Anti-inflammatory	Reduces muscular spasms and tension	
21. St.John's Wort									Wound healing	Antidepressant	Inflammation of stomach and intestines	
22. Korean Ginseng									Adaptogenic tonic			
23. Goldenrod	Ca								Diuretic	Skin disorders	Cough medicine	Anti-rheumatic
24. Gotu kola	Ca	Co	Hg	Se	K	Mn	Zn	Na	Multifunctional general tonic	Wound healing	Venous insufficiency	
25. Horsetail	Ca	K							Diuretic	Skin disorders	Stops bleeding	
26. Rosemary									General tonic	Reduces muscular spasms and tension	Antimicrobial	Carminative
27. Boswellia	Cr	Mo	As	K					Stops infection	Expectorant	Sedative	
28. Bilberry									Urinary tract infections			
29. Passion flower	Ca	Se	Zn						Sedative	Restlessness and sleeplessness	Nervous gastrointestinal disorders	
30. Peppermint	Ca	K	Cr						Choleretic	Carminative	Antibacterial	Reduces muscular spasms and tension

Table 27: Element healing capacity.

Al	Bone formation
As	Proper growth, development and reproduction.
Ca	Formation and maintenance of bones, muscular growth and normal blood clotting
Cd	Maximize growth
Co	Myelin formation to support the manufacture of red blood cells, metabolism of fats, carbohydrates and synthesis of proteins.
Cr	Anti-inflammatory properties, normal blood sugar and fat metabolism
Cu	Wound healing, formation of oxygen-carrying molecule hemoglobin, formation red blood cells and bones.
Fe	Production of hemoglobin and myoglobin, energy production, oxygenation of red blood cells and healthy immune system.
Hg	Nervous system, renal system functioning and tooth filling amalgams.
K	Normal blood pressure, building muscles, transmission of nerve impulses and heart activity
Mn	Protein metabolism, bone formation, carbohydrate (glucose) metabolism, blood clotting, activates enzyme responsible for formation of urea, breast milk in females, DNA and RNA synthesis.
Mo	Normal uric acid levels, anti-cancer properties anti-oxidative properties and protect cells from free radicals.
Na	Acts as an electrolyte, required in the manufacture of hydrochloric acid in the stomach and for normal kidney functioning.
Se	Anti-aging properties, prevent cancer, stimulates anti-body response to infections, fight cold sores and shingles and reduce excessive mercury levels.
Th	No data available
V	Stabilize blood sugar levels, formation of bones and teeth.
Zn	Immune system, synthesis of the nucleic acids RNA and DNA, protect cells from free radicals, normal growth and reproductive development.

Table 28: The results of medicinal plants trace elements in µg/ℓ using ICP-MS.

Element	1. African potato	2. Calendula	3. Comfrey	4. Feverfew	5. Horehound	6. Melissa	7. Milk Thistle	8. Nettle Root	9. Rosemary	10. Thyme	11. Yarrow
Al	0.2	0.2	<1.50E-01	0.3	<1.50E-01	<1.50E-01	0.2	<1.50E-01	0.6	<1.50E-01	0.5
As	0.2	<1.50E-01	<1.50E-01	0.3	<1.50E-01	0.2	<1.50E-01	<1.50E-01	<1.50E-01	0.2	0.2
B	0.3	1.3	0.5	1.2	0.9	0.8	0.4	0.4	0.4	1	1.1
Cu	0.3	0.6	1.6	0.3	0.4	0.2	1.1	0.2	0.2	0.2	0.3
Sn	0.3	0.2	<1.50E-01	0.3	0.2	0.3	0.1	<1.50E-01	0.2	0.3	0.2
Mn	0.4	0.7	0.15	4	0.8	1	0.15	0.5	0.6	0.6	2
Fe	0.8	0.9	0.4	3.1	0.5	<1.50E+00	0.3	0.2	1.8	0.5	2
Si	3.8	8.3	15.2	19.8	9.6	21.9	10.2	17.4	8.9	15.2	17.4
Ca	22	86.3	11.7	123	19.9	0.8	22.2	47.9	2.3	128	154
P	141	294	68	854	372	162	163	122	11	98	194
Mg	176	185	104	353	362	987	79	204	243	529	387
Na	193	340	201	17	321	288	12	286	108	89	156
K	836	1650	4020	3850	2450	1540	356	928	1030	1370	1750
Hg	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00
Li	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00
U	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00
Zn	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00	<1.50E+00
Mo	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01
Cr	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01
Se	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01
Ni	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01
Cd	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01
Th	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01
V	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01
Pb	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01	<1.50E-01
Co	<7.50E-01	<7.50E-01	<7.50E-01	<7.50E-01	<7.50E-01	<7.50E-01	<7.50E-01	<7.50E-01	<7.50E-01	<7.50E-01	<7.50E-01

APPENDIX I: Some of the medicinal plants commonly used in South Africa [2,3].

Image of a medicinal plant.	Scientific name (Family), Common name, Trace elements present and Main medicinal uses.
	***Achillea millefolium*** *(Asteraceae)*, Yarrow **Medicinal uses:** *Antispasmodic*, *astringent*, bitter tonic, increases sweating, lowers blood pressure, reduces fever, *anti-inflammatory*, *diuretic* and anti-arthritic. **Trace elements:** No data available. **Dosage:** 4.5g yarrow herb, 3g yarrow flowers, 3 teaspoons of fresh juice and for external use 100g of herb in 20 liters of water.
	***Allium sativum*** *(Alliaceae)*, Garlic **Medicinal uses:** Lipid lowering, *antibiotic*, *expectorant*, *diaphoretic*, *antispasmodic*, common cold and lowering of blood pressure. **Trace elements:** Ca, Fe, K, Mg, Mn, P, Se and Zn. **Dosage:** 2-4g of fresh cloves of garlic per day.
	***Artemisia absinthium*** *(Asteraceae)*, Wormwood **Medicinal uses:** Indigestion, *carminative*, muscle relaxer, *choleretic*, anti-inflammatory, eases stomach pains, mild *antidepressant*, *antiseptic* and bitter tonic. **Trace elements:** No data available. **Dosage:** 3g of dry herb per day.
	***Arctium lappa*** *(Asteraceae)*, Burdock **Medicinal uses:** Diuretic and treatment of skin disorders. **Trace elements:** Ca, Cr, Cu, Fe, K, Mg, Mn, P, Se, Si and Zn. **Dosage:** No data available.

	***Astragalus membranaceus*** *(Fabaceae)*, Astragalus **Medicinal uses:** Traditional tonic and immune stimulant. **Trace elements:** Ca, Cu, Fe, K, Mg, Mn and Zn. **Dosage:** 10-30g of dried root per day, 4-8ml of 1:2 liquid extract per day.
	***Boswellia sacra*** (Burseraceae), Boswellia **Medicinal uses:** *Antiseptic, expectorant* and *sedative*. **Trace elements:** No data available. **Dosage:** No data available.
	***Calendula officinalis*** (Asteraceae), Calendula **Medicinal uses:** Anti-inflammatory, antispasmodic. **Trace elements:** Ca. **Dosage:** About 1-2g of dried flowers in a cup of boiling water 2 or 3 times per day.
	***Centella asiatica*** *(Apiaceae), Gotu kola* **Medicinal uses:** Wound healing, venous insufficiency and general tonic. **Trace elements:** Ca, Fe, K, Mg, Mn, P, Se and Zn. **Dosage:** 0.6g dry herb taken 3 times a day.

Chrysanthemum parthenium *(Asteraceae),* Feverfew
Medicinal uses: Migraine *prophylactic*, anti-inflammatory, fever, *rheumatic,* skin conditions and gynaecological disorders.
Trace elements: Ca, Fe, K, Mg, Mn, P, Se and Zn.
Dosage: 0.7-2ml of 1:1fresh plant tincture per day, 1-2ml of 1:5 dried plant tincture per day.

Cynara scolymus (Asteraceae), Artichoke
Medicinal uses: Choleretic, liver-protectant and *lipid* lowering
Trace elements: No data available.
Dosage: A daily dose of 6g of the drug is recommended.

Echinacea purpurea *(Asteraceae),* Echinacea
Medicinal uses: Immunostimulant, skin *inflammation*, wounds, ulcers, colds and infections.
Trace elements: Ca, Fe, K, Mg, Mn, P, Se and Zn
Dosage: 1.5-4.5g of dried root per day, 2.5-6g dried aerial parts per day, 3-9ml of 1:2 liquid extract per day,
3-5.5ml of 1:1 fresh plant tincture per day.

Equisetum arvense *(Equisetaceae),* Horsetail
Medicinal uses: Diuretic, slow wound healing and skin disorders.
Trace elements: Ca, Fe, K, Mg, Mn, P, Se, Si and Zn.
Dosage: A daily dose for internal use is 2-4g of the dried herb. External use of 10g per liter of water is recommended.

Eugenia caryophyllus *(Myrtaceae)*, Cloves
Medicinal uses: *Anaesthetic*, *antiseptic, analgesic*, *antibacterial*, carminative, mind and body stimulant.
Trace elements: Ca, Fe, K, Mg, Mn, P and Zn.
Dosage: No data available.

Euphrasia officinalis *(Scrophulariaceae)*, Eyebright
Medicinal uses: Anti-inflammatory and eye complaints such as *conjunctivitis*.
Trace elements: Ca, Cr, Fe, K, Mg, Mn, P, Se and Zn.
Dosage: 2-4g of dried herb 3 times a day, 2-4ml of 1:2 liquid extract 3 times a day, 2-6ml of1:5 tincture 3 times a day.

Foeniculum vulgare *(Apiaceae)*, Fennel
Medicinal uses: Expectorant, digestion, antispasmodic, anti-inflammatory, aromatic, eyewash and carminative.
Trace elements: Ca, Fe, K, Mg, Mn, P, Se and Zn.
Dosage: 900-1800mg of dried fruit per day, 3-6ml of 1:2 liquid extract per day, 7-14ml of 1:5 tincture per day.

Glycyrrhiza glabra *(Fabaceae)*, Liquorice
Medicinal uses: Expectorant, anti-inflammatory and antispasmodic.
Trace elements: Ca, Fe, K, Mg, Mn, P and Zn.
Dosage: 2-6ml of 1:2 liquid extract per day.

	***Hydrangea arborescens*** (Hydrangaceae) Hydrangea **Medicinal uses:** Diuretic. **Trace elements:** No data available. **Dosage:** No data available.
	***Hypericum perforatum*** *(Hypericaceae)*, St. John's wort **Medicinal uses:** *Antidepressant*, antispasmodic, astringent, sedative, relieves pain, *antiviral* and wound healing. **Dosage:** 2-5g of dried herb per day, 3-6ml of 1:2 liquid extract per day, 7.5-15ml of 1:5 tincture per day.
	***Hypoxis hemerocallidea*** *(Hypoxidaceae)*, African potato **Medicinal uses:** Tonic, prostate problems, improvement of the immune system for Human Immune virus (HIV) and cancer. **Trace elements:** No data available. **Dosage:** A daily dose of 2-4g of dry root.
	***Marrubium vulgare*** *(Lamiaceae)*, White horehound **Medicinal uses:** Choleretic, digestive complaints, dry cough and expectorant. **Trace elements:** Fe and K. **Dosage:** A daily dose of 4.5g of dried herb is recommended.

	***Medicago savita*** (*Fabaceae*), Alfalfa **Medicinal uses:** Tonic and cholesterol lowering. **Trace elements:** Ca, Cu, Fe, K, Mg, Mn, P, Si and Zn. **Dosage:** 5-10g of the dried herb is taken 3 times a day.
	***Melissa officinalis*** *(Lamiaceae), Melissa* **Medicinal uses:** *Sedative*, increases sweating, nerve tonic, carminative, *spasmolytic* and antiviral. **Trace elements:** No data available. **Dosage:** 1.5-4.5g of dried leaf may be taken several times a day.
	***Mentha x piperita*** *(Lamiaceae), P*eppermint **Medicinal uses:** Digestive disorders, choleretic, *antiseptic*, increases sweating, carminative, spasmolytic and *cholagogue.* **Trace elements:** Ca, Cu, Fe, K, Mg, Mn, P, Se and Zn. **Dosage:** 6-9g of dried leaf per day, 1.5-4ml of 1:2 liquid extract per day, 3.5-11ml of 1:5 tincture per day.
	***Olea europaea*** *(Oleaceae),* Olive tree **Medicinal uses:** *Anti-hypertensive*, anti-inflammatory, diuretic, cholagogue, mild laxative, *demulcent* and *emollient.* **Trace elements:** Ca **Dosage:** A daily dose of about 1-2g of olive leaf. For internal use, 15-30ml of oil is taken with meals.

	***Panax ginseng*** *(Araliaceae)*, Korean ginseng **Medicinal uses:** *Adaptogenic tonic.* **Trace elements:** No data available. **Dosage:** A daily dose of 1-2g (up to 3g) of dried root.
	***Passiflora incarnata*** *(Passifloraceae)*, Passion flower **Medicinal uses:** Mild sedative to treat nervousness, restlessness, sleeplessness, and nervous gastrointestinal disorders, anti-inflammatory as well as antispasmodic. **Trace elements:** Ca **Dosage:** Tea made with 2g of finely chopped herb taken 3 times a day. Maximum daily dose is 4-8g of herb.
	***Rosmarinus officinalis*** *(Lamiacaeae)*, Rosemary **Medicinal uses:** General tonic, *antimicrobial*, spasmolytic, astringent, anti-inflammatory, carminative and *stomachic.* **Trace elements:** Ca, Cr, Fe, K, Mg, Mn, P, Se, Si and Zn. **Dosage:** 2g of dry herb is taken 3 times a day. For internal use, a maximum of 20 drops of oil (1ml) pr day.
	***Silybum marianum*** *(Asteraceae)*, Milk-thistle **Medicinal uses:** Digestive tonic and *hepatoprotective* to toxic liver damage. **Trace elements:** No data available. **Dosage:** A daily dose of about 12-15g of dry fruits is recommended while extract containing 200-400mg of silymarin is considered an effective dose.

Solidago virgaurea *(Asteraceae)*, Goldenrod
Medicinal uses: Diuretic, cough medicines, *anti-rheumatic*, sores and slow healing wounds.
Trace elements: No data available.
Dosage: Tea made from 2-3g of the herb steeped in a cup of boiling water taken 3-5 times a day. The recommended daily dose is 6-12g.

Sutherlandia frutescens *(Fabaceae)*
Sutherlandia
Medicinal uses: Adaptogenic tonic, reducing the feelings of stress, increasing energy and stimulation the immune system.
Trace elements: No data available.
Dosage: A daily dose of about 1-2g of the dry herb is taken as tea or decoction. One or two tablets containing 300mg of the dry herb 3 times per day.

Symphytum officinale *(Boraginaceae)*, Comfrey
Medicinal uses: Wound healing, anti-inflammatory, lung disorders, stomach ulcers and bleeding.
Trace elements: Ca, Fe, K, Mg, Mn, P, Se and Zn.
Dosage: A decoction of 1 part dry roots to 10 parts of water is a suitable alternative. A daily dose of 5-10g of dry roots taken in the form of tea.

Thymus vulgaris *(Lamiaceae)*, Thyme
Medicinal uses: Expectorant, spasmolytic, antiseptic, tonic and *antibiotic*.
Trace elements: Ca, Fe, K, Mg, Mn, P, Se and Zn.
Dosage: 2-6ml of 1:2 liquid extract per day, 5-15ml of 1:5 tincture per day.

Urtica dioica *(Urticaceae),*
Stinging nettle
Medicinal uses: Anti-rheumatic, inflammation of urinary tract, tonic, astringent, *anti-allergic*, reduces prostate enlargement and skin conditions.
Trace elements: Ca, Cu, Fe, K, Mg, Mn, P, S, Se and Zn.
Dosage: 8-12g of dried herb per day, 4-6g of dried root per day, 3-6ml of 1:2 herb liquid extract per day, 4-9 ml of 1:2 root liquid extract per day,
7-14ml of 1:5 tincture per day.

Vaccinium myrtillus *(Ericaceae),* Bilberry
Medicinal uses: Anti-diarrhoeal.
Trace elements: Ca, K, Mg, Mn, P, S, Se, Si and Zn.
Dosage: 3-6ml of 1:1 liquid extract per day. The daily dose of 20-60g dried berries is recommended.

Valeriana officinalis *(Valerianaceae),*
Valerian
Medicinal uses: Lower blood pressure, relieves muscle *spasms,* sedative for restlessness, sleeplessness and minor nervous conditions.
Trace elements: Ca, Fe, K, Mg, Mn, P, Se and Zn.
Dosage: 3-9g of dried root/rhizome per day, 2-6ml of 1:2 liquid extract per day, 5-15ml of1:5 tincture per day.

Zingiber officinale *(Zingiberaceae),* Ginger
Medicinal uses: *Anti-emetic*, *diaphoretic*, circulatory stimulant, inhibits coughing, anti-inflammatory, antiseptic, carminative, cholagogue and antispasmodic.
Trace elements: Ca, Fe, K, Mg, Mn, P, Se and Zn.
Dosage: 500-1000mg of fresh fruit 3 times a day, 500mg of dried root 2-4 times a day, 0.7-2ml of 1:2 liquid extract per day, 1.7-5ml of 1:5 tincture per day.

GLOSSARY OF CHEMICAL, MEDICAL AND PHARMACEUTICAL TERMS

Adaptogen- a substance with a non-specific action that causes improved resistance to physical and mental stress
Adequate Intake (AI)- a recommended average daily nutrient intake level based on observed or experimentally determined approximation or estimates of nutrient intake by a group of apparently healthy people that are assumed to be adequate; used when a RDA cannot be determined
Albumin- any protein with water solubility, moderately soluble in concentrated salt solutions and experiences heat protein denaturation
Alzheimer's disease- is a disorder of loss of mental functions resulting from brain tissue changes for example memory loss
Amino acid- chemical substances that form the building blocks of proteins
Anaemia- reduced number of red blood cells in the blood
Anaesthetic- a substance that causes localized or general loss of sensation
Analgesic- a substance that relieves pain
Antiallergic- a relief from itching and swelling caused by other substances
Antibacterial- a substance that kills or inhibits the growth of bacteria
Antibiotic- a substance that kills or inhibits the growth of micro-organisms
Antidepressant- a substance that alleviates depression
Antiemetic- a substance that prevents vomiting
Antifungal- a substance that kills or inhibits the growth of fungi
Antihypertensive- a substance that reduces high blood pressure
Anti-inflammatory- a substance that causes symptomatic relief of inflammation
Antimicrobial- a substance that kills or inhibits the growth of micro-organisms
Antioxidant- a substance that is able to protect cells or counteract the damage caused by oxygen free radical or oxidation
Antirheumatic- a substance that relieves the symptoms of rheumatism
Antiseptic- a substance that stops or inhibits infection
Antispasmodic- a substance that reduces muscular spasms and tension
Apathy- state of indifference, when an individual has an absence of interest/concern to certain aspects of emotional, social or physical life
Arthritis- inflammation of joints
Astrigent- a substance that reacts with proteins in wounds, on the surface of cells or membranes, resulting in a protective layer and causing contraction
Blood clotting- the solidification of blood which stops the system from losing blood when blood vessels are damaged or cut
Carcinogenic- a substance that causes cancer
Carminative- a substance that that reduces flatulence
Cholagogue- a substance that stimulates the flow of bile from gall bladder
Choleretic- a substance that stimulates the liver to produce bile
Cholesterol- steroid fat-like material found in the human body associated with increased risk of coronary diseases
Cocaine- crystalline alkaloid that is obtained from the leaves of the coca plant from Erythroxylaceae family
Codeine- alkaloid used for its pain killing and as a cough suppressant
Conjunctivitis- inflammation of the thin clear membrane over the white part of the eye
Demulcent- a substance that soothes the mucous membranes
Demyelination- is any disease of the nervous system in which the myelin sheath of neurons is damaged
Diaphoretic- a substance that increases sweating
Diarrhoea- abnormally frequent discharge of watery stool for more than 3 times per day
Diuretic-a substance that increases the volume of urine
DNA (Deoxyribonucleic acid)- double stranded nucleic acid that contains genetic instructions of all living organisms and some viruses
Edema- is the increase of interstitial fluid in any organ causing swelling
Emollient- a substance that soothes and softens the skin
Enzyme- protein that catalyses a chemical reaction

Expectorant- a substance that increases mucous secretion or its expulsion from the lungs
Fatigue- tiredness in human beings
Flatulence- accumulation of excessive gas in the intestines
Flavoids- falls under a class of plant secondary metabolites and have antioxidant activity
Fluorosis- health condition caused by an overdose of fluorine
Glycoside- a chemical substance that yields at least one simple sugar upon hydrolysis
Goiter- swelling of the neck due to enlarged thyroid gland
Hemoglobin- is an iron-containing oxygen transport metalloprotein in the red blood cells
Hepatoprotective- protecting the liver from inflammation
Hypertension- high blood pressure (>140/90 mm Hg)
Hyperthyroid- disease state in humans caused by excessive amount of thyroid hormone that circulate in the blood
Hypothyroid- disease state in humans caused by insufficient production of thyroid hormone by thyroid gland
Immune stimulant- a substance capable of improving the immune system
Inflammation- localized swelling, redness and pain as a results of an infection or injury
Insomnia- a sleeping disorder characterized by persistent difficulty falling asleep or staying asleep despite the opportunity
Kidney stones- are solid concretions of dissolved minerals in urine forming inside the kidneys or bladder
Lipid- a substance soluble in non-polar solvents and insoluble in water
Lipid lowering- a substance that lowers cholesterol levels in blood
Metabolic alkosis- is a metabolic condition in which the PH of the blood is elevated beyond the normal range
Morphine- a highly potent pain killer drug
Mutagenic- a physical or chemical agent that changes the genetic information usually DNA of an organ
Myelin- is an electrically-insulating phospholipid that surrounds only the axon of many neurons
Myoglobin-protein in heart and skeletal muscles that has oxygen bound to it thus providing extra reserve that can maintain high level of activity for a longer period
Nickeloplasmin- a nickel containing protein found in human sera
Osteomalacia- softening of bones due to defective bone mineralization, for example rickets in children
Osteoporosis- a reduction in bone mass resulting in fractures
Prophylactic- a substance that prevents disease
Prostate- a gland at the base of the male bladder that secretes a fluid that forms part of semen
Pulmonary- having to due with the lungs from Latin pulmo for lungs
Quinines- a natural white crystalline alkaloid having fever reducing, pain killing and anti-inflammatory properties and a bitter taste
RDA (Ribonucleic acid)- single stranded nucleic acid that contains genetic materials and is a central to the synthesis of proteins
Recommended Dietary Allowance (RDA)- the average daily nutrient intake level sufficient to meet the nutrient requirements of nearly all 98% of healthy individuals in a particular life stage and gender group
Reserpine- drug that is used for control of high blood pressure
Rheumatism- general term referring to painful joints
Sedative- a substance that calms down the nerves
Seizures- excessive neuronal activity that is usually self-limiting, it can manifest as an alteration in mental state and muscle movements
Spasm- involuntary contraction of muscles
Stomachic- a substance that promotes appetite and digestion
Tannins- secondary metabolites with several phenolic OH-groups, that can form hydrogen and ionic bonds with proteins thereby altering their conformation
Tolerable Upper Intake Level (UL)- the highest average daily nutrient intake level likely pose no risk of adverse health effects to almost all individuals in the general population
Tonic- a substance that maintains health
Ulcer- a lesion on the skin or mucous membrane
Uric acid- minor end-product of nitrogen metabolism in the human body and is found in small amounts in urine