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M.Sc. Thesis on:

Determination of selected Essential and Toxic Metals in the Leave of Moringa Oleifera of Pawi District, Benishangul Gumuz Regional State, Ethiopia

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Jimma, Ethiopia

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A Thesis Submitted to School of Graduate Studies Jimma University in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Chemistry

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This is to certify that a thesis submitted by **Habtamu Mosisa Senbeta**: Determination of Selected Essential and Toxic Metals in the Leave of *Moringa Oleifera* of Pawi District, Benishangul Gumuz Regional State, Ethiopia submitted in partial fulfillment of the Requirements for the Degree of Masters of Science in Chemistry compiles with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Adugna Boke (M.Sc.) Signature _____ Date _____

Abbreviations

WHO	World Health Organization
FAO	Food and Agricultural Organization
LOD	Limit of Detection
LOQ	Limit of Quantification
%R	Percentage Recovery
S_b	Standard Deviation of the Blank
FAAS	Flame Atomic Absorption Spectroscopy
RSD	Relative Standard Deviation
SD	Standard Deviation
IDL	Instrumental Detection Limit
DNA	Deoxy Ribonucleic Acid
SOD	Superoxide Dismutase

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ABSTRACT

Leave of Moringa oleifera was collected from Pawi district of Metkel Zone, Benishangul Gumuz Regional state,Ethiopia. It was digested by wet digestion method using mixture of HNO_3, H_2O_2 and HCl and Concentration of selected essential (Fe, Cu, Zn & Mn) and toxic heavy metals (Cd & Pb) were determined by flame atomic absorption spectroscopy (FAAS). For quantitative determination of the target analytes, external calibration curves utilized were exhibited acceptable coefficient of deterimination ,r^2,0.996 or better. The mean concentration of the metals in the studied Moringa Oleifera ranges from 0.05 - 0.17 mg/kg, 2.92 - 4.98 mg/kg, 0.04 - 0.06 mg/kg, 0.16 - 0.38 mg/kg, 0.40 - 0.54 mg/kg for Pb, Fe, Cu, Zn and Mn, respectivily; however Cd was not detected.The efficiency of the method was also evaluted using recovery studies by spiking known amount of the standered to the sample before wet digestion and satisfactory recoveries in the range of 86-116% were obtained. The obtained results indicated that the concentrations of the studied metals are below the primissible limit of WHO/FAO.Therefore,the consumption of the Moringa leave of the study area could not posse health problem to the consumers.

Key words: Moringa Oleifera, essential elements, toxic heavy metals, FAAS, wet digestion.

1. INTRODUCTION

1.1. Background

Moringa plant has 13 varieties of species. They are Moringa aborea, Moringa oleifera, Moringa hildebrandtii, Moringa ovalifolia, Moringa douhardii, Moringa peregrina, Moringa borziana, Moringa arivea, Moringa pymaea, Moringa concansis, Moringa ruspoliana, Moringa Longituba and Moringa Stenopetala [1]. Among these *Moringa oleifera* is the most common species, for its nutritous and many medicinal uses [1,2]. It belongs to kingdom plantea, sub-kingdom tracheabinata super-division spermatophyta, division mangliophyta, class mangliopisida, sub-class diiliniida, order caparless, family morgrancea, genus moringa and species oliferia [3]. It is very important plant, native to Agra and Qudh in North Western Region of India, South of Himaliyan Mountain, spread in many countries of tropics and subtropics [4]. The plant is fast growing, drought resistance, branched out when it is cut back several times, and easily cultivated from seed or cutting [5]. It is small sized tree with approximately 10-12 m height. Commonly named as Sahjana, Sainjana in Hindi, Sajina in Bengali, Ben oil tree, Miracle tree and Mother best friend, Drumstick and Horseradish in English. The plant is rich in proteins, vitameins, minerals, folic acid and β-carotene. Different parts of *Moringa oleifera* are used as food having high nutritional value [6].

Moringa oleifera consists of 92 nutrients and 46 antioxidant. It is considered to be cure three hundred disease [7]. Its numeruous parts such as leaf, roots, barks, flowers, and immature pods act as cardiac and circulatory stimulants, posses antihypertensive, antispasmodic, antitumor, diuretic and antiulcer [8]. Other useful medicinal properties of the plant include antidiabetic [9], anti-inflammatory [10], wound healing, antipyretic [11], hepatoprotective [12], antiasthmatic [13], antioxidant [14], antiepileptic [15], antimicrobial [3], anticancer [16], anticholestermic [17], antibacterial and antifungal [18, 19] activity.

The popular uses of leaves and seeds from *Moringa oleifera* raise the question about safety and health of their products, especially due to the heavy metal concentrations. Daily exposure to heavy metals above the permissible limits has been associated with mental retardation, cancer, neuropathy, hepatic dysfunction and renal failure. Heavy metals are released into the environment through antropogenic and natural activities [20]. Plants take up and accumulate

these metals in their tissues and then transferred to human being thruogh food chain ,indicating their importanc of deterimination in different parts of plants [21]. In indigenous medicinal plants major and trace elements have significant roles in preventing a variety of human ailments and diseases [22]. Heavy metals are considered as potential carcinogens and are associated with varies diseases, such as cardiovascular, bone, kidney, and gastrointestinal diseases and as well as they also reduced general intellectual capacity [22]. Consumption of contaminated herbal plants can cause serious health problems as a result of excessive accumulation of heavy metals. In herbal plants, the permissible limits of varies heavy metals have been set by WHO/FAO [6].

Several works have been done, on the levels of nutrients including minerals in *Moringa oleifera* [23,24]. Generally the nutritional contents of plants deponds on the geographical location and the soil properties where the plant grows [24]. Therefore, deterimination of the level of different metals in Moringa oleifera leave which are grown in different geographical origion is crucial to evaluate the risk to human health [23]. In this study, the concentration of some selected essential and toxic heavy metals in Moringa oleifera leave grown in Pawi district,Metkel Zone, Beneshangul Gumuz regional state of Ethiopia were determined.

1.2. Statement of the Problem

Human beings are commonly exposed to different trace metals through food, water and air. These trace metals play critical role in different metabolic processes, but they are toxic to human beings if exceed above certain limits. So, there is increasing interest to determine the levels of varies elements in different herbal plants [25]. Analysis of the metal contents in the leave of *Moringa Oleifera* is important to known whether the levels of the metals are risk to life or not. Although several studies have been conducted on the determinations of metals in *Moringa Olefeira* leave grown in different part of the world, no has been conducted on *Moringa Olefeira* leave of grown in from pawi district, Metkel Zone, BenishangulGumuz regional state of Ethiopia. So in this study, the concentration of some selected essential and toxic heavy metals in the leave of *Moringa Oleifera* leave were determined and the obtained results were compared with metals content of other areas *Moringa Oleifera* plant.

1.3. Objectives of the study

1.3.1. General objective

The main objective of this study is to determine the levels of selected essential and toxic heavy metals in *Moringa oleifera* leave grown in Pawi Dstrict, Metekel Zone, Benishangul Gumuz Regional state, Ethiopia.

1.3.2. Specific objectives

- To determine the concentration of some selected essential (Fe, Cu, Zn & Mn) and toxic heavy metals (Cd & Pb) in Moringa oleifera.
- To compare the concentration of the target metals in *Moringa Oleifera* leave samples collected from different areas of the district as well as with commercial one.
- To compare the levels of the metals in *Moringa oleifera* leave of the study area with the reported other areas Moringa and /or similar plant leaves.

1.4. Significance of the Study

Plants are the basic sources of knowledge of medicine . *Moringa oleifera* has an impressive range of medicinal uses with high nutrational value [26]. In addation, it was found that it can work as biosorbent for heavy metals in water treatment [20]. It is considered as a natural product and enviromentally friendly material [27]. The plant leave have a lot of economic value such as medicinal, nutritional and pesticidal values. However, these values have not been clearly justified from the area of which this research was conducted. Therefore , the finding of this study would indicate the amount of the selected metals, help in promoting awarness regarding the safty of *Moringa Oleifera* leave and ultimatly aid in safegurding public health. It also serves as asecondry sources of information for other researchers who want to do on this or simliar plants.

2.LITERATURE REVIEW

2.1. Moringa Oleifera

Moringa oleifera is the most cultivated species of a monogeneric family the *Moringaceae* that is native to the sub-Himalayans regions of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree, also known as the Horseradish tree, Drumstick tree, Benzolive tree, Kelor, Marango, Baijhan, Mulangay, Sajna or Ben oil tree, was utilized by the ancient Romans, Greeks and Egyptians. In Africa and Indonesia; *Moringa oleifera* leaves are given to nursing mothers in the belief that they increase lactation in nursing mothers. Traditionally, the leaves, fruits, flowers, and immature pods of this tree are edible; they are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, the Philippines, Hawaii, and some African nations. In developing nations, *Moringa oleifera* is used as an alternative to imported food supplements to treat and combat malnutrition, especially among infants and nursing mothers, by virtue of its chemical constituents. The Moringa tree has been praised for its nutritional and medicinal properties, and many claims have been made. All parts of the tree including leaves, pods, bark, root has medicinal value, hence, it was called the miracle tree. It is now widely cultivated in the tropical and subtropical regions [29]. Picture of *Moringa Oleifera* taken from Almu keble is presented in fingure 1 below.

Figure 1: Picture of *Moringa oleifera* taken at Almu keble,Pawi disrtict in may 2009 .

2.1.1. Plant Classification (Taxonomy)

The botanical classification of *Moringa Oleifera* is listed in table 1 below.

Table 1: Botanical Classification of *Moringa oleifera*

Kingdom	Plantae- plants
Sub kingdom	Tracheobionta- vascular plant
Super division	Spermatophyta- seed plant
Division	Magnoliophyta-flowering plant
Class	Mangoliopsida-dicotyledon
Sub class	Dilleniodae
Order	Cappareles or violes
Family	Morngacae-hores radish tree
Genus	Moringadam-moringa
Species	*Moringa oleifera*-hares radish tree[4]

2.1.2. Origin and Habitat

Moringa oleifera, (Drumstick tree, Horseradish tree) is an indigenous tree from north-western India, now it is widely cultivated and has become naturalized in many locations in the tropics [30]. It is a native tree in arid and semi-arid regions in the southern Rift valley of Ethiopia. The local farmers use the species as one of the major arable tree inter-crop in multi-storey system especially by the Konso people in Gamogofa. *Moringa oleifera* has a wide range of adaptation from arid to humid climates, grows in a rainfall range from 300-1400 mm. It is widely distributed in south western part of Ethiopia at an altitude range of about 1100 - 1600 m. The major growing areas are Arbamich, Negelle and Wellayta sodo. *Moringa oleifera* is commonly called Shiferaw in Amharic [25].

2.1.3.Description of Mornga Oliefera

Fingure 2, shows the immature pods, leave and flowers of Moringa Oleifera.

Figure 2 picture of (A): Immature pods (B): Leave (C):Flowers

Moringa oleifera is a softwood tree that branches freely, fast-growing, a small-to-medium-size tree with about 10 m height and diameter of 0.2 - 0.4 m. The stem is short, normally straight but occasionally is poorly formed that reaches a height of l.5 - 2 m before it starts branching but can reach up to 3 m. The leaf are alternate, twice or thrice pinnate grow mostly at the branch tips, 20 - 70 cm long, greyish downy when young, long petiole with 8 -10 pairs of pinnae each bearing two pairs of opposite, elliptic or obovate leaflets and one at the apex, 1- 2 cm long. The white-to-cream coloured flowers are conspicuous, lightly fragrant borne on inflorescences 10 - 25 cm long, 2.5 cm in diameter, borne in sprays with five at the top of the flower, although they can be tinged with pink in some varieties. The five-reflexed sepals are linear-lanceolate. The five petals are slender-spatulate, surround the five stamens and five staminodes and are reflexed except for the lowest. The fruits are trilobed capsules, frequently referred to as pods. Immature pods are green and in some varieties have some reddish colour. Pods are pendulous, brown, triangular, splitting lengthwise into three parts when dry, 30 - 120 cm long, 1.8 cm wide containing about 20 seeds embedded in the pith, pod tapering at both ends, 9 -ribbed.The seeds are round with a brownish semi-permeable seed hull, with 3 papery wings. Seed hulls are generally brown to black, but can be white if kernels are of low viability [29].

2.1.4. Common Name of Moringa Oleifera

There are varieties of names for *Moringa Oleifera* trees as given by different continents and countries in the world [26]. The names of *Moringaoleifera* called by some countries are listed in the Table 2.

language	Name	Language	Name	Language	Name
Latin	Moringa Oleifera	Tamil	Amokira	Bengali	Sojenedanta
English	Horse radish tree	Malayalam	Muringa	Telgo	Mulakkaya
Hindi	Sahjan	Gujarati	Saragvo	Nepali	Swejan
Oriya	Sajan	Kannada	Karamaddinagaddi	Sinhalese	Murunga
Punjabi	Surajana	Marathi	Shevga	Amharic	Shifraw[25]

Table 2: Moringa oleifera's name given by some countries.

2.1.5. Ecology and Cultivation

Moringa oleifera is fast growing tree and can tolerate drought, sandy soil, bacteria and fungai, thrives dry to moist climates, tolerate annual temperature of 19 to 28 ^{0}C [27], annual rainfall of 250-1500 mm, pH of 4.5-9 [28]. It is a wild plant and cultivated through out the plains, especially in hedges and in house yards [26]. Moringa oleifera is easiy cultivated by cutting or seeds [27].

2.1.6. Traditional Use

Tradditionally, *Moringa oleifera* tree is known as a miracle tree as almost all parts(tissues) of the tree have their own medicinal properties and are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant [1]. Leave of the plant have anti-oxidant properties and are known to cures hallucinations, dry tumors, hiccup and asthma.The root and bark are useful in treatment of heart complaints, eyedisease, inflammation, dyspepsia and enlargement of spleen. Its flowers is used to cure inflammation and muscle diseases. Seed oil is useful in the treatment of leprousulcers [1]. Generally*Moringa oleifera* have known for its coagulative properties on waste water, cardiac and circulatory stimulant, antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol

lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial, antifungal activities and have lots of macro and microelements and various forms of nutrient [33].

2.1.7. Chemical Composition of Moringa Oleifera

Moringa oleifera leaf are a rich source of β-carotenes, amino acids, phenolic compounds, vitamins, and minerals [23]. Have almost all the vitamins found in fruits and vegetables. Vitamins that are found in *Moringa* are, vitamin A (Beta carotene), vitamin B_1 (Thiamine), vitamin B_2 (Riboflavin), vitamin B_3 (Niacin), Vitamin B_6 (Pyrodixine), vitamin B_7 (Biotin), vitamin C (Ascorbic acids), vitamin D (cholecalciferol), vitamin E (Tocopherol)andvitamin K [7].

Moringa oleifera also contains phytochemicals like phenolic compounds, carbohydrate [2], phytosterol, phenols [5,], quinine, protein, sterol, sugar [35], glycosides [5], alkaloids, terpenoids, saponin, flavonoids, and tannin [5]. Several valuable reviews of the ethnobotanical uses of Moringa oleifera are available. Moringa has been found to be a good source of polyphenols and antioxidants. Phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin have been reported in its flowers, roots, fruits, and seeds. The leaves, in particular, have been found to contain phenolics and flavonoids; these compounds have various biological activities, including antioxidant, anticarcinogenic, immunomodulatory, antidiabetic, antiatherogenic, and hepatoprotective functions and the regulation of thyroid status. Moreover, leaves contain trace elements that are essential to human health. For instance, magnesium, iron, selenium, and zinc play an important role in metabolism, and interest in these elements is increasing together with reports relating trace element status and oxidative diseases [7].

In the west, one of the best known use for Moringa is utilization of powdered seeds to flocculate contaminates to purify drinking water. The seeds are also eaten green, roasted, powdered and steeped for tea or used in curries. Recently, this tree has been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, vitamin C, and carotenoids suitable for utilization in many of the so – called "developing" regions of the world where under nourishment is a major concern. The seeds of Moringa oleifera are particularly rich in proteins, lipids and minerals (Mg and Zn). Seeds of Moringa oleifera had also the strongest radicals scavenging activity (99.74 %) and flavonoids content found that the dried leaves had crude protein levels of 30.0 % and 19 amino acids. The dried leaves had the following mineral contents: calcium,

phosphorus, magnesium, potassium, sodium, sulphur, zinc, copper, manganese, iron and selenium. The values of amino acids, minerals and vitamins profiles reflect desirable nutritional balance [4]. Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and that the protein quality of Moringa leaves rivals that of milk and eggs. These aforementioned biological activities of *Moringa oleifera* confirmed its medicinal uses and potentials [79].

2.1.8. Pharmacological properties

A number of medicinal properties have been ascribed to various parts of *Moringa oleifera* plant. Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders [34]. *Moringa oleifera* roots, ethanol extract of leaf and its constituents have been reported to posses antispasmodic activity [35]. Various parts of the plant such as roots,fruit, leaf, flowers, gum and the aqueous infusion of seeds, the crude extract of leaf have diuretic activity and the diuretic components are most likely play a complementary role in the overall blood pressure lowering effect of the plant [28, 34].

The active phytochemicals nitrile, mustard oil glycosides and thiocarbamate glycosides have been isolated from *Moringa* leaf are responsible for the blood pressure lowering effect [28,34]. Niazinin A, niazinin B, niazimicin and niazinin A + B which are found in *Moringa* leave have shown a blood pressure lowering effect in rats mediated possibly through a calcium antagonist effect [28]. The ethanol extract of *Moringa oleifera* pods also has led to the isolation of Methyl p-hydroxybenzoate, β-sitosterol, thiocarbamate and isothiocyanate glycosides have anti-hypertensive activity [36]. The ethanolic, petroleum ether, solvent ether and ethyl acetate extracts of *Moringa oleifera* seeds showed significant antipyretic activity in rats [28].

There are many causes of blindness. Vitamin A deficiency causes impaired dark adaption and night blindness. *Moringa* leaf, pods and leaf powder contain high proportion of Vitamin A and their consumption can help to prevent night blindness and eye problems in children.consumption of *Moringa oleifera* leave (β- carotene and leutin) with oil helps in improving Vitamin A

nutrition and perhaps delays the onset of cataract. Also the juice can be instilled into eyes in cases of conjunctivitis [28].

Numerous parts of *Moringa olifera* have cosmetic value. *Moringa* seed oil, known as Behen oil is widely used as a carrier oil in cosmetic preparations. The healing properties of *Moringa* oil were documented by ancient cultures. *Moringa* oil posesses exceptional oxidative stability. It contains high amount of oleic acid similar to olive oil. *Moringa* oil is light and spreads easily on the skin. It is good oil for use in massage and aromatherapy applications. It can be used in body and hair care as a moisturizer and skin conditioner. Other uses include soap making and for use in cosmetic preparations such as lip balm and creams. Its butter, a semisolid fraction of *Moringa* oil, is used in baby products to contribute a free radicle resistant emollient with exceptionally long lasting skin softening and soothing effects [28]. Aqueous leaf extracts regulate thyroid hormone and can be used to treat hyperthyroidism, purification of water, microbial elimination and biosorbent [35].

Processing of moringa, most plants lose their nutritive properties when processed. When compared, the nutritive content of raw, germinated and fermented moringa seed flour, it was found that phytochemicals were higher in raw seed flour and amino acid content was at its peak in fermented and germinated seed flour. This can be a result of the biochemical activities during germination and microbial activity during fermentation. However, a study reviewed the effect of boiling, simmering and blanching to see the retention of nutrient content of moringa leaves. Interestingly, boiling was the most effective of all the techniques as it reduced the cyanide, oxalate and phytate contents, more significantly than the other two methods. The presence of phytate and other anti-nutrients can reduce the bioavailability of certain nutrients and processing can hence be done for maximum utilization of required nutrients from the seeds and leaves. Yang et al. reported that boiling increased the availability of iron and antioxidant content. Hence, the processed moringa seed flour can be used to treat malnutrition problems. However, some studies have shown that children refuse to take in moringa due to its slight bitter taste. Some researchers designed moringa noodles by three methods of cooking noodles, sautéing, steaming and boiling. These noodles were tested on rats and the effects on mammary glands were studied. Interestingly, the sautéed noodles had a better effect on the mammary glands of rats and improved milk production. The effect of sautéing on the noodles improved lactogogum values, because the oil used was rich in sterols [70].

2.2. Heavy metals

Agroup of metals with density greater than 5 g/cm^3 and atomic number above 20 are refered to heavy metals [37], these metals can be released into the environment by human activities or through natural constituents of the earth's crust [20]. Some of these metals are essential to growth and production of bones, teeth, hair, blood, nerves, skin, vitamins, enzymes and hormones.They may also play a major role in nerve transmission, blood circulation, cellular integrity, energy production and muscle contraction. Several trace elements are essential constituents of enzymes and play vital role in human metabolism. Those all nutriants (essential and non essential)elements are primarily supplied through diet. The amount needed depends on age, sex, and health status, geographical and climatic conditions [25].

Heavy metals are not easily biodegradable and consequently can be accumulated in human vital organs leading to unwanted side effect. Dietary exposure to heavy metals like Cd, Pb, Zn, Pb , Co, Mn, Fe and Cu may bring risks to human health. Intake of heavy metal contaminated food has serious impact on reducing some essential nutrients in the body causing intrauterine growth retardation, impaired psycho-social behaviour, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer [38]. Reports have shown that high intake of heavy metals can lead to metal poisoning where as low intake levels can lead to deficiency effects [21].

2.2.1 Essentiality and Toxicity of Metals in Plants and Animals

Living things store and then transport mineral nutrients to use in physiological processes and protection to toxic effects of the metals. Metals have primary role in building body tissue and regulating different physiological processes. They are also basic components of enzymes and hormones [39]. From the mineral nutrients K, Na, Mg, Ca, P, S and N are macro-nutrients, because they are required by greater amount than others [40]. Among these macro-nutrients K, N and P are primary nutrients as well as Ca, Mg and S are secondary nutrients [40]. Other mineral nutrients including Fe, Mn, B, Mo, Cu, Zn, Cl and Co are micro-nutrients, because they are required in relatively smaller amounts than macro-nutrients [40].

Essential mineral nutrients are necessary for growth and development of a plant. There are three basic criteria for a metal to act as essential mineral nutrients .These are (1) the plant must be

unable to complete either vegetative or reproductive stages in the absence of such mineral nutrient, (2) its function is specific for that mineral nutrients and cannot be replaced by another mineral nutrients and (3) the metal must be directly involved in a plant metabolic or structural process [25]. However, both essential and non-essential metals are toxic if consumed in high amounts for long periods of time. The toxicity and optimal intake to meet physiological needs for essential metals is not similar for all metals [39]. Some heavy metals like Pb and Cd are non-essential and have no biological role in plant metabolism, and are toxic even at trace amounts [41, 43]. Essential metals may cause unfavorable health effects at some amounts below or beyond the level required for optimum nutrition [39].

Mineral uptake by plants can be influenced by mineral concentrations in soils, soil pH; cations exchange capacity, organic matter content, types and varieties of plants and age of the plants. Humans would take these minerals by eating or drinking plants products that grow in mineral rich soils [42]. For instance, elements such as Fe, Zn and Mn are essential micro-nutrients for both animals and plants [40]. They react with vitamins, produce enzymes and also used in every biological activity of organisms like photosynthesis, respiration and hormone and deoxyribonucleic acid (DNA) synthesis [44].

Fe: is vital element for almost all living organisms, participating in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. High concentrations of Fe may lead to tissue damage, as a result of the formation of free radicals. Disorders of iron metabolism are among the most common diseases of humans, encompassing a broad spectrum of diseases with diverse clinical manifestations ranging from anemia to Fe overload and, possibly, to neurodegenerative conditions [45].

Mn: is an essential micro-nutrient for both plants and animals. In plants it is used for chloroplast formation, nitrogen metabolism and structure of photosynthetic proteins and enzymes [46, 47]. It is also required for normal growth, development and cellular homeostasis [48]. But when it is consumed in excess amount it disturbs photosynthetic process. In animals it is used in bone formation, fat and carbohydrate metabolism, blood sugar regulation, Ca absorption and as cofactor of several enzymes [48, 49]. It activates numerous enzymes and a constituent of some enzymes [49, 50] needed for energy and protein metabolism, regulation of cell metabolism as well as it act as antioxidants and prevent oxidative stress by neutralizing oxidants produced

under different stresses like environmental or production stress or stress related to infections or diseases [49, 51]. Mn also plays an essential role in connective tissue growth and blood clotting [50]. Its deficiency lead to skeletal abnormalities, postural defects, impaired growth , disturbances in lipid and carbohydrate metabolism, poor bone formation, reduced fertility and birth defects , and impaired insulin synthesis and action [48 – 50, 52].

Zn: is also another micro-nutrient. In plants it is used to produce chlorophyll. Plant leaves get discolor when the soil is deficient in Zn and plant growth is stunted [53]. It also affects several metabolic processes of plants [54, 56]. High levels of Zn in soil inhibit many plant metabolic functions; resulting in retarded growth and cause senescence. Zn toxicity in plants limits (rescues) the growth of both root and shoot [55]. Its toxicity also causes chlorosis in the younger leaves, which can extend to older leaves after prolonged exposure to high soil Zn levels [56]. The chlorosis may arise partly from an induced Fe deficiency as hydrated Zn^{+2} and Fe^{+2} ions have similar radii [57]. Excess Zn can also give rise to Mn and Cu deficiencies in plant shoots. Such deficiencies have been attributed to a hindered transfer of these micronutrients from root to shoot. This hindrance is based on the fact that the Fe and Mn concentrations in plants grown in Zn rich media are greater in the root than in the shoot [56].

Cu: is another micronutrient which is used in a number of essential enzymes including super oxide dismutase (SOD), cytochrome, c-oxidase lysyloxidase and ceruloplasmin, but its ion is potentially toxic because it is a potent generator of free radicals [58]. Cu plays role in common neurological conditions such as Alzheimer's disease, the prion disease, Wilson's disease and amyotrophic lateral sclerosis [59]. Cu toxicity is related to the induction of reactive oxygen species in the Fenton type reactions causing breaks of DNA strands as well as damage to membranes and mitochondria [60].

Pb and Cd: are non-essential for both plants and animals. Although toxicity and the resulting threat to human health of any contaminant is a function of concentration, it is well-known that chronic exposure to Cd and Pb can cause adverse effects [61,62]. Pb is known to induce a broad range of physiological, biochemical, and behavioral dysfunctions [63]. Pb-induced oxidative stress contributes to the pathogenesis of Pb poisoning by disrupting the delicate pro-oxidant/antioxidant balance that exists within mammalian cells [61, 62]. Cd is also a potent cell poison, which causes different types of damage, including cell death or increase in cell proliferation. Oral exposure to Cd may result in adverse effects on a number of tissues [58]. Cd

also affects the nervous system, and neurological disorders such as learning disabilities and hyperactivity in children. In neuronal cells, Cd induces oxidative stress, which produces protein damage and subsequently neurodegeneration. It is also known to enhance the production of free radicals in the brain and to interfere with the cellular defense mechanism against oxidation [58].

2.2.2. Some different studies conducted on moringa Oleifera plants.

Diffent studies have been performed by different reseachers on the deterimination of the concentration of essential and toxic heavey metals on different part of Moringa oleifera plants from different countries with different analytical techniques. The reported amounts of the selected elements in each study was different as they are different in their geographical location, climatic condition, the soil type,age of the plant,the solvent used during digestion, human activities, availibilty of the targeted metals and even analytical technique (AAS, ICP- OES, ICP-Ms etc, used for the analysis of selected analytes.

In Africa, many studies have indicated that a vast number of indigenous wild plants play a significant role in the diet of the population [64]. Vegetables are the cheapest and the most available sources of important nutrients, supplying the body with minerals, salts, vitamins and certain hormone precursors, protein energy and essential amino acids [65].

Moringa oleifera and *Moringa stenopetala* are the two most common species among the 13 species of the Moringa family. *Moringa oleifera* originates from the Himalaya and *M. stenopetala* is endemic to East Africa [66]. All parts of the tree are edible, providing a highly nutritious food for both humans and animals. *Moringa stenopetala* is one of the most frequently cultivated indigenous species for its palatable leaves in the semiarid areas of Konso, Derashe and Arbaminch Zuria districts of the Southern Rift Valley of Ethiopia and locally called as “Haleko” or “Shiferaw.” It is also cultivated from the lower Omo Valley to the North and in the neighboring regions of South Omo, Gamo-Gofa and Borena [67]. A study conducted by Melesse et al., indicated that the leaves of *Moringa stenopetala* are rich in protein (28.2%) and contain reasonable amounts of essential amino acids of which some are comparable with those found in soybean meal. It is a multipurpose tree that is cultivated both for human food and animal feed in Southern Ethiopia [68].

Studies from other countries indicate that the leaves have immense nutritional value such as phytochemicals, vitamins, minerals, and amino acids [69]. As such, the leaves have been used to combat malnutrition, especially among infants and nursing mothers. It has been reported that *Moringa oleifera* leaves product, especially leaf powder, is becoming increasingly popular in Ethiopia because of its outstanding indigenous nutritive and medicinal value [70].

It has been reported that in many developing countries, the supply of minerals is inadequate to meet the mineral requirements of farm animals and rapidly growing population. Mineral composition of a plant plays significant role in its nutritional, medicinal and therapeutic values [71]. Different reported studies indicated that, Moringa *oleifera* contains several elements which are the basic building block of matter. Some of the elements are calcium, magnesium, potassium, sodium and the minor elements are iron, zinc, copper and manganese [72].

Researcher from India has reported the concentration of Cd, Co, Cu, Fe, Ni, Pb and Zn as 0.01mg/g, 0.003 mg/g, 0.57 mg/g, 7.42 mg/g, 1.64 mg/g, 0.11 mg/g, and 3.30 mg/g respectively, in *Moringa oleifera,* Drumstick Fruit, by analyzing the sample with Inductive Coupled Plasma Analyzer (ICPA) [73]. Other researchers from Ethiopia, has reported the concentration of Cu, Ni and Mn in the leave part of moringa oleifera using atomic absorption spectroscopy from Wukro Agricultural College which is located in northern part of Ethiopia in Tigray National Regional state, Eastern Tigray Zone, Wukro woreda as 2.87 mg/kg, 3.20 mg/kg and 1.10 mg/kg respectively [25].

From Legon-accra, researchers has reported the concentration of Cu, Fe, Mn and Zn as 0.13 mg/kg, 0.29 mg/kg, 0.0005 mg/kg and 0.02 mg/kg in the dried leave sample of Moringa oleifera by using the fast sequential Atomic Absorption Spectrometer (AAS) technique (Varian AA240FS) [74] respectively. On the other hand from Backyard at Badagry, logos state researchers has assessed the variations in mineral composition and heavy metal content of Moringa oleifera leave, such as Mn, Fe, Zn, Pb, and Cd, using atomic observation spectrophotometry accordingly, the reported values from the studies were 63 mg/kg - 86 mg/kg for Mn, 154 mg/kg – 214 mg/kg for Fe, 11 mg/kg – 18 mg/kg and Pb and Cd as non detected [24]. Study conducted in Thailand determined 11 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in Leaves of *Moringa oleifera*, collected from urban and rural gardens in sites by using inductively coupled plasma - mass spectrometry (ICP-MS) and the reported result by the researcher shows the difference in the amount of selected metals from rural and urban garden.

For example, the amount of Zn was reported in urban garden as 14.32 mg/kg maximum and 1.10 mg/kg minimum in rural garden, Cu was reported with maximum value of 8.46 mg/kg in urban garden and with minimum value of 0.97 mg/kg in rural garden. In most of the studied sites the concentration of Cd was not detected [23].

Some study from India determined the amount of heavy metals in some medicinal plants collected from local market in Salem,Tamil nadu. Of those medicinal plants Moringa oleifera is the one and they reported the amount of heavy metals in the leave parts of Moringa oleifera using atomic absorption spectrophotometer as 298.09 mg/kg for Fe, 96.37 mg/kg for Mn, 0.68 mg/kg for Cd, 11.51 mg/kg for Cu, 14.67 mg/kg for Zn and Pb was not detected [22]. More over the study conducted from Agra and Gwalior reported the amount of trace elements such as Zn, Fe, Ni, Mn, K, Ca, Mg, Co, Cr, Cu, Cd, Pb and As using atomic absorption spectroscopy in Leaves of *Moringa oleifera as* 12.32 ppm for Zn in Agra and 9.428 ppm in Gwalior , as 54.96 ppm for Fe in Agra and 56.98 ppm in Gwalior, as 48.157 ppm for Mn in Agra and 43.02 ppm in Gwalior, as 4.62 ppm for Cu in Agra and 6.46 ppm in Gwalior, as 0.005 ppm for Cd in Agra and ND in Gwalior, as 1.56 ppm for Pb in Agra and 2.11 ppm in Gwalior [75].

3 MATERIALS AND METHODS

3.1 Chemicals and Reagents

Chemicals and reagents used in this work are of analytical grade. HCl (37%), HNO_3 (70%) and H_2O_2 (30%) were obtained from Blulux (Faridabad, Haryana, India). Stock standard solution of 1000 mg/L in HNO_3 (2%) of the metals Fe, Mn, Zn, Cu, Cd and Pb were also obtained from Blulux. Double distilled water was used for sample preparation, dilution and rinsing of the apparatus throughout the work. For each element working solutions were prepared by diluting its stock solution.

3.2. Apparatus and Instruments

Flame atomic absorption spectrophotometer (FAAS) (buck scientific model PG instrument AA500, USA) fitted with deuterium background corrector and air acetylene flame atomizer was used for the analysis of the target metals. Apparatus such as mortar and pestle were used for grinding and homogenizing the Moringa Oleifera leave samples; analytical digital balance (KERN ABJ220-4NM, German); for weighing sample, 0.5 mm mesh size sieve for sieving.

3.3. Cleaning of Apparatus

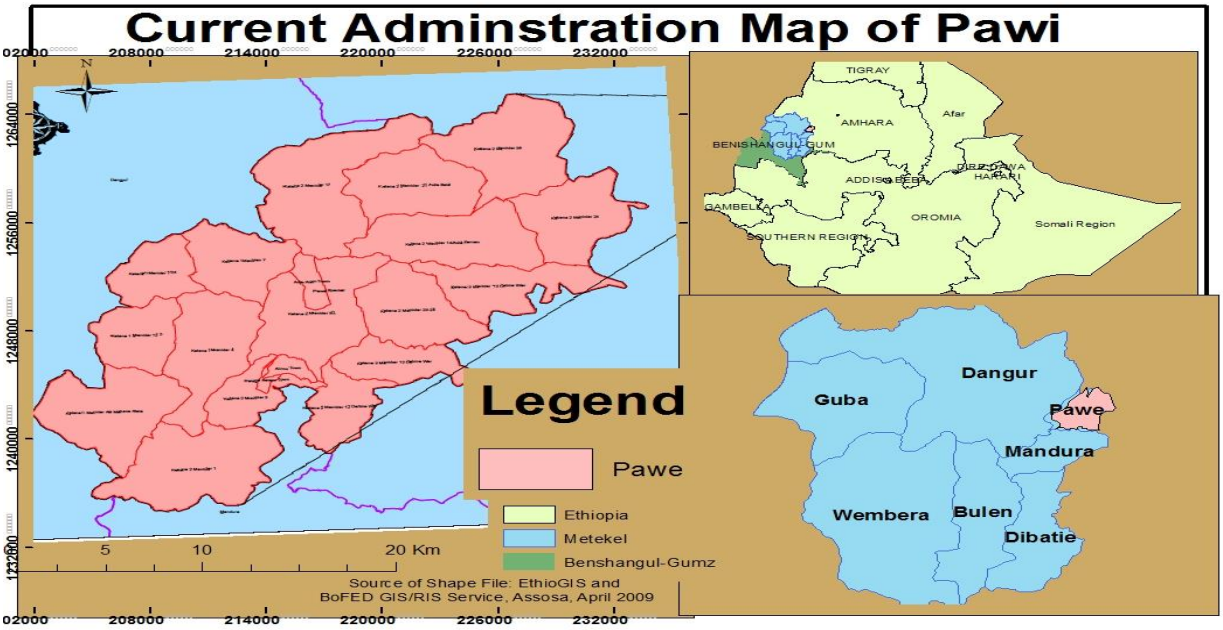
Apparatus such as plastic bottles, glassware, volumetric flasks, measuring cylinder, beakers, watch glass and other necessary materials used in this study were washed with tap water using detergents followed by distilled water. The apparatus were then soaked in 10 % nitric acid for 24 h. Eventually they were rinsed with double distilled water for several times to make them of the acid free. Then the apparatus were dried in oven and kept in dust free place for use.

3.4. Sample Collection and Preparation

3.4.1. Description of the study area

Pawi District is one of the twenty woreda of Benishangul Gumuz regional state. The center of woreda, Almu town, is located at 570 km North West of Addis Abeba and 916 km from Jimma town. Pawi is bounded with Dangur woreda in the west and North West, Mandura woreda in the south, Aalfa woreda (in Amhara regional state) in the north east, Gwangwa woreda (in Amhara regional state) in the south east and Dangila woreda (in Amhara regional state) in the east. Pawi woreda has an area of 567.51 square kilometer. It lies between $36^0$25’00.66’’east longitude and

$11^0$19’59.47’’north latitude. It is characterized by hot and wet climate, with an average temperature of 25 ^{0}C; climate variation in temperature goes from 8 ^{0}C in the coldest (July and august) to above 40 ^{0}C in the hottest period (March and April); the average annual minimum and maximum temperature ranged between 16.2^0 C and 32.25^0 C respectively. The total mean annual rainfall during crop season is 1502 mm. It is found at an altitude of 1000-2000 m above sea level [76].The map representing the study area is presented as follows below.



Source; Ethio-GIS and BOFED GIS service Assossa, April 2009

3.4.2. Sample Collection

The Moringa oleifera leave samples were collected from three different localities of the district based on peoples need towards consuming the leave part of this particular plant (Almu, Mendar-2 and Agenta). More over one commercial sample were also collected from Jimma Town supper markets. The collected samples were packed in to clean polyethylene plastic bags, labeled and transported to Jimma university laboratory for further treatment.

3.4.3. Sample Preparation

First the leave samples were washed thoroughly with distilled water to remove surface contaminants like soil, dust and spray residues. The samples were air dried for about three weeks and then they were dried in the oven at a temperature of 80 ^{o}C for 8 h. The dried sample were ground and homogenized into fine powder to obtain fine particles that pass through a 0.5 mm mesh size sieve and kept dry in a polyethylene bag in dust free place until the digestion. The commercial sample was actually in the form of powder but the size of the powder is not as such enough to pass through the sieve. Therefore, it was placed in oven for a certain time to remove the moisture content. After that it was further grinded to obtain homogenized fine particle size that can pass through 0.5 mm mesh size sieve.

3.5. Digestion of Moringa Oleifera Leaves Sample

For the digestion of Moringa oleifera leave samples, the procedure reported by Pequeru.et.al [77] was used with some modification; accordingly 0.5 g of the fine ground samples was weighed and transferred to the conical flask. Then 5 mL of HNO_3 was added with continuous stirring followed by addition of 4 mL of H_2O_2 .The mixture was then shaken carefully until the solid material was dissolved. Thereafter, the content was boiled for 15 minutes by placing on the hot plate. Then after cooling 5 m L of HNO_3 was added and the content boiled again for about 10 minute. Again after cooling the solution, 5 m L of 1:1 (HCl: HNO_3) solution was added and the solution was then boiled followed by dropwise addition of H_2O_2 until color less solution was obtained. Eventually, after cooling the obtained colorless solution, the content was filtered by whatman No 42 filter paper and transferred to 50 mL volumetric flask. The filtrate was then diluted to the mark with double distilled water, transferred to polyethylene plastic cups and kept in refrigerator until analysis. Each sample was digested in triplicate. The digestions of blank reagents were also performed using all reagents used above except the samples.

3.6. Determination of Metals in the sample

Quantitatively the targeted metals were determined using FAAS. Before the analysis the instrument was calibrated by preparing series of standard solutions of the target analytes. The operating conditions of FAAS employed for each analyte are given in Table 3**.**

Table 3: Instrumental operating conditions for determination of metals using FAAS

Analytes	**silt(nm)**	**peak(nm)**	**Energy (%)**	**lamp current(mA)**	**high voltage(V)**
Pb	0.4	283.21	99.6	5	293
Fe	0.2	248.16	99.6	5	395.5
Cu	0.4	324.64	101.6	5	261.5
Zn	0.4	283.21	99.6	3	292.5
Mn	0.4	283.21	99.6	5	289.75
Cd	0.4	283.21	99.6	2	387.5

3.7.Evaluation of Analytical method performance validation

Method validation is the process used to confirm that the analytical procedure used for a specific test is suitable for its intended use. Results from method validation can be used to evaluate the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. The parameters: accuracy, precision, limit of detection and quantitation were used for method validation.

3.7.1 Precision and Accuracy

In this study, the precision of the result was evaluated by the standard deviation of the results of triplicate samples (n = 3), analyzed under the same condition. Alternatively, the accuracy of the results was evaluated by recovery studies. To perform a recovery study, a known amount of analyte was added into Moringa Oleifera leave samples. Known concentration of standard solutions (1000 mg/L of Pb, Cd, Cu, Mn, Zn and Fe) were taken. From these standard solutions, 100 ppm of(Fe,Mn,Cu and Zn) and 10ppm of Pb and Cd intermediate standard solutions were prepared in 50 ml and then 1000 µL of the intermediate standard solution were added (spiked) to

0.5 g of Moringaoleifera leave samples. Then they were digested with the same digestion method as Moringaoleifera leave samples. After diluting the spiked Moringaoleifera leave samples to the required volume (50 mL) with double distilled water, they were analyzed with the same method used for the analysis of the Moringaoleifera leave sample. Triplicate samples were prepared and analyzed. Percentage recovery is then determined by the following formula.

$$\% \text{ Recovery} = \frac{\text{Metal conc. in spiked sample - Metal conc. in un spiked sample}}{\text{Concentration added}} \times 100$$

3.7.2 Determination of Detection Limits

There are different methods of determining detection limit (LOD). The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD is the concentration that gives a signal three times the standard deviation of the response divided to the slope of the calibration curve [78]. $LOD = 3S_a / b$. Where S_a is the standard deviation of the blank response and b is the slope of the calibration curve.

3.7.3 Determination of Quantitation Limits

Quantitation limit (LOQ) is the level above which quantitative results may be obtained with a specified degree of confidence. The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ is the concentration that gives a signal ten times the standard deviation of the response divided to the slope of the calibration curve [78]. $LOQ = 10S_a / b$. Where S_a is the standard deviation of the blank response and b is the slope of the calibration curve.

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4. RESULTS AND DISCUSION

Before quantitative determination of the target metals; the instrument was calibrated for each target analyte using five series of standard concentration. The calibration curves were plotted as function of observance versus concentration of the standard solution. The correlation coefficients (r^2) of calibration curve were 0.996 or better, showing good linearity over the concentration ranges used. Table 4 shows information regarding calibration curves

Table 4: Regression equation and coefficient of determination of the calibration curves.

Analyte	Standard concentrations(mg/kg)	Regression equation	r^2
Pb	0.08, 0.16, 0.32, 0.64, 1.28	Y = 0.0172x + 0.0001	0.999
Fe	0.03, 0.12, 0.48, 2.8, 5.6	Y = 0.0697x + 0.0058	0.999
Cu	0.018, 0.054, 0.162, 0.486, 2.458	Y = 0.1109x - 0.0003	0.999
Zn	0.01, 0.04, 0.16, 0.64, 2.56	Y = 0.2495x +0.0394	0.996
Mn	0.01, 0.04, 0.14, 0.64, 2.56	Y = 0.1073x + 0.0004	0.999
Cd	0.06, 0.15, 0.375, 0.938, 2.344	Y = 0.2832x - 0.0060	0.999

4.2. Analysis of the traget metals in Moringa Oleifera leave

Mean concentration(μ) of the target metals in Moringa oleifera leave sample were presented in table 5.

Table 5: Mean concentration (mg/kg)(μ± RSD; n=3) of selected essential and toxic metals**.**

Sample area	Pb	Fe	Cu	Zn	Mn	Cd
Almu	0.05 ± 12.40	2.92 ± 0.80	0.05 ± 5.16	0.16 ± 1.66	0.54± 7.11	ND
Mender-2	0.11 ± 1.91	3.55 ± 0.73	0.06 ± 4.44	0.38 ± 1.53	0.40± 1.34	ND
Agenta	0.17 ± 7.06	4.41 ± 1.07	0.06 ± 4.63	0.36 ± 1.34	0.48± 1.44	ND
Commercial	0.17 ± 5.24	4.98 ± 1.08	0.04 ± 6.11	0.22 ± 1.39	0.44 ± 0.28	ND
FAO/WHO*	0.10 -10.00	15.00	3.00	27.40	2.50	0.20 – 0.81

*ND: not detected; *Permissible limit*.

As can be observed from the table above mean concentration of the target metals in moringa oleifera leave samples were 0.05 – 0.17, 2.92 – 4.98, 0.04 – 0.06, 0.16 – 0.38 and 0.40 – 0.54 mg/kg for Pb, Fe, Cu, Zn and Mn respectivly. But Cd was not detected.

The obtained concentration of Fe in the stuidied Moringa oleifera leave ranges from 2.92 – 4.98 mg/kg. The highest Fe concentration (4.98 mg/kg) was observed in comercial sample where as the least (2.92 mg/kg) was measured in Almu. Such variation observed might be due to sampling cites differences, difference in climatic condition , soil type, plant age, and/or different human activities [24]. Different studies have been carried out on the level of Fe in Moringa oleifera plants in different area by different researchers ,using different analytical techniques and different results were obtained. For instance according to, E. T. Gyamfi, et.al the amount of Fe in dried Moringa leave sample in Legon-Accra, were reported to be 0.29 mg/kg [47]. Other researcher, Fakankun, O.A,et.al at Nigeria also reported the amount of Fe in the leave of Moringa Oleifera as, 214.00 mg/kg [24]. Limmat.et.al in, Thailand has reported the concentration of Fe in the Moringa Oleifera leave ranging from 22.13 mg/kg – 65.35 mg/kg [23]. R.Subramanianet.al from India has also reported the leave part of Moringa Oleifera accordingly; the value was reported to be 298.09 mg/kg [22]. So generally the result obtained in this study is lower (except the one by E.T Gyamfi) than the other reported values this may be because of the aforementioned reasons above.

The studied Moringa Oleifera leaf contains Cu from 0.04 mg/kg – 0.06 mg/kg. The higher concentration was found in Mender- 2 and Agenta (0.06 mg/kg) and the lower was observed in Almu (0.05 mg/kg). Other studies have also indicated that Cu content of Moringa oleifera leaf varied with their origion [4, 22]. Cu plays role in the synthesis of haemoglobin and some neurogical conditions like Alzeimer's disease, wilson's disease, prion disease . But,when it exceed certain concentration limit it couses deramititis, metallic teste in the mouth, hair and skin discoloration [6]. The normal daily intake of Cu is 2 – 5 mg per day [6]. Different studies have also reported the concentration of Cu in leave part of Moring oleifera in different part of the world. For instance, Subramanian, et.al from India, Limmat.et.al in, Thailand, Kassa Belay and Hailay Kiros in wukiro woreda and E. T. Gyamfi et.al determined the amount of Cu in dried Moringa leave sample in Legon-Accra, also determined the amount of Cu in Moringa Oleifera and they have reported the value as follows; 0.13 mg/kg, 0.79 mg/kg - 8.49 mg/kg, 11.51 mg/kg, 2.87 mg/kg respectively [22, 23, 25, 74]. Generally those reported values of Cu in the leave sample of Moringa Oleifera plant from different area are higher than the value obtained in this

study. In edible plants the permissible limit set by FAO/WHO is 3.00 mg/kg [6]. Thus ,the obtained concentration of Cu in Moringa oleifera leaf sample in this study is below the permissible limit.

The concentration of Zn in the studied sample was varies 0.16 mg/kg – 0.38 mg/kg. The highest concentration where found in Mender-2 (0.38 mg/kg) and the lowest concentration was found in Almu (0.16 mg/kg). While the concentration of Zn in Agenta and comercial sample was 0.36 mg/kg and 0.22 mg/kg respectivly. But ,the concentration of Zn obtained in this study is lower then the values reported in moringa leave of kashare by Abdulkadir Ahmed Gidad et.al (3.40 mg/kg), India by R. Subramanian et .al (14.66 mg/kg), from central india, KarunaSVand RajniN (15.96mg/kg) and Mamatha P.et.al also determined the amount of Zn in the leave sample of Moring oleifera plants and reported the result to be 10.03 mg/kg [82 , 22, 31, 38]. Zn is found in more than 200 proteins and enzymes as well as it is also required in forebrain development, DNA synthesis ,steroidogensis bone formation and wound healing [6]. But, its high concentration is neurotoxin. Normal daily in take of Zn is 12.00 -15.00 mg per day. The perimissible limit set by FAO/WHO in edible plant was 27.40 mg/kg [6].

The concentration of Mn in the studied sample is from 0.40 mg/kg – 0.54 mg/kg . Relativily the highest concentration was found in Almu (0.54 mg/kg) and the lowest was found in Mender-2 (0.40 mg/kg). Mn activates many metalloenzymes like arginase, carboxylase, and pyruvate. Its dificiency results in several skeletal and reproductive abnormalities. But excess of manganse couse adverse effects on the lungs and brain [6]. Thus the estimated dietary in take of Mn in adults is 11.00 mg per day[6]. Different studies have been conducted on the levels of Mn in the leave of moringa oleifera plants and the following results were reported. According to Kassa Belay and Hailay Kiros in wukiro woreda, the amount of Mn were reported as 1.10 mg/kg [25] .On the other hand Limmat et.al in, Thailand, has reported the Mn concentration as 32.12 mg/kg [23]. Moreover, Jaya Gupta and Amit Gupta from Gwalior also reported the amount of Mn as 43.02 mg/kg [83]. Permissible levels in food as per WHO & FAO for Mn is 2.50 mg/kg [25]. The results obtained in this study are lower than the reported values by other researchers from different areas as mentioned above and the concentration of Mn was found below estimated dietary intake hence the consumption of leave of Moringa from the study area is safe with regard to Mn.

The highest concentration of Pb was obtained in Agenta (0.17 mg/kg) and the least concentartion was observed in Almu (0.05 mg/kg),while both Mender-2 and comrcial samples contained 0.11 mg/kg. Different reasearches also indicated that Moringa leaf contain certain amount of Pb. For instance ,according to fowotade and abdellah Moringa oleifera in kazure town ship,jigawa state of nigeria contain 0.16 mg/kg Pb [21]. Karuna and Rajina also reported that Moringa oleifera leafe of central india contain 0.04 mg/kg of Pb [31] and mashier et al.[82] also reported as Moringa leaf contain 0.87 mg/kg Pb. Pb is non–essential element and has no benifilal effects in humans. Exposure to Pb can bring health problems such as abnormal brain, chronic nephritis of the kidneys, anemia, oxidative stress etc,when its intake is exceeding certain concentration limit [6]. However, in the current study the concentration of Pb is with in the tolarable range i.e, 0.10 – 10.00 mg/kg as seted by WHO in edible plants [6].There for the consumption of Moringa oleifera leave in the study area(Pawi) is safe with respect to Pb.

In the stuidied Moringa oleifra leave samples ,Cd was not detected. However, other reports revealed that Moringa oleifera leave contains some amount of Cd, 0.16 mg/kg,0.07 mg/kg and 0.68 mg/kg [21, 22, 50] respectivly. On the other side Jaya Gupta and Amit Gupta, reported the amount on Cd Moringa oleifera (leafs) of Agra as 0.005 mg/kg [83]. It is known that Cd is very hazardoues to human .It causes increase blood pressure, damage kidneys and liver [6].According to WHO the permissible limit of Cd is 0.20 – 0.81 mg/kg in edible plants [6].

4.2. Comparison with previously reported values

In this study the obtained results were compared with different reported literature values on the same plants from different countries. The comparison result is summarized in table 6 as follows.

Table 6: Comparison of results obtained with literature reports

Analyte	Previously reported (mg/kg)	In this study (mg/kg)	Reference
Fe	298.09, 22.13, 214, 0.29	2.92 – 4.98	[22,23,24, 74]
Cu	11.51, 0.79, 2.87, 0.13	0.04– 0.06	[22, 23, 25,74]
Zn	14.66, 15.96, 10.03,3.40	0.16 – 0.38	[22, 31, 38 79]
Mn	32.12, 1.10, 43.02	0.40 – 0.54	[23.25, 80,]
Pb	0.16, 0.04, 0.87	0.05 – 0.17	[21, 31, 82]
Cd	0.16, 0.036, 0.68	ND	[21, 22, 38] respectively.

As can be absorbed from the table above, the reported values as well as the results of this study are different from each other. The results of the current study are almost lower than the other literature values. There for, the possible reasons for such variation could be either because of, the difference in geographical location, soil type , climatic condition, availability of metals, digestion procedure, nature of solvent, different human activities, plant age, instrument used and the like.

4.3 Method validation

The efficiency of the method used was evaluated by determination of LOD and LOQ as well as performing recovery studies. The LOD and LOQ of the method were determined based on the regression equation of the calibration curves and their values are given in table 7.

Table 7: LOD and LOQ of the method

Analyte	**IDL (mg/kg)**	**LOD (mg/kg)**	**LOQ(mg/kg)**
Pb	0.012	0.017	0.058
Fe	0.005	0.012	0.043
Cu	0.004	0.013	0.045
Zn	0.003	0.040	0.132
Mn	0.002	0.003	0.009
Cd	0.003	0.035	0.116

As has been previously mentioned recovery study was conducted by spiking the Moring Oleifera leave sample with known amount of analytes. The obtained results are presented in Table 8.

Table 8: Recovery (%R) of selected essential and toxic heavy metals.

Sample area		Elemets				
		Pb	Fe	Cu	Zn	Mn
Almu	Conc in sample(mg/kg)	0.05	2.92	0.05	0.16	0.54
	Added concentration(mg/kg)	0.2	2	2	2	2
	Conc.inspiked sample(mg/kg)	0.28	4.93	1.82	2.20	2.51
	Precent recovery(%)	116	101	89	102	99
Mender-2	Conc. in sample(mg/kg)	0.11	3.55	0.06	0.38	0.40
	Added concentration(mg/kg)	0.2	2	2	2	2
	Conc in spiked sample(mg/kg)	0.33	5.39	2.16	2.41	2.19
	Precent recovery(%)	108	92	106	102	90
Agenta	Conc in sample(mg/kg)	0.17	4.41	0.06	0.36	0.48
	Added concentration(mg/kg)	0.2	2	2	2	2
	Conc in spikedsample(mg/kg)	0.34	6.27	1.99	2.31	2.23
	Precent recovery(%)	87	93	97	97	87
Comercial	Conc in sample(mg/kg)	0.17	4.98	0.04	0.22	0.44
	Added concentration(mg/kg)	0.2	2	2	2	2
	Conc in spiked sample(mg/kg)	0.34	6.76	2.08	2.14	2.18
	Precent recovery(%)	87	89	102	96	87

The (% R) of the target metals were ranging from 87 – 116 %. But %R of Cd was not determined since it was not detected in the sample. The observed %R was in acceptable acceptance ranges from 80 – 120 % [84].Therefore, this indicates that the procedure used in this method is valid to determine the targeted analytes.

4.4. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a widely used statistical method to compare group means. The one way ANOVA can compare the mean of more than two groups of samples. ANOVA Uses the F-statistics to compare whether the difference between sample means are significant or not. The variation in the sample mean of the analyte was tested by using ANOVA, to determine whether the source for variation was from experimental procedure or difference in mineral content of the soil, PH of the soil, water, atmosphere, variation in application of agrochemicals like fertilizers, pesticides, herbicides etc or other variation in cultivation procedures. So the ANOVA result shows that there exists statistically significant difference at 95 % confidence level in the mean concentration of all the metals

Table 9: ANOVA between and within moringa leave sample at 95% confidence level

Metal	Source of variation	Degree of freedom	F calculated	p-value
Pb	Between sample	3	73,924	0.000
	Within sample	8		
	Total	11		
Fe	Between sample	3	163,660	0.000
	Within sample	8		
	Total	11		
Cu	Between sample	3	5,568	0.000
	Within sample	8		
	Total	11		
Zn	Between sample	3	2,774	0.000
	Within sample	8		
	Total	11		
Mn	Between sample	3	1973	0.000
	Within sample	8		
	Total	11		

5. CONCLUSSION AND RECOMMENDATION

5.1 Conclusion

The amount of heavy metals were determined by using flame atomic absorpation spectrometry.The mean concentration of the metals obtained were 0.05 mg/kg - 0.17 mg/kg for Pb, 2.92 mg/kg - 4.98 mg/kg for Fe, 0.04 mg/kg - 0.06 mg/kg for Cu, 0.16 mg/kg - 0.38 mg/kg for Zn and 0.40 mg/kg - 0.54 mg/kg for Mn and the concentration of Cd was not detected. In all studied sites the concentration of the selected elements are in the order of Cu < Pb < Zn < Mn < Fe. The results obtained shows that trace metals found in the leave of Drumstick, *Moringa oleifera leave* samples were generally below the WHO and FAO permissive level and Therefore, according to this study, consumption of *Moringa Oleifera* leaves is recommended because of the less concentration of these trace heavy metals that cannot lead health problem to the consumer. Based on the current standing, eating *Moringa* contributes appreciable amount of trace metals for the individuals. The result obtained in the current study were also compared with eairly reported values.The variation in the heavy metal contents of *Moringa* oleifera leave found when compared to earlier studies could be due to climatic and edaphic factors, solvents used for the analysis, the cultivation method used and age of the plant and the instrument used. In addition, the method has been validated in terms of percent recovery, detection limit and limit of quantification, precision and accuracy. The statistical analysis performed using one way ANOVA, indicates the presence of significant difference ($p < 0.05$) between the mean concentrations of analytes of the study areas.

5.2 Recommendation

The following recommendations are made as a result of the outcome of this study

- Monitoring the levels of heavy metals in *Moringa* should be encouraged.
- We recommended that research shoud be carried out on other part of *Moringa oleifera.*
- Study might be conducted on the metal contents of soil on which the Moringa plants grows.
- It might be repeated with other analytical instruments (ICP-OES or ICP-MS) to draw strong conclusion about the metals content of Moringa in the studied areas.

6. REFERENCES

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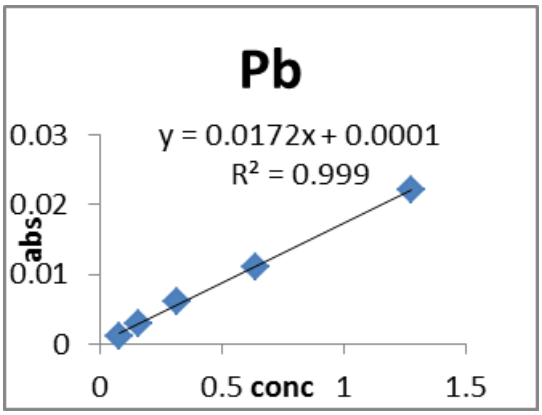
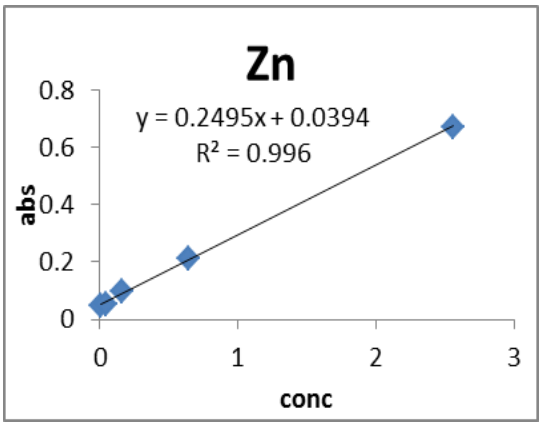
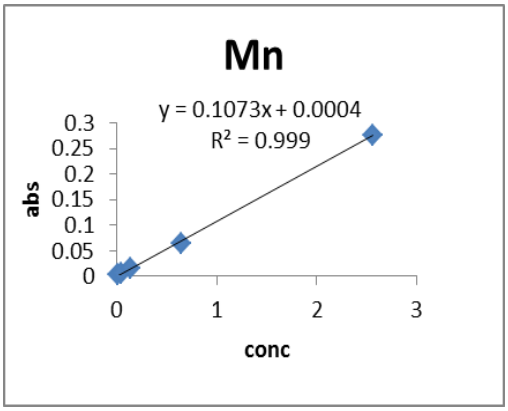
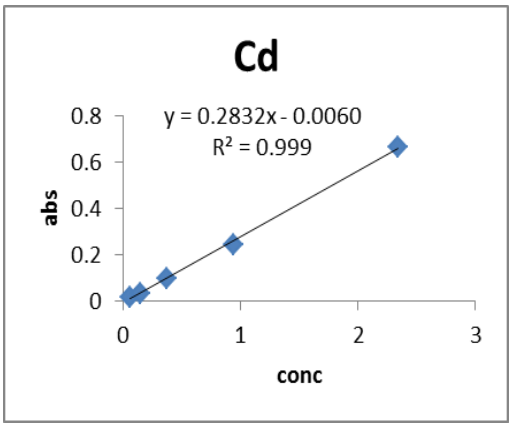
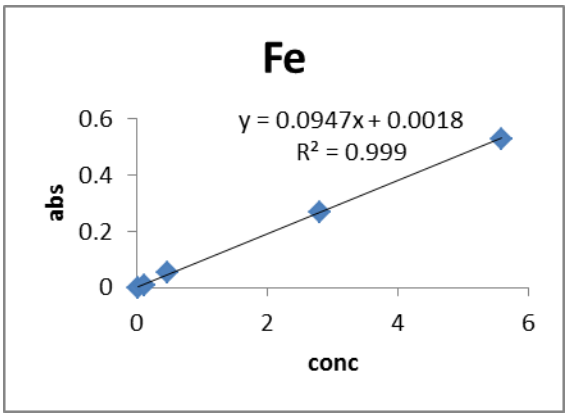
APPENDEX: calibration curves for each analytes.










Fe
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R² = 0.999
abs
conc
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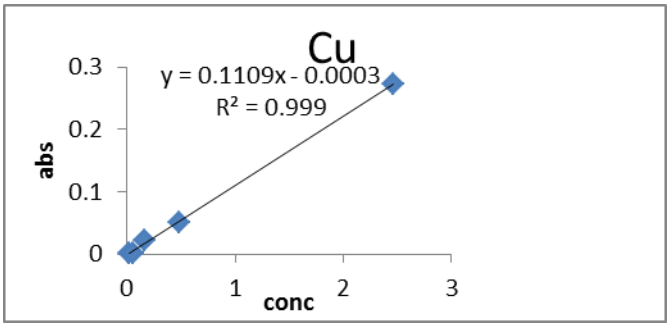
0.4
0.2
0
0
2
4
6


Cu
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R² = 0.999
abs
conc
0.3
0.2
0.1
0
0
1
2
3