

**CHEMICAL ANALYSES OF *CERATOTHECA SESAMOIDES*
ENDL, *VERNONIA AMYGDALINA* DEL, *CORCHORUS*
OLITORIOUS, *CORCHORUS TRIDENS*, *MOMORDICA*
CHARANTIA AND *SENNA OCCIDENTALIS* LINN**

BY

**Umar Gwarzo SANI B. Sc. (BUK) 1992, M. Sc. (ABU) 2006
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DECLARATION

I declare that, the work in this dissertation entitled “Chemical analyses of *Ceratotheca sesamoides* Endl, *Vernonia amaygdalina* Del, *Corchorus olitorius*, *Corchorus tridens*, *Momordica charantia* and *Senna occidentalis* Linn” has been carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Sani, Umar Gwarzo

Signature

Date

CERTIFICATION

This thesis entitled “CHEMICAL ANALYSES OF *CERATOTHECA SESAMOIDES* ENDL, *VERNONIA AMAYGDALINA* DEL, *CORCHORUS OLITORIUS*, *CORCHORUS TRIDENS*, *MOMORDICA CHARANTIA* AND *SENNA OCCIDENTALIS* LINN” by Sani, Umar Gwarzo meets the regulations governing the awards of the degree of Doctor of Philosophy in Analytical Chemistry of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof. C.E. Gimba

Chairman Supervisory Committee

Signature

Date

Dr. D. J. Adeyemo

Member Supervisory Committee

Signature

Date

Dr. E.D. Paul

Member Supervisory Committee

Signature

Date

Prof. V.O. Ajibola

Head of Department

Signature

Date

Prof. A.A. Joshua

Dean School of Postgraduate Studies

Signature

Date

DEDICATION

This research work is dedicated to my father (Mal. Muhammad Sani Adamu), late mother (Malama Rabiya Sani), brother (Mal. Ismail Sani) and late sister (Malama Fatima Sani).

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ABSTRACT

Vegetables viz. *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Ceratotheca sesamoides* Endl, *Vernonia amygdalina* Del, *Mormodica charantia* Linn and *Senna occidentalis* Linn were analyzed for their nutrient, anti-nutrient, vitamin, major, minor, trace and ultra-trace metal contents. Standard analytical methods approved by the (Association of Official Analytical Chemists) were used for the nutrient analysis. Spectrophotometric methods were used for the determination of vitamins A and B in the samples while iodine method was used for the determination of vitamin C respectively. Instrumental neutron activation analysis (INAA) was used for the determination of major, minor, trace and ultra-trace elements contents. The levels of various anti-nutrients in the leaves were determined by appropriate methods. Results of the nutrient composition analyses showed that moisture content ranged 46.90 – 75.53%; ash (2.48 – 11.95%); crude protein (3.19 – 19.36%); crude lipid (3.33 – 6.67%); carbohydrate (5.69 – 10.38%) and calorific value of (93.71 – 163.90Kcal/100g). These values conform to normal values found in other exotic vegetables. Results of vitamins analyses revealed that vitamin A has the highest concentration in *Vernonia amygdalina* Del (379.46mg/100g); *Corchorus tridens* Linn (345.84mg/100g); *Ceratotheca sesamoides* Endl and least in *Mormodica charantia* Linn (0.06mg/100g). Also, vitamin C amounts in the analyzed plants ranged 17.63 – 255.61mg/100g which lies within amounts found in some of the highly utilized leafy vegetables. For the B vitamins, only B₁ was found in relatively high amounts in *Corchorus olitorious* Linn (154.125mg/100g) and *Vernonia amygdalina* Del (170.30mg/100g) while the rest (B₂ and B₃) determined in this work were in small amounts (0.08 – 1.98mg/100g and 0.50 – 7.916mg/100g) respectively. In addition to that, results of the analyses for anti-nutrients showed that cyanide content are low and less than the 10mg/kg Codex standard

for cyanide in dried cassava. Likewise, the amounts of phytates, oxalates and tannins are low. Moreover, results for the metal analyses in dry weight basis of the plant samples indicates different pattern of concentrations in the various plants and their parts. Out of the twenty nine elements determined; K, Ca, Na, Fe, Al were found in higher concentrations in the range of 10040.0 – 67270.0, 2802.0 – 26950.0, 36.0 – 3520.0, 145.0 – 22215.0, and 52.0 – 2262.0ppm while Rb, Mn, and Zn at lower amounts in the range of 3.9 – 41.0, 3.19 – 326.78, 14.0 – 166.0ppm and the rest either at BDL levels or in traces. Student's t-test statistical analysis ($p < 0.05$) on the various parameters indicated varied relationships. The results revealed that these plants contain nutrients, vitamins, and mineral elements and low levels of toxicants. Therefore the plants should be included in diets and will enable consumers, health professionals and regulatory bodies to document and make reference to the data obtained in this work.

ABBREVIATIONS

AAS:	Atomic Absorption Spectrometry
AES:	Atomic Emission Spectrometry
AOAC:	Association of Official Analytical Chemists
ATP:	Adenosine Triphosphate
CERT:	Center for Energy Research and Training
DNA:	Deoxyribonucleic acid
GABA:	Gamma-aminobutyric acid
IAEA:	International Atomic Energy Agency
ICP:	Inductively Coupled Plasma
INAA:	Instrumental Neutron Activation Analysis
LD ₅₀ :	Median lethal dose
MNSR:	Miniature Neutron Source Reactor
MS:	Mass Spectroscopy
NAA:	Neutron Activation Analysis
NAD:	Nicotinamide Adenine Dinucleotide
NADP:	Nicotinamide Adenine Dinucleotide Phosphate
NAFDAC:	National Agency for Food and Drugs Administration and Control
NFPA:	National Food Processors Association
NIRR-1:	Nigerian Research Reactor 1
PAs:	Proanthocyanidins
PDCAAS:	Protein Digestibility-Corrected Amino Acid Score
PPM:	Parts Per Million
RDAs:	Recommended Dietary Allowances

WHO: World Health Organization

XRF: X-Ray Fluorescence Analysis

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CHAPTER ONE

1.0 Introduction

Less utilized green leafy vegetables are plants consumed in relatively small quantities as a side dish or relish with the staple food. Usually these vegetables are the leaves, roots or stems of herbaceous plant. Their flower, calyces and immature seeds or fruits may also be consumed. They are also used as boiled vegetables or added to soups and stews (Tomori and Obijole, 2000) and their consumption is considered inferior in taste and nutritional value when compared to their cultivated counterparts (Vainio-Matilla, 2000).

However, with persistence of malnutrition in the developing countries in spite of increased basic food production, micronutrient deficiencies and other nutrition related diseases still afflict over 2 billion people worldwide (Tomori and Obijole, 2000; Ejoh *et al.*, 2005). These had become a source of interest to nutritionist and other concerned scientific researchers. In fact, several studies (Osaki *et al.*, 2003, Fasakin, 2004; Alabi *et al.*, 2005, Antia *et al.*, 2006; and Ekop, 2007) showed that many species of wild green leafy vegetables are rich sources of nutrients.

Leafy vegetable are also known to add taste and flavor as well as substantial amount of protein, fiber, minerals and vitamins to the diet (Oyenuga and Fetuga, 1975; Adeyemi, 1987, Fasakin, 2004; Alabi *et al.*, 2005; Ejoh *et al.*, 2005). In addition, some plants with promising bioactive properties also contain useful minerals and food value for human and animal consumption (Alabi *et al.*, 2005).

Moreover, leafy vegetables are the cheapest and most available sources of substantial amount of vitamins A and C to the most vulnerable group, viz: rural populace. However, in tropical Africa millions of people still suffer from vitamin A and C deficiency despite the increased consumption of leafy vegetables (Ejoh *et al*, 2005). These leafy vegetables are relatively inexpensive, easily and quickly cooked and rich in several nutrients especially β -carotene and vitamin C which are essential for human health (Tomori and Obijole, 2000).

Several vegetable species abound in Nigeria and most West African countries are used partly as condiments or spices in human diets or as supplementary feeds for livestock such as rabbits, poultry, swine and cattle (Aletor and Adeogun, 1995). These vegetables are harvested at all stages of growth and fed either as processed, semi-processed or fresh to man while they are usually offered fresh to livestock. The nutritional interest in some of these vegetable species stems from their rich contents of essential amino acids, vitamins and minerals. Further to their rich content of the mentioned nutrients above, it is established that green vegetable leaves are also source of proteins because of their ability to synthesize amino acids from a wide range of virtually every available primary materials such as water, carbon dioxide, and atmospheric nitrogen (as in legumes).

However, the presence of inherent toxic factors or anti-nutritional components in plants has been one major obstacle in harnessing the full benefits of the nutritional value of plant foods, vegetables inclusive (Liener, 1969; Nwokolo and Bragg, 1977; Lewis and Fenwick, 1987). Although the presence of these anti-nutritional factors is always in trace quantities, they have been established to play significant roles in the nutritional quality of food. However, many food-processing techniques have been highlighted as possible means of

reducing or eliminating the anti-nutrient levels in plant food sources to innocuous levels that can be tolerated by animals particularly in monogastric nutrition (Fasuyi and Aletor, 2005).

Available medical literature reveals that plants have been used for curative purposes (Ming, 1999). There are many plants whose green leaves, roots and stems are used in herbal preparations for the treatment of various ailments (Ekop, 2007). Most of the times the potency of those plants is accounted for in terms of their organic constituents but, it is an established fact that there is a high relationship between the chelation of metals and some chemotherapeutic agents. The role of inorganic elements in animal and plant metabolism has long been established but the effect and influence of these elements on administration of medicinal plants has received relatively little attention (Gwarzo *et al.*, 2006). Also there is an increased awareness of the value of leafy vegetables in contributing to a balanced diet particularly in areas where animal protein is deficient (Tomori and Obijole, 2000)

However, the consumption of vegetables and basic foodstuffs from plants and medicinal plants traditionally has been largely indiscriminate without due regard to possible side effects. Indeed, diet has long been considered as the major source of human exposure to trace and ultra-trace elements and consequently their levels in basic food stuff determines the quantity available to man. Because, it was postulated that, any element absent in plants is probably not essential for man (Saiki *et al.*, 1990, Tomori and Obijole, 2000, Gwarzo *et al.*, 2006). Nevertheless, some elements in the body can play a protective role in decreasing the risk of some diseases. Consequently the levels of minerals in basic food stuff are of great interest from toxicological and nutritional point of view.

Other items obtainable on consumption of green leafy vegetables as earlier stated are the anti-nutritional factors. When consumed in higher doses, they may pose problems to the person concerned. For example, high values of cyanides has been implicated for cerebral damage and lethargy in animals and man; Oxalate can form complexes with most essential trace metals, thereby making them unavailable for enzymatic activities and other metabolic activities (processes) (Akwaowo *et al.*, 2000) and formation of kidney stones (Liebman and Costa, 2000). In addition, presence of tannins, phytic acids and HCN may make the plants potent poisons (Antia *et al.*, 2006)

The data obtained however, from the analysis of chemical composition of food items is important to both consumers and health professional alike. The recent campaigns, the setting up of bodies and legislations by health ministries that regulates food and drug administration highlighted this need. In addition, the data obtained from plants (vegetables) are mainly used to monitor such aspects of life of man like medicine, nutrition, pollution (environment) and toxicology of the substance in the long-term consumption of the plant. Researchers have developed methods and instrumentations for the measurement of constituents of food items and other materials of interest because of their impact on human health. Their focus is on the measurement of analytes that are either beneficial or detrimental to human health (Miller-Ihli, 1996, Paul *et al.*, 2011 and 2013).

In Nigeria, the National Agency for Food and Drugs Administration and Control (NAFDAC) has been set up and charged with the responsibility of protecting public health by promoting wholesomeness, quality and efficacy of processed foods, medicines, cosmetics, medicinal devices, chemicals and prepackaged water through an effective

quality assurance system and public enlightenment. In addition, there are inspectorates and enforcement agencies as part of prevention of illnesses. These bodies or agencies are also charged with the responsibility of formulating regulations and compilation of standard specifications for compliance by manufacturers, importers and exporters of regulated products and instituting labels depicting information about nutritional qualities of both processed and unprocessed foods (NAFDAC, 2003; AOAC, 1993 and NFPA, 1994).

It is a common knowledge that administration of plants claimed to have medicinal properties has largely been indiscriminate without due regard to possible side effects. It is thus important to determine the chemical content of these plants for pharmacological assays and other probable roles in the curative process. In addition to that, plants are generally the major sources of food and minerals to both man and animals. The different species of plant plays an important role in the diet of the inhabitants of arid, semi-arid and savanna countries of Africa including Nigeria. They contain minerals in quantities compared favorably with foods of W.H.O standard (Barminas *et al.*, 1998). Of particular importance among the parts of the plant are the leaves and the seeds containing trace elements at varying levels depending on many factors such as species variety, stage of maturity and environment (Daun and McGregor, 1991).

Therefore, since minerals, organic constituents and anti-nutritionals are very important to man, accurate determination of them in wild plants, utilized and less utilized plants will help nutritionist to recommend a list of plants for food supplementation and estimate daily aggregate allowance from sources for meeting daily human requirements.

From the environmental, pollution and toxicological point of view, many minerals including those that are essential to life can be toxic at high doses or in certain compound formulation. So the amount of minerals in living organism normally correlate significantly with amounts in the environment. Also heavy metals do not degrade but accumulate in food and are a serious threat to human health. In fact, there are certain plants known to have a special ability which enable them to take elements with the tendencies of accumulating same to dangerous levels, thus making the plants toxic (WHO, 1980; IRI, 1989; Szefer and Szefer, 1994 and Last, 1995). Likewise, it is a known fact that different parts of plants contain trace elements that are harmful to the body of man at a certain concentrations.

Sewage from homes, industrial effluents into aquatic environment, smoke from industries and vehicles, parent materials from which the soil is formed; types of agrochemicals used in farming and waste disposal systems all contribute to the elemental composition of plants.

Therefore, there is a greater tendency for the accumulation of a particular element as suggested by Kovacs (1979) and Whitton (1989) and the extent of accumulation is a reflection of the concentration of such materials in the environment as well as the species of the plant in use. It is an established fact that metals in particular are present in both plants and animals as components of simple salts and complexes performing various functions (Yalwa, 2002). They are required by plants in amounts that result in their classification as essential, beneficial and non-essential for their proper growth and development (Daniel, 1990).

Moreover, metals and in particular trace metals are a major class of contaminants in our present world arising principally from natural and anthropogenic sources. The

anthropogenic sources are primarily from industrialization and mining activities which are of paramount importance to ecosystem sustainability (Okorie and Egila, 2012). In addition to that, advancement in technology as well as increase in population have led to the environmental concern emanating from indiscriminate dumping of refuse and discharge of industrial effluents, petroleum waste and crude oil spills replete with most common trace metals in our environments (Okorie and Egila, 2012).

Likewise, it is a fact that, mineral composition of plants found in a particular location reflects the type of activities of man around the location, the soil type, chemical nature of rocks underneath, specie of the plant, part of the plant, variety, stage of maturity and to some extent the inherent property of plant (Pritchard,1999). It has also been found that most plants in the vicinity of rivers fed with industrial sewage have been contaminated by toxic micro metals and also plants growing on industrial effluent-contaminated soil accumulate potentially toxic elements from the soil (Dike *et.al*, 2005).

However, Tomori and Obijole (2000) stated that, it is of interest to periodically assess the mineral content of foods especially vegetables which supply micronutrients because of their nutritional implications. Indigenous vegetables and the exotic ones (e.g Cabbage, Brocoli and Lettuce etc) also play important roles in human diets. They supply the body with minerals, vitamins and certain hormone precursors in addition to protein, taste, flavor, fiber and energy (Fasakin, 2004 and Ndolovu and Afolayan, 2008).

1.1.0 Objectives of the research

The objectives of this research are to:

- i. Determine qualitatively and quantitatively the major, minor, trace and ultra trace elements in *Vernonia amygdalina* Del, *Corchorus olitorius* Linn, *Corchorus tridens* Linn, *Ceratotheca sesamoides* Endl, *Senna occidentalis* Linn and *Momordica charantia* Linn.
- ii. Determine the nutritive (proteins, lipids, carbohydrates, fibers and vitamins) and anti-nutritive (cyanides, tannins, oxalates and phytates) factors in the above listed plants.
- iii. Assess the nutritional qualities of the plants and

1.2.0 Justification of the research

Previous research on the above mentioned plants were mainly on their phytochemistry, bioactivities of their extract, their proximate composition and essential elements. Therefore, this work shall focus on the multi-element analysis and nutritional quality of the plants.

CHAPTER TWO

2.0 Literature review

2.1 *Corchorus olitorius* Linn

The plant *Corchorus olitorius* Linn belongs to the family *Tiliaceae*. In Nigeria it has vernacular names include malafiya or molikhiya-Hausa; oyo, eyo (Abeokuta) eyo-ga-nbe,

ayo (Ijebu-Ode), Yoyo (Ilesha), ewedu (Lagos), ewedu-ga-nbe; sobo, oyoyo- Yoruba; ahu mara (Owerri), aheheara, ahihira or agheregha (Umuahia)-Igbo (Dalziel, 1955).

It is a mucilaginous pot herb with young leaves for spinach for Europeans. There are three or four varieties cultivated locally some with deeply cut fairly broad leaves. In West Africa, it is apparently used only as a vegetable. In India, apart from its fiber, it is used as medicine as well as a vegetable. As by-products of the jute industry, the butts or root ends and cuttings of the plant can be used for making paper-pulp and are suggested as possible source of motor spirit (Dalziel, 1955; Watt and Breyer-Brandwijk, 1962).

Phytochemical investigation of the plant has shown the presence of large amounts of carbohydrates and proteins with tannis, flavonoids, oils, steroids and terpenoids in significant concentrations (Akaneme, 2008). Infact the protein content was found to be about 20% of the leaves (Vainio-matilla, 2000) and quoted at about $58.88 \pm 0.064 - 127.5 \pm 1.05$ mg/g by the plant was found to be about $571.53.01$ mgg⁻¹.

Other medicinal uses to which the plant *C. oltoriosis* Linn is put in to are the softening and drawing the breast, abdominal diseases, and effective galactogogue, as a purgative and for lungs trouble (Watt and Breyer-Brandwijk, 1962). These uses may be attributed to the presence of such components of the plant like the saponins in the seeds, hydrocyanic acid in the vegetative and reproductive organs, β - sitosterol, corchoralic acid in the corchorin and corchororgenin (Watt and Breyer-Brandwijk, 1962). The toxicity of the plant has been ascribed to its corchorin content, but corchororgenin is more powerful in pharmacological action.

Also paper chromatography of the unfermented seeds has disclosed the presence of two glucosides one of which is olitorium (Watt and Breyer-Brand-Wijk, 1962). Further studies on the plant revealed two new digital glucoside from *C. olitorious* Linn and *C. capsularies* which were named corchoroside A (M. pt 188 – 190 °c) and corchoriside B (M.pt 224 – 240 °c). The average lethal dose for cat was 0.053-0.0768 mgkg⁻¹ in the case of the latter. In addition to the cardiac glucosides the seed contains raffinose and a tasteless resin. The fixed oil content of the seed is 11.3-14.76 %, with the constituents, physical and chemical constants documented (Watt and Breyer-Brandwijk, 1962). The mucilage of the plant has also been studied and the leaf contain oxydase and chlorogenic acid (Watt and Breyer-Brandwijk, 1962)

Likewise, in an effort to report the proximate composition of *C. olitorius* Linn, the ash content was found to be 14.4 ± 0.38 - 29.9 ± 0.67 mgg⁻¹ while moisture was not more than 794.0 ± 5.41 mgg⁻¹ (Ijomah *et al.*, 2000). The iron and calcium contents were also reported to be high (Vaino-Matilla, 2000).

2.2 *Corchorus tridens* Linn

The plant *Corchorus tridens* Linn belong to the family *Tiliaceae*. Its common names include turgunnuwa, or Lalo-Hausa/Fulani (Dalziel, 1955)

The leaves and young shoots of the plant are used as a vegetable and soup herb, and are also a good fodder for camels and other domestic stock. It yields a good fiber used for fishing lines in northern Nigeria and elsewhere. When long, the stems are used for horizontal ties of conical hut roots (Dalziel, 1955).

On the analysis of the proximate composition of *C. tridens* Linn, indicated the presence of high amount of fiber, calcium, iron and vitamin C.

2.3 *Ceratotheca sesamoides* Endl.

The plant *Ceratotheca sesamoides* Endl belongs to the family *Pedaliaceae*. It has vernacular names include Yaido, yando, Karkashi or Kalhashi-all Hausa; Eku or E-kuku and e kuku ile-Yoruba (Dalziel, 1955).

The plant is wild, brought in to cultivation in various part of Africa, and is variable. In some places, only the leaves are used while in others the seeds. It is sold as a soup vegetable and used along with other foodstuffs for the sake of its mucilaginous quality. The smooth seeds vary in color from yellowish to black-brown with a narrow radiate zone (Dalziel, 1955).

An analysis of the seeds showed 37.3 % oil (Dalziel, 1955). The leaves mixed with chopped grass and clay makes it adhesive for building huts. Also in the preparation of shea butter the juice is added to the boiling pot to accelerate the separation of the fat. In northern Nigeria, the peeled root is applied to the eye for style while in southern Nigeria the leaves are rubbed on the foot before putting on an Ivory anklet (Dalziel, 1955). The leaves of the plant are eaten in central Chad, while the seeds pods and leaves are ground into powder in the Sudan for the preparation of a dish waika (Freedman, 1998).

Proximate analyses of seeds of *C. sesamoides* Endl (Fasakin, 2004) have indicated the presence of high levels of crude protein (21.32-22.15 %); oil (17.25-21.00 %); crude fiber (25.75-29.75 %); calcium (2.65-3.15 %) and phosphorus (0.53-0.54 %). While the leaves

contains low calories soluble carbohydrates and fats, high levels of protein (29.35-29.85 %) calcium (2.60-2.62 %) and phosphorus (0.25-0.27 %) which suggested a potent supplement to the starchy staple foods that are traditionally consumed (Fasakin, 2004).

Moreover, previous studies have also demonstrated the presence of crude protein (8.5%) soluble carbohydrate (8.2%); crude fibre (8.5%); fat (1.5%) and ash (20.2) (Freedman, 1998). Various amino acids that are present in the seeds and leaves of *C. sesamoides* Endl includes aspartic acid, threonine, Serine, glutamic acid, proline, glycine, alanine, valine, cysteine, methionine, phenylamine, lysine, histamine and arginine (Freedman, 1998); minerals determined also include sulphur (0.23 %); potassium (0.23 %); Magnesium (0.48 %); calcium (1.48 %); manganese (123 mgg⁻¹) and copper (15 mgkg⁻¹).

2.4. *Momordica charantia* Linn

The plant *Momordica charantia* Linn belong to the family *Cucubitaceae* and its common names are balsam pear, bitter lemon or African cucumber. It is a prostrate having perennial herb with long simple hairs, climbing by means of tendrils and reproduces from seeds. The stem is angled, more or less hollow and with minute hairs. The leaves are alternate up to 5cm long, with a spiral tendril at opposite sides. They are unevenly lobed and the lobes are more or less notched. The petioles are 4-5 cm long or short and pubescent (minutely hairy) and produce an offensive smell when crushed. The inflorescence consists of male and female flowers on the same plant; a conspicuous bract on the peduncle subtends the male flower. The corolla is bright yellow and measures about 2-5 cm across. The fruit is a warty gourd, usually with longitudinal furrows, orange-yellow when ripe up to 15 cm long,

splitting to expose the seed that are red and about 2mm across (Akobundu and Agyakwa, 1998; Dalziel, 1955).

The plant (*M. charantia* Linn) is a weed of cultivated fields, bush re-growths and waste areas, although widely cultivated in Africa, Asia and South America for its valuable medicinal properties. Indigenous people are over the world employ the fruit juice or leaf tea for diabetes, colic, wounds, infections; hepatitis, laxatives and stimulant (Lothkar and Rajarama, 1966; Platel and Srinivasan, 1997; Umukaro and Ashorobi, 2006). *M. charantia* Linn is also useful in the treatment of inflammatory conditions such as rheumatism and gout (Umukaro and Ashorobi, 2006).

The extract from the leaves of *M. charantia* Linn was reported to exhibit hypoglycemic activity comparable to tolbutamide (Platel and Srinivasan, 1997) and treatment with the plant was found to lower blood glucose levels in animals and human studies (Lothkar and Rajarama, 1996). It also demonstrated antibacterial, antifungal, antineoplastic, antiviral, antimutagenic, purgative properties produced contractors of the guinea ilium, anti-inflammatory and membrane stability properties (Jilka *et al.*, 1983; Guevara *et al.*, 1990; Sofowora; 1979; Umukaro and Ashorobi, 2004 and Barca *et al.*, 2004). Results showed that oral administration of ethanol extract of *M. charantia* Linn seed (200 and 400 mg/kg) significantly suppressed the development of egg albumin-induced paw edema ($P < 0.01$). In the chronic test, the ethanol extract of seed (200 and 400 mg/kg) showed significant reduction in granuloma weight of rats ($P < 0.05$). The anti-inflammatory effect of the ethanol extract of *M. charantia* Linn seed was similar to that of indomethacin (10 mg/kg).

Moreover, the free radical scavenging activities of *M. charantia* Linn were investigated using the DPPH test. Only the ethanol extract of the *M. charantia* Linn seed showed a moderate free radical scavenging effect on DPPH (IC₅₀, 198.69 µg/ml) when compared with the positive control, V_E, (IC₅₀, 44.91 µg/ml). The results of the study provide a scientific basis to explain, in part, the popular use of seed of *M. charantia* Linn in Tibetan folk medicine as “Bolengguazi”. The study also supports the claims by the traditional Tibetan medicine practitioners about the use *M. charantia* Linn seeds in inflammatory diseases, such as “Chiba” (Umukaro and Ashorobi, 2006, Fang *et al.*, 2007).

Phytochemical analysis of *M. charantia* Linn revealed the presence of alkaloids, saponins, glycosides, phenolics, reducing sugars, free acids, essential oils and hydroxytryptamine (Dhalla *et al.*, 1981, Barca *et al.*, 2004). The essential oils were analysed by GC/MS and 90.9 % of them were analyzed. The major constituents were trans-nerolidol, apiole, cis-dihydrocarveol and germacrene (Barca *et al.*, 2004).

2.5 *Senna occidentalis* Linn

The plant *Senna occidentalis* Linn belongs to the family *Caesalpinioideae* and has a common name of coffee senna. It has an erect, hairless undershrub, annual or biennial growing to about 100 cm high and reproduces from seeds. The stem is ribbed, woody below and loosely branched. The leaves are compound, alternate and 10-15 cm long. The leaflets are 4-6 pairs, broadly- lanceolate, 2.5-7.5 cm long and 1-2 cm wide. The inflorescence is an axillary raceme with yellow flowers. The fruit is flat slightly curved and green in color. The pods are smooth, 15cm long and 6mm wide with 20-30 brown, ovate seeds that are about 3mm across (Akobundu and Agyakwa, 1998).

S. occidentalis Linn is a common weed in field crops, waste areas and roadside in West Africa (Akobundu and Agyakwa, 1998). Its ethnobotanical studies have revealed the potent cathartic effect of the leaves (Wang *et al.*, 2002), used as tea for constipation, treatment of eczema and other skin disorders, treatment for smallpox and measles, potent cure for gonorrhoea and pile, control insects and treatment for fevers (Ogunkunle and Ladejobi, 2006; Idu *et al.*, 2006). Also reported is the use of decoction of the *S. occidentalis* Linn leaves and flowers as an expectorant in bronchitis and dyspnoea, an astringent and as a mouthwash in stomatis.

Phytochemical studies of *S. occidentalis* Linn showed the presence of alkaloids, flavonoids, cardiac glycoside, tannins, phlobatanins, saponins and anthraquinones (Ogukunle and Ladejobi, 2006). Likewise antimicrobial activity and volatile oils were also reported (Idu *et al.*, 2006).

However toxicity of the seed of the seed of *S. occidentalis* Linn has been demonstrated in children and body system affected are hepatic, skeletal muscles and the brain with exact toxic principles yet to be determined (Vashishtha *et al.*, 2007).

2.6 *Vernonia amygdalinia* Del

The plant *Vernonia amygdalinia* Del belong to the family *compositae*. Its common name is bitter leaf. While vernacular names in Nigeria are Shuwaka-Hausa, Ewuro-Yoruba and Olubu or Onubu-Igbo (Dalziel, 1955). The root of the plant is commonly used in the form of a piece from which the bark has been removed after scorching. In some places the twig

is used. It is the most valued of the tooth cleaners and is chewed mostly as a stomachic tonic and appetizer. The Hausas use it mixed with red natron medicinally for gastro intestinal troubles. The bitter leaf is used in soup and is antiscorbutic and a digestive tonic. The leaves are rubbed on the body for itching conditions, parasitic skin disease, ringworm or cold infusion used as a wash. The leaves act as dewormer in infants, rubbed on the breast to assist weaning, tonic after fever, cough medicine, purgative and laxatives (Dalziel, 1955; Adekunle and Odukoya, 2008).

The different species of Vernonia analysed are good source of vitamin A and C and carotenoids but processing leads to significant losses (Ejoh *et al.*, 2005). In addition to that chemical and nutritional investigation of the leaves of the plant indicates the presence of seven (7) amino acids, carbohydrate, proteins, Lipids, acids, Iodine, hydrocyanic acid and oxalates. Other components detected and determined are Na, K, P, Mg, Mn, Fe, Cu, Zn and fibres (Locket *et al.*, 2000; Alabi *et al.*, 2005).

Phytochemical investigation of the aqueous ethanol extracts of the leaves of *V. amygdalina* Del indicated the presence of anthraquinones, anthocyananins Flavonoids, phlobatannis, saponins and steroids (Adekunle and Odukoya, 2008).

2.7.0 Chemical Composition of Leafy Vegetables

Vegetables like all other things contain a very wide range of different chemical compounds and show varies in composition and structure. Apart from the obvious inter-specific differences, no two individuals, weather animals or vegetables are exactly the same or for that matter any two parts. An individual vegetable, being largely composed of living tissues

which are metabolically active is constantly changing in composition and the rate and extent of such change depends on the physiological role and stage of the organ concerned (Kader, 2002).

2.7.1 Moisture content

As expected, fresh leafy vegetables have high moisture content ranging from 72 % in cassava leaves to 92 – 93 % in Indian spinach and bitter leaf. The amount in individual samples will of course, depend on several factors including age, agronomic practices prevailing during cultivation and freshness (Oguntona, 1998).

2.7.2 Carbohydrate

Simple sugars are the immediate products of the process of photosynthesis and it is hardly surprising that the structural frame work of plant tissue is largely composed of complex molecules built up from monosaccharides and closely related compounds such as uronic acid (Murano, 2003). Green vegetables have little carbohydrate in them, and those that are there are packed in layers of fiber, which made them very slow to digest. The proportions of different carbohydrate constituent can change due to the metabolic activity of the plant. The amounts of carbohydrates in leafy vegetables are usually less than 9 % (Oguntona, 1998).

2.7.3 Protein

Proteins are considered as structural constituent since they are the major sole component of the cytoplasm of living cells. Some leafy vegetables and sweet corn contain over 4 % of protein but in most other products the level is below 3 % including enzyme systems which are of primary importance in the physiology and post mortem behavior (Oguntona, 1998).

2.7.4 Lipids

The lipids of vegetables are like the proteins largely confined to the cytoplasmic layer in which they are especially associated with the surface membranes (Kader, 2002). Leafy vegetables are known to be poor sources of fat. Among the proximate components of vegetables fat content represents the lowest in this category of foods. It is in fact unusual to find leaves with ether extract exceeding 1 % in fresh leafy vegetables, although contents of dry sample can range from 1-30 % (Oguntona, 1998).

2.7.5 Pigments

The natural coloring matter in vegetables include a large number of individual chemical compounds which fall naturally into three main groups namely the chlorophyll, the carotenoids and the flavonoids pigments (Luh and Woodroof, 1975). Chlorophylls are the normal green pigments in plants which play important role in photosynthesis and are widely distributed in all green plants tissues. The chlorophyll amount to an extent of about 0.1% of the fresh weight in green leaves, and are localized in special plastic called chloroplast. Carotenoids are yellow, orange-red pigments, all soluble in fat. They are found along with chlorophylls, in green leaves. An important member of the family is β -carotene. This is a brilliant orange-yellow pigment, which is closely related to vitamin A, because it is easily changed into that vitamin on being eaten. Flavonoid pigments, are water-soluble, and unlike the other two are found in the water in the cell-sap. A subgroup called anthocyanins is responsible for reds, blues and violets colourations found in a wide variety of fruits and vegetables (Ihekoronye and Ngoddy, 1985).

2.7.6 Mineral Elements

The total amount of mineral matter in vegetable is represented by the ash content. The mechanism of mineral uptake by plant is such that element other than those which are necessary for normal development are absorbed from the soil. As a result, plants usually contain in varying proportions, the full range of mineral element which are present in the soil in which they are grown (Luh and Woodroof, 1975). Among the factors influencing the mineral composition of leafy green vegetables, soil fertility (or type and quality of fertilizer used) is perhaps the most important. Most of the earlier studies have shown that Nigerian leafy vegetables contain appreciable amounts of minerals. Specifically, these vegetables are low in sodium but relatively high in potassium (Oguntona, 1998).

2.7.6.1 Some major, minor and trace elements of interest

2.7.6.1.1 Chromium

Chromium exists in micro and macro quantities in soils but in micro quantity in plants. Cruf (1964), Johnson and Simons (1977) described procedures that permit rapid multi-element analysis of plant materials by direct reading emission spectroscopy, the method does not require any sample treatment and allows analysis of twenty-one elements. Its discovery and nutritional importance was first recognized in 1954 by Curran when he found that ,the metal enhanced the synthesis of cholesterol and fatty acids and was later identified as the active component of the glucose tolerance factor, which alleviate the impaired glucose tolerance in rats fed certain diets apparently deficient in chromium (Schwatz and Mertz, 1959)

Even though essentiality of the element chromium has not been biochemically defined, there are clinical evidences that showed the element might be responsible for some cases of impaired glucose tolerance, high blood glucose, low blood glucose and refractoriness to insulin ultimately diabetes (Kiple and Coneeorles, 2000).

Several spectroscopic techniques like colorimetric and AAS (Lerner *et al*, 1976); direct reading spectrometry and ICP excitation (Moselly *et al.*, 1978); neutron activation analysis (Rustamov *et al.*, 1974), XRFs (Schneider, 1970) for environmental analysis of chromium have been reported for chromium analysis. Plants and animals have also been recognized to contain the element as a cellular component. Its toxicity in plant leaves starts from 1 – 4 ppm (Anderson *et al.*, 1997) while it is twenty times more in the roots. Also chromium concentrations were determined together with other elements in both plants and animals by Boamin (1988), Cao and Ying (1994) and Longerich (1995) using the methods of AAS AES and XRFs respectively.

2.7.6.1.2 Copper

The presence of copper in plant animal tissues was recognized early in the nineteenth century but this was thought to be the result accidental contamination and the concentration is about 100ppm in both mentioned tissues, Earth crust (Kiple and Coneeorles, 2000). The element was also analyzed colorimetrically (Chiba and Watanabe, 1972) in plant and soil samples. The universal distribution of the element in plants prompted the hypothesis that copper was a catalyst participating in life process (Kiple and Coneeorles, 2000) and this led to some spectrochemical studies of the metal (Kashun, 1969 and Naidina and Treshchenco, 1972).

Some other methods that have for the determination of copper for possible sources of the metal due its dietary importance include neutron activation anlysis (Funtua *et al*, 2003; Dim *et al*, 2004); the dropping mercury electrode technique (Reed and Cummings, 1941); X-ray spectrography (Pazsit and Nagy, 1965) and Scott (1960) who used flame atomic absorption photometry.

2.7.6.1.3 Lead

Lead is usually found in soil and plant samples and the mean concentration in the Earth Crust is about 16ppm (Turekian and Scott, 1967). In the presence of other elements lead has been determined gravimetrically, titrimetrically, electrolytically, colorimetrically, spectrographically, polarographically, linear sweep oscillo-polarographically and spot- test respetively (Ekwumemgbo, 2003). Others are laser induced breakdown spectrometry (Wisbrun *et al.*, 1993 and applied instrumental techniques such as XRFs, ICP-AES and ICP-MS (Fujikawa *et al.*, 1995).

2.7.6.1.4 Manganese

Manganese was found to be a constant component of plants, animals and soil and has been shown to be an essential requirement by plants and microorganisms. It has a mean concentration in the Earth Crust of about 900-1000ppm (Ekwumemgbo, 2003). Attempts to demonstrate its essentiality to laboratory animals proved difficult but the metal was determined colorimetrically in soil and plant samples (Dobritsykaya, 1969).

The use of such methods like gravimetry, electrolysis, polarography, spectrography, absorptiometry, AAS and X-ray fluorescence (Ekwumemgbo, 2003) for the analysis manganese in soil and plants led to the identification of specific functions of the metal in plants and animals. It was observed that the spectrographic methods are more suitable than the chemical methods for the analysis of manganese in soil samples (Burrell *et al.*, 1954). Likewise (Gouhui *et al.*, 1994; Simabuco *et al.*, 1994) used XRFs to analyze the element in plant and soil samples while (Cao and Ying, 1994) used AES techniques.

2.7.6.1.5 Nickel

Nickel is usually found in soil and plant samples and the mean concentration in the Earth Crust is about and was colorimetrically determined (Chiba and Yoshishica, 1970) in soil and plant samples. Other reported methods are spectroscopic analysis, chromatography, polarography and linear oscillography while recent analysis of nickel used techniques like XRFs, ICP-AES and ICP-MS (Ekwumemgbo, 2003; Fujikawa *et al.*, 1995; Gouhui *et al.*, 1994 and Longerich, 1995).

2.7.1.6.6 Selenium

Plant and soil samples usually contain selenium in trace amounts and the mean content in soil samples is about 0.3ppm (Aubert and Pinta, 1977). The more important forms of selenium in soil are basic selenite (SeO_3^{-2}), calcium selenate (CaSeO_4) and organic selenium compounds derived from decayed vegetation (Williams and Byers, 1936).

Ravokovitch and Margolin (1957) reported the highest concentration of selenium top soil, while Lane (1966) showed that fluorescence analysis of selenium gave results, which were excellent agreement with those obtained by neutron activation analysis, AAS (Stanton,

1974); ICP-AES (Mcquaker *et al*, 1979); XRFs and ICP-MS (Simabuco and Filho, 1994 and Fujikawa *et al*, 1995) are some of the techniques reported on the analysis selenium in environmental samples.

2.7.6.1.7 Vanadium

Numerous biochemical and physiological functions for vanadium have been suggested based on its *in vitro* actions on cells and pharmacological actions in animals. The actions (Willsky, 1990) include insulin mimetic properties, numerous stimulatory effects on cell proliferation and differentiation, effects on glucose and ion transport across the plasma membrane, effects of cell phosphorylation – dephosphorylation and effects on oxidation – reduction process. The above observations and suggestions came into being from the knowledge of the existence of vanadium in soil and plant samples in trace quantities. Various methods of vanadium analysis like colorimetry and spectrography (Naidina and Tereschenco, 1970 and Ekwumemgbo, 2003) were reported for soil and plant samples. Spectrophotometry (Moselhy *et al*, 1978); neutron activation analysis (Funtua *et al.*, 2003 and Dim *et al.*, 2004) and XRF (Ekwumemgbo, 2003) are some of the usual methods for its analysis.

2.7.6.1.7 Zinc

Zinc is widely distributed in nearly all plants and its concentration in soil varies widely. The mean concentration in the concentration in the Earth crust is 500ppm and electrolytic methods, titrimetry, polarography colorimetry, turbidimetry and spectrochemical (Ekwumemgbo, 2003) are some of the reported methods of zinc analysis. Scott (1960)

observed flame atomic absorption photometry has a greater sensitivity than flame photometry for zinc analysis.

Other reported methods for zinc analysis in soil and plant samples are XRFs (Pazsit and Nagy, 1979 and Ekwumemgbo, 2003), neutron activation analysis (Funtua *et al*, 2003 and Dim *et al* 2004); radioactivation (Yamada, 1964); AAS (Kadow and Rabban, 1970); atomic fluorescence and flame photometry and XRF (Simabuco and Filho, 1994; Gouhui *et al*, 1994 and Longerich, 1995).

2.7.7 Vitamins

Vitamins can be defined as complex organic compounds required in catalytic amounts for the proper functioning of living cells and not synthesized in amounts to meet physiological needs. The quantities required may range from micrograms per day e.g. vitamins B₁₂ to milligrams per day e.g. vitamin C (Aurand *et al.*, 1987). In contrast to other food constituents, vitamins are not used for structural or energy requirements, or as raw materials for synthesizing other compounds.

However, in complete absence of a vitamin, clinical conditions known as deficiency diseases develop sometimes with fatal consequences (Okaka *et al.*, 2002). Although it is convenient to list specific symptoms resulting from an inadequate intake of an individual vitamin, deficiencies due to a single vitamin are seldom encountered in practice. A diet that is deficient in one vitamin is usually deficient in several vitamins. As a result, the vitamin that is lowest in amount relative to its dietary requirements will probably determine the major signs of a deficiency but the symptoms of other deficiencies will also be present.

Deficiency diseases, therefore, are the most frequently clinical manifestations of several vitamin deficiencies (Aurand *et al.*, 1987).

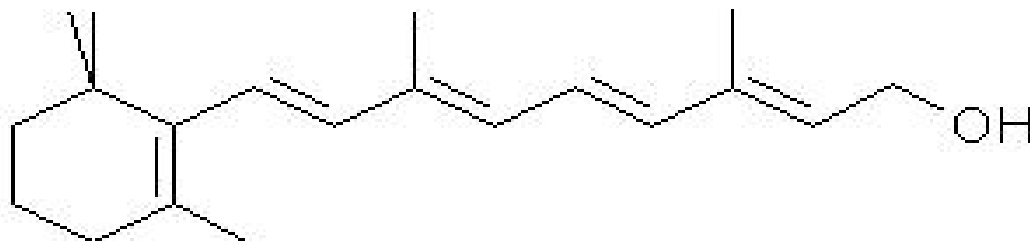
As with other nutrients many factors are known to influence the amount of vitamins in leafy vegetables. In particular, cultivar and maturity are important factors, while light can also sometimes be important. It is known, for example, that crops that mature during autumn contain higher vitamin A and their precursors, than those that mature in poorer light of winter. Also, the richest vegetable sources of thiamin include leafy green vegetables and it is known that this vitamin is retained at high levels in the leaves before being transferred to the seed or root at maturity. Green leafy vegetables contain small quantity of riboflavin but niacin and folate can be present in reasonable amounts. Green leafy vegetables are good sources of vitamin C and this component of Nigerian green leafy vegetables has received considerable attention from Nigerian scientists over the years (Aletor and Adeogun, 1995; Oguntona, 1998).

2.7.7.1 Classification of Vitamins

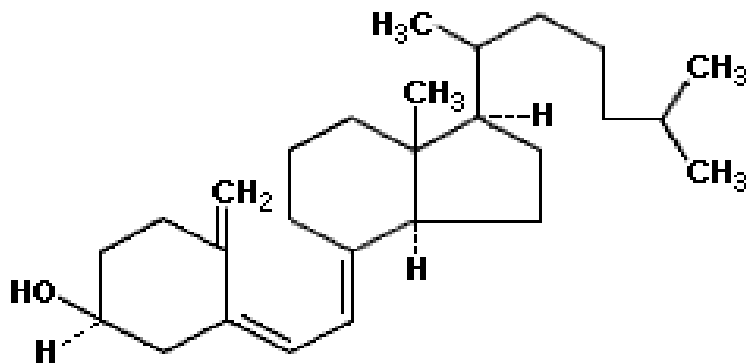
Vitamins are classified based on solubility. Some are soluble in fat while some in water. Vitamins that are fat soluble are stored in the body and can accumulate. Water soluble vitamins are flushed out by the kidneys. Additionally, some classify vitamins as to whether they are obtained naturally from food or from supplements. However, this becomes somewhat complicated as many foods are vitamin fortified (Farris, 2011).

2.7.7.1.1 Fat-Soluble Vitamins

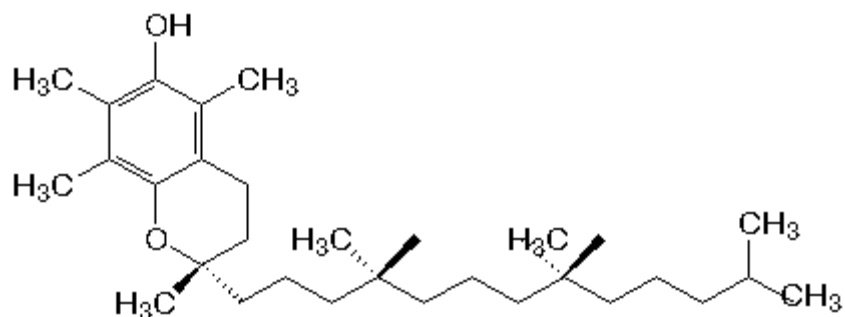
Vitamin A, D, E and K are stored in fat and liver cells in the body. Vitamin A is important for vision, especially night vision, bone growth and mucous membranes. As antioxidants, it may reduce the risk of some form of cancer. Vitamin D aids in the absorption of calcium and phosphorus. Teeth, bones and cartilages requires vitamin D. Vitamin E is also an antioxidant and helps to generate red blood cells and prevents blood from clotting. Vitamin K also works with the blood, aiding in the normal clotting process and bone maintenance (Orazulike, 2003).



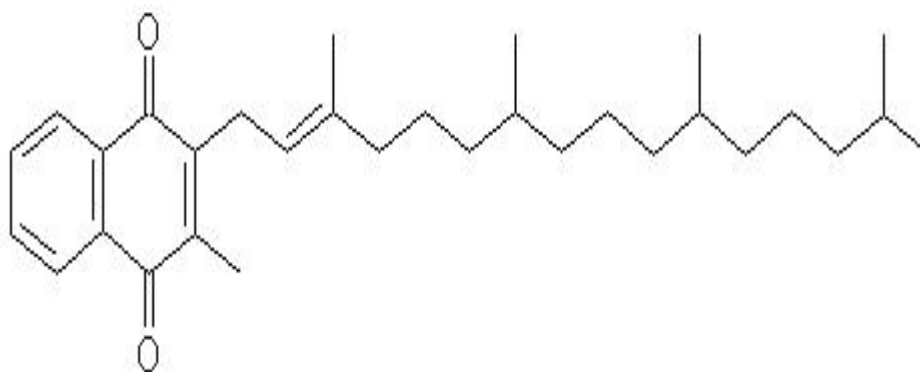
Vitamin A (Retinol)



Vitamin D₃



Vitamin E (α -tocopherol)



Vitamin K (Phylloquinone)

2.7.7.1.2 Water-Soluble Vitamins

The water-soluble vitamins have many properties in common. They have similar distribution in foods, which explains why a deficiency of several factors is observed more frequently than a deficiency of a single vitamin. This group of vitamins must be supplied in the diet every day because dietary excesses are excreted in urine and not stored to an appreciable extent. The water-soluble vitamins function as co-enzymes for specific enzyme systems essential for various metabolic processes of the cell (Aurand *et al.*, 1987). Some

water-soluble vitamins are partly lost in cooking procedures. This factor has to be kept in mind while meeting their requirements (Mudambi and Rajagopal, 2008).

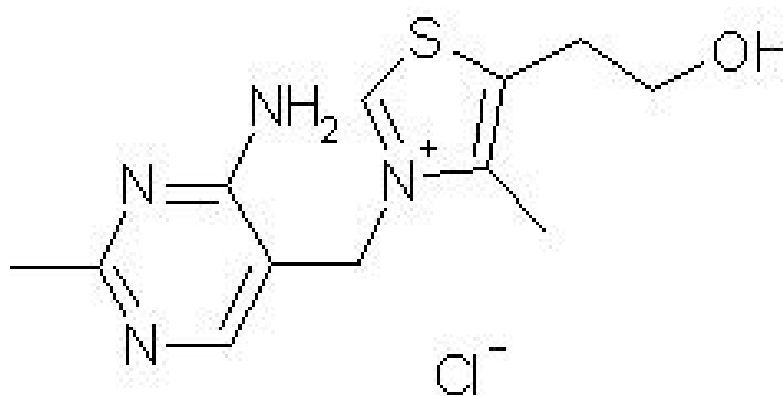
- ***Thiamin (Vitamin B₁)***

Thiamin was first isolated in 1926 from rice polishings by Jansen and Donath. It is a white, water-soluble crystalline solid which is relatively heat stable in dry form but heat labile in solution, particularly when it is alkaline (Begun, 2007). It is named as the thio vitamin (sulfur containing vitamin). First, it was named aneurin for the detrimental neurological effect when it is not present in the diet. It was eventually assigned the generic descriptor name vitamin B₁. Its phosphate derivatives are involved in many cellular processes. The best characterized form is thiamin-pyrophosphate (TPP), a coenzyme in the catabolism of sugars and amino acids. In yeast, TPP is also required in the first step of alcoholic fermentation (Ensminger *et al*, 1995).

All living organisms use thiamin in their biochemistry, but it is only synthesized in bacteria, fungi and plants. Animals must obtain it from their diet, and, thus for them, it is a vitamin. Insufficient intake in birds produces a characteristic polyneuritis, and in mammals, results in a disease called beriberi affecting the peripheral nervous system and/or the cardiovascular system, with fatal outcome if not cured by thiamin administration. In less severe deficiency, nonspecific signs include malaise, weight loss, irritability and confusion. The recommended dietary allowance of thiamin in most countries is set at about 1.4mg (Fox and Cameron, 1989). Thiamin in the food is released by the action of phosphatase and pyrophosphatase in the upper small intestine. At low concentrations, the process of absorption is a carrier-mediated, and at high concentrations, absorption occurs via passive

diffusion. Active transport is greatest in the jejunum and ileum. Decline in thiamin absorption occurs at intakes above 5mg (Orazulike, 2003). Thiamin is found in a wide variety of foods at low concentrations. Yeast, yeast extracts and pork are most highly concentrated sources of thiamin. In general, cereal grains are the most important dietary sources of thiamin. Whole grains contain more thiamin than refined grains.

Some other foods rich in thiamin are oat meal, flax, sunflower seeds, brown rice, asparagus, cauliflower, potatoes, cabbage etc (Combs, 2008).



Vitamin B₁ (Thiamine Chloride)

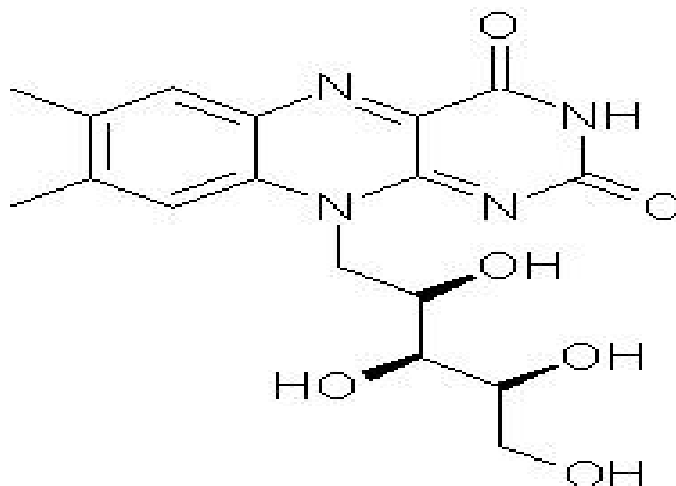
- *Riboflavin (Vitamin B₂)*

Riboflavin, also known as vitamin B₂, or additive E 101, is an easily absorbed micronutrient with a key role in maintaining health in humans and animals. It is the central component of the cofactors FAD and FMN, and is therefore required by all flavoproteins. As such vitamin B₂ is required for a wide variety of cellular processes. It plays a key role in energy metabolism, and for the metabolism of fats, ketone bodies, carbohydrates and

proteins. The recommended dietary allowance of riboflavin for adults is 1.2mg (Bender and Bender, 1999).

The name riboflavin comes from ribose (the sugar whose reduced form, ribitol, forms part of its structure) and 'flavin' the ring moiety which imparts the yellow colour to the oxidized molecule (from Latin flavins – yellow). Riboflavin is best known visually as the vitamin which imparts the orange colour to solid B-vitamins preparations, the yellow color to vitamin supplement solutions, and the usual fluorescent yellow color to the urine of persons who supplement with overdose B complex preparations (Combs, 2008).

Riboflavin deficiency has profound effect on the metabolism of carbohydrates, fats and proteins. All three of these basic food elements require riboflavin if they are to be properly utilized by the body. A deficiency of vitamin B₂ may result in blood shot eyes, abnormal sensitivity to light, itching and burning of the eyes, inflammation in the mouth, a sore and burning tongue, and the malfunctioning of the adrenal glands (Sizer and Whitney, 2003). Regarding the occurrence and sources of riboflavin, yeast extract is considered to be exceptionally rich source. Wheat bran, eggs, meat, milk and cheese are important sources in diets containing these foods. Cereal grains contain relatively low concentrations of flavins, but are important sources in those parts of the world where cereal constitute the staple diet. The milling of cereals results in considerable loss (up to 60 %) of vitamin B₂. Riboflavin is generally stable during heat processing and normal cooking of foods if light is absent (Fox and Cameron, 1989).

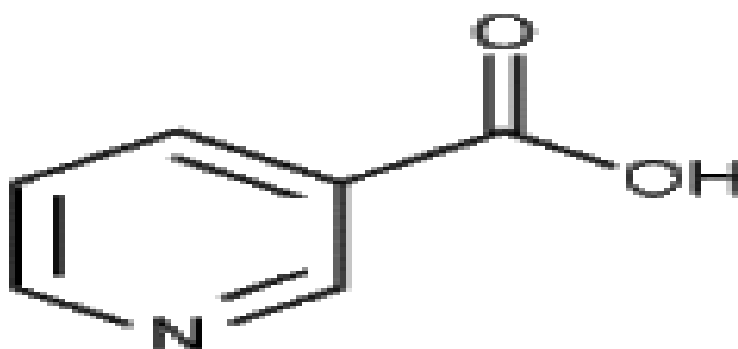


Vitamin B₂ (Riboflavin)

- ***Niacin (Vitamin B₃)***

Niacin (also known as vitamin B₃, nicotinic acid and vitamin PP) is a colourless, water-soluble solid derivative of pyridine. Other forms of vitamin B₃ include the corresponding amide, nicotinamide (niacinamide), as well as more complex amides and a variety of esters. Niacin cannot be directly converted to nicotinamide, but both compounds could be converted to NAD and NADP *in vivo*. Although, the two are identical in their vitamin activity, nicotinamide does not have the same pharmacological effects (lipid modifying effects) as niacin. Nicotinamide does not reduce cholesterol or cause flushing. Nicotinamide may be toxic to the liver at dose exceeding 3g/day for adults. The recommended dietary allowance of niacin is 14mg per day (Whitney and Rolfes, 1998). Niacin is a precursor to NAD + /NADH and NADP +/NADPH, which play essential metabolic roles in living cells. It is involved in both DNA repair and the production of steroid hormones in the adrenal gland. Niacin has been used to increase levels of HDL cholesterol in the blood and has been found to modestly decrease the risk of cardiovascular

events in a number of controlled human trials. Niacin is one of five vitamins associated with a pandemic; vitamin C deficiency (scurvy), thiamin deficiency (beriberi), vitamin D deficiency (rickets), vitamin A deficiency (night blindness) and niacin deficiency (pellagra). Niacin is found in a variety of foods, including liver, chicken, beef, fish, cereal, pea nuts and legumes, and is also synthesized from tryptophan, which is found in meat, dairy and eggs (Combs, 2008).

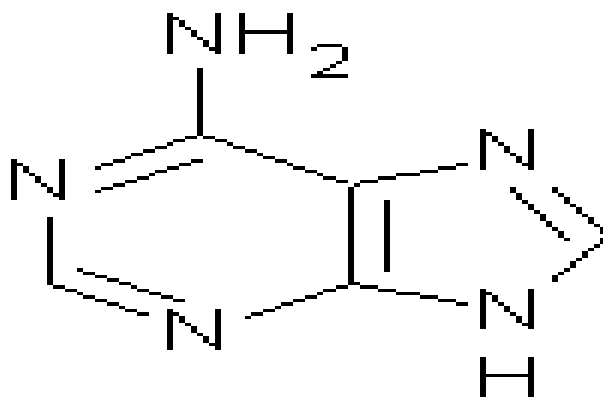


Vitamin B₃ (Niacinamide)

- Adenine (Vitamin B₄)

Adenine is one of the five nitrogenous bases (cytosine, guanine, adenine, thymine and uracil) that helps make up the code in DNA and RNA. Adenine is also an important part of adenosine triphosphate or ATP. Adenine forms adenosine, a nucleoside, when attached to ribose, and deoxyadenosine when attached to deoxyribose, and it forms adenosine triphosphate (ATP), a nucleotide, when three phosphate groups are added to adenosine. ATP is used in cellular metabolism as one of the basic methods of transferring chemical

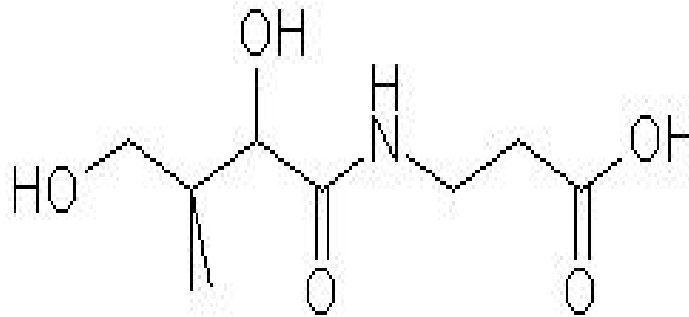
energy between reactions. When administered intravenously, adenosine causes transient heart block in the AV node of the heart. In individuals suspected of suffering from a supraventricular tachycardia (SVT), adenosine is used to help identify the rhythm. Adenine is found in brewer's yeast, whole grains (bread and cereals), raw unadulterated honey, bee pollen, royal jelly, most fresh vegetables, most fresh fruits etc (Ensminger *et al.*, 1995).



Vitamin B₄ (Adenine)

- Pantothenic Acid (Vitamin B₅)

Pantothenic acid is a water-soluble vitamin. For many animals, pantothenic acid is an essential nutrient. Animals require pantothenic acid to synthesize coenzyme-A (CoA), as well as to metabolize proteins, carbohydrates and fats. Its name derives from the Greek pantothen (meaning-from everywhere) and a small quantity of pantothenic acid for adults is 5mg per day (Gopper *et al.*, 2009).

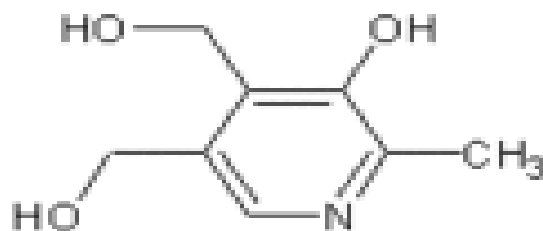


Vitamin B₅ (Pantothenic Acid)

- Pyridoxine (Vitamin B6)

Pyridoxine or vitamin B6 is a water-soluble vitamin and is part of the vitamin B-complex. Several forms of the vitamin are known, but pyridoxal phosphate (PLP) is the active form as a cofactor in many reactions of amino acid metabolism, including transamination, deamination, and decarboxylation. PLP also is necessary for the enzymatic reaction governing the release of glucose from glycogen. The recommended dietary intake for adults is 1.3mg per day (Okaka *et al.*, 2002).

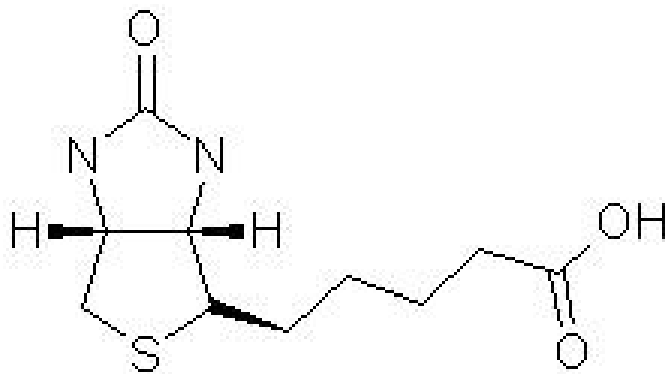
Vitamin B₆ is widely distributed in foods in both its free and bound forms. Good sources include meats, whole grain products, vegetable nuts and bananas. Cooking, storage and processing losses of vitamin B₆ vary and in some foods may be more than 50 %, depending on the form of the vitamin present in the food. Plant foods lose the least during processing as they contain mostly pyridoxine which is far more stable than the pyridoxal or pyridoxamine found in animal foods (Gopper *et al.*, 2009).



Vitamin B₆ (Pyridoxine)

- *Biotin (Vitamin B7)*

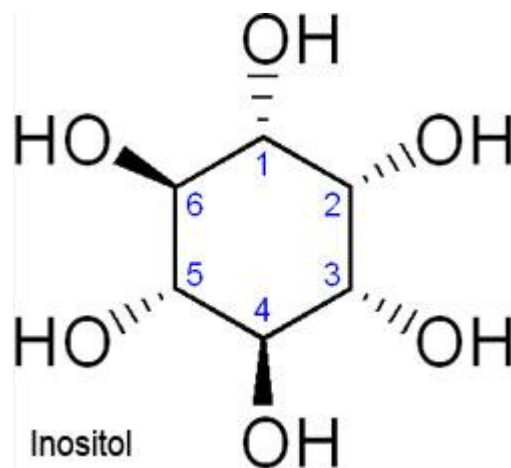
Biotin is a water-soluble B-complex vitamin which was discovered by Bateman in 1916. Biotin is necessary for cell growth, the production of fatty acids, the metabolism of fats and amino acids. It plays a role in the citric acid cycle, which is the process by which biochemical energy is generated during aerobic respiration. Biotin not only assists in various metabolic reactions but also helps to transfer carbon dioxide. Biotin may also be helpful in maintaining a steady blood sugar level. Biotin is often recommended for strengthening hair and nails. As a consequence, it is found in many cosmetics and health products for the hair and skin, though it cannot be absorbed through the hair or skin itself (Whitney and Rolfes, 1998). Biotin deficiency is rare because, in general, intestinal bacteria produce biotin in excess of the body's daily requirements. For that reason, statutory agencies in many countries, for example the USA and Australia, do not prescribe a recommended daily intake of biotin, a number of metabolic disorders in which an individual's metabolism of biotin is abnormal exist (Begun, 2007).



Vitamin B₇ (Biotin)

-Inositol (Vitamin B₈)

Inositol, sometimes called vitamin B₈, is a widely-occurring nutrient generally considered to fall somewhere within the broad B-complex family of vitamins. Foods rich in inositol include unprocessed whole grains, most citrus fruits, unrefined molasses, brewer's yeast, cabbage, lima beans, some nuts, etc (Gopper *et al.*, 2009). While the specifics of this vitamins action in human body remains a mystery, experiments conducted on animals have resulted in the identification of this compound is necessary for the normal growth and survival of organisms (Combs, 2008).

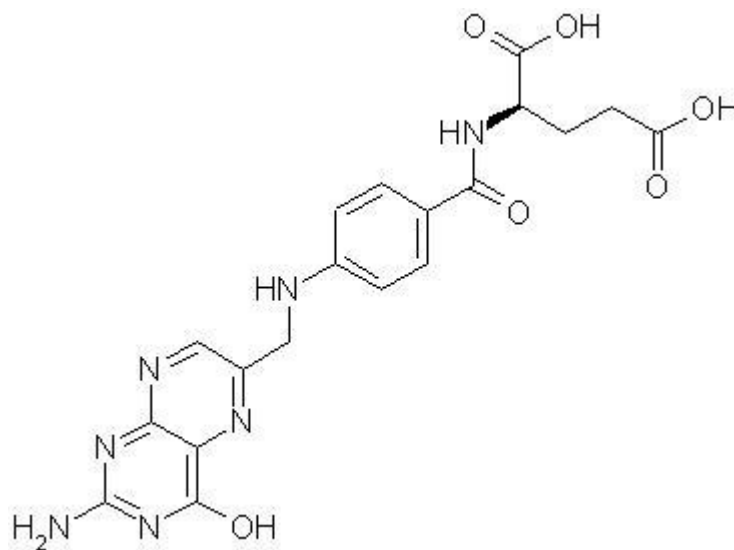


Vitamin B₈ (Inositol)

- Folic Acid (Vitamin B₉)

Folic acid (also known as vitamin B₉, vitamin Bc or folacin) and folate (the naturally occurring form), as well as pteroyl-L-glutamic acid, pteroyl-L-glutamate, and pteroylmonoglutamic acid are forms of the water-soluble vitamin B₉. Folic acid is itself not biologically active, but its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver (James, 1995).

Folic acid is essential to numerous bodily functions. The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in biological reactions involving folate. It is especially important in aiding rapid cell division and growth, such as in infancy and pregnancy, as well as in ‘feeding’ some cancers. While normal diet also high in natural folates may decrease the risk of cancer, there is diverse evidence that high folate intake from supplementation may actually promote some cancers as well as precancerous tumours and lesions. Children and adults both require folic acid to produce healthy red blood cells and prevent anemia. The recommended dietary intake of folic acid for adults is 400µg per day. Green vegetables are the good source of folic acid (Brown, 2004).

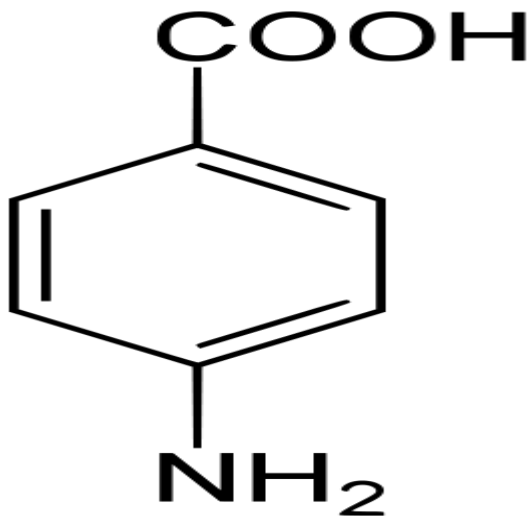


Vitamin B₉ (Folic acid)

- *Para amino benzoic acid (Vitamin B₁₀)*

Vitamin B₁₀, also known as factor R was later determined to be pteroylmonoglutamic acid mixed with other B-vitamins. Its deficiency caused slowed growth and deteriorated feather development in chicks, along with blood problems. It was believed by some researchers to apply to humans. Some early researchers used the term vitamin B₁₀ to denote para-amino-

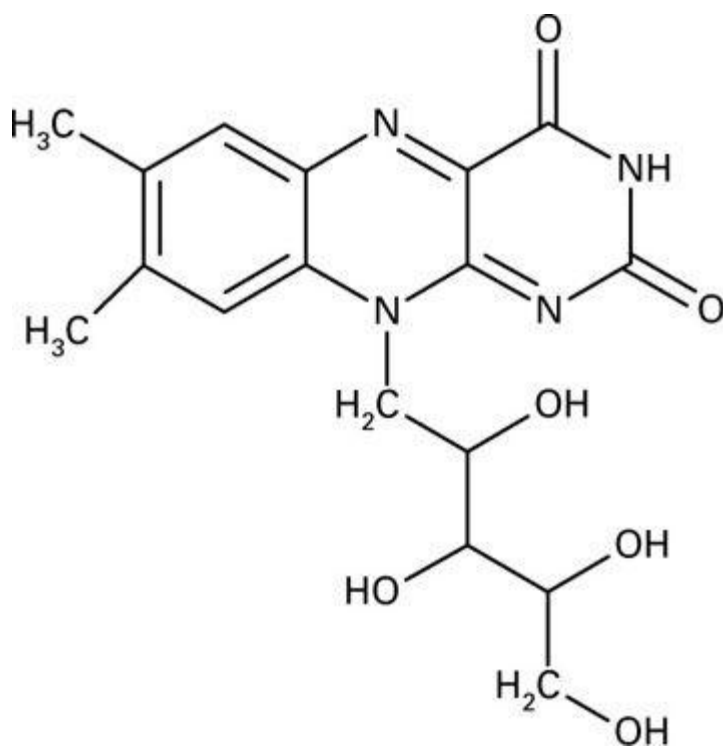
benzoic acid. Vitamin B₁₀ is increasingly used as a permanent skin protection against the damages caused by pollution and the sun. It also has alleged anti-inflammatory and anti-allergic effects. This B-complex vitamin is important for the skin's growth and normal colour. Natural sources of vitamin B₁₀ are bran, kidney, liver, molasses, wheat germ, yoghurt etc (Gopper *et al.*, 2009).



Vitamin B₁₀ (Para amino benzoic acid)

- ***Vitamin B₁₁ (Pteryl-Hepta-Glutamic Acid)***

Vitamin B₁₁ is a form of folic acid (one of five folates necessary for humans) and is now known as 'chick growth factor'. Vitamin B₁₁ functions in DNA and RNA syntheses, cell division and development of the fetus nervous system. The deficiencies of vitamin B₁₁ include lack of appetite, weight loss, nausea and vomiting, diarrhea, low immunity etc. Sources of vitamin B₁₁ include leafy vegetables, eggs, lentils, rice, wheat germ, liver, kidneys, bananas, oranges etc. (Gopper *et al.*, 2009).

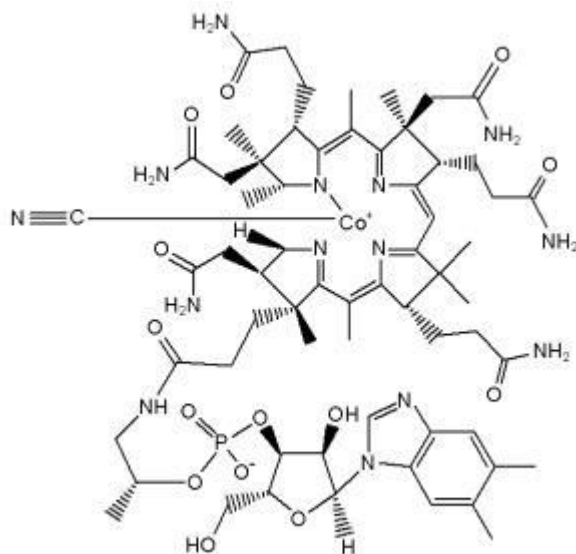


Vitamin B₁₁

- *Cobalamin (Vitamin B₁₂)*

Vitamin B₁₂ also called Cobalamin is a water-soluble-vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is normally involved in the metabolism of every cell of the human body, especially DNA synthesis and regulation, fatty acid synthesis and energy production. Vitamin B₁₂ consists of a class of chemically-related compounds (vitamers), all of which have vitamin activity. It contains the biochemically rare element cobalt. Biosynthesis of the basic structure of the vitamin in nature is only accomplished by simple organisms such as bacteria and algae, but conversion between different forms of the vitamin can be accomplished in the human body. The recommended dietary intake for an adult for this vitamin ranges from 2 to 3µg per day (Combs, 2008). Vitamin B₁₂ deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels only slightly lower than

normal, a range of symptoms such as fatigue, depression and poor memory may be experienced. However, these symptoms by themselves are too nonspecific to diagnose deficiency of the vitamin. Vitamin B₁₂ is found in foods that come from animals, including fish and shellfish, meat (especially liver), poultry, eggs, milk and milk products etc (Srilakshmi, 2006).



Vitamin B₁₂ (Cobalamin)

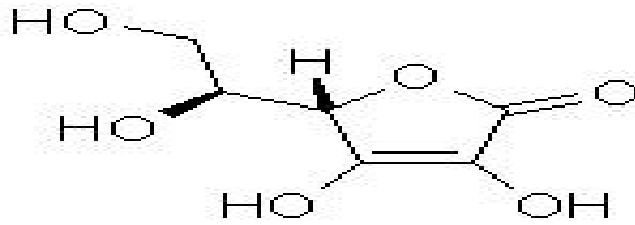
- ***Vitamin C (Ascorbic Acid)***

Vitamin C or ascorbic acid is a white water-soluble solid of formula C₆H₈O₆. The vitamin isolated and its chemical structure elucidated in 1932 by C.G. King (Mudambi and Rajagopal, 2008). In spite of the name ascorbic acid, the molecule does not contain a free carboxyl group. It is really a lactone formed from the free acid by loss of water between a carboxyl group on one carbon atom and a hydroxyl group on another. Lactones behave much like acids and for many purposes can be regarded as acids. Ascorbic acid has the sharp taste usually associated with acids and form salts (Fox and Cameron, 1989).

Vitamin C is essential to a healthy diet as well as being a highly effective antioxidant, acting to lessen oxidative stress; a substrate for ascorbate peroxidase in plants, and an enzyme cofactor for the biosynthesis of many important biochemicals. Vitamin C acts as an electron donor for important enzymes. Ascorbic acid performs other numerous physiological functions in the human body. These functions include the synthesis of collagen, carnitine and neurotransmitters, the synthesis and catabolism of tyrosine and the metabolism of microsome (Brown, 2004).

Scurvy, the most severe form of vitamin C deficiency is relatively rare throughout the world. Faulty cooking habits and inadequate intake of fresh vegetables and fruits are the major causes of dietary deficiency of vitamin C. Early symptoms are non specific, but include listlessness, fatigue, and weakness, shortness of breath, muscle cramps, aching bones, joints and muscles and loss of appetite. The clinical features of scurvy are characterized by gingivitis (bleeding gums), petechiae (small haemorrhagic spots), arthralgia (pain in the joint), a depression, postural hypotension and a marked limitation to perform strenuous work. Delayed wound healing occurs due to reduced synthesis of connective tissue (Srilakshmi, 2006).

The outstanding sources of vitamin C are the citrus fruits (oranges, lemons, limes and grapefruit), other fresh fruits, and green leafy vegetables. Tomatoes, cabbage, cauliflower, broccoli provide liberal quantities of ascorbic acid. Potatoes, green beans, peas and apples are important sources of the vitamin because they are consumed in large quantities (Kareem and Adebawale, 2007).



Vitamin C (Ascorbic acid)

2.7.8.0 Anti-nutritional factors

The main problems in nutritional exploitation of green leafy vegetables are the presence of anti-nutrients and toxic principles. These factors are usually present in trace amount but they do have profound effects on the nutritional potential of the vegetables. The presence of anti-nutritional components such as phytic and oxalic acids in green leafy vegetables has impairment on the efficient utilization of calcium and magnesium by formation of indigestible complexes with the mineral elements. Saponin and tannin are other anti-nutritional components of interest in vegetables studies (Adebayo and Babajide, 2007).

These substances render some important food components to be not available to consumers by binding with them. They include phytates, oxalates, tannins, cyanides, glucosinolates and hemagglutinins depending on the type of plant (Gilani *et al*, 2007).

Digestibility of protein in traditional diets from developing countries such as India, Guatemala, and Brazil has been found to be considerably lower compared to that of protein in typical North American diets (54–78 versus 88–94%). This presence of less digestible protein fractions, high levels of insoluble fiber, and high concentrations of anti-nutritional factors in the diets of developing countries, which are based on less refined cereals and grain legumes as major sources of protein, are responsible for poor digestibility of protein

(Gilani *et al.*, 2007). The effects of the presence of some of the important antinutritional factors on protein and amino acid digestibility of food and feed products are discussed elsewhere (Fasuyi, 2007; Omoruyi *et al.*, 2007; Gilani *et al.*, 2005 and Abdullahi *et al.*, 2007).

Food and feed products may contain a number of antinutritional factors that may adversely affect protein digestibility and amino acid availability. Antinutritional factors may occur naturally, such as glucosinolates in mustard and rapeseed protein products, trypsin inhibitors and hemagglutinins in legumes, tannins in legumes and cereals, phytates in cereals and oilseeds, and gossypol in cottonseed protein products. Antinutritional factors may also be formed during heat/alkaline processing of protein products, yielding many compounds, oxidized forms of sulfur amino acids, D-amino acids, and lysinoalanine (LAL, an unnatural amino acid derivative). The presence of high levels of dietary trypsin inhibitors from soybeans, kidney beans, or other grain legumes can cause substantial reductions in protein and amino acid digestibilities (up to 50 %) in rats and pigs. Similarly, the presence of high levels of tannins in cereals, such as sorghum, and grain legumes, such as fababean (*Vicia faba* L.), can result in significantly reduced protein and amino acid digestibilities (up to 23 %) in rats, poultry, and pigs. Studies involving phytase supplementation of production rations for swine or poultry have provided indirect evidence that normally encountered levels of phytates in cereals and legumes can reduce protein and amino acid digestibility by up to 10%. D-amino acids and L-alanine formed during alkaline/heat treatment of proteins such as casein, lactalbumin, soy protein isolate, or wheat proteins are poorly digestible (less than 40 %), and their presence can reduce protein digestibility by up to 28 % in rats and pigs (Gilani *et al.*, 2005) . A comparison of the

protein digestibility determination in young (5-week) versus old (20-month) rats suggests greater susceptibility to the adverse effects of anti-nutritional factors in old rats than in young rats. Therefore, the inclusion of protein digestibility data obtained with young rats, as the recommended animal model, in the calculation of PDCAAS (Protein Digestibility-Corrected Amino Acid Score) may overestimate protein digestibility and quality of products, especially those containing anti-nutritional factors, for the elderly. For products specifically intended for the elderly, protein digestibility should be determined using more mature rats (Fasuyi, 2007; Omoruyi *et al.*, 2007; Gilani *et al.*, 2005 and Abdullahi *et al.*, 2007).

2.7.8.1 Phytic acid

It is an acid with the formula $(C_6H_6[OPO(OH)_2]_6)$ and found in seeds of plants as the insoluble calcium magnesium salt (phytin); derived from corn steep liquor; inhibits calcium absorption in intestine; used to treat hard water, to remove iron and copper from wines, and to inactivate trace-metal contaminants in animal and vegetable oils (Rimbach *et al.*, 1998). It is a combination of inositol (a substance related to hexose sugars) and phosphorous. In the gut, phytic acid reduces the absorption of some minerals (e.g. calcium, iron, magnesium, and zinc) by forming insoluble salts with the minerals. Relatively high concentrations of phytic acid occur in wholegrain cereals and some other foods rich in fiber. Phytic acid (known as inositol) hexakisphosphate or phytate when in salt (form) is the principal storage form of phosphorus in many plant tissues, especially branch and seeds. Phosphorus in this form is generally not bioavailable to non-ruminant animals because they lack the digestive enzyme, phytase, required to separate phosphorus from the phytate

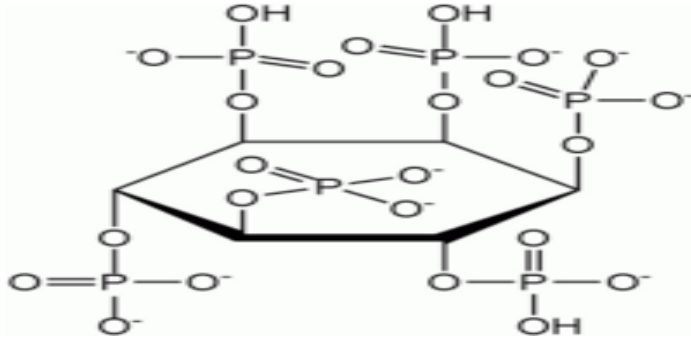
molecule (Rimbach *et al.*, 1998). On the other hand, ruminants readily utilize phytate because of the phytase produced by rumenmicroorganisms. Phytic acid (myo-inositol 1,2,3,4,5,6 hexakisdihydrogenphosphate), the main phosphorus storage of cereals, legumes and oilseeds, is known to bind essential divalent cations such as calcium, magnesium, iron, zinc and manganese, forming largely insoluble complexes, and thereby decreasing their bioavailability in humans and other monogastric animals (Zhou and Erdman, 1995). In contrast to the anti-nutritive effects, potential benefits of PA and myo-inositol were observed in azoxymethane- (Shamsuddin and Ullah, 1989) and dimethylhydrazine- (Shamsuddin *et al.*, 1989) induced colon cancer, pulmonary adenoma (Estensen and Wattenberg, 1993) and, in the case of transplanted fibrosarcoma (Vucenik *et al.*, 1992), in mice and rats. Furthermore, in a wide-spectrum organ carcinogenesis model, Hirose *et al.*, (1991) showed that a supplement of 2% phytic acid inhibited development of neoplastic lesions in the liver and pancreas. In vitro studies clearly demonstrated that phytic acid also reduced cell proliferation rate in different cell lines, including erythroleukemia (Shamsuddin *et al.*, 1992) and human mammary cancer (Shamsuddin *et al.*, 1996).

The basic mechanisms of the anti-carcinogenic effect of phytic acid are still not clear. Phytic acid may exert anticarcinogenic benefits in part because of its antioxidant capability (Thompson and Zhang, 1991). A function of phytic acid as a natural antioxidant was first postulated by Graf *et al.*, (1984). Experimental evidence indicates that in the presence of phytic acid, iron-catalyzed generation of hydroxyl radicals may be blocked (Graf *et al.*, 1987). Hydroxyl radical formation was measured spectrophotometrically by the formation of formaldehyde in the presence of dimethylsulfoxide (Graf *et al.*, 1984, Hawkins *et al.*, 1993). However, the most direct technique available for the detection of free radicals is

electron spin resonance spectroscopy (ESR). Spin trapping involves the addition reaction of the free radical of interest to a diamagnetic compound (spin trap) to produce a relatively stable spin adduct. The information about the radical trapped is contained in the hyperfine splitting of the spin adducts, whereas the amplitude of the signal enables the amount of free radicals generated to be quantified (Buettner, 1987). There is a lack of information on the effect of phytic acid on hydroxyl and superoxide radical formation using ESR. Furthermore, most in vivo experiments showing anti-carcinogenic effects of PA failed to consider the mineral supply and indicators of oxidative damage in the experimental animals as pointed out previously (Pallauf and Rimbach 1997; Zhou and Erdman 1995).

Calcium is first added to flour because of the suspicion that high levels of phytic acid in bread interfere with calcium absorption and cause rickets (Zhou and Erdman, 1995). However, the link between rickets and foods rich in phytic acid is far from conclusive and it is not certain to what extent these foods interfere with mineral absorption. There is some evidence that those who regularly eat high fibre diets adapt to the high phytic acid content by secreting an enzyme which can break phytic acid down into inositol and phosphorus (Pallauf and Rimbach, 1997).

However, a component of some high-fiber foods, including many cereal grains that may, in excessive amounts, cause constipation or interfere with the body's ability to absorb minerals.



Structure of phytic acid

In most commercial agriculture, non-ruminant livestock such as swine and poultry are fed mainly grains such as soybeans and maize. Because phytate from these grains is unavailable for absorption, the unabsorbed phytate passes through the gastrointestinal tract, elevating the amount of phosphorus in the manure. Excess phosphorus excretion can lead to environmental problems such as eutrophication.

The bioavailability of phytate phosphorus can be increased by supplementation of the diet with phytase enzyme. Also, viable low phytic acid mutant lines have been developed in several crop species in which the seeds have drastically reduced levels of phytic acid and concomitant increases in inorganic phosphorus. However, reported germination problems have hindered the use of these cultivars thus far.

Phytic acid is found within the hulls of nuts, seeds, and grains. In-home food preparation techniques can reduce the phytic acid in all of these foods. Simply cooking the food will reduce the phytic acid to some degree. More methods that are effective are soaking in an acid medium, lactic acid fermentation, and sprouting.

Phytic acid is a strong chelator of important minerals such as calcium, magnesium, iron and zinc and can therefore contribute to mineral deficiencies in people whose diets rely on these foods for their mineral intake such as those in developing countries. In this way, it is an anti-nutrient.¹ For people with a particularly low intake of essential minerals, especially young children and those in developing countries, this effect can be undesirable.

Dietary mineral chelators helps, through reducing the available minerals, to prevent over-mineralization of joints, blood vessels, and other parts of the body, which is most common in older persons. They do not correct the disorder that causes this negative distribution of substances though, and can reduce the availability of these minerals for other essential processes.

Some of the therapeutic uses of phytic acid are that it may be considered as phytonutrient, providing an antioxidant effect. Phytic acid's same mineral binding properties may also prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract. It may also reduce the risk of colon cancer (Rimbach *et al.*, 1998). Researchers now believe that phytic acid, found in the fiber of legumes and grains, is the major ingredient responsible for preventing colon cancer and other cancers (Rimbach *et al.*, 1998).

Phytic acid is often considered as an anti-nutrient because it forms insoluble complexes with minerals such as zinc, calcium, magnesium and iron. Phytic acid has a structure similar to that of myo-inositol, which has been demonstrated to reduce hepatic lipid levels. The addition of 5 to 10 percent phytic acid resulted in a very depressed growth and food intake. These levels are very high compared to the 0.035 % phytic acid in the Western diet. Hepatic levels of triglyceride and cholesterol and lipogenic enzymes activity were reduced

with increasing dietary phytate level. The addition of 10 % sodium phytate drastically depressed growth, food intake, and serum triglyceride and cholesterol levels. The lower level of phytic acid (0.013 %) significantly reduced hepatic serum levels of lipids and triglycerides. Intake of phytic acid resulted in reduced hepatic cholesterol concentrations, increased fatty acid synthetase and reduced hepatic malic enzyme activity. Serum lipid levels were not much influenced by phytic acid intake. The study concluded that dietary intake of phytic acid at a level of 0.035 % may protect against a fatty liver resulting from elevated hepatic lipogenesis, and that the anti-nutrient effect of phytic acid on mineral absorption will only occur at 10 fold higher levels. The researchers even speculated that phytic acid might be considered more like a vitamin than an anti-nutrient (Onomi *et al.*, 2004).

Phytic acid's chelating effect may serve to prevent, inhibit, or even cure some cancers by depriving those cells of the minerals (especially iron) they need to reproduce. The deprivation of essential minerals like iron would, much like other broad treatments for cancers, also have negative effects on non-cancerous cells. It is unknown if this would affect other cells in the body that require iron (such as red blood cells) or if the deprivation of minerals is more localized to the internal colon region.

Phytic acid has no known toxicity and is not known to cause mutagenic activity. It may have more therapeutic value when added to water rather than when naturally absorbed in foods as it is difficult to free from fiber (Rimbach *et al.*, 1998).

Phytic acid is one of the few chelating therapies for uranium removal. As a food additive, phytic acid is used as a preservative.

Food must be well cooked in order to free from the fiber and enable it to be absorbed in the system. IP6 rarely appears in soluble fiber. It's usually attached to the bran, the hard (insoluble) fiber, which is difficult to digest. IP6 is found in legumes, peas, wheat, barley, and oats. Of any studied legumes, whole soybeans have the highest levels of phytic acid. Those who argue for the beneficial effects of phytic acid, and freeing it up for interaction with the system through cooking; do not argue that cooking destroys the phytic acid. This is a major deviation between those arguing for the merits of processed grain products.

2.7.8.2 Oxalic Acid

Oxalic acid (chemical formula HOOC-COOH) is a strong organic acid, which is widely distributed in nature in both plants and animals with more in plants than animals. The name comes from the plant *Oxalis* (wood sorrel) from which it was first isolated. Oxalic acid has the ability to form a strong bond with various minerals, such as sodium, potassium, magnesium, and calcium. When this occurs, the compounds formed are usually referred to as oxalate salts. Thus, "oxalate" usually refers to a salt of oxalic acid, one of which is calcium oxalate (Lieberman and Costa, 2000). Although both sodium and potassium oxalate salts are water soluble, calcium oxalate is practically insoluble, which is why calcium oxalate, when present in high enough levels, has the propensity to precipitate (or solidify) in the kidneys or in the urinary tract to form calcium oxalate crystals. Calcium oxalate crystals, in turn, contribute to the formation of kidney stones. Approximately 75 % of all kidney stones are composed predominantly of calcium oxalate. For reasons not yet fully understood, women have a much lower incidence of kidney stone (Lieberman and Costa, 2000).

It has been found that most foods do not contain significant amounts of oxalate. The primary sources of dietary oxalate are plants and plant products. Although the physiological role of oxalate in plants is not clearly understood, it is well established that a number of plants have the ability to synthesize oxalate. Seeds and leafy plants related to spinach and rhubarb contain the most oxalate. Many plant foods contain very low levels of oxalate. Foods of animal origin contain negligible amounts of oxalate (Menon and Mahle, 1982).

2.7.8.3 Tannins

Tannins are generally defined as naturally occurring polyphenolic compounds of high enough molecular weight that form complexes with proteins. These are classified into two groups based on their structural types a) hydrolysable tannins and b) condensed tannins. Tannins are one of the many types of secondary compounds found in plants which are oligomeric in nature with multiple structure units having free phenolic groups, molecular weight ranging from 500 to >20,000, soluble in water, with exception of some high molecular weight structures and an ability to bind proteins and form insoluble or soluble tannin-protein complexes.

The subdivision in to the groups is also considered as: hydrolysable tannins (HT) and proanthocyanidins (PA) (often called Condensed Tannins).The hydrolyzable tannins are molecules with a polyol (generally D-glucose) as a central core. The hydroxyl groups of these carbohydrates are partially or totally esterified with phenolic groups like gallic acid (gallotannins) or ellagic acid (ellagitannins) and are usually present in low amounts in plants.

Some authors define two additional classes of hydrolysable tannins: taragallotannins (gallic acid and quinic acid as the core) and caffetannins (caffeic acid and quinic acid) (Hagenah and Gross, 1993; Niemetz and Gross, 2001).

- *Gallotannins*

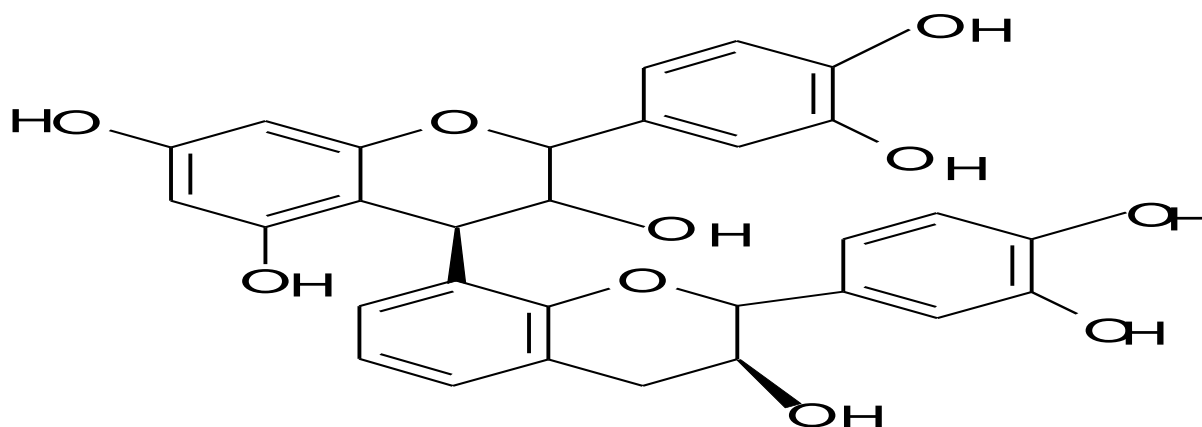
The phenolic groups that esterify with the core are sometimes constituted by dimers or higher oligomers of gallic acid (each single monomer is called galloyl). Each HT molecule is usually composed of a core of D-glucose and 6 to 9 galloyl groups in nature, there is abundance of mono and di-galloyl esters of glucose (MW about 900). They are not considered to be tannins. At least 3 hydroxyl groups of the glucose must be esterified to exhibit a sufficiently strong binding capacity to be classified as tannin. The most famous source of gallotannins is tannic acid obtained from the twig galls of *Rhus semialata*. It has a penta galloyl-D-glucose core and five more units of galloyl linked to one of the galloyl of the core (Hagenah and Gross, 1993; Niemetz and Gross, 2001).

- *Ellagitannins*

The phenolic groups consist of hexahydroxydiphenic acid, which spontaneously dehydrates to the lactone form, ellagic acid. Molecular weight range: 2000-5000. Properties Hydrolysable tannins (HT): mild acids or mild bases to yield carbohydrate and phenolic acids hydrolyze them. Under the same conditions, proanthocyanidins (condensed tannins) do not hydrolyze. HTs are also hydrolyzed by hot water or enzymes (i.e. tannase).

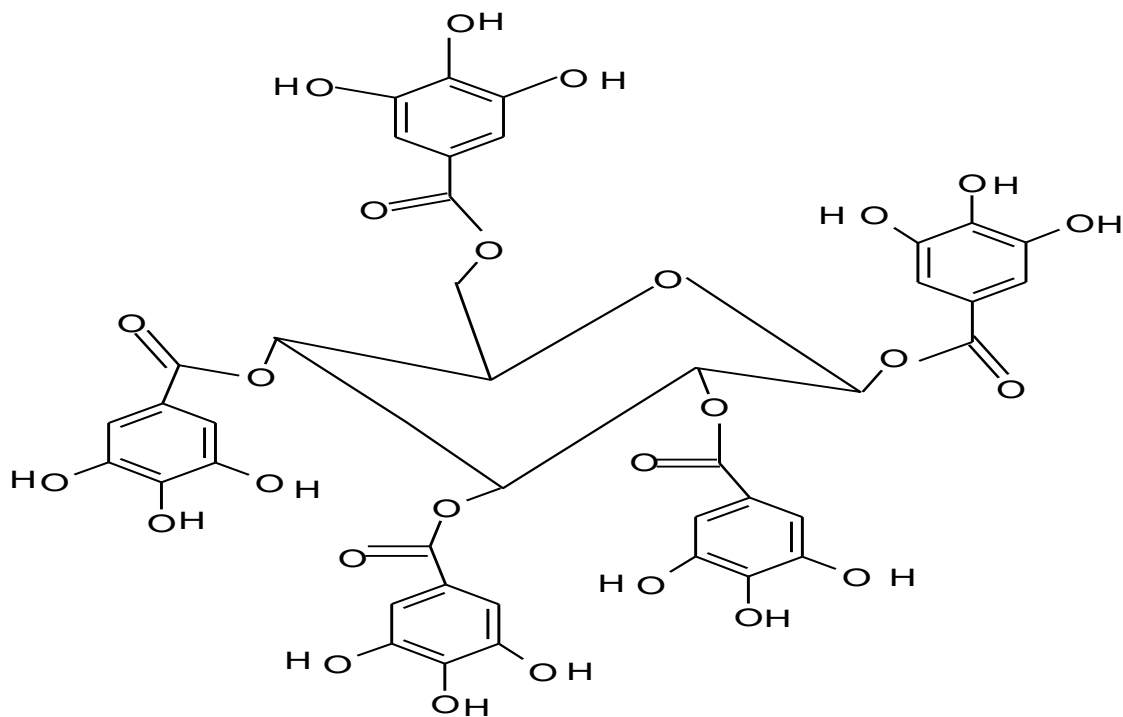
- *Proanthocyanidins (condensed tannins)*

PAs are more widely distributed than HTs. They are oligomers or polymers of flavonoid units (i.e. flavan-3-ol) linked by carbon-carbon bonds not susceptible to cleavage by hydrolysis. PAs are more often called condensed tannins due to their condensed chemical structure.



Epicatechin

However, HTs also undergo condensation reaction. The term, condensed tannins, is therefore potentially confusing. The term, proanthocyanidins, is derived from the acid catalyzed oxidation reaction that produces red anthocyanidins upon heating PAs in acidic alcohol solutions. The most common anthocyanidins produced are cyanidin (flavan-3-ol, from procyanidin) and delphinidin (from prodelphinidin) (Stafford, 1983).



1,2,3,4,6-pentagalloyl-o-D-glucose

PAs may contain from 2 to 50 or greater flavonoid units; PA polymers have complex structures because the flavonoid units can differ for some substituents and because of the variable sites for interflavan bonds.

Anthocyanidin pigments are responsible for the wide array of pink, scarlet, red, mauve, violet, and blue colors in flowers, leaves, fruits, fruit juices, and wines. They are also responsible for the astringent taste of fruit and wines.

PA carbon-carbon bonds are not cleaved by hydrolysis. Depending on their chemical structure and degree of polymerization, PAs may or may not be soluble in aqueous organic solvents. Tannins attract the interest of plant breeders because of two contradictory reasons: their negative effect on the nutritional quality of the varieties of plants produced, and the

role they play in certain defense mechanisms against pathogens and pests (Broadhurst and Jones, 1978). The former make it an imperative to analyze the green leafy vegetables in our localities for tannin contents.

2.7.8.4 Hydrocyanic acid

Hydrocyanic acid (prussic acid) is an aqueous, colorless solution with a characteristic almond odor, and contains hydrogen cyanide (HCN) at a concentration of 2 to 4 %w/w (Martindale, 1989, Martindale, 1977). Hydrocyanic acid is listed also as a synonym for hydrogen cyanide (Myers, 1998), which is used as a gas for the eradication of pests and is intensely poisonous (Martindale, 1989).

Very small levels of hydrocyanic acid are found in many plants, being produced as a breakdown product from a type of secondary metabolite known as cyanogenic glycosides.

2.7.8.4.1 Toxicity of cyanides

Hydrocyanic acid and its vapor are intensely poisonous, and signs and symptoms of toxicity are the same as those for cyanide (Ellenhorn and Banceloux, 1998).

Cyanide inhibits the mitochondrial respiratory chain enzyme cytochrome oxidase, an enzyme necessary for cellular oxygen transport. Poisoning by cyanides may occur from inhalation of the vapor, ingestion, or absorption through the skin. Poisoning may arise from cyanide pesticides, industrial accidental exposure, or the inhalation of fumes from some burning plastics. Poisoning may also occur from 'cyanide-containing' plants or fruits (Martindale, 1998).

Signs of acute cyanide poisoning include headache, vertigo, agitation, confusion, coma, convulsions and death. When large doses of hydrocyanic acid are taken, unconsciousness occurs within a few seconds and death within a few minutes (Martindale, 1989). Death through inhalation occurs in 1 hour at 100 ppm, while 300 ppm exposures prove fatal within several minutes. The fatal dose of orally ingested hydrocyanic acid for adult humans is considered to be about 50 mg, and of the K or Na cyanide salts, 200 – 500 mg (Myers, 1998). The calculated lethal dose of KCN in children is 1.2 – 5 mg/kg. In the U.K. the control exposure limit of hydrogen cyanide is 10ppm (short-term), and the recommended exposure limit of cyanides (as CN^-) is 5mg per m^3 (long-term) (Martindale, 1989, Ellenhorn and Baceloux, 1998).

The LD_{50} of hydrogen cyanide is listed as being 0.5 mg/kg (Martindale, 1989, Myers, 1998), meaning that a child weighing 10kg could be killed by a 5 mg dose of hydrogen cyanide .

Cyanide is primarily considered as a neurotoxin, and various neurological conditions including ataxia, hypertonia, neuromyopathies and blindness as well as impaired thyroid function have been associated with chronic exposure (Martindale, 1989, Ellenhorn and Baceloux, 1998). With smaller toxic doses the symptoms, which occur within a few minutes, may include constriction of the throat, nausea, vomiting, giddiness, headache, palpitation, hyperpnoea then dyspnoea, bradycardia (initially tachycardia may occur), unconsciousness, and violent convulsions, followed by death. The characteristic smell of bitter almonds may not be obvious. Cyanosis is not prominent. Similar but usually slower effects occur with cyanide salts (Myers, 1998).

Bioavailability of hydrocyanic acid in animals or humans is generally assessed through measuring plasma thiocyanate levels (Myers, 1998, Vettler, 2000).

2. 7. 8.4. 2. Cyanogenic glycosides

Cyanogenic glycosides occur in at least 2,500 known plants (Vettler, 2000), and many of these belong to the *Fabaceae*, *Rosaceae*, *Linaceae*, *Asteraceae* families. They are composed of an alpha-hydroxynitrile type aglycone and a sugar moiety (mostly D-glucose). A large number of the most important human food plants contain cyanogenic glycosides, and these phytochemicals appear to have a natural function as chemoprotective agents against herbivores (Jones, 1988). Cyanogenic glycoside containing plants widely used in phytotherapy include Wild Cherry bark (*Prunus serotina*), which contains prunasin), Elder (*Sambucus spp*) which contains sambunigrin, Linseed (*Linum spp*), which contains linustatin and neolinustatin (Cunnane, 1993), and various species of Clover (*Trifolium spp*) (Buhrmester, 2000). As is common in the plant kingdom, widespread variability in levels of these secondary metabolites is known to occur within different populations of the same species (Buhrmester, 2000).

One of such widely used root crops is cassava (*Manihot esculenta*), and is a staple plant food for more than 500 million people in developing countries particularly in Africa. The root of this plant contains a glucoside with a high content of the cyanogenic glycoside linamarin (also found in lima beans), as well as protein and other nutrients (Carlsson, 1999, Carod-Artal, 1999).

Orally ingested linamarin is relatively poorly bioavailable, about one-quarter being excreted unchanged and another quarter metabolized into a yet unknown compound (Carlsson, 1999, Carod-Artal, 1999).

Excessive intake of Cassava which has not been properly processed to reduce hydrocyanic acid content, especially in diets based almost exclusively on it for a long period of time, is associated with elevated levels of plasma thiocyanate, various neurological syndromes, hypothyroidism and goitre (Kamalu and Agharanya, 1991, Carod-Artel, 1999, Kuti and Kuti, 1999, and Vettler, 2000).

Hydrocyanic acid levels of 12 leafy vegetables were measured by Nigerian researchers, who found that the values ranged from 4.32 – 23.8 mg per 100g of dried mass (Udosen and Ukpahah, 1993). Hydrocyanic acid content of Cherry Laurel leaves is reported as being in the region of 50-210 mg/100 gm, or 0.05 to 0.2 % by the most recent source (Myers, 1998), although other authors report levels ranging from 0.1 to 5 %, young leaves apparently yielding more than old (Evans 1996, Grieve, 1931).

2.8 Neutron activation analysis

Neutron activation analysis (NAA) may be defined as a method of measuring concentrations of constituents in a given sample by measuring the characteristic radiations emitted by the radioactive nuclides resulting from selected nuclear transformations occurring in a neutron flux. Though the existence of neutrons was predicted by Rutherford in 1920 and first observed by Chadwick in 1932 with great penetrating power and undeflected by both electric and magnetic fields (neutral) and was found to have

approximately the mass as the proton its analytical significance was not realized by then .

It was soon after the discovery of induced radioactivity, Hevesy and Levi in 1936 applied neutron activation to an analytical problem. From this beginning the technique grew, gaining momentum with the development of nuclear reactors by Fermi in 1942 with available neutron fluxes of the order of 10^{12} neutrons $\text{cm}^{-2} \text{ s}^{-1}$. By the early 1950s review papers outlining basic principles of the method, applications and experimental data were available. Since this development, a lot of studies had been carried out using neutron activation analysis. These studies include among others: determination of elements in plants and life sciences (Nadkarni and Ehman 1969; Ondov, 1975; IAEA, 1967; Bowen *et al.*, 1963; Nadkarni and Morrison, 1974 and Wilkinson, 1969).

Likewise, NAA has become a routine method for elemental analysis in many fields of human endeavor like archeology, where use of trace element patterns are used to link artifacts and their sources and Geochemistry where trace elements in rocks, mineral exploration (Graham *et al.*, 1982). Others are biomedical sciences where trace elements linked to specific diseases and the monitoring of trace elements in preventive medicine (Rapaport *et al.*, 1979; Sivasankara, 1975) and material science where trace elements are measured in high purity semi-conductors and alloys.

Some of the analytical works by NAA technique are as follows: the determination of platinum in biological tissue (Haveland and Wiebe, 1991); determination of Br, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V in cereals, oils, sweeteners and vegetables (Soliman, 1999); multi-element instrumental neutron activation analysis of biological tissue using a

single comparator standard and processing by computer (Lineken, 1973); determination of nanogram of quantities of gold in biological tissues (Sparaque *et al.*, 1979); rapid determination of antimony in biological and environmental samples using instrumental neutron activation analysis (Valente *et al.*, 1978) and environmental pollution study by multi-elemental neutron activation analysis of garden soil samples from Nagpur city, India (Chutke *et al.*, 1994).

Few recent works by NAA are analysis of *Guera Senegalensis* (combrataceae) - a tropical medicinal plant (Funtua *et al.*, 2003); determination of some elements in *Ageratum conyzoides* Linn - a tropical medicinal plant (Dim *et al.*, 2002); an experiment using neutron activation analysis and a rare earth element to mark cotton plants and two insects that feed on them (Showler, 2006); neutron activation analysis of leaf samples from different parts of Abuja Metropolis (Kogo *et al.*, 2009); the analysis of chrome tanned leather shavings using neutron activation analysis (Achi, *et al.*, 2012); analysis of essential elements for plants growth using instrumental neutron activation analysis (Njinga, 2013) and instrumental neutron activation analysis of honey samples from Lokoja and Suleija , North Central Nigeria (2013).

2.8.1 Principles and practice of NAA

NAA technique is based on the principles that whenever neutrons impinge on any ordinary material, some of the neutrons are captured by the constituent elements of the material and become radioactive (Haskin and Ziege, 1971). The interaction of the neutrons with matter is almost exclusively by collision. When an interaction takes place the energy of the neutron is rapidly distributed throughout the target nucleus resulting in the formation of a

compound nucleus in an excited state. The excitation energy arises from the binding energy roughly 8 MeV of the added neutron in the given nucleus and also from the kinetic energy of the captured neutron which may range from several MeV for fast neutron down to a fraction of 1 eV for a thermal neutron. The mode of de-excitation depends on the energy of the incident neutron. There are three (3) neutron reactions that are of interest in neutron activation analysis: capture reaction, transmutation and inelastic scattering.

2.8.1.1 Reactor Neutron Activation Analysis

This is a method in which neutrons in the reactor with certain amount of energy are used to bombard a sample, making stable isotopes of the elements to be determined in the sample become radioactive. Thus the qualitative and quantitative analyses of the elements are determined by measuring the delayed gamma (γ) radiation released from the decay of the radioactive nuclide produced in nuclear reaction. Reactor neutrons have a wide energy spectrum but they can be divided in to three kinds: thermal, medium energy and fast neutrons.

The thermal neutron activation analysis also belongs to the category of the trace element analysis. When compared with the other analytical methods, it has the following advantages:

- a) High sensitivity: Sensitivity refers to the ability to measure a small in concentration.

The analytical sensitivity for the most elements in the periodic table ranges from 10^{-6} (ppm) to 10^{-13} g.

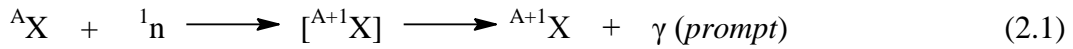
- b) High accuracy and precision: Accuracy indicates the closeness of the experimental value to the real value. While precision, indicates the discrepancies in a series of experimental values that is repeatedly obtained for the same parameter. Simply,

accuracy refers to reliability of the result or how close the result is to the true value while precision refers to repeatability and reproducibility of the result.

- c) No reagent contamination: in non destructive activation analysis, no chemical treatment of the sample is made before irradiation. Therefore there is no sample contamination caused by reagents. Non-destructive means, the physical and chemical state of the sample is not altered.
- d) A wide range of elements are determined: up to thirty four elements can be measured in the same sample (simultaneous analysis of multi-component system).
- e) Nondestructive activation analysis can be used, in many occasions to perform the simultaneous determination of many elements, thus avoiding the errors caused by the sample dissolution and separation.
- f) A small base effect: the activation analysis is suitable for various samples with complicated chemical composition. For example, if many elements with similar chemical properties coexist in a sample, it can be difficult to analyze them by conventional chemical methods simultaneously.
- g) A small sample or quantity is needed for the analysis

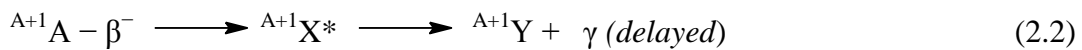
2.8.1.2 Capture Reactions

This is the most widely applied nuclear reactions for activation analysis. It is (n,γ) reaction in which, a low energy or thermalized neutron is absorbed by a nucleus resulting in the formation of a compound nucleus. The de-excitation of the compound nucleus results in the emission of prompt gamma rays.



Stable isotope (compound nucleus)

As a result of this reaction, the neutron to proton ratio is increased to form a nucleus of the same element. This increase in neutron to proton may be sufficient to produce an unstable nucleus which then most probably decays by the emission of an electron (beta negative decay),



The beta decay may be accompanied by gamma ray emission (delayed) if the product isotope following beta emission is left in excited state. Neutrons of any energy may participate in this type of reaction but thermal neutrons predominate since the absorption cross section in this region is inversely proportional to the neutron velocity for most nuclei (i.e. the so-called 1/V law). The energy reactor thermal neutron is fraction of 1eV (0.025 eV).

2.8.1.3 Transmutation

It is the second most common activation reaction in which there is the absorption of a neutron followed by subsequent release of a charged particle most often a proton. Other reactions that occur, though with less probability, are (n, d), (n, α), (n, t) and (n, ${}^3\text{He}$) in each of which the chemical identity of the target nucleus is altered. In some cases fast neutrons are required to supply sufficient energy to the compound nucleus to permit (n, p) and (n, α) reactions). This is because the reactions are threshold reactions where the mass

balance between the reactants and the products, the Q-value is such that extra energy is needed before the reaction can proceed.

Moreover, any charged particle must have sufficient energy to overcome the coulomb potential barrier before emission can proceed. Here it is rare for slow neutrons to be involved in this type of reaction, the reason being that a positively charged particle can be expelled from the nucleus only if it has sufficient energy required to detach it from the nucleus. It is only for few elements, of low atomic number, for which the nuclear electrostatic potential is small, that charged particle emission is possible after the capture of slow neutrons. An example of this is the interaction of slow neutrons with ${}^6\text{Li}$ and ${}^{10}\text{B}$ both resulting in the emission of alpha particles.

2.8.1.4 Inelastic scattering

Inelastic scattering occurs when a neutron bombards a nucleus and comes off with less energy than that of the incident neutron, the difference in energy going in to an excited state of the residual nucleus and appearing as a prompt gamma-ray. Such scattering may be noted as (n, n^1) or $(n, n\gamma)$ reactions. The occurrence of inelastic depends upon there being an excited state at low enough energy so that the incident neutron can supply this energy of excitation. In most heavy elements the first excited state is within reach of fast neutrons, being about 100 KeV for most elements, thus fast neutrons scattering in heavy reaction to proceed the incident neutron must have kinetic energy greater than the binding energy of the neutron in the target nucleus which makes it possible for the emission of two neutrons to take place. With increasing neutron energy the $(n, 2n)$ process is observed with a higher yield until competition from the higher energy $(n, 3n)$ reaction takes place.

2.8.2 Neutron Sources and Energy Spectra

There are many techniques available for the production of neutrons in fluxes suitable for activation analysis. These include nuclear reactors, isotopic sources, high energy charged particle accelerators, low energy deuteron accelerators (neutron generators). However, due to the fact that only neutrons from nuclear reactors were used for this work, it is the only one that will be highlighted.

The neutrons emitted from a nuclear fall into three main classes based on their energies.

- (i) Fast fission neutrons;
- (ii) Epithermal neutrons and
- (iii) Thermal neutrons.

Fast fission neutrons, i.e. neutrons which are yet to enter in to collision with moderators, have energy in the range 0 – 25 MeV for the ^{235}U fission with an average energy of 2 MeV (Akanle *et al.*, 1987). The fission flux as a function of energy has been approximated by several semi-empirical formulae by Watt and reported by Akanle *et al.*, 1987 as:

$$N(E)dE = \left(\frac{2}{11e}\right)^{\frac{1}{2}} e^{-E} \sin(2E)^{\frac{1}{2}} dE \quad (2.3)$$

$N(E)dE$ is the number of neutrons in the interval between E and $(E + dE)$.

Modification to the equation was proposed by Cranberg *et al.*, (1956) and studied experimentally in the energy region 180KeV – 12MeV by Akanle *et al.*, 1987 to give

$$N(E)dE = 0.42527e^{(-E/0.96)} \text{Sinh}(2.29E)^{1/2} dE \quad (2.4)$$

The neutrons are slowed down by elastic collisions with the moderator in the intermediate region, they show an energy distribution which varies as $1/E$, for a medium showing negligible absorption and leakage.

When the neutrons reach thermal equilibrium with the moderator, the thermal neutron flux as a function of energy is described by a Maxwellian energy distribution given by

$$M(E)dE = \frac{2}{\sqrt{\pi}} E^{1/2} \frac{1}{T} (E)^{1/2} e^{[-E/ET]} dE \quad (2.5)$$

Where, $E_T = kT$ and T is the absolute temperature of the medium while k is Boltzmann's constant.

$M(E)dE$ is the flux in the energy interval E and $(E+dE)$.

At 293K, the most probable velocity is $V_0 = 2200 \text{ ms}^{-1}$ corresponding to an energy of 0.025 eV. It is also assumed that there is no absorption and leakage from the system.

2.8.3 Reaction Rate

Since neutrons of various energies are available for interaction in a reactor and as the cross section for any process is energy dependent, the reaction rate which is defined as the number of events occurring in unit time per target nucleus is given by

$$R = \int_0^{\infty} \varphi(E)\sigma(E)dE \quad (2.6)$$

Where

- $\varphi(E)dE$ is the flux of neutron between energy E and $(E +dE)$
- $\sigma(E)$ is the microscopic cross section of neutrons at energy E .

$$\text{Number of target nuclei/volume} = \rho \frac{N_0}{A} \quad (2.7)$$

Where N_0 = Avogadro's number

ρ = Density

A = Atomic Mass

$$\text{Total target area} = f \frac{mN_0}{A} \quad (2.8)$$

Where

f = Isotopic abundance

m = Mass

A = Mass number

N_0 = Avogadro number

Therefore, total rate of reaction is given as

$$\text{Rate} = f \frac{mN_0}{A} \int_0^{\infty} \phi(E) \sigma(E) dE \quad (2.9)$$

In radioactive capture the contribution to the reaction rate from raw fission neutrons is small and thus can be neglected. The reaction rate R is thus expressed as:

$$R = \phi_{th} \sigma_c + \phi_c I_0 \quad (2.10)$$

Where

- ϕ_{th} is the conventional thermal neutron flux up to Cadmium cut-off energy E_{Cd}
- σ_{th} is the cross section for the nuclide obeying the "1/V" law at 2200 mS⁻¹.
- ϕ_c is the epithermal neutron flux per unit logarithmic energy interval.
- I_0 is the infinitely dilute resonance integral defined by:

$$I_0 = \int_{E_{Cd}}^{\infty} \sigma(E) \frac{dE}{E} \quad (2.11)$$

I_0 can be obtained for a number of nuclides from the literature.

However for threshold reactions whether transmutation or inelastic scattering where fast neutrons are needed for the reaction to take place, the reaction is defined as:

$$R = \int_0^{\infty} \sigma(E)f(E)dE \quad (2.12)$$

$$= \int_{E_T}^{\infty} \sigma(E)f(E)dE$$

$F(E)$ represents the fraction of the fission neutron flux between E and $(E + dE)$

- E_T is the threshold energy.

A fission neutron spectrum average cross section is given as

$$\bar{\sigma} = \frac{\int_0^{\infty} \sigma(E)f(E)dE}{\int_0^{\infty} f(E)dE} \quad (2.13)$$

And the equivalent fission flux ϕf is given as:

$$R = \bar{\sigma}\phi f \quad (2.14)$$

Values of $\bar{\sigma}$ can be found in tabulated data, which are calculated with respect to a well defined unperturbed fission neutron spectrum, usually that of ^{235}U .

The rate, at which a nuclear reaction proceeds, therefore, depends on three parameters:

- The number of target atoms present
- The cross section, and
- The number of neutrons incident per unit area of the target material per unit time.

The relationship is expressed by:

$$R = N\phi\sigma \quad (2.15)$$

Where, R = the reaction rate (sec^{-1})

N = number of target nuclei present

ϕ = the neutron flux density ($\text{n}/(\text{cm}^2\text{-sec}^{-1})$)

σ = the reaction cross section (cm^2)

The magnitude of the reaction cross section depends on the nature of the target nuclide and on the energy of the incident neutrons. With thermal (low) neutrons (n, γ) reactions generally have large cross sections, although there are some exceptions.

2.8.4 Decay Modes of Radioisotopes

Radioactive nuclei produced by neutron activation disintegrate by beta (β), gamma (γ) and in some cases alpha (α) emission which may be associated with x-ray and electron emission. Alpha (α) emission is common for heavy elements where binding energies are lower. Beta emission whether negative (β^-) or positive (β^+) emission is typical of nuclei containing excess neutrons or protons. Negative (β^-) emission is emitted with continuous spectrum and is usually by a neutrino which carries away the excess energy of these beta particles, having energies less than the maximum. The positive beta emissions are accompanied by annihilation radiation of 0.511 MeV energy. Orbital electron capture (k-capture) is typical of nuclei containing excess protons when enough energy is not available to permit positron emission. K-capture leaves a vacancy in the k-shell of electrons; another orbital electron drops down to fill this vacancy thereby emitting characteristic x-rays of the product atom. The characteristic x-rays emitted can be used for elemental identification provided a low energy photon detector is employed.

Gamma-ray emission occurs whether a nucleus finds itself in an excited state following beta decay. The energy of the gamma-ray is equal to the difference in energy between the initial and final nuclei. The gamma-ray appears with a half-life characteristic of the parent beta decay, but with energy that reflects the energy level structure of the daughter nucleus. This property makes gamma radiation useful for identifying specific radio nuclides

especially in the presence of any other radio nuclides. This property makes gamma radiation useful for identifying specific radio nuclides especially in the presence of any other radio nuclides. This is an important aspect of activation analysis. If de-excitation from the excited state to the ground state takes place within a life-time that is measurable, i.e longer than a few milliseconds, such radio nuclides are known as metastable isotopes and the transition is called isomeric transition (IT). Another common way for a nucleus to lose excess energy is by an electromagnetic interaction between the nucleus and orbital electron which results in the emission of an electron whose kinetic energy is equal to the nuclear transition energy, minus the binding energy of the emitted electron. This electron then appears with an energy given by:

$$E_{e^-} = E_{ex} - E_b \quad (2.16)$$

E_b is the binding energy in the original electron shell. This process called internal conversion (I.C), competes with gamma-ray emission as a de-excitation process.

A rough analogue of internal conversion is auger electrons. This is when the excitation energy originates in the atom rather than the nucleus. A preceding process (such as electron capture) may leave the atom with a vacancy in a normally complete electron shell. This vacancy is often filled by neutrons from outer shells of the atom with the emission of characteristic x-ray photon. Alternatively, the excitation energy of the atom may be transferred directly to one of the outer electrons causing it to be ejected from the atom. This electron is called auger electron and appears with an energy given by the difference between the original atomic excitation energy and the binding energy of the shell from which the electron was ejected. In all cases, the auger electron's energy is relatively low compared with beta (β) or conversion electrons, particularly, because its emission is

avored only in low z-elements for which electron binding energies are small. Typical Auger electrons with a few KeV energy are subject to pronounced self-absorption within the source and are easily stopped by very thin source covers or detector entrance window.

2.8.5 Analysis Procedure

Neutron activation analysis has been more extensively used in the last three decades with the wide availability of high resolution Ge(Li) or hyper purity Ge detectors. In practice, about a gram or less of sample sealed in polythene rabbit for short irradiation, or high purity quartz capsule for long irradiation, is irradiated along with the elemental standards of interest. Short irradiation last about 1 to 5 minutes while long irradiation last to a week. After each irradiation the samples are counted on a Ge(Li) detector connected to a multichannel analyzer for varying time periods. Taking the advantage of different half-lives and activation cross section of the elements:

- (i) The sample is exposed to neutron (irradiated) for a pre-determined length of time taking in to consideration the half-life of the element of interest (t_{ic}).
- (ii) It is then transferred to the counting station and allowed to cool or decay for a definite length of time (t_d).
- (iv) The gamma-ray spectrum is acquired for counting time (t_c) and
- (v) The area under the full energy peak (FEP) of interest is calculated.

From the element of interest in the standard, the relative FEP areas of the sample and standard, the relative neutron fluxes used for irradiating the sample and the standard and the times involved, the amount of the element in the sample can be calculated.

2.8.6 Calculation:

The calculation is fairly straight forward. For example, assuming that, the mass of the element in the unknown sample is W_u grams and that one irradiates the sample for $t_{i(u)}$ sec., allows it to decay for $t_{d(u)}$ sec. and counts the emission for $t_{c(u)}$ sec. The activity of the radionuclide of interest at the end of the irradiation is given by:

$$A_u^0 = N\varphi_u \sigma(1 - e^{-\lambda t_i(u)}) \quad (2.17)$$

$$= 6.02 \times 10^{23} \frac{1}{M} \varphi_u (1 - e^{-\lambda t_i(u)}) \quad (2.18)$$

Where

A_u^0 = Activity of the unknown sample

I = the isotope abundance of the element

M = the atomic mass of the element

φ_u = the neutron flux density in n/ (cm²-sec)

N = the number of atoms of the specific target isotope present.

Most tables list I in units of atom present, in which case M should be the atomic mass of the element, not the mass of the individual isotopes.

When the acquisition of the spectrum begins, the activity will be:

$$A_u = A_u^0 e^{-\lambda t_d(u)} \quad (2.19)$$

The detector will detect only a fraction of this activity. Only those events that register in the FEP are of interest. These factors are accounted for by the detector photopeak efficiency ξ . The count rate registered by the detection in the FEP at the instant when counting starts is therefore given as:

$$C_u = \xi A_u^0 e^{-\lambda t_d(u)} \quad (2.20)$$

The analyzer will integrate the count rate for the period of time t_c . At the end of this time, total counts registered in the FEP excluding any background effects will be

$$C_u = \frac{\xi}{\lambda} \frac{1}{m} \varphi_u \sigma (1 - e^{-\lambda t_d(u)}) e^{-\lambda t_d(u)} (1 - e^{-\lambda t_d(u)}) W_u (6.02 \cdot 10^{23}) \quad (2.21)$$

If the exact values of the parameters in the above equation were known, W_u could be calculated directly. However, because of uncertainties in the numerical values of ξ , φ and σ , it is more convenient to employ a comparative method whereby one also irradiates a sample of known content of the element of interest, and then we shall have:

$$\frac{C_u}{C_S} = \frac{\xi_u \varphi_u (1 - e^{-\lambda t_d(u)}) e^{-\lambda t_d(u)} (1 - e^{-\lambda t_d(u)}) W_u}{\xi_s \varphi_s (1 - e^{-\lambda t_d(s)}) e^{-\lambda t_d(s)} (1 - e^{-\lambda t_d(s)}) W_s} \quad (2.22)$$

Therefore,

$$W_u = \frac{C_u}{C_s} \frac{\varphi_s}{\varphi_u} W_s \quad (2.23)$$

When employing nuclear reactors or isotopic source the value of φ_u and φ_s may also be the same, and a further simplification results.

$$W_u = \frac{C_u}{C_s} W_s \quad (2.24)$$

Basically, neutron activation analysis is a technique which enables multi-element analysis to be done in a variety of matrices. The sample is bombarded with neutrons from a reactor or neutron source and the radioactive species of a given element is produced depending on two main parameters:

- (i) The half life of the nuclide
- (ii) The activation cross section of the element. This is the probability that the element will absorb neutrons and become radioactive.

The equation of activation analysis is:

$$A_o = \frac{\varepsilon\sigma_Q\rho W_Q\phi}{M_Q} = N_A(1 - e^{-\lambda t_i})ds^{-1} \quad (2.25)$$

Where

A_o = activity of element Q at the end of the irradiation (ds^{-1}).

ε = fraction of total counting rate is measured photo peak.

σ_Q = neutron capture cross section of element (m^2).

W_Q = weight of element Q irradiated (Kg).

P = fractional abundance of particular isotope of the element (Q).

ϕ = flux of neutrons used in irradiation ($m^{-2}s^{-1}$).

M_Q = atomic weight of element (Q) to be measured.

N_A = Avogadro number (mol^{-1}).

λ = decay constant of induced radionuclide (s^{-1}).

$$\lambda = \frac{0.693}{T_{\frac{1}{2}}} \quad (2.26)$$

Where $T_{\frac{1}{2}}$ = half life of induced radionuclide.

t_i = irradiation time(s)

After irradiation the induced radioactivity decays according to

$$A_t = A_o e^{-\lambda t_d} \quad (2.27)$$

Where:

t_d = time after end of irradiation, i.e. decay time.

Measurements are usually made using the comparator method which means that the unknown sample and a standard of known concentration are irradiated under identical experimental conditions such as ϵ , σQ , ρ , NA and MQ are the same.

Then,

$$\frac{A_S}{A_r} = \frac{W_S}{W_r} \quad (2.28)$$

Where,

S = sample

r = standard

All results must be referred to the same time after irradiation, i.e. zero taken as the time when the sample is removed from the reactor.

The integral count must be corrected for the following:

- (a) **Dead time** – Which is the minimum amount of time that will separate two events in order that they are recorded as two separate pulses. This varies depending on the activity of the sample. For samples with high initial dead time, this falls rapidly during the counting interval. The print out gives a live time, indicative of when the analyzer is working and a real time which is the counting interval. The dead time is therefore given by (real time – live time) and the figure on the print is an overall value for the whole counting interval.

However, if all the samples are of similar concentration the dead time variations are approximately the same for all the samples.

If life time = t_l secs

And real time = t_r secs

If the integral counts in the peak = c

Then,

$$\text{True counts} = \frac{C \times t_r}{t_1} = c_1 \text{ counts} \quad (2.29)$$

The sample is however decaying as the counting proceeds, therefore the initial count rate at the beginning of the counting interval is given by:

$$\frac{0.693C_t}{T_1(1-e^{-0.693t_r/T_1})} \quad (2.30)$$

$$\text{Or count rate initially} = \frac{C\lambda}{1-e^{-\lambda}} = \frac{C \times 0.693}{T_1(1-e^{-0.693t_r/T_1})} \quad (2.31)$$

(b) **Delay time:** If the delay between the end of the irradiation and the start of counting is t_d secs, then the above count rate must be corrected by a factor,

$$e^{-\frac{0.693t_d}{T_1}} = e^{-\frac{0.693t_d}{T_1}} \quad \text{-----} \quad (2.32)$$

When all the integral counts have been corrected to the same point in time, then the concentration of the element can be calculated by simple proportion using comparator method with the chemical standard of known concentration.

2.8.7 Capabilities and Limitations of Neutron Activation Analysis:

The NAA technique is one of the modern techniques of analysis. Its fundamental limitation is its inability to distinguish among different chemical form or oxidation states of an element. It also suffers from possible interferences and matrix effects. However, since biological materials are essentially a C-H-O-N-P and S matrix that does not get activated

during thermal neutron irradiation, there is little background interference. However some interference can occur from the production of the same daughter nuclei from competing (n,p) or (n, α) reactions or from nuclei with the same or similar gamma (γ) rays. One of the most important advantages of INAA over other techniques of analysis is that it is essentially non destructive, that is to say that, very often, a complete analysis can be performed without altering the physical or chemical nature of the sample. It also involves minimum sample manipulation and therefore the sample the sample is not contaminated by reagents and containers as could occur in destructive wet-chemical techniques. Neutron activation also has a high degree of sensitivity for the majority of elements. A very important advantage of neutron activation analysis over some other analytical methods is that simultaneous analysis of multi-component systems is easy to perform.

The availability of high flux thermal neutron irradiation facilities and high resolution intrinsic Ge and Li drifted germanium (Ge(Li)) or silicon Si(Li) detectors has made neutron activation analysis a very attractive tool for determining trace elements in plants and plant products.

The electronic components needed for processing the detector signals have evolved in to standardized modular units, and are relatively simple to select. One important factor in using a multichannel analyzer is the analyzer dead time. A multichannel analyzer receives a pulse, digitizes that pulse, and stores it in the proper memory channel. During this time, the analyzer is dead to any incoming pulses. If the sample has a high activity level, this could result in an appreciable loss of counts. Most modern analyzers keep track of dead time and automatically lengthen the counting time to compensate for its effect (dead time is the minimum amount of time that must separate two events in order that they are recorded as

two separate pulses in nearly all detector systems). The internal correction works best when the counting time is short compared to the half-life of the radionuclide of interest.

This normally followed by the emission of gamma (γ) radiation as shown in equation (2.33) below



NAA utilizes the fact that the number of atoms of the radioisotope produced (${}^{A+1}\text{X}$), depends on the affinity (cross –section) of neutron-nuclei reaction, duration of irradiation and the half-life of the radioisotope produced (Racovic, 1970)

The activity (A) that can be produced in material is given by

$$A = N\sigma\phi (1-\exp-\lambda t_i) \quad (2.34)$$

And $\lambda=0.693$ (2.35)

Where, A = induced activity at the end of irradiation

N = number of target atoms of elements in the material

σ = cross-section (cm^2) which is the probability that the material will undergo a reaction with the bombarding neutrons and the isotopic abundance of the element in the material.

ϕ = irradiation flux, neutron/ cm^2 /second and the higher the flux, the greater the induced activity for a given element and irradiation time.

t_i = irradiation time

$T_{1/2}$ = half life of the radioisotope produced

λ = decay constant of radioisotope produced

The term $(1-\exp-\lambda t_i)$ in equation in equation (2) is called saturation factor, when the rate of formation of radioisotope is exactly equal to its decay. As the time, t_i becomes large

compared to the half-life of the product ($T_{1/2}$), the saturation factor approaches unity so that (5.2) becomes

$$A = N\sigma\phi \quad (2.36)$$

Where N can be expressed in the form

$$N = N_{av}Wk/A_t \quad (2.37)$$

N_{av} = Avogadro's number; 6.023×10^{23}

W = weight of element in g

A_t = Atomic weight of the element

K = Fractional abundance of given target nuclide in the sample of the material.

However, for absolute determination of a given element in the material, the values of σ , ϕ , $T_{1/2}$ and t_i must be known and the experimental determination of A. This is indeed a difficult task for a rapid determination of elements in the material. Also cross-section of radionuclide known are no better than 5 to 15% and irradiation fluxes may vary in the sample, hence a difficult for activity measurement (Racovic, 1970)

It is because of the above reasons that the comparator technique is employed. Here the material sample and a standard containing the sought elements are irradiated and counted under identical conditions.

$$\text{Thus, } \text{CPM}[\text{Sample}]/\text{CPM}[\text{standard}] = \mu\text{g} [\text{Sample}] / \mu\text{g}[\text{Sample}] \quad (2.38)$$

CPM = Counts per minute (count rate)

Similarly, a routine, rapid and sensitive quantitative analysis can be made using the techniques of NAA. This is because most radionuclide exhibits a characteristic beta- and gamma-ray energies and half-lives. When the radiations are characterized using appropriate detection apparatus, an idea of the material composition can be obtained (Ross, 1970).

For the comparative method discussed above, no detailed knowledge of the neutron flux in the irradiation site nor of the nuclear data of the isotope is required. The disadvantages of this method include, however, the difficulty in maintaining, and ensuring the stability of the chemical standard and the extra time required to irradiate and count the standard. Furthermore, differences in matrix composition between the sample and the standard will contribute an additional uncertainty. These drawbacks have prompted an investigation into what information about neutron flux in the irradiation position, and what nuclear data concerning the target and product nuclides would be required to calculate element masses directly from the gamma ray spectrum, hence the use of absolute neutron activation method.

In the application of absolute method, it has been pointed that uncertainties in nuclear data were the major of systematic errors in this activation analysis. Evidence shows the induced error amounts to ~20%. Half life data for most nuclides, in general, are known with uncertainty of ~1%. However analyses are not usually sensitive to errors in half life unless the irradiation, decay and counting times are significant compared to half life. The recommended values of gamma ray emission probabilities in the literature are also known to within ~1% with few exceptions. In general the errors in decay scheme data still are not the main factors which limit the accuracy of the method. Thermal neutron cross sections which basically determine the reaction rate in mainly thermal neutron spectra have uncertainties ranging from ~5 – 10%. From the latest compilations, it is believed that the major to poor accuracy is due to the scatter in the resonance integral data (Kafala and Macmahon, 1993). Deviations from the commonly 1/E shaped epithermal neutron spectrum

frequently occur in thermal reactors, and can lead to severe changes in the apparent resonance integrals.

In a research reactor, it is usual to characterize the neutron flux distribution using three parameter, namely: (i) the thermal neutron flux, (ii) the epithermal to thermal neutron flux ratio, and (iii) a parameter measuring the deviation of the epithermal neutron spectrum from a 1/E shape. The best method of estimating these parameters is one which utilizes more measured reaction rates than the minimum in order to produce an over – determined set of simultaneous equations. The solution of this set can be obtained by applying the method of generalized least squares which provides the best values of flux parameters and improved estimates of nuclear data.

2.8.7 Neutron flux convention

The convention expresses the reaction rate for any irradiated element in terms of neutron flux and nuclear data parameters. The calculated saturated specific gamma ray emission rate for an (n, γ) reaction in a thermal reactor is:

$$R_s = \eta \cdot N_a \cdot \left\{ \phi_{th} + \phi_e \left[\frac{w'(\alpha)}{g} + f_1(\alpha) \left(\frac{1}{E_r} \right)^\alpha A = \pi r^2 + \left(\frac{1}{E_r} \right)^\alpha \left(\frac{I_o}{g\sigma_o} - f_2(0) + f_2(\alpha) \right] \right\} \quad (2.39)$$

$$\text{Where } R_s = \frac{N_c}{m\epsilon\gamma SD C t_1} \text{ (experimental saturated specific gamma ray emission rate)} \quad (2.40)$$

$$\eta = g\theta P_{\gamma\sigma_o}/M \quad (2.41)$$

N_c is net peak area corrected for pulse losses

M is atomic weight

m is element mass

N_A is Avogadro's number

θ is natural abundance of target isotope

P_γ is gamma ray emission probability

ϵ_γ is photopeak efficiency

S is saturation factor $S = (1 - e^{-\lambda t_i})$, t_i is irradiation time

D is decay factor $D = e^{-\lambda t_d}$, t_d is decay time between end of radiation and start of counting

C is decay during counting factor, $C = (1 - e^{-\lambda t_c})$, t_c is real counting time

t_i is live counting time

Φ_{th} is the conventional Maxwellian thermal flux

Φ_e is the epithermal flux component per unit $\ln E$ at 1eV

α is the epithermal shape parameter

g is the Westcott factor, a measure of deviation of cross section from $1/v$ law

σ_0 is 2200m/s cross section

I_0 is infinitely dilute resonance integral

\bar{E}_r is the effective resonance energy (Kafala and Macmahon, 1993)

$$\text{Also, } I_0 = \int_{E_{cd}}^{\infty} \sigma(E) \frac{1}{E} dE, \quad f_1(\alpha) = \int_{\mu kT}^{E_{cd}} \frac{v_0}{v} \frac{E_1^\alpha}{E^{1+\alpha}} dE \text{ and } f_2(\alpha) = \int_{E_{cd}}^{\infty} \frac{v_0}{v} \frac{E_1^\alpha}{E^{1+\alpha}} dE \quad (2.42)$$

μkT is the joining energy point between thermal and epithermal flux being $\mu \sim 5$ for most water or heavy water reactors, μkT is 0.13eV at room temperature. To deal with the integral from μkT to E_{cd} , a dimensionless quantity $w'(\alpha)$ is introduced:

$$w'(\alpha) = \frac{1}{\sigma_0} \int_{\mu kT}^{E_{cd}} (\sigma(E) - g \frac{\sigma_0 V_0}{V} \frac{E_1^\alpha}{E^{1+\alpha}}) dE \quad (2.43)$$

it is a measure of the deviation of the cross section from $1/v$ – law in the energy range μkT to E_{cd} . $w'(\alpha)$ is a small quantity and its dependence on α can normally be neglected (Kafala and Macmahon, 1993); i.e $w'(\alpha) \sim w(0)$.

2.8.9 HPGe detector efficiency

The calculation of the saturated activity requires the absolute counting efficiency of the gamma ray detector. A set of standard sources with an energy of 60 ~ 1408 keV were measured independently on a HPGe detector at 5 and 13cm from the detector end cap. The experimental efficiency data were fitted by a poly-log function with five parameters (Kafala and Macmahon, 1993).

$$E_\gamma(E) = (P_1 + P_2 (\ln E) P_3 (\ln E)^2 + P_4 (\ln E)^3 + P_5 (\ln E)^5)/E, E \text{ is in MeV} \dots\dots (2.44)$$

2.8.10 Instrumentation in NAA

The equipment needed for neutron activation analysis (NAA) consists of neutron source and radiation detectors. The easy and sensitivity of the analysis depend mostly on the degree of sophistication of those devices (Haskin and Ziege, 1971). The choice of neutron source for analysis is based on availability, required neutron flux and neutron source energy. The neutron source includes nuclear particles like photons, deuterium and alpha particles to strike targets with production of neutrons (Jonah *et al.*, 2006).

The reactor for this work is The Nigeria Research Reactor-1 (NIRR-1) installed at Center for Energy Research and Training (CERT), Ahmadu Bello University, Zaria. It is a

Miniature Neutron Source Reactor (MNSR) and has a tank-in-pool structural configuration with a nominal thermal power rating of 31kW. NIRR-1 was acquired for an extensive soil fertility mapping project of the arable land in Nigeria aimed at improving food production. Like all MNSR facilities, NIRR-1 is specifically designed for neutron activation analysis (NAA) for the analysis of trace, minor and major elements indifferent matrices (Jonah, *et al.*, 2006).

NIRR-1 is a low-power nuclear reactor, which has highly enriched uranium fuel, light water as moderator and beryllium as reflector. The associated facility for radioactivity measurements is a gamma-ray data acquisition system. It consists of a horizontal dip-stick High purity Germanium (HPGe) detector with a relative efficiency of 10% at 1332.5 keV gamma ray line, the MAESTRO emulation software compatible with the ADCAM[®] multi-channel analyzer (MCA) card, associated electronic modules all made by EG&G ORTEC and a personal computer. The efficiency curves of the detector system at near and far source-detector geometries have been determined by standard gamma-ray sources in the energy range of 59.5 – 2254keV and were extended to 4000keV by a semi-empirical method. For data processing, gamma-ray spectrum analysis software WINSPAN 2004 was used. The software requires that calibration factors be pre-determined by a multi-element standard reference material for elements of interest using adopted irradiation and counting regimes. In addition to NAA calculations, WINSPAN 2004 performs peak analysis, remote control of the MCA and other auxiliary functions such as efficiency calibration and nuclear data generation.

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 Sample identification and collection

The plants in this work (*Ceratotheca sesamoides* Endl, *Mormodica charantia* Linn, *Vernonia amagadlina* Del, *Senna occidentalis* Linn, *Corchorus olitorious* Linn and *Corchorus tridens* Linn) were identified at the Department of Biological Sciences of Ahmadu Bello University (ABU) Zaria and voucher specimens are kept there.

Samples of the plant materials (roots, stems, fruits and leaves) were collected from Karkari village, Gwarzo Local Government Area of Western part of Kano State, Nigeria and transported in polythene bags.

3.2 Sample treatment

After collection, the samples were washed twice with tap water and rinsed with deionised water. They were first air dried for 7 days and then further dried in an oven at 60 °C for 12 hours. After drying, the samples were ground using pestle and mortar and sieved (125 µm mesh sieve).

3.3 Neutron activation analysis measurement

The ground sample was mixed well and approximately 150 mg sub-sample was weighed and wrapped in polyethylene films. For the elements leading to short-lived activation products, the sample was packed and sealed in 7 cm³ rabbit capsules. Irradiation with thermal neutrons was carried out at an outer irradiator channels (i.e., B₄) of the Nigeria Research Reactor-1 (NIRR-1) operating at a thermal neutron flux setting of 2×10¹⁰ n/cm²s, which corresponds to a neutron flux of 1×10¹⁰ n/cm²s in the outer channels. After the

irradiation, a computer (PC-) based gamma ray spectrometry set-up performed measurement of induced radionuclide. This consists of a HPGG detector coupled to a computer based Multi-Channel Analyzer (MCA) via electronic modules. The relative efficiency of detector is 10 % and an energy resolution of 1.95 KeV at gamma-ray energy of 1332 KeV belonging to ^{60}Co . Through appropriate choice of cooling time, detector's dead time was controlled to be less than 10 %. Identification of gamma ray of product radionuclides through their energies and quantitative analysis of their concentrations was achieved using the gamma ray spectrum analysis software, WINSPAN - 2004. The certified reference material IAEA – soil - 7 was used as the standard, while other two certified reference materials, GSD - II and GSR-5 were used as analytical quality control materials to validate the procedure for all the elements.

3.4 Preparation of reagents

3.4.1 Aqueous ammonia (6 M)

375 cm³ of concentrated ammonia was taken in to 1000 cm³ volumetric flask and then made to the mark with distilled and deionized water.

3.4.2 Boric acid (2.5 %)

5.0 g of Boric acid was weighed in to 100 cm³ volumetric flask then made to the mark with water.

3.4.3 Orthophosphoric acid

The concentrated acid is used.

3.4.4 Potassium Iodide (5 %)

5.0 g of potassium Iodide was weighed into a 100 cm³ volumetric flask then made to the mark with water.

3.4.5 Silver nitrate (0.02 M)

0.85 g of AgNO₃ was weighed into a 250 cm³ beaker already containing little amount of water. This was transferred into a 250 volumetric flask and the beaker washed with fresh water, transferred in to flask and then made to the mark.

3.4.6 Hydrochloric acid (20 %)

20 cm³ of HCl were taken into a 100 cm³ flask and then made to the mark.

3.4.7 Ammonium thiocyanate (0.3 %)

0.3 g of ammonium thiocyanate was weighed into a 100 cm³ volumetric flask and then made to the mark.

3.4.8 Starch solution (1 %)

1.0 g of water-soluble starch was weighed into a 100 cm³ volumetric flask and then made to the mark with distilled and deionized water.

3.4.9 Iron (III) Chloride (0.1 M)

4.0625 g Of Iron (III) chloride was weighed into a 250 cm³ volumetric flask and then made to the mark with water.

3.4.10 Hydrochloric acid (0.05 N)

2.10 cm³ of hydrochloric acid was measured into a 500 cm³ volumetric flask and made to the mark with water.

3.4.11 Hydrochloric acid (0.1 N)

4.17 cm³ of hydrochloric acid was measured into a 500 cm³ volumetric flask and then made to the mark with water.

3.4.12 Hydrochloric acid (6 M)

125 cm³ of hydrochloric was measured into a 250 cm³ volumetric flask and then made to the mark with distilled and deionized water.

3.4.13 Potassium permanganate (0.1 N)

3.16 g of potassium permanganate was weighed in 1-liter volumetric flask and then made to the mark with water.

3.4.14 Ethanol (50 %)

500 cm³ of ethanol was taken in to 1-liter volumetric flask and then made to the mark with water.

3.4.15 Potassium permanganate (5 %)

50.0 g of potassium permanganate was measured in to 1-liter volumetric flask and then made to the mark with water.

3.4.16 Potassium permanganate (0.5 %)

0.5 g of potassium permanganate was measured into a 100 cm³ volumetric flask and then made to the mark with distilled and deionized water.

3.4.17 Potassium dichromate (0.5 %)

0.5g of potassium dichromate was measured into a 100 cm³ volumetric flask and then made to the mark with water.

3.4.18 Hydrogen peroxide (30 %)

30.0 cm³ of hydrogen peroxide was measured in to 1-liter volumetric flask and then made to the mark with water.

3.4.19 Sodium sulphate (40 %)

40.0 g of sodium sulphate was measured in to 1-liter volumetric flask and then made to the mark with water.

3.4.20 Ethanolic Sodium hydroxide

4.2 g of sodium hydium hydroxide was dissolved in to 5 cm³ of water and sufficient aldehyde - free ethanol was added to make it to 1000 cm³ mark. The resultant solution was tightly stoppered and allowed to stand for 24 hours. After that, the supernatant solution was decanted in to another suitable and tightly-closed container.

3.4.21 Sulphuric acid (0.02 N)

0.56 cm³ of concentrated sulphuric acid was measured into 1-liter volumetric flask and then made to the mark with water.

3.5.22 Sulphuric acid (1.24 %)

3.125 cm³ of concentrated sulphuric acid was measured into a 250 cm³ volumetric flask and then made to the mark with distilled and deionized water.

3.4.23 Sulphuric acid (1 N)

27.78 cm³ of concentrated sulphuric acid was measured into a 1-liter volumetric flask and then made to the mark with water.

3.4.24 Methyl red Indicator

1.0 g of solid methyl red was dissolved in 600 cm³ of ethanol in a 1-liter volumetric flask and made up to the mark with 400 cm³ of distilled water.

3.4.25 Methylene blue Indicator

1.0 g of solid methylene blue indicator was dissolved in 600 cm³ of ethanol in a 1-liter volumetric flask and made up to the mark with 400 cm³ of distilled water.

3.4.26 Potassium Cyanide (0.1 N)

1.625 g of potassium cyanide was weighed into a 250 cm³ volumetric flask and then made to the mark with water.

3.4.27 Iron (III) chloride (1.04 %)

1.04 g Of Iron (III) chloride was measured into a 100 cm³ volumetric flask and then made to the mark with water.

3.4.28 Calcium Chloride (5 %)

5.0 g of Calcium Chloride was weighed into 100 cm³ volumetric flask and then made to the mark with water.

3.4.29 Sulphuric Acid (25 %)

25 cm³ of concentrated Sulphuric acid was measured into a volumetric flask and then made to the mark with water.

3.5.0 Determination of Proximate Composition

The samples were analyzed for proximate composition (moisture, ash, crude protein, lipids, soluble carbohydrates, crude fiber and vitamins).

3.5.1 Determination of Moisture Content

The moisture content was determined by drying the fresh sample at 110 °C to a constant weight in an oven.

$$\text{Percentage moisture content (\%)} = \frac{\text{Original weight} - \text{Final weight} \times 100}{\text{Original weight}}$$

3.5.2 Determination of Ash Content

The ash content was determined by incineration of the sample in a muffle furnace at 550 °C for 8 hours. The Association of Official Analytical Chemists (AOAC, 1980) method was used. Porcelain crucibles were washed and dried in an oven to a constant weight at 110 °C for 10 min. They were allowed to cool in a desiccator, and weighed. 2.0 g of each sample were weighed into the porcelain crucibles and reweighed. The crucibles containing the samples were transferred into a muffle furnace, which was set at 550 °C for 8 h to ensure proper ashing. They were then removed and allowed to cool in the desiccators then finally weighed. The percentage ash content was calculated.

$$\text{Percentage ash content (\%)} = \frac{\text{Weight of ash residue} \times 100}{\text{Dry weight of sample}}$$

3.5.3 Determination of Crude Fat

This was done by exhaustive Soxhlet extraction of a known amount (2.0 g) of each plant sample with pet-ether (40 – 60°C) and methanol mixed in the ratio 1:1 for 2 hours (AOAC, 1980). The detailed procedure is as follows; 2 g of each sample were placed into separate extraction thimbles and then covered with cotton wool. The extraction thimbles containing the samples were placed in the extraction jacket. Clean dried 500 ml round bottom flasks containing few anti-bumping granules were weighed and 150 ml of petroleum ether and 150ml methanol was poured into each flask fitted with Soxhlet extraction units. The round bottom flasks and the condenser were connected to the Soxhlet extractor and cold-water circulation was put on. The heating mantle was switched on; the heating rate was adjusted until the solvents were refluxing at a steady rate. Extraction was carried out for 2 hours.

The solvents were recovered and the oil was dried in the oven at 70°C for 1 h. The round bottom flask and oil were cooled and then weighed. The lipid content was calculated.

$$\text{Percentage fat (\%)} = \frac{\text{Weight of the fat} \times 100}{\text{Weight of the sample}}$$

3.5.4 Determination of Crude Fiber

This was done by obtaining the loss in weight on ignition of the residue of dried plant sample after digestion of fat-free samples with 1.25 % each of sulphuric acid and sodium hydroxide solution under specified condition.

$$\text{Percentage crude fibre(\%)} = \text{Wt. of dried fat free sample} - \text{Wt. of ash} \times 100$$

3.5.5 Determination of Crude Protein

This was done by the use of the microkjedahl nitrogen method as described by AOAC (1980), which involves the digestion of a given weight of the sample with concentrated H₂SO₄ and a catalyst to convert any organic nitrogen to ammonium sulphate in solution, followed by the decomposition of ammonium sulphate with NaOH. The ammonia liberated was distilled in to 5 % boric acid. The nitrogen from ammonia was deduced from titration of the trapped ammonia with 0.05 N HCl using methylene red and methylene blue (double indicator solution) indicators.

$$\text{Percentage Nitrogen (\%)} = \frac{\text{Vol. acid(ml)} - \text{Normality of acid} \times 1.4}{\text{Weight of sample}}$$

$$\text{Therefore, Percentage Crude prtein(\%)} = \% \text{Nitrogen} \times 5.3$$

3.5.6 Determination of carbohydrate content

The percentage carbohydrate contents of the samples were determined by difference as:

Percentage (%) Carbohydrate content = 100 – (% Moisture + % Fat + % Ash + % Crude Protein + % Crude fiber)

3.5.7 Determination of Calorific Value

This was done by summing the multiplied values for crude protein, fat or lipid and carbohydrate (excluding crude fiber) by the following factors (4, 9 and 4).

3.6.0 Determination of Anti - nutritional Content

The samples were analyzed for anti nutritionals (Hydrogen cyanide, oxalates, phytates, and tannins).

3.6.1 Determination of Crude Hydrogen Cyanide

This was done by the alkaline titration method (A.O.A.C, 1995). 10.0 g of ground sample was soaked in a mixture of 200 cm³ of distilled and 10cm³ of orthophosphoric acid. The mixture was left over night to release all bound hydrocyanic acid. The mixture was distilled until 150cm³ of the distillate was collected. 20 cm³ of the distillate was taken into a conical flask containing 40 cm³ of distilled water. 6 cm³ of 6 moldm⁻³ aqueous ammonia and 2 cm³ of 5% potassium iodide solution were added. The mixture was titrated against 0.02 moldm⁻³ silver nitrate to faint but permanent turbidity.

Result was calculated as 1ml 0.02 mol/dm³ AgNO₃ = 1.08 gHCN

3.6.2 Determination of Oxalate

Total oxalate was determined as described by Day and Underwood (1986). 1.0 g of sample was weighed into 100 ml conical flask. 75 ml H₂SO₄ (3 mol/L) was added and stirred for 1 h with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25 ml of the filtrate was then taken and titrated while hot against 0.05 mol/L of KMnO₄ solution until a faint pink color persisted for at least 30 s.

The oxalate content was then calculated by taking 1 ml of 0.05 mol/L of KMnO₄ as equivalent to 2.2 mg oxalate (Ihekoronye and Ngoddy, 1985; Chinma and Igyor, 2007 and Dahouenon-Ahoussi *et al.*, 2012).

3.6.3 Determination of Tannins

This was done by boiling 2.0 g of the dried samples with 300 cm³ of distilled water. This was diluted in a volumetric flask and then filtered through a non-absorbent cotton wool. 25 cm³ of this infusion was measured in to a 2-litre porcelain dish and titrated with 0.1 N potassium permanganate until blue solution changed to green, then 10 drops of 0.1 N potassium permanganate was added (A.O.A.C, 1980).

3.6.4 Determination of Phytates

This was done by soaking 4.0 g of the ground sample in 100 cm³ of 20 % hydrochloric acid for 5 hours and filtered. 25 cm³ of the filtrate was placed in a conical flask and 5 cm³ of 0.3 % ammonium thiocyanate solution was added. The mixture was titrated with standard Iron (III) chloride solution until a brownish-yellow color persisted for 5 minutes (Reddy *et al.*, 1982).

Results were calculated from 4 mols of Fe: 6 mols of Phytic acid.

3.6.5 Determination of Vitamins

3.6.5.1 Determination of Vitamin A (β – Carotene)

The level of β -carotene in the samples was determined by spectrophotometric method (Onwuka, 2005). Exactly 10g of the fresh leaves was macerated with 200 cm³ distilled water in a blender. The bulk liquid was then filtered and 200 μ l of the filtrate was saponified with 200 μ l alcoholic KOH and evaporated in a water bath for 2 hours. The carotene was extracted by adding 200 μ l xylene-kerosene mixture (1:1). The extract was centrifuged at 1000 rpm for 5 minutes. The aliquot was measured in a spectrophotometer at 460 nm. The extracts were then allowed to bleach by placing the test tubes near the window for UV light radiation. The level of β -carotene was calculated as follows:

$$\beta - \text{Carotene } (\mu\text{g/ml}) = A_0 (460) - A_1 (480)$$

Where: A_0 = initial absorbance

A_1 = absorbance after bleaching

3.6.5.2 Vitamin B₁(Thiamin)

5.0g of the sample were homogenized with 50 cm³ ethanolic sodium hydroxide. It was filtered in to a 100 cm³ flask. 10 cm³ of the filtrate was pipetted and the color developed by the addition of 10 cm³ of potassium dichromate was read at 360 nm. A blank sample was prepared and the color developed and read at the same wavelength (Okwu and Josiah, 2006)

3.6.5.3 Vitamin B₂ (Riboflavin)

5.0 g of the sample was extracted with 100 cm³ of 50 % ethanol solution and shaken for 1 hour. This was filtered into a 100 cm³; 10cm³ of the extract was pipetted in to 50 cm³

volumetric flask. 10 cm³ of 5 % potassium permanganate and 10 cm³ 30 % H₂O₂ were added and allowed to stand over a hot water bath for 30 minutes and 2cm³ of 40 % sodium sulphate added. This was made to 50 cm³ mark and the absorbance measured at 510 nm in a spectrophotometer (Okwu and Josiah, 2006).

3.6.5.4 Determination of Vitamin B₃ (Niacin)

5.0 g of the sample was treated with 50 cm³ of 1 N sulphuric acid and shaken for 30 minutes. 3 drops of ammonia solution were added to the sample and filtered. 10 cm³ of the filtrate was pipetted into a 50 cm³ volumetric and 5 cm³ of potassium cyanide was added. This was acidified with 5 cm³ 0.02 N H₂SO₄ and absorbance measured in the spectrophotometer at 470 nm wavelength (Okwu and Josiah, 2006).

3.6.5.5 Determination of vitamin C (Ascorbic Acid)

Vitamin C in the samples was determined by iodine titration method (Aurang, *et al.*, 1987).

Exactly 10 g of fresh samples were blended in 100 cm³ distilled water and then filtered.

Five milliliter of the supernatant was transferred into 120 cm³ flask. Twenty milliliter of distilled water and 2 cm³ of 1 % starch was added to each sample. The samples were then titrated with 0.01 N iodine solution containing 16 g potassium iodide.

1ml iodine solution = 0.88 mg vitamin C

3.7 Data analysis

The data obtained from the elemental analyses of the plants were analyzed by both univariate and multivariate statistical methods and relationships between the measured parameters were examined by analysis of variance (ANOVA).

CHAPTER FOUR

4.0 RESULTS

This research demonstrates the amounts of major, minor, trace and ultra trace elements in *Vernonia amygdalina* Del, *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Ceratotheca sesamoides* Endl, *Senna occidentalis* Linn and *Momordica charantia* Linn, nutritive (proteins, lipids, carbohydrates, fibers and vitamins) and anti- nutritive (cyanide, tannin, oxalate and phytate). For the concentration of the major, minor, trace and ultra-trace metals in the analyzed plants, instrumental neutron activation analysis (INAA) was used. The irradiation and counting schemes together with the WINSPAN multi-element gamma-ray analysis software have been validated using the standard reference materials. Results shown in Table 4.1 give the routine irradiation and measuring regimes developed for NIRR-1 facilities. Table 4.2 gives comparison of certified values with our results in ppm or as indicated in % for certified biological reference, IAEA-336 (Lichen) and IAEA-359 (Cabbage). Table 3 gives nuclear data and limits of detection for the elements of interest using adopted experimental conditions while Tables 4.4 – 4.9 give the analytical results of INAA of the analyzed plants.

4.1 Elemental composition of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl *Mormodica charantia* Linn and *Senna occidentalis* Linn

The results for the elemental composition of the whole plants in this work are presented in Tables 4.4 – 4.9. The results show that amounts of Al ranged from 52.0 - 2262.0 ppm. These amounts are higher in the leaves of the plants (1013.0 – 2262 ppm) followed by the roots (128.0 – 1567.0 ppm), stems (52.0 – 1215.0ppm) and then the fruits (53.0 – 346.0) as

the least. For As, the element was only found in the roots of *S. occidentalis* Linn at 1.08 ppm and absent in the other plants while that of Ba, Br and Ca are 5.0 – 583.0 ppm, 1.3 – 6.50 ppm and 2802.0 – 26950.0 ppm.

However, the amounts of Co, Cr, Cs, Cu, Dy and Eu are low or at below detection limit (BDL) in most of the plants. For Co, the amount is 0.36 – 0.76 ppm in the stems and leaves of *C. tridens* Linn, leaves of *C. sesamoides* Endl, roots and stems of *M. charantia* Linn and roots of *S. occidentalis* Linn while for Cr, the range is 2.90 – 66.08 ppm in the fruits of *M. charantia* Linn, roots and leaves of *C. sesamoides* Endl. Copper was only found in roots of *C. sesamoides* Endl at 61.95 ppm and fruits of *S. occidentalis* Linn at 74.52 ppm while Eu is only found in the roots of *V. amygdalina* Del at 0.031 ppm.

The amounts of Fe in all the samples analyzed ranged from 145.0 – 1727.0 ppm with the level higher in the leaves. Thus, the amount in the roots of *C. olitorious* Linn is 283.0 ppm, absent in the stems and 563.0 ppm in the leaves. In *C. olitorious* Linn, the concentration of Fe is as follows: 376.0 ppm (roots), BDL (stems) and 1130.0 ppm in the leaves respectively. These values are higher than what was obtained in the roots (197.0 ppm), stems (145.0 ppm) and leaves (611.0 ppm) of *V. amygdalina* Del. All these values are lower than what was obtained in *C. sesamoides* Endl, *M. charantia* Linn (except in the stems) and *S. occidentalis* Linn (except in the leaves).

However, the concentrations of Hf, La, Lu, Mg, Sb, Sc, Sm, Th, U, and Yb in the analyzed plants are either at BDL levels or trace amounts as indicated in Table 10. For example, 0.24 – 2.70 ppm La in *C. olitorious* Linn; 1.90 ppm Hf in the leaves of *C. tridens* Linn; 0.023

ppm Lu in the leaves of *C. sesamoides* Linn; 0.29 – 0.42 ppm Sb in the leaves of *M. charantia* Linn, *V. amygdalina* Del, *C. sesamoides* Endl and *S. occidentalis* Linn respectively. For Sc, the amounts are 0.02 – 0.07 ppm in *C. olitorious* Linn, 0.043 – 0.33 ppm in *C. tridens* Linn, 0.31 – 0.95 ppm in *V. amygdalina* Del, 0.62 ppm in the leaves of *C. sesamoides* Endl, 0.06 – 0.33 ppm in *M. charantia* Linn and 0.03 – 0.06 in *S. occidentalis* Linn.

Moreover, the concentrations of K in *C. olitorious* Linn, *C. tridens* Linn, *V. amygdalina* Del, *M. charantia* Linn and *S. occidentalis* Linn ranged from 4052.0 – 67270.0 ppm and the values significantly varied ($p < 0.5$) in the roots, stems, leaves and fruits of each of the plants. For example, the amount of K in the roots of *C. olitorious* Linn is 10040.0 ppm, that of the stem is 22950.0 ppm and the leaves 33870.0 ppm indicating an increase in concentration of the metal. In *C. tridens* Linn, the values are; 21110.0 ppm (roots), 54290.0 ppm and (stems), 43120.0 (leaves) while that of *V. amygdalina* Del are; 4042.0 ppm (roots), 29210.0 ppm (stems) and 37710.0 ppm (leaves), and 7949.0 ppm (roots), 16090.0 ppm (stems), 33690.0 ppm (leaves) and 15850.0 ppm in *S. occidentalis* Linn indicating the absence of regular pattern of increase or decrease.

The results also show that amount of Mn in the analyzed plants ranged from 3.19 – 326.78 ppm in all the parts of the plants. Higher concentration were found in the fruits of *M. charantia* Linn (326.78 ppm) followed by 292.43 ppm in the roots of *C. sesamoides* Endl and the least (3.19 ppm) in the stems of *C. olitorious* Linn. Pattern of distribution of Mn in the parts of the individual plants differs significantly in the analyzed plants except *C. olitorious* Linn.

Meanwhile, the amounts of Rb in analyzed plants are as follows: 6.60 and 14.0 ppm in the stems and of leaves of *C. olitorious* Linn with BDL levels in roots; 3.9, 20.0 and 23.0 ppm in the roots, stems and leaves of *C. tridens* Linn; 4.9 and 41.0 ppm in the roots and leaves of *V. amygdalina* Del with BDL levels in the stems; BDL, 26.0 and 11.0 ppm in the roots, stems and leaves of *C. sesamoides* Endl; 12.0, 15.0 ppm and BDL in the roots, stems and leaves of *M. charantia* Linn and 8.4, BDL, 24.0 and 5.5 ppm in the roots, stems, leaves and fruits of *S. occidentalis* Linn. Extent of distribution of Rb in the different parts of all the plants are not significantly different ($p < 0.5$) except in *M. charantia* Linn.

The concentration of V in the analyzed plants is higher in the roots of *C. sesamoides* Endl (107.0 ppm) followed by the fruits of *M. charantia* Linn (80.33 ppm) and leaves of *C. sesamoides* Endl (3.1 ppm) while the values in the other parts are in traces.

For the concentration and distribution of Zn in the analyzed plants as depicted in Tables 4.4 – 4.9 has shown that significantly higher values ($p < 0.5$) are found in *M. charantia* Linn (44.0, 62.3 and 166.3 ppm) in the stems, leaves and fruits followed by *C. sesamoides* Endl's roots (101.2 ppm), stems (33.0 ppm) and leaves (42.0 ppm). The amounts of Zn in *C. olitorious* Linn are 13.0, BDL and 37.0 ppm in the roots, stems and leaves; *C. tridens* Linn are 14.0, 22.0 and 51.0 ppm in the roots, stems and leaves; *V. amygdalina* Del is 42.2 ppm in leaves and BDL levels in other parts of the plant and *S. occidentalis* Linn are 82.0, BDL, 60.0 and 35.0 ppm in the roots, stems, leaves and fruits. The concentrations are not significantly different ($p < 0.5$) in the different parts of all the plants except *C. sesamoides* Endl and *S. occidentalis* Linn.

4.2 Translocation factors of elements in the parts of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl *Momordica charantia* Linn and *Senna occidentalis* Linn

The results for the translocation factors of the determined elements between the roots to the stems ($T_{S/R}$) or shoots to the leaves ($T_{L/S}$) are shown in Table 10. The $T_{S/R}$ values for Al in the analyzed plants are as follows: *C. olitorious* Linn (2.35), *C. tridens* Linn (1.59), *V. amygdalina* Del (0.36), *M. charantia* Linn (0.16), and *S. occidentalis* Linn (2.46) while that of Ba are 1.00 for *V. amygdalina* Del, 0.37 for *M. charantia* Linn and not determined for *C. olitorious* Linn, *C. tridens* Linn, *C. sesamoides* Endl and *S. occidentalis* Linn and meanwhile, that of Br is 1.00 for *C. olitorious* Linn and *V. amygdalina* Del.

Also the $T_{S/R}$ values for Ca in the plants are 0.89 for *C. olitorious* Linn, 0.31 for *C. tridens* Linn, 0.40 for *V. amygdalina* Del, 0.86 for *C. sesamoides* Endl and 0.19 for *S. occidentalis* Linn. The $T_{S/R}$ values for K ranged from 0.14 in *V. amygdalina* Del to 1.19 in *C. sesamoides* Endl while that of La is 0.18 in *M. charantia* Linn to 8.00 in *V. amygdalina* Del. For Mn the value is significantly higher ($p < 0.05$) in *M. charantia* Linn (20.03) followed by *C. olitorious* Linn (4.42), *S. occidentalis* Linn (0.95), *V. amygdalina* Linn (0.85), *C. tridens* Linn (0.72) and the least found in *M. Charantia* Linn (0.27) while the $T_{S/R}$ values of Na is highest in *V. amygdalina* Del (9.14) and the least found in *M. charantia* Linn (0.20)

However, for the translocation factor between the stems and the leaves ($T_{L/S}$), the highest value is found in *M. charantia* Linn (4.77) while the least value is found in *C. tridens* Linn (0.01). Likewise the same table (Table 10) showed that some transfer factors values are

indicated as not determined (Nd) indicating the concentrations of those metals are at BDL (below detection limit) levels.

4.3 Bioavailability of calcium, iron, manganese and zinc in the leaves of of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl *Mormodica charantia* Linn and *Senna occidentalis* Linn

The results for the ratio of anti-nutrient molar concentrations to the metal molar concentrations are indicated in Table 11. For [Phytate]/[Ca] and [Oxalate]/[Ca], *C. olitorious* Linn values (7.91×10^{-3} and 4.11×10^0) are significantly higher ($p < 0.05$) compared to *V. amygdalina* Del (7.57×10^{-3} and 8.79×10^{-3}), *M. charantia* Linn (2.87×10^{-3} and 2.28×10^{-3}), *C. sesamoides* Endl (1.81×10^{-3} and 9.03×10^{-2}), *C. tridens* Linn (1.34×10^{-3} and 3.80×10^{-1}) and *S. occidentalis* Linn (1.86×10^{-1} and 0.1×10^0).

In addition to that, the for [Phytate]/[Fe] and [Oxalate]/[Fe] are as follows: *C. olitorious* Linn (3.28×10^{-1} and 1.76×10^2), *C. tridens* Linn (2.46×10^{-2} and 6.94×10^{-1}), *V. amygdalina* Del (9.37×10^{-1} and 2.79^{-3}), *C. sesamoides* Endl (1.88×10^{-2} and 9.37×10^{-1}), *M. charantia* Linn (1.89×10^{-2} and 9.42×10^{-1}) and *S. occidentalis* Linn (6.33×10^{-2} and 3.16×10^0) respectively.

Moreover, for [Phytate]/[Mn] and [Oxalate]/[Mn], the values for *C. olitorious* Linn (2.62×10^0 and 1.36×10^3) are significantly higher ($p < 0.05$) than the rest of the analyzed plants viz: *C. tridens* Linn (4.37×10^{-1} and 1.24×10^1), *V. amygdalina* Del (1.81×10^0 and 2.11×10^0), *C. sesamoides* Endl (3.24×10^{-1} and 1.60×10^1), *M. charantia* Linn (5.68×10^{-2} and 4.50×10^1) and *S. occidentalis* Linn (4.90×10^{-1} and 2.63×10^{-1}).

However, values for [Ca][Phytate]/[Zn] and [Ca][Oxalate]/[Zn] for the plants are as follows: *C. oleriosus* Linn (2.34×10^0 and 1.21×10^4), *C. tridens* Linn (2.33×10^1 and 6.58×10^0), *V. amygdalina* Del (1.28×10^0 and 1.47×10^0), *S. occidentalis* Linn ($1.67 \times 10^{10-1}$ and 8.99×10^0) respectively.

4.4 Proximate composition of *Corchorus oleriosus* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl, *Mormodica charantia* Linn and *Senna occidentalis* Linn

The proximate composition of *C. oleriosus* Linn, *C. tridens* Linn, *V. amygdalina* Del, *C. sesamoides* Endl, *M. charantia* Linn and *S. occidentalis* Linn is shown in Table 4.12. The result shows that moisture content is higher in *C. oleriosus* Linn (75.53 %) followed by *C. tridens* Linn (67.62 %) and the least is *M. charantia* Linn (46.90 %). For ash, the order is *M. charantia* Linn > *C. sesamoides* Endl > *V. amygdalina* Del > *S. occidentalis* Linn > *C. tridens* Linn > *C. oleriosus* Linn (11.95 %, 10.74 %, 10.01 %, 8.34 %, 4.27 % and 2.48 %) respectively.

However, the amount of crude protein is highest in *M. charantia* Linn (19.36 %) followed by *V. amygdalina* Del (10.64 %) and the least is *C. oleriosus* Linn (3.18 %). For crude lipid the amounts are in the order of (5.15 %) in *C. oleriosus* Linn, (4.70 %) in *C. tridens* Linn, (3.33 %) in *V. amygdalina* Del, (6.67 %) in *C. sesamoides* Endl, (5.34 %) in *M. charantia* Linn and (5.00 %) in *S. occidentalis* Linn respectively. In addition to that, the amount of crude fiber in the analyzed plants is in the order of *M. charantia* Linn (10.49 %) > *C. tridens* Linn (8.40 %) > *S. occidentalis* Linn (8.00 %) > *V. amygdalina* Del (7.70 %) > *C. oleriosus* Linn > (5.00 %) > *C. sesamoides* Endl (3.50 %).

However, the carbohydrate content is highest in *V. amygdalina* Del (10.38 %) followed by *S. occidentalis* Linn (9.31 %), *C. sesamoides* Endl (9.13 %), *C. olitorious* Linn (8.65 %), *C. tridens* Linn (7.29 %) and the least, *M. charantia* Linn (5.69 %) respectively.

4.5 Vitamin composition of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl, *Mormodica charantia* Linn and *Senna occidentalis* Linn

The results of the vitamin content of the above listed plants are presented in Table 13. The results show that, the amount of vitamin A is significantly higher ($p < 0.05$) in *V. amygdalina* Del (379.46 mg/100g), *C. tridens* Linn (345.84 mg/100g), *S. occidentalis* Linn (310.28 mg/100g) and *C. sesamoides* Endl (327.84 mg/100g) and lower in *C. olitorious* Linn (2.18 mg/100g) and *S. occidentalis* Linn (0.06 mg/100g).

For vitamin B₁, the concentration is highest in *V. amygdalina* Linn (170.30 mg/100g) followed by *C. olitorious* Linn (154.3 mg/100g), *S. occidentalis* Linn (1.40 mg/100g), *C. sesamoides* Endl (0.89 mg/100mg), *M. charantia* Linn (0.72 mg/100g) and least, *C. tridens* Linn (0.68 mg/100g). Moreover, the amounts of vitamin C in *C. sesamoides* Endl (255.61 mg/100g), *V. amygdalina* Del (200.49 mg/100g) and *C. tridens* Linn (178.33 mg/100g) are significantly higher than amounts ($p < 0.05$) in *C. olitorious* Linn (94.92 mg/100g), *M. charantia* Linn (53.17 mg/100g) and *S. occidentalis* Linn (17.63 mg/100g) respectively.

However, the amounts of vitamin B₂ and B₃ are generally low ($p < 0.05$) in all the analyzed plants compared to vitamins A, B₁ and C. For *C. olitorious* Linn the values are (0.73 and 7.91 mg/100g), *C. tridens* Linn (1.07 and 0.72 mg/100g), *V. amygdalina* Linn (1.98 and

2.46 mg/100g), *C. sesamoides* Endl (1.14 and 0.94 mg/100g), *M. charantia* Linn (0.08 and 0.50 mg/100g) and *S. occidentalis* Linn (0.38 and 2.13 mg/100g).

4.6 Anti-nutritional composition of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl, *Mormodica charantia* Linn and *Senna occidentalis* Linn

The antinutrients contents of the above listed plants are presented in Table 14. The results show that phytates (2.18 mg/100g), oxalates (15.40 mg/100g), tannins (0.73 mg/100g) and cyanides (7.92 mg/100g) are significantly higher ($p < 0.05$) than phytates (0.33, 1.65, 0.35, 0.21 and 0.27 mg/100g), oxalates (1.26, 0.26, 2.41, 2.41 and 1.98 mg/100g) and cyanides (1.11, 0.54, 0.79, 0.56 and 0.23 mg/100g) in *C. tridens* Linn, *V. amygdalina* Del, *M. charantia* Linn and *S. occidentalis* Linn. The above trend is only different in the case of tannins where *C. sesamoides* Linn contains higher value than (1.81 mg/100g) *C. olitorious* Linn.

Table 4.1: Routine irradiation and measuring regimes developed for NIRR-1 facilities

Neutron Flux/irradiation					
Channel	Procedure	T_{irr}	T_d	T_m	Activation products
$1 \times 10^{11} \text{ n/cm}^2\text{/s}$					
outer irradiation	S1	2min	2 – 15min	10min	^{28}Al , ^{27}Mg , ^{38}Cl , ^{60}Cu , ^{51}Ti , ^{52}V , $^{116\text{m}}\text{In}$
Channels (B4, A4)	S2	2min	3 – 4h	10min	^{24}Na , ^{42}K , ^{165}Dy ^{56}Mn , ^{142}Eu
$5 \times 10^{11} \text{ n/cm}^2$					
Inner radiation					
Channels (B1, B2, B3 and A1)					
	L1	6h	4 – 5d	30min	^{24}Na , ^{42}K , ^{76}As , ^{52}Br ^{140}Lu , ^{153}Sm , ^{198}Au ^{239}Np (U), ^{72}Ga , ^{122}Sb
	L2	6h	10 – 15d	60min	^{46}Sc , ^{141}Ce , ^{50}Co , ^{51}Cr ^{134}Cs , ^{152}Eu , ^{177}Lu , ^{131}Ba ^{86}Rb , ^{182}Th , ^{160}Tb , ^{175}Yb ^{233}Pa (Th), ^{65}Zn , ^{59}Fe , ^{181}Hf

Source: Jonah *et al*, 2006 and Achi *et al*, 2012

Table 4.2: Comparison of certified values (CV) with our results in ppm or as indicated in % for the certified reference materials, IAEA-336 (Lichen) and IAEA-359 (Cabbage)

Element	IAEA-336 (Lichen)		IAEA-359(Cabbage)	
	This work (TW)	CV	This work (TW)	(CV)
Al	709±35	570-790	175± 13	-
As	BDL	0.55-0.71	BDL	0.0960.104
Ba	BDL	5.3-7.5	BDL	10.5-1.5
Br	11.2±1.3	11.2-14.6	8.0±3.0	-
Ca(%)	BDL	-	1.97 ±0.12	1.8-1.9
Ce	BDL	1.11-1.45	BDL	-
Co	BDL	0.24-0.34	BDL	-
Cr	BDL	0.89-1.23	BDL	1.24-1.36
Cs	BDL	0.097-0.123	BDL	-
Cu	BDL	144	BDL	78.6
Eu	BDL	0.019-0.027	BDL	-
Fe(%)	BDL	0.038- 0.048	BDL	0.014-0
K(%)	BDL	0.16-0.21	3.3±0.4	3.18-3.32
La	0.66±0.04	0.56-0.76	0.27±0.05	-
Lu	BDL	0.004-0.009	BDL	-
Mg(%)	BDL	BDL	0.21-0.22	-
Mn	61±2	56-70	29.4±0.8	31.3-32.5
Na	317±16	280-360	676±30	567-601

Rb	BDL	1.54-1.98	BDL	-
Sb	BDL	0.063-0.083	BDL	-
Sc	0.16±0.03	0.15-0.17	BDL	-
Sm	BDL	0.092-0.12	BDL	-
Tb	BDL	0.012-0.016	BDL	-
Th	BDL	0.12-0.16	BDL	-
V	BDL	1.25-1.69	BDL	-
Yb	BDL	0.025-0.049	BDL	-
Zn	BDL	27.0-33.8	BDL	-

BDL – below detection limit

Table 4.3: Nuclear data and limits of detection for the elements of interest using adopted experimental conditions

Target isotope	Product isotope by (n, γ) reation	Half-lfe	Gamma-energy KeV	LOD(ppm)
²³ Na	²⁴ Na	14.96h	1368.60	40(L1)
²⁶ Mg	²⁷ Mg	9.46min	1014.4	7250(S1)
²⁷ Al	²⁸ Al	2.24min	1778.9	17(S1)
³⁷ Cl	³⁸ Cl	37.24	1624.7	2900(S)
⁴¹ K	⁴² K	12.36h	1524.58	2400(S2)
⁴⁵ Sc	⁴⁶ Sc	83.81d	889.28	0.2(L2)
⁴⁸ Ca	⁴⁹ Ca	8.72min	3084.54	6600 (S1)
⁵⁰ Ti	⁵¹ Ti	5.76min	329.08	2500(S1)
⁵⁰ Cr	⁵¹ Cr	27.7d	320.98	23(L2)
⁵¹ V	⁵² V	3.75min	1434.08	15(S1)
⁵⁵ Mn	⁵⁶ Mn	2.58h	846.76	0.9(S2)
⁵⁸ Fe	⁵⁹ Fe	44.5d	1099.25	829(L2)
⁵⁹ Co	⁶⁰ Co	5.27y	1173.2	3.0(L2)
⁶⁵ Cu	⁶⁶ Cu	5.10min	1039.2	172(S1)
⁶⁴ Zn	⁶⁵ Zn	243.9d	1115.55	120(L2)
⁷¹ Ga	⁷⁴ Ga	14.1h	834.1	1.0(L1)
⁷⁵ As	⁷⁶ As	26.32h	559.10	1.2(L1)
⁸¹ Br	⁸² Br	35.3h	776.5	3.0(L1)
⁸⁵ Rb	⁸⁶ Rb	18.8d	1076.6	3.0(L2)

¹¹⁵ In	^{116m} In	54.15	1097.3	0.5(S1)
¹²¹ Sb	¹²² Sb	64.8h	564.24	0.5(L1)
¹³³ Cs	¹³⁴ Cs	2.06y	795.85	1.7(L2)
¹³⁰ Ba	¹³¹ Ba	11.8d	496.3	264(L2)
¹³⁹ La	¹⁴⁰ La	40.3h	1596.21	0.2(L1)
¹⁴⁰ Ce	¹⁴¹ Ce	32.5d	145.44	14(L2)
¹⁵¹ Eu	¹⁵² Eu	13.3y	1408.5	0.6(L2)
¹⁵² Sm	¹⁵³ Sm	46.27h	103.18	0.1(L1)
¹⁵⁹ Tb	¹⁶⁰ Tb	72.3d	879.38	1.1(L2)
¹⁶⁴ Dy	¹⁶⁵ Dy	2.33h	94.70	0.7(S2)
¹⁷⁴ Yb	¹⁷⁵ Yb	4.19d	396.33	0.9(L1)
¹⁷⁶ Lu	¹⁷⁷ Lu	6.71d	208.36	0.1(L2)
¹⁸⁰ Hf	¹⁸¹ Hf	42.4d	482.2	1.1(L2)
¹⁸¹ Ta	¹⁸² Ta	115d	1221.4	1.0(L2)
¹⁹⁷ Au	¹⁹⁸ Au	2.7d	411.8	0.02(L1)
²³² Th	²³³ Pa	27.0d	312.01	1.2(L2)
²³⁸ U	²³⁹ Np	2.36d	277.60	1.5(L1)

S1, S2, L1 and L2 represent irradiation and counting schemes adopted for the respective elements.

Table 4.4: Elemental concentration of *Corchorus olitorius* Linn in ppm

Element	Roots (COLR)	Stems (COLS)	Leaves (COLL)
Al	491±40†	209±19†	1043.0±88.0†
Ba	BDL	12±2†	12.3±2.6†
Br	1.3±0.4†	1.3±0.3†	8.04±0.89
Ca	5541±820†	6242± 924†	16670.0±2434†
Co	BDL	BDL	BDL
Cr	BDL	BDL	BDL
Cs	NA	NA	NA
Cu	BDL	NA	BDL
Dy	BDL	BDL	BDL
Eu	BDL	BDL	BDL
Fe	283±53‡	BDL	563±52‡
Hf	NA	NA	BDL
K	10040± 121‡	22950±184‡	33870±2370‡
La	0.93±0.04†	0.24±0.02†	2.70±0.16†
Lu	BDL	BDL	BDL
Mn	14.1±0.6†	3.19±0.14†	68.0±3.0†
Na	786±2.0‡	236±1.0‡	3520±.0‡
Rb	BDL	6.6±0.8†	14.0±1.0†
Sb	BDL	BDL	BDL
Sc	0.07±0.01†	BDL	0.02±0.02†
Sm	0.14±.01	BDL	BDL

Ta	NA	NA	NA
Th	BDL	BDL	0.95±0.16
U	NA	NA	NA
V	BDL	BDL	1.47±0.31
Yb	BDL	BDL	BDL
Zn	13.0±3.0†	BDL	37.0±3.3†

BDL - Below detection limit; NA – Not analyzed; †Elements determined in the parts of a plant are not significantly different while ‡ are different at p<0.5.

Table 4.5: Elemental concentration of *Corchorus tridens* Linn in ppm

Element	Roots (CTLR)	Stems (CTLS)	Leaves (CTLL)
Al	334.0±29.0†	209.0±19.0	453±119.0†
As	BDL	BDL	BDL
Ba	BDL	111.0±18.0	138.0±19.0
Br	BDL	3.5±0.6†	5.0±1.0†
Ca	3788.0±591.0‡	12350±1803‡	14750±21390‡
Co	BDL	0.36±0.12†	0.46±0.11†
Cr	BDL	BDL	BDL
Cs	BDL	BDL	BDL
Cu	NA	NA	NA
Dy	BDL	BDL	BDL
Eu	BDL	BDL	BDL
Fe	376.0±45.0‡	BDL	1130±73‡
Hf	NA	BDL	1.9±0.1
K	21110.0±1457.0‡	54290.0±326.0‡	43120.0±57.0‡
La	1.7±0.0†	0.034±0.002†	2.72±0.04†
Lu	BDL	BDL	BDL
Mg	NA	NA	NA
Mn	23.0±1.0‡	32.0±1.0‡	62.3±2.6‡
Na	315.0±8.0‡	198.0±2.0‡	416.0±3.0‡
Rb	3.9±0.8†	20.0±3.0†	23.0±2.0†
Sb	BDL	BDL	BDL

Sc	0.11±0.01†	0.043±0.008†	0.33±0.01†
Sm	BDL	NA	NA
Ta	NA	NA	NA
Th	7.0±1.0†	BDL	1.1±0.1†
U	NA	NA	BDL
V	0.7±0.2‡	BDL	1.9±0.3‡
Yb	BDL	BDL	BDL
Zn	14.0±3.0†	22.0±3.0†	51.0±4.0†

BDL – Below detection limit; NA – Not analyzed; †Elements determined in the parts of a plant are not significantly different while ‡ are different at p<0.5.

Table 4.6: Elemental concentration of *Vernonia amygdalina* Del in ppm

Element	Roots (VADR)	Stems (VADS)	Leaves (VADL)
Al	440.0±36.0‡	1215.0±114‡	657.0±56.0‡
As	BDL	BDL	BDL
Ba	5.4±1.5‡	44.0±2.0‡	BDL
Br	2.7±0.4†	2.7±0.5†	6.5±0.7†
Ca	2802±443.0†	7028.0±1040.0†	13250.0±1934.0†
Co	BDL	BDL	BDL
Cr	BDL	BDL	BDL
Cs	NA	NA	NA
Cu	NA	NA	NA
Dy	BDL	BDL	BDL
Eu	0.031±0.008	BDL	BDL
Fe	197.0±36.0†	145.0±41.0†	611.0±62.0†
Hf	NA	BDL	BDL
K	4052.0±122.0‡	29210.0±234.0‡	37710.0±453.0‡
La	3.20±0.05†	0.40±0.08†	2.39±0.05†
Lu	BDL	BDL	BDL
Mg	NA	NA	NA
Mn	11.6±0.5‡	13.7±0.6‡	76.0±3.0‡
Na	731.0±2.0†	80.0±1.0†	414.0±3.0†
Rb	4.9±0.8†	BDL	41±3.0†
Sb	BDL	BDL	0.42±0.07

Sc	0.95±0.07†	BDL	0.31±0.01†
Sm	BDL	0.03±0.01‡	0.28±0.01‡
Ta	NA	NA	NA
Th	0.41±0.06†	BDL	0.35±0.06†
U	NA	BDL	BDL
V	0.42±0.09	BDL	BDL
Yb	BDL	BDL	BDL
Zn	BDL	BDL	42.2±3.8

BDL – Below detection limit; NA – Not analyzed; †Elements determined in the parts of a plant are not significantly different while ‡ are different at p<0.5.

Table4.7: Elemental concentration of *Ceratotheca sesamoides* Endl in ppm

Element	Roots (CSLR)	Stems (CSLS)	Leaves (CSLL)
Al	1567.0±79.0‡	BDL	2262.0±188.0‡
As	BDL	BDL	BDL
Ba	193.0±7.0‡	BDL	5.0±1.0‡
Ca	13930.0±569.0†	16140.0 ±2744.0†	11850.0±754.0†
Co	BDL	BDL	0.5±0.1
Cr	65.08±8.2‡	BDL	2.9±0.5‡
Cs	BDL	BDL	NA
Cu	61.95±2.1	BDL	BDL
Dy	BDL	BDL	BDL
Eu	BDL	BDL	BDL
Fe	1727.0±67.0†	BDL	1598.0±43.0†
Hf	BDL	BDL	NA
K	23250.0±832.0†	30120.0±422.0†	22650.0±1586.0†
La	BDL	0.41±0.04†	6.8±0.34†
Lu	BDL	BDL	0.023±0.005
Mg	NA	NA	NA
Mn	292.43±5.8‡	14.6±0.6‡	92.0±4.0‡
Na	BDL	68.0±4.0‡	493.0±28.0‡
Rb	BDL	26.0 ±3.0†	11.0±1.0†
Sb	BDL	BDL	0.4±0.05
Sc	BDL	BDL	0.62±0.04

Sm	BDL	0.06±0.006	BDL
Ta	BDL	BDL	NA
Th	BDL	BDL	4.7±0.7
U	BDL	BDL	BDL
V	107.0 ±2.6‡	BDL	3.1±0.4‡
Yb	BDL	BDL	0.35±0.05
Zn	101.2±6.0‡	33.0±5.0‡	42.0±4.0‡

BDL – Below detection limit; NA – Not analyzed; †Elements determined in the parts of a plant are not significantly different while ‡ are different at p<0.5.

Table 4.8: Elemental concentration of *Mormodica charantia* Linn in ppm

Element	Stems (MCLS)	Leaves (MCLL)	Fruits (MCLF)
Al	165.0±14.0†	1013.0±83.0†	346.0±12.0†
As	BDL	BDL	BDL
Ba	215.0±17.0†	583.0±29.0†	122.0±3.6†
Br	BDL	2.54±0.06	BDL
Ca	5096.0±759.0†	26950.0 ±3782.0†	4697.0±228.0†
Co	0.55±0.12†	0.76±0.11†	BDL
Cr	BDL	BDL	66.83±8.7
Cs	BDL	BDL	BDL
Cu	BDL	BDL	74.52±6.4
Dy	BDL	BDL	BDL
Eu	BDL	BDL	BDL
Fe	213.0±47.0‡	1066.0±79.0‡	1590.0±234.0‡
Hf	BDL	1.45±0.12	BDL
K	51490.0±309.0†	43090.0±474.0†	67270.0±455.0†
La	0.44 ±0.02†	2.47±0.06†	BDL
Lu	BDL	BDL	BDL
Mg	NA	NA	BDL
Mn	11.3±0.5‡	41.4±1.7‡	326.78±38.0‡
Na	170±1.0†	835±5.0†	BDL
Rb	12.0±2.0‡	15.0±2.0‡	BDL
Sb	BDL	0.40±0.06	BDL

Sc	0.06±0.01‡	0.33±0.01‡	BDL
Sm	0.05±0.01‡	0.37±0.01‡	BDL
Ta	BDL	BDL	BDL
Th	BDL	0.75±0.09	BDL
U	BDL	BDL	BDL
V	BDL	1.93±0.22‡	80.33±9.1‡
Yb	BDL	BDL	BDL
Zn	44.0 ±4.0†	62.3±4.4†	166.3±11.0†

BDL – Below detection limit; NA – Not analyzed; †Elements determined in the parts of a plant are not significantly different while ‡ are different at p<0.5.

Table 4.9: Elemental concentration of *Senna occidentalis* Linn in ppm

Element	Roots (SOLR)	Stems (SOLS)	Leaves (SOLL)	Fruits (SOLF)
Al	128.0±11.0†	52.0±5.0†	380.0±38.0†	53.0±5.0†
As	1.08±0.04	BDL	BDL	BDL
Ba	BDL	BDL	81.0±18.0	BDL
Br	BDL	BDL	4.0±0.8	BDL
Ca	4336.0±659‡	8867.0 ±1293‡	18310.0±2665‡	8800.0±1293.0‡
Co	0.41±0.09	BDL	BDL	BDL
Cr	BDL	BDL	BDL	BDL
Cs	BDL	BDL	BDL	NA
Cu	NA	NA	BDL	BDL
Dy	BDL	BDL	BDL	BDL
Eu	BDL	BDL	BDL	BDL
Fe	22215.0±89.0‡	BDL	390.0±55.0‡	BDL
Hf	BDL	BDL	BDL	NA
K	7949.0±119.0‡	16090.0±290.0‡	33690.0±438.0‡	15850.0±127.0‡
La	0.74±0.03‡	0.16±0.02‡	1.23±0.02‡	0.14±0.01‡
Lu	BDL	BDL	BDL	BDL
Mg	NA	NA	NA	BDL
Mn	7.1±0.3‡	7.5±0.3‡	46.0±2.0‡	13.0±1.0‡
Na	278.0±2.0‡	131.0±2.0‡	319.0±3.0‡	36.2±0.5‡
Rb	8.4±1.7†	BDL	24.0±2.0†	5.5±0.5†
Sb	BDL	BDL	0.29±0.05	BDL

Sc	0.03±0.01†	BDL	0.06±0.01†	BDL
Sm	0.12±0.01†	BDL	0.11±0.01†	BDL
Ta	NA	NA	BDL	NA
Th	BDL	BDL	0.17±0.05	BDL
U	BDL	BDL	BDL	BDL
V	BDL	BDL	BDL	NA
Yb	BDL	BDL	BDL	BDL
Zn	82.0±5.0‡	BDL	60.0±5.0‡	35.0±3.0‡

BDL – Below detection limit; NA – Not analyzed; †Elements determined in the parts of a plant are not significantly different while ‡ are different at p<0.5.

Table 4.10: Translocation factors of the elements between roots to the stems (TS/R) and stems to the leaves (TL/S) of the plants below

Element	<i>C. olerifolius</i> Linn		<i>C. tridens</i> Linn		<i>V. amygdalina</i> Del		<i>C. sesamoides</i> Endl		<i>M. charantia</i> Linn		<i>M. occidentalis</i> Linn	
	S/R	L/S	S/R	L/S	S/R	L/S	S/R	L/S	S/R	L/S	S/R	L/S
Al	2.35	0.20	1.59	0.14	0.36	1.85	Nd	Nd	0.16	2.93	2.46	0.14
Ba	Nd	0.98	0.80	0.12	Nd	Nd	Nd	Nd	0.37	4.77	Nd	Nd
Br	1.00	0.16	Nd	0.70	1.00	0.42	Nd	Nd	Nd	Nd	Nd	Nd
Ca	0.89	0.37	0.31	0.84	0.40	0.53	0.86	1.36	0.19	5.54	0.49	0.48
Co	Nd	Nd	Nd	0.78	Nd	Nd	Nd	Nd	0.72	Nd	Nd	Nd
Cr	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Cu	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Fe	Nd	Nd	Nd	Nd	1.36	0.24	Nd	Nd	0.20	0.67	Nd	Nd
K	0.44	0.68	0.39	1.26	0.14	0.77	0.77	1.33	1.19	0.64	0.49	0.48
La	3.88	0.09	50.0	0.01	8.00	0.17	Nd	0.06	0.18	Nd	4.63	0.13
Mn	4.42	0.05	0.72	0.51	0.85	0.18	20.03	0.16	0.27	0.13	0.95	0.16
Na	3.33	1.50	1.59	0.48	9.14	0.19	Nd	1.39	0.20	Nd	2.12	0.41

Rb	Nd	0.47	0.20	0.87	Nd	Nd	3.36	0.80	Nd	Nd	Nd	Nd
Sc	Nd	Nd	2.56	0.13	Nd	Nd	Nd	0.18	Nd	Nd	Nd	Nd
Sm	Nd	Nd	Nd	0.14	Nd	0.11	Nd	Nd	0.16	Nd	Nd	Nd
Th	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
V	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	0.02	Nd	Nd
Zn	Nd	Nd	0.64	2.32	Nd	Nd	3.07	0.79	0.71	0.37	Nd	Nd

TF values > 1 signifies good transfer of the elements while TF values <1 signifies retention of that particular element in the affected parts.

Table 4.11: Bioavailability of some of the metals in the leaves of *Corchorus Olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl, *Mormodica charantia* Linn and *Senna occidentalis* Linn

Parameter	<i>C. olitorious</i> Linn	<i>C. tridens</i> Linn	<i>V. amygdalina</i> Del	<i>C. sesamoides</i> Endl	<i>M. charantia</i> Linn	<i>S. occidentalis</i> Linn	Critical value
[Phytate]/[Ca]	7.91×10^{-3}	1.34×10^{-3}	7.57×10^{-3}	1.81×10^{-3}	2.87×10^{-3}	1.86×10^{-3}	0.24
[Phytate]/[Fe]	3.28×10^{-1}	2.46×10^{-2}	2.30×10^{-1}	1.88×10^{-2}	1.89×10^{-2}	6.33×10^{-2}	0.40
[Phytate]/[Mn]	2.62×10^0	4.37×10^{-1}	1.81×10^0	3.24×10^{-1}	5.68×10^{-2}	4.90×10^{-1}	NA
[Phytate]/[Zn]	4.02×10^0	6.31×10^{-1}	3.86×10^0	8.29×10^{-1}	1.32×10^{-1}	7.60×10^{-1}	1.50
[Oxalate]/[Ca]	4.11×10^0	3.80×10^{-1}	8.79×10^{-1}	9.03×10^{-2}	2.28×10^{-1}	0.10×10^0	2.50
[Oxalate]/[Fe]	1.70×10^2	6.94×10^{-1}	2.69×10^{-1}	9.37×10^{-1}	9.42×10^{-1}	3.16×10^0	NA
[Oxalate]/[Mn]	1.36×10^3	1.24×10^1	2.11×10^0	1.60×10^1	4.50×10^1	2.63×10^{-1}	NA
[Oxalate]/[Zn]	2.95×10^3	1.78×10^1	4.48×10^0	4.14×10^1	1.05×10^1	4.09×10^1	NA
[Ca][Phytate]/[Zn]	2.24×10^0	2.33×10^1	1.28×10^0	1.72×10^1	1.55×10^{-2}	1.67×10^{-1}	0.50
[Ca][Oxalate]/[Zn]	1.21×10^2	6.58×10^0	1.49×10^0	1.12×10^1	1.22×10^0	8.99×10^0	NA

Source: Umar *et al.*, 2005, Frontela2008 *et al.*, 2008 and Mitchkpe *et al.*, 2008. NA: Not available.

Table 4.12: Proximate composition of the leaves of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia Amygdalina* Del, *Ceratotheca sesamoides* Endl, *Momordica charantia* Linn and *Senna occidentalis* Linn in percentage (%) unless otherwise stated.

Parameter	Moisture (%)	Ash (%)	Crude protein (%)	Crude Lipid (%)	Crude fiber (%)	Carbohydrate (%)	Calorific value (Kcal/100g)
<i>Corchorus olitorious</i> Linn	75.53±1.09	2.48±0.97	3.19±0.43	5.15±0.67	5.00±0.34	8.65±0.94	93.71
<i>Corchorus tridens</i> Linn	67.62±0.41	4.27±1.56	7.72±1.80	4.70±0.12	8.40±0.51	7.29±0.07	100.20
<i>Vernonia amygdalina</i> Del	57.94±0.68	10.01±1.33	10.64±0.36	3.33±0.24	7.70±0.65	10.38±0.45	114.07
<i>Ceratotheca sesamoides</i> Endl	63.84±1.49	10.74±0.50	6.12±0.17	6.67±0.26	3.50±0.21	9.13±0.64	121.03
<i>Momordica charantia</i> Linn	46.90±0.32	11.95±0.31	19.36±0.84	5.34±0.51	10.49±0.78	5.69±0.16	163.90
<i>Senna occidentalis</i> Linn	65.51±2.7	8.35±0.38	3.83±0.27	5.00±0.49	8.00±0.66	9.31±0.75	97.48
RDA value	N.A	N.A	56g/day	30g/day	21-38%	130g/day	2600-2800Cal.

Source of RDA values: NRC (National Research Council) (1989)

Table 4.13: Anti-nutritional factors of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotherca sesamoides* Endl, *Momordica charantia* Linn and *Senna occidentalis* Linn leaves in mg/100g unless otherwise stated.

Anti-nutrient	Phytates	Oxalates	Tannins	HCN
<i>Corchorus olitorious</i> Linn	2.18±0.02	15.4±1.25	0.73±0.31	7.92±1.11
<i>Corchorus tridens</i> Linn	0.33±0.04	1.26±0.32	0.59±0.13	1.11±0.09
<i>Vernonia amygdalina</i> Del	1.65±0.67	0.26±0.95	0.41±0.08	0.54±0.25
<i>Ceratotherca sesamoides</i> Endl	0.35±0.13	2.41±0.41	1.81±0.53	0.79±0.12
<i>Momordica charantia</i> Linn	0.21±0.08	2.41±0.27	0.87±0.23	0.58± 0.06
<i>Senna occidentalis</i> Linn	0.27±0.08	1.98±0.17	0.63±0.11	0.23±0.05
Critical Values	400.0 – 800.0mg	71.0mg	50.0mg	0.5 – 3.5mg

Source of critical values of anti-nutritionals: Munro and Bassir, 1973 and Ikediobi *et al.*, 1980

Table 4.14: Vitamin content of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl, *Momordica charantia* Linn and *Senna occidentalis* Linn plant leaves in mg/100g unless otherwise stated with their RDA values.

Vitamin	Vitamin A (Retinol)	Vitamin B ₁ (Thiamin)	Vitamin B ₂ (Riboflavin)	Vitamin B ₃ (Niacin)	Vitamin C (Ascorbic acid)
<i>Corchorus olitorious</i> Linn	2.18±0.02	154.13±2.76	0.73±0.31	7.92±1.111	94.95±2.61
<i>Corchorus tridens</i> Linn	345.84±0.21	0.68±0.35	1.07±0.22	0.72±0.02	178.33±5.57
<i>Vernonia amygdalina</i> Del	379.46±0.42	170.30±0.76	1.98±0.06	2.46±0.34	200.49±12.5
<i>Ceratotheca sesamoides</i> Endl	327.84±0.72	0.89± 0.26	1.14±0.04	0.94±0.01	255.61±5.2
<i>Momordica charantia</i> Linn	0.06±0.012	0.72±0.001	0.08±0.001	0.5±0.001	53.17±0.58
<i>Senna occidentalis</i> Linn	310.28±1.68	1.40±0.02	0.38±0.01	2.13±0.05	17.63±1.56
RDA Value	900.0µg	1.2mg	1.3mg	90.0mg	15.0mg

Source of RDA values: U.S. food and Nutrition board, 2001

CHAPTER FIVE

5.0 DISCUSSION

5.1 Elemental composition of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl, *Mormodica charantia* Linn and *Senna occidentalis* Linn

5.1.1 Aluminium

The concentration of Aluminium in the analyzed plants ranged from 52.0 – 2262.0 ppm as seen in tables 4.4 – 4.9. In the leaves the range is the highest (1013.0 – 2262 ppm) while for the roots the range is 128.0 ppm in *S. occidentalis* Linn to 440.0 ppm in *V. amygdalina* Del with the element not detected in *M. charantia* Linn.

Beside ranking as the third most abundant element in the Earth's crust in the form of silica and aluminosilicate, only a small quantity of it exist in a soluble form which is capable of influencing biological systems (Silva, 2012). Its bioavailability is mainly restricted to soils that are acidic in nature there by making the aluminosilicate clays and aluminium hydroxide minerals to dissolve there by releasing aluminium cations available to plants grown on such soils (Silva, 2002). In fact, this observation reflects the high concentration of aluminium in *C. sesamoides* Endl leaves (2262.0 ppm) known to grow well on sandy soils. This may be the consequences of natural development of soil acidification when basic cations are leached from soils or accelerated by some farming practices (Delhaizie and Ryan, 1995).

A value of the amount of Aluminium above 150 mg/KgDm in sub clover plant leaves may indicate toxicity (Bouma *et al.*, 1981). The signs of aluminum toxicity may resemble those

of phosphorus deficiency (overall stunting; small; abnormally dark green leaves; purpling of stems, leaves and leaf veins; and yellowing and death of leaf tips). Aluminium injured roots are characteristically stubby and spatulate while the root tips are thickened and may be brown. The root system as a whole has many stubby lateral roots but lacks fine branching. Aluminium damaged roots are inefficient in absorbing water and nutrients (Cregan, 1980).

5.1.2 Calcium

Calcium in the form of calcium pectate participates in cell wall structure and associates as Ca^{+2} with cell membrane phospholipids and is necessary for maintenance of permeability of cells in the plant. It also acts activators of some enzymes; amylases, ATPases and phospholipase (White and Broadly, 2003) in living systems. This may be the reason why calcium has high concentration in all the samples. In the roots of these plants the levels of Ca ranged from 2802.0 – 13930.0 ppm while for the stems the range is 6242.0 – 16140.0 ppm and the leaves 11850.0 – 26950.0 ppm. This value is inconformity with the values obtained elsewhere (Ndlovu and Afolayan, 2008). The trend of the concentration of the element is in the order of roots < stems < leaves with the overall highest in the leaves of *M. charantia* Linn (26950.0 ppm).

Calcium roles in the body of man include components of the skeleton, teeth and essential constituent of cells and tissue fluids. It is also concerned with the activity of enzymes especially those necessary for the transmission of nerve impulses, coagulation of blood and for the contractile properties of muscles. Therefore with these high concentrations of Ca in the leaves of these plants that are edible will greatly supplement the mineral needs for

animals when they consume these plants. The high concentrations may also be attributed to the presence of large tissue fluids in the stems and leaves since calcium is more of structural component. It is required for various structural roles in the cell wall and membranes; it is also a counter-ion for inorganic and organic anions in the vacuole and cytosolic Ca^{+2} concentrations ($[\text{Ca}^{+2}][\text{Cyt}]$) is an obligate intracellular messenger coordinating responses to numerous developmental cues and environmental challenges (White and Broadly, 2003).

5.1.3. Vanadium

For *C. oltorius* Linn element vanadium was only detected and determined in the leaves of and absent in the other parts of the plant analyzed. For *Corchorus tridens* Linn the concentrations are 0.70 ppm in the roots and 1.90 ppm in the leaves and absent in the stems. Similar pattern was observed in the other plants analyzed in this work (*Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl and *Momordica charantia* Linn) with little variation in *Ceratotheca sesamoides* Endl leaves having the highest values. The amounts of vanadium in these plants are very much lower compared to those reported in plants of 251.0 – 452.0 ppm (Gwarzo *et al.*, 2006), 96.70 – 112.0 ppm (Ekwumengbo, 2002) and (Macdonald and Edwards, 1987).

5.1.4 Copper

The element copper is at below detection limit (BDL) concentration in *C. oltorius* Linn, *C. tridens* Linn, *S. occidentalis* Linn and *V. amygdalina* Del but found in the roots of *C. sesamoides* Endl at 61.95 ppm levels and that of *M. charantia* Linn at 74.52 ppm level. Though the element (Cu) is at BDL levels in most of the plants analyzed, conforms the

notion researchers have on concentrations of metal ions increasing from roots to the stems, to the leaves and then to flowers/fruits (Allen *et al.*, 1974). The presence of copper in plants may not be unconnected with the roles it plays in photosynthesis, respiratory electron transport chains, ethylene sensing, cell wall metabolism, oxidative stress protection and biogenesis of molybdenum cofactor and its average content in plant tissue is 10 ppm with critical free copper concentration in nutrient (below which deficiency occurs) ranges from 10^{-14} to 10^{-16} M (Yruela, 2009).

5.1.5 Manganese

Manganese concentration in *C. olitorious* Linn ranged from 3.19 – 68.0 ppm. The pattern of accumulation is from stems to the roots, and then to the leaves. For *C. tridens* Linn similar concentrations were observed and ranged from 23.0 – 62.3 ppm. The pattern of accumulation is that, leaves > stems > roots. In addition to that, the amount of manganese in the other plants analyzed in this work did not follow a particular pattern as against what was obtained elsewhere in which the roots have the highest, followed by the leaves and the least are the stems (Salami and Non, 2002). This work (Table 4.4 – 4.9) has also indicated lower values of manganese compared to other studies elsewhere 17.3 – 356.0 ppm in *Leptadenia lanceifolia* Decne (Gwarzo *et al.*, 2012). It was also found that, the element (Mn) is at 7.5 – 46.0 ppm level in *S. occidentalis* linn. The apparent presence of manganese in all the plants analyzed in this work is against what was obtained elsewhere (its absence in *Moringa oleifera* Lam), (Gwarzo, *et al.*, 2006) and this showed a phenomenon of selective absorption and accumulation of certain metals by certain species of plants as observed by in spinach (Folanranmi *et al.*, 2002).

5.1.6 Sodium

The concentration of sodium in *C. olitorious* Linn ranged from 236.0 – 786.0 ppm, 243.757 – 416.0 ppm in *C. tridens* Linn, 80.0 – 731.0 ppm in *V. amygdalina* Del, 493.0 – 686.0 ppm in *C. sesamoides* Endl and 170.0 – 835.0 ppm in *M. charantia* Linn and 36.2 – 278.0 ppm in *S. occidatalis* Linn as observed in tables 4.4 – 4.9. The amounts of the element (Na) in the roots of *C. olitorious* Linn is highest followed by the leaves and then the roots. This trend contradicts other finds elsewhere (Folaranmi *et al.*, 2002) who found that, the decrease is in the order of roots > stems > leaves. For *C. tridens* Linn also the order is leaves > roots > leaves while for *V. amygdalina* Del the order is the same as that of *C. olitorious* Linn. But for *C. sesamoides* Endl, the stems have the highest amount of Na while the leaves have while the leaves have the least. Sodium is the key element in maintaining turgor within the plant's stem. With high Na concentrations in the stems, osmotic pressure increases and water flows in to the stems to maintain concentration equilibrium (Subbarao *et al.*, 2003; Rodriguez-Navarro and Rubio, 2006).

5.1.7 Potassium

Potassium is the chief cation of protoplasm with important functions in the plant cell as charge balancer, component of potassium-enzyme complex and an activator of other enzymes (Rodriguez-Novarro and Rubio, 2006)). Tables 4.4 – 4.9 depicts the concentration of potassium in the analyzed plants. Values for the element in *C. olitorious* Linn ranged from 10040.0 – 33870.0 ppm with the highest in the leaves the roots contain the least. The concentration of potassium in *C. tridens* Linn ranged from 21110.0 – 54290.0 ppm and it is in the order of stems > leaves > roots. For *V. amygdalina* Del; the highest concentration of potassium is in the leaves while the least is in the roots in the order roots >

stems > leaves. Also the concentration of potassium in *C. sesamoides* Endl ranged from 22650.0 – 30120.0 ppm with the stems having the highest amount and the roots with the least. *M. charantia* Linn fruits surprisingly in this work exhibit unusually high concentration of the metal (67270.0 ppm) followed by the roots (51490.0ppm) and the leaves having the least value (43090.0 ppm).

For *S. occidentalis* Linn the leaves have the highest amount of the element (33690.0ppm) followed by the stems (16090.0 ppm) then the roots (7949.0 ppm) and the element detected at 15850.0 ppm in the fruits of the plant.

Generally the amounts of potassium in all the samples analyzed especially the leaves of these plants that are edible are high. In fact the levels found in this work exceed the amounts found elsewhere (8650 – 18100 ppm in *M. oleifera* Lam) (Gwarzo *et al.*, 2006). Therefore this work suggests that, the consumption of the leaves of these plants will contribute to the healthy living of humans and animals as the element (K) plays an important role in nerve and muscle excitability and also for carbohydrate metabolism (Abbrecht, 1969).

5.1.8 Lanthanum

The concentrations of La in the analyzed plants are in traces with the range of 0.034 – 6.80 ppm. In *C. olerifera* Linn the amounts of the element is in the order of leaves > roots > stems which follows the same trend in *C. tridens* Linn and *V. amygdalina* Del. But in *C. sesamoides* Endl the leaves have the highest concentration of the element (6.80 ppm) followed by the stems (0.41 ppm) with none in the roots of the plant. Likewise in *M.*

charantia Linn the highest concentrations found in the leaves (2.47 ppm) followed by the stems (0.44 pm) and none detected in the fruits of the plant. Lanthanum is also found in traces in all the parts of *S. occidentalis* Linn decreasing in the order of leaves > roots > stems > fruits.

The low concentration of La in the analyzed plants in this work follows the general trend of low concentration of rare earth elements in green plants, particularly grain crops and vegetables. The rare earth element concentration patterns of leaf, stem and root are generally very similar to each other and to the host soil (Wang *et al.*, 1997).

Although lanthanum has no known biological role, its pharmacological effects on several receptors and ion-channel, its specificity for GABA receptors is unique among divalent cations. It acts at the same rate on the GABA receptor as zinc (Bolydyrera, 2005) and was found in the form of La^{+3} to inhibit both seimonastic and nyctinasic closure but did not affect the reopening process of the leaves of *Mimosa pudica* L., thus mimicking calcium ions (Cambell and Thomas, 1977). Also lanthanum treatment to growing plants (e.g Cucumber) have been to lead the change in the uptake of other trace elements which suggests that a rare earth element is directly or indirectly involved in the ion transport of the plant and affects plant growth by regulating the uptake and distribution of elements that influence the plant cell physiology and biochemistry (Zeng *et al.*, 2006).

However, lanthanum has been found to have low to moderate toxicity because on injection of its solution to animal's body produces hyperglycemia, low blood pressure, degeneration

of the spleen, hepatic alteration and impaired memory and learning abilities (Feng *et al.*, 2006). Therefore, consumption of such plants for a long time may have negative impact.

5.1.9 Samarium

Samarium has no biological role but it has been noted to stimulate metabolism. Soluble samarium salts are mildly toxic by ingestion and therefore health hazards associated with it includes skin and eye irritations (Haley *et al.*, 1961). In this work the element has been detected and determined only in the roots of *C. olitorious* Linn with the concentration at 0.14 ppm. Also, in *C. tridens* Linn it was found in the stems and leaves and its concentration ranges from 0.056 ppm to 0.39 ppm. The element amounts to 0.03ppm in the stems and 0.28 ppm in the leaves of *V. amygdalina* Del but found in the stems of *C. sesamoides* Endl at 0.60 ppm levels. Moreover, in *M. charantia* Linn, samarium is found in the stems and leaves in the order 0.05 – 0.37 ppm. Likewise it is only found in the roots and leaves of *S. occidentalis* Linn at trace levels (0.11 ppm and 0.12 ppm). The values obtained in this work is higher than the amounts found in the work of Zhang *et al.*, 2013 in the analysis of Sm and Eu in wheat by ICP-MS method (0.0029 – 0.192 ppm) and it was in the order of roots > leaves > stems > seeds.

5.1.10 Scandium

The concentration of scandium in *C. olitorious* Linn in the roots and leaves is at 0.07 ppm and 0.22 ppm. For *C. tridens* Linn, it ranged from 0.043 – 0.33 ppm with a decrease in the order of leaves > roots > stems and absent in the fruits. But for *V. amygdalina* Del the element ranged from 0.13- 0.95 ppm with the element not detected in the stems.

Also the range of Sc concentrations in *M. charantia* Linn is 0.06 – 0.33 ppm and none detected in the fruits of the plant. However the element is only detected and determined in the leaves of *C. sesamoides* Endl at 0.62 ppm levels. This trend was also observed in *Senna occidentalis* Linn with the element absent in the stems and fruits of the plant. Where it is detected and determined (roots and leaves) the range is 0.03 – 0.06 ppm. The low concentration of the element in the analyzed plants and its absence in some of them is inconformity with the claim that only about 3.0 % of plants that were analyzed for scandium show its presence and even those amounts were tiny, with vegetables having only 5.0 ppb and grasses with 70.0 ppb levels. The element (Sc) has no biological role and only trace amounts of it reach the food chain. It is non-toxic, though there have been suggestions that some of its compounds might be carcinogenic (Shtangeeva *et al.*, 2006) and with water animals, scandium causes damage to cell membranes which have several negative influences on reproduction and on the functions of the nervous system.

5.1.11 Chromium

Chromium's concentrations in *C. olitorious* Linn, *V. amygdalina* Del and *S. occidentalis* Linn are at below limit of detection (BDL) while it is only detected in the roots of *C. tridens* Linn and *M. charantia* Linn at 3.48 ppm and 66.83 ppm levels as indicated tables 4.4 – 4.9. For *C. sesamoides* Endl, the element is only detected in the roots and leaves at 2.9 – 65.08 ppm but absent in the stems.

From these tables (4.4 – 4.9) it could be seen that *M. charantia* Linn has the highest Cr concentration and this conforms with the local uses to which the leaves and fruits of this

plant as an anti-diabetic drug. This is because Cr is an essential trace element associated with some biological pathways related glucose tolerance (Felman and Braganca, 1988).

Moreover, the relatively high concentration of Cr in these plants may not be unconnected to being a component of several enzymes in living systems (Kiple and Coneorles, 2000) and conforms to normal concentration range in plants of around 1.0 – 100.0 ppm (Allen *et al.*, 1974).

5.1.12 Iron

Concentration of iron in all the samples analyzed is high and ranged from 145.0 – 1727.0 ppm. This high concentration of iron is attributed to the fact that, it is basically a structural component of the plant pigments, enzymes and cytochromes. It functions as component of blood hemoglobin that transport oxygen around the body to every cell for the production of energy and the maintenance of all cell functions, DNA replication, RNA synthesis and making of bone marrow when taken by animals. Its deficiency results in anemia (Hamilton *et al.*, 1988).

In *C. olitorious* Linn it ranged from 283.0 – 563.0 ppm and the accumulation is in the order leaves > roots > stems. For *C. tridens* Linn, the range is 376.0 – 1130.0 ppm with pattern of accumulation, decreasing from the leaves to the roots and then stems. This is in line with some other observations elsewhere (Folaranmi *et al.*, 2002).

But *V. amygdalina* Del, the concentrations of iron ranges from 145.0 – 611.0 ppm with the leaves having the highest followed by the roots and the stems having the least concentrations as observed in *C. tridens* Linn and in *Amaranthus tricolor* (Folaranmi *et al.*,

2002). Also in *C. sesamoides* Endl, the concentration of iron ranged from 1598.0 – 1727.0 ppm in the order root > leaves and none in the stems of the plant.

However, the order of the concentration in *M. charantia* Linn is in the order of stems, fruits > leaves > stems. The unusual high concentration of iron in the fruits of *Momordica charantia* Linn may not be unconnected to the fact that, it was the unripe fruits that were analyzed and contains high amount of chlorophyll and the element is associated with pigments and cytochromes (Erdal *et al.*, 2008).

The concentration of iron in *S. occidentalis* Linn ranged from 390.0 – 2215.0 ppm with the highest in the roots followed by the leaves and absent in stems and fruits. This observation seems to differ with the trends observed in the other plants analyzed in this work and may be an inherent property of the plant which requires further clarification.

5.13 Cobalt

Cobalt concentrations in some of the plants analyzed in this work are in traces and none detected in some of them. Its range in all the plants is 0.36 – 0.76 ppm. These amounts are in conformity with normal levels of the element in plants which is 0.10 – 0.60 ppm (Allen *et al.*, 1974).

Cobalt's concentration in *C. tridens* Linn ranged from 0.36 – 0.46 ppm and increased from the stems to the roots and none detected in the roots and fruits of the plant. Also the element was only found in the leaves of *C. sesamoides* Endl at 0.50 ppm levels and absent

in other parts of the plant. The same pattern was found in *S. occidentalis* Linn but only detected and determined in the roots of the plant at 0.41 ppm levels.

However in *M. charantia* Linn the element was found in the stems and leaves in the range 0.55 – 0.76 ppm with the leaves having the highest amount. It could be noted that the element was not detected in *C. olitorious* Linn and *V. amygdalina* Del.

5.1.14 Zinc

Zinc metal was detected and measured in the roots, stems and leaves of *C. olitorious* Linn at 14.0 – 51.0 ppm levels. The leaves contain the highest followed by the stems while the roots contain the least concentrations. In *C. tridens* Linn the range of Zn concentration is 51.0 – 115.50 ppm and the pattern of bioaccumulation is in the order of roots > stems > leaves and absent in the fruits. Also in *V. amygdalina* Linn the element is only determined in the leaves of the plant at 42.20 ppm levels but at below detection limit (BDL) in the other parts of the plant. For *C. sesamoides* Endl the range is 33.0 – 101.2 ppm and is in the order of roots > leaves > stems. The same trend was observed in *M. charantia* Linn and the range is 44.0 – 166.3 ppm. This relatively high concentration of zinc in this plant is similar to what was obtained elsewhere (Bakare *et al.*, 2010) and conforms to the local use of the plant in the treatment of diabetes. Because, it is known that inorganic mineral elements such as potassium, calcium and zinc play important roles in the maintenance of normal glucose-tolerance and in the release of insulin from beta cells of islets of Langerhans (Choudhary and Bandyopadhyay, 1999). Zinc present in the plant also is beneficial to prevention and treatment of diarrhoeal episode, it is also involves in normal functioning of immune system.

However, in *S. occidentalis* Linn the amounts of Zn ranged from 35.0 – 82.0 ppm and the element absent in the stem of the plant. Its concentration is in the order of roots > leaves > fruits. This does not reflect the pattern of accumulation obtainable in *C. tridens* Linn, *V. amygdalina* Del and *C. sesamoides* Endl in this work (roots > stems > leaves > fruits) as seen in table 4.4 – 4.9. The ranges are in conformity with what is obtainable as natural concentration of Zn in plants which is 15.0 – 100.0 ppm (Allen *et al.*, 1974).

5.1.15 Bromine

Bromine concentration in *C. olitorious* Linn ranged from 1.30 – 8.04 ppm. For *C. tridens* Linn the levels of bromine is 3.50 – 5.00 ppm with none in the roots and fruits of the plant. In *V. amygdalina* Del the range of Br is 2.70 – 6.50 ppm. But in *C. sesamoides* Endl the element is only detected in the leaves at 5.00 ppm and absent in the other parts of the plant. The same trend was observed in *M. charantia* Linn and *S. occidentalis* Linn (presence of bromine in the leaves and none in other parts). The pattern of accumulation in all the samples decreases from the roots to the stems, the leaves and then the fruits as seen in tables 4.1 – 4.6. These values conformed to what was obtained in a similar study; 1.35 – 4.76 ppm (Ekwemegbo, 2002) and they lies within normal Br concentrations (1.00 – 10.00 ppm) in soils (Lindsay, 1979).

5.1.16 Rubidium

The concentration of rubidium in *C. olitorious* Linn and ranged from 6.60 – 14.00 ppm in the stems and leaves with none in the roots and fruits. For *C. tridens* Linn the range is 3.90 – 23.00 ppm and is in the order of leaves > stems > roots while in *V. amygdalina* Del the level is in the range of 4.90 – 41.0 ppm and these amounts were observed in the roots and

leaves with none in the stems. For *C. sesamoides* Endl and *M. charantia* Linn, Rb is only found in the stems and the leaves with none in the roots of the plant at 11.00 – 26.00 ppm and 12.00 – 15.00 ppm levels.

But in *S. occidentalis* Linn, it was only detected and measured in the roots, leaves and fruits but absent in the stems and in the range of 5.50 – 24.00 ppm with pattern of accumulation in the order of leaves > roots > fruits. The usual mean of this element in soil is 100.0 ppm (Webber and Jellama, 1965) while in plants is 0.20 – 194 ppm in plants (Wyttenbatch *et al.*, 1995) and the values obtained in this work are relatively small, since plants usually obtain their mineral elements from the soil (Dim *et al.*, 2004). Though Rb is a trace element, it plays important roles in maintaining good health of animals when they consume plants containing the metal. It alters the function and replication of cancer cells leading to its arrest and hence used in cancer treatment (Altius directory, 2013).

4.1.17 Barium

The levels of accumulation of Ba in *C. olitorious* Linn ranged from 12.00 – 12.30 ppm which varies as leaves > roots and none in the stems of the plant. For *C. tridens* Linn, the level is in the range of 111.0 – 138.0 ppm in the order of stems > leaves and none detected in the roots and fruits. Also, the amount of Ba in *V. amgdalina* Del is in the order of root > stems and ranged from 5.40 – 44.00 ppm and not detected in the leaves of the plant. In *C. sesamoides* Endl the level of accumulation of Ba is 193.2 ppm in the roots but at BDL levels in the stems and leaves. For *M. charantia* Linn, the range is 122.0 – 583.0 ppm and in the order of leaves > stems > fruits. The element (Ba) in *S. occidentalis* Linn is only observed in the leaves (81.0 ppm) and absent in the roots, stems and the fruits. The

relatively low concentration of Ba in the analyzed plants may not be due to its high concentration in the soil where the plants were grown but it has been found that relative to the amount of Ba found in soils, little is typically bioconcentrated by plants (Schroeder, 1970). However, there are some plants, such as legumes, forage plants, Brazil nuts and mushrooms that accumulate barium (Aruguete *et al.*, 1998).

Despite being at low concentration in the analyzed plants, long term consumption of them may result in long term bioaccumulation of Ba and reach toxic level and the toxicity is usually attributed to barium ions (Ba^{2+}) (ATSDR, 2007). The intestinal mucosa of mammals is highly permeable to barium ions which are readily absorbed from the gastrointestinal tract and lung. Soluble barium salts move rapidly in to blood (CCME, 2013). The mechanism of barium toxicity is related to its ability to substitute for calcium. Toxicity results from stimulation of smooth muscles of the intestinal tract, the cardiac muscle and the voluntary muscles resulting in paralysis (CCME, 2013). Toxicity can also result from barium acting as a physiological antagonist to potassium ions which possess a similar effect ionic radius. Hypokalaemia is associated with the ability of barium ions to block potassium channels (Koch *et al.*, 2003).

5.1.18 Hafnium

The element Hf is only detected and measured in the leaf samples of *C. tridens* Linn and *M. charantia* Linn at 1.90 ppm and 1.45 ppm levels while absent in their roots, stems and fruits. The element was also not detected in the other analyzed plants (*C. olitorious* Linn, *C. sesamoides* Endl, *V. amygdalina* Del and *S. occidentalis* Linn). This observation conforms with the common knowledge that distribution and level of elements in plants depends

largely on its availability in the soil, parent material underneath, activities of man around premises and type and amount of fertilizer or manure applied to the plant (Gwarzo *et al.*, 2006). Although present at trace levels in the leaves of some of the plants analyzed in this work, the fear of long accumulation of the element on consumption of the plants by man and any his animals should not be anticipated because there appears to be no evidence of biochemical or biological activity of hafnium in living organisms. Furthermore, the metal is nontoxic, as are its compounds, unless the latter hydrolyze to yield acidic solutions or vapors (nautilus.fis.uc.pt/.eo7240.html, 2013).

5.1.19 Thorium

In *C. olitorious* Linn, *C. sesamoides* Endl, *M. charantia* Linn and *S. occidentalis* Linn the element thorium was only detected and quantified in their leaves and is the range of 0.17 – 4.70 ppm. But the element was found in the roots and leaves of *C. tridens* Linn and *V. amygdalina* Del in the order of roots > leaves. The amounts of Th in *C. tridens* Linn is 1.10 – 7.0 ppm while it is 0.35 – 0.411 ppm in *V. amygdalina* Del. However the element Th was only detected in the leaves of *S. occidentalis* Linn. Although, very few data exist about the concentrations of Th in foods, fresh fruits, tea and vegetables, very low concentrations were found in the work of Kobashi and Tominiga, 1985 Viz; Cabbage $\leq 3.3 \times 10^{-3}$ Ci/g; Green tea, $2.0 \times 10^{-3} - 3.0 \times 10^{-3}$ Ci/g; Broccoli, $\leq 3.6 \times 10^{-3}$ Ci/g and Cucumber $\leq 2.9 \times 10^{-3}$ Ci/g.

5.1.20 Europium

Europium was only detected and quantified in the roots of *V. amygdalina* Del at 0.031 ppm levels and leaves of *C. sesamoides* Endl at 0.03 ppm but none in the other parts. Also the element was not detected in the samples of *C. olitorious* Linn, *C. tridens* Linn, *M.*

charantia Linn and *S. occidentalis* Linn. The element has no known environmental and biological role (no threat to plants and animals) and its salts could be mildly toxic by ingestion, but its toxicity has not been fully investigated (Lenntech, 2013).

5.1.21 Antimony

Concentrations of antimony in the leaves of *V. amygdalina* Del, *C. sesamoides* Endl, *M. charantia* Linn and *S. occidentalis* Linn ranged from 0.29 – 0.42 ppm is in the order of *V. amygdalina* Del > *C. sesamoides* Endl = *M. charantia* Linn > *S. occidentalis* Linn. The element was not detected in the roots, stems and fruits of these plants and none in *C. olitorious* Linn and *C. tridens* Linn. The low concentration of antimony in some of plants analyzed and its apparent absence in others conforms to its low concentration in the earth's crust (about 0.2 ppm) (Baroni *et al.*, 2001). It is usually more concentrated in soil than in the parent materials (Kabata-Pendias and Pendias, 1984). Antimony is considered geochemically immobile (Ainsworth *et al.*, 1990, 1991) and when it occurs in soluble forms can be readily absorbed by plants.

Indeed, a similar radiological multi-element assessment of plants like this one showed a lower concentration of antimony in the roots, older leaves and lower part of the shoots (Coughtrey *et al.*, 1984). Antimony is also a non essential element in plants, by competing with essential metabolites, can even be phytotoxic (Foy *et al.*, 1978, Bowen, 1979). In mature leaves, a concentration of 150 mg/Kg has been considered phytotoxic (Kabata-Pendias and Pendias, 1984). Nevertheless, this and other aspects ie biological role of antimony, its transfer in to food chains and its behavior in the pedosphere are not well known. In terrestrial vascular plants, the background antimony concentration was found to

be similar to this work (0.29 – 0.42 ppm) and ranged from 0.2 – 50 ppm (Brooks, 1972, Bowen, 1979, Coughtrey *et al.*, 1983). Higher contents from 7.00 – 50 mg/Kg were found in mineralized areas (Shacklette *et al.*, 1978) and 110 – 900 mg/Kg in grasses growing around Lead and antimony smelters (Ragaini *et al.*, 1977 and Ainsworth *et al.*, 1991).

5.1.22 Ytterbium

The element ytterbium is detected and quantified in the leaves of *C. sesamoides* Endl at 0.35 ppm levels and is at below detection limit (BDL) levels in the other parts of the plant. The element is also found at BDL levels in the other plants analyzed in this work. The health effect of Yb is that, it has no biological role, but has been noted that its salts stimulate metabolism. It is a skin and eye irritant and is suspected tetratogen (Lenntech, 2013).

5.1.23 Arsenic

Arsenic is only found in the root of *S. occidentalis* Linn at 1.08 ppm levels and absent in other part of the plant. It is also absent the rest of the plants analyzed in this work. This low concentration of Arsenic in *S. occidentalis* Linn and apparent absent (at BDL levels) in the rest of plants analyzed may be due to the fact that, its origin from both natural and anthropogenic sources is at trace levels everywhere in nature (Lombi *et al.*, 2002). Arsenic is a toxic metalloid and in fact, WHO has set a limit of 10 µg/L for the element (As) above which health problems may occur. It has been postulated that, tens of millions of people are exposed to this risk by drinking contaminated water or ingesting cereal crops cultivated in polluted soils. A long lasting exposure to this highly toxic metalloid could affect the

gastrointestinal transit, the kidney, liver, the lungs, and the skin and increases the risk of cancer (Song *et al.*, 2010).

Though, the concentration of As in *S. occidentalis* Linn is low, its long term consumption may lead to its bioaccumulation to reach toxic level and in fact some cases of poisoning or even death have been attributed to the consumption of the plant and arsenic may be the cause (Peres *et al.*, 2010).

5.1.24 Lutetium

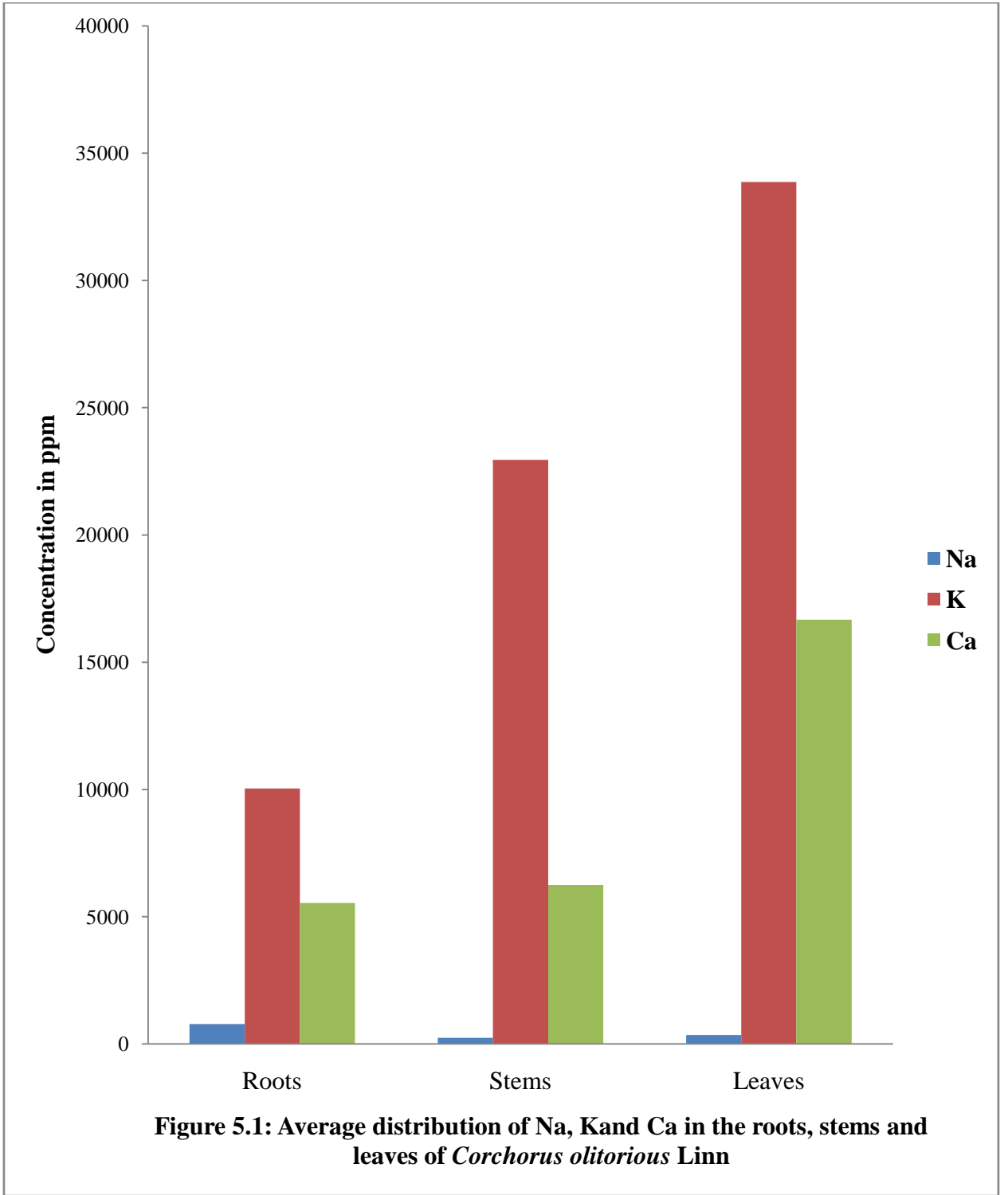
The element (Lu) is absent in all the plants analyzed except in the leaves of *Ceratotherca sesamoides* Endl at 0.023 ppm levels. This is because uptake of rare earth elements (REEs) much faster through the roots and is not a characteristic only for the REEs, since the ions of other elements have far more faster uptake through the leaf and are included in the metabolism of a plant than when the uptake goes through the root (Kastori, *et al.*, 2010). This leads to only tiny amounts of it are taken up by plant roots, so little gets in to the human food chain. Lutetium is judged to be mildly toxic by ingestion, but it is estimated that vegetables have as little as 10ppt in their dry weight. The element is also believed to have no biological role (Kastori, *et al.*, 2010, Wimpeny, *et al.*, 2013).

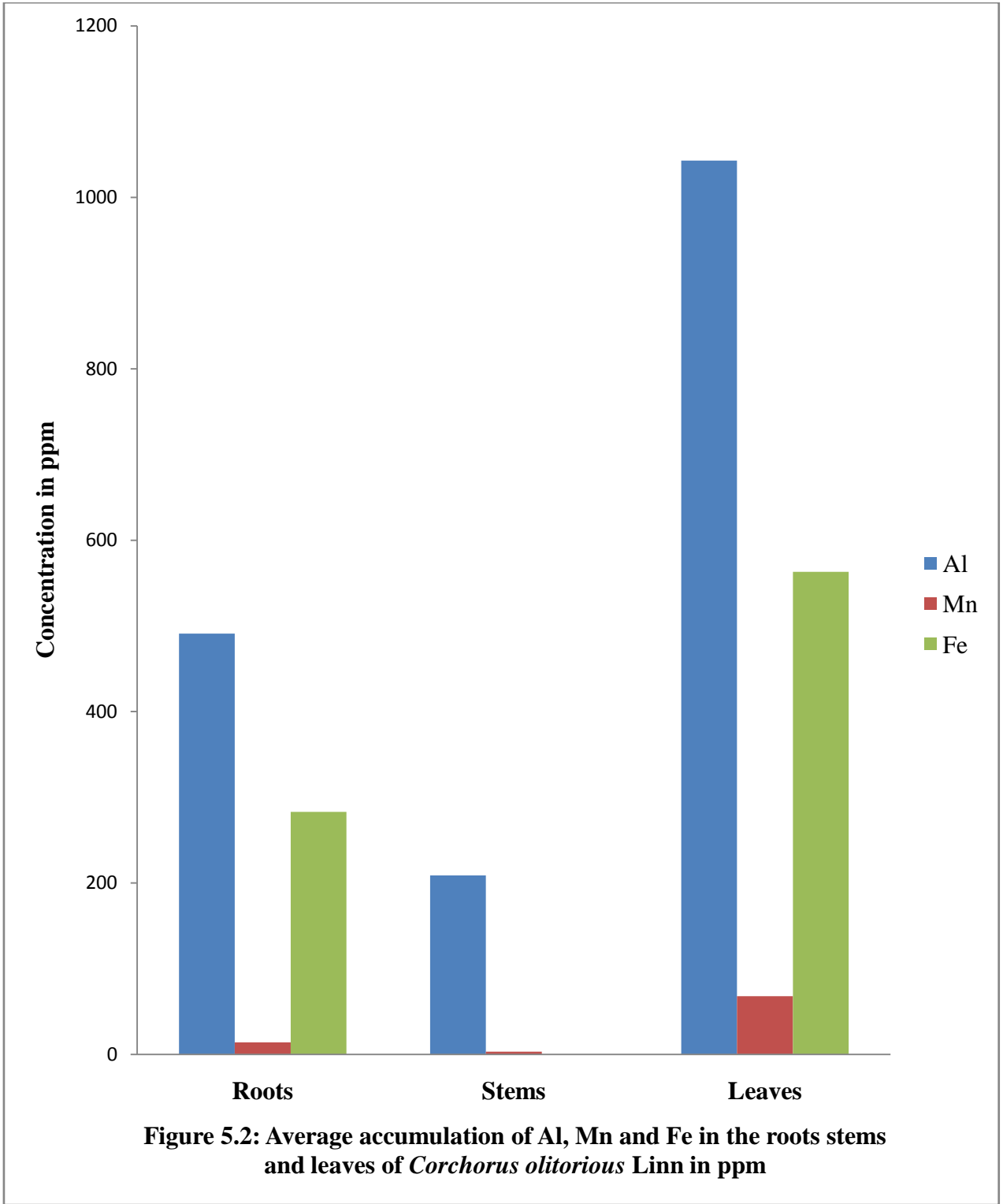
5.2.0 Variations in the distribution of the elements detected in the parts of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Endl, *Mormodica charantia* Linn and *Senna occidentalis* Linn

5.2.1 Variation in the distribution of the elements detected elements in the roots, stems, leaves and fruits of *C. olitorious* Linn.

Figure 5.1 below indicates the variation in the distribution of Na, K and Ca in the roots, stems and leaves of *C. olitorious* Linn. K is found to be increasing in the order roots < stems < leaves while the concentration of Ca is almost constant in the roots and stems but increased in the leaves of the plant. This signifies that, the leaf of *C. olitorious* Linn serves as a repository of these metals and consumption of it will serve as a good source of them. For Na metal, though at lower concentrations compared to the other two metals (K and Ca), its concentration is almost constant with the roots having the highest.

Figure 5.2 below signifies the variation in concentration of Al, Mn and Fe in the roots, stems and leaves of *C. olitorious* Linn. Fe is having the highest concentration and is observed to increase from the roots to the stems and fall to traces in the leaves of the plant. This high concentration of iron is attributed to the fact that, it is basically a structural component of the plant pigments, enzymes and cytochromes. Though not essential to the survival of the plants some trace amounts of Al were detected and determined in *C. olitorious* Linn and found to decrease from the roots to the stems and then increase to the leaves and absent in the fruits of the plant.

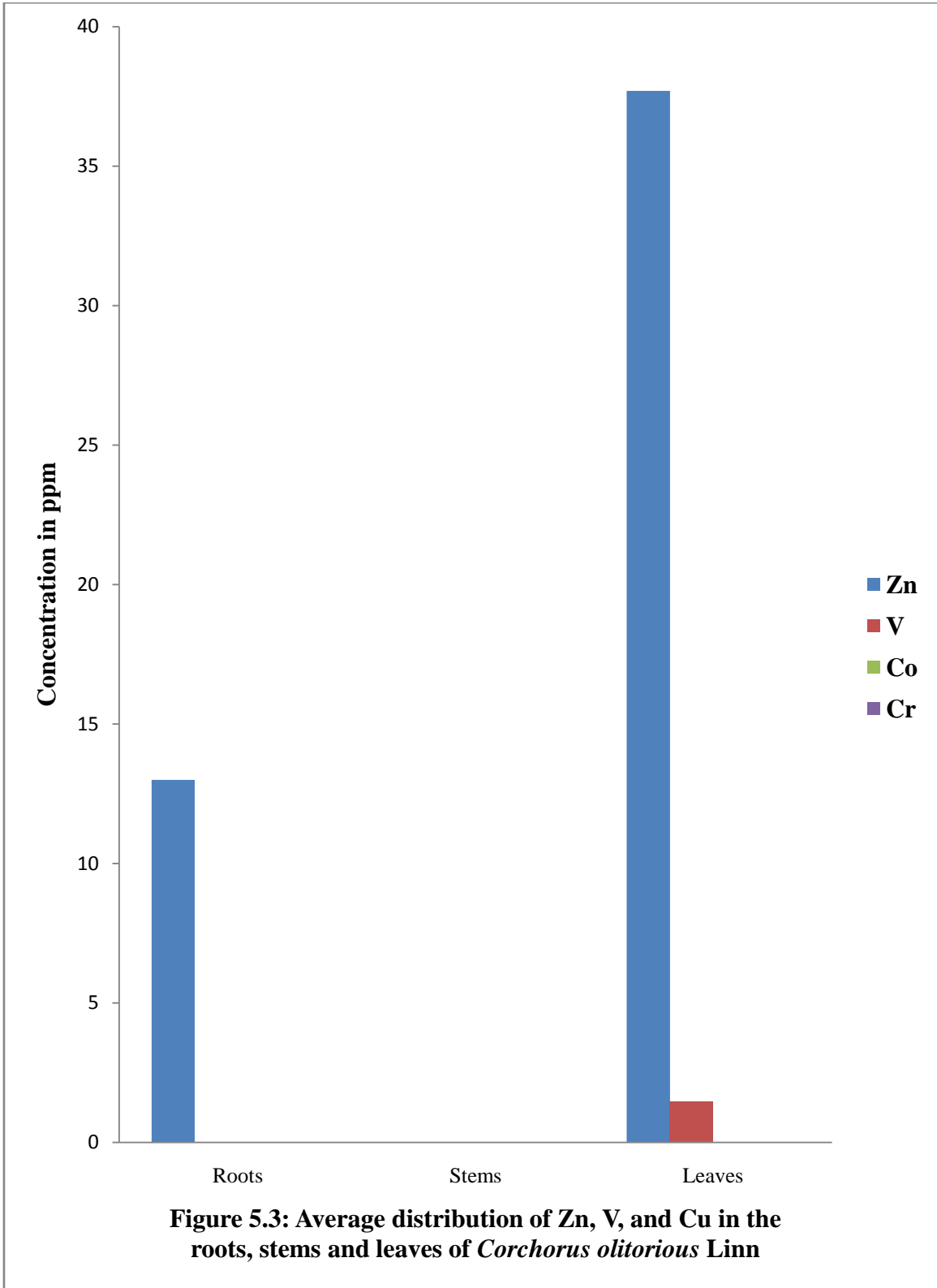


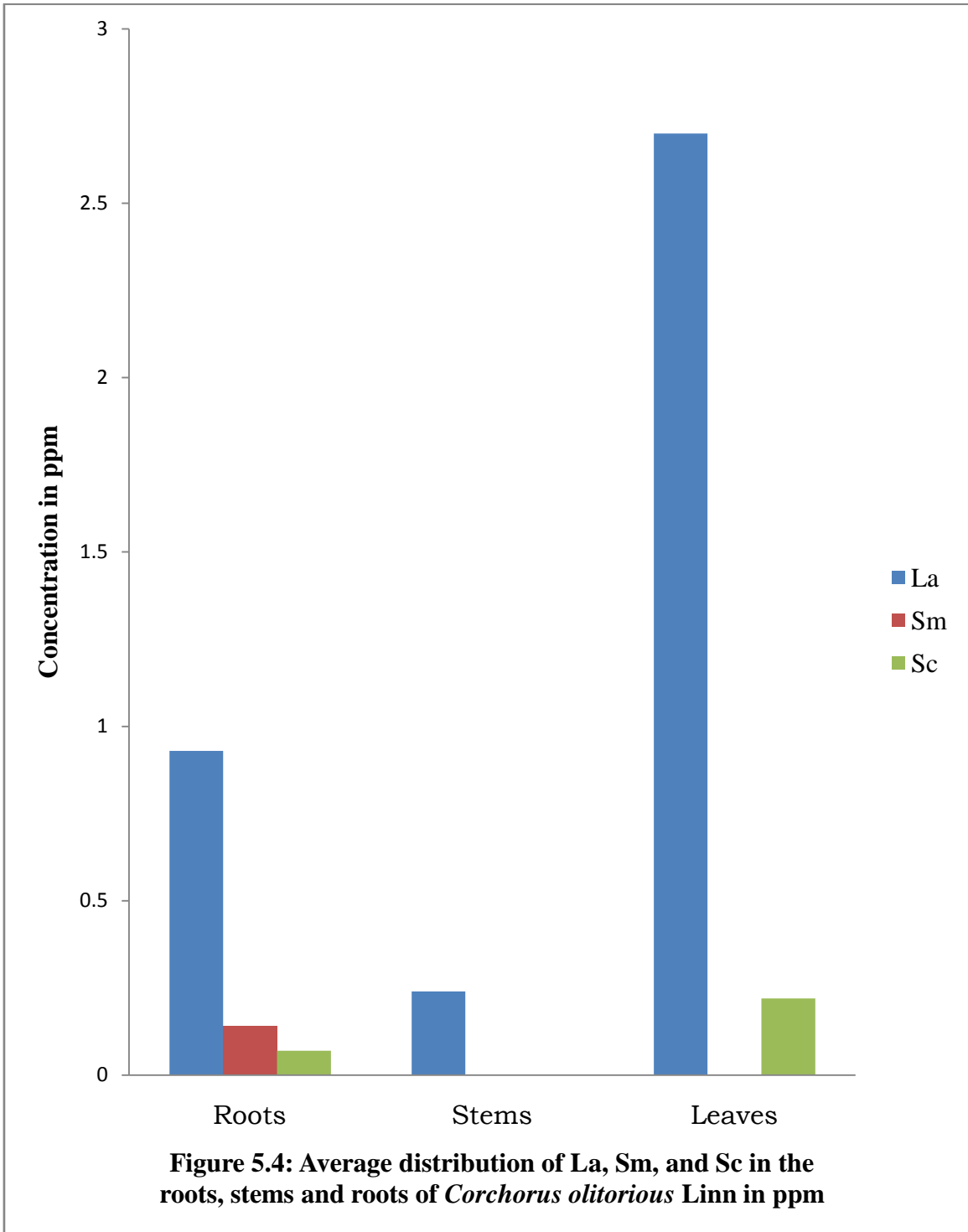


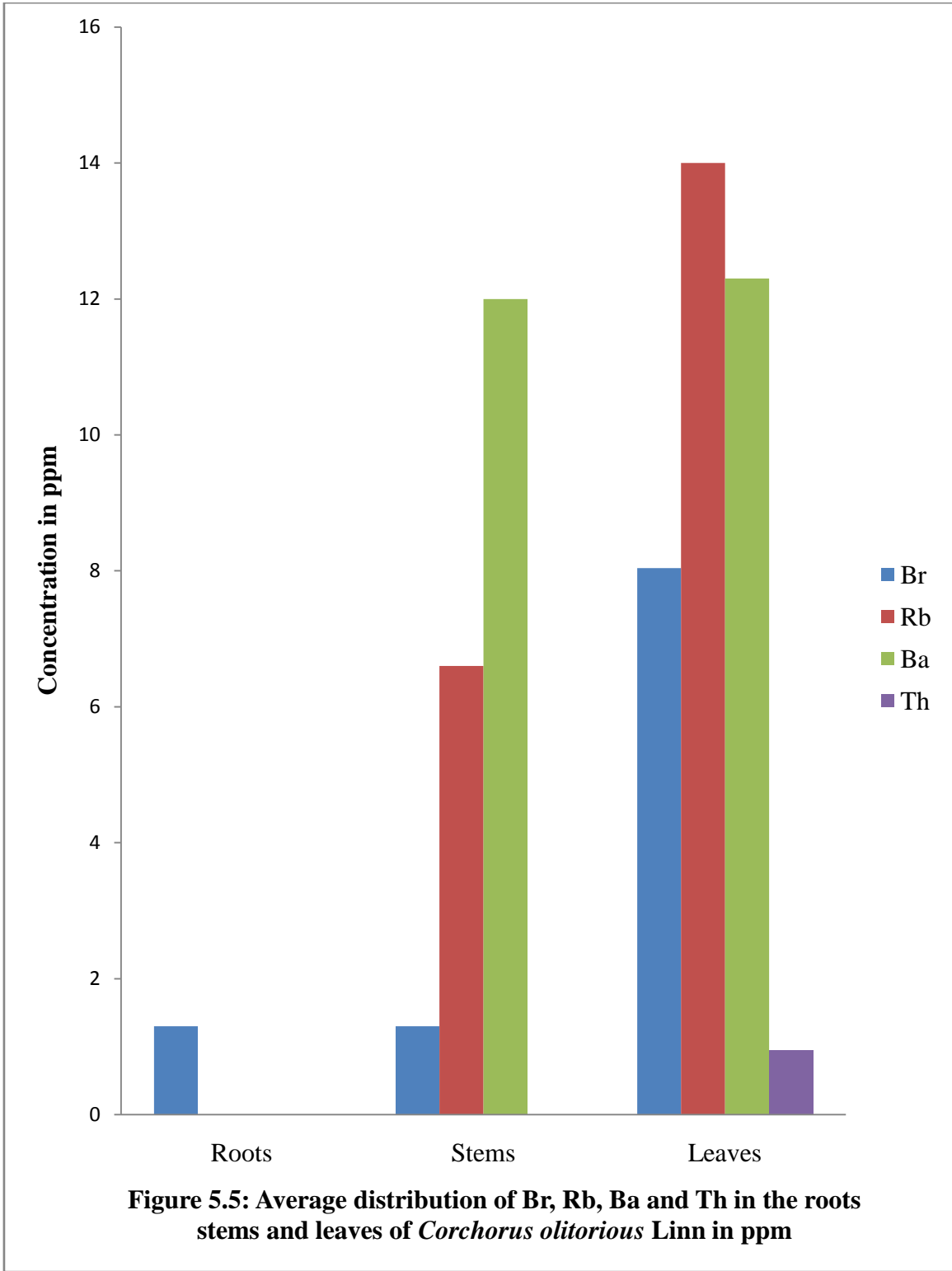
The figure below (Figure 5.3) depicts the pattern of accumulation of Zn, V, Cr and Cu in the roots, stems, leaves and fruits of *C. olitorious* Linn. Cu and Zn decrease from the roots to the stems, increases to the leaves and then later decreased to the fruits while V and Cr increase from the roots to the stems and then increase to the leaves and levels fall to the fruits.

Figure 5.4 below displays the distribution of La, Sm and Sc in the roots, stems, leaves and fruits of *C. olitorious* Linn. La concentration decreases from the roots to the stems, increase to the leaves and absent in the fruits. For Sm, the element decreases from the roots to the stems and absent in the leaves and fruits as seen in figure 4.4 while Sc is only detected in the roots and leaves of the plant and missing in the stem and fruits.

The pattern of distribution of Br, Rb, Ba and Th in the roots, stems, leaves and fruits of *C. olitorious* Linn is shown in figure 5.5 below. Concentration of Ba and Th increases from the roots to the stems and decreased to the leaves and absent in the fruits. But for Br the concentration remained significantly constant in the roots and stems, increased to the leaves and then missing in the fruits. For Rb, the element is absent in the roots of the plant but its concentration increased from the stems to the leaves and absent in the fruits of the plant.





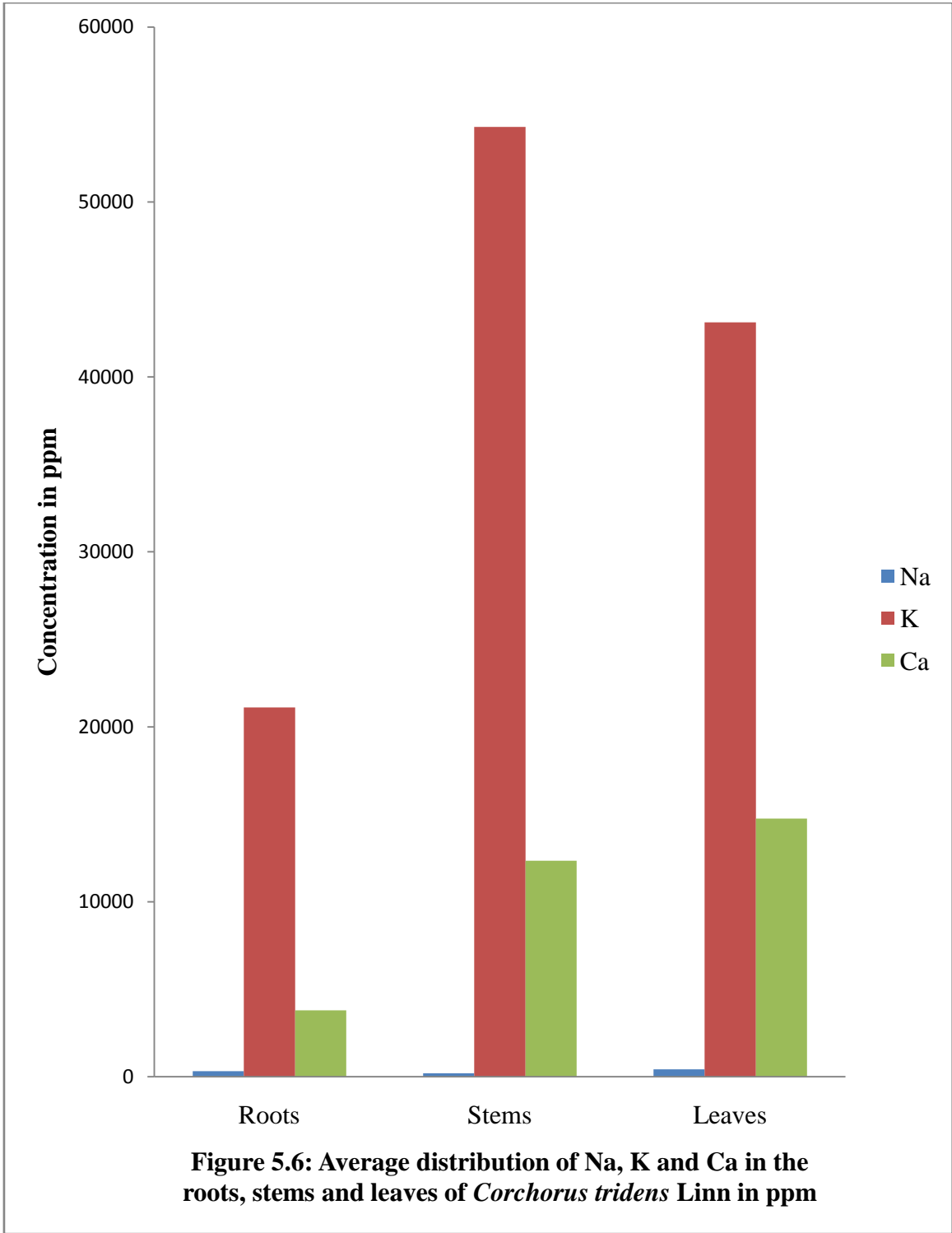


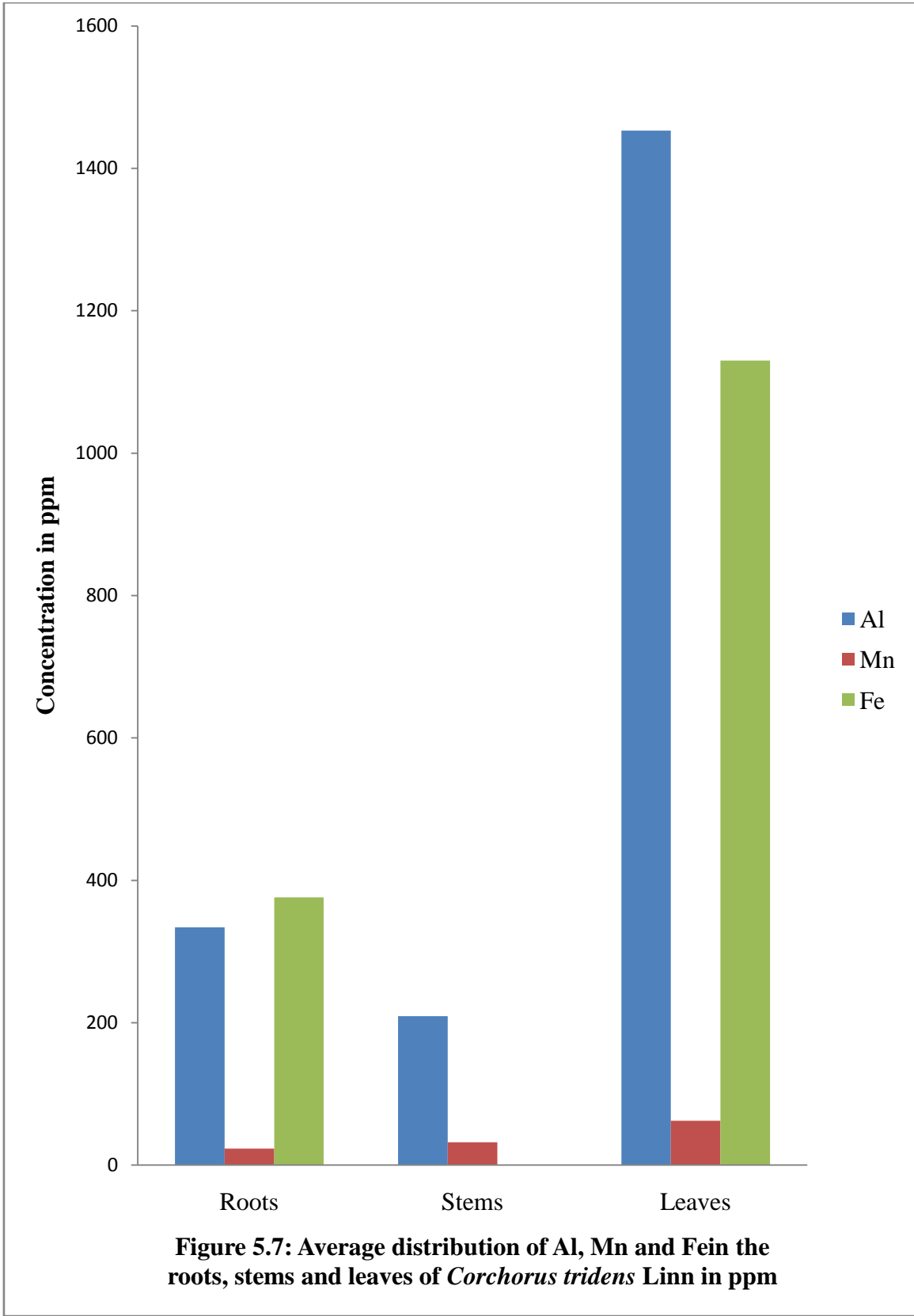
5.2.2 Variation in the distribution of the elements detected in the roots, stems and leaves of *Corchorus tridens* Linn.

Figure 5.6 below show the distribution pattern of Na, K and Ca in the roots, stems and leaves of *C. tridens* Linn. For Na, the concentration increases from the roots to the stems and then decreases to the leaves while for K and Ca their bioaccumulation decreases from the roots to the stems and finally to the leaves which conforms to what was obtained in plants elsewhere (Gwarzo, 2006)

Figure 5.7 below indicates the distribution of Al, Mn and Zn in the roots, stems and leaves of *C. tridens* Linn and in relatively high amounts. Al's concentration decreases from the roots to the stems and then increases to the leaves while that of Fe increases from the roots to the stems and then decreases the leaves. For Mn, the accumulation decreases from the roots to the stems and decreases to the leaves.

The figure below (Figure 5.8) depicts the distribution pattern of Zn, V and Cr in the roots, stems and leaves of *C. tridens* Linn. The concentration of Zn metal in the plant increases from the roots to the stems and then to the leaves while that of V increases from the leaves to the stems then decreases to the leaves. But for Cr, the bioaccumulation of the metal increases to the stems and significantly remained the same in the leaves of the plant.





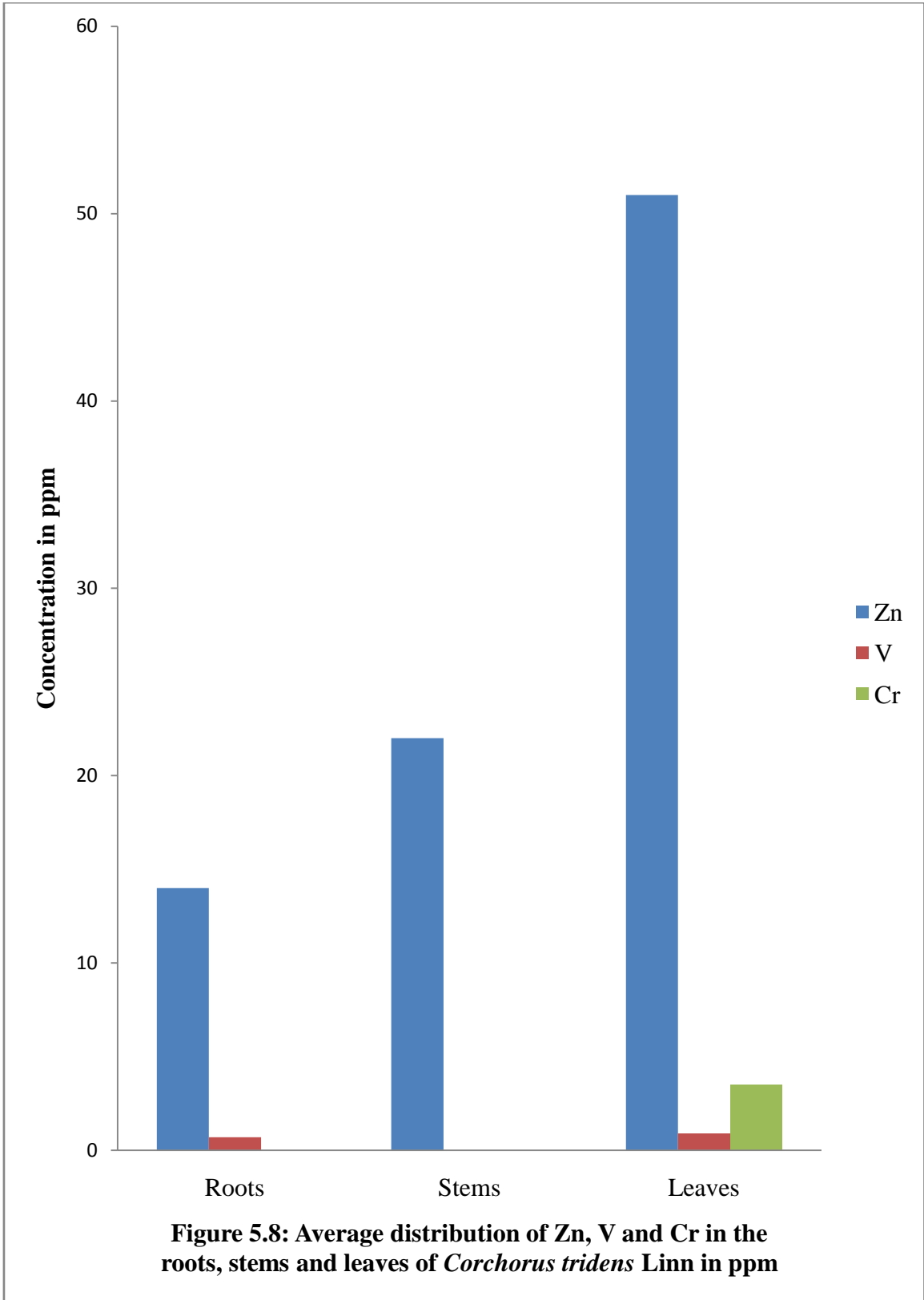
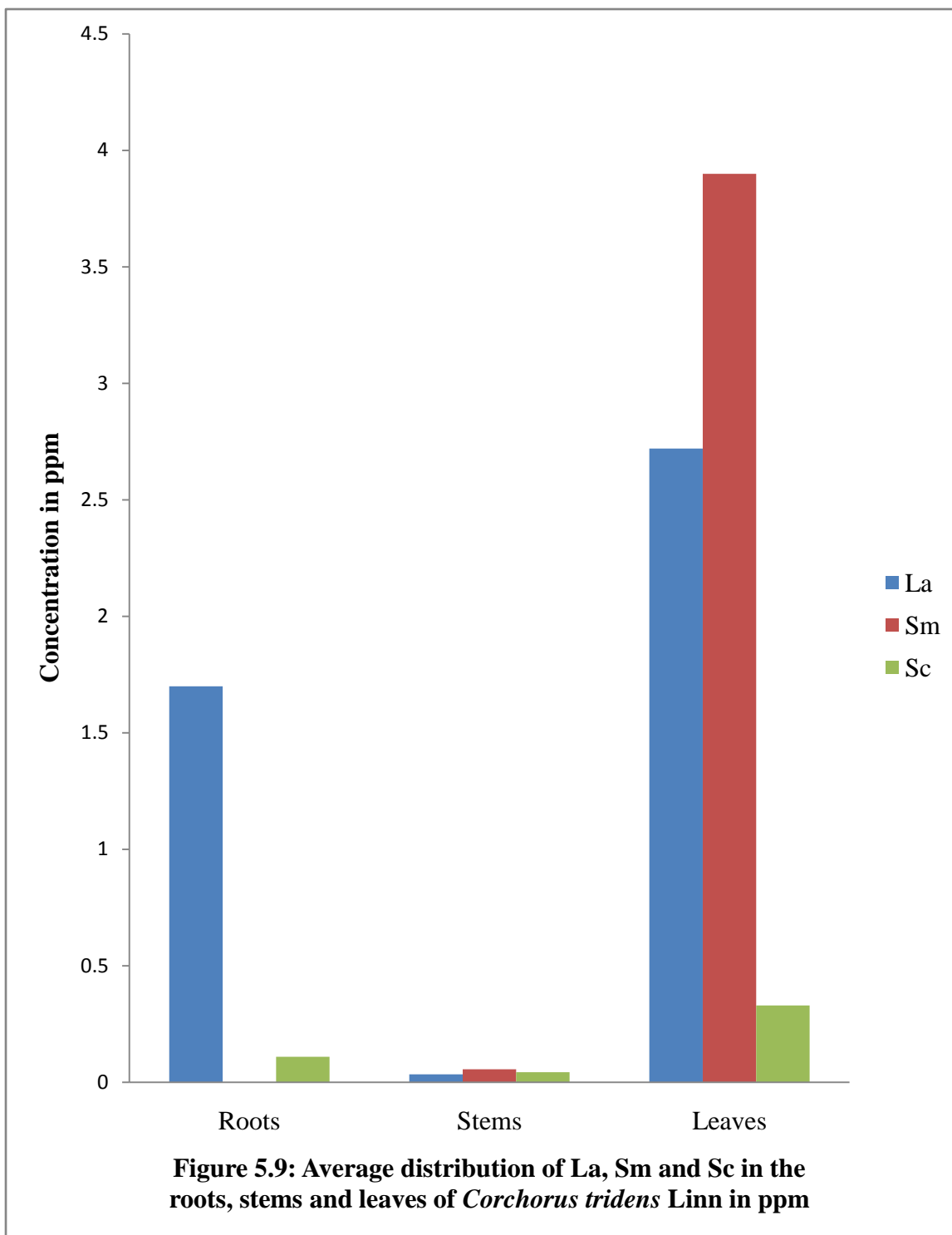
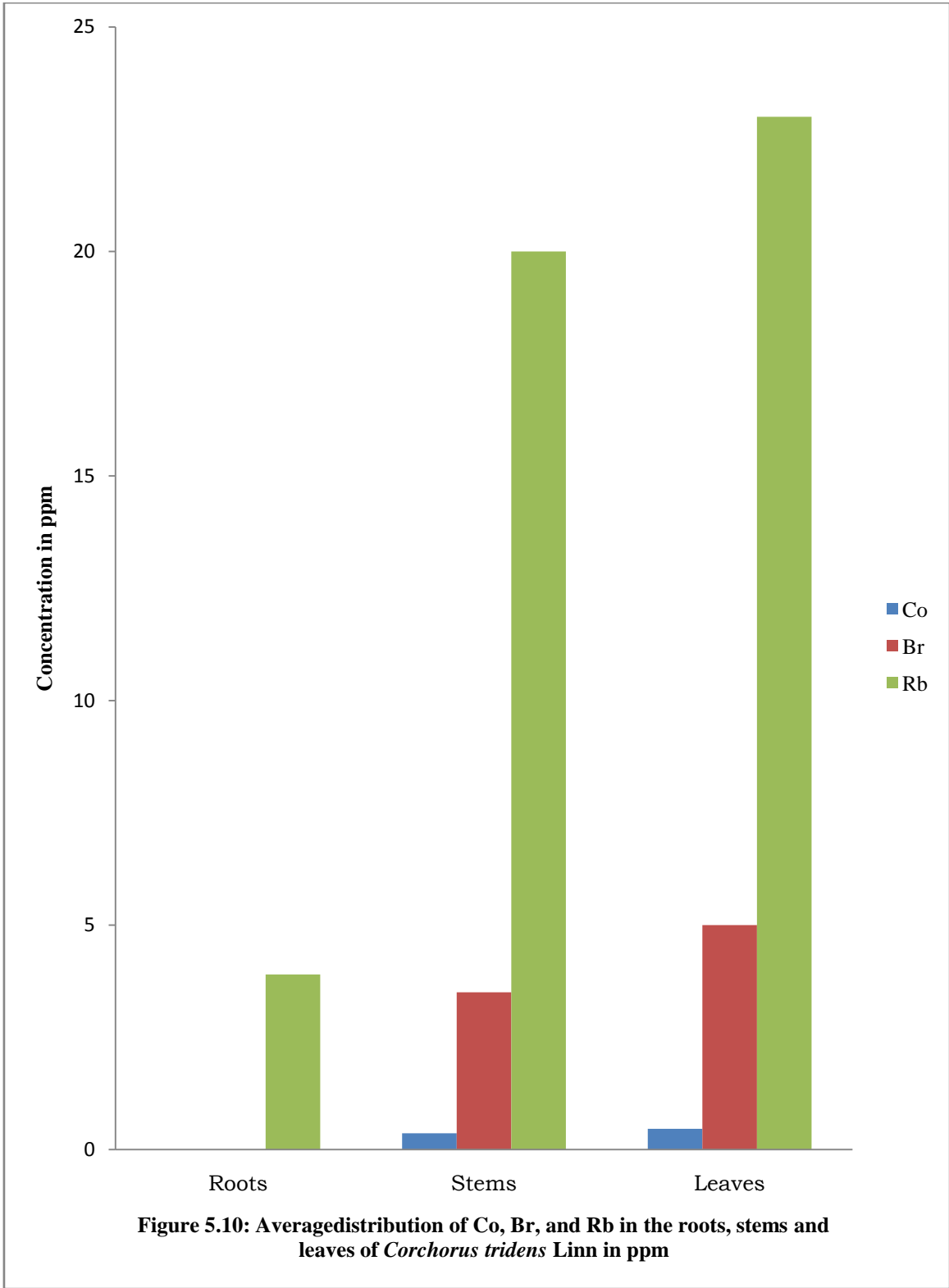


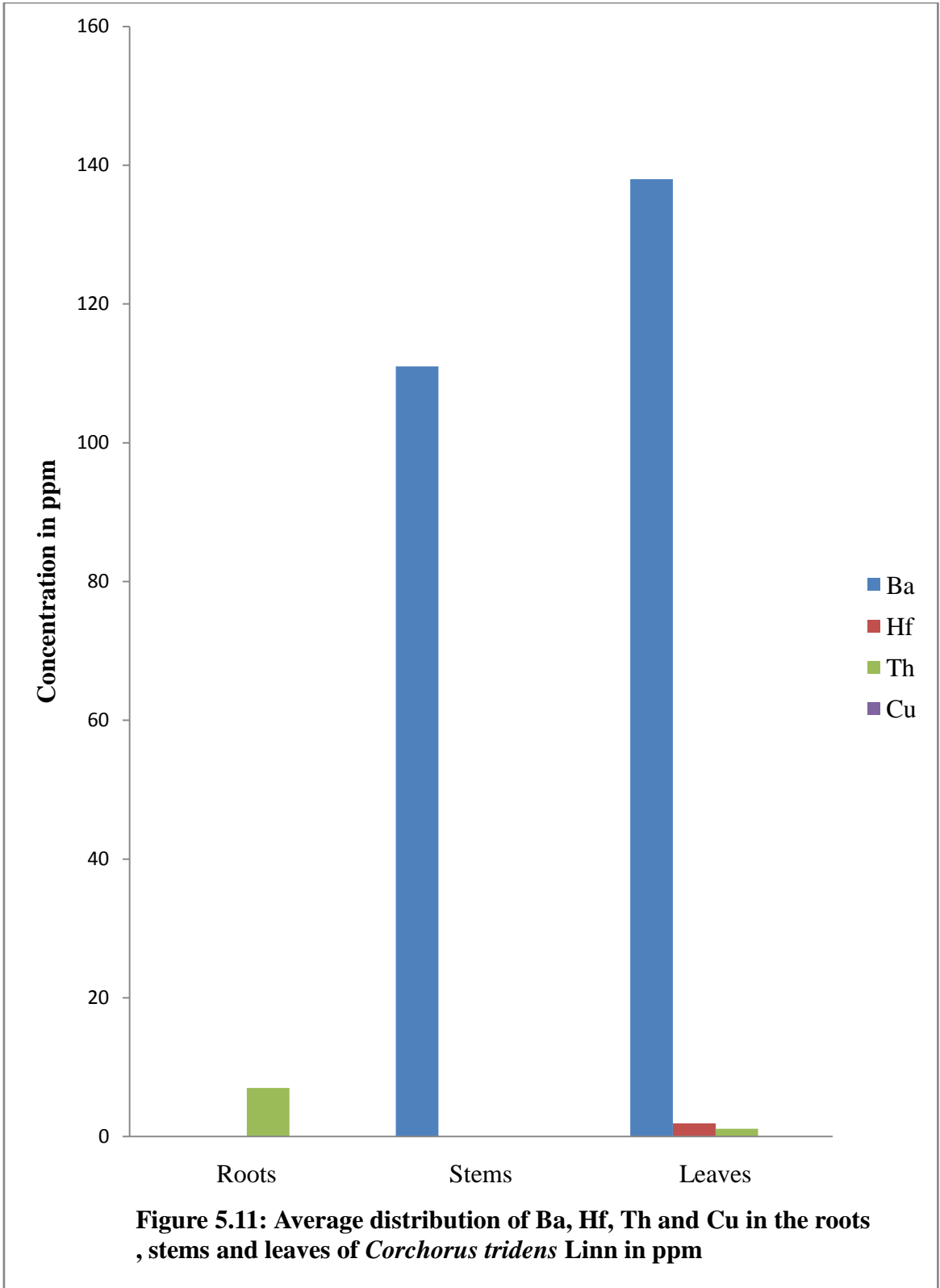
Figure 5.9 below is depicting the pattern of distribution of La, Sm and Sc in the roots, stems and leaves of *C. tridens* Linn. All the element's concentrations increases from the stems to the roots and then to the leaves except Sm that increases from the roots to the stems and then to the leaves.

Figure 5.10 below depicts the distribution of Co, Br and Rb in the roots, stems and leaves of *C. tridens* Linn. Thee to their concentrations decrease from the roots to the stems and then to theleaves.

The fiigure below (5.11) shows the pattern of distribution of Ba, Hf, Th and Cu in the roots, stems and leaves of *C. tridens* Linn. Ba concentration increase from the roots to the stems and decrease to the leaves. Hf was only detected and determined in the roots of the plant but absent in the stems and leaves. But for Th, the pattern of bioaccumulation is that the concentration decreases from the roots to the stems and then later increases in the leaves of the plant. However, the trend of accumulation of Cu is that, the amount of the metal decreases from the roots to the stems and then to the leaves of the plant.







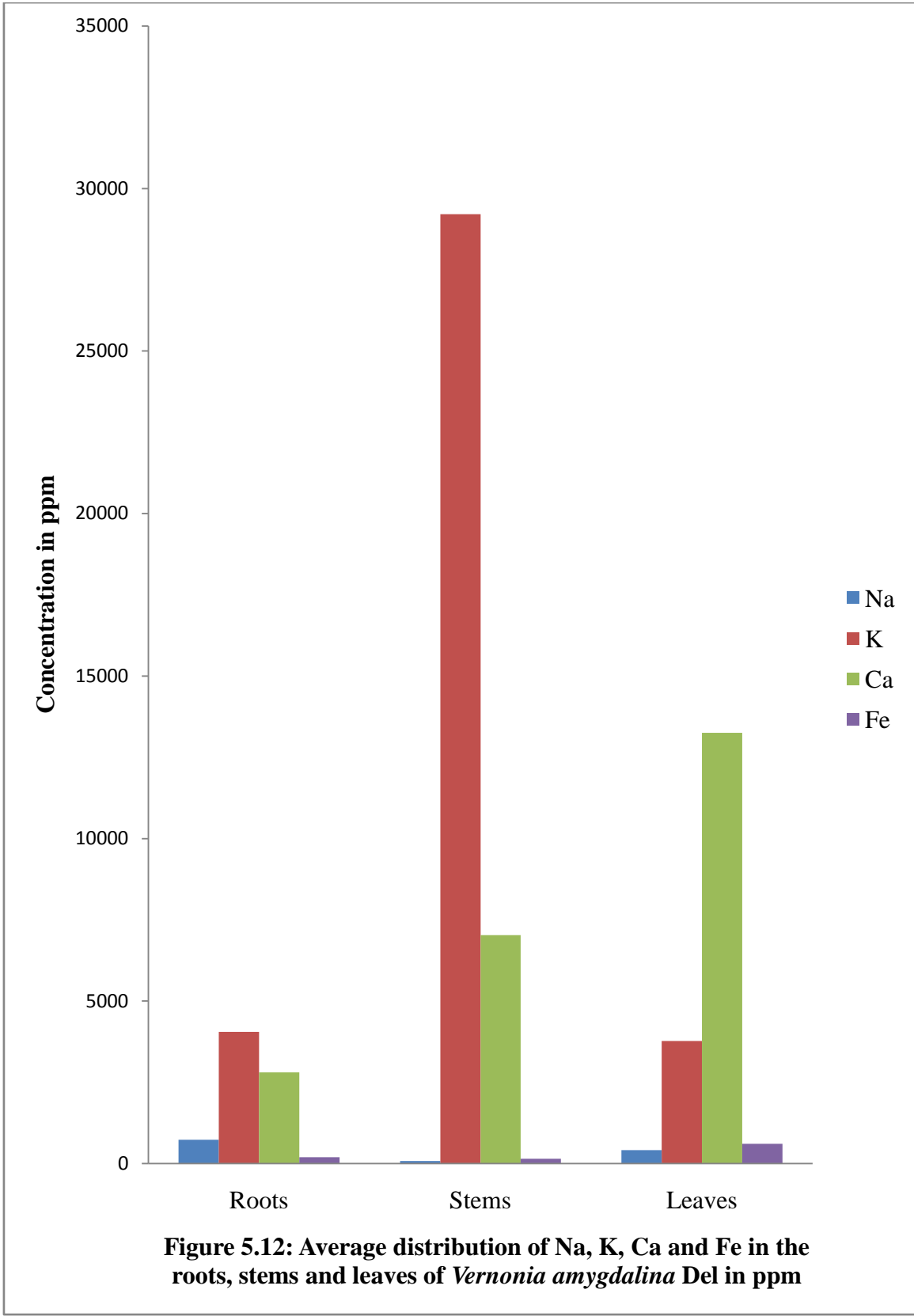
5.2.3 Variation in the distribution of the elements detected in the roots, stems, and leaves *Vernonia amygdalina* Del

Figure 5.12 below depicts the pattern of distribution of Na, K, Ca and Fe in the roots; stems and the leaves of *V. amygdalina* Del. Na and Ca concentrations are found to increase from the roots to the stems and to the leaves while that of Fe was found to be highest in the stems followed by the roots and the leaves having the least. However, Na has the least concentration in *V. amygdalina* Del and its pattern of accumulation is that, it decreases from the roots to the stems and increases to the leaves.

The figure below (Figure 5.13) signifies the distribution pattern of Al, Mn and Cu in the roots stems and leaves of *V. amygdalina* Del. Al's concentration increases from the roots to the stems and fell drastically to the leaves of the plant. But for Mn, the bioaccumulation decreases from the roots to the stems and decreases to the leaves while Cu is absent in all the parts of the plant.

Figure 5.14 below shows the bioaccumulation of V, Cr and Zn in the roots, stems and roots of *V. amygdalina* Decne. V and Cr concentrations in the parts of this plant are that they increase from the roots to the stems and then decreases to the leaves. But that of Al decreased drastically from the roots to the stems and then increased to the leaves.

Figure 5.15 below shows the distribution of La, Sc and Br in the roots, stems and roots of *V. amygdalina* Del. La and Sc's concentrations decreases from the roots to the stems and then increases to the leaves while that of aluminum remained approximately constant in the roots and stems and then finally decreased to the leaves.



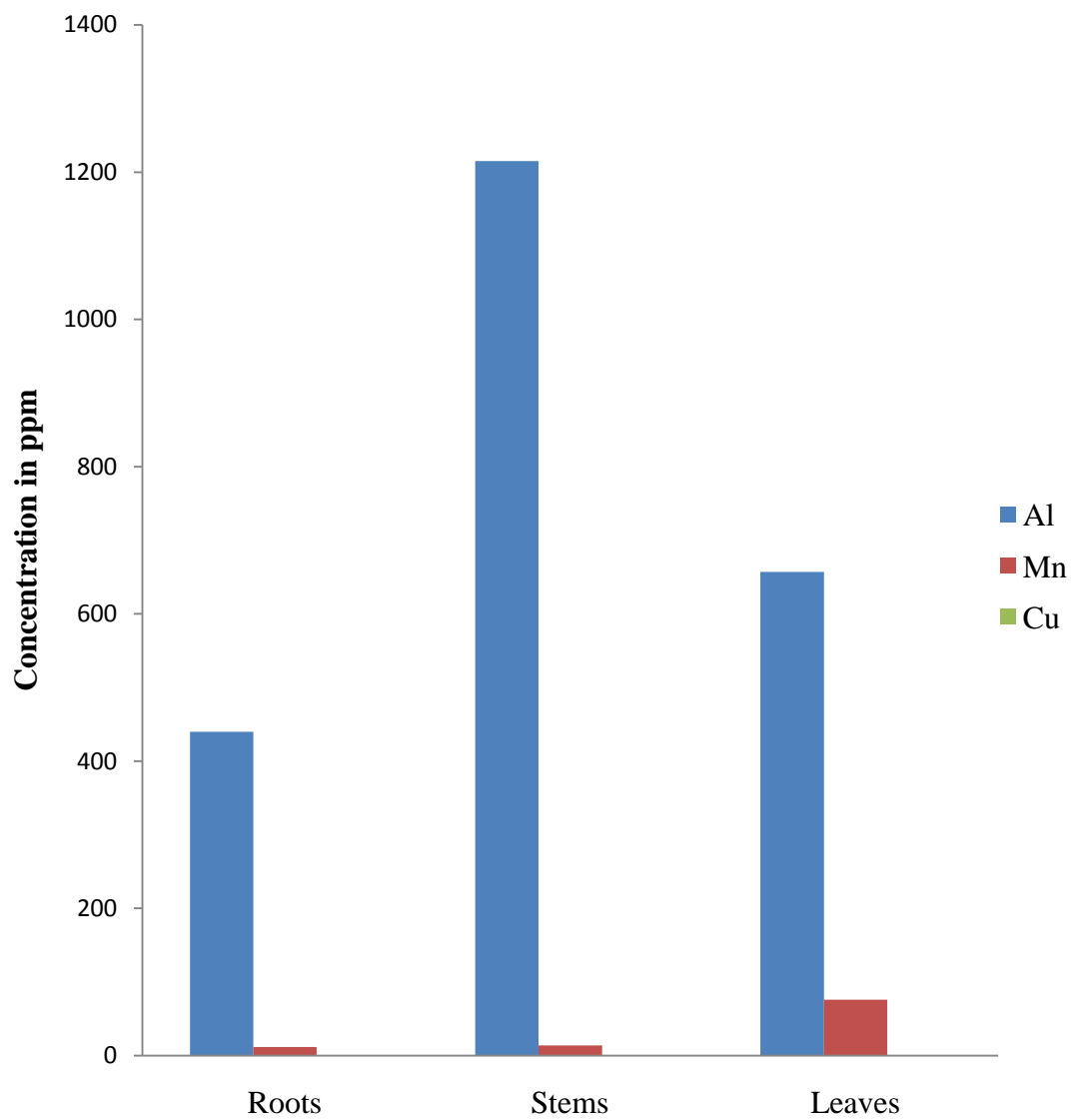
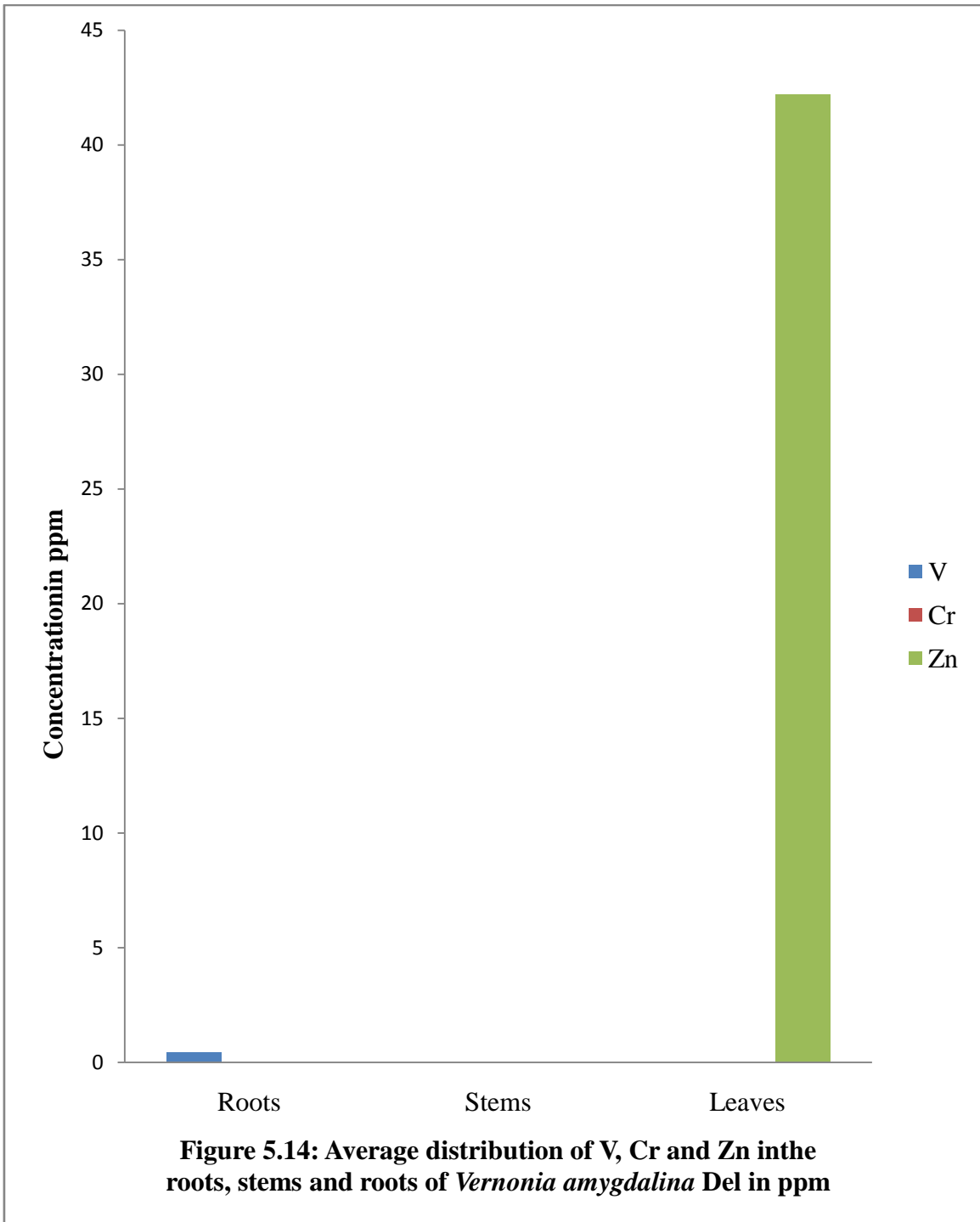


Figure 5.13: Average distribution of Al, Mn and Cu in the roots, stems and leaves of *Vernonia amygdalina* Del in ppm



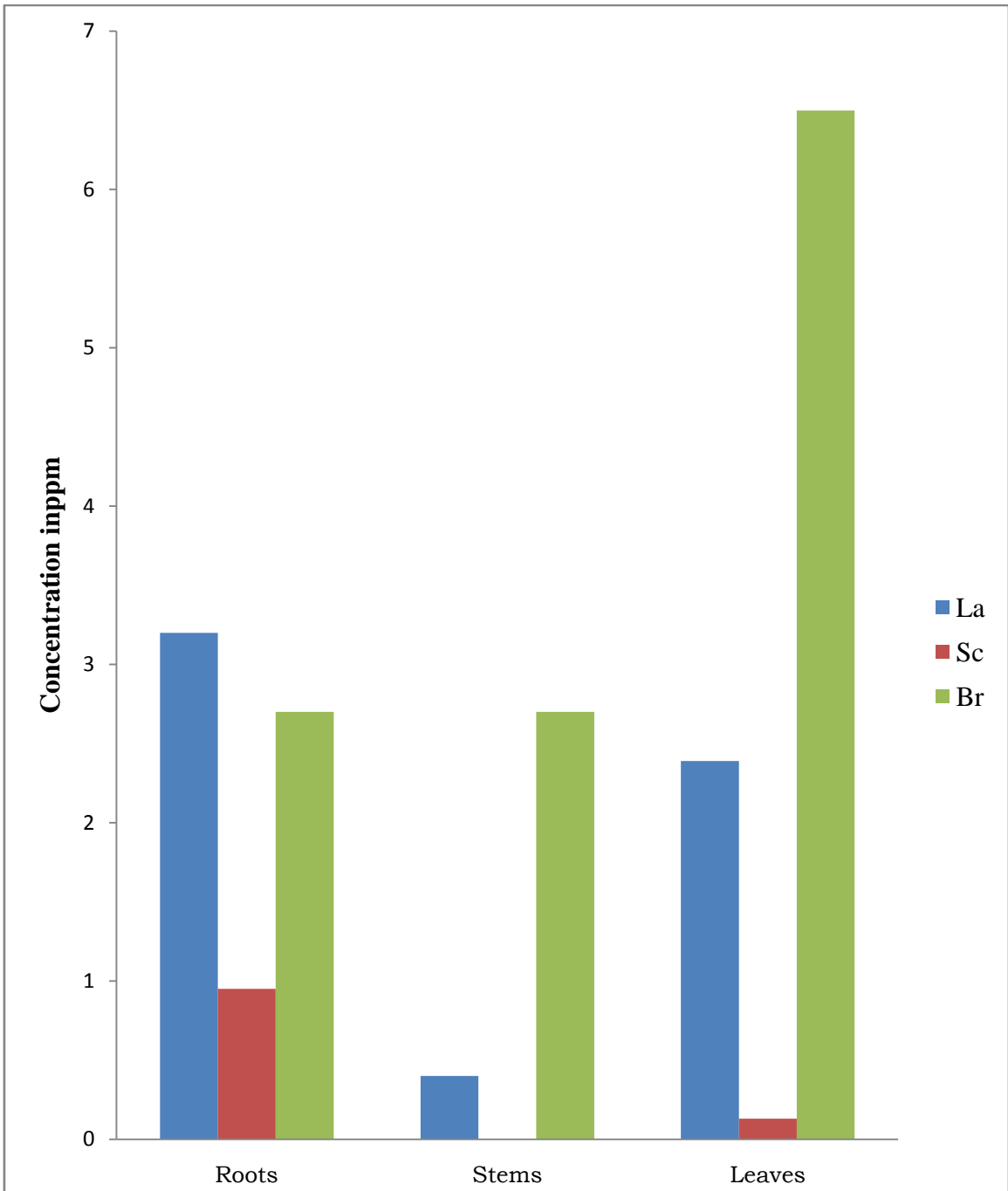


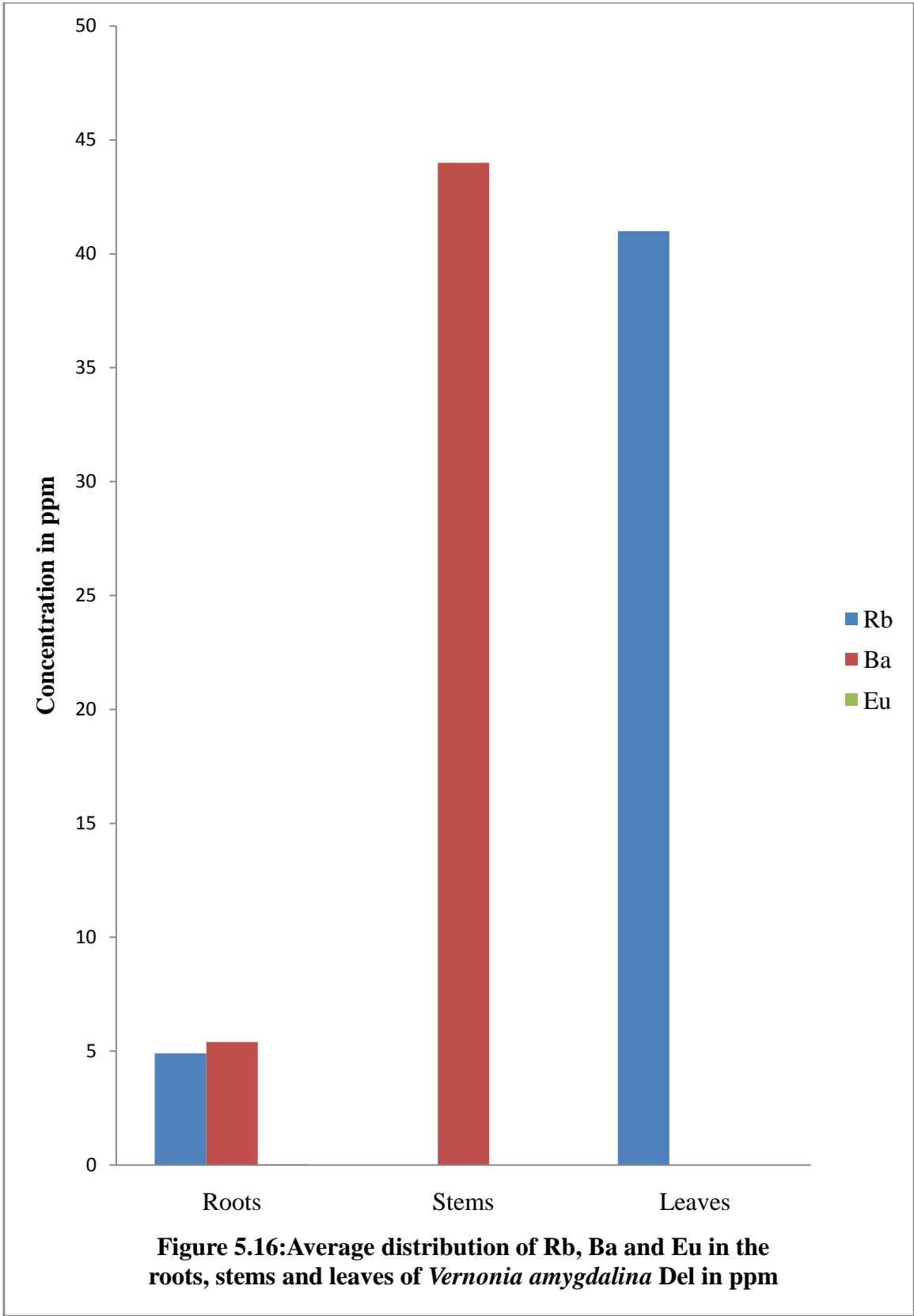
Figure 5.15: average distribution of La, Sc and Br in the roots, stems and leaves of *Vernonia amygdalina* Del in ppm

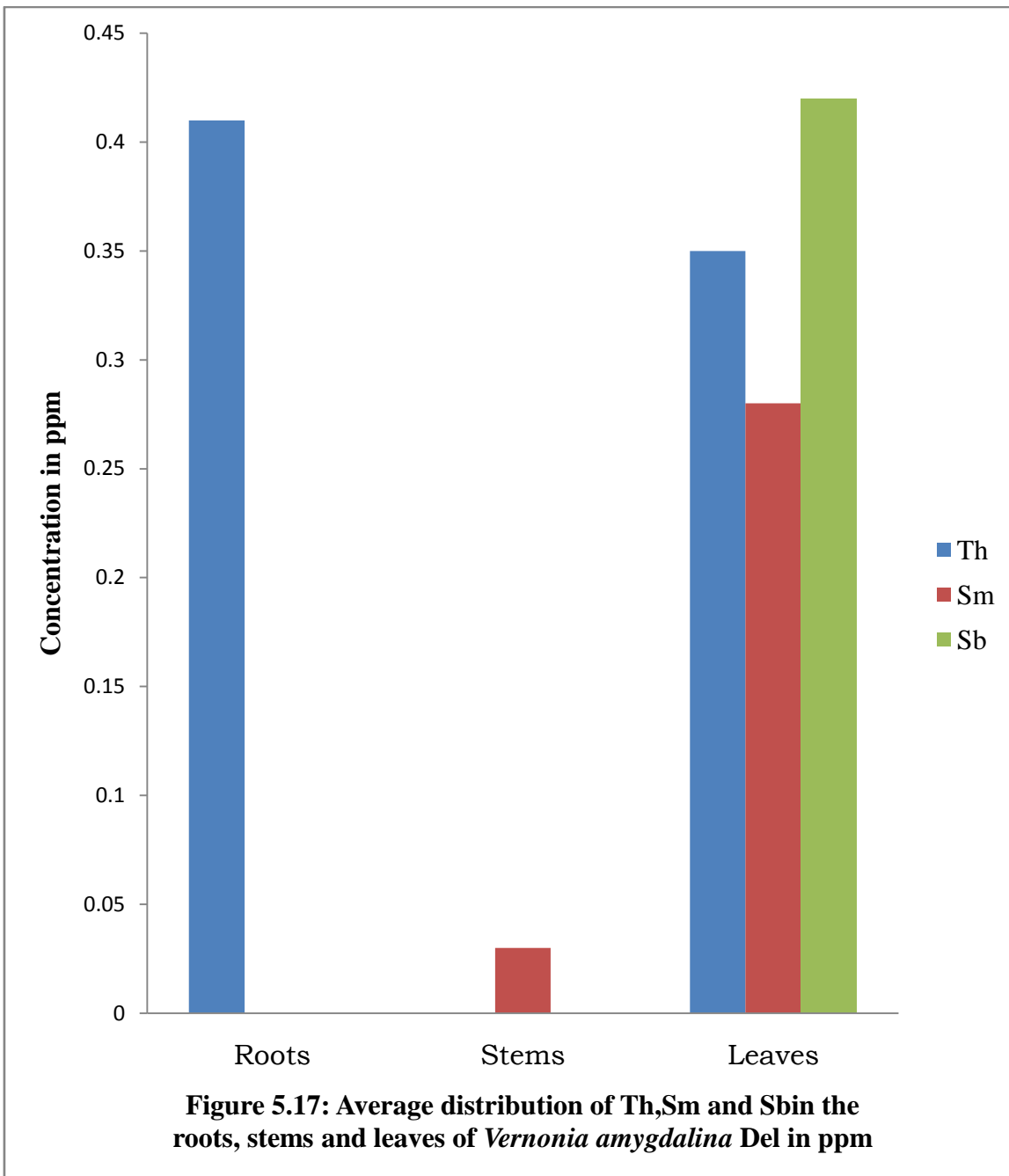
Figure 5.16 below depicts the pattern of distribution of Rb, Ba and Eu in the roots, stems and leaves of *V. amygdalina* Del. Ba concentration decreases from the roots to the stems then to the leaves. While Rb concentration decreases from the roots to the stems and then increase in the leaves of the plant. However, for Eu the concentration decreases from the roots to the stems and then to the leaves

Figure 5.17 below indicates the pattern of distribution of Th, Sm and Sb in the roots stems and leaves of *V. amygdalina* Del. Th and Sb concentration decrease from the roots to the stems and increases to the leaves while Sm concentration increases from the stems and the leaves.

5.2.4 Variation in the distribution of the elements detected in the roots, stems and roots of *Ceratotheca sesamoides* Endl

Figure 5.18 below show the distribution pattern of Na, K, Ca, Al and Fe in the roots, stems and leaves of *C. sesamoides* Endl. Na concentration increases from the roots to the stems and the decreases to the leaves. But K that is having the highest concentration in this plant has its amounts increasing from the roots to the stems and increased extensively to the leaves. But for Ca, the bioaccumulation pattern increases from the leaves to the stems and then decreased to the leaves. However for Fe, the accumulation pattern decreases progressively from the roots to the stems and finally to the leaves. Moreover, that of Al increases from the roots, to the stems and then finally to the leaves.





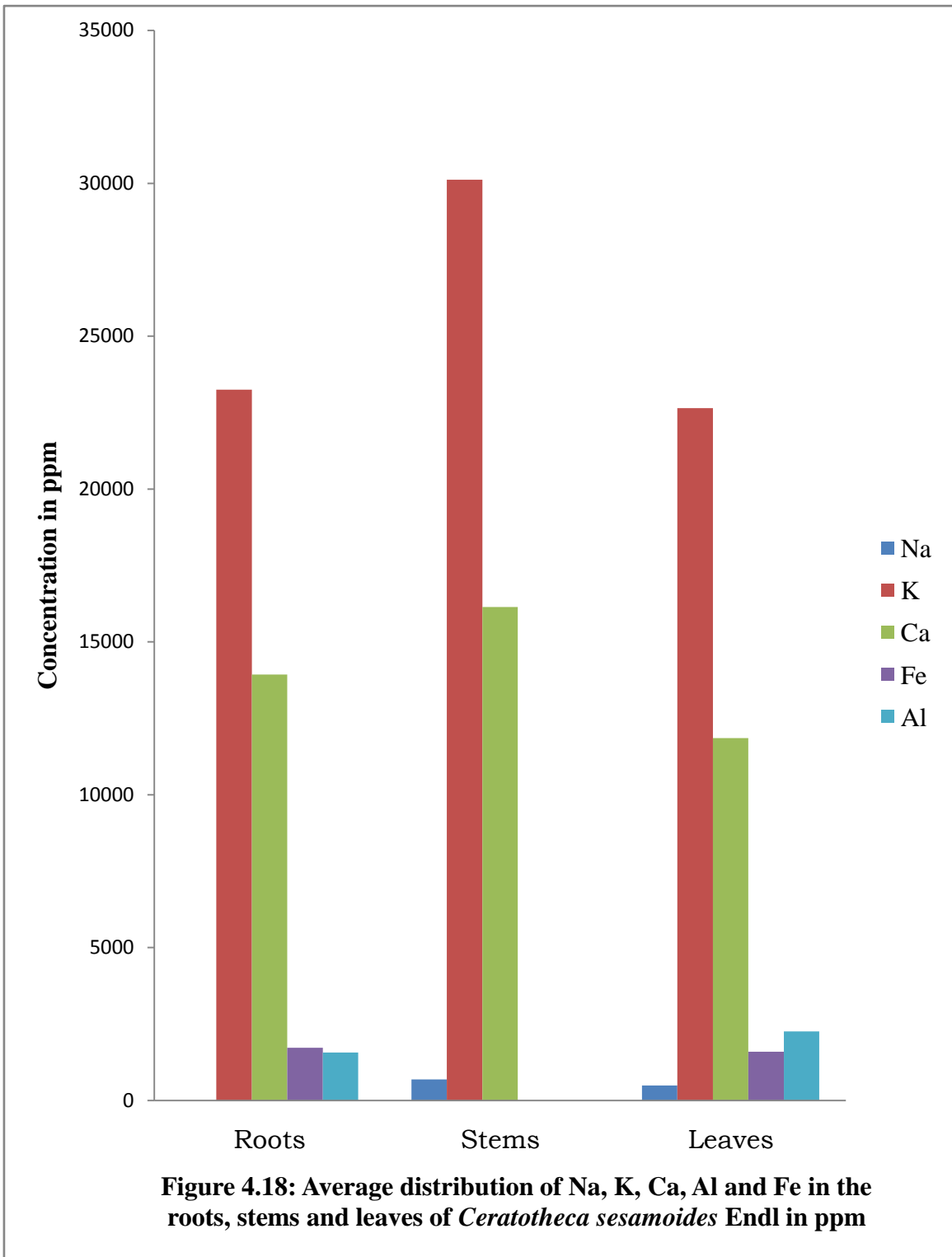
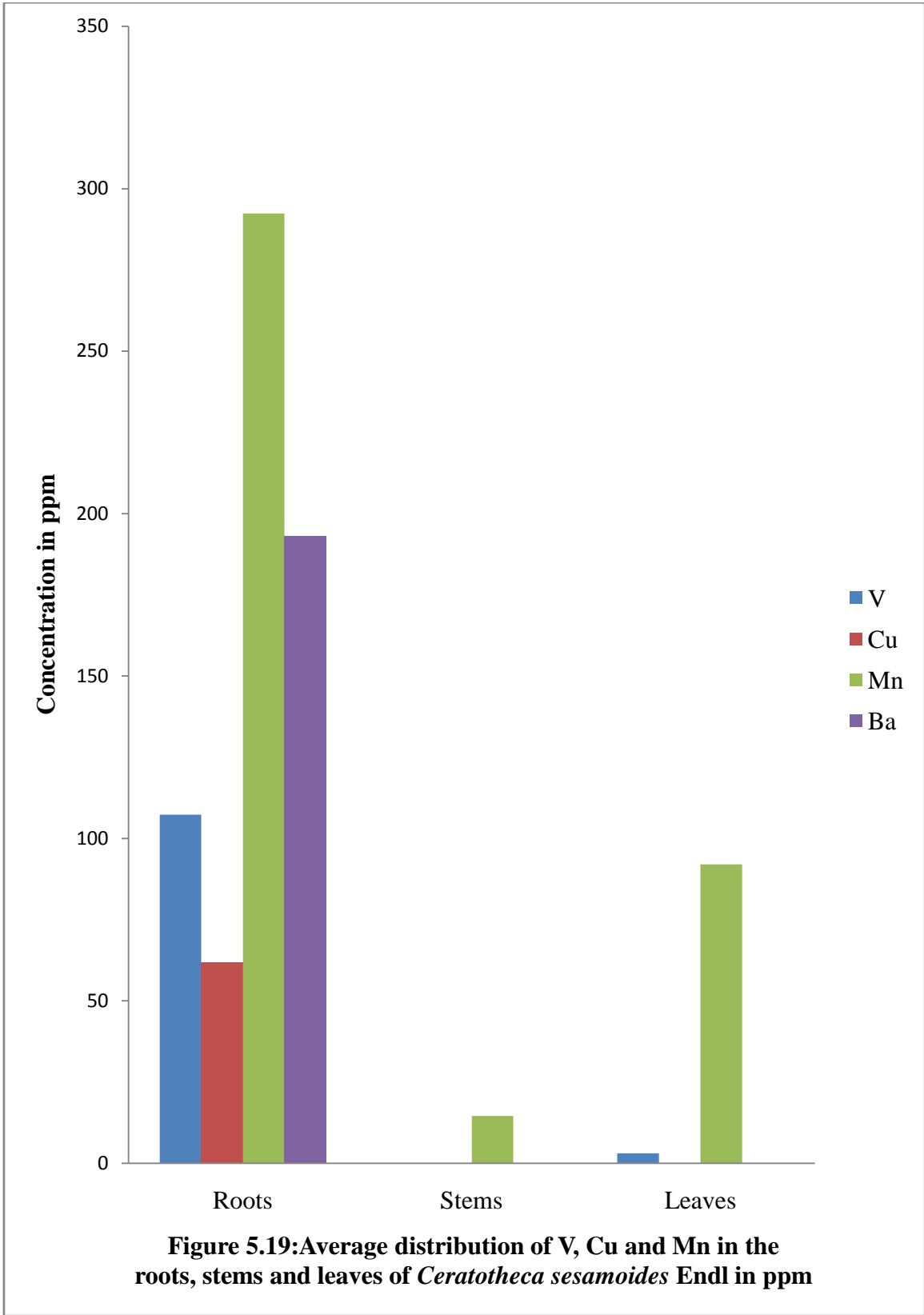
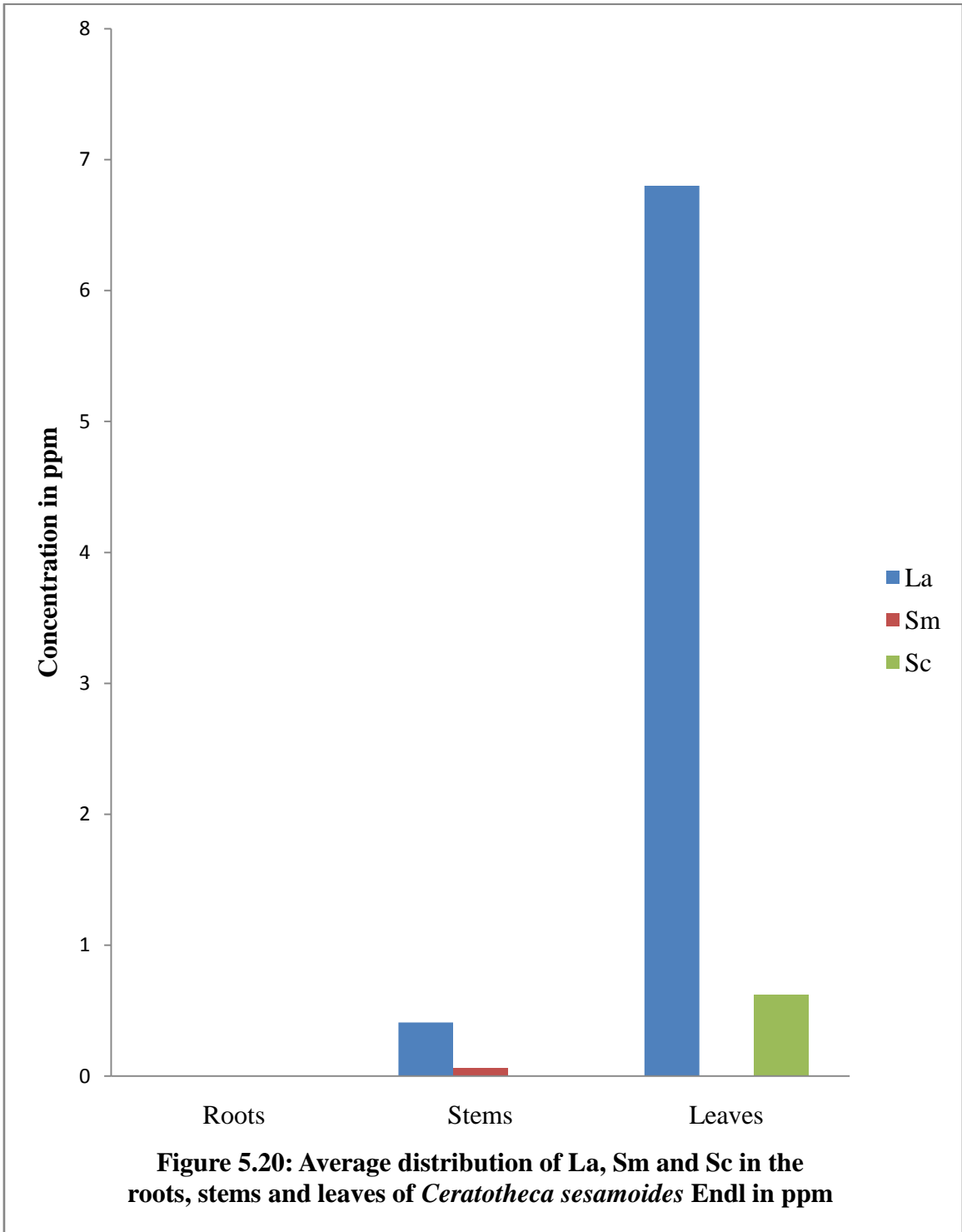


Figure 5.19 below indicates the distribution of pattern V, Cu, Ba and Mn in the roots stems and leaves of *C. sesamoides* Endl. Cu, Ba and Mn concentrations decreases from the roots, to the stems and then to the leaves. It could also be seen from the figure that Mn concentration is the highest among those described in this figure. However V concentration is found to increase from the roots to the stems and then finally decreases to the leaves.

Distribution of La, Sm and Sc in the roots, stems and leaves of *C. sesamoides* Endl is depicted in figure 5.20 below. Though all of them are in traces, La concentrations are higher and it is found to increase from the roots to the stems and then to the leaves. Similar pattern was observed with Sc. However Sm concentration is significantly the same throughout the plant.

Figure 5.21 indicate the distribution pattern of of Cr, Zn and Rb in the roots stems and leaves of *C. sesamoides* Endl. All the elements follow similar pattern accumulation of increasing from the roots to the stems and then decrease in the leaves of the plant.





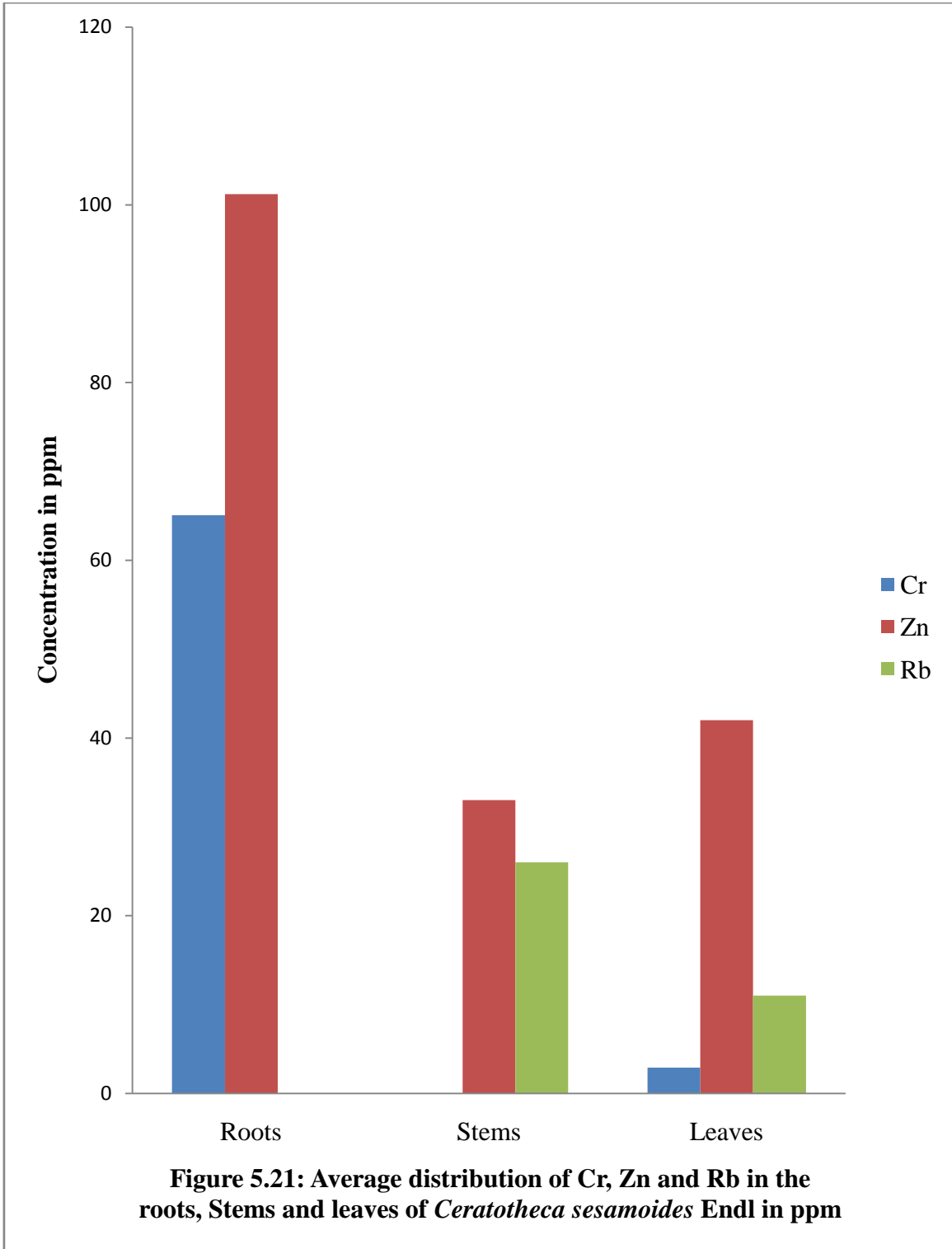


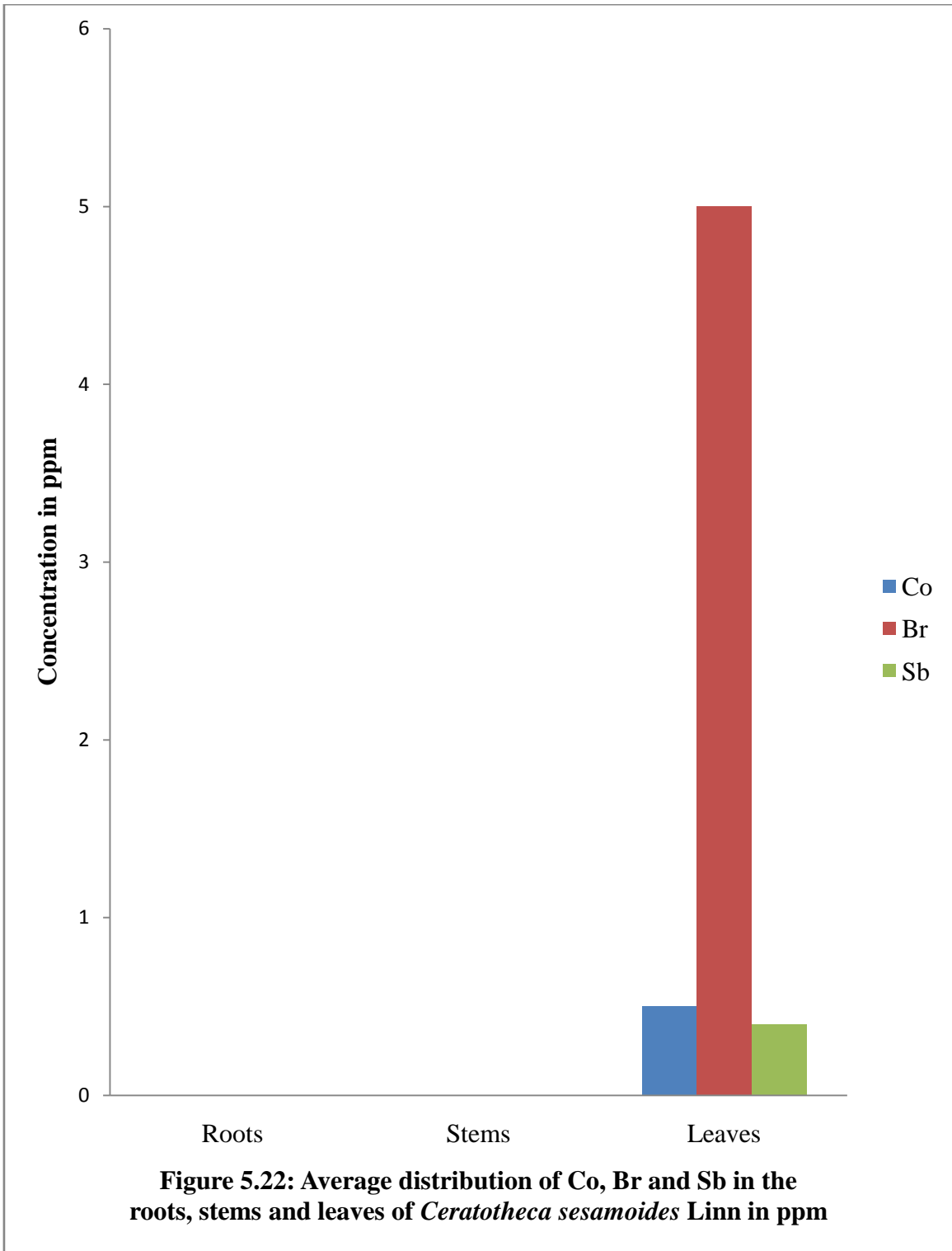
Figure 5.22 indicates the distribution pattern of Co, Br and Sb in the roots, stems and leaves of *C. sesamoides* Endl. All the elements follow similar pattern accumulation of increasing from the roots to the stems and then to the leaves of the plant.

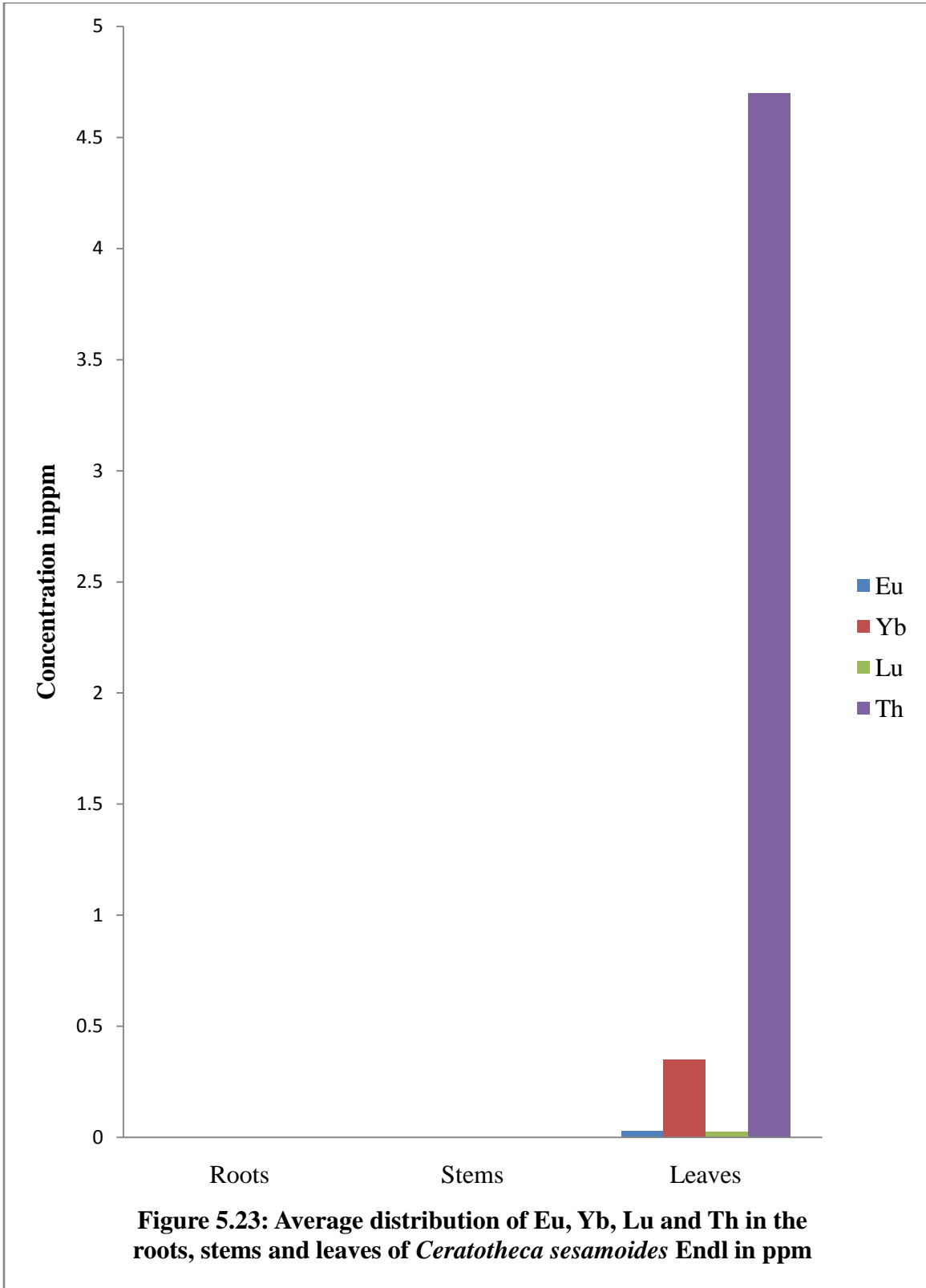
Figure 5.23 indicates the bioaccumulation pattern of Eu, Yb, Lu and Th in the roots, stems and leaves of *C. sesamoides* Endl. All the elements follow similar pattern of accumulation of increasing from the roots to the stems and then decrease in the leaves of the plant and are all at trace levels.

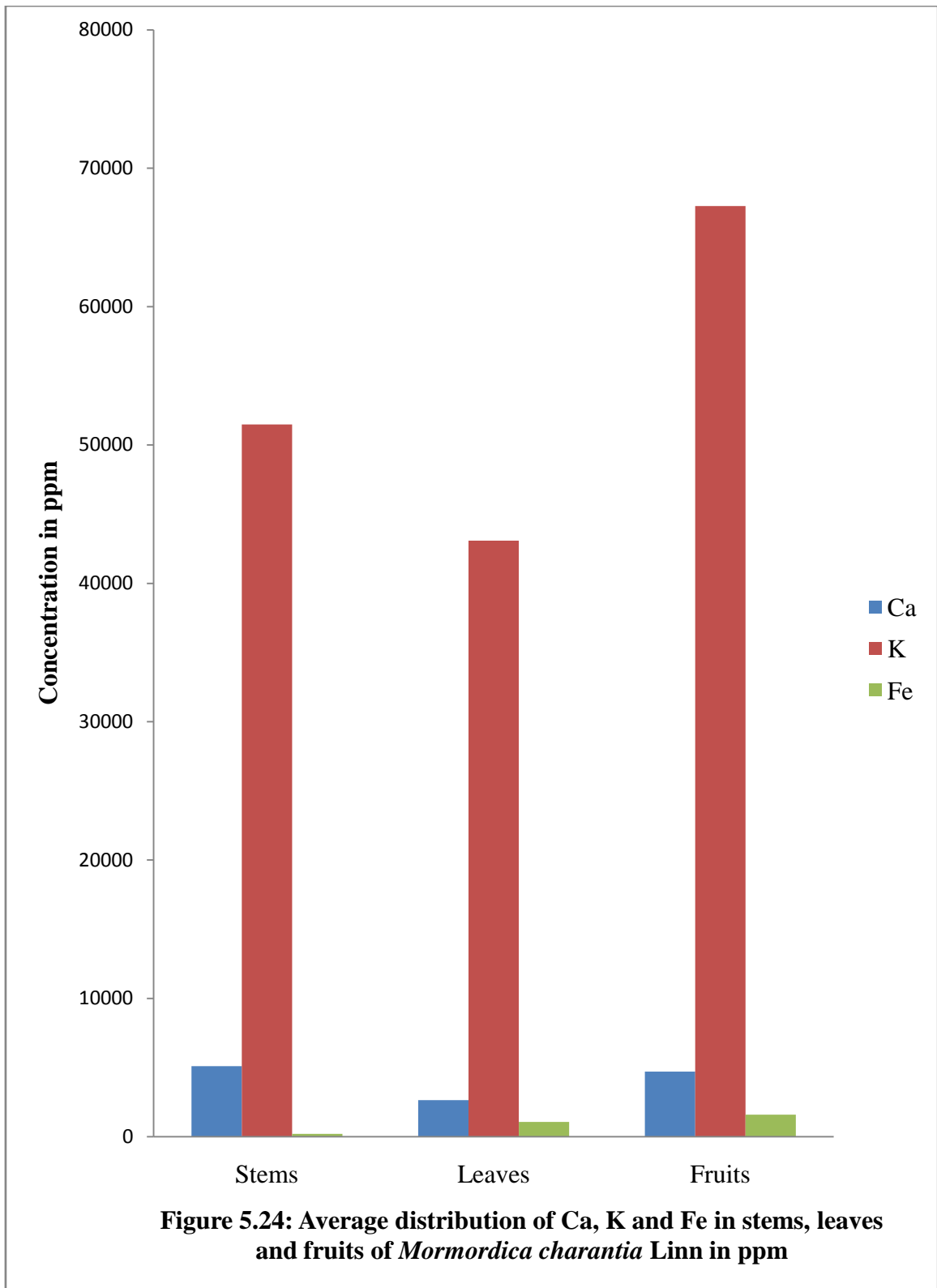
5.2.5 Variation in the distribution of detected elements in the stems, leaves and fruits of *Momordica charantia* Linn

Figure 5.24 below depicts the distribution pattern of the essential elements that are generally known to be in higher concentration in plants (Ca, K and Fe) in the roots, stems and leaves of *M. charantia* Linn. K concentration decrease from the roots to the stems and then increases in the leaves. But, however the pattern of bioaccumulation of Ca and Fe is that, they increase from the roots to the stems and declined in the leaves of this plant.

Figure 5.25 below indicates the distribution pattern of V, Cu and Mn in the roots, stems and leaves of *M. charantia* Linn. Though, their concentrations is in the order $Mn > Cu > V$, their bioaccumulation pattern is that, they decreases from the roots to the stems and then to the leaves.







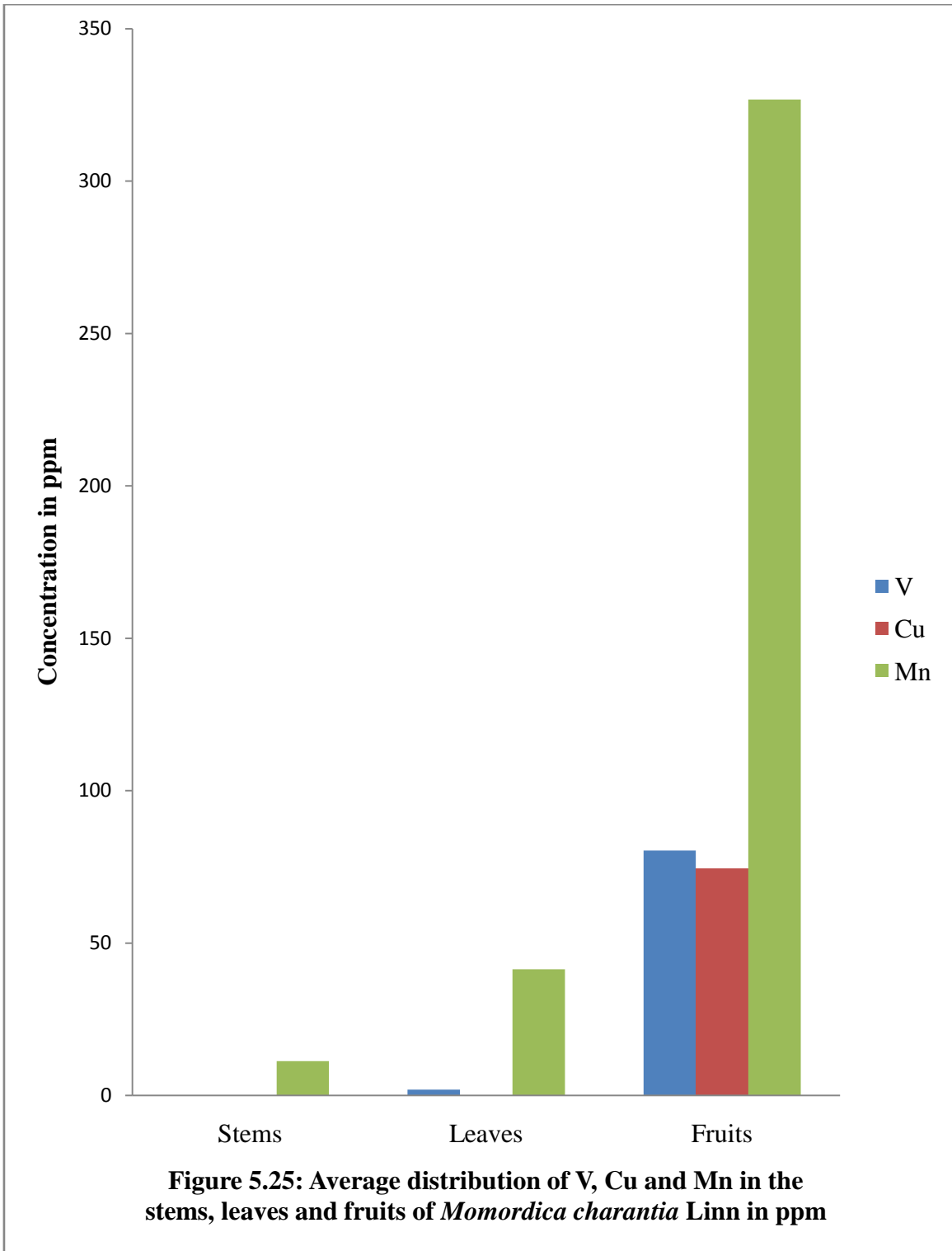
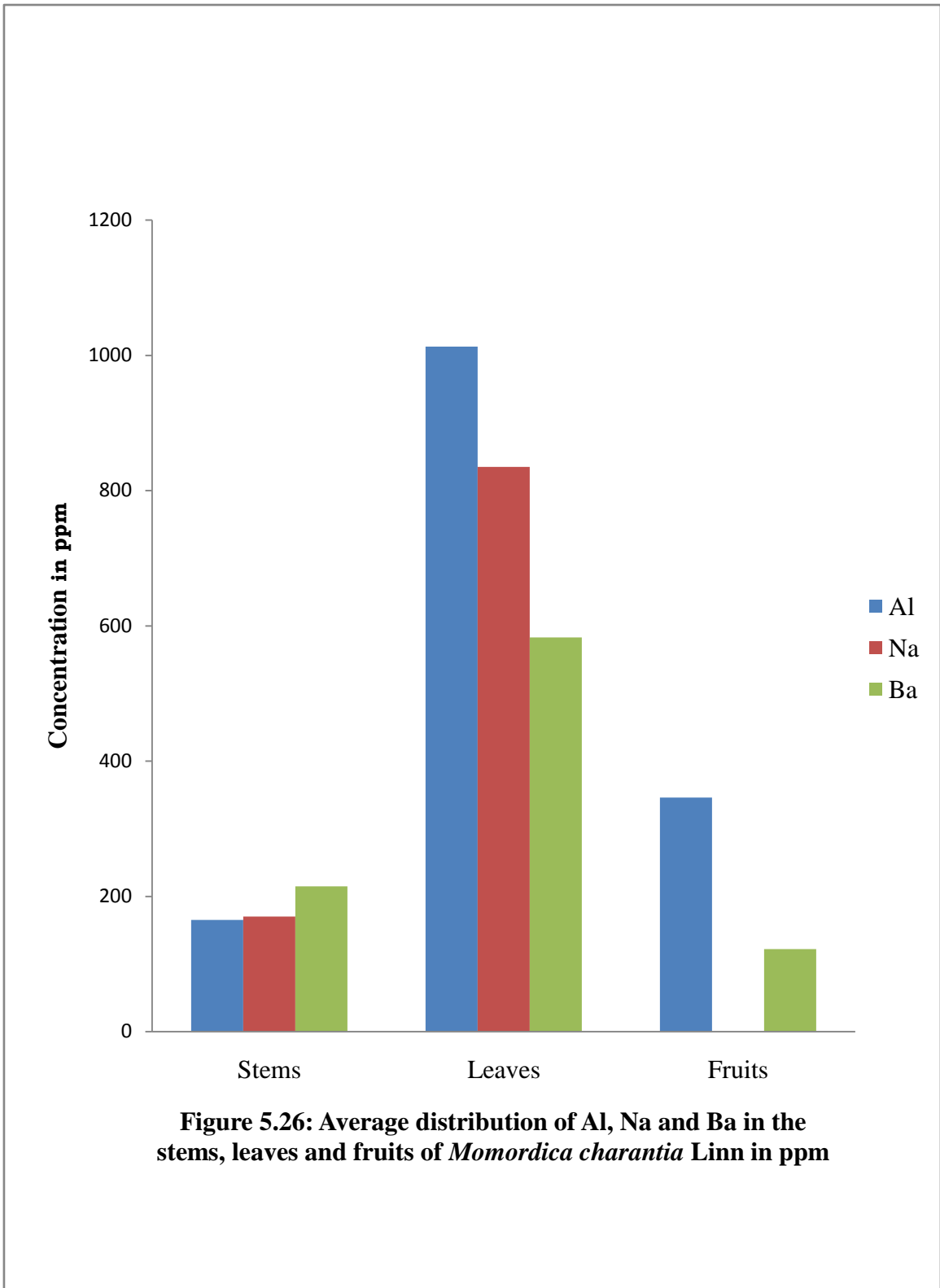


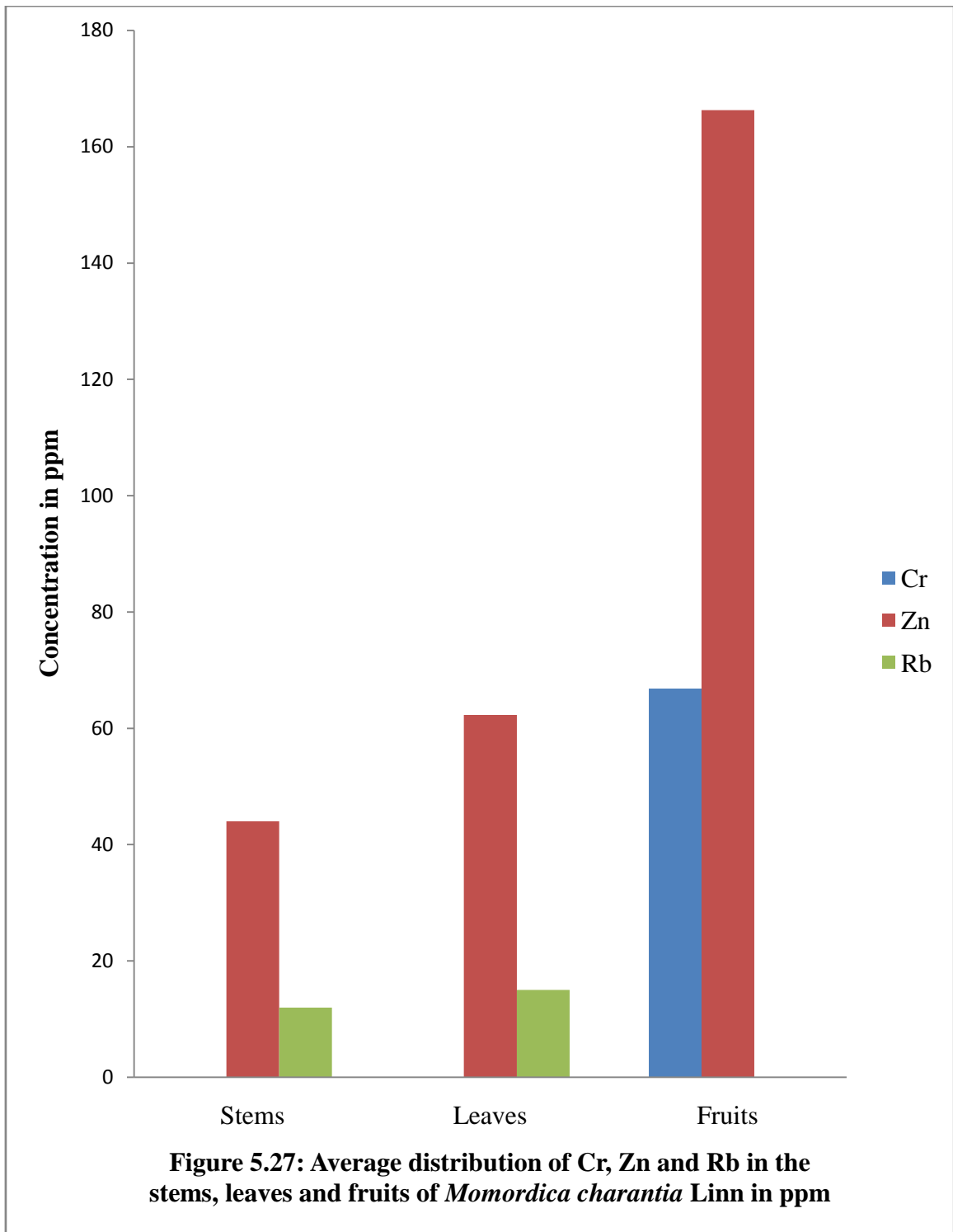
Figure 5.26 below signifies the distribution pattern of Al, Na and Ba in the roots, stems and leaves of *M. charantia* Linn. The concentration of all the stated elements increases from the roots to the stems and then to the leaves. The average concentration of Al metal is greater than that of Na and followed by Ba with the least.

The figure below (5.27) is revealing the pattern of distribution of Cr, Zn and Rb in the roots, stems and roots of *M. charantia* Linn. The concentration of all the stated elements increases from the roots to the stems and then to the leaves.

The figure below (5.28) is revealing the pattern of distribution of La, Sm, Sc and Co in the roots, stems and roots of *M. charantia* Linn. The concentrations of La, Sm and Sc increases from the roots to the stems and then to the leaves while that of Co increases from the stems further increase to the leaves.

Figure 5.29 below is showing the pattern of distribution of Br, Sb, Hf and Th in the roots, stems and roots of *M. charantia* Linn. The concentration of Sb, Br and Hf increases from the roots to the stems and then decreases to the leaves while that of Th increases from the stems further increase to the leaves.



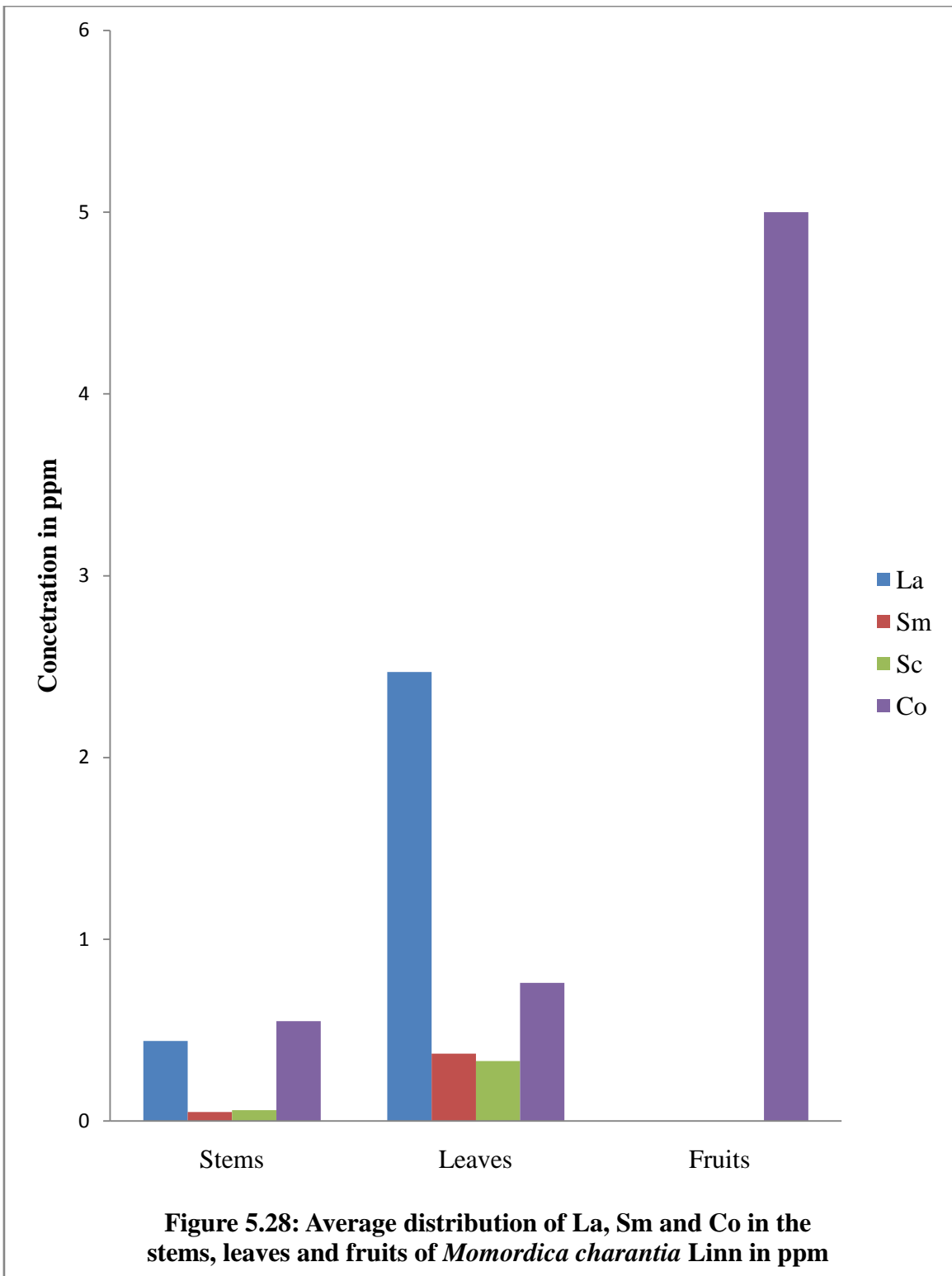


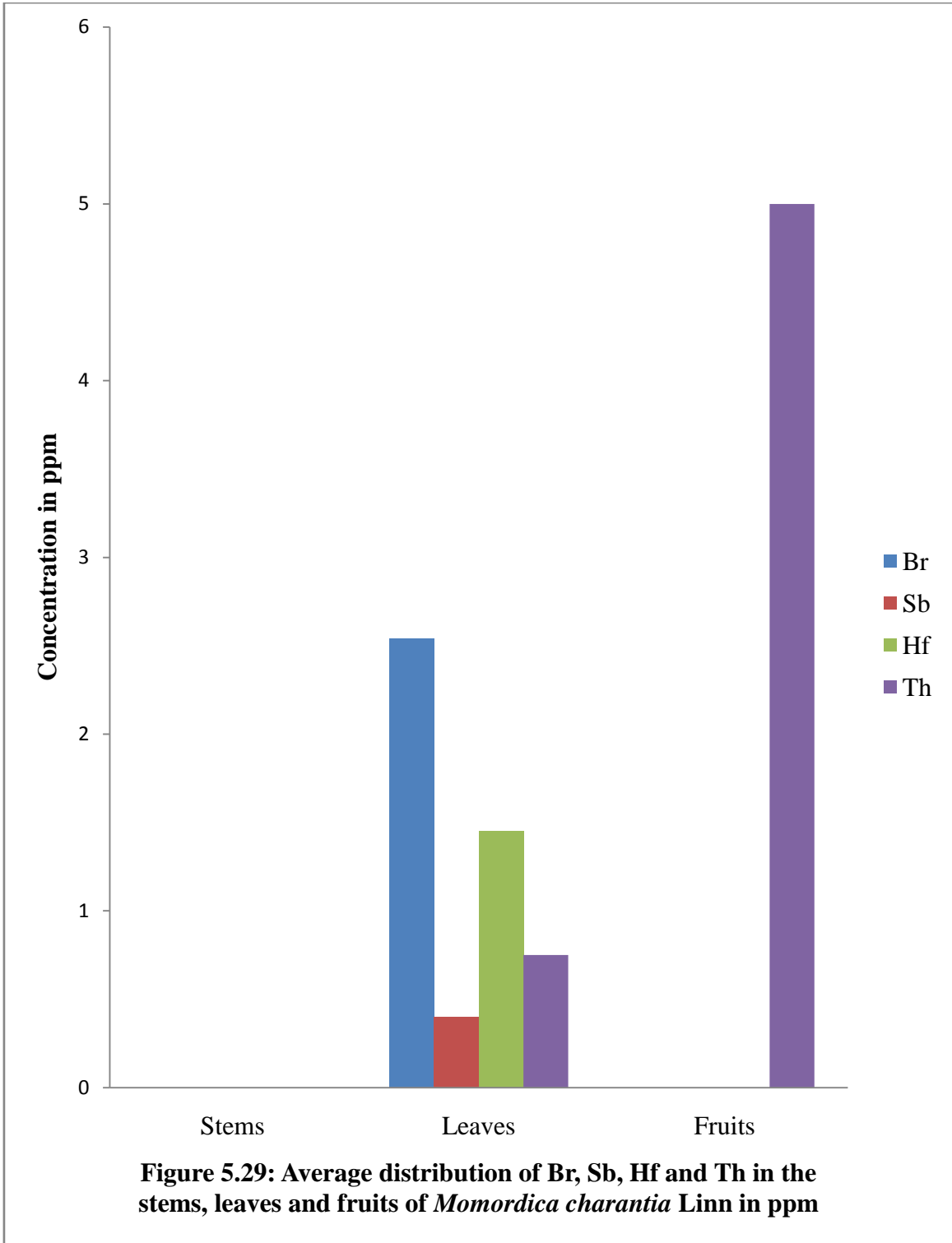
The figure below (5.28) is revealing the pattern of distribution of La, Sm, Sc and Co in the roots, stems and roots of *M. charantia* Linn. The concentrations of La, Sm and Sc increases from the roots to the stems and then to the leaves while that of Co increases from the stems further increase to the leaves.

Figure 5.29 below is showing the pattern of distribution of Br, Sb, Hf and Th in the roots, stems and roots of *M. charantia* Linn. The concentration of Sb, Br and Hf increases from the roots to the stems and then decreases to the leaves while that of Th increases from the stems further increase to the leaves.

5.2.6 Variation in the distribution of the detected elements in the roots, stems, leaves and fruits of *Senna occidentalis* Linn

Figure 5.30 below depicts the distribution pattern of Ca, K and Fe in the roots, stems, leaves and fruits of *S. occidentalis* Linn. Ca concentration increases from the roots to the stems, decreases to the leaves and then to the fruits. Also, the concentration of K increases from the roots of the plant to its stems to the leaves and declined to the fruits. However, the concentration of Fe decreases progressively from the roots, stems, leaves and finally to the fruits.





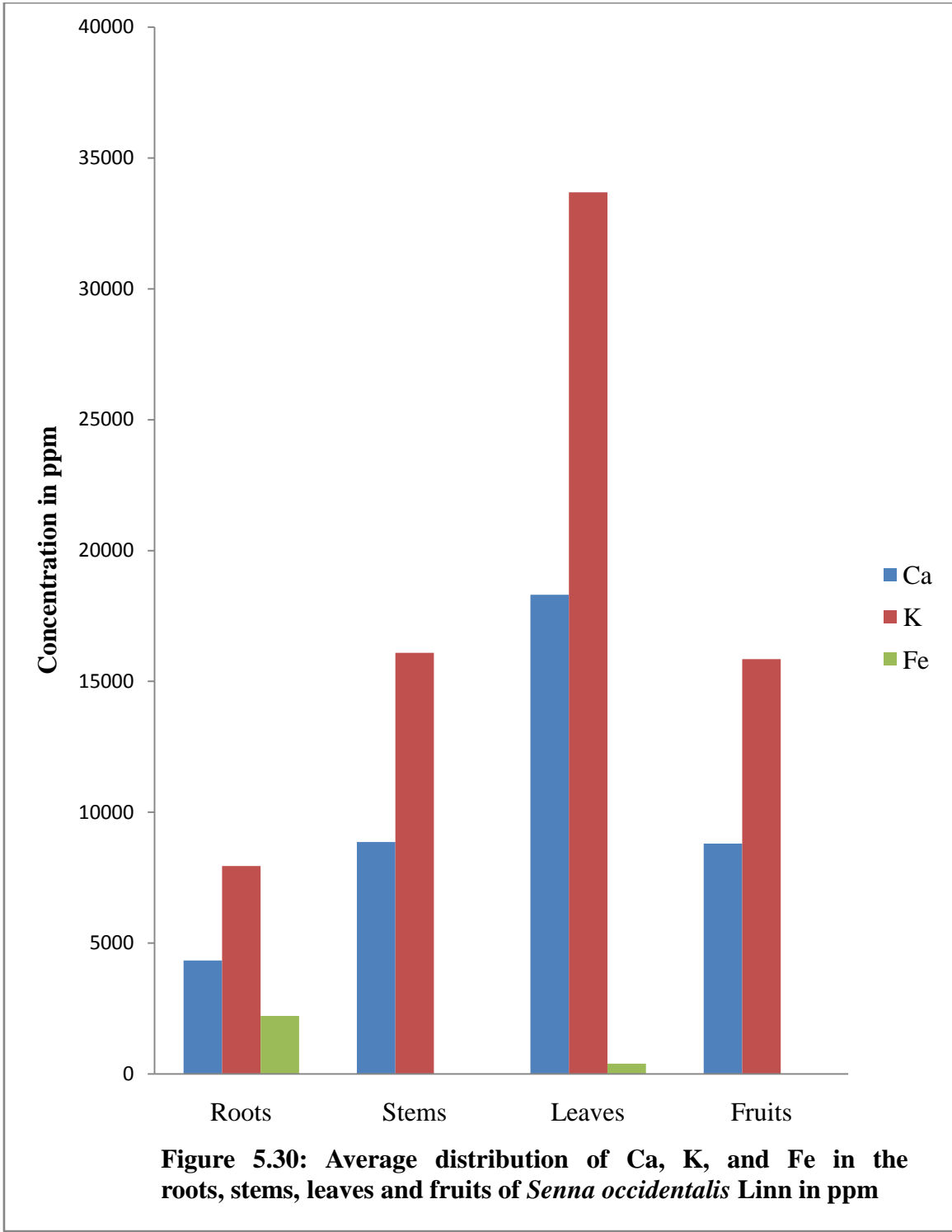
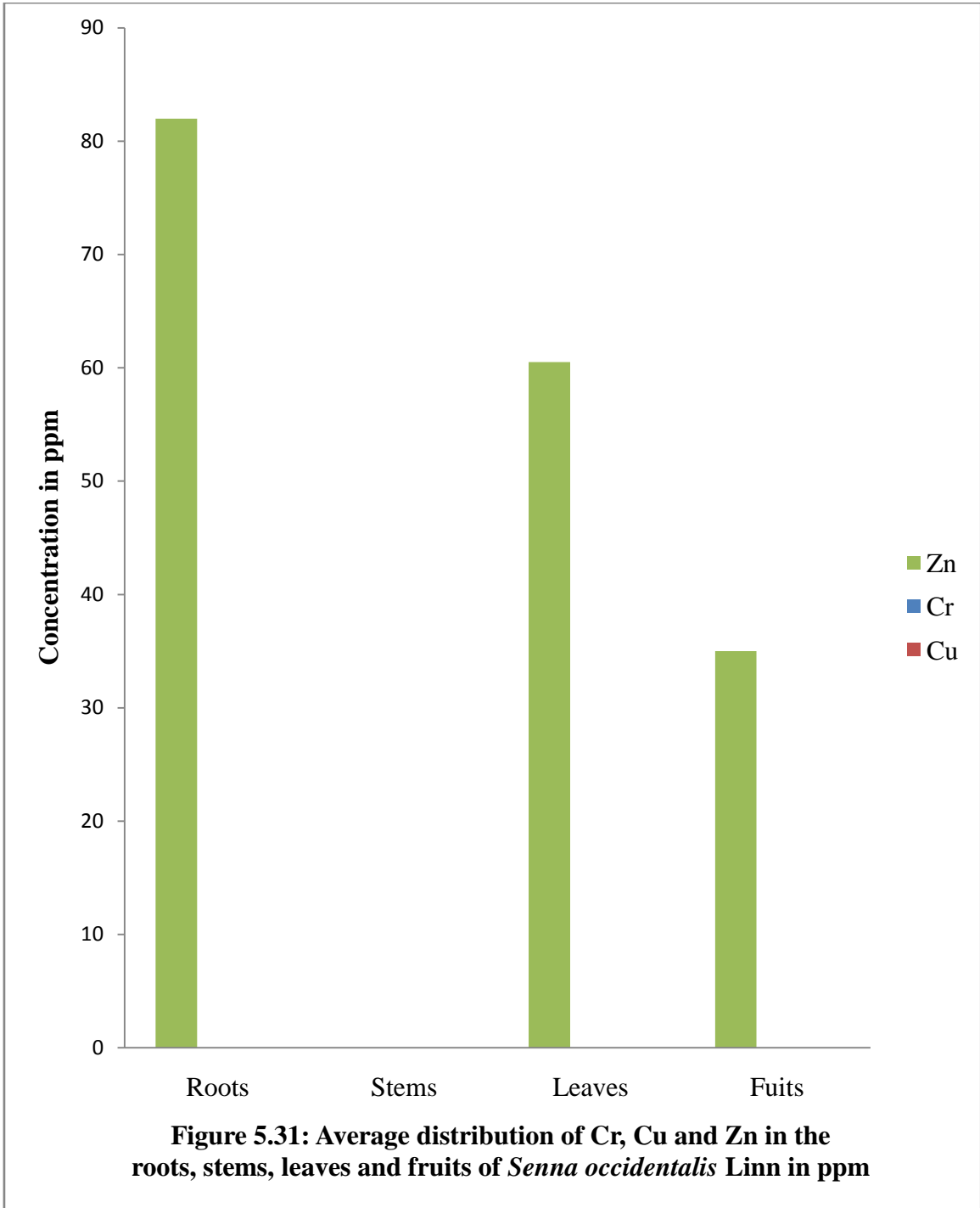


Figure 5.31 below shows the distribution pattern of Cr, Cu and Zinc in the roots, stems, leaves and fruits of *S. occidentalis* Linn. The concentrations of Cr in this plant increases from the roots to the stems while it decreases from the stems leaves and then increases to the fruits. But in Cu, the accumulation pattern is that, the roots have the highest concentration which decreases in the stems and then rose in the leaves and then decreases in the fruits. However, similar pattern of bioaccumulation of Cr was observed in the case of Zn with it increasing from the roots to the stems then decreases to the leaves and declined in the fruits of this plant.

The figure (5.32) below shows the distribution pattern of Sb, Br and Co in the roots, stems, leaves and fruits of *S. occidentalis* Linn. Though these set of elements are usually found at trace and ultra-trace levels in living tissues, Br is found to be highest in the leaves of this plant. Its concentration remained the same in the roots and stems and then increased sharply in the leaves and fell drastically in the fruits of the plant. For Sb, the element was not detected in the roots but in the stems which increased to the leaves and then decreased in the fruits. However, the concentration of Co in this plant is highest in the roots which decreased in the stems and remained approximately constant in the leaves and absent in the fruits.



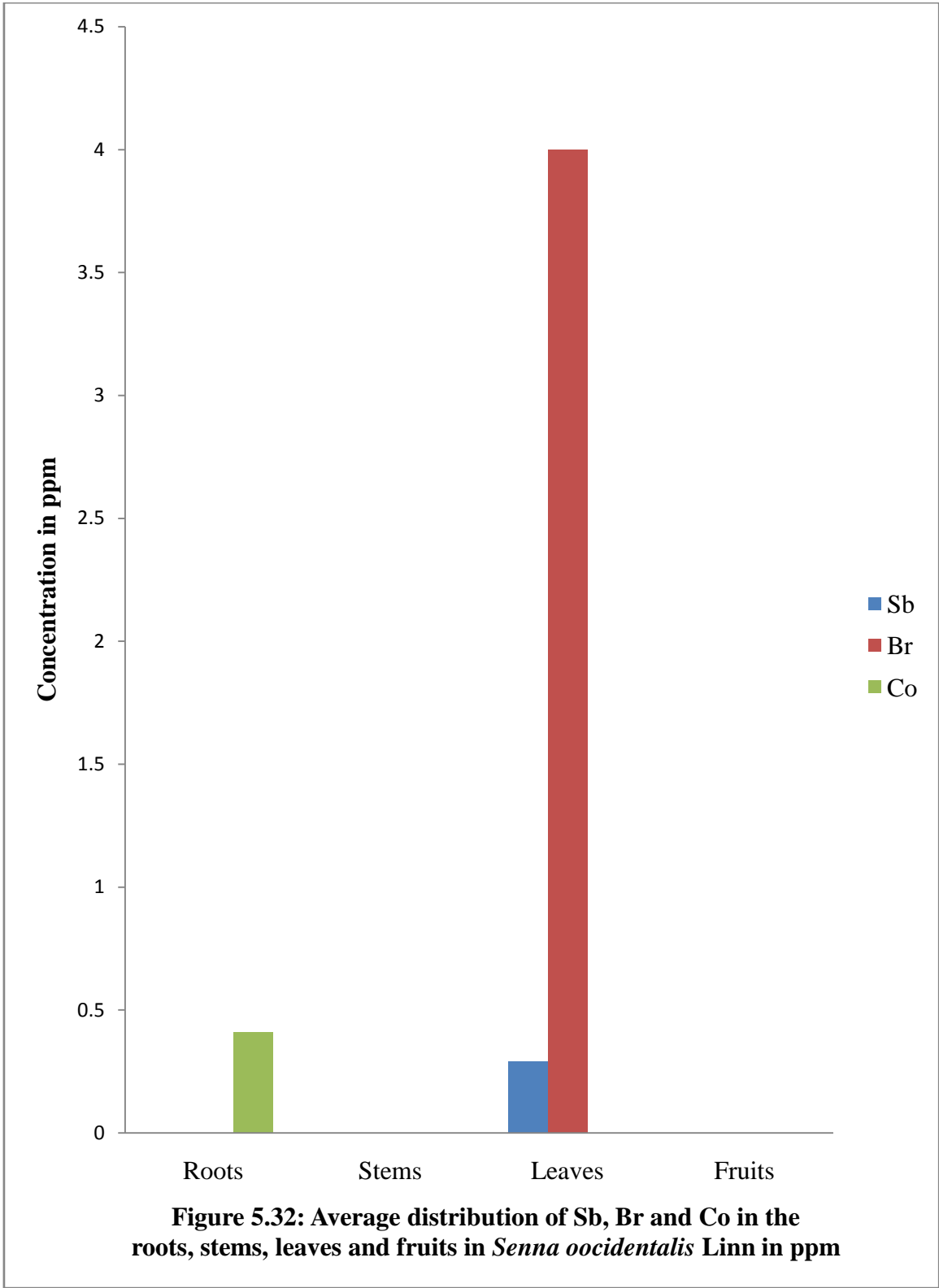


Figure 5.33 below signifies the bioaccumulation pattern of As, La, Sm and Sc in the roots, stems, leaves and fruits of *S. occidentalis* Linn. Despite being at trace level in this plant, As is only found in the roots but missing in the other parts of the plant. For La, the pattern is that it decreases from the roots to the stems, to the leaves and increase slightly in the fruits of the plant. Similar pattern was observed is observed in the bioaccumulation of Sm for the roots and stems but differs in the leaves by increasing in amounts and decreasing in the fruits. However, Sc concentration decreases from the roots then increases in the leaves and then decreases in the fruits.

The figure below (5.34) indicates the distribution pattern of Al, Ba and Rb in the roots, stems, leaves and fruits of *S. occidentalis* Linn. Al concentration decreases from the roots to the stems, increases to the leaves and then increases to the leaves and declined to the fruits. However, Ba was not detected in the roots of *Senna occidentalis* Linn but was observed to be absent in the stems, the fruits and only found in the leaves of the plant. Moreover, the pattern of bioaccumulation of Rb is approximately constant with the element slightly higher in the leaves.

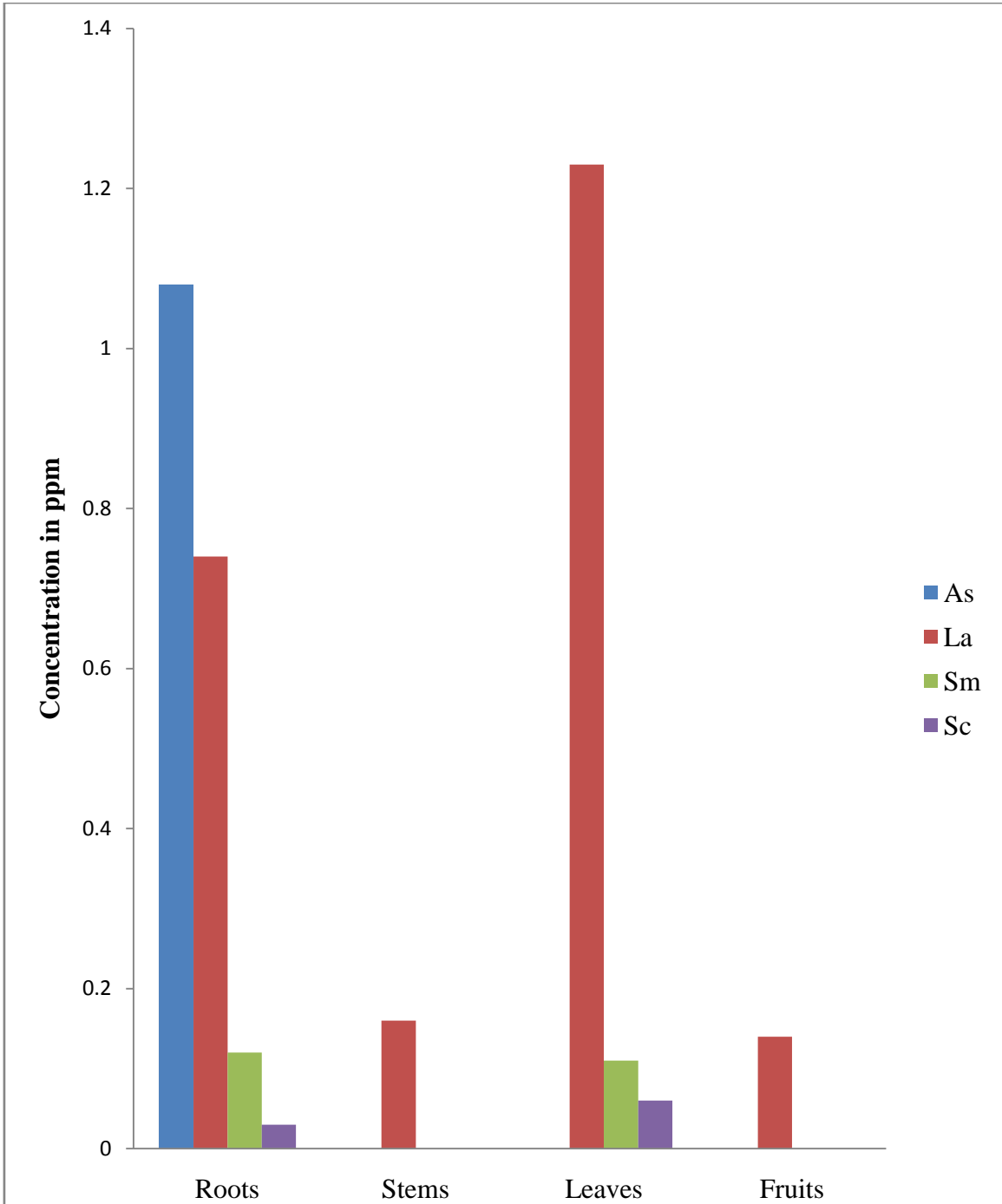
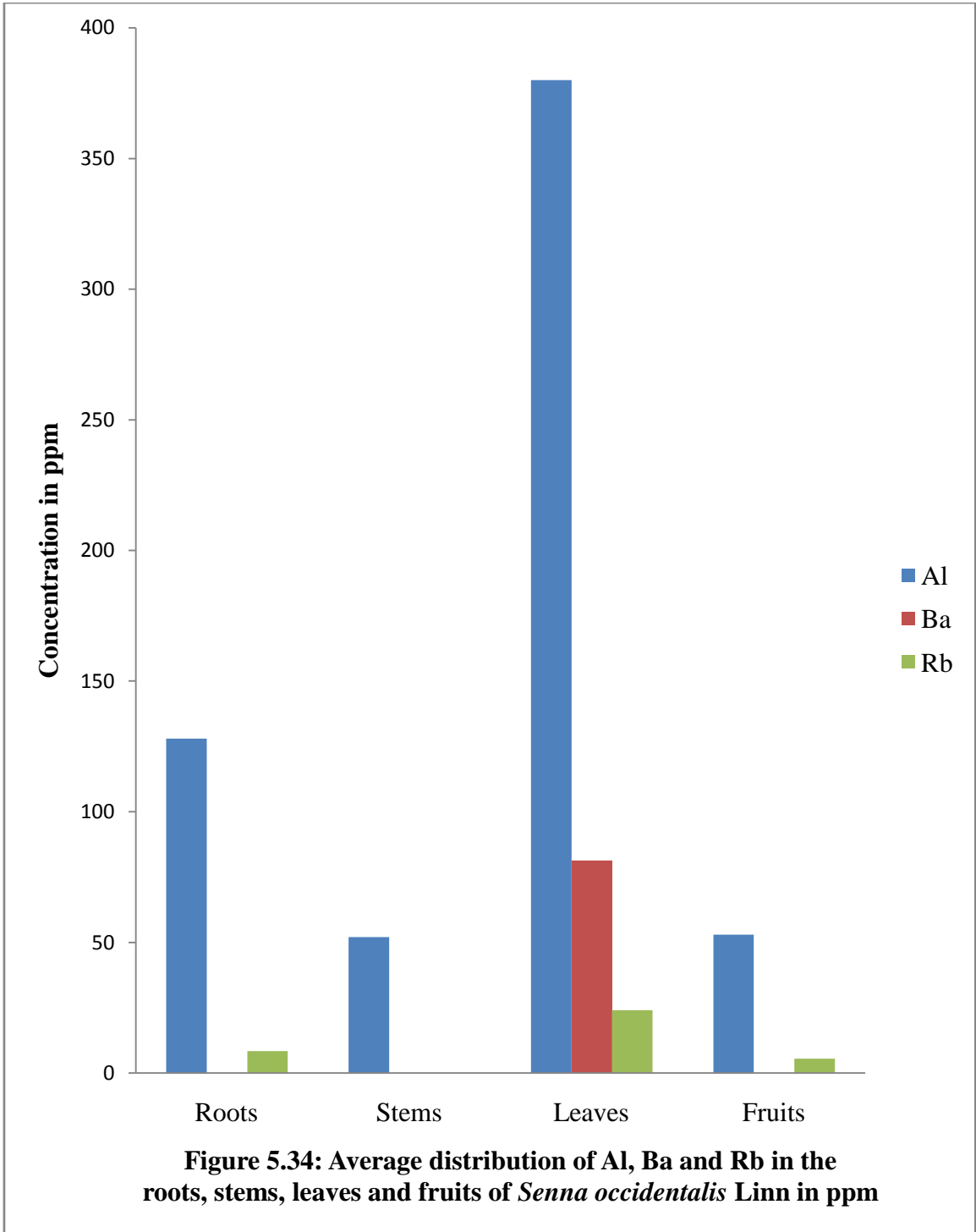


Figure 5.33: Average distribution of As, La, Sm and Sc in the roots, stems, leaves and fruits of *Senna occidentalis* Linn in ppm



5.3.0 Translocation factors of the elements in *Corchorus olitorius* Linn, *Corchorus tridens* Linn, *Vernonia amygdalina* Del, *Ceratotheca sesamoides* Linn, *Mormodica Charantia* Linn

Table 4.10 shows the translocation factors (TFs) of some of the analyzed elements in the plants. It can be either the ratio of metal concentration in the shoots to the roots ($[\text{Metal}]_{\text{Shoot}}/[\text{Metal}]_{\text{Root}}$) or ($[\text{Metal}]_{\text{Leaf}}/[\text{Metal}]_{\text{Shoot}}$) that show the metal translocation properties from roots to shoots or shoots to leaves (Nour *et al.*, 2009). TF factor >1 of a particular element indicates that it is translocated effectively from one organ to the other (Bu-olayan and Thomas, 2009, Baker and Brooks, 1989, Zhang *et al.*, 2002 and Fayiga and Ma, 2006.). In this work, the BF has been categorized in to $\text{BF}_{\text{S/R}}$ and $\text{BF}_{\text{L/S}}$, not determined for some of the elements in some organs of the plants analyzed and values were found to vary without any particular pattern.

Although, most plants species have a translocation factor of <1 , depicting bioaccumulation in the order of roots $>$ stems $>$ roots (Rezvani and Zaefarian, 2011) it is found to range from 0.02 – 20.03 in this work. Values >1 for a particular element indicates that, the plant translocate that particular element effectively from one organ to another (Bu-olayan and Thomas, 2009). For Al, the $\text{TF}_{\text{S/R}}$ in *C. olitorius* Linn, *C. tridens* Linn and *S. occidentalis* Linn are all >1 indicating a good transfer of the metal from the roots to the stems (biomagnification of the metal in the stems) while it is <1 in *V. amygdalina* Del and *M. charantia* Linn indicating retention of it in the roots. However, all the $\text{TF}_{\text{L/S}}$ values for Al in the analyzed plants are <1 except that of *V. amygdalina* Del and *M. charantia* Linn indicating an inverse relationship between $\text{TF}_{\text{S/R}}$ and $\text{TF}_{\text{L/S}}$.

Moreover, the significance of establishing pattern of translocation of metals from roots to other parts of a plant species can be very useful in biological monitoring of heavy metal contamination as well as selection of metal accumulators or tolerant species. It can also be useful in selecting part of a plant for consumption or rejection in terms of quantities of minerals contained so that toxicity may be avoided or otherwise. That is why the metal translocation process in plant species is a crucial factor in determining the metal distribution in different plant tissues (Xiong, 1998). A number of factors including anatomical, biochemical and physiological factors (Salt *et al.*, 1995) contribute to metal accumulation and distribution in the upper vegetative parts. The mechanism of metal translocation begins with metals being mobilized and taken by root cells from soil, bound by cell wall and then transported across the plasma membrane, driven by ATP-dependent proton pumps that catalyses H^+ extrusion across the membrane. Along with cationic nutrients, plant transporters are also involved in shuttling potentially toxic cations across plant membranes (Maser *et al.*, 2001 and Singh *et. al.*, 2003).

Usually, the tolerance of plants to increasing levels of metals especially the toxic ones can result from the exclusion of toxic elements or their metabolic tolerance to specific elements. The major mechanism in tolerant species of plants appears to be compartmentalization of metal ions ie sequestration in the vacuoles compartment, which excludes them from cellular sites where processes such as cell division and respiration occur, thus proving to be as an effective protective mechanism (Chaney *et al.*, 1997, Hall, 2002 and Singh *et al.*, 2010).

In addition to that, the $TF_{S/R}$ and $TF_{L/S}$ values of barium in all the plants analyzed in this work are < 1 except $TF_{L/S}$ of *M. charantia* Linn which indicates that Ba is

hyperaccumulated by the leaves of this plant while bromine is well translocated by all the plants analyzed because TF values are either <1 or $=1$. A key trait of metal hyperaccumulators is the efficient metal transport from roots to stems or stems to leaves characterized by the TFS/R or $FTL/S >1$ (Zhao *et al*, 2006). For calcium, it is *C. sesamoides* Endl and *M. charantia* Linn that bioaccumulates it well in their leaves because their $TF_{L/S}$ values are >1 .

Moreover, from Table 10 it could be seen that most of $TF_{S/R}$ and $TF_{L/S}$ values for some elements were assigned Nd (not determined) because their concentration in the affected parts of the plants were at below detection limit (BDL), hence could not be determined. Such elements include Co, Fe, Rb, Sc, Sm, V and Zn. For a particular plant whose $TF_{S/R}$ or $TF_{L/S}$ is determined, the value is < 1 except for Fe in *V. amygdalina* Del ($TF_{S/R} = 1.36$), Rb in *C. sesamoides* Endl ($TF_{L/S} = 2.36$), Sc in *Corchorus tridens* Linn ($TF_{S/R} = 2.56$) and Zn in *Corchorus tridens* Linn ($TF_{S/R} = 2.32$) and *C. sesamoides* Endl ($TF_{S/R} = 3.07$).

However, the TF values for K, La, Mn, and Na varied in the plants like the previous elements discussed. $TF_{S/R}$ and $TF_{L/S}$ values of the elements for the plants analyzed are either > 1 indicating bioaccumulation of that particular element or < 1 showing the ability to translocate it well.

5.4.0 Bioavailability of the determined elements

Bioavailability of metals is the extent to which bioaccessible metals adsorb on to or absorb in to and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time

and under defined conditions (Drexler *et al.*, 2003). Table 4.11 above signifies the bioavailability levels of some of the elements in the analyzed plants. Although, present in substantial amounts in indigenous leafy vegetables, many nutrients such as vitamins, antioxidants, minerals, and important proteins (Akula *et al.*, 2007 and Mariga *et al.*, 2012) and their availability to man and his animals is restricted due to the presence of in built anti-nutritionals (phytates, oxalates, trypsin inhibitors, cyanides and ployphenols) in the plants (Nadeem *et al.*, 2010).

To predict the bioavailability of metals (effect of antinutrients especially phytate and oxalate) anti-nutrient to metal ratios are calculated. The calculated molar ratios of phytate to calcium ([Phytate]/[Ca]) in the analyzed plants ranged from 1.34×10^{-4} – 8.85×10^{-2} in the order of *C. tridens* Linn < *M. charantia* Linn < *C. sesamoides* Endl < *C. olitorious* Linn < *S. occidentalis* Linn < *V. amygdalina* Del as shown in Table 13 and are below the critical level of 0.5 as outlined by Hassan *et al.*, (2011), Frontela *et al.*, (2008) and Mitchkape *et al.*, 2008. This indicates that, the presence of phytate in the analyzed plants will not hinder the bioavailability of calcium to the organisms that consume the plants.

Also the phytate to iron molar ratios ([Phytate]/[Fe]) calculated for the analyzed plants as indicated in table 4.13 above ranged from 1.88×10^{-2} – 3.28×10^{-1} in the order of *C. olitorious* Linn > *V. amygdalina* Del > *C. tridens* Linn > *M. charantia* Linn > *C. sesamoides* Endl > *S. occidentalis* Linn and these values are lower than the crital value (0.4)(Hassan *et al.*, 2011, Frontela *et al.*, 2008 and Mitchkape *et al.*, 2008). This signifies that, the phytate content in the analyzed plants would not hinder bioavailability of iron when consumed.

Likewise, the [Phytate]/[Mn] values for the analyzed plants are generally higher than that of Ca and Fe and ranged from 0.056 – 2.62 with *C. olitorious* Linn having the highest while *M. charantia* Linn the least. For [Phytate]/[Zn] in the analyzed plants the range is 0.14 – 4.02 and is in the order of *C. olitorious* Linn (4.02) > *V. amygdalina* Del (3.86) > *C. sesamoides* Endl (0.83) > *S. occidentalis* Linn (0.76047) > *C. tridens* Linn (0.63146) > *M. charantia* Linn (0.13195). And since the critical ratio for [Phytate]/[Zn] is 1.5 (Hassan *et al*, 2011, Frontela *et al*, 2008 and Mitchkape *et al*, 2008) the metal could still be available on consumption of the plants. Where the ratio is higher than the critical value, is an indication that the phytate may hinder the bioavailability of Zn (Umar, 2005) and could be improved on subjecting the plant leaves to certain treatments (Enneking and Wink, 2000, Kantar, 1994, Kala and Mohan, 2012, Ramakrishna, *et al.*, 2010 and Nadeem *et al.*, 2010). However, it has been found that [Ca][Phytate]/[Zn] ratio is a better measure of Zn bioavailability than [Phytate]/[Zn] ratio (Obah and Amusan, 2009). The plant samples analyzed have [Ca][Phytate]/[Zn] ratios of 2.24 (*C. olitorious* Linn), 23.29 (*C. tridens* Linn), 1.28 (*V. amygdalina* Del), 17.227 (*C. sesamoides* Endl), 0.015 (*M. charantia* Linn) and 0.17 (*S. occidentalis* Linn) as seen in table 4.13 which are below and above the critical level (0.5).

Moreover, the result in table 13 has also showed that the [Oxalate]/ [Ca] ratios in the analyzed plants except that of *C. olitorious* Linn (4.1092) are below the critical level (2.5) known to impair calcium bioavailability (Hassan *et al*, 2007). For Fe, Mn and Zn, the values of the ratios ranged from 0.26304 – 1359.1 and since the critical values for them are not known, there is no conclusion that could be drawn and therefore calls for further

research. This is also applicable to $[M][\text{Oxalate}] / [\text{Zn}]$ obtained for Fe, Mn and Zn which are having relatively low values.

Though, the calculated $[\text{Anti-nutritional}] / [M]$ showed that, the metals can be bioavailable, improvement can still be achieved by subjecting the plant materials to processes that have been found to reduce the amounts of anti-nutritionals which subsequently make the metals available for absorption. These anti-nutritionals known to decrease the bioavailability of minerals especially Ca, Mg, Fe and Zn include phytate, oxalates and tannins (Agte *et al.*, 1999, Oatwy *et al.*, 2001, and Bhandari and Kawabata, 2004), while others include L-Dopa, phenolics, haemagglutinin, trypsin and chymotrypsin inhibitors, anti-vitamins, protease inhibitors, flatulence factors, saponins and hydrogen cyanide (Emenalom and Udedibie, 1998; Vadivel and Pugalenthil, 2008).

Some of the processing methods for the removal of anti-nutritional factors include heating (cooking, boiling, frying and roasting), soaking, germination, fermentation and genetical modification (Calvalho, 2013, Enneking and Wink, 2000, Ekpo *et al.*, 2004, Ekpo, *et al.*, 2005b and Ramakrishna, *et al.*, 2006).

5.5 Proximate composition of *Corchorus olitorious* Linn, *Corchorus tridens* Linn, *Vernonia Amygdalina* Del, *Ceratotheca sesamoides* Endl, *Momordica charantia* Linn and *Senna occidentalis* Linn

Table 4.12 indicates the proximate composition of *C. olitorious* Linn, *C. tridens* Linn, *V. amygdalina* Del, *C. sesamoides* Endl, *M. charantia* Linn and *S. occidentalis* Linn. Usually,

proximate composition analysis is used to estimate the relative amounts of protein, lipid, water, ash, dietary fiber and carbohydrate in an organism.

5.5.1 Moisture

Vegetable cells contain important quantities of water. It plays a vital role in the evolution and reproductive cycle and in physiological process. It has effects on the storage period length and on the consumption of tissue reserve substances. Levels obtained in the analyzed plants ranged from 46.90 – 75.53 % with *C. olerifolius* Linn having the highest and the other plants in the order of *C. tridens* Linn > *S. occidentalis* Linn > *C. sesamoides* Endl > *V. amygdalina* Del > *M. charantia* Linn. The value obtained for *C. olerifolius* Linn (75.53 %) is very much similar to another work on the plant elsewhere (Adebayo, 2010) who obtained 85.78 %. But Onwordi *et al.*, 2009 and Mohammed and Mann, 2012 got lower values (30.90 and 55.60 %).

Moisture content of *C. tridens* Linn is 67.62 % and this value is lower than the value usually declared for fresh vegetables (90.96 %) although very much lower values were obtained for the same plant (7.98 %) for dried samples (Nkafamiya *et al.*, 2010).

For *V. amygdalina* Del, the moisture is 57.94 %. This value is lower than those obtained by Aliero and Abdullahi, 2009 (75.00 %) and Sengupta and Pal, 1980 (86 – 88 %). These variations may be largely attributed to the different sampling locations. In addition to that, the moisture content of the fresh leaves of *C. sesamoides* Endl obtained in this work (63.84 %) is similar to the other values obtained elsewhere (78.23 - 83.08 %) by Fasakin, 2004.

In addition to that, the moisture content of *M. charantia* Linn obtained in this work (46.90 %) is also lower than the values obtained elsewhere for *M. balsamina* Linn leaves (71.00 %) by Hassan and Umar, 2006 and very much smaller values (17.97 %) were obtained by Bakare *et al.*, 2010. It should be noted that moisture contents of the fruits of *M. charantia* Linn (91.92 – 993.80 %) are very much higher than that of the leaves (Islam *et al.*, 2011 and Ullah *et al.*, 2011).

However, the moisture content of *S. occidentalis* Linn leaves in this work is 65.51 %. This value is higher than other values obtained elsewhere; 8.0 % and 46.01 % (Daniyan *et al.*, 2011 and Allismith, 2009).

Water in vegetables is in the following forms:

1. Bound water or dilution water which is present in the cell and forms true solutions with minerals or organic substances;
2. Colloidal bound water which is present in the membrane, cytoplasm and nucleus and acts as a swelling agent for these colloidal structure substances during drying/dehydration processes;
3. Constitution water directly bound on the chemical component molecules and which is removed with difficulty;

These forms of water that is determined as moisture content generally constitute about 90.96 % of vegetables weight (FAO, 2013).

Moisture content is one of the most commonly measured properties of food materials. It is important to food scientists for a number of reasons:

i. Legal and Labeling Requirements. There are legal limits to the maximum or minimum amount of water that must be present in certain types of food.

ii. Economic. The cost of many foods depends on the amount of water they contain - water is an inexpensive ingredient, and manufacturers often try to incorporate as much as possible in a food, without exceeding some maximum legal requirement.

ii. Microbial Stability. The propensity of microorganisms to grow in foods depends on their water content. For this reason many foods are dried below some critical moisture content.

iii. Food Quality. The texture, taste, appearance and stability of foods depend on the amount of water they contain.

iv. Food Processing Operations. Knowledge of the moisture content is often necessary to predict the behavior of foods during processing, *e.g.* mixing, drying, flow through a pipe or packaging.

v. Aids digestion and lowers shelf life. Moisture content of vegetables makes them easy to digest and reduce shelf life because moisture content facilitates bacterial action resulting in spoilage (Olaiya and Adigun, 2010).

It is therefore important for food scientists to be able to reliably measure moisture contents of plants foods especially vegetables.

5.5.2 Ash content

When organic compounds are heated at about 500 – 600 °C they decompose and the remaining residue is the ash. It consists of oxides and salts containing anions such as

phosphates, chlorides, sulfates and other halides and cations such as sodium, potassium, calcium, magnesium, iron and manganese. During the ashing process organic salts decompose, losing the carbon-containing moiety. The metal from such salts forms oxides or reacts with other ions of the matrix. Some metals (e.g. cadmium and lead) may be volatilized during ashing. Therefore, measurement of ash contents suggests the amounts of inorganic matter present in the vegetable which is converted to metal oxides, water and carbon dioxide from the organic components of the vegetables (Olaiya and Adigun, 2010).

Table 4.12 indicates the amounts of ash in the analyzed samples, with *M. charantia* Linn having the highest (11.95 %) followed by *C. sesamoides* Linn (10.74 %), *V. amygdalina* Linn (10.01 %), *S. occidentalis* Linn (8.35 %), *C. tridens* Linn (4.27 %) and then *C. olitorious* Linn. Some of these values conform to other works elsewhere while others differ. For example, Bakare *et al.*, 2010, Hassan and Umar, 2006, got higher ash contents for *M. charantia* Linn (18.00 %, 15.42 %) compared to this work while some others got lower values like Hussain *et al.*, 2011(10.15 %). The same was observed for all the vegetables analyzed. For *C. olitorious* Linn, values higher than that obtained in this work (2.48 %) includes Omale and Ogwu, 2011 (5.00 %), Adebayo, 2010 (3.27 %), Ndolovu and Afolayan, 2008 (10.5 %) and Onwordi, 2009 (20.20 %) while those lower are Islam *et.al*, 2004 (1.80 %) and Adeniyi, 2012 (0.64 %). Also for *V. amygdalina* Del, values higher than that obtained in this work (10.01 %) are Atangwho *et al.*, 2009 (11.93 %), Asaolu *et al.*, 2012 (9.56 %) and Adegunwa *et.al*, 2011 (17.00 %) while lower value is Aliero and Abdullahi, 2009 (9.56 %).

Amount of ash in *C. tridens* Linn is 4.27 %. This value is higher than that obtained by Asibey-Berko and Tayie, 1999 (1.5 %). For *C. sesamoides* Linn, the amount of ash obtained for this work is 10.74 %. This value is higher than the average value (10.25 %) obtained in some cultivars of *C. sesamoides* Endl (Fasakin, 2004) but lower than the work of Freedman, 2012 (20.2 %) who determined the ash from the mixture of the leaves and fruits of the plant. In addition to that the, the level of ash in the leaves of *S. occidentalis* Linn is about 8.35 %. This value is higher than that obtained by Daniyan *et al.*, 2011 (5.9 %) but lower than that for *S. siamea* leaves (17.93 %) by Allismith, 2009 and *S. obtusifolia*, (12.80 %) by Ogunsan *et al.*, 2011. These variations of ash content in the analyzed plants may not be unconnected to the different sources of the samples because different locations contain varied minerals and in different concentrations.

5.5.3 Crude protein

Table 4.12 above indicates the protein content of the analyzed plants. The protein content of *C. olitorious* Linn determined in this work is 3.19 % which is lower than that determined by Onwordi, 2009 (11.20 %), Adeniyi, 2012 (6.21 %), Mohammed and Mann, 2012 (8.25) and higher than that determined by Adebayo, 2010 (1.82 %), Islam *et al.*, 2004 (2.56 %), Ndlovu and Afolayan, 2008 (1.63 %), Omale and Ugwu, 2011 (0.80 %). Likewise that of *C. tridens* Linn is 7.72 % which is higher than that obtained Asibey-Berko and Tayie, 1999 (2.7 %). For *V. amygdalina* Del, the protein content is 10.64 % which is very much higher than that obtained by Saidu and Jideobi, 2009 (2.10 %), Sengupta, and Pal, 1980 (2.1 – 2.36 %), Aliero and Abdullahi, 2009 (5.12 %), James and Emmanuel, 2011(2.27 %). Values of protein higher than that obtained in this work are includes; Belewu *et al*, 2009 (28.88 %), Atangwho, 2009 (23.25 %) and Asaolu *et al.*, 2012 (50.64 %).

Also, the amount of crude protein contained in *C. sesamoides* Endl determined in this work is 6.12 %. This value is much lower than that obtained by Fasakin, 2004 (28.83 %). For *M. charantia* Linn the amount of protein is 14.36 % and this value is much lower than 27.46 % obtained by Bakare *et al.*, 2010. Values obtained lower than that in this work are, 11.28 % (Hassan and Umar, 2006) and 1.58 % (Ullah *et al.*, 2011). The protein content of *S. occidentalis* Linn determined, however is 14.36 % which is much higher than that obtained by Daniyan *et al.*, 2011 (2.3 %), Alli smith,2009 (4.01%) but lower than that obtained by Ogunsan *et al.*, 2011 (15.22 %) and Ingweye *et al.*, 2010 (29.54 %). These differences observed might be due to variation in the environmental factors and agronomic practices at the various locations that the analyzed plant leaves were harvested Shokanbi *et al.*, 2011.

5.5.4 Crude Lipid

Lipid content is used to denote a mixture of both oil and fats occurring widely in organic tissue especially in the adipose tissue of animals and in the seeds, nuts, fruits and leaves of plants. The quantity of crude lipid determined in *C. olitorious* L. (5.15 %) and other plants analyzed in this work is depicted in Table 12. This amount is much higher than that determined by Ndlovu and Afolayan, 2008 (1.72 %), Onwordi, 2009 (0.32 %), Adeniyi *et al.*, 2011 (5.07 %), Islam *et al.*, 2002 (5.07 %) and Mohammed and Mann, 2012. For *C. tridens* Linn, the lipid content is 4.74 % and this value is lower than the obtained by Asibey-Berko and Tayie, 1999 (1.40 %).

Likewise, the lipid content of *V. amygdalina* Del determined in this work is 3.333 % which is very much lower than the one obtained by Aliero and Abdullahi, 2009 (0.5 %) and lower than the one obtained by Shokanbi *et al.*, 2011 (6.04 %), Ejoh *et al.*, 2007 (4.70 %), Asaolu

et.al., 2010 (9.05 %), Saidu and Jideobi, 2009 (0.7 %) and Sengupta and Pal, 1980 (0.3 – 1.0 %)

However, the lipid content of *C. sesamoides* Endl obtained in this work is 6.67 % which is higher than the one obtained by Fasakin, 2004 (4.00 – 5.63 %) and Ijeoma, 2012 (1.74 %). Also the lipid content of *M. charantia* Linn is 5.34 % which is higher than the one obtained by Hassan and Umar, 2006 (2.66 %), Bakare *et.al*, 2010 (3.68 %), Hussain *et al.*, 2011(1.75 %). For *S. occidentalis* Linn, the lipid content in this work is 5.004 % which is very much lower than the amount determined by Daniyan *et al.*, 2011(14.9 %), Allismith, 2009 (12.02 %), Ingweye *et.al*, 2010 (2.31%) and Ogunsan *et al.*, 2011(6.78 %).

5.5.5 Crude fiber content

Table 12 above depicts the amounts of crude fiber determined in the analyzed plants. In *C. olitorious* Linn, the crude fiber is at 5.00 mg/100 g which is lower than the amount obtained by Yekeen *et al.*, 2013 (7.40 g/100 g) and higher than that obtained by Ndlovu and Afolayan, 2008 (20.30 g/Kg) in the same plant. For *C. tridens* Linn, the crude fiber content is 4.70 g/100g which is lower than what was obtained by Asibey-Berko and Tayie, 1999 (8.1 g/100g). Likewise, the crude fiber content of *V. amygdalina* Del is 7.70g/100g which is lower than the values obtained by Owen, 2011(13.10 %DM), Yekeen *et al.*, 2011 (9.82 %DM) and higher than that obtained by Igile *et al.*, 2013 (7.63 g/100g).

Moreover, *C. sesamoides* Endl has the least amount of crude fiber in this work which amounts to 3.5 mg/100g and is lower than 7.25 g/100g obtained by Fasakin, 2004. Also the fiber content of *M. charantia* Linn analyzed in this work is 10.49 g/100g and is higher

than that obtained by Bakare *et al.*, 2010 (3.31 g/100g) and lower than the value got by Hassan and Umar (2006) in *M. balsamina* Linn (29.00 g/100g). However, the crude fiber content of *S. occidentalis* Linn found in this work is 8.00 g/100g which is lower than the amount found in *S. siamea* (12.36 g/100g) by Alli-Smith, 2009, *S. alata* petals (25 g/100g), *S. hirstuta* petals (40 g/100g), *S. obtusifolia* petals (30 g/100g) by Essiett and Bassey (2013) and higher than that in *S. obtusifolia* (2.58g/100g).

There are several types of fiber in plant materials that function differently and provide distinctive health benefits. They may either be soluble or insoluble. Soluble fibers bind with fatty acids and slow digestion so blood sugars are released more slowly into the body. These fibers help lower cholesterol and help regulate blood sugar levels for people with diabetes. Insoluble fibers help to hydrate and move waste through the intestines and control the pH levels in the intestines. These fibers help prevent constipation and keep one regular. However, there is evidence indicating that these complex carbohydrates (fibers) directly interact with the food antioxidants and interfere with the adequate assimilation of these compounds (Montagne, *et al.*, 2003, Cummings *et al.*, 2004, DeVries, 2004 and Palafox-carlos, *et al.*, 2011).

5.5.6 Carbohydrate content

Table 4.12 above also indicates the amounts of carbohydrate in the plants analyzed in this work. For *C. olitorious* Linn the amount determined amounts to 8.65 g/100g which is lower than that found by Yekeen *et al.*, 2013 (48.17 %), Idris *et al.*, 2009 (19.58 %) and Ndlovu and Afolayan, 2008 (69.5 g/100g) but higher than that determined by Adeniyi *et al.*, 2012

(6.25 g/100g). For *C. tridens* Linn, the carbohydrate content amounts to 7.29 g/100g which is similar to was obtained in the same plant by Asibey-Berko and Tayie, 1999 (8.7 g/100g). However, for *V. amygdalina* Del, the carbohydrate content is 10.377 g/100g) which is lower than the amounts obtained elsewhere (Yekeen *et al.*, 2011) who got 35 % and higher what was obtained by Alabi *et al.*, 2005 (4.31 mg/100 and Igile *et al.*, 2013 (20.80 mg/100g). Likewise, the carbohydrate content of *C. sesamoides* Endl in this work is 9.13 g/100g which is lower than what was obtained by Fasakin, 2004 (39.95 g/100g) and Ijeomah *et al.*, 2012. But for *M. charantia* Linn, the carbohydrate content is 5.69 g/100g which is lower than what was obtained by Bakare *et al.*, 2010 (32.34 g/100g), Hussain, *et al.*, 2009 (56.02 g/100g) and lower than what was obtained by Bangash *et al.*, (2011).

However, the carbohydrate content of *S. occidentalis* Linn is 9.307 g/100g which is higher than that obtained in *S. siamea* (Alli Smith, 2009) but similar to the amount obtained by Odhav *et al.*, 2007(9.37 g/100g) and lower than found in *S. alata*, *S. hirstuta* and *S. Obtusifolia* (53.70, 42.0 and 40g /100g) by Essiet *et al.*, 2013.

Though vegetables are never good sources of carbohydrate, these plants have appreciable quantities which ranged from 5.69 – 10.38 g/100g and could serve as good sources since the RDA value for this category of food is 7.0 – 100 mg/g (Ijeomah *et al.*, 2012).

5.5.7 Calorific value

Table 4.12 depicts the calorific values of the analyzed plants in this work. These values are all between 93.71 and 163.90 Kcal/100g of dry sample. These levels are generally low due to low crude lipid, crude protein and carbohydrate but high moisture (Ejoh *et al.*, 2007). For *C. olitorious* Linn, the amount is 93.71 Kcal/100g is lower than what was ought to be

obtained elsewhere (Ndlovu and Afolayan, 2008 and Yekeen *et al.*, 2013). But for *C. tridens* Linn, the energy content found in this work is 100.20 Kcal/100g which is lower what was obtained elsewhere (Asibey-Berko and Tayie, 1999) who obtained 283.1 Kcal/100 for the same plant. In addition to that, for *V. amygdalina* Del the energy value is 114.07 Kcal/100g which is also lower than what was obtained by Ejoh *et al.*, 2007 in bitter and non bitter species of *Vernonia* (365.05 – 392.67 Kcal/100g), Igile *et al.*, 844.49 KJ/100g and Owen, 2011 (527.8 Kcal/Kg).

Moreover, the calorific value for *C. sesamoides* is 121.03 Kcal/100g and this value is low and comparable to the amounts obtained in the plants analyzed in this work. Also the value for *M. charantia* Linn is 163.90kcal/100g which is lower than what was obtained by Hussain *et al.*, 2009 (370 Kcal/100g) and Bakare *et al.*, 2010 (213 Kcal/100g) but higher than found in the fresh leaves of *M. balsamina* (52.0 Kcal/100g) Odhav, 2007. For *S. occidentalis* Linn the energy value is 97.48 Kcal/100g which is higher than the amount in the fresh leaves of the same plant (84.0/100g) (Odhav *et al.*, 2007).

The calorific values obtained for the vegetables in this work and found in the literature are generally low compared to 248.8 – 351.30kcal/100g in some Nigerian vegetables (Isong *et al.*, 1999, Antia *et al.*, 2006 and Akubugwo *et al.*, 2007).

5.6 Vitamin content of *C. olitorious* L., *C. tridens* L., *V. amygdalina* D., *C. sesamoides* E, *M. charantia* Linn and *S. occidentalis* L.

Vitamins are potent organic compounds found in certain foods and perform specific and vital functions in body chemistry (Nkafamiya *et al.*, 2010, Asensi-Fabado and Munne-

Bosch, 2010). Human body needs them for growth, function, energy, tissue repair and waste removal. Except for a few, they cannot be manufactured or synthesized by organisms and their absence results in specific deficiency diseases. One of the excellent sources of vitamins has been found to be green leafy vegetables in addition to minerals and dietary fiber and water to aid digestion (Ijeomah *et al.*, 2012).

5.6.1 Vitamin A (Retinol)

The amount of vitamin A determined in *C. olerifolius* Linn in this work is 2.18 mg/100g which is an important parameter in determining food quality of vegetables. In fact it is one of the important reasons why the plant is widely consumed as a vegetable among rural communities in most parts of Africa (Adebayo, 2010) and it is known to contain a high level of protein, vitamin C, iron and folate which are good in the prevention of anemia while that of *C. tridens* Linn is 345.84 mg/100g. For *V. amygdalina* Linn the vitamin A content is 379.46 mg/100g which is higher than the amount obtained by Atanghwo *et al.*, 2009 (1.05 mg/100g) and Eyong *et al.*, 2011 (0.0927 and 0.0645 mg/100g) in the roots and stems stem bark extract. The vitamin A content of *C. sesamoides* Linn in this work is also 327.84 mg/100g while that of *M. charantia* Linn is 0.06 mg/100g which is very much lower than preceding plants. Likewise for *S. occidentalis* Linn, the vitamin A content is 310.28 mg/100g which is very much higher than that found in the seeds (0.06408 mg/100g) of *S. obtusifolia* (Ingweye *et al.*, 2010).

5.6.2 Vitamin B₁ (Thiamin)

Vitamin B₁ content of *C. olerifolius* Linn determined in this work is 15.4 mg/100g while that of *C. tridens* Linn is 0.68 mg/100g. These values are good enough to indicate high

vitamin B₁ content because plants are generally known to contain minute amounts of vitamins. Also the vitamin B₁ content of *V. amygdalina* Del is 17.03 mg/100g which is higher than what was obtained in the root and stem bark extract of the plant (0.37 and 0.50 mg/100g) by Adegunwa *et.al*, 2011 and that of *C. sesamoides* Endl is 0.89 mg/100g. For *M. charantia* Linn the vitamin B₁ is 0.72 mg/100g and that of *S. occidentalis* Linn is 1.40 mg/100g.

5.6.3 Vitamin B₂ (Riboflavin)

The vitamin B₂ content of *C. olitorious* Linn as determined in this work is 0.73 mg/100g while that of *C. tridens* Linn is 1.07 mg/100g. Also the amount found in *Vernonia amygdalina* Del is 1.98 mg/100g and that of *C. sesamoides* Endl is 1.14 mg/100g. However that of *M. charantia* Linn is 0.08mg/100g while that of *S. occidentalis* Linn is 0.38 mg/100g.

5.6.4 Vitamin B₃ (Niacin)

The vitamin B₃ content of *C. olitorious* Linn is 7.92 mg/100g which is very much higher than what is contained in *C. tridens* Linn (0.72 mg/100g). The amount in *V. amygdalina* Del is 2.46 mg/100g which is very much higher than the amounts in *C. sesmoides* Endl. However, the vitamin B₃ content of *M. charantia* Linn is higher than the amount obtained by Islam *et al.*, 2011 (0.04 mg/100g) and significantly lower than the content of all the analyzed plants in this work (0.5 mg/100g) because even that found in of *S. occidentalis* Linn is 2.13 mg/100g).

5.6.5 Vitamin C (Ascorbic acid)

The vitamin C content of *C. olerifolius* Linn determined in this work is 94.95 mg/100g which is higher than values obtained by Ogunlesi *et al.*, 2010 (44.74 mg/100g) and Adebayo *et al.*, 2010 (27.13 mg/100g). This is an indication that when the plant is consumed would provide more than a daily dietary amount of vitamin C in a 100 g sample of the food for an average man or for a woman during pregnancy and lactation (60 mg) (Food and Nutrition Board, 1969). For *C. tridens* Linn the content is 178.33 mg/100g and indicates that the plant is containing higher values of vitamin C when compared to the more exotic vegetables like amaranths (25.40 mg/100g) Akubugwo *et al.*, 2007, spinach (130.0 mg/g) Tressler *et al.*, 1936 and lettuce and significantly similar to different varieties of cabbage as obtained Mariga *et al.*, 2012 (57.9 – 234.2 mg/100g).

Moreso, vitamin C content of *V. amygdalina* Del in this work is 200.49 mg/100g which is also higher than the amounts determined elsewhere by Ogunlese *et al.*, 2010 (125 mg/100g) and Adegunwa *et al.*, 2011 (49.3 mg/100g) in the same plant. Similar comparative analysis conducted by Atangwo *et al.*, 2009 on *V. amygdalina* Del, *Azadirachta indica* and *Gongronema latifolium* and found values of vitamin C similar to this work (202.40 mg/100g). But lower values of vitamin C were found in the stem and root bark extracts of *V. amygdalina* Del by Eyong *et al.*, 2011 (4.90 and 10.3 mg/100g). For *C. sesamoides* Endl, the vitamin content is 255.61 mg/100g which is the highest amount found in the plants analyzed this work. However moderate amount of the vitamin was determined in *M. charantia* Linn (53.17 mg/100g) which is significantly similar to the value obtained by Ogunlesi *et al.*, 2009 (40.20 mg/100g), Islam *et al.*, 2011 (50.0 mg/100g) but lower the amount obtained by Bakare *et.al*, 2010 (66000 ppm) and lower than amount obtained Ullah

et al., 2011 (11.77 %). But low amount of the vitamin was found in the *S. occidentalis* Linn sample analyzed in this work (17.63 mg/100g). This value is still higher than the value found in *S. obtusifolia* seeds (11.88 mg/100g).

5.7.0 Antinutritional factors of *Corchorus olitorius* Linn, *Corchorus tridens* Linn, *Vernonia Amygdalina* Del, *Ceratotheca sesamoides* Endl, *Momordica charantia* Linn and *Senna occidentalis* Linn

These anti-nutritional factors include phytates, oxalates, tannins and hydrogen cyanide unless destroyed by heat or some other suitable treatment can exert adverse physiological effects when ingested by man and animals (Liener, 1980). Oxalates and phytates binds trace elements and macro elements such zinc, calcium, magnesium and iron in the gastrointestinal tract and making these dietary minerals unavailable for absorption and utilization by the body while tannins makes proteins unavailable to the body and cyanides are poisonous to man and animal (Kala and Mohan, 2012).

5.7.1 Phytates

The amount of phytate in *C. olitorius* Linn is 2.18 g/ 100g and is the highest among the plants analyzed in this work. This amount is higher than the amount obtained Ndlovu and Afolayan, 2008 (11.71 g/100Kg) and Agbaire, 2012 (9.39 µg/g) while in *C. tridens* Linn the amount is 0.327 g/100g which is lower than the amount obtained by Asibey- Berko and Tayie, 1999 (2.7 g/100g). For *V. amygdalina* Del the amount of phytate is 1.654 g/100g which is higher than that obtained by Agbaire, 2012 (8.5 µg/g), Shokanbi *et al.*, 2011 (1.13 g/100g) and Yakubu *et al.*, 2012 (169.0 mg/100g). The values of the factor that is higher than those above, was found by Oboh, 2006 (1015.4 mg/100g). Likewise, in *C. sesamoides* Endl the phytate content is 0.36 g/100g, *M. charantia* Linn (0.21 g/100g) and *S.*

occidentalis Linn, 0.27 g/100g. The value obtained for *S. occidentalis* Linn in this work is higher than the one obtained by Ingweye *et al.*, 2010 (240 mg/100g) in *Senna obtusifolia* seeds and phytate was also qualitatively detected in the leaves *senna siamea* (AlliSmith, 2009).

5.7.2 Oxalates

The amount of oxalate determined in the leaves of *C. olitorious* Linn is 15.4 mg/100g which is very much lower than that obtained by Agbaire, 2012 (0.80 µg/g); *C. tridens* Linn, 1.26 mg/100g, *V. amygdalina* Del, 0.26 mg/100g lower than that obtained by Agbaire, 2012 (2.30 µg/g); *C. sesamoides* Endl, 2.41mg/100g, *M. charantia* Linn, 2.41mg/100g and *S. occidentalis* Linn, 1.98 mg/100g.

5.7.3 Tannins

Tannin content of *C. olitorious* Linn determined in this work is 0.73 mg/100g which is very much lower than that obtained by Agbaire, 2012 (0.12 µg/g). For *C. tridens* Linn the tannin content is 0.59 mg/100g. For *V. amygdalina* Del is 0.41 mg/100g which is very much lower than that obtained by Agbaire, 2012, *C. sesamoides* Endl, 1.18 mg/100g, *M. charantia* Linn, 0.87 mg/100g and *S. occidentalis* Linn is 0.63 mg/100g.

5.6.4 Hydrogen cyanide

Amount of hydrogen cyanide determined in this work is 7.92 mg/100g and is lower than that determined by Agbaire, 2012 is lower than that of *Corchorus tridens* Linn is 1.11 mg/100g, *Vernonia amygdalina* Del, 0.54 mg/100g lower than that obtained Agbaire, 2012,

Ceratotheca sesamoides Endl 0.79 mg/100g, *Mormodica charantia* Linn, 0.58 mg/100g
and 0.23 mg/100g.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Many local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials. Though several works reporting compositional evaluation and functional properties of various types of edible wild plants in use abound in the literature, much still need to be done. Many workers have reported some compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries (Jimoh *et al.*, 2011). Vegetables have also been found to contain valuable food ingredients which can be used as energy sources, body building, regulatory and protective materials. They are valued mainly for their higher carbohydrate, vitamin and mineral contents. Vegetables may be edible roots, stem, leaves, fruits or seeds. Each group contributes to diet in its own way (Robinson, 1990). Vegetables also act as buffering agents for acidic substances produced during the digestion process (Fayeman, 1999). Vegetables contain both essential and toxic elements over a wide range of concentrations (Ajewole, 1999). The concentration of these elements is a function of the concentration in the soil on which the vegetable is planted (Adeniyi *et al.*, 2011).

In this work, *C. olitorious* Linn, *C. tridens* Linn, *V. amygdalina* Del, *C. sesamoides* Endl, *M. charantia* Linn and *S. occidentalis* Linn were analyzed for their major, minor, trace and ultra-trace elements, proximate composition, vitamins and anti-nutritional factors. Bioavailabilities of some of the metals were ascertained by calculating the ratios of concentration of anti-nutritionals to metal concentrations in various organs of the plants (Table 4.11) and plotting the concentration of the metals against the various parts of the

analyzed plants as shown in figures 5.1 – 5.34. Though the concentration of major elements including K and Ca known to be in high concentrations in tissues of plants because of the roles they play as components of simple salts and complexes performing various functions (Yalwa, 2002), their concentrations in the analyzed plants samples (10040 – 67270 ppm and 2802 ppm) are relatively higher than normal concentrations found in most plants (10 – 100 ppm and 50 – 60 ppm) (Allen *et al.*, 1974).

For microelements such as Ba, Fe, Mn, Zn, Rb and Co, their concentrations are also high and in fact some values of the elements such as Fe (145 – 2215 ppm), 5.0 – 583.0 ppm (Ba), 11.3 – 326.78 ppm (Mn), 13.0 – 166.30 ppm (Zn), 3.9 – 41.0 ppm (Rb), and 0.32 – 0.76 ppm (Co) may prompt the researcher to declare the analyzed plants as indispensable sources of micronutrients to man and his animals when consumed. This is because the normal concentrations of these metals in plants are Fe (40.0 – 500.0 ppm), Ba (9.2 – 131.9 ppm in Sedge and Nutgrass), Mn (50.0 – 356.0 ppm), Zn (15.0 – 100.0 ppm), Rb (0.2 – 194.0 ppm), and Co (0.1 – 0.60 ppm) and the above values of the metals determined are within normal range.

Moreover, for the ultra-trace elements; Sc, Sm, Th, Br, V, La, Cu, Sb, Lu and Eu, their concentrations are relatively within normal levels. This is evident when concentrations of these elements in this work are compared to normal levels in plants. For example, the range of Sc concentration in this work is 0.03 – 0.95 ppm while normal range in vegetables is 5.00 ppb and 70.0 ppb in grasses, Sm in this work is 0.03 – 0.37ppm while 0.0029 – 0.192 ppm was found by Zhang *et al.*, 2013. Also Th in this work is 0.17 – 7.0 ppm while normal range in plants is 2.0×10^{-3} – 3.6×10^{-3} Ci/g, V in this work is 0.7 – 10.7 ppm while normal

range in plants is 96.0 – 112.0 ppm which signifies low concentration of the element in the analyzed plant. For lanthanum however, the range is 0.034 – 6.80 ppm while normal range in plants is generally low (Wang *et al.*, 1997). In addition to that, the concentration range of copper in this work is 61.95 – 74.52 ppm which is higher than normal concentrations (10.0ppm) of the element in plants while antimony's concentration found in the plants analyzed in this work is 0.29 - 0.42 ppm which is within normal background Sb concentration (0.29 – 50.0 ppm) in vascular plants. Though found only in *C. sesamoides* Endl leaves, Lu concentration is at 0.023 ppm which conforms to the amounts found in plants because it has been estimated that vegetables have as little as 10 ppt in their dry weight(Tyler, 2004).

Moreover, the concentration of Al in the plants analyzed is relatively and ranged from 53.0 – 2262.0 ppm and it was only in the stems of *C. sesamoides* Endl that the metal was at BDL level. Though the metal (Al) concentration in the analyzed plants is high only minute quantities of it is needed by the plants for photosynthesis and minute quantities may be poisonous to the plants. The toxicity of Al is usually manifested as inhibition of root and shoots growth (Eisenmenger, 1935 and Mosser-Pietraszewska, 2001).

In addition to that, appendix ii – vii shows the comparison of the average concentrations of some of the determined elements in this work, their average normal concentration in plants and their recommended daily allowances which may be useful for assessment of nutritional quality of the plants at a glance.

Since the motive for elemental analysis of green plants, food and feed samples is mainly aimed to investigate nutritional quality or contamination, one of the indices for such is transfer factor (TF). In this work the TF has been categorized as either $TF_{\text{Shoot/Root}}$ ($TF_{S/R}$) or $TF_{\text{Shoot/Leaf}}$ ($TF_{S/L}$) and most plant species have been found to have $TF > 1$. For Al, the $TF_{S/R}$ for *C. olitorious* Linn, *C. tridens* Linn, and *S. occidentalis* Linn which signifies good transfer of the element from roots to shoots of the plants while it is found to be < 1 in *V. amygdalina* Del and *M. charantia* Linn which indicates accumulation of the element in the roots of the said plants. But for $TF_{L/S}$ all values are < 1 except that of *M. charantia* Linn (2.93) signifying retention of the element in the stems.

However, the TF values for Ba, Br, Ca, K, La, Mn, Na, and Rb in the analyzed plants varied like the previous elements discussed. $TF_{S/R}$ values of the elements for all the plants except *C. olitorious* Linn and *Momordica charantia* Linn are > 1 and ranged 1.25 – 2.24 while that of $TF_{L/S}$, in *C. olitorious* Linn and *C. sesamoides* Endl that is < 1 and values for the rest of the analyzed plants is > 1 and ranged from 1.19 – 3.09 as depicted in Table 4.10.

For the proximate analysis of the plants, results have shown that, moisture content which depends on environmental conditions such as humidity, temperature, harvest time, and climate as well as storage conditions is highest in *C. olitorious* Linn (75.53 %) while *M. charantia* Linn has the least (46.90 %). These values correlate well with the fiber content in the plants as found in five medicinal plants of Pakistan (Hussain *et al.*, 2010). Though the moisture contents of the plants analyzed in this work are lower than what was obtained elsewhere in some vegetables (91.6 % in *Talinum triangulare* by Saidu and Jideobi, 2009;

Tindall, 1988), the values could supply the 20 % total water consumption of individuals expected to come from food items (FNB, 2005 and Khan *et al.*, 2013).

The ash content of the analyzed plants ranged 2.48 – 11.95 % which indicates substantial amount of minerals in the plants. These values are either higher than reported values for some plants or lower for some vegetables in Nigeria (Saidu and Jideobi, 2009; Nwauzoma and Dawari, 2013 and Jonathan, 2013). Likewise, the crude protein content of the analyzed plants varied among the analyzed plants. For example, the crude protein content of *C. olitorious* Linn is 3.19 %, *C. tridens* Linn is 7.72 %, *V. amygdalina* Del is 10.64 %, *C. sesamoides* Endl is 6.12 %, *M. charantia* Linn, 19.95% and *S. occidentalis* Linn is 8.35%. Therefore, consumption of these plants could serve as good source of protein and could provide some portion of the recommended daily requirement (RDAs) for adults (63.0 g), women (50.0 g) and children (28.0 g) (Akindahunsi and Salawu, 2005, NNSP, 2011 and Khan *et al.*, 2013). Also the crude lipid content of the analyzed vegetables ranged 3.33 % in *V. amygdalina* Del to 6.67 % in *C. sesamoides* Endl which is considered as a chief source of storage form of energy, essential fatty acids and fat soluble vitamins and precursors of vitamins. These vegetables are good sources of lipids. Lipids are essential fats that play a very important role in the human body (Saidu & Jideobi, 2009) which include help in brain function, joint mobilization and even energy production. They also help the body to absorb fat-soluble vitamins such as vitamins A and E (Osborne & Voogt, 1978). The values obtained are lower than 11.0 % in water spinach leaves, 12.0 % in *S. obtusifolia*, 11.0 % in *A. caudatus* leaves, 28.2 % in *Centilla asiatica* leaves, 29 % in *Bahunian purpurea* leaves, and 60% in *A. hybridus*, but higher than 0.47 % in *A. viridus*, 0.54 % in *Chenopodium murale* and 0.63 % in *Scandex pectenvenaris* leaves (Imran *et al.*, 2007).

Likewise the fiber content of the analyzed plants ranged from 3.5 % in *C. sesamoides* Endl to 10.49 % in *M. charantia* Linn. These values are relatively low when compared with the RDA value of 109.63 mg/g. The plants would be better consumed in combination with other high fiber food to meet the daily fiber requirement. Dietary fiber, though non-nutritive, confer laxative effect in the gastrointestinal tract, thereby shortening transit time of food in the tract, and increasing water-holding capacity of faeces. As a result, the likely incidence of the disorders such as constipation, diverticulitis, irritability bowel syndrome, gall stone and colorectal cancer is prohibited (Leveile, 2003; Anderson *et al.*, 2003; Keys *et al.*, 2006; Shailong and Ugwuona, 2011 and Ijeomah *et al.*, 2012). Also, Dietary fiber reduces the risks of cardiovascular diseases. Reports have shown that increase in fiber consumption might have contributed to the reduction in the incidence of certain diseases such as diabetes, coronary heart disease, colon cancer and various digestive disorders (Badau *et al.*, 2013). Fiber consumption also soften stools and lowers plasma cholesterol level in the body (Pillai and Nair, 2013). Maintenance of internal distension for a normal peristaltic movement of the intestinal tract is a physiological role played by crude fiber in addition to fiber reduces tracolonic pressure which is beneficial in diverticular disease (Belewu *et al.*, 2009). Plants with high fiber are adequate for better rumination and digestion in ruminant animals (NRC, 1978). However, it is reported that when a vegetable has very high fiber content, it may cause intestinal irritation and a decrease of nutrient bioavailability (Pillai and Nair, 2013).

The carbohydrate contents of the analyzed plants in this work are considerably small (5.69 – 10.377 %) when compared to what is obtainable in some other vegetables. For example 61.03 % in *A. caudatus* leaves, 55.67 % in *T. terrestris*, 54.2% in water spinach leaves, and

75.0 % in sweet potato leaves. But higher when compared with *A. viridus*, *C. murale* and *N. officinale* leaves, that is, 4.74, 3.41 and 3.38 and 7.32 %, respectively (Imran *et al.*, 2007). Carbohydrates are principal and indispensable source of energy. The RDA for carbohydrates is 130.0 g (FAO, 1998); while in Pakistan 349 g of carbohydrate intake is reported. Therefore, due to the carbohydrate contents of the analyzed plants, they can be a good food source.

The calorific value of the analyzed plants ranged from 93.71 – 163.90 Kcal/100g and is smaller than what was obtained in some other plants (Isong *et al.*, 1999, Hussain *et al.*, 2010 and Hussain, *et al.*, 2013). The individual values are as follows; 93.71 Kcal/100g for *C. olitorious* Linn, 100.20 Kcal/100g for *C. tridens* Linn, 114.065 Kcal/100g for *V. amygdalina* Del, 121.03 Kcal/100g for *C. sesamoides* Endl, 163.90 Kcal/100g and 97.484 Kcal/100g for *S. occidentalis* Linn. The low calorific values are to be expected as a result of low carbohydrate content of the analyzed plants and this implies that consumption of such plants alone could not meet the calorific needs of individuals.

However, for the anti-nutritional factors evaluated in this work, the levels are generally low and within manageable level when the plants items are subjected certain treatments before consumption. The phytate contents of the plants varied between 0.2705 mg/100g in *S. occidentalis* Linn to 2.175 mg/100g in *C. olitorious* Linn which is higher compared to 8.24mg/100g in *Cassia siamea* leaves (Ngaski, 2006) and 2.13 ± 0.51 mg/100g in *Parkia biglobosa* (McDonald *et al.*, 1995) but lower than 392.23 mg/100g in egg plant and 1214 ± 15.56 mg/100g in *T. terrestris* (Hassan *et al.*, 2007). One of the effects of phytic acid is that, it can bind to mineral elements such as calcium, zinc, manganese, iron and magnesium

to form complexes that are indigestible, thereby decreasing the bioavailability of these elements for absorption. The low phytate content in the leaves of the plants analyzed indicate that the consumption of their leaves will not affect the bioavailability of the elements especially Ca, Fe, Cu, Mn and Zn for absorption, because the [Phytate]/[Metal] molar ratios for all are the plants are below the critical levels of 0.24, 0.4 and 1.5 (Frontela *et al.*, 2008 and Mitchkpe *et al.*, 2008). Hence the leaves are said to exhibit good Ca, Fe, Cu, Mn and bioavailability.

The oxalate content of the analyzed plants ranged from 0.262 mg/100g in *V. amygdalina* Del to 154.125 mg/100g in *C. oltoriosis* Linn while 2.4075 mg/100g is in *C. sesamoides* Endl and *M. charantia* Linn while its levels are 1.26 mg/100g in *C. tridens* Linn and 1.98 mg/100mg in *S. occidentalis* Linn. These values are low compared to some other plants and advantageous because when in large amounts would bind calcium and some other metals to form complexes (e.g Calcium oxalate crystals). These oxalate crystals formed prevents the absorption and utilization of calcium by the body causing diseases such as rickets and osteomalacia (Ladeji *et al.*, 2004 and Umaru *et al.*, 2007). The calcium crystals may also precipitate around the renal tubules thereby causing renal stones. The formation of crystal is said to take place in the digestive tract (Thompson and Yoon, 1984).

In addition to that, the tannin contents of the plants analyzed in this work are also low (0.41 – 1.812 mg/100g) when compared to the contents of some other plants (Umaru *et al.*, 2007) and similar to what is obtainable in *A. hybridus* (Akubugwo *et al.*, 2007). Tannins bind with carbohydrates (Swain, 1965a) and with metal ions (Srikantia, 1976), and more importantly inactivate digestive enzymes (Huisman and van der Poel, 1989). They also damage gut cells (Bernays *et al.*, 1989) and are responsible for astringent taste (Marquardt, 1983) due to

a cross-linking between tannins and proteins and glycoproteins (Goldstein and Swain, 1965).

The hydrogen cyanide content of the analyzed plants is also low (0.2344 – 7.916 mg/100g) when compared with some other reported values in *V. amygdalina* Del, *Eucalyptus globulus*, *O. gratissimum*, *Gnetum Africana* and *A. africanum* leaves (Ifon and Bassir, 1979, Alabi *et al.*, 2005 and Akubugwo *et al.*, 2007). Hydrogen cyanide in plants is an extremely poisonous substance formed by the action of acids on metal cyanides and it has been reported that large doses of it can cause death within minutes (Gettle and Bane, 1938) while smaller doses may result to stiffness of the throat, chest, palpitation and muscles weakness. The result obtained in this work falls within the threshold value (0.51 – 3.5 mg/g) reported for safety (Anhawange, *et al.*, 2009).

The vitamin composition in mg/100g of the analyzed plants is presented in Table 4.14 and it could be seen that the samples contained Vitamin A (Retinol) in the range of 0.06 mg/100g in *M. charantia* Linn to 379.46 mg/100g in *V. amygdalina* Del which showed that the samples were a good source of vitamin A with the exception of *M. charantia* Linn since the RDA for vitamin A is 1.5 mg / 100. Vitamin A is necessary for vision process and also plays a role in skin mucosa, bones, teeth, hair, normal reproductive capabilities and is an important anti-oxidant (FAO 2001). The samples also contained 0.68 – 170.30 mg/100g of vitamin B₁ (Thiamin). The RDA for thiamin is 1.1 mg and the results showed that the samples were a rich source of thiamin. The functions of thiamin in the body are for proper functioning of nervous system and helps release energy from carbohydrates. Riboflavin (Vitamin B₂) is present in the plants in the range of 0.08 – 1.98 mg/100g and functions in

human body as co-enzyme that acts as hydrogen acceptor in amino acid metabolism (FAO, 2001), helps in the release of energy from foods and is essential for healthy eyes, skin, nails and hair. The riboflavin contents were lower than *A. hybridus* L. (Akubugwo *et al.*, 2007), *Sonchitanus eruca* and *Sonchus asper* (Hussain *et al.*, 2010) but could still serve as a good source of the vitamin.

Likewise, the vitamin C (Ascorbic acid) of the analyzed plants ranged from 17.63 mg/100g in *S. occidentalis* Linn leaves to 255.61 mg/100g *C. sesamoides* Endl leaves as presented in Table 4.14. These values are considerably high when compared to some other vegetables and could serve as a good source of vitamin C. The vitamin (C) is necessary for healthy teeth, gums, and bones and is essential for proper functioning of adrenal and thyroid glands. Also, vitamin C (Ascorbic acid) is an anti-oxidant and such acts as a general de-toxicant. The amounts of Vitamin B₃ (Niacin) in the analyzed plants is also relatively high (0.50 – 7.92 mg/100g) and compares favorably well with other vegetables. Niacin or nicotinamide is a coenzyme, which is involved in many biochemical processes including the detoxification of foreign compounds in the body (Njoku & Akumefula, 2007 and Igile *et al.*, 2013).

6.2 Recommendation

From the results obtained on the analysis of *C. olitorious* Linn, *C. tridens* Linn, *V. amygdalina* Del, *C. sesamoides* Endl, *M. charantia* Linn and *S. occidentalis* Linn, it is recommended that;

- i. The above plants can be explored as an alternative food for malnutrition in developing countries.

- ii. Further studies are recommended in order to exploit their food value to promote human health and nutritional quality of the consumers especially in the area of other anti-nutrients that have not been determined (e.g saponins, lectins, saponins, protease inhibitors, goitrogens, gossypol, chlorogenic acid and amylase inhibitors).
- iii. Amino acid profile
- iv. Fatty acid profile
- v. It is also important to determine the composition of these plants that are collected different locations in Nigeria.

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APPENDIX I



Reactor Engineering Section, Centre for Energy Research and Training
Ahmadu Bello University, P.M.B. 1014, Zaria NIGERIA

Our Ref.NIRR-1/JC/10/105

Date: 2011-08-15

The analytical result of instrumental neutron activation analysis of seventeen (17) biological sample for SANI UMAR GWARZO are presented below

The reported uncertainty was calculated mainly from coming statistics and is not the normal standard deviation on replicate analyses.

A SUMMARY OF ANALYTICAL RESULT

ELEMENT	COLL	CSLL	SOLL	CSLS	MCLS	VADS
Mg (ppm)	NA	NA	NA	NA	NA	NA
Al (ppm)	1043±88	2262±188	380±38	BDL	165±14	1215±114
Ca (ppm)	16670±2434	11850±754	18310±2655	16140±2744	5096±759	7028±1040
Ti (ppm)	Na	Na	Na	Na	Na	Na
V (ppm)	1.47±0.31	3.1±0.4	BDL	BDL	BDL	BDL
Cu (ppm)	BDL	BDL	BDL	BDL	BDL	BDL
Mn (ppm)	68±3	92±4	46±2	14.6±0.6	11.3±0.5	13.7±0.6
Dy (ppm)	BDL	BDL	BDL	BDL	BDL	BDL
Na (ppm)	353±20	493±28	319±3	686±4	170±1	80±1
K (ppm)	33870±2370	22650±1586	33690±422	51490±422	51490±309	29210±234
As (ppm)	BDL	BDL	BDL	BDL	BDL	BDL
La (ppm)	2.70±0.16	6.8±0.3	1.23±0.02	0.41±0.04	0.44±0.02	0.40±0.08
Sm (ppm)	BDL	BDL	0.11±0.01	0.06±0.006	0.05±0.01	0.03

U (ppm)	NA	NA	BDL	BDL	BDL	BDL
Sc (ppm)	0.022±0.02	0.62±0.04	0.06±0.01	BDL	0.06±0.01	BDL
Cr (ppm)	BDL	2.9±0.5	BDL	BDL	BDL	BDL
Fe (ppm)	563±52	1598±143	390±55	BDL	213±47	145±41
Co (ppm)	BDL	0.5±0.1	BDL	BDL	0.55±0.12	BDL
Zn (ppm)	37.7±3.3	42±4	60±5	33±5	BDL	2.7±0.5
Br (ppm)	8.04±0.89	5±1	4.0±0.8	BDL	BDL	BDL
Rb (ppm)	14±1	11±1	24±2	26±3	12±2	BDL
Sb (ppm)	BDL	0.40±0.05	0.29±0.05	BDL	BDL	BDL
CS (ppm)	NA	NA	BDL	BDL	BDL215±17	44±12
Ba (ppm)	12.3±2.6	BDL	81±18	BDL	BDL	BDL
Eu (ppm)	BDL	0.03±0.01	BDL	BDL	BDL	BDL
Yb (ppm)	BDL	0.35±0.05	BDL	BDL	BDL	BDL
Lu (ppm)	BDL	0.023±0.005	BDL	BDL	BDL	BDL
Hf (ppm)	BDL	NA	BDL	BDL	BDL	BDL
Ta (ppm)	NA	NA	NA	BDL	BDL	BDL
Th (ppm)	0.95±0.16	4.7±0.7	0.17±0.05	BDL	BDL	BDL

ELEMENT	MCLL	SOLS	CTLL	VADL	COLR	SOLF
Mg(ppm)	NA	NA	NA	NA	NA	NA
Al (ppm)	1013±83	52±5	1453±119	657±56	491±40	53±5
Ca (ppm)	26950±3782	887±1293	14750±2139	13250±1934	5541±820	8800±1293
Ti (ppm)	NA	NA	NA	NA	NA	NA
V (ppm)	1.93±22	DBL	1.9±0.3	BDL	BDL	BDL
Mn (ppm)	41.4±1.7	7.5±0.3	62.3±2.6	76±3	14.1±0.6	13±1
Dy (ppm)	BDL	DBL	BDL	BDL	BDL	BDL
Na (ppm)	835±5	131±2	416±3	414±3	786±2	36.2±0.5
K (ppm)	43090±474	16090±290	43120±517	37710±453	10040±121	15850±127
As(ppm)	BDL	DBL	BDL	BDL	BDL	BDL
La (ppm)	2.47±0.06	0.16±0.02	2.72±0.04	2.39±0.05	0.93±0.04	0.14±0.01
Sm (ppm)	0.37±0.01	BDL	0.39±0.01	0.28±0.01	0.14±0.01	BDL
U (ppm)	BDL	BDL	BDL	BDL	NA	NA
Sc (ppm)	0.33±0.01	BDL	0.33±0.01	0.13±0.01	0.07±0.01	BDL
Cr (ppm)	BDL	BDL	3.48±1.02	BDL	BDL	BDL
Fe (ppm)	1066±79	BDL	1130±73	611±62	283±53	BDL
Co (ppm)	0.76±11	BDL	0.46±0.11	BDL	BDL	BDL
Zn (ppm)	62.3±4.4	BDL	51±4	42.2±3.8	13±3	35±3
Br(ppm)	2.54±0.06	BDL	5±1	6.5±0.7	1.3±0.4	BDL
Rb(ppm)	15±2	BDL	23±2	41±3	BDL	5.5±0.5
Sb (ppm)	0.40±0.06	BDL	BDL	0.42±0.07	B DL	BDL
CS (ppm)	BDL	BDL	BDL	BDL	N A	NA
Ba(ppm)	583±29	BDL	138±19	BDL	BDL	BDL
Eu (ppm)	BDL	BDL	BDL	BDL	BDL	BDL
Yb (ppm)	BDL	BDL	BDL	BDL	BDL	BDL
Lu (ppm)	BDL	BDL	BDL	BDL	BDL	BDL
Hf (ppm)	1.45±.012	BDL	1.9±0.1	BDL	NA	NA
Ta (ppm)	BDL	BDL	BDL	BDL	NA	NA
Th (ppm)	0.75±0.09	BDL	1.1±0.1	0.35±0.06	BDL	BDL

ELEMENT	VADR	COLS	CTLL	SOLR	CTLR
Mg(ppm)	NA	NA	NA	NA	NA
Al (ppm)	44±36	109±19	209±19	128±11	334±29
Ca (ppm)	2802±443	6242±924	12350±1803	4336±659	3788±591
Ti (ppm)	NA	NA	NA	NA	NA
V (ppm)	0.42±0.09	BDL	BDL	BDL	0.7±0.2
Mn (ppm)	11.6±0.5	3.19±0.14	32±1	7.1±0.3	23±1
Dy (ppm)	BDL	BDL	BDL	BDL	BDL
Na (ppm)	731±2	236±1	198±2	278±2	315±18
K (ppm)	4052±122	22950±184	54290±326	7949±119	21110±1457
As(ppm)	BDL	BDL	BDL	1.08±0.04	BDL
La (ppm)	3.20±0.05	0.24±0.02	0.034±0.002	0.74±0.03	1.7±0.1
Sm (ppm)	BDL	BDL	0.056±0.006	0.12±0.01	BDL
U (ppm)	NA	NA	BDL	BDL	NA
Sc (ppm)	0.95±0.07	BDL	0.043±0.008	0.03±0.01	0.11±0.01
Cr (ppm)	BDL	BDL	BDL	BDL	BDL
Fe (ppm)	197±36	BDL	BDL	2215±89	376±45
Co (ppm)	BDL	BDL	0.36±0.12	0.41±0.09	BDL
Zn (ppm)	BDL	BDL	22±3	82±5	14±3
Br(ppm)	2.7±0.4	1.3±0.3	3.5±0.6	BDL	BDL
Rb(ppm)	4.9±0.8	6.6±0.8	20±3	8.4±1.7	3.9±0.8
Sb (ppm)	BDL	BDL	BDL	BDL	BDL
CS (ppm)	NA	NA	BDL	BDL	BDL
Ba(ppm)	5.4±1.5	12±2	111±18	BDL	BDL
Eu (ppm)	0.031±0.008	BDL	BDL	BDL	BDL
Yb (ppm)	BDL	BDL	BDL	BDL	BDL
Lu (ppm)	BDL	BDL	BDL	BDL	BDL
Hf (ppm)	NA	NA	BDL	BDL	NA
Ta (ppm)	NA	NA	BDL	BDL	NA
Th (ppm)	0.41±0.06	BDL	BDL	BDL	7±1

BDL: Below Detection Limit NA:- Not Analyzed

Please contact the undersigned for further clarification



Dr. G. I. Balogun
(Head of Section and Reactor Manager)

APPENDIX II

Comparison of the average concentration of some of the elements determined in *Corchorus olitorious* Linn, their average concentration in plants and their recommended daily allowance (RDA) in ppm unless otherwise stated.

Concentration (ppm)			
Element	Concentration in the analyzed samples	Normal concentration in plants	Recommended daily allowance (RDA)
Al	581.00	200.00	N.A
Ba	12.15	0.50-40.00	N.A
Br	3.55	2.83	N.A
Ca	9484.33	55.00	1000.00mg
Co	BDL	0.40	0.03mg
Cr	BDL	1.00-100.00	40.00µg
Cu	BDL	13.75	2.00mg
Fe	423.00	275.00	10.00mg
K	1046.00	55.00	3500
La	1.29	<10.00	N.A
Mn	39.95	203.00	5.00mg
Na	458.33	N.A	1.50g
Rb	10.30	47.78	N.A
Sc	0.145	N.A	N.A.
Th	0.95	N.A	N.A
V	1.47	104.50	1.80mg
Zn	25.35	57.50	15.0mg

N.A=Not available; BDL= Below detection limit

Sources: United State Food and Nutrition's Recommended Daily Dietary Allowance, revised 1973, The value of Food, 1979 and the Cambridge World History of Food, 2000.

APPENDIX III

Comparison of the average concentration of some of the elements determined in *Corchorus tridens* Linn, their average concentration in plants and their recommended daily allowance (RDA) in ppm unless otherwise stated.

Concentration in ppm			
Element	Concentration in the analyzed samples	Normal concentration in plants	Recommended daily allowance (RDA)
Al	665.33	200.00	N.A
Ba	124.50	0.50-40.00	N.A
Br	4.25	2.83	N.A
Ca	10296.00	55.00	1000.00mg
Co	0.41	0.40	0.03mg
Cr	3.48	1.00-100.00	40.00µg
Cu	BDL	13.75	2.00mg
Fe	753.00	275.00	10.00mg
K	39506.67	55.00	3500
La	1.48	<10.00	N.A
Mn	39.10	203.00	5.00mg
Na	309.67	N.A	1.50g
Rb	15.63	47.78	N.A
Sc	0.161	N.A	N.A.
Th	4.05	N.A	N.A
V	1.30	104.50	1.80mg
Zn	29.00	57.50	15.0mg

N.A=Not available; BDL= Below detection limit

Sources: United State Food and Nutrition's Recommended Daily Dietary Allowance, revised 1973, The value of Food, 1979 and the Cambridge World History of Food, 2000.

APPENDIX IV

Comparison of the average concentration of some of the elements determined in *Vernonia amygdalina* Del, their average concentration in plants and their recommended daily allowance (RDA) in ppm unless otherwise stated.

Concentration (ppm)			
Element	Concentration in the analyzed samples	Normal concentration in plants	Recommended daily allowance (RDA)
Al	770.67	200.00	N.A
Ba	24.70	0.50-40.00	N.A
Br	3.97	2.83	N.A
Ca	7693.33	55.00	1000.00mg
Co	BDL	0.40	0.03mg
Cr	BDL	1.00-100.00	40.00µg
Cu	BDL	13.75	2.00mg
Fe	317.67	275.00	10.00mg
K	14890.67	55.00	3500
La	1.997	<10.00	N.A
Mn	68.57	203.00	5.00mg
Na	408.33	N.A	1.50g
Rb	22.95	47.78	N.A
Sc	0.54	N.A	N.A.
Th	0.38	N.A	N.A
V	0.42	104.50	1.80mg
Zn	42.2	57.50	15.0mg

N.A=Not available; BDL= Below detection limit

Sources: United State Food and Nutrition's Recommended Daily Dietary Allowance, revised 1973, The value of Food, 1979 and the Cambridge World History of Food, 2000.

APPENDIX V

Comparison of the average concentration of some of the elements determined in *Ceratotheca sesamoides* Endl, their average concentration in plants and their recommended daily allowance (RDA) in ppm unless otherwise stated.

Concentration (ppm)			
Element	Concentration in the analyzed samples	Normal concentration in plants	Recommended daily allowance (RDA)
Al	1914.50	200.00	N.A
Ba	193.20	0.50-40.00	N.A
Br	5.00	2.83	N.A
Ca	13973.33	55.00	1000.00mg
Co	0.50	0.40	0.03mg
Cr	33.99	1.00-100.00	40.00µg
Cu	61.95	13.75	2.00mg
Fe	1662.50	275.00	10.00mg
K	25340.00	55.00	3500
La	3.81	<10.00	N.A
Mn	133.01	203.00	5.00mg
Na	589.50	N.A	1.50g
Rb	18.50	47.78	N.A
Sc	0.62	N.A	N.A.
Th	4.70	N.A	N.A
V	55.21	104.50	1.80mg
Zn	58.73	57.50	15.0mg

N.A=Not available; BDL= Below detection limit

Sources: United State Food and Nutrition's Recommended Daily Dietary Allowance, revised 1973, The value of Food, 1979 and the Cambridge World History of Food, 2000.

APPENDIX VI

Comparison of the average concentration of some of the elements determined in *Momordica charantia* Linn, their average concentration in plants and their recommended daily allowance (RDA) in ppm unless otherwise stated.

Concentration (ppm)			
Element	Concentration in the analyzed samples	Normal concentration in plants	Recommended daily allowance (RDA)
Al	508.00	200.00	N.A
Ba	306.67	0.50-40.00	N.A
Br	2.54	2.83	N.A
Ca	12247.67	55.00	1000.00mg
Co	0.655	0.40	0.03mg
Cr	66.83	1.00-100.00	40.00µg
Cu	74.52	13.75	2.00mg
Fe	956.33	275.00	10.00mg
K	53950.00	55.00	3500
La	1.455	<10.00	N.A
Mn	126.49	203.00	5.00mg
Na	502.50	N.A	1.50g
Rb	13.50	47.78	N.A
Sc	0.195	N.A	N.A.
Th	0.75	N.A	N.A
V	41.13	104.50	1.80mg
Zn	70.20	57.50	15.0mg

N.A=Not available; BDL= Below detection limit

Sources: United State Food and Nutrition's Recommended Daily Dietary Allowance, revised 1973, The value of Food, 1979 and the Cambridge World History of Food, 2000.

APPENDIX VII

Comparison of the average concentration of some of the elements determined in *Senna occidentalis* Linn, their average concentration in plants and their recommended daily allowance (RDA) in ppm unless otherwise stated.

Concentration (ppm)			
Element	Concentration in the analyzed samples	Normal concentration in plants	Recommended daily allowance (RDA)
Al	153.25	200.00	N.A
Ba	81.00	0.50-40.00	N.A
Br	4.00	2.83	N.A
Ca	10078.25	55.00	1000.00mg
Co	0.41	0.40	0.03mg
Cr	BDL	1.00-100.00	40.00µg
Cu	BDL	13.75	2.00mg
Fe	1302.50	275.00	10.00mg
K	18394.75	55.00	3500
La	0.57	<10.00	N.A
Mn	18.40	203.00	5.00mg
Na	191.05	N.A	1.50g
Rb	12.63	47.78	N.A
Sc	0.045	N.A	N.A.
Th	0.17	N.A	N.A
V	BDL	104.50	1.80mg
Zn	59.00	57.50	15.0mg

N.A=Not available; BDL= Below detection limit

Sources: United State Food and Nutrition's Recommended Daily Dietary Allowance, revised 1973, The value of Food, 1979 and the Cambridge World History of Food, 2000.