

**ADDIS ABABA UNIVERSITY  
SCHOOL OF GRADUATE STUDIES**



**DETERMINATION OF ESSENTIAL, NON-ESSENTIAL AND TOXIC  
METALS IN *CROTON MACROSTACHYUS* LEAVES AND ITS INFUSION  
(A TRADITIONAL MEDICINAL PLANT IN ETHIOPIA)**

**BY**

**AMARE AREGAHEGN**

**JULY, 2010**

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IN CHEMISTRY**

**BY**

**AMARE AREGAHEGN**

**ADVISOR: PROF. B. S. CHANDRAVANSHI**

**JULY, 2010**

## **Dedication**

To My Mother Worke Degefu, My uncle Wondossen Beyene,

My Sisters Buziye Belachew, Yeshimebet Belachew and

My Friend Fikre Shiferaw.

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## **LIST OF ACRONYMS AND ABBREVIATIONS**

FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agricultural Organization
WHO	World Health Organization
TM	Traditional Medicine
ANOVA	Analysis of Variance
ND	Not Detected
RSD	Relative Standard Deviation
SNNPRS	South Nations Nationalities and Peoples Regional State

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**Abstract**

The concentration of nine essential metals (Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr, Ni) and two non-essential and toxic metals (Cd, Pb) were determined in leaves of the same species of *Croton macrostachyus* collected from four different areas (Akaki, Abomsa, Bonga and Dilla) and infusions of leaves collected from Akaki using flame atomic absorption spectrometry (FAAS). An optimized digestion procedure was selected based upon less reagent consumption, digestion time and mass of sample using 2 mL of HNO<sub>3</sub> and 2 mL of HClO<sub>4</sub> with 2:30 hours total time at temperature round 270 °C for digestion of 0.5 g of powder sample while 4 mL of HNO<sub>3</sub> and 1 mL of HClO<sub>4</sub> with 2:30 hours total time for 25 mL infusion evaporated up to 3 mL. The validity of the optimized procedure was evaluated by the analysis of spiked samples whose recovery was in the range 92-103% for the Croton leaves powder and 94-105% for the infusion samples. The mean concentration range of each metal in Croton leaves powder samples were Ca (1202–7040 µg/g), Mg (271–2961 µg/g), Fe (169–581 µg/g), Mn (157–1776 µg/g), Zn (19.7–60.5 µg/g), Cu (6.31–18.6 µg/g), Co (19.7–34.5 µg/g), Cr (21.3–87.5 µg/g), Ni (2.65–26.2 µg/g), Cd (1.08 – 0.75 µg/g) and Pb (10.5–21.9 µg/g) for the Croton leave powders and Ca (467–1087 µg/g), Mg (13.9–52.6 µg/g), Fe (3.43–6.53 µg/g), Mn (2.17–3.42 µg/g), Zn (2.24–9.51 µg/g), Cu (2.17–2.54 µg/g), Co (2.15–2.83 µg/g), Cr (2.61–13.44 µg/g), Ni (2.17–2.80 µg/g), Cd (0.207 µg/g) and Pb (0.371–2.07 µg/g) for the infusion samples. The Croton leave powders contained more amounts of metals than the infusions and it was rich in Ca to a largest extent followed by Mg and Fe for Akaki and Abomsa and for Bonga and Dilla sites, respectively. Fe concentration was determined to be the highest followed by Cr and Zn out of the trace microelements Croton leaves powder taken from Akaki and Abomsa whereas Mn concentration was the second next to Ca for Bonga and Dilla sites. The metals were observed to leach in to the infusions at a rate highest for Ca (61.5%) in the 24 h infusion and lowest for Fe (1%) in the 3 h infusion. The concentrations of metals in the Ethiopian *Croton macorostachyus* are comparable to values for other medicinal plants reported in other parts of the world. Poor correlation was observed between Mn as well as

Co with almost all metals Croton leave powder samples and good correlation between all metals in Croton leaves infusion was observed.

***Key Words:* Croton macrostachyus, Croton leaves powder, Croton leaves infusion, Essential metals, Non-essential and Toxic metals, FAAS.**

## 1. INTRODUCTION

The use of plants as treatment of diseases and food dates beyond recorded history perhaps as old as the history of mankind. Old civilisations in China, India, Egypt and Greece had a rich knowledge of its utility and expertise in using many types of plants. It has been estimated that more than 80% of the world's population utilizes plants as their primary source of medicinal agents, largely due to the high cost of Western pharmaceuticals, but also because the traditional medicines are generally more acceptable from a cultural and spiritual perspective. Even in the Western world, the use of herbal medicines is steadily growing with approximately 40% of the population reporting use of herbs to treat medical illness [1, 2].

It is known that many countries in Africa, Asia and Latin America use traditional medicine (TM) to meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in the industrialized countries. In China, for example, traditional herbal preparations account for 30-50% of the total medicinal consumption. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home [3].

Over a comparatively short period of time, modern medicine has developed powerful methodologies for proving efficacy, ensuring quality, standardizing good manufacturing practices, testing for safety, and conducting post-marketing surveillance for adverse effects. Many, but not all, traditional medicines have an inadequate evidence base when measured by these standards. During last century, the use of medicinal plants declined with the development of synthetic drugs, especially in developed countries. Notwithstanding, the use of easily accessible and low-cost medicinal herbs continued, co-existing with modern medicine. Lately, there has been a revival of social interest in the use of herbal products because of their observed and proven efficacy and being free from some toxic effects associated with synthetic drugs. Nowadays, plant materials are widely used throughout developed and developing countries as home remedies, nutritional supplements or as raw materials for the pharmaceutical industry, representing a substantial proportion of the global drug market. For many millions of people,

often living in rural areas of developing countries, herbal medicines, traditional treatments, and traditional practitioners are the main – sometimes the only – source of health care. This is care that is close to homes, accessible, and affordable. In some systems of traditional medicine, such as traditional Chinese medicine and the Ayurveda system historically rooted in India, traditional practices are supported by wisdom and experience acquired over centuries.

Many of the plants species used for this purpose have been found to contain therapeutic substances which can be extracted and used in preparation of drugs, but the plant itself can also be used either directly or as an extract for medication, a practice that is particularly popular in developing countries. Medicinal herbs and their preparations (hot and cold infusions, decoction, and tinctures) are widely used by human beings all over the world [4, 5].

## **1.2. The Traditional Medicine in Ethiopia**

### **1.1.1. History of Traditional Medicine in Ethiopia**

Ethiopia has a long history of traditional medicine and has developed ways to combat disease through it. The ways are also as diverse as the different cultures, language and belief. In Ethiopia up to 80% of the population uses traditional medicine due to the cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine and difficult access to modern health facilities. Healing in Ethiopian traditional medicine is not only concerned with curing of diseases but also with the protection and promotion of human physical, spiritual, social, mental and material wellbeing. It is widely believed in Ethiopia that the skill of traditional health practitioners is 'given by God' and knowledge on traditional medicines is passed orally from father to a favorite child, usually a son or is acquired by some spiritual procedures [3]. Traditional healing knowledge is guarded by certain families or social groups. Healers obtain their drugs mainly from natural substances and in descending order of frequency these constitute plants, animals and minerals. Drugs are prepared in various dosage forms including liquids, ointments, powders and pills. Drugs are also prescribed in a nonformulated form and additives are usually incorporated and more than one drug is used in a single dosage form. Drugs were administered using different routes, the main ones being, topical, oral and respiratory. When side

effects became severe, antidotes were claimed to be used. The healers imposed restriction when certain types of drugs were taken by patients. Drugs are stored usually in containers such as bottles, papers, pieces of cloth, leaves and horns, and were kept anywhere at home [7].

### **1.1.2. Uses of Traditional Medicines in Ethiopia**

In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of the cultural beliefs and attitudes [8]. Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief and treating skin infections. Antimicrobial and wound healing plants are among some of the major medicinal plants that are commonly available in markets [9]. Despite its significant contribution to society, TM has received very little attention in modern research and development and less effort has been exerted to upgrade the traditional health practices in the country. But, the long history of use of medicinal plants in Ethiopia and its huge biotic riches can be of paramount importance in future research and drug discovery [10]. Even today, this huge traditional knowledge of medicinal plants is playing an important role in the development of new drugs. An example of drugs discovered based on information derived from an ethnobotanical investigation is aspirin from *Filipendula ulmar*, morphine from *Papaver somniferum*, ephedrin from *Ephedra sinica* to name a few [11].

### **1.1.3. Policy Development in Connection to TM in Ethiopia**

Formal recognition to TM in Ethiopia was given in 1942 (Proc. 27) where the legality of the practice is acknowledged as long as it does not have negative impact on health. This was reaffirmed in the 1943 and 1948 (Proc. 100) Medical Registration Proclamations. Articles in the Ethiopian Penal Code (512/1957) and the Civil Code (8/1967) provide guidelines for the practice of traditional medicine. But they did not stipulate any requirement for registration. Registration and licensing was introduced in 1950. During the 1970s and 1980s the country's health policy emphasized disease prevention and health service development in the rural areas. It was followed by official attention to the promotion and development of traditional medicine, particularly after the adoption of the Primary Health Care Strategy in 1978. In November 1979, the Office for the

Coordination of Traditional Medicine was established. It conducted chemical assays and biomedical studies of some herbal medicines and a total of 6,000 traditional practitioners were registered and monograph describing 260 medicinal plants was prepared. Meetings and workshops were organized that brought together traditional and modern medical practitioners. In different areas of the country healers also formed their own professional associations. However, these lacked guidance, funds and personnel to help them move forward.

The National Policy of Traditional Medicine under the current Federal Democratic Republic of Ethiopia was issued as part of the Health, Drug, and Science and Technology Policy issued in 1993. Traditional medicine is placed as one of the eight priorities of the current Health Policy. It was reported that due attention shall be given to the development of the beneficial aspects of traditional medicine including related research and its gradual integration into Modern Medicine. The general strategies adopted include identifying and encouraging the utilization of its beneficial components, coordinating and encouraging research including its linkage with modern medicine and developing appropriate regulation and registration of practitioners. The 10 targets of the drug policy include conducting coordinated research on traditional medicines and striving for development into pharmaceutical drugs. The same document outlines general strategies for strengthening the health sector through research and development, creating favorable conditions for the development of safe and effective drugs and involving private providers. Currently there is no registered traditional practitioner and way of registration in the Federal Ministry of Health though herbal medicines are sold on the streets with medical claims. No regulatory requirements exist for the manufacturing or safety assessment of traditional medicines and herbal medicines are not included in the essential medicines list. There is neither a post market surveillance system, a restriction on the sale of herbal medicines nor a guideline for clinical trials using traditional medicines.

In 1986, over 6000 practitioners of traditional medicine were registered with the Ethiopian Ministry of Health. At present according to the Association Chairman, the Association has a membership of 9000 healers, vendors and collectors. This includes not all parts of the country, but only a few areas. If all traditional medicine practitioners, vendors and collectors are

registered in the country under the association, it is expected to be more than 80,000 in number [11].

In recent times the Ministry of Health has been making an effort to integrate traditional medicine into the general network of health services, particularly since the skills of certain healers are known to be effective. The Ethiopian flora is estimated to contain between 6500 and 7000 species of higher plants of which about 12% are endemic [10]. Of the many medicinal plant in Ethiopia, *Croton macrostachyus* is the most common.

## **1.2. *Croton macrostachyus***

### **1.2.1. Botanical Aspect of *Croton macrostachyus***

*Croton macrostachyus* Hochst. ex Del. is commonly known as rushfoil or broad-leaved Croton (English), Bisana (Amharic), Makanissa, Bakkanissa, Badessa, Alaleh, Dogoma (Oromo), Wusha, Masincho (Sidama) [12], Bisana (Shinasha), Islami, Tambuk and Tambush (Tigrigna) [13]. It belongs to the Euphorbiaceae, a very large family with 300 genera and 8,000 to 10,000 species, and is the most numerous in the tropics [14, 15]. The name of the genus *Croton* comes from a Greek word *Kroton*, which means ticks, because of the seeds' resemblance to ticks [16]. The genus contains over 1,200 species, which are distributed throughout the world (Berry, 2000). Eight of these species (*C. dichogamus*, *C. zambesicus*, *C. menyhartii*, *C. somalense*, *C. schimperianus*, *C. sylvaticus*, *C. lobatus*, and *C. macrostachyus*) are found in Ethiopia, but the most common species is *Croton macrostachyus* [17]. The specific epithet is from the Greek macro – (large) and – stachyus (relating to spike) hence “with a large spike”.

### **1.2.2. Ecological and Geographical Distribution of *Croton***

*Croton macrostachyus* is native to Eritrea, Ethiopia, Kenya, Tanzania, Uganda and Nigeria. It is a medium sized deciduous tree of East Africa particularly wide spread between 200-2500 m in mountainous forests and savannah of the tropical regions and ever green bush land areas that receive between 700-2000 mm rainfalls annually [18, 19]. These trees experience extended flowering seasons in most areas, peaking in March-June and May-July, providing excellent bee forage. For instance, in Kenya, flowering is observed in Kakamega District in March and April; in Nyeri, Meru and Kericho Districts in June and July; and in Pokot District in August and September. In Nigeria, flowering occurs in March to May and fruiting from January to March. After pollination by insects, fruit development takes place 3-5 months. *Croton macrostachyus* is 3-25 m high, although more commonly 6-12 m. It is common in secondary forests, on forest edges along rivers, woodlands, wooded grasslands or clump bush land and along road sides. It is associated with *Janiperus* *Podocarpus* habitats and occurs in the warmer parts of the montane rain forests and semi-tropical rain forests. Outside the forests, in wetter areas, the species is widely distributed. In Ethiopia, *C. macrostachyus* grows between 500 and 3400 m (more frequently between 1100 m and 2700 m) a.s.l. The species occurs in forest margins, along edges of roads, mostly in moist lowlands, both dry and moist midlands, and highlands areas of Ethiopia. *C. macrostachyus* also occurs as a pioneer species commonly on degraded mountain slopes, on disturbed areas, in borders of cultivated fields, on waste ground, along river habitats [20, 21].

### **1.2.3. Uses of *Croton macrostachyus***

*Croton macrostachyus* is employed in soil conservation, trees are commonly planted for the useful shade that they provide, leaf fall provides mulch and green manure; the cream-coloured, soft wood is used for indoor carpentry, furniture, veneers, tool handles, boxes and crates. Many parts of the *Croton macrostachyus* have medicinal value including boiled leaf decoction is drunk or ashes taken orally as treatment for cough; juice from fresh leaves is applied on wounds to hasten clotting. Roots are used as an anthelmintic for tapeworm, for malaria, venereal diseases, as antidiabetic, and the seeds are widely used as purgative, for constipation and for stomach

worms. Bark from stems and roots are boiled in water and most newly born babies are bathed in the mixture as a remedy for skin rash. The leaves of the tree are also used for fodder and the tree is used for shade. As *Croton macrostachyus* is available almost in all part of Ethiopia, people are using it for as a medicine for treatment of snakebite, malaria, headache, internal worms, rabies, gonorrhoea, ascariasis, sexually transmitted diseases and tinea versicolor for human being in which the traditional medician ordered the patients to take unlimited dosage of the plants part like to take two, three or even more glasses of medicinal plant solution orally. The plant is also used for fever and wounds of domestic animals [21].

#### **1.2.4. Phytochemical Study on the Genus *Croton* and Constituent of *Croton* species**

Phytochemical study on the genus *Croton* has lead to the isolation and characterization of different classes of secondary metabolites. Terpenes, flavonoids and alkaloids have been isolated from the different *croton* species. Terpenoids are the predominant secondary metabolite constituents in the genus. The most common class of compounds of *Croton* is represented by diterpenoids. Apparently, clerodane is the widest spread class of diterpenoids in croton, which has been found in species from America (e.g. *C. cajucara*), Africa (e.g. *C. macrostachyus*) and Asia (e.g. *C. tiglium*). The genus is also rich in constituents with biological activities, chiefly diterpenoids such as labdane (**1**), clerodane (**2**), kaurane (**3**), trachylobane (**4**) and pimarane (**5**).

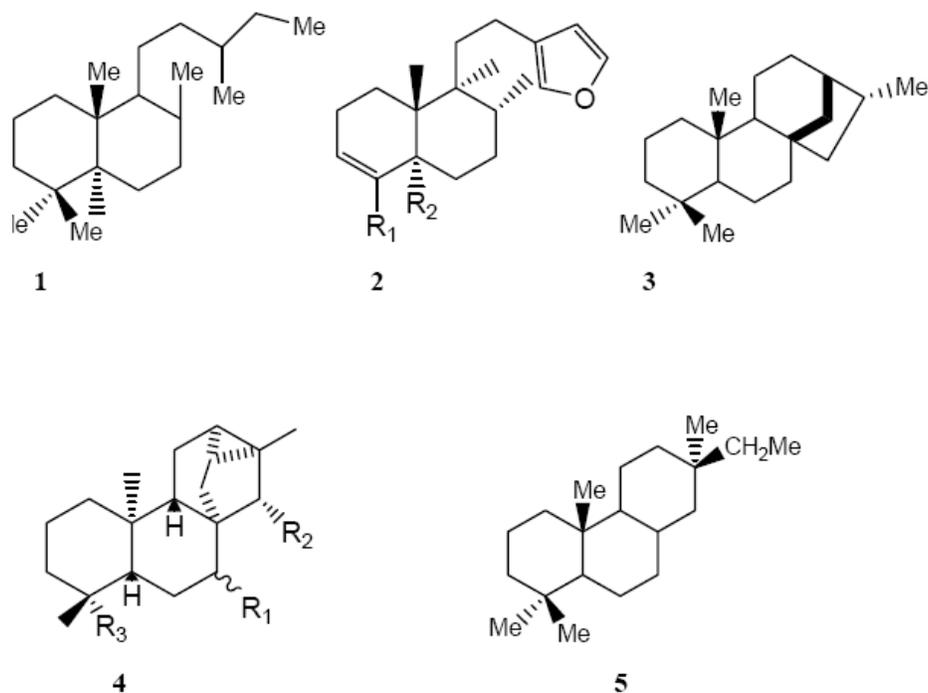


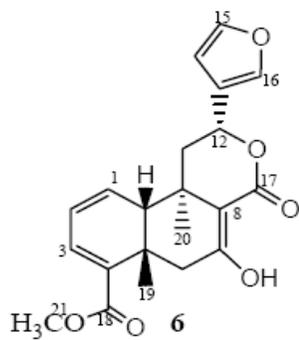
Figure 1. Structure of different diterpenoids.

Several species of the genus are aromatic, indicating the presence of volatile oil constituents. As most Euphorbiaceae, croton species may contain latex, which is red-coloured in some species, a characteristic usually with medicinal properties. Several croton species have a long role in the traditional use of medicinal plants in Africa, Asia and South America. Popular uses include treatment of cancer, constipation, diabetes, digestive problems, dysentery, external wounds, fever, leukemia, balsamic, narcotic, rheumatism, stomachic and tonic, bronchitis, diarrhea, leprosy, psoriasis, urticaria, hypercholesterolemia, hypertension, inflammation, intestinal worms, malaria, pain ulcers and weight loss. Some of the croton species which are used for the treatment of such diseases traditionally in different worlds include: *C. cajucara* Benth., popularly known as “sacaca”, *C. celtidifolius* Baill., commonly known as “sanguede-adave”, *C. eluteria* Benett., commonly known as “cascarilla”, *C. malambo* Karst., *C. nepetaefolius* Baill., *C. palanostigma* Klotzsch, *C. schiedeanus* Schlecht., *C. uraucurana* Baill., *C. zehntneri* Pax. Et Hoffm. (in South America), *C. arboreous* Millsp, *C. californicus* Mull. Arg., *C. draco* Cham. & Schldl. (in North America and Central America), *C. macrostachyus* Hochst. ex Rich., *C. zambesicus* Mull. Arg (synonyms to *C. amabilis* Mull. Arg.; *C. gratissimus* Burch.) (in Africa), *C. kongensis* Gagnep., *C. oblingifolius* Roxb., popularly known as “chukka”, *C. sublyratus* Kurz., *C. tigilium* L., and *C.*

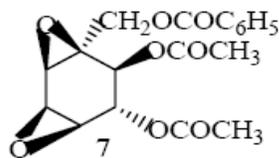
*tonkinensis* Gagnep, popularly called “Kho sam Bac Bo” (in Asia). The parts of those croton species which are used for medicinal purpose for treatment of different kinds of diseases are the leaves, the roots, the stem barks and the fruits.

Table 1. Some terpenes isolated from *Croton macrostachyus*

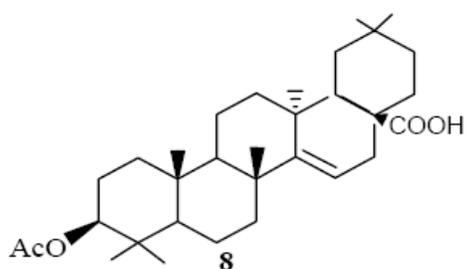
No.	Name of the compound	Structure	The source	Reference
1.	Crotomacrine	6	Fruits	22
2.	Crotopoide	7	Fruits	22
3.	3 $\beta$ -Acetoxy tetraer-14-en-28-oic acid	8	Roots	23
4.	Trachyloban-19-oic acid	9	Roots	23
5.	Trachyloban-18-oic acid	10	Roots	23
6.	Neoclerodan-5,10-en-19,6 $\beta$ ; 20, 12-diolie	11	Roots	23
7.	3 $\alpha$ , 19-dihydroxy trachyloban	12	Roots	23
8.	3 $\alpha$ ,18,19-trihydroxy trachyloban	13	Roots	23
9.	Lupeol	14	Root bark	24



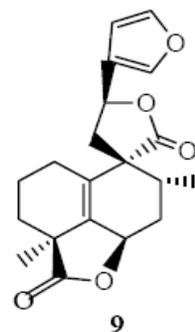
**Crotomacrine**



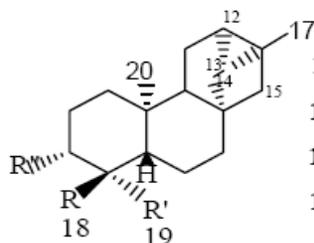
**Crotepoxide**



**3β-acetoxy tetraer-14-en-28- oic acid**



**Trachyloban-19-oic acid**

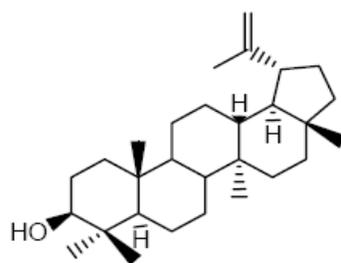


**10.** R= CH<sub>3</sub>, R'= COOH, R''=H

**11.** R= COOH, R'= CH<sub>3</sub>, R''=H

**12.** R=CH<sub>3</sub>, R'= CH<sub>2</sub>OH, R''=OH

**13.** R= CH<sub>2</sub>OH, R'= CH<sub>2</sub>OH, R''=OH



**14 (Lupeol)**

Figure 2. Structure of some terpenes isolated from *Croton macrostachyus*.

### 1.2.5. Elemental Analysis in Traditional Medicine

Although, the organic components of *Croton macrostachyus* have been investigated, the literature survey shows that little or nothing is acknowledged about the elemental uptake and distribution in the plant. The same broadly applies to many plants of known medicinal value. Based on the requirements for the human body, elements are classified as the macro- and micro-nutrients and others as non-essential and toxic elements. The micro-nutrients, when present in amounts exceeding the required levels, can be toxic. Many non-essential elements (As, Pb, Hg and Cd) even at very lower levels than the trace elements are toxic [12]. Toxicity can also be viewed as an element that exceeds the required amount. A toxic element could damage an enzyme system or DNA through complexation. The main factors that govern the toxicity are the exposure or dose and the susceptibility of the organism/tissue to a particular element [13]. In this study the elemental uptake and distribution of selected macro, micro and toxic elements (Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr, Ni, Cd, and Pb) in Croton leaves and its infusions in Ethiopian *Croton macrostachyus* plant will be investigated.

Plants constitute an important link by the transfer of trace elements from soil to man. The level of elements in plants varies, the content being affected by the geochemical characteristics of a soil and by the ability of plants to selectively accumulate some of these elements. Bioavailability of the elements depends on the nature of their association with the constituents of a soil. Plants readily assimilate elements through the roots. Additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers that can be absorbed through the leaf blades [25, 26].

Trace elements play an important role in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living cells of plants, animals and human beings. More than sixty (60) elements are found in human body in various forms among which twenty-five (25) are considered essential to human health out of which fourteen (14) exist usually less than  $1 \mu\text{g g}^{-1}$  of tissue and so termed trace. Cobalt is essential component of vitamin B<sub>12</sub>; zinc is found in several enzymes and genetic material transcription; copper is key component of redox enzymes and

chromium has a role in glucose metabolism, iron in oxygen transport and so enables metabolism [27, 28].

Though required in very small amount, deficiency of trace elements cause diseases, whereas their presence in excess may result in toxicity to human life disturbing normal functioning of organs and central nervous system. Anemia from iron deficiency affects more than half of pregnant women and at least one-third of children under five years. In Ethiopia the Food and Agricultural Organization estimate indicated prevalence of iron deficiency is 85% among children and 58% among pregnant women while in Kenya similar survey estimated 60% and 70%, respectively [29, 30]. In view of the above facts, the medicinal plants studied are a source of biologically important elements, which may play a part in the observed therapeutic properties of these plants. Hence it is expected that plants with high concentrations of the above-mentioned macro and micronutrients, which in most cases are present in permissible levels, might play an important role in maintenance of human health.

On the other hand, trace metals like lead, cadmium and mercury are known for their detrimental health effect. Cadmium, for example, has been considered as an extremely significant pollutant even in small amount, affecting all forms of life because of its high toxicity and great solubility in soil and water. No level of lead in blood as well should be considered safe for children due to its neurotoxicity [31, 32].

The society, in addition to their daily food intake, these will be the major intake source of toxic trace metals by human beings. More over the problem of environmental pollution due to rapid discharge of industrial, agricultural and swage effluents into water bodies, lands and air are the major source of toxic trace metals for the society [33].

The concern about the quantitative estimation of various essential, non-essential and toxic element concentrations is important for determining the effectiveness of the medicinal plants in treating various diseases and also to understand their pharmacological action. Moreover, trace elemental analysis of medicinal plants can be used to decide the dosage of the herbal drugs prepared from these plant materials.

The research also helps to develop a system to conserve medicinal plants by propagation and local cultivation and to provide opportunities for sustainable harvesting from the wild. Though some phytochemical investigations are reported, so far no study on the plant species, *Croton macrostachyus* has showed determination of essential, non-essential and toxic metals. Hence this research project is intended to determine levels of essential, non-essential and toxic metals in Ethiopian freshly collected *Croton macrostachyus* leaves and its infusion using flame atomic absorption spectroscopy.

At present, there are no general established guideline values for the permissible levels of the main part of metals or essential elements in medicinal herbs. The World Health Organization cites maximum permissible levels in raw plant materials only for cadmium ( $0.3 \text{ mg kg}^{-1}$ ), arsenic ( $1 \text{ mg kg}^{-1}$ ) and lead ( $10 \text{ mg kg}^{-1}$ ). The continuity of such research endeavours, in terms of periodical assessment of these and other metal concentration in all the known herbal plants used in traditional medicine, would go a long way toward predicting the quality assurance and safer use of herbal products.

### **1.3. Statement of the Problem**

*Croton macrostachyus* is very important to cure a number of diseases and hence used by most of individuals in the country in a regular basis. It is also known that the source of mineral nutrients for human being is plant materials consumed in the form of food or medicine. Thus, it is very important to assess the essential, non-essential and toxic mineral nutrients that can be accumulated in the stated plant species so as to address the individual daily intake of mineral nutrients. Furthermore, dosages of traditional medicines are not precisely understood. So, individuals may take these traditional medicines in larger quantity. Therefore, beside toxicity from the active ingredients, the individuals may suffer from trace toxic metal toxicity and hence their normal body function will be affected as trace metals are responsible for most of our body enzymatic activity. Therefore, determination of trace metals in the plant extract is very important to ensure individuals health status. Furthermore, the result of this study may help to propose the maximum dosage of the plant for normal body function in terms of trace metal content. Based on

this finding the local expertise will try to manage the normal dosage by integrating their experience with the one we are going to report optimum quantity.

Since *Croton macrostachyus* is one of the main traditional medicine and serve to cure different diseases for human being and domestic animals, the knowledge of their mineral concentrations are of particular interest. However, information on the contents of essential and non-essential trace elements in the different parts of the plant extract is scarce in the literature.

#### **1.4. Purpose of the Study**

The purpose of this study is to determine the essential and non-essential trace elements in the leaves powder and its infusion of the plant extract and to propose optimum normal dosage in terms of its active constituent and trace metal composition. Furthermore, the findings of this study will provide adequate information on the distribution of major, minor and trace metals in the leaves and infusion of the plant extract and it will ensure the health dietary safety of individuals who use this plant as source of medicine.

#### **1.5. Objectives of the study**

##### **1.5.1. General Objective**

The main objective of this project is to determine the level of essential, non-essential and toxic metals in the leaves and its infusion of *Croton macrostachyus* grown in Ethiopia.

##### **1.5.2. Specific Objectives**

- i. To develop an optimum working procedure for digestion and analysis of leaves and infusions of the same species of *Croton macrostachyus* for their essential, non-essential and toxic metal contents by FAAS.
- ii. To determine essential (Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr), non-essential and toxic metals (Ni, Cd, Pb) in Croton leaf and its infusions by flame atomic absorption spectrometer

- iii. To determine essential (Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr), non-essential and toxic metals (Ni, Cd, Pb) in leaves of *Croton macrostachyus* grown in Ethiopia by flame atomic absorption spectroscopy.
- iv. To compare the levels of essential (Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr), non-essential and toxic (Ni, Cd, Pb) metals in the leaves and infusions of *Croton macrostachyus*.
- v. To estimate the amount of elements released from plants into the water extracts.
- vi. To look some correlation trends among the element concentrations in the different area of study by applying simple statistical tools.

## **2. EXPERIMENTAL**

### **2.1. Instrument and Apparatus**

Stainless steel axe and Teflon (PTFE) knife were used to cut the Croton leaves in to pieces while air-circulating oven (Digitheat, J.P. Selecta, Spain) was used for drying the samples placed on porcelain. Blending device (Moulex, France), ceramic pestle and mortal were used for grinding and homogenizing the Croton samples. Digital analytical balance (Mettler Toledo, Model At250, Switzerland) was used for weighing the Croton samples. Round bottom flasks with grounded glass (100 mL) fitted with reflux condenser were employed in digesting the bulb sample on Kjeldahl heating apparatus (Gallenhamp, England). Borosilicate volumetric flasks (25, 50, 100 and 250 mL) were used during dilution of sample and preparation of metal standard and infusion solutions. Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA), micropipettes (Dragonmed, 1-10  $\mu$ L, 100-1000  $\mu$ L, Shangai, China) were used during measuring different quantities of volumes of sample solution, acid reagents and metal standard solutions. Metals' concentration determination was done by flame atomic absorption spectrophotometer (Buck Scientific, Model 210VGP AAS, East Norwalk, USA) equipped with deuterium background corrector and hollow cathode lamps with air-acetylene flame.

## **2.2. Chemicals and Reagents**

Reagents that were used in the analysis were all analytical grade. 69-70% HNO<sub>3</sub> (Supreme Enterprises Cantt, India) and 70% HClO<sub>4</sub> (Aldrich, UK) were used for the digestion of the Croton leaves and infusion samples. Lanthanum nitrate hydrate, (99.9%, Aldrich, USA) was used to prevent the chemical interference on Ca and Mg in the sample solution during the analysis of Croton samples. Stock standard solution of concentration 1000 mg/L in 2% HNO<sub>3</sub> of the metals Ca, Mg, Mn, Fe, Zn, Cu, Zn, Cr, Ni, Co, Cd and Pb (Buck Scientific Puro-Graphic™) from which 10 mg/L of intermediate standard obtained were used for the preparation of the calibration standards of each metal. Working standards were prepared from intermediate standards of each metal. Deionized water (chemically pure: 1.5 μs/cm and below) was used for sample preparation, dilution, and rinsing apparatus prior to analysis.

## **2.3. Procedure**

### **1.3.1. Cleaning Apparatus**

Apparatus such as glassware, plastic containers and polyethylene bags were washed with the tap water using detergent followed by deionized water rinsing. The apparatuses were then soaked with about 10% (v/v) nitric acid for 24 h followed by rinsing with deionized water five times. The apparatus were then dried in hot air oven and kept in dust free place until analysis begins.

### **2.3.2. Description of the Study Area**

The study was conducted in selected sites of four different regions namely: Abomsa, a town in central Ethiopia – located in the Arsi Zone of the Oromia region, administrative center of Merti Woreda, Dilla, a market town in southern Ethiopia – the administrative center of the Gedeo Zone in the Southern Nations, Nationalities and People Region (SNNPR), and located on the main road from Addis Ababa to Nairobi, Akaki Kaliti, one of the outskirts sub-cities of Addis Ababa and Bonga the administrative center of Kefa zone in the Southern Nations, Nationalities and People Regional state. The selection of these sites was based on some reasons.

Firstly, availability of ethnobotanical information, i.e, we have got literature which has been done on this plant from Bonga, Ethiopia. Secondly, we wanted to compare metal accumulation in this plant from industrial and agriculturally polluted areas with that of none polluted environment. Thirdly, we have selected the stated areas based on availability of the plant and its popularity in using as medicinal plant by the local peoples around. Thus having these three reasons, we have selected the stated areas for our study.

Their geographical location and distance from Addis Ababa (capital city of Ethiopia) are given in Table 2.

Site	Latitude	Longitude	Altitude (m)	Distance from Addis Ababa (km)
Abomsa	8 <sup>0</sup> 35' N	39 <sup>0</sup> 51' E	1438	202
Dilla	6 <sup>0</sup> 24' 30'' N	38 <sup>0</sup> 18' 30'' E	1570	359
Akaki Kality	9 <sup>0</sup> N	38 <sup>0</sup> 45' E	2200-2800	23
Bonga	6 <sup>0</sup> 15' to 8 <sup>0</sup> 08' N	35 <sup>0</sup> 30' to 36 <sup>0</sup> 46' N	500 - 3350	440

### 2.3.3. Sampling

Sampling plan is composed of three components; (i) sampling, (ii) sample preparation, and (iii) analysis. Nature of the analyte of interest, distribution of the analyte throughout the lot, physical characteristics of the product, accessibility of the product to random representative sampling, sampling procedure, and size of sample are some of the factors affecting the ability of the sampling plan to accomplish the certain objective.

#### 2.3.3.1. Sample Collection, Preservation and Handling

Depending on the availability of Croton plant, representative amount of leaves were collected from four different regions. As mentioned above, these regions are located in eastern, northern, central and southeastern parts of Ethiopia. The leaves were collected from a minimum of 16 mature plants, four plants per region and eight leaves per plant starting from the bottom to the tips by stalk position. The samples were packaged into polyethylene plastic bags, labeled and

transported to laboratory for further treatment. The Croton samples were put in clean plastic bags labeled according to their region and brought to the lab for further pretreatment. The leaves of the *Croton macrostachyus* were separated with stainless steel and Teflon knives, washed with a running tap water so as to remove adsorbed soil particulates and then rinsed with distilled deionized water and air dried. About 300 g of different region of the Croton leaves were put on acid-washed porcelain labeled according to the samples region and dried in air oven at 80 °C for 48 h till it got brittle and crisp. Cooling to ambient temperature, the dried samples were pound in to fine powder with blending device, mortar and pestle, and sieved (1 mm). The powdered sample was then placed in pre-cleaned screw capped polyethylene container and stored in desiccators with calcium chloride to keep to constant dry weight till digestion.

#### **2.3.4. Sample Preparation**

Sample preparation is a critical step in any analytical sequence. Some of the aspects that should be taken into consideration in a sample preparation procedure include sample size, number and concentration of analytes, compatibility to the measurement equipment, required analytical blanks, time, reagent consumption and suitable precision and accuracy. In the present study, the Croton samples were made ready for the analysis after digestion using the Kjeldahl digester heating block.

##### **2.3.4.1. Croton Leaves Powder Samples**

A bulk sample, for each region and each sample type, was prepared by taking 10 g of Croton powder from each four plants and mixed for homogeneity using an electronic blender. Thus, the amount needed for analysis was taken from the 40 g of the bulk Croton powder sample prepared.

#### **2.3.4.2. Croton Leaves Infusion Samples**

The Croton leaves infusion was made from Croton leaves taken from Akaki site only. By varying the time, four Croton leaves infusion samples were prepared. As there is no specific method of preparing the Croton leaves infusion in Ethiopia, usually twenty five gram of fresh leaves (8 to 14 leaves) were grinded with mortar and pestle and were added in to a 250 mL tap water (at room temperature, 25 °C). This was done on the habits of traditional medician, i.e. they keep the leaves in the water for half a day or for complete day depending on the ages of the patient. So, in this study, to compare and consider the amount of metals extracted in to the supernatant, the infusions were done for 3, 6, 12 and 24 hours. Finally, pure infusion sample was obtained by filtering and the decrease in volume was compensated by adding water up to the mark.

#### **2.4.5 Optimization of the Working Procedure**

It is important to develop an optimum working procedure in order to get a reliable result from an analytical experiment. Thus, to prepare a clear colorless sample solution that is suitable for the analysis using AAS different working procedures for the digestion of Croton leaves and Croton leaves infusion were assessed using the HNO<sub>3</sub> and HClO<sub>4</sub> acid mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature. By examining the nature of the final digests obtained by varying the above parameters, the optimized procedure was selected depending up on the clearness of the digests, less digestion time, less reagent volume and simplicity.

Table 3. Different methods tested during the optimization of procedures for Croton leaves powder

No	<sup>a</sup> Weight of CLP (g)	Reagent volumes (mL)			Maximum temperature (°C)	Time (min)	Result
		HNO <sub>3</sub>	HClO <sub>4</sub>	Total volume			
1	0.5	4	3	7	300	180	Colorless but turbid
2	0.5	5	2	7	300	180	Clear but yellowish
3	0.5	6	1	7	300	180	Clear and colorless
4	0.5	5	1	6	300	180	Clear but yellowish
5	0.5	5	1	6	300	180	Clear and colorless
6	0.5	3	2	5	300	180	Clear but yellowish
7	0.5	4	1	5	300	180	Clear and colorless
8	0.5	2	2	4	300	150	Clear and colorless
9	<b>0.5</b>	<b>2</b>	<b>2</b>	<b>4</b>	<b>270</b>	<b>150</b>	<b>Clear and colorless</b>
10	0.5	2	2	4	270	120	Clear and yellowish
11	0.5	2	2	4	240	180	Clear and light yellow
12	0.5	3	1	4	270	150	Clear and light yellow

<sup>a</sup> Croton leaves powder.

Optimization of the digestion step for the Croton leaves infusion was also made by varying the parameters used for digestion of the Croton leaves powder. The volume of the Croton leaves infusion taken was 25 mL but to avoid the dilution of the conc. HNO<sub>3</sub> and HClO<sub>4</sub> the volume of the Croton leaves infusion sample was decreased by evaporating to around 3 mL on the hot plate.

Table 4. Different methods tested during the optimization of procedures for Croton leaves infusion samples.

No	<sup>a</sup> Volume of <sup>b</sup> CLI (mL)	Reagent volumes (mL)			Maximum temperature (°C)	Time (min)	Result
		HClO <sub>4</sub>	HNO <sub>3</sub>	Total volume			
1	25	1	2	3	300	180	Yellowish
2	25	2	2	4	300	180	Yellowish and turbid
3	25	1	3	4	300	180	Yellowish with no turbidity
4	25	1	4	5	300	180	Clear and colorless
5	25	2	3	5	300	180	Clear and colorless
6	25	3	3	6	350	180	Clear but yellowish
7	25	2	4	6	300	180	Clear and yellowish
<b>8</b>	<b>25</b>	<b>1</b>	<b>4</b>	<b>5</b>	<b>240</b>	<b>150</b>	<b>Clear and colorless</b>
<b>9</b>	25	1	4	5	240	120	Clear and turbid
10	25	1	4	5	210	120	Clear but yellowish
11	25	1	4	5	270	210	Clear but yellowish
12	25	1	4	5	350	180	Clear and turbid

<sup>a</sup> Volume of the sample was reduced to about 3 mL by evaporating.

<sup>b</sup> Croton leaves infusion.

Optimization procedure No. 9 in Table 3 and No. 8 in Table 4 were chosen for the digestion of Croton leaves and Croton leaves infusion samples, respectively. The choice was made by noticing that the final solution should be clear and colorless without any suspended matter.

### 2.3.5.1. Digestion of the Croton Leaves

Exactly 0.5 g of the dried and grounded Croton sample was accurately weighed on a digital analytical balance and transferred quantitatively in to a 100 mL round bottom digestion flask. To the Croton leave samples, 4 mL of freshly prepared 2:2 mixture of 70% of conc. HNO<sub>3</sub> and 70%

of conc.  $\text{HClO}_4$  was added, respectively. The mixture was then heated on Kjeldahl heating apparatus fitting the flask to a reflux condenser for the first 30 min by setting the temperature to dial at 4 ( $120^\circ\text{C}$ ) and followed by dialing at 9 ( $270^\circ\text{C}$ ) as maximum temperature for the other 2:00 hours.

The digested solutions were allowed to cool for 20 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution, two 5 mL portions of distilled-deionized water were added to dissolve the precipitate formed on cooling and gentle swirled to reduce dissolution of the filter by digest residue. The cooled digested samples were filtered into a 50 mL standard flask with a Whatman filter paper (110 mm, diam.) to remove any suspended or turbid matter. Subsequent rinsing the filtrate with 5 mL distilled-deionized water followed until the volume reaches the mark. For each bulk samples, triplicate digestions were carried out. The digested and diluted sample solutions stored in tightly capped polyethylene bottles and kept in refrigerator until analysis time.

#### **2.3.5.2. Digestion of the Croton Leaves Infusion Samples**

To determine the amount of metals extracted from the Croton leaves to the leaves infusion (to the water at room temperature,  $25^\circ\text{C}$ ) 25 mL of the leave infusion was transferred quantitatively to the 100 mL round bottom flask and heated on the hot plate to evaporate until the sample was decreased to around 3 mL. This was done to avoid dilution of the acid mixture added for digestion. The solution was allowed to cool before the addition of the acid mixture to avoid explosion due to contact between the organic matter and conc.  $\text{HClO}_4$

5 mL of 4:1  $\text{HNO}_3$  and  $\text{HClO}_4$  was added and swirled gently to homogenize then fitted to a reflux condenser and digested continuously for two and half hours on a Kjeldahl digestion block. The temperature was adjusted at  $120^\circ\text{C}$  for the first 30 min and finally it was adjusted to the maximum temperature  $240^\circ\text{C}$  for 2:00 hours. The digested solutions were allowed to cool for 20 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution, two 5 mL portions of distilled-deionized water were added to dissolve the precipitate formed on cooling and gentle swirled to reduce dissolution of the filter

by digest residue. The cooled digested samples were filtered into a 50 mL standard flask with a Whatman filter paper (110 mm, diam.) to remove any suspended or turbid matter. Subsequent rinsing the filtrate with 5 mL deionized water follow until the volume reaches the mark. The digestion gave a clear colorless solution. Each Croton leaves infusion sample was digested in triplicate and hence a total of twelve digest was made for the four types of Croton leaves infusion samples. All the final digest solutions were kept in a refrigerator until the analysis for the metals.

### **2.3.5.3. Digestion of the Blank Samples**

Digestion of a reagent blank was also performed in parallel with the Croton samples keeping all digestion parameters the same. For the analysis of the Croton samples 9 reagent blank samples were prepared. All the digested samples were stored in refrigerator until analysis using AAS. The solutions were used to determine concentration of the 11 elements by AAS.

## **2.4. Instrument Operating Conditions**

Intermediate standard solutions containing 10 mg/L were prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L. These intermediate standards were diluted with distilled-deionized water to obtain four working standards for each metal of interest. In this study a total of 11 metals were analyzed using flame atomic absorption spectrophotometer (Buck Scientific Puro-Graphic<sup>™</sup>) equipped with deuterium arc background corrector and air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument Three replicate determinations were carried out on each sample. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. All the eleven metals (Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr, Ni, Cd and Pb) were analyzed by the absorption mode of the instrument.

The same analytical procedure was employed for the determination of elements in nine-digested blank solutions. Three readings were recorded for each digests using a three points external

calibration curve obtained by optimizing the different FAAS conditions shown in Table 5 to give the maximum signal intensity.

Table 5. Instrumental operating conditions for the determination of metals in Croton leaves and leave infusion samples using flame atomic absorption spectrophotometer.

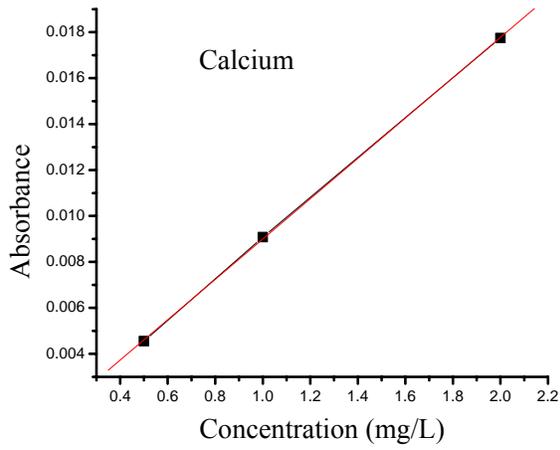
Element	Wavelength (nm)	Detection limit (mg/L)	Slit width (nm)	Energy (eV)
Mg	285.2	0.001	0.7	3.994
Ca	422.7	0.01	0.7	3.606
Cu	324.7	0.02	0.7	3.327
Fe	248.3	0.03	0.2	3.256
Mn	279.5	0.001	0.7	3.971
Co	240.7	0.05	0.2	2.746
Ni	341.5	0.04	0.2	2.928
Cr	357.9	0.05	0.7	3.536
Zn	213.9	0.005	0.7	3.047
Pb	217.0	0.1	0.7	3.49
Cd	228.9	0.005	0.7	3.129

#### 2.4.1. Instrument Calibration

Calibration curves were prepared to determine the concentration of the metals in the sample solution. The instrument was calibrated using three series of working standards. The working standard solutions of each metal were prepared from the 10 mg/L intermediate standard solutions of their respective metals as mentioned previously under section 2.3.6. Wavelengths, concentration of the intermediate standards, working standard solutions and the correlation coefficients of the calibration curve for each of the metals are presented in Table 6. The calibration graph of each of metals of interest is shown in Figure 3.

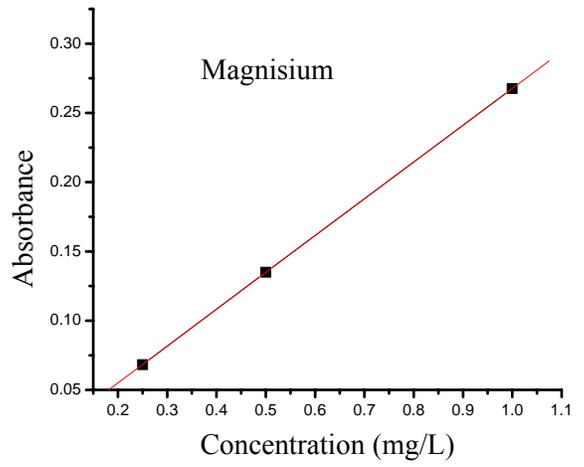
Table 6. Concentrations of working standard solutions and correlation coefficients of the calibration curves.

Metal	Wavelength (nm)	Conc. of intermediate standard solution (mg/L)	Conc. of working standard (mg/L)	Correlation coefficient
Ca	422.7	10	0.50, 1.0, 2.0	0.99994
Mg	285.2	10	0.25, 0.50, 1.0	1.0
Fe	248.3	10	0.50, 1.0, 1.5	0.99997
Mn	279.5	10	0.25, 0.50, 1.0	0.99993
Cu	324.8	10	0.50, 1.0, 1.5	0.99952
Zn	213.9	10	0.20, 0.40, 0.80	0.99924
Co	240.7	10	0.25, 0.50, 1.0	0.99953
Ni	341.5	10	0.25, 0.50, 1.0	0.99999
Cr	357.9	10	0.50, 1.0, 1.5	0.99933
Cd	228.9	10	0.25, 0.5, 1.0	0.99981
Pb	217.0	10	0.2, 0.4, 0.8	0.99999



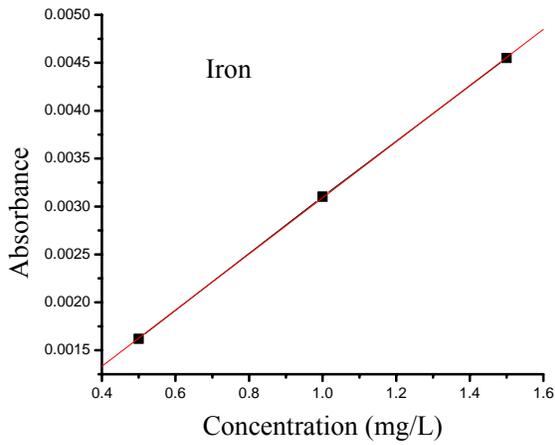
$$Y = 2.165E^{-4} + 0.00878X$$

$$R = 0.99994$$



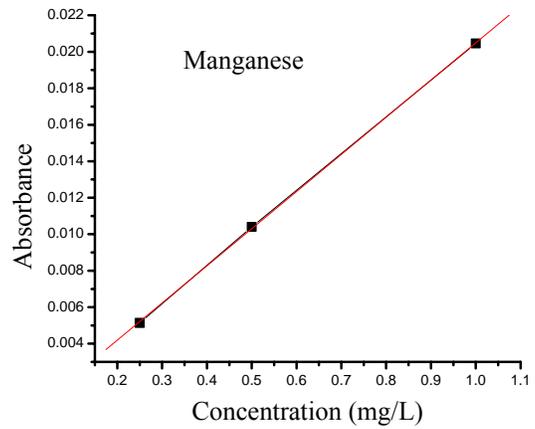
$$Y = 0.00195 + 0.26571X$$

$$R = 1$$



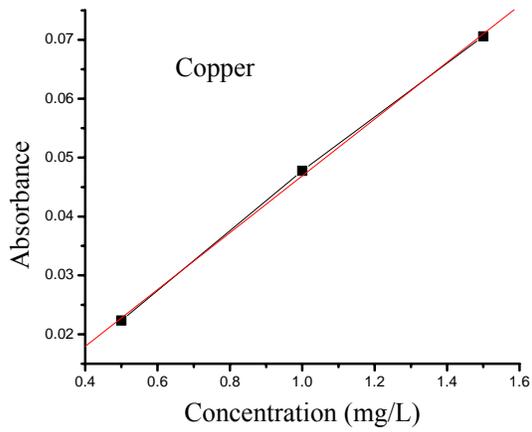
$$Y = 1.61E^{-4} + 0.00293$$

$$R = 0.99997$$



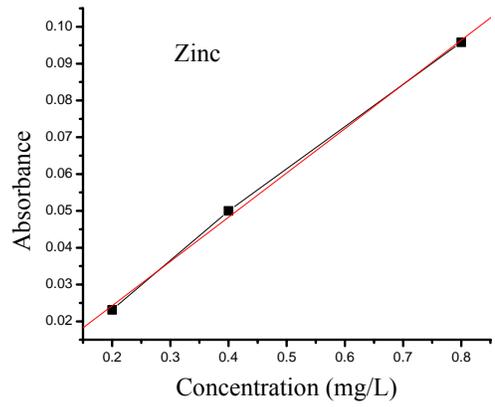
$$Y = 1.135E^{-4} + 0.02038$$

$$R = 0.99993$$



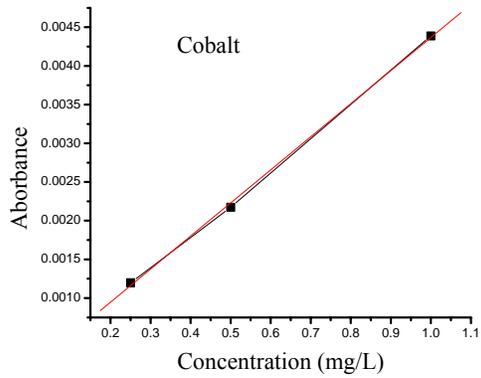
$$Y = -0.00134 + 0.04824$$

$$R = 0.99952$$



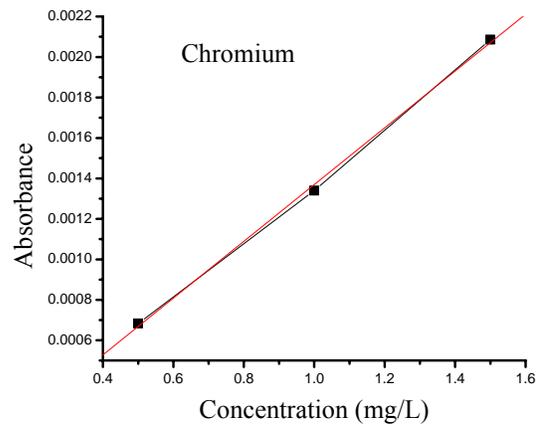
$$Y = 2.18E^{-4} + 0.12019$$

$$R = 0.99924$$



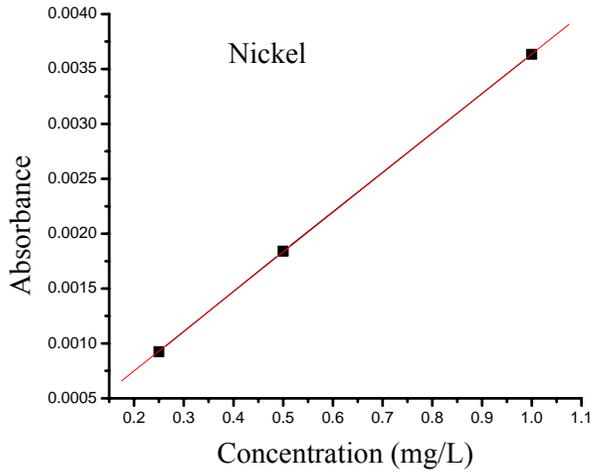
$$Y = 9.1E^{-5} + 0.00428$$

$$R = 0.99953$$



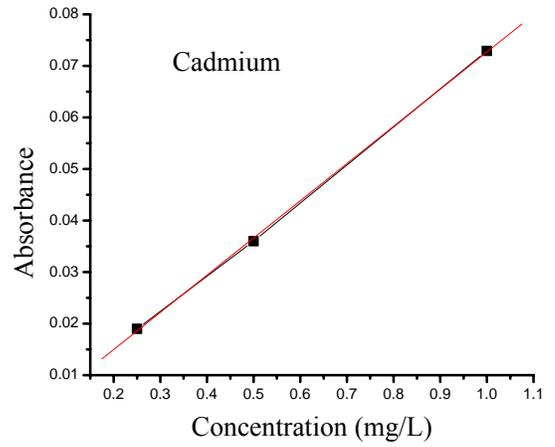
$$Y = -3.33333E^{-5} + 0.0014$$

$$R = 0.99933$$



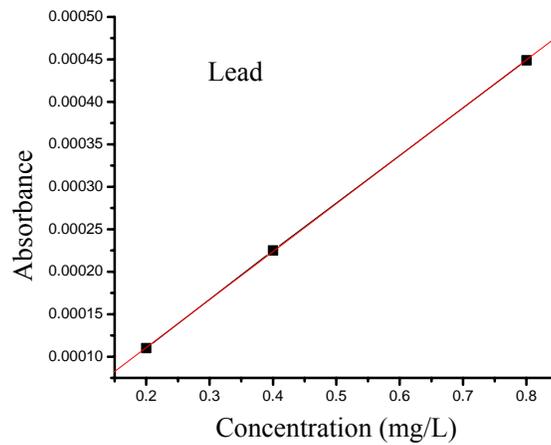
$$Y = 2.85E^{-5} + 0.00361$$

$$R = 0.99999$$



$$Y = 5.26E^{-4} + 0.07218$$

$$R = 0.99981$$



$$Y = -2E^{-6} + 5.64286E^{-4}$$

$$R = 0.99999$$

Figure 3. Calibration curves of metals standard solutions.

## 2.5. Method Detection Limit

Nine blank samples were digested following the same procedure as the samples and each of the samples were analyzed for metal concentrations of Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr, Ni, Cd and Pb by AAS. The standard deviations for each element were calculated from the nine blank measurements to determine method detection limit of the instrument.

Method detection limit is defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero [34]. In other words, it is the lowest analyte concentration that can be distinguished from statistical fluctuations in a blank, which usually correspond to three times the standard deviation of the blank ( $3\delta_{\text{blank}}$ , where  $\delta$  = standard deviation of the blanks). As can be seen from Table 7, the method detection limit of each element is above the instrument detection limit.

Table 7. Method detection limits for Croton leaves, and leaves infusion samples

Metal	*MDL for Croton leaves samples ( $\mu\text{g/g}$ )	*MDL for Croton leaves infusion samples ( $\mu\text{g/g}$ )
Ca	3.1	5.2
Mg	2.6	4.1
Fe	0.05	0.07
Mn	1.1	2.0
Zn	0.4	0.5
Cu	1.2	1.5
Co	1.0	1.4
Cr	0.8	1.2
Ni	1.7	1.8
Cd	0.06	0.08
Pb	0.3	0.31

\*MDL = method detection limit.

## **2.6. Precision and Accuracy**

The errors in analytical results are most often expressed using accuracy and precision. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions whereas the accuracy of an analytical procedure expresses the closeness of measurements to the true value. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

In the present study, the precision of the results were evaluated by the indicated pooled standard deviation and relative standard deviation of the results of three samples ( $n = 3$ ) with a triplicate measurement of each sample meaning that a total of 9 measurements for a given bulk sample. These parameters are useful in estimating and reporting the probable size of random error. The results of the present analysis are reported with corresponding pooled standard deviation of nine measurements for a bulk sample of triplicate reading per sample and relative standard deviation. Table 10 and 11 shows % RSD of each metal in each sample.

## **2.7. Method Validation**

Method validation is the process of providing that analytical method is acceptable for its intended purpose. Because of the absence of certified reference material for the Croton samples in our laboratory, the validity of the optimized digestion procedure was assured by spiking the samples with a standard of known concentration of the analyte metals. Thus, the efficiency of the optimized procedure was checked by adding 704  $\mu\text{L}$  of 1000 mg/L Ca and 296  $\mu\text{L}$  of 1000 mg/L of Mg into a 0.5 g of Croton leaves powder samples. 58  $\mu\text{L}$  Fe, 28  $\mu\text{L}$  Mn, 150  $\mu\text{L}$  of Zn, 125  $\mu\text{L}$  of Cu 150  $\mu\text{L}$  of Co, 225  $\mu\text{L}$  of Cr, 125  $\mu\text{L}$  of Ni, and 13.5  $\mu\text{L}$  of Cd were added at once into a 0.5 g of Croton leaves powder samples in one digestion flask from each 40 mg/L standard solutions in presence of 4.4  $\mu\text{L}$  of 1000 mg/L of Pb.

Since the concentration of metal in the Croton leaves infusion is lower than that of the Croton leaves powder, the recovery test for the Croton leaves infusion was done by adding smaller amount of the

spiked metals than that of the Croton leaves powder. 52.6  $\mu\text{L}$  of 1000 mg/L of Mg, 108.7  $\mu\text{L}$  of 1000 mg/L of Ca together with 0.98  $\mu\text{L}$  of 1000 mg/L of Fe, 0.34 $\mu\text{L}$  of 1000 mg/L of Mn, 1.2  $\mu\text{L}$  of 1000  $\mu\text{L}$  of Zn, 19  $\mu\text{L}$  of 40 mg/L of Cu, 14.1  $\mu\text{L}$  of 40 mg/L of Co, 50.4  $\mu\text{L}$  of 40 mg/L of Cr, 14  $\mu\text{L}$  of 40 mg/L of Ni, 2.6  $\mu\text{L}$  of 40 mg/L of Cd and 0.8  $\mu\text{L}$  of 1000 mg/L of Pb were spiked at once in to the Croton leaves infusion sample in one digestion flask.

The spiked samples were digested in triplicate following the same digestion procedure developed previously. The digested spiked samples were finally analyzed for their respective metals using FAAS.

Table 8. Recovery test values for Croton leaves sample.

Metal	<sup>a</sup> Conc. in sample ( $\mu\text{g/g}$ )	Amount added ( $\mu\text{g/g}$ )	<sup>b</sup> Conc. in spiked sample ( $\mu\text{g/g}$ )	<sup>c</sup> Recovery (%)
Ca	7040	1408	8406 $\pm$ 12	97 $\pm$ 7
Mg	2961	592	3511 $\pm$ 8	93 $\pm$ 2
Fe	581	116	698 $\pm$ 5	102 $\pm$ 3.5
Mn	283	56	335 $\pm$ 3	98 $\pm$ 2.5
Zn	60.5	12	71.3 $\pm$ 1.5	94 $\pm$ 1.4
Cu	18.6	10	27.6 $\pm$ 0.8	96 $\pm$ 0.25
Co	21.8	12	71.4 $\pm$ 4	95 $\pm$ 4.3
Cr	87.5	18	104 $\pm$ 5	92 $\pm$ 8
Ni	26.2	10	35.8 $\pm$ 0.3	98 $\pm$ 5.1
Cd	1.08	1.08	2.19 $\pm$ 0.4	103 $\pm$ 6.2
Pb	21.9	8.8	30.2 $\pm$ 5.1	94 $\pm$ 2.6

<sup>a</sup> Mean concentration of samples analyzed in triplicate.

<sup>b</sup> Mean concentration  $\pm$  SD of samples spiked in triplicate.

<sup>c</sup> Mean recovery  $\pm$  SD of percentage recoveries of triplicate analyses.

Table 9. Recovery test values for Croton leaves infusion sample

Metal	<sup>a</sup> Conc. in sample (µg/g)	Amount added (µg/g)	<sup>b</sup> Conc. in spiked sample (µg/g)	<sup>c</sup> Recovery (%)
Ca	1087	217	1300 ± 4	98 ± 1.3
Mg	52.6	10.5	62.8 ± 2.5	97 ± 3
Fe	6.53	1.96	8.37 ± 2.8	94 ± 1.5
Mn	3.42	0.68	4.14 ± 5	105 ± 5
Cu	2.54	1.52	4.05 ± 7	99 ± 0.8
Zn	9.51	2.38	11.8 ± 3.5	95 ± 4
Co	2.83	1.13	3.94 ± 2	98 ± 2.5
Cr	13.4	4.03	17.31 ± 1.5	96 ± 1.8
Ni	2.80	1.12	3.84 ± 5	93 ± 2
Cd	0.21	0.21	0.41 ± 4.5	97 ± 3.5
Pb	2.07	1.66	3.65 ± 3.52	95 ± 2.4

<sup>a</sup> Mean concentration of samples analyzed in triplicate.

<sup>b</sup> Mean concentration ± SD of samples spiked in triplicate.

<sup>c</sup> Mean recovery ± SD of percentage recoveries of triplicate analyses.

The percentage recovery lies within the range 92-103% for the Croton leaves powder and 93-105% for the Croton leaves infusion, which are within the acceptable range for all metals as shown in Table 9 and 10. This confirms that the method is of good accuracy.

### 3. Results and Discussion

#### 3.1. Determination of Essential, Non-essential and Toxic Metals

The accuracy and precision of the method were tested by spiking the samples with a standard of known concentration of the analyte metals. The results indicated that the concentrations of elements determined by the present FAAS method are in agreement ( $100 \pm 10\%$ ) within the acceptable range for all metals. Thus, in the present work, the concentration of the eleven metals, essential (Ca, Mg, Mn, Fe, Cu, Zn, Co, Cr, Ni) and non-essential (Pb, Cd) in the Croton leaves and their infusions were determined by FAAS using three point external calibration curve. The results showed that the samples had variable composition of each analyte metals with wide concentration range. Among the identified eleven elements, all were found to be above the method detection limit except Cd not found in Dilla and Bonga sites in the Croton leaves powder. On the other hand most metals were not detected in the infusion samples prepared for six and three hours. The results are given in Table 10 and 11 with their respective % RSD.

Table 10. Mean concentration ( $X \pm SD$ ,  $n = 9$ ,  $\mu\text{g/g}$  dry weight) and relative standard deviation (% RSD) of essential and toxic elements in Croton leaves samples.

Metal	Concentration of metal ( $\mu\text{g/g}$ ) dry weight basis			
	Akaki leave	%RSD	Abomsa leave	%RSD
Ca	$7040 \pm 84$	1.2	$5976 \pm 37$	0.6
Mg	$2961 \pm 32$	1.1	$1361 \pm 54$	3.9
Fe	$581 \pm 40$	6.9	$329 \pm 7.1$	2.2
Mn	$283 \pm 5$	1.7	$157 \pm 7.5$	4.7
Zn	$60.5 \pm 2.3$	3.8	$34.5 \pm 2.6$	7.6
Cu	$18.6 \pm 0.7$	3.8	$8.20 \pm 0.70$	8.6
Co	$21.8 \pm 0.7$	3.8	$34.5 \pm 2.6$	7.6
Cr	$87.5 \pm 6.6$	7.5	$81.5 \pm 2.2$	2.7
Ni	$26.2 \pm 1.1$	4.3	$2.65 \pm 0.16$	6.2
Cd	$1.08 \pm 0.09$	8.76	$0.75 \pm 0.002$	7.4
Pb	$21.9 \pm 0.9$	4.1	$11.5 \pm 0.2$	2.1

Metal	Concentration of metal ( $\mu\text{g/g}$ ) dry weight basis			
	Bonga leave	%RSD	Dilla leave	%RSD
Ca	1823 $\pm$ 81	4.4	1202 $\pm$ 43	3.5
Mg	384 $\pm$ 29	7.6	271 $\pm$ 2	0.7
Fe	169 $\pm$ 6	3.7	232 $\pm$ 4	1.9
Mn	180 $\pm$ 7	3.9	421 $\pm$ 9	2.2
Zn	33.6 $\pm$ 1.1	3.3	19.7 $\pm$ 0.6	3.1
Cu	7.75 $\pm$ 0.59	7.6	6.31 $\pm$ 0.48	7.6
Co	33.6 $\pm$ 1.1	3.3	19.7 $\pm$ 0.6	3.1
Cr	21.3 $\pm$ 0.9	4.3	60.2 $\pm$ 2.6	4.3
Ni	3.16 $\pm$ 0.26	8.2	11.8 $\pm$ 1.0	8.7
Cd	ND		ND	
Pb	20.7 $\pm$ 1.2	5.9	10.5 $\pm$ 0.1	1.3

The concentration of metals in the Croton leaves powder was higher than that in the Croton leaves infusion either in dry weight or wet weight basis. Metals at high concentration in the Croton leaves powder had higher percentage of leaching in to the infusions.

Table 11. Mean concentration ( $X \pm SD$ ,  $n = 9$ ,  $\mu\text{g/g}$  wet weight) and relative standard deviation (% RSD) of essential and toxic elements in Croton leaves infusion tap water

Meta l	Concentration of metals			
	24 Hours infusion	%RSD	12 Hours infusion	%RSD
Ca	1087 $\pm$ 41	3.8	687 $\pm$ 40	5.8
Mg	52.6 $\pm$ 2.2	4.1	28.1 $\pm$ 0.5	1.8
Fe	6.53 $\pm$ 0.24	3.7	5.14 $\pm$ 0.06	1.3
Mn	3.42 $\pm$ 0.04	1.2	3.00 $\pm$ 0.07	2.2
Zn	9.51 $\pm$ 0.39	4.1	4.64 $\pm$ 0.30	6.5
Cu	2.54 $\pm$ 0.11	4.3	2.47 $\pm$ 0.004	0.2
Co	2.83 $\pm$ 0.08	2.7	2.42 $\pm$ 0.013	0.6
Cr	13.4 $\pm$ 0.9	6.8	6.53 $\pm$ 0.18	2.7
Ni	2.80 $\pm$ 0.013	0.48	2.51 $\pm$ 0.095	3.7
Cd	0.21 $\pm$ 0.007	3.23	0.16 $\pm$ 0.007	7.1
Pb	2.07 $\pm$ 0.043	2.08	1.58 $\pm$ 0.039	2.4

Meta l	Concentration of metals			
	6 Hours infusion	%RSD	3 Hours infusion	%RSD
Ca	577 $\pm$ 29	5.1	467 $\pm$ 19	4.1
Mg	15.1 $\pm$ 1.2	7.9	13.9 $\pm$ 0.9	6.7
Fe	4.21 $\pm$ 0.16	3.69	3.43 $\pm$ 0.17	4.89
Mn	2.66 $\pm$ 0.02	0.8	2.17 $\pm$ 0.03	1.2
Zn	3.26 $\pm$ 0.19	5.9	2.24 $\pm$ 0.02	1.05
Cu	2.33 $\pm$ 0.014	0.60	2.17 $\pm$ 0.007	0.32
Co	2.23 $\pm$ 0.01	0.5	2.15 $\pm$ 0.01	0.2
Cr	5.69 $\pm$ 0.12	2.13	2.61 $\pm$ 0.015	0.59
Ni	2.49 $\pm$ 0.082	3.31	2.17 $\pm$ 0.0038	0.18
Cd	0.11		0.11	
Pb	1.04 $\pm$ 0.066	6.36	0.371 $\pm$ 0.015	4.04

Table 12. Mean concentration ( $X \pm SD$ ,  $n = 9$ ,  $\mu\text{g/g}$ ) and relative standard deviation (%RSD) of essential and toxic elements in tap water.

Metal	Mean concentration ( $X \pm SD$ , $n = 9$ ) of metals in tap water		%RSD
	Conc. in $\mu\text{g/mL}$	Conc. in $\mu\text{g}/10 \text{ mL}^*$	
Ca	$4.81 \pm 1.60$	$48.1 \pm 1.6$	3.3
Mg	$0.42 \pm 0.04$	$4.2 \pm 0.4$	0.9
Fe	$0.33 \pm 0.15$	$3.3 \pm 0.15$	4.4
Mn	ND	ND	ND
Zn	$0.16 \pm 0.01$	$1.6 \pm 0.1$	0.5
Cu	$0.018 \pm 0.009$	$0.18 \pm 0.009$	4.8
Co	$0.056 \pm 0.009$	$0.56 \pm 0.009$	1.5
Cr	$0.118 \pm 0.021$	$1.18 \pm 0.021$	1.7
Ni	$0.015 \pm 0.004$	$0.15 \pm 0.004$	2.8
Cd	$0.011 \pm 0.002$	$0.11 \pm 0.002$	1.7
Pb	$0.022 \pm 0.001$	$0.22 \pm 0.001$	0.6

\* 25 g leaves were used to prepare 250 mL infusion, i.e. 1 g leave was infused in 10 mL tap water.

Table 13. Mean concentration of metal ( $\mu\text{g/g}$ , wet weight) in infusion minus mean concentration of metal in tap water.

Metal	24 hour infusion	12 hour infusion	6 hour infusion	3 hour infusion
Ca	1039	639	528	419
Mg	48.4	23.9	10.9	9.71
Fe	3.2	1.81	0.88	0.1
Mn	3.42	3	2.66	2.17
Zn	7.95	3.05	1.70	0.68
Cu	2.36	2.29	2.15	1.99
Co	2.27	1.86	1.67	1.59
Cr	12.3	5.35	4.51	1.43
Ni	2.65	2.36	2.34	2.02
Cd	0.098	0.05	ND	ND
Pb	1.85	1.36	0.82	0.15

### 3.2. Distribution of Metals in Different Croton Samples

Plant constitute is an important link by the transfer of trace elements from soil to man. The level of essential elements in plants varies, the content being affected by the geochemical characteristics of a soil and by the ability of plants to selectively accumulate some of these elements. Bioavailability of the elements depends on the nature of their association with the constituents of a soil. Plants readily assimilate elements through the roots. Additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers that can be absorbed through the leaf blades [37].

The essential and metals in *Croton macrostachyus* leaves and their infusion will be affected by different factors. Physical and chemical properties of the soil, taking medicinal samples from the farm areas (where application of natural and artificial fertilizers taking place), increasing industrialization and associated pollution of the biosphere, storage and processing of Croton samples, climatic condition of the region and other factors are the main contributors for the mineral contents of Croton leaves and their infusion.

#### 3.2.1. Concentration of Metals in Croton Leaves powder

Table 10 shows the mean concentrations of various metals, in the same Croton species plant in different part of the country. From the study, it was revealed that all the metals were accumulated to greater or lesser extents by plant leave studied.

#### **Calcium**

From Table 10, it is clear that calcium is present in all the samples collected from four different regions with concentration ranging from  $1202 \pm 43 \mu\text{g/g}$  to  $7040 \pm 84 \mu\text{g/g}$ . Among all these regions the highest concentration of calcium was found in Akaki Croton leave powder and the lowest in Dilla Croton leave powder. The concentration of calcium found in Bonga and Dilla Croton leaves powder were  $1823 \pm 81 \mu\text{g/g}$  and  $1202 \pm 43 \mu\text{g/g}$ . The high concentrations of Ca are very significant because Ca is known to enhance the qualities of bones and teeth and also of neuromuscular systemic and cardiac functions.

## **Magnesium**

The magnesium concentration varied from  $2961 \pm 32$  to  $271 \pm 2$   $\mu\text{g/g}$ . Dilla Croton leaves had the lowest Mg concentration ( $271 \pm 2$   $\mu\text{g/g}$ ) while Akaki Croton leaves had the highest ( $2961 \pm 32$   $\mu\text{g/g}$ ). The magnesium concentration of Abomsa and Bonga Croton leaves were  $1361 \pm 54$  to  $384 \pm 29$   $\mu\text{g/g}$ . Like calcium, magnesium is critical to many cell functions. It assists in the operation of more than 300 enzymes, is needed for the release and use of energy from the energy-yielding nutrients, and directly affects the metabolism of potassium, calcium and vitamin D. Magnesium acts in the cells of all the soft tissues, where it is part of the protein-making machinery and is necessary for the release of energy.

The abundance of Ca and Mg in the Croton leaves is also in agreement with the previous studies, which indicated that these two elements were the most abundant elements in many medicinal plants and tea leaves [39, 40].

## **Iron**

Iron plays a unique role in the metabolic process. The role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells [41]. Iron deficiency is the most prevalent nutritional deficiency in humans. The Fe concentration varies in the range from  $169 \pm 6$   $\mu\text{g/g}$  in Bonga Croton leaves to  $581 \pm 40$   $\mu\text{g/g}$  in Akaki Croton leaves. The higher concentration of Fe in Akaki suggests the possible use of this medicinal plant to compensate for an iron deficiency. The concentration of iron found in Abomsa and Dilla Croton leaves powder were  $329 \pm 7$   $\mu\text{g/g}$  and  $232 \pm 4$   $\mu\text{g/g}$ , respectively.

The plant in all the four regions accumulate Fe above the limits proposed by FAO/WHO in edible plants (20  $\mu\text{g/g}$ ). However, for medicinal plants the WHO limits not yet been established for Fe. Sheded *et al.* reported that the range of Fe in their study was between 261 to 1239  $\mu\text{g/g}$  in selective medicinal plants of Egypt [42], so that the present result is in good agreement with this range.

## **Manganese**

Coming to the case of manganese, an appreciable amount of Mn is present in all the areas of the study, its concentration is maximum in Dilla Croton leaves  $421 \pm 9 \mu\text{g/g}$  and minimum in Abomsa Croton leaves powder ( $157 \pm 7 \mu\text{g/g}$ ). The concentration of manganese found in Akaki and Bonga Croton leaves powder were  $283 \pm 5 \mu\text{g/g}$  and  $180 \pm 7 \mu\text{g/g}$ , respectively.

Manganese is an antioxidant nutrient and is important in the breakdown of amino acids and production of energy. It is essentially required for the metabolism of Vitamin B<sub>1</sub>, C and E and for the activation of various enzymes which are important for proper digestion, utilization of foods and hence in regulating immune response of the body. It helps in eliminating fatigue and reduces nervous irritability.

Similar to Fe, the plants in all the four regions accumulate Mn above the limits proposed by FAO/WHO in edible plants ( $2 \mu\text{g/g}$ ). However, for medicinal plants the WHO limits not yet been established for Mn, the range of Mn in five medicinal plants studied in Spain had concentration ranging from 46 to 2134 mg/kg [41].

## **Zinc**

From Table 10, it is clear that zinc is present in all the medicinal plants with concentration ranging from  $19.7 \pm 0.6 \mu\text{g/g}$  to  $60.5 \pm 2.3 \mu\text{g/g}$ . Among all these study areas the highest concentration of zinc is found in Akaki Croton leaves powder ( $60.5 \pm 2.3 \mu\text{g/g}$ ) and the lowest concentration is found in Dilla Croton leaves powder ( $19.7 \pm 0.6 \mu\text{g/g}$ ). The concentration of zinc found in Abomsa and Bonga Croton leaves powder are comparable,  $34.5 \pm 2.6 \mu\text{g/g}$  and  $33.6 \pm 1.1 \mu\text{g/g}$ , respectively.

Zn is a dietary trace mineral that, in addition to its many essential functions in growth and developments, is essential for the function of the immune system cells and Zn has been shown to be effective in the treatment of the common cold. Zinc is also an important element responsible for many enzymatic processes and is involved in working of genetic materials, proteins, immune reactions, wound healing, development of the foetus, and sperm production. It has been suggested that normal levels of Zn can prevent diarrhea [44].

The permissible zinc limit set by FAO/WHO in edible plants was 27.4  $\mu\text{g/g}$ . After comparison, metal limit in the studied medicinal plant with those proposed by FAO/WHO it is found that only Dilla Croton leave is within this limit while all others study area accumulate Zn above this limit. However, for medicinal plants the WHO limits not yet been established for Zn. According to Bowen and Allaway, the range of Zn in agricultural products should be between 15 to 200  $\mu\text{g/g}$  [43].

### **Copper**

Copper was detected in all samples with varying concentrations. Leaves powder of Akaki contain the highest amount ( $18.6 \pm 0.7 \mu\text{g/g}$ ) of copper while least concentration was exhibited in the leaves powder of Dilla ( $6.31 \pm 0.48 \mu\text{g/g}$ ). Abomsa and Bonga leaves powder contains comparable amount of copper,  $8.20 \pm 0.70 \mu\text{g/g}$  and  $7.75 \pm 0.59 \mu\text{g/g}$ , respectively.

Cu is essential for a variety of biochemical processes and is needed for certain critical enzymes to function in the body. Copper is also involved in the functioning of the nervous system, in maintaining the balance of other useful metals in the body such as Zn and molybdenum, and is necessary for the normal function of the immune system [44].

Although the results are not in the permissible limit of copper set by FAO/WHO in edible plants 3.0  $\mu\text{g/g}$ , the results obtained from our medicinal plant are in the permissible limit of copper set by China and Singapore in medicinal plants, which were 20  $\mu\text{g/g}$  and 150  $\mu\text{g/g}$  respectively. Reddy and Reddy also reported that the range of Cu contents in the 50 medicinally important leafy material growing in India were 17.6  $\mu\text{g/g}$  to 57.3  $\mu\text{g/g}$  [45].

### **Cobalt**

The Co content was observed to be the highest,  $34.5 \pm 2.6 \mu\text{g/g}$  in Abomsa Croton leaves powder and relatively comparable in Bonga Croton leaves powder ( $33.6 \pm 1.1 \mu\text{g/g}$ ). The concentration of cobalt found in Akaki and Dilla Croton leaves powder are  $21.8 \pm 0.7 \mu\text{g/g}$  and  $19.7 \pm 0.6 \mu\text{g/g}$ , respectively.

Cobalt is an essential element for plants having the capability to fix nitrogen in root tubercles and it is essential for the B<sub>12</sub> vitamin and the thyroid metabolism; the daily ingestion must be around 3 µg [46].

There are no established criteria for Co in medicinal plants. Basgel and Erdemoglu (2006) determined Co concentration ranged between 0.14 to 0.48 µg/g in seven herbs in Turkey [47].

### **Chromium**

Another essential element, Cr, acts a co-factor in the insulin synthesis and in the cholesterol and blood triglycerides control. The Cr content was observed to be the highest,  $87.5 \pm 6.6$  µg/g in Akaki Croton leaves powder and relatively comparable in Abomsa Croton leaves powder ( $81.5 \pm 2.2$  µg/g). The concentration of chromium found in Bonga and Dilla Croton leaves powder are  $21.3 \pm 0.9$  µg/g and  $60.2 \pm 2.6$  µg/g, respectively.

Chromium plays an important role in diabetes treatment. Chromium is found in the pancreas, which produces insulin. Chromium deficiency may also be a risk factor in arteriosclerotic disease [48]. We found that the chromium content was maximum,  $87.5 \pm 6.6$  µg/g in Akaki Croton leaves powder. Hence the use of this medicinal plant may be advised for the treatment and control of diabetics. However, chronic exposure to Cr may result in liver, kidney and lung damage.

### **Nickel**

Abomsa Croton leave accumulate lowest Ni that is,  $2.65 \pm 0.16$  µg/g and Akaki Croton leave accumulate maximum that is  $26.2 \pm 1.1$  µg/g. The concentration of nickel found in Bonga and Dilla Croton leaves powder were  $3.16 \pm 0.26$  µg/g and  $11.78 \pm 1.02$  µg/g. The permissible limit set by FAO/WHO in edible plants was 1.63 µg/g. However, for medicinal plants the WHO limits not yet been established for Ni. Ni toxicity in human is not a very common occurrence because its absorption by the body is very low [49].

### **Cadmium and Lead**

Other metals such as Pb and Cd had no biochemical or physiological importance, so they are considered as very toxic pollutants. In this study, cadmium was not detected in Dilla and Bonga

Croton leaves powder. Elevated Cd concentrations were determined in Abomsa Croton leave  $0.75 \pm 0.024 \mu\text{g/g}$  next to Akaki Croton leave  $1.08 \pm 0.09 \mu\text{g/g}$ .

The permissible limit for Cd set by FAO/WHO in edible plants was  $0.2 \mu\text{g/g}$ . However, for medicinal plants the permissible limit for Cd set by WHO, China and Thailand was  $0.3 \mu\text{g/g}$ .

The content of Pb in the Croton leaves analyzed varied between 10.5 and  $21.9 \mu\text{g/g}$  which is higher than the permissible limit for medicinal plant set by China, Malaysia, Thailand and WHO ( $10 \text{ mg kg}^{-1}$ ). The maximum concentrations of Pb were determined in Abomsa Croton leaves ( $11.5 \pm 0.2 \mu\text{g/g}$ ) next to Akaki Croton leaves ( $21.9 \pm 0.9 \mu\text{g/g}$ ). The concentration of Pb found in Bonga and Dilla Croton leaves were  $20.7 \pm 1.2 \mu\text{g/g}$  and  $10.5 \pm 0.1 \mu\text{g/g}$ , respectively.

The relatively high concentrations of Pb and Cd found in the Croton samples are certainly due to industrial pollution as well as the addition of some fertilizers and herbicides on the farm and farm sides. In addition, it was reported that the contamination of plants with Pb depends on several factors, for example, traffic densities and distance from the road. On the other hand heavy metal contents of medicinal plants depend on the plant species and climatic factors.

The level of metals in the Croton leave sample areas are shown in Figure 4 and 5 indicating the decreasing concentration order of metals  $\text{Ca} > \text{Mg} > \text{Fe} > \text{Mn} > \text{Cr} > \text{Zn} > \text{Pb} > \text{Ni} > \text{Co} > \text{Cu} > \text{Cd}$  for Akaki,  $\text{Ca} > \text{Mg} > \text{Fe} > \text{Mn} > \text{Cr} > \text{Zn} = \text{Co} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Cd}$  for Abomsa,  $\text{Ca} > \text{Mg} > \text{Mn} > \text{Fe} > \text{Zn} = \text{Co} > \text{Cr} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Cd}$  and  $\text{Ca} > \text{Mn} > \text{Mg} > \text{Fe} > \text{Cr} > \text{Zn} = \text{Co} > \text{Ni} > \text{Pb} > \text{Cu} > \text{Cd}$  for Dilla.

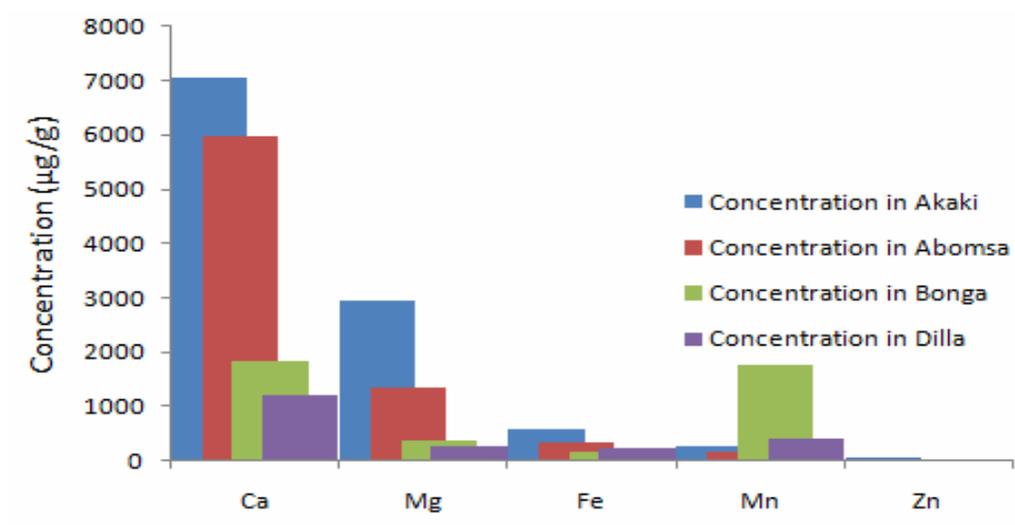


Figure 4. Concentration ( $\mu\text{g/g}$ ) of metals in different Croton leaves sample.

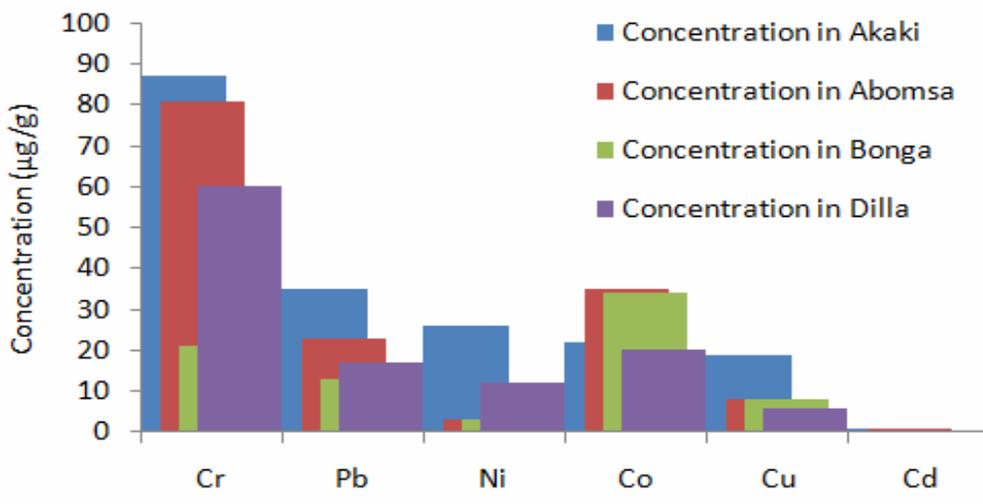


Figure 5. Concentration ( $\mu\text{g/g}$ ) of microelement in different Croton leaves sample.

### **3.2.2. Concentration of Metals in Croton Leaves Infusion Samples**

The element concentrations in Croton leaves infusion were determined to assess the actual amount of exposure to these elements by drinking the extract. The concentration of 11 elements determined for four different time of Croton leave infusions were calculated on the basis of 25 g of fresh Croton leaves in 250 mL tap water. This was done on the basis of what most people in the rural area do by counting the number of leaves (from 8 to 14 leaves) and taking it into about two cups of water (approximately 250 mL water), i.e. the infusion samples were obtained while taking into account preparation habits by the traditional medicians. The results indicated that there was a large variation of the element concentrations in different time of infusions. The highest average levels of metals in infusion were found in the 24 h infusion, whereas the lowest average levels of metals were detected in the 3 h infusion.

#### **3.2.2.1. Concentration of Metals in a 24 and 12 Hours Infusion**

Table 11 shows the levels of metals measured in Croton leaves infusion prepared for 24, 12, 6 and 3 hours. Ca was observed in the largest amount  $1087 \pm 41 \mu\text{g/g}$  followed by Mg,  $52.6 \pm 2.2 \mu\text{g/g}$  in the 24 hour infusion sample. Zn was found in larger amount than Fe with concentrations ( $9.51 \pm 0.39$ ,  $6.53 \pm 0.24 \mu\text{g/g}$ ), respectively. Mn ( $3.42 \pm 0.04 \mu\text{g/g}$ ) was found in appreciable amount. Among the trace metals Cr were detected in larger amount ( $13.4 \pm 0.9 \mu\text{g/g}$ ). With the exception of Cr, less amount of trace metals were detected in the 24 hours infusion of which Co ( $2.83 \pm 0.08 \mu\text{g/g}$ ) was in slightly higher concentration than others. The concentrations of Ni ( $2.80 \pm 0.013 \mu\text{g/g}$ ) found were greater than the concentration of Cu ( $2.54 \pm 0.11 \mu\text{g/g}$ ).

The toxic heavy metals Cd and Pb were also detected in the 24 hour infusion sample. The amount of Cd and Pb detected in this infusion sample were  $0.21 \pm 0.007 \mu\text{g/g}$  and  $2.07 \pm 0.043 \mu\text{g/g}$ , respectively.

Similar to the 24 hours infusion, the concentration of Ca ( $687 \pm 40 \mu\text{g/g}$ ) was greater than Mg ( $28.1 \pm 0.5 \mu\text{g/g}$ ). Among the trace metals Cr ( $6.53 \pm 0.18 \mu\text{g/g}$ ) was the first followed by Fe ( $5.14 \pm 0.06 \mu\text{g/g}$ ) and Zn ( $4.64 \pm 0.30 \mu\text{g/g}$ ), respectively. The amount of Co ( $2.42 \pm 0.013$

$\mu\text{g/g}$ ), Cu ( $2.47 \pm 0.004 \mu\text{g/g}$ ) and Ni ( $2.51 \pm 0.095 \mu\text{g/g}$ ) detected were smaller than the amount of Mn ( $3 \pm 0.07\mu\text{g/g}$ ). The amount of toxic metals (Cd and Pb) detected in the 12 hours infusion samples ( $0.16 \pm 0.007 \mu\text{g/g}$  and  $1.58 \pm 0.039 \mu\text{g/g}$ , respectively) were smaller than the amount detected in the 24 hours infusion.

The levels of the metals are indicated in Figure 6 and 7 showing decreasing concentration trend of Ca > Mg > Cr > Zn > Fe > Mn > Co > Ni > Cu > Pb > Cd for the 24 h infusion and Ca > Mg > Cr > Fe > Zn > Mn > Ni > Cu > Co > Pb > Cd for the 12 h infusion respectively.

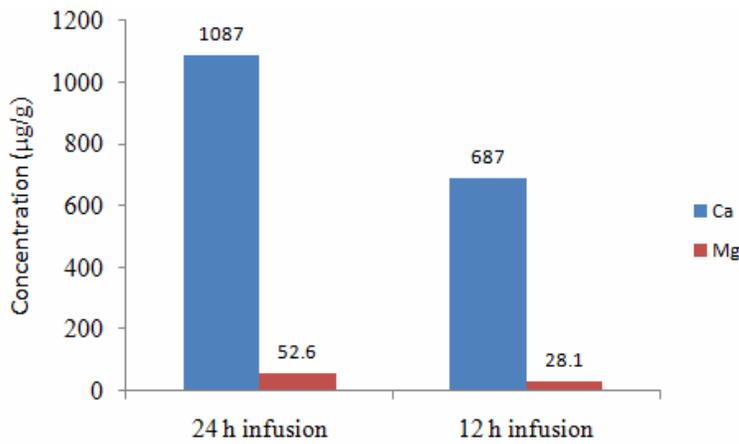


Figure 6. Concentration ( $\mu\text{g/g}$ ) of the essential macroelemnts in 24 h and 12 h infusion.

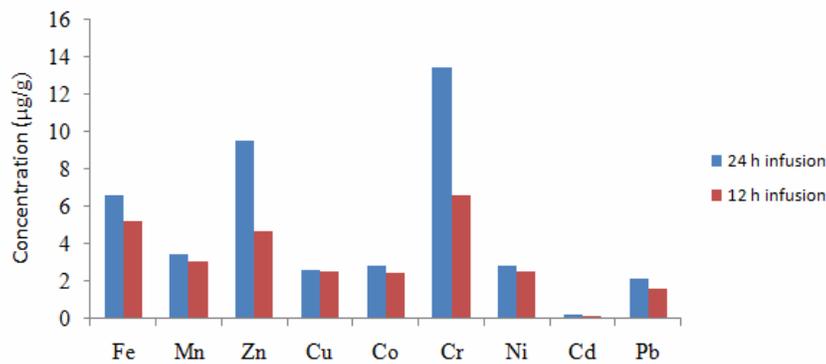


Figure 7. Concentration ( $\mu\text{g/g}$ ) of the essential and non-essential microelements in 24 h and 12 h infusion.

### 3.2.2.2. Concentration of Metals in 6 and 3 Hours Infusion

Similar to the 24 h and 12 h infusion metal extraction into the supernatant solution, there is a great variation in the 6 h and 3 h infusion. In both cases, Mg was the first next to Ca. However, the concentration of Cr was the third for the 6 h infusion while Fe was the case for the 3 h infusion. Beside these, there was a difference in the degree of extraction of metal in the 6 h and 3 h infusions as shown below indicating decreasing order of (Ca > Mg > Cr > Fe > Zn > Mn > Ni > Cu > Co > Pb for 6 h and Ca > Mg > Fe > Cr > Zn > Ni = Co = Mn > Co > Pb for 3 h infusions) metal concentration.

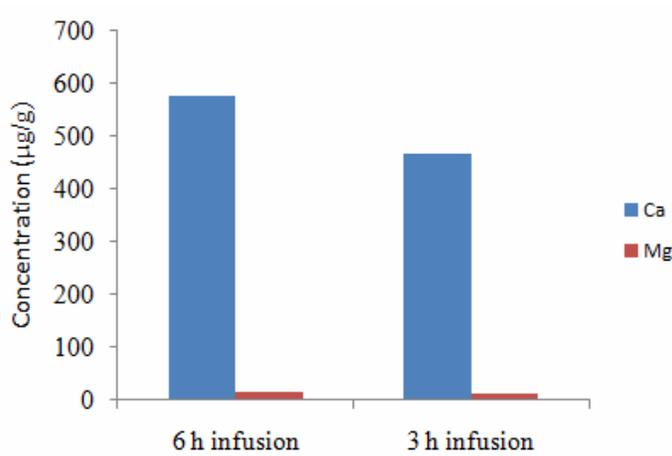


Figure 8. Concentration (µg/g) of the essential macroelemnts in 6 h and 3 h infusion.

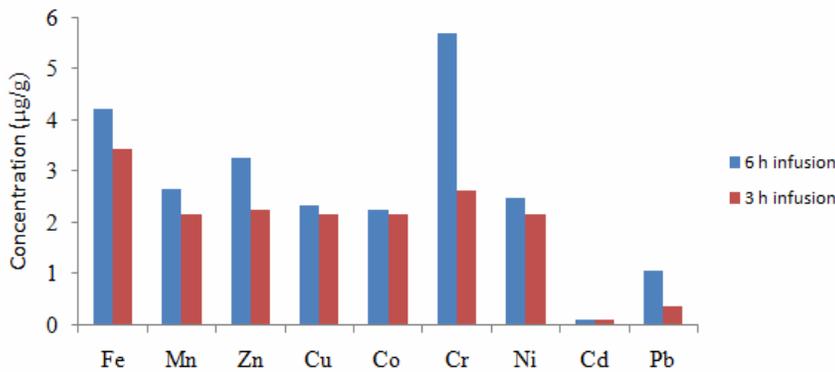


Figure 9. Concentration (µg/g) of the essential and non-essential microelements in 6 h and 3 h infusion.

### 3.7. Comparison of Metals in the Croton Leaves Powder and Infusion Samples

The concentrations of the elements in the analyzed samples were quite varied. The order of the metal concentration for Akaki Croton leaves powder and Abomsa Croton leaves powder is almost similar except that the Akaki Croton leave powder with higher mean concentration of Ni than Mg and lower Co concentration than Pb was observed to differ. Beside these the concentration of Zn and Co in Abomsa Croton leave powder are equal which was not true in Akaki Croton leaves powder. The trend in metal concentration for that of the Bonga Croton leaves powder is similar to Dilla Croton leaves powder except that the concentration of Cr is greater than that of Zn and Co which were equal in Dilla Croton leave powder. Ca was found at highest concentration than other metals in all the four sample sites, with the mean concentration ranging from 1202  $\mu\text{g/g}$  (Dilla Croton leaves powder) to 7040  $\mu\text{g/g}$  (Akaki Croton leaves powder). Mg was the next highest than other metals for Akaki (2961  $\mu\text{g/g}$ ) and Abomsa (1361  $\mu\text{g/g}$ ) sites. But Mn was the next highest than the other metals for Bonga (180  $\mu\text{g/g}$ ) and Dilla (420  $\mu\text{g/g}$ ) sites.

Cr was found in highest amount out of the microelements with mean concentration ranging from 60.2  $\mu\text{g/g}$  (Dilla Croton leaves powder) to 87.5  $\mu\text{g/g}$  (Akaki croton leaves powder) followed by Pb with mean concentration range 10.5  $\mu\text{g/g}$  (Dilla Croton leaves powder) to 21.9  $\mu\text{g/g}$  (Akaki Croton leaves powder). The lowest concentration observed for all the four sites was that of Cd, ranging from 0.03  $\mu\text{g/g}$  (Abomsa Croton leaves powder) to 1.08  $\mu\text{g/g}$  (Akaki Croton leaves powder). Overall, the concentrations of each metal in the four sites are comparable.

In the infusion samples, Ca was also observed at higher concentration than other metals with concentration range from 419  $\mu\text{g/g}$  (12 h infusion) to 1040  $\mu\text{g/g}$  (24 h infusion). Unlike the powder samples for Bonga and Dilla, Mg with mean concentrations ranging from 9.71  $\mu\text{g/g}$  (3 h infusion) to 48.4  $\mu\text{g/g}$  (24 h infusion) was coming next to Ca similar to the powder samples for Akaki and Abomsa.

Unlike the powder samples, the mean concentration of Cr ranging from 1.43 µg/g (3 h infusion) to 12.3 µg/g (24 h infusion) was higher than that of Fe with mean concentration range 0.10 µg/g (3 h infusion) to 3.20 µg/g (24 h infusion) and Zn with range of 0.68 µg/g (3 h infusion) to 7.95 µg/g (24 h infusion) was higher than Cu with range 1.99 µg/g (3 h infusion) to 2.36 µg/g (24 h infusion).

Table 14. Metal concentrations (µg/g) of Croton leaves powder in wet weight basis and range of concentration of metals (µg/g) in infusion samples. \*The moisture content of the plant was 76%.

Metal	<sup>a</sup> Croton leaves powder	<sup>b</sup> Croton leaves infusion
Ca	1688	419 – 1040
Mg	710	9.71 – 48.4
Fe	139	0.10 – 3.2
Mn	67.9	2.17 – 3.42
Zn	14.6	0.68 – 7.95
Cu	4.56	1.99 – 2.36
Co	5.28	1.59 – 2.27
Cr	20.9	1.43 – 12.3
Ni	6.24	2.02 – 2.65
Cd	0.26	0.05 – 0.10
Pb	5.27	0.15 – 1.85

<sup>a</sup> Croton leaves powder in wet weight basis. <sup>b</sup> Croton leaves infusion in absence of tap water.

As time of infusion decreases from 24 h to 3 h, the toxic heavy metals Pb and Cd were found to be too low to be detected by the available method. The concentrations were observed to be below the method detection limit for Cd in the 12 h to 3 h infusion of time.

Generally, Akaki Croton leaves powder contained higher concentration of all metals than other Croton leaves samples but its Mn and Co contents were least. Abomsa Croton leaves powder

contained higher concentration of Ca, Mg, Fe, Zn, Cu, Cr and Cd next to Akaki Croton leaves powder while Dilla Croton leaves powder contained least amount of Ca, Mg, Zn, Cu, Cr, Cd and Pb as can be seen from Table 15. This trend of concentration is also similar for the other Croton leaves samples.

Table 15. Order for the level of concentrations of metals in the Croton leaves powder.

Metal	Order for concentration level			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Ca	Akaki	Abomsa	Bonga	Dilla
Mg	Akaki	Abomsa	Bonga	Dilla
Fe	Akaki	Abomsa	Dilla	Bonga
Mn	Bonga	Dilla	Akaki	Abomsa
Zn	Akaki	Abomsa	Bonga	Dilla
Cu	Akaki	Abomsa	Bonga	Dilla
Co	Abomsa	Bonga	Akaki	Dilla
Cr	Akaki	Abomsa	Bonga	Dilla
Ni	Akaki	Dilla	Bonga	Abomsa
Cd	Akaki	Abomsa	ND	ND
Pb	Akaki	Bonga	Abomsa	Dilla

### 3.8. Comparison of the Metal Content in Croton Leaf Sample with Other Medicinal Plants

Table 16. Comparison of metal concentration ( $\mu\text{g/g}$ ) in Croton leaves with other medicinal plant leaves in worldwide.

Medicinal Plant	Origin	Part used	Concentration ( $\mu\text{g/g}$ ) of metals in medicinal plants					Ref.
			Ca	Mg	Mn	Fe	Zn	
<i>Taraxacum officinale</i> Weber	Spain	Leaves	29247	4461	101	853	68	43
<i>Eucalyptus globulus</i> Labill	Spain	Leaves	18621	1616	2134	89	23	43
<i>Plantago major</i> L.	Bulgaria	Leaves	48022	6405	46	373	56	43
<i>Mentha piperita</i> L.	Bulgaria	Leaves	21131	5483	116	376	45	43
<i>Matricaria chamomilla</i> L.	Egypt	Leaves	926	2642	76	701	49	43
<i>Calotropis procera</i> Ait.	Nigeria	Leaves	18900	-	231.5	1871.5	71.7	47
<i>Acalypha wilkensiana</i>	Nigeria	Leaves	28400	-	44.4	760.1	75.3	47
<i>Euphorbia hirta</i> Linn.	Nigeria	Leaves	8120	-	52	535.7	191.1	47
<i>Pelargonium graveolens</i> L.	Egypt	Leaves	-	-	26.4	516	12.1	49
<i>Marjorana hortensis</i> L.	Egypt	Leaves	-	-	28	671	10.59	49
<i>Gynostemma pentaphyllum</i>	Thailand	Leaves	5583- 34070	1756-7739	43.42-259.4	125.5-2321	25.43-61.95	40
<i>Camellia sinensis</i>	Thailand	Leaves	1384-6550	783.6-2549	229.4-1512	20.91-318.3	10.13-55.40	40
<i>Morus alba</i>	Thailand	Leaves	15286-25182	3078-5188	75.27-352.7	89.46-408.2	19.16-34.42	40
<i>Croton macrostachyus</i>	Ethiopia	Leaves	1201-7040	271-2961	157-420	167- 581	20-61	This study

Medicinal Plant	Origin	Part used	Concentration ( $\mu\text{g/g}$ ) of metals in medicinal plants					Ref.
			Cu	Ni	Cr	Cd	Pb	
<i>Taraxacum officinale</i> Weber	Spain	Leaves	27	-	-	-	7	43
<i>Eucalyptus globulus</i> Labill	Spain	Leaves	10	-	-	-	6	43
<i>Plantago major</i> L.	Bulgaria	Leaves	13	-	-	-	6	43
<i>Mentha piperita</i> L.	Bulgaria	Leaves	19	-	-	-	8	43
<i>Matricaria chamomilla</i> L.	Egypt	Leaves	20	-	-	-	3	43
<i>Calotropis procera</i> Ait.	Nigeria	Leaves	137.8	235.2	198.6	-	-	47
<i>Acalypha wilkensisiana</i>	Nigeria	Leaves	146.1	203.6	34.6	-	-	47
<i>Euphorbia hirta</i> Linn.	Nigeria	Leaves	234.8	241.1	-	-	-	47
<i>Marjorana hortensis</i> L.	Egypt	Leaves	3.95	25.25	2.15	2.05	14.4	49
<i>Gynostemma pentaphyllum</i>	Thailand	Leaves	5.141-15.48	0.530-6.600	0.434-12.42	0.021-4.772	0.361-64.40	40
<i>Camellia sinensis</i> ,	Thailand	Leaves	3.075-22.42	2.281-9.194	0.205-10.54	0.002-0.100	0.060-53.89	40
<i>Morus alba</i>	Thailand	Leaves	5.724-11.09	0.368-2.171	0.250-1.419	0.001-0.022	0.118-1.185	40
<i>Croton macrostachyus</i>	Ethiopia	Leaves	6 - 19	3 - 26	21 - 87	0.75 - 1.08	10 - 22	This study

Comparison of levels of metals in *Croton macrostachyus* leaves with other medicinal plants reported in various parts of the world are summarized in Table 16. The results of present study are in good agreement with the most of reported values.

### 3.9. Comparison of the Metal Content in Croton Leaves Infusion with Infusion of Other Medicinal Plants

Table 17. Comparison of metal contents of Croton leaves infusion with other medicinal plant leaves infusion in worldwide.

Medicinal Plant	Concentration ( $\mu\text{g/g}$ ) of Metals in <sup>a</sup> infusion of Medicinal Plants					
	Ca	Mg	Mn	Fe	Zn	Ref.
<i>Taraxacum officinale</i> Weber	134	53	0.566	0.13	0.262	43
<i>Eucalyptus globulus</i> Labill	24	27	19.085	0.06	0.123	43
<i>Plantago major</i> L.	528	114	0.498	0.11	0.494	43
<i>Mentha piperita</i> L.	171	99	1.206	0.10	0.181	43
<i>Matricaria chamomilla</i> L.	82	41	0.319	0.50	0.380	43
<i>Gynostemma pentaphyllum</i>	6272-40011	2153-9610	48.26-276.6	5.481-59.55	7.517-50.19	40
<i>Camellia sinensis</i> ,	136.0-751.5	764.3-2152	137.5-741.7	4.444-21.62	5.947-43.32	40
<i>Morus alba</i>	1576-8506	887.6-5218	9.572-94.96	0.00-37.61	2.747-37.60	40
<i>Croton macrostachyus</i>	419-1039	9.71- 48.4	2.17-3.42	0.1-48.39	0.68-7.95	This study

Medicinal Plant	Concentration ( $\mu\text{g/g}$ ) of Metals in <sup>a</sup> infusion of Medicinal Plants				
	Cu	Cr	Cd	Pb	Ref.
<i>Taraxacum officinale</i> Weber	0.182	-	-	0.011	43
<i>Eucalyptus globulus</i> Labill	0.068	-	-	0.049	43
<i>Plantago major</i> L.	0.105	-	-	0.007	43
<i>Mentha piperita</i> L.	0.112	-	-	0.025	43
<i>Matricaria chamomilla</i> L.	0.207	-	-	0.036	43
<i>Gynostemma pentaphyllum</i>	1.622-7.993	0.00-1.405	0.1025-2.307	0.1126-8.017	40
<i>Camellia sinensis</i> ,	1.201-8.427	0.00-0.6911	0.004-0.0237	0.0004-3.162	40
<i>Morus alba</i>	1.478-7.385	0.00-0.3968	0.00-0.0284	0.0075-0.1510	40
<i>Croton macrostachyus</i>	1.99-2.36	1.43-12.3	0.098	0.16-1.85	This study

Comparison of levels of metals in *Croton macrostachyus* leaves infusion with other medicinal plants infusion reported in various parts of the world are summarized in Table 17. The results of present study are in good agreement with the most of reported values.

### **3.6. Leaching of the Metals in to Croton leaves Infusion**

As can be seen from Table 16, each metal is extracted from the Croton leaves to the water at different rate. In addition to its highest concentration in the Croton leaves powder, Ca (61.5%) was found in large amount for the 24 h infusion with more extraction efficiency followed by Cr (58.8%), Zn (54.3%) and Cu (51.7%) extracted to more than 50%. Exactly 50% of Mn was extracted for the 24 h infusion. In the 24 h infusion, less than half of the amount in the fresh Croton leaves was extracted for the case of Co (43.0%), Ni (42.4%), Cd (37.8%) and Pb (35.0%). In the 24 h infusion only 6.80% of Mn and 2.30% of Fe were released into the water extract.

For the 12 h infusion, the extraction efficiency of Ca (37.8%), Ni (37.8%) and Co (35.3%) were the highest next to Cu (50.2%), respectively. Pb (25.7%), Cr (25.6%) and Zn (20.8%) have comparable extraction efficiency at the 12 h infusion. The smallest extraction efficiency was observed for Fe (2.40%), Mg (3.40%) and Mn (4.40%), respectively.

Although a very high Ca concentration was present in all infusion samples, the percence of Ca released was very low, especially for the 6 h and 3 h infusions.

Similarly maximum extraction efficiency of Cu (47.1%) and (43.6%) was observed for the 6 h and 3 h infusions respectively. Next to Cu, Ca showed maximum extraction efficiency both in the 6 h and 3 h infusions. There were no differences in the percent of infusions of Fe for 24 h and 12 h as well for 6 h and 3 h. For the four type of infusion done on Croton fresh leave, Mg and Fe had the lowest percent releases in infusions.

Although the concentrations of Cd and Pb seem to be lower, these toxic elements can be easily released at the higher rate in the infusions within longer period of time, i.e. the toxic elements,

Cd and Pb, were leached from fresh Croton leaves samples at highest rates, (37.8%) and (35.0%), respectively, in the 24 h infusion. So one can minimize the percentage of leaching of these toxic metals by decreasing time of infusion.

The decreasing rate of extraction of metals in the 24 h, 12 h, 6 h and 3 h Croton leave infusion samples are shown in Figures 20-24.

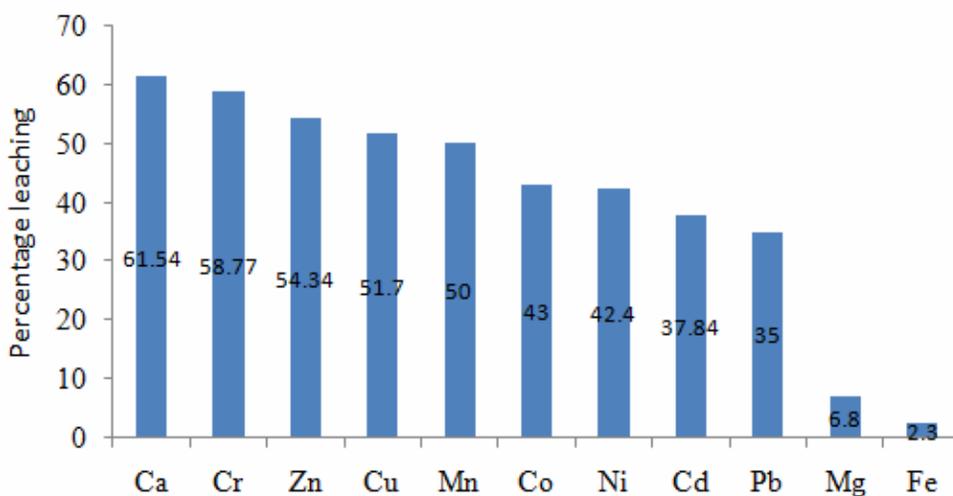


Figure 10. Percentage leaching of metals in a 24 h Croton leaves infusion.

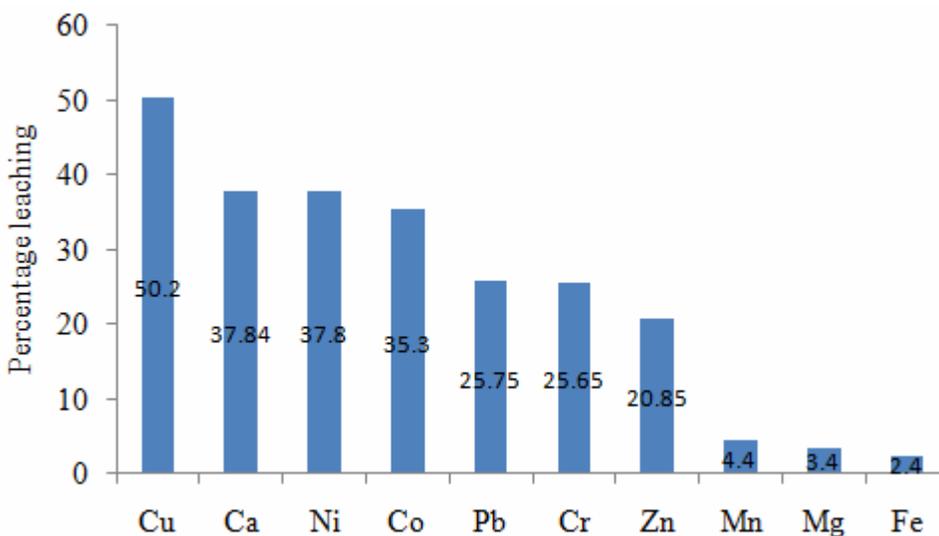


Figure 11. Percentage leaching of metals in a 12 h Croton leaves infusion.

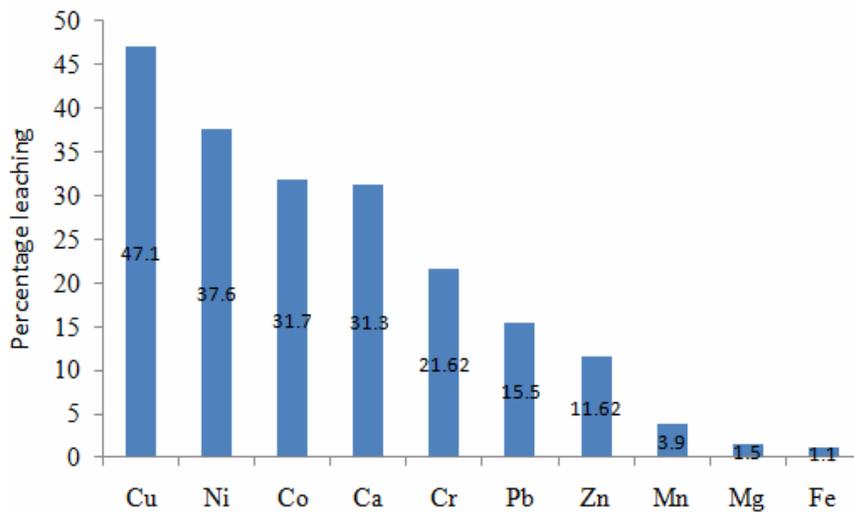


Figure 12. Percentage leaching of metals in a 6 h Croton leaves infusion.

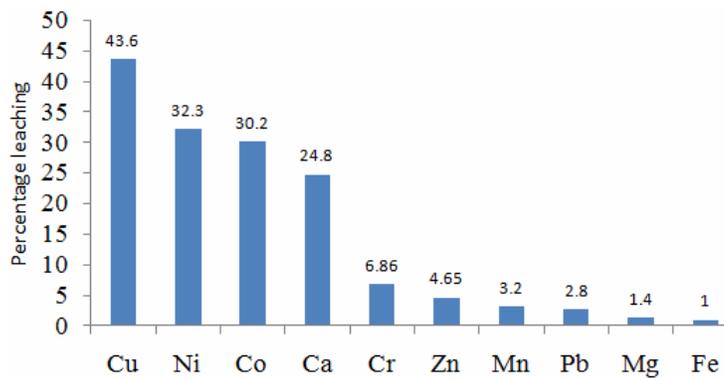


Figure 13. Percentage leaching of metals in a 3 h Croton leaves infusion.

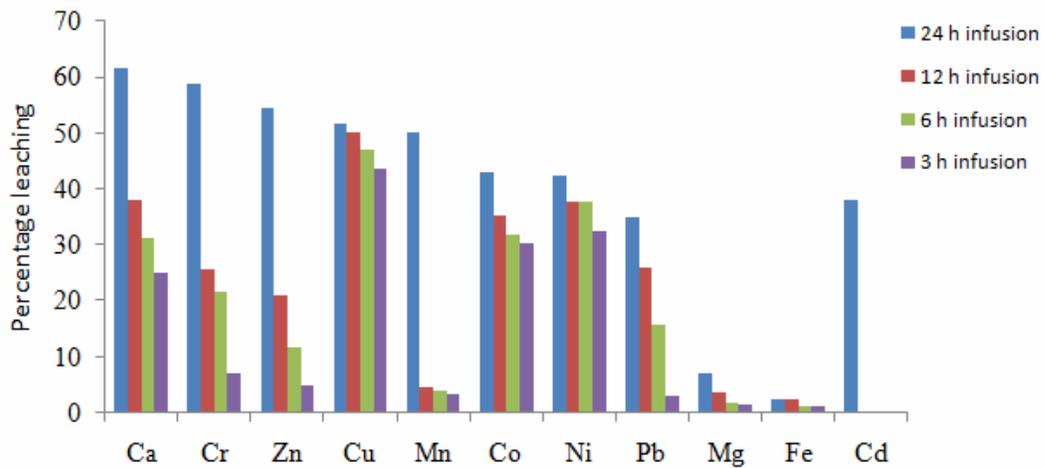


Figure 14. Percentage leaching of metals in 24 h, 12 h, 6 h and 3 h Croton leaves infusions.

Table 18. Efficiency of extraction of metals in to the Croton leave infusion from wet (fresh) Croton leaves at different time interval.

Metal	Percentage leaching (%)			
	24 hour infusion	12 hour infusion	6 hour infusion	3 hour infusion
Ca	61.5	37.8	31.3	24.8
Mg	6.80	3.40	1.50	1.40
Fe	2.30	2.40	1.10	1.00
Mn	50	4.40	3.90	3.20
Zn	54.3	20.9	11.62	4.65
Cu	51.7	50.2	47.1	43.6
Co	43.0	35.3	31.7	30.2
Cr	58.8	25.7	21.6	6.86
Ni	42.4	37.8	37.6	32.3
Cd	37.8	19.3	ND	ND
Pb	35.0	25.7	15.5	2.80

### 3.7. Statistical Analysis

Analysis of variance (ANOVA) is a powerful statistical technique which can be used for the separation and estimation of the different causes of the variation of the more than one means obtained for different experiments. As the sample means vary from one sample to another, ANOVA tests whether there is a difference between the samples means and thus enabling to explain the cause of error. ANOVA is used to test hypothesis about differences between two or more means [34]. For the present study, the significance of variation between samples was analyzed using one-way ANOVA which can be made using detail calculations following a statistical formula or by computer using excel, Minitab and SPSS software. For the present study SPSS (SPSS 13.0 for window, The Apache software foundation, 2000) was used for statistical analysis to know the presence or absence of significant difference in mean concentration of each metal between the analyzed Croton samples.

Except between Abomsa/Dilla Croton leave powder samples, there is a significant difference ( $p < 0.05$ ) in mean concentrations of Ca at 95% confidence interval between all the four samples of Croton leave powder (Akaki/Abomsa, Akaki/Bonga, Akaki/Dilla, Bonga/Abomsa and Bonga/Dilla). For Mg, Fe Mn and Cd there is significant difference ( $p < 0.05$ ) at 95 % confidence interval was observed in their mean concentrations between all the four Croton leave powder samples (Akaki/Abomsa, Akaki/Bonga, Akaki/Dilla, Abomsa/Dilla, Bonga/Abomsa and Bonga/Dilla).. The mean concentrations of Zn and Co differ significantly ( $p < 0.05$  at 95 % confidence interval) for Akaki and Abomsa Croton leave powders while it does not differ significantly ( $p > 0.05$  at 95 % confidence interval) for Akaki compared to Bonga and Dilla. Similarly, Zn and Co have a mean concentration that doesn't differ significantly between the Abomsa/Bonga, Abomsa/Dilla and Bonga/Dilla. The mean concentration of both Ni and Cu are significantly different between all the sample sites. However, the mean concentration of Ni and Cu do not differ significantly ( $p < 0.05$  at 95% confidence interval) between Abomsa and Dilla. Except Akaki compared with Abomsa and Akaki compared with Bonga for Cr and Pb respectively, there is a significant difference ( $p < 0.05$ ) at 95% confidence level in the mean concentration between all sites.

The Croton leave infusion samples were also analyzed using the same statistical analysis technique. The mean concentrations of Ca, Mg, Fe, Mn, Cr, Cd and Pb are significantly different

( $p < 0.05$ ) at 95% confidence interval between all the infusion samples. For Zn and Ni, there is no significant difference ( $p > 0.05$ ) at 95% confidence interval in mean concentration between infusions prepared for 12 h and 6 h. However there is a significant difference in mean concentrations between the other infusion samples (24 h infusion/12 h infusion, 24 h infusion/6 h infusion, 24 h infusion/3 h infusion, 12 h infusion/3 h infusion and 6 h infusion/3 h infusion. 12 h infusion and 6 h infusion and 6 h infusion and 3 h infusion showed insignificant difference in the mean concentration of Co and significant difference for the remaining pair wise.

Absence of insignificant difference in some mineral nutrient among samples from Akaki, Abomsa, Bonga and Dilla may indicate that since these areas are under the same geographical location and share common climatic conditions. Similarly presence of significant difference in concentration for some minerals of Croton leaves powder and their infusion at different time might arise from the chemical composition of the leaves itself, the specific environmental conditions in which the plant was grown or the studied area might contains higher concentration of mineral nutrient in the soil.

### 3.8. Pearson Correlation of Metals

The Pearson correlation matrices using correlation coefficient (r) for the samples were shown in Table 17 and 18 for Croton leaves powder and Croton leaves infusion samples, respectively.

Table 19. Correlation matrices for metals in Croton leaves powder

	Ca	Mg	Fe	Mn	Zn	Cu	Co	Cr	Ni	Cd	Pb
Ca	1										
Mg	0.919	1									
Fe	0.871	0.982	1								
Mn	-0.590	-0.510	-0.612	1							
Zn	0.821	0.939	0.876	-0.185	1						
Cu	0.750	0.944	0.927	-0.275	0.965	1					
Co	0.071	-0.234	-0.398	0.415	-0.086	-0.343	1				
Cr	0.795	0.739	0.805	-0.954	0.466	0.523	-0.351	1			
Ni	0.465	0.757	0.840	-0.440	0.679	0.846	-0.790	0.564	1		
Cd	0.993	0.959	0.923	-0.592	0.865	0.819	-0.033	0.803	0.568	1	
Pb	0.301	0.497	0.388	0.489	0.760	0.697	0.099	-0.217	0.380	0.351	1

Table 20. Correlation matrices for metals in Croton leave infusion samples.

	Ca	Mg	Fe	Mn	Zn	Cu	Co	Cr	Ni	Pb
Ca	1									
Mg	0.987	1								
Fe	0.948	0.944	1							
Mn	0.929	0.901	0.987	1						
Zn	0.999	0.989	0.935	0.911	1					
Cu	0.873	0.855	0.978	0.988	0.995	1				
Co	0.996	0.996	0.959	0.929	0.995	0.883	1			
Cr	0.993	0.962	0.942	0.943	0.989	0.883	0.980	1		
Ni	0.930	0.872	0.940	0.977	0.913	0.938	0.911	0.961	1	
Pb	0.919	0.898	0.991	0.998	0.896	0.994	0.924	0.927	0.961	1

In Croton leave powder sample, for all the metals analyzed except manganese, cobalt and chromium (with Zn, Cu, Co, Ni and Pb) the correlation was very high (close to 1 or -1). This poor relationship may be due to the fact that we are considering different size of leaves, soil type, environmental conditions and capacity of the plant to accumulate specific metal. The high association between metals, evidenced by high positive correlation coefficient, can arise from common anthropogenic or natural sources as well as from similarity in chemical properties. Lead was the least associated with the other metals which might imply different sources. In infusion samples, correlations were again significant among all the metals.

#### 4. Conclusion

In this work, a study of the metal content in the Ethiopian traditional medicinal plant - *Croton macrostachyus* leaves powder and their infusion has been carried out. The concentration of eleven elements Ca, Mg, Fe, Mn, Zn, Cu, Cr, Co, Ni, Cd and Pb have been analyzed by flame atomic absorption spectrometry (FAAS). The wet digestion in a digester heater block using Kjeldahl method developed in this study provides an effective method for the digestion of both the Croton leaves powder and its infusions. This was revealed by the excellent recoveries (93-105%) obtained which were found in the acceptable range for the analyzed metals.

The non-essential toxic metals Cd was not detected in Dilla and Bonga Croton leave samples revealing that they are free from this trace heavy metal. Generally, the Croton leave powders contained more amounts of metals than the infusions and it was rich in Ca to a largest extent followed by Mg and Fe for Akaki and Abomsa and for Bonga and Dilla sites, respectively. Fe concentration was determined to be the highest followed by Cr and Zn out of the trace microelements Croton leaves powder taken from Akaki and Abomsa whereas Mn concentration was the second next to Ca for Bonga and Dilla sites. The Croton leaves infusion prepared from the fresh Croton leaves sample was observed to contain the essential and non-essential elements which could also serve as a medicinal value depending on the amount consumed and their mineral concentrations

Statistical analysis indicates that there were significant variation in the level of some metals for the samples studied which could be mainly arised from the chemical composition of the leaves itself, the specific environmental conditions in which the plant was grown or the studied area might contains higher concentration of mineral nutrient in the soil. The present study also shows that the levels of the metals analyzed are generally comparable well with levels in other medicinal plants from other parts of the world. Poor correlation was observed between Mn as well as Co with almost all metals Croton leave powder samples and good correlation between all metals in Croton leaves infusion was observed

The mineral composition data here obtained proves that the Croton leave powder taken from polluted area and infusion done for average time could be source of some essential metals and thus the medicinal plant infusion and powder taken at right dose is functional. Therefore, it may produce health risks for human consumption, if people take medicinal plants from polluted area than the unpolluted areas. On the other hand they have medicinal value if they are taken from the unpolluted areas at the right dose. As the metals are extracted to the infusions at different rate, further research is suggested on the kinetics of the infusion and form of existence of the metals in different kinds of medicinal plants in Ethiopia

To sum up, *Croton macrostachyus*, which is widely taken in different parts of Ethiopia as medicine, contained both essential and toxic elements in a wide range. Some of them (Akaki and Abomsa sites) can be used as beneficial sources for Ca, Mg, and Fe. At the same time, they may also contain high levels of some toxic elements such as Cd and Pb over the standard limited values for medicinal plants. Although the intakes of Cd from Bonga and Dilla sites and infusion done for average time, in the present study is below the standard limits and may not constitute a health risk from toxic elements originating from these sample sites, it is absolutely essential to have good quality control of plant raw materials and to determine the presence of some contaminants, especially toxic elements, to avoid overconsumption and their cumulative toxicities in long-term use.

## 5. References

1. Lanfranco, G. Invited review article on traditional medicine. *Electronic Journal of Biotechnology*, **1999**, 2, 1–3.
2. Demma, J.; Engidawork, E.; Hellman, B.; Potential genotoxicity of plant extracts used in Ethiopian traditional medicine. *Journal of Ethnopharmacology* **2009**, 122, 136–142.
3. Kassaye K. D.; Amberbir. A.; Getachew. B.; Mussema. Y. A historical overview of traditional medicine practices and policy in Ethiopia. *Ethiopian Journal of Health Development* **2006**, 20, 127-134
4. World Health Organization. *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*. WHO, Geneva, Switzerland, **2001**.
5. Baquar, S. R. The role of traditional medicine in rural environment. In: Issaq, S. (Ed.), *Traditional Medicine in Africa*. East Africa Educational Publishers Ltd., Nairobi, **1995**, pp. 141–142.
6. World Health Organization. *National Policy on Traditional Medicine and regulation of Herbal medicines*, Report of WHO Global Survey, Geneva, Switzerland May **2005**.
7. Abebe, W. Traditional pharmaceutical practices in Gondar Region, northwestern Ethiopia. *Ethnopharmacol* **1984**, 11, 33-47.
8. Abebe, D. The role of herbal remedies and the approaches towards their development. In: *Proceedings of the Workshop on Development and Utilization of Herbal Remedies in Ethiopia*, Nazareth, **1996**, p. 29.
9. Tadeg. H.; Mohammed E.; Asres K.; Gebre-Mariam T. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of Ethnopharmacology* **2005**, 100, 168–175.
10. Gidey, M. An ethanobotanical study of medicinal plants used by the Zay people in Ethiopia. CBM: Skriftserie 3: 81-89 Uppsala **2001**.
11. Desissa D.; Binggeli P. Uses and conservation status of medicinal plants used by the Shinasha people **2000**.
12. Breitenbach, F.V. *The Indigenous Trees of Ethiopia*, Second Revised and Enlarged Edition, Ethiopian Forestry Association, Addis Ababa, **1963**, pp. 306.

13. Gidey, M.; Teklehaymanot, T.; Animut, A.; Mekonnen, Y. Medicinal plants of the Shinasha, Agew and Amhara peoples in the northwest Ethiopia, *Journal of Ethno pharmacology*, **2007**, 110, 516-525.
14. Shukla, P.; Misra, S.P. *An Introduction to Taxonomy of Angiosperms*. Vikas Publishing House Pvt. Ltd., Delhi, **1979**, pp. 556
15. Heywood, V.H. *Flowering Plants of the World*. Andromeda Oxford Ltd., Oxford, **1993**, pp. 335.
16. Berry, P.E. *Floristics and molecular phylogeny of a giant genus – Croton* (Euphorbiaceae) **2000**, [Http://www.botany.wisc.edu/berry/bio.htm](http://www.botany.wisc.edu/berry/bio.htm). Accessed on July 20, 2009.
17. Gilbert, M.G. *Euporbiaceae*. In: *Flora of Ethiopia and Eritrea. Canalicaceae to Euporbiaceae*, (Edwards, S.; Tadesse, M.; Hedberg, I.; Eds.). Vol. 2, Part 2. Addis Ababa, Ethiopia, Uppsala, Sweden, **1995**.
18. Kapingu, M.C.; Guillaume, D.; Mbwambo, Z.H.; Moshi, M.J.; Uliso, F.C.; Mahunnah, R.L.A. *Phytochemistry*, **2000**, 54, 767-770.
19. Tane, P.; Tatsimo, S.; Connolly, J.D. *Tetrahedron Letters*, **2004**, 45, 6997-6998.
20. Wakjira, K. *M. Sc. Thesis, Seed germination physiology and nursery establishment of Croton macrostachyus* Hocht. Ex Del, Addis Ababa University, Department of Chemistry, Addis Ababa, **2007**, pp. 5 – 9.
21. Yibralign, Z. *M. Sc. Thesis, Phytochemical investigation on the stem bark of Croton macrostachyus* (Bisana), Addis Ababa University, Department of Chemistry, Addis Ababa, **2007**, pp. 9, 10.
22. Tane, P.; Tatsimo, S.; Connolly, J. D. *Tetrahedron Letters*, **2004**, 45, 6997-6998.
23. Menberu, D. *M. Sc. Thesis, Chemical Composition of Croton Macrostachyus*, Addis Ababa University, Department of Chemistry, Addis Ababa, **1981**, pp. 2, 16.
24. Lokeshwari, H; Chandrappa, G.T. Impact of heavy metal contamination of Bellandur lake on soil and cultivated vegetation. *Current Science*, **2006**, 91, 622-627.
25. Olajire, A.A.; Ayodele, E.T. Study of atmospheric pollution levels by trace elements analysis of tree bark and leaves. *Bulletin of Chemical Society of Ethiopia*, **2003**, 17, 11-17.
26. Cundeve, K.; Pavlovovska, G; Stafilov, T. *Journal of Brazilian Chemical Society*, **2007**, 18(6), 1207-1214.
27. . Williams, D.R. *The Metals of Life: The Solution Chemistry of Metal Ions in Biological*

- System*, Van Nostrand Reinhold, Great Britain. **1971**, pp 2-19.
28. Hawkes, C.; Ruel, M.; Babu, S. Agriculture and Health, *Food and Nutrition*, **2007**, 28(2), 227-275.
  29. Deckelbaum, R.J.; Palm, C.; Mutno, P.; Declerck, F. Econutrition: Implementation models from the millenium villages project in Africa, *Food and Nutrition Bulletin*, **2006**, 27(4), 335-342.
  30. Liu, D.; Kottke, I. Subcellular localization of cadmium in the roots of allium cepa by electron energy loss spectroscopy and cytochemistry, *Journal of Bioscience*, **2004**, 29(3), 329-335.
  31. Kumar, A.; Pastore, P. Lead and cadmium in soft toys. *Current Science*, **2007**, 93(6), 812-822.
  32. Kocak, S.; Tokusoglu, O.; Aycan, S. Some heavy metals and trace essential elements detection in canned vegetable foodstuffs by differential pulse polarography, DPP, *Electronic Journal of Environmental Agricultural Food Chemistry*, **2005**, 4(2), 871-878.
  33. Hashmi, D.R.; Ismail, S.; Shaikh, G.H. Assessment of the level of trace metals in commonly edible vegetables locally available in the markets of Karachi City, *Pakistanian Journal of Botany*, **2007**, 39(3), 747-751.
  34. Miller, J.N.; Miller, J.C. *Statistics and Chemometrics for Analytical Chemistry*, 5<sup>th</sup> ed Pearson, Harlow, England, **2005**.
  35. Butcher, D.J.; Sneddon, J. *A Practical Guide to Graphite Furnace Atomic Absorption Spectroscopy*, John Wiley and Sons, New York, **1998**, pp 34-149.
  36. Zief, M.; Mitchell, J.W. *Contamination Control in Trace Element Analysis Chemical Analysis Vol. 47*, New York, Wiley, **1976**.
  37. 36. Harris, D.C. *Quantitative Chemical Analysis*, 4<sup>th</sup> ed., W.H. Freeman and Company, New York, **1982**, p. 84.
  38. Nomita, K.; Nandakumar, S.; Sanjiv, K. Estimation of essential and trace elements in some medicinal plants by PIXE and PIGE techniques Nuclear Instruments and Methods in Physics Research B, **2008**, 266, 1605–1610.
  39. Piotr, K.; Zbigniew, F.; Anna, D.; Peter, O. Determination of selected microelements in polish herbs and their infusions. *Science of the Total Environment*, **2007**, 381, 99–104.

40. Al Moaruf, O.; Muibat O.; Asiata O.; Isiaka A.; Nureni, O. Heavy trace metals and macronutrients status in herbal plants of Nigeria. *Food Chemistry*, **2004**, 85, 67–71.
41. Sumontha, N.; Nuchanart, R.; Jutamaad, S. Determination of Trace Elements in Herbal Tea Products and Their Infusions Consumed in Thailand. *J. Agric. Food Chem.* **2006**, 54, 6939-6944.
42. Shazia, J.; Muhammad Tahir, S.; Sardar, K.; Muhammad Qasim, H.; Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. *Journal of Medicinal Plants Research*, **2010**, 4(7), 559-566.
43. Rashed, M. Trace elements in some wild plants from the shores of the high dam lake and adjacent desert, as determined by Atomic Absorption Spectroscopy. *Journal of Arid Enviroments*, **1995**, 29, 185-197.
44. Queralt, I.; Ovejero, M.; Carvalho, M.; Marques, A.; Llabre, J. Quantitative determination of essential and trace element content of medicinal plants and their infusions by XRF and ICP techniques. *X-Ray Spectrometry*, **2005**, **34**, 213–217.
45. Garg, A.; Kumar, A.; Nair, G.; A. V. R. Reddy, A.; Analysis of some Indian medicinal herbs by INAA. *Journal of Radioanalytical and Nuclear Chemistry*, **2007**, 271(3) 611–619.
46. Sussa, F.; Silva, E.; Damatto, S.; Fa'varo, D.; Mazzilli, B. Radioactive and stable elements' concentration in medicinal plants from Brazil. *Journal of Radioanalytical and Nuclear Chemistry*, **2009**, 281, 165–170.
48. Obiajunwa, E.; Adeleke, A.; Olanrewaju, O. Essential and trace element contents of some Nigerian medicinal plants. *Journal of Radioanalytical and Nuclear Chemistry*, **2002**, 252(3), 473–476.
49. Lokhande, R.; Singare, P.; Andhele, M.; Acharya, R. Analysis of Mineral Content of Some Medicinal Plants by NAA and AAS Techniques. *Radiochemistry*, **2009**, 51(3) 321–325.
50. Basgel, S.; Erdemog, S. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Science of the Total Environment*, 2006, 359, 82– 89.
51. Abou-Arab, K.; Abou Donia, K. Heavy metals in Egyptian spices and medicinal plants and the effect of processing on their levels. *Journal of Agriculture and Food Chemistry*, **2000**, 48, 2300-2304.