LEVELS OF ESSENTIAL NUTRIENTS AND TOXIC METALS IN FRUITS OF AWARA MELKA AND NURA ERA FARMS, ETHIOPIA

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DEDICATION

This work is sincerely dedicated to:

Ato Guta Yami, W/o Abaru Fayisa, My Brothers and Sisters for Their Support and True love, and All African Children Suffering from Malnutrition

Shambel Guta

DECLARATION

I, the undersigned, declare that this is my original work and has not been submitted for a degree in any other university, and that all sources of material used for the thesis have been duly acknowledged.

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TABLE OF CONTENTS

Page

Acknowledgements..... i

Table of content..... ii

Lists of Tables..... iv

Lists of Figures..... v

Abstract..... vi

1. INTRODUCTION 1

1.1 General Background of the Study..... 1

1.2 Fruit Production in Ethiopia 2

1.3 Role of Fruit Nutrients in Human Nutrition..... 6

1.3.1 Role of vitamin C in human health..... 6

1.3.2 Role of metals in fruits in human health..... 8

1.3.3 Physicochemical forms of metals in environmental samples..... 12

1.3.4 Factors affecting fruits nutrient compositions..... 13

1.4 Objectives 13

1.5 Analysis of Nutrients in Fruits 14

1.5.1 Analysis of vitamin C in fruits 15

1.5.2 Analysis of metals in fruits 15

1.5.2.1 Sample decomposition 16

1.5.2.2 Determination of concentration of metals..... 17

2. EXPERIMENTAL..... 18

2.1 Equipments and Reagents..... 18

2.1.1 Equipments 18

2.1.2 Reagents..... 18

2.2 Sample Collection and Pretreatment..... 19

2.2.1 Cleaning sample containers..... 19

2.2.2 Fruits sampling and pretreatment..... 19

2.2.3 Soil sampling and pretreatment..... 21

2.2.4 Water sampling and pretreatment 21
2.3 Digestion of Fruits and Extraction of Metals in Soils 21
2.3.1 Digestion of fruits for metal concentration determination 22
2.3.2 Extraction of soil samples..... 23
2.4 Determination of Metals in Fruits, Soils, and Water Samples..... 23
2.5 Extraction and Determination of Vitamin C..... 24
2.6 Determination of pH of Soils 25
2.7 Determination of Electrical Conductivity 26
3. Results and Discussion..... 27
3.1 Moisture Content of Fruits 27
3.2 Validation of Analytical Procedure 28
3.2.1 Accuracy of procedures in the determination of metals 28
3.2.1.1 Recovery of metals in spiked fruit samples..... 29
3.2.1.2 Recovery of metals in spiked soils samples..... 30
3.2.2 Assessment of accuracy of procedures for the determination of total ascorbic acid 32
3.3 Method Detection Limits (MDL) 33
3.4 Concentration of Metals in Fruits 34
3.5 Soil Analysis 38
3.5.1 Some characteristics of soil 38
3.5.2 Concentrations of metals in the soils 43
3.6 Concentration of Metals in the Water..... 48
3.7 Correlations among Level of Metals in Fruits, Soils and Irrigation 50
3.8 Total Ascorbic Acid in the Fruits Obtained From the Selected Farms 50
4. Conclusions and Recommendations..... 53
4.1 Conclusion..... 53
4.2 Recommendations..... 54
5. References..... 55

LIST OF TABLES

Page

Table 1. Location of Awara Melka and Nura Era Farms..... 4

Table 2. Climatic information on Awara Melka and Nura Era Farms..... 5

Table 3. Digestion procedures tested for decomposing fruit samples..... 22

Table 4. The moisture content of fruits 28

Table 5. Recoveries of metals in fruit samples spiked with metal standards..... 30

Table 6. Recoveries of metals in soil samples obtained by spiked with metal standards 32

Table 7. Method detection limits of the soil and fruits 33

Table 8. Metal level of fruit samples collected from the Farms 37

Table 9. Some characteristics of the soils collected from the Awara Melka and Nura Era farms 42

Table 10. Average metal concentration in soils from Awara Melka and Nura Era Farms 44

Table 11. Average metal concentration and their ranges in water samples collected from Rivers and Channels used to irrigate Awara Melka and Nura Era Farms..... 49

Table 12. Average concentrations of total ascorbic acid in the fruits..... 51

LIST OF FIGURES

Page

Figure 1. Map of Ethiopia showing the location of Nura Era Farm..... 5

Figure 2. Reaction of $AscH^-$ with free radicals 7

Figure 3. Average metal concentrations in the fruits collected from Awara Melka and Nura Era Farms 38

Figure 4. Average metal concentrations in the soils with respect the lands on which fruits planted on 43

Figure 5. Working curve for vitamin C 52

ABSTRACT

LEVELS OF ESSENTIAL NUTRIENTS AND TOXIC METALS IN FRUITS OF AWARA MELKA AND NURA ERA FARMS, ETHIOPIA

By: Shambel Guta

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Levels of selected essential (Na, K, Ca, Mg, Fe, Cu, Zn, Mn) and non–essential (Pd and Cd) elements were determined in banana, grape, guava, mandarin and orange. Bananas were collected from Awara Melka Farm, and the remaining from Nura Era Farm, Ethiopia. The freeze dried fruits were digested by wet digestion using HNO_3 and $HClO_4$. The levels of the above elements were determined using flame atomic absorption spectrometer. The highest amount of K (348) and Mg (28.12 mg/100 g edible portion) were obtained in banana compared to other analyzed fruits. Total ascorbic acid of those fruits were also determined by 2,4-dinitrophenylhydrazine method. Appreciable amount of total ascorbic acid was found in guava (117.1 mg/100 g edible portion) compared to that of other analyzed fruits. Further more, soils and irrigation waters were analyzed for the above metals.

Key words: Banana, Grape, Guava, Mandarin, Orange, Total ascorbic acid, Flame atomic absorption spectroscopy.

Therefore, interest in the role of antioxidants in the human health has prompted research in the fields of horticulture and food science to assess fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved through cultivars development, maturity, harvesting methods, post-harvest procedures, processing technologies and storage conditions [6].

Furthermore, biological materials, such as fruits, often serve as sensitive indicators of pollution. They are employed for studies on the uptake of toxic materials like uranium, thorium, cadmium, mercury and lead [7].

Therefore, analysis of nutrients in fruits can be seen from environmental and nutritional points of views, because the results can indicate the extent of pollution of the environment and nutritional status of human. Accurate and adequate food composition data are invaluable for estimating the adequacy of intakes of essential nutrients and assessing exposure risks from intake of toxic non-essential elements. Moreover, the data can help in recommending daily intakes of fruits locally.

In light of the above, it is worthwhile to determine the concentrations of some minerals (Na, K, Ca, Mg, P, Fe, Cu, Zn, Mn, Pb, and Cd) and nutrients (vitamin C) in fruits cultivated and consumed in Ethiopia. In Ethiopia, as in many less developed countries, such data are not readily available.

1.2 Fruit Production in Ethiopia

About 0.23 million tons of fruits are estimated to be produced annually in Ethiopia [8]. Apple, apricot, avocado, banana, guava, grape, grapefruit, lemon, mango, mandarin, orange, papaya, peach and pineapple are major fruits produced in different parts of Ethiopia mostly for local consumption. Much of fruits in Ethiopia are produced in gardens and commercial farms such as

Awara Melka, Nura Era, Metehara, Shewa Robit, Error Gota and Hurso Military Training Center Farms etc. In areas suitable for fruit production, some farmers produce fruits for local consumption [9].

Ethiopian Fruit and Vegetables Marketing Enterprise (Etfruit) is the leading organization that distributes citrus fruits in many Ethiopian cities. Etfruit sold about 142.9, 7.5, 1.4, 276.1, 1623, 2.0, 2.3, 4.9 and 0.8 thousand quintals of banana, grapefruit, lemon, mandarin, orange, grapevine, guava, mango, and papaya, respectively and got 244.9 million Birr from 1988-1992 Ethiopian Calender. Upper Awash Agro-industry Enterprise is the main supplier of citrus (orange, mandarin, grapefruit, lemon and lime) to Etfruit [9].

Quality Control Service of the Etfruit evaluates the physical appearance of fruits. It has no laboratory to evaluate internal quality (chemical composition). At present, producers are not paid by the quality of fruits they produce. On the other hand, consumers do not get quality fruits even if they are willing to pay. This has negative effect on the fruit industry. A joint effort to study the existing situation by concerned institutions such as Etfruit, Ethiopian Quality and Standards Authority, Ethiopian Health and Nutritional Research Institute, Ethiopian Agricultural Research Organization, other researchers and fruit producing farms may help in improving the quality standard of fruit production in Ethiopia. This will help the country in general and fruit producers in particular to increase the production of fruits for local consumption and export of fresh and processed fruit products. Quality is important particularly for export markets where competition is very keen [9].

Mineral and nutritional analysis has been done on apple, avocado, apricot, and banana that have been collected from Tigray, Gonder, Wello, Arsi, Harar, and Sidamo and average data for each fruit have been given [10]. Variations of nutrient composition of fruits on characteristics of the land, climate, cultivation conditions, and stage of maturation, and the role of fertilization and

irrigation water have been overlooked. In addition, only few fruits were analyzed even though the composition varies within species.

Generally, there is no exhaustive work that has been done on fruits cultivated and consumed in Ethiopia. In the present work, it was planned to analyze most essential and toxic nutrients in selected varieties of fruits mainly to assess nutritional impact on health. This is essential in view of the existing knowledge gap about the composition of fruits produced in Ethiopia. Furthermore, research endeavors of this nature mark the bottom line, as studies linking nutrient composition of fruits to farmland and irrigation water quality in Ethiopia are non-existent. Besides such study creates public awareness (consumers, merchants and producers) on the composition of foodstuff that is produced and consumed in Ethiopia.

In this study, banana from Awara Melka Farm and grape, guava, mandarin, and orange from Nura Era Farm were collected. Soil and water samples were collected from both farms. Locations and climatic conditions are shown in Tables 1 and 2, respectively, for the above farms. In addition Figure 1 shows the map of Ethiopia to locate Nura Era Farm from which most of the fruits were collected.

Table 1. Location of Awara Melka and Nura Era Farms [9].

Farms	Location			Distance from Addis Ababa (km)	Nearest big town
	Region	Zone	District		
Awara Melka	A.N.R.S.	Awash Fentale	Awash Fentale	235	Metehara
Nura Era	O.N.R.S.	East Shewa	Boset (Walenchiti)	188	Metehara

A.N.R.S.: Afar National Regional State; O.N.R.S.: Oromiya National Regional State.

Table 2. Climatic information on Awara Melka and Nura Era Farms [9].

Farms	Altitude (m)	Annual rain fall (mm)	Temperature (°C)		Agro-ecological zones
			Maximum	Minimum	
Awara Melka	750	250-300	39.0	8.0	A_1
Nura Era	1100-1205	316	37.5	10.4	SM_2

A_1: Hot to warm arid low land plains.

SM_2: Tepid to cool sub moist mid highland.

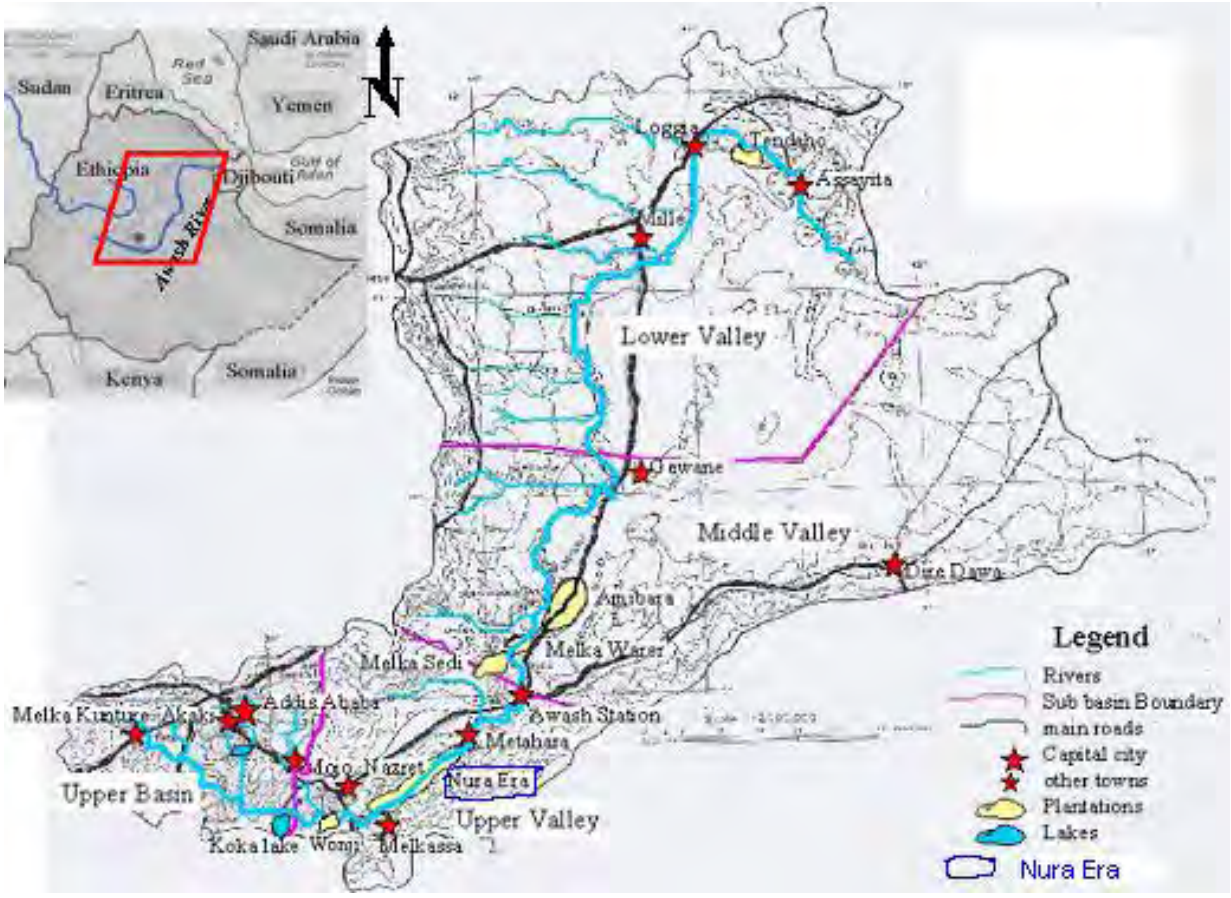

Figure 1. Map of Ethiopia showing the location of Nura Era Farm.

1.3 Role of Fruit Nutrients in Human Nutrition

Fruits are known to have included in the human diet since prehistoric time and are now in the Western and developing countries there is a habit to take fresh fruits after lunch or dinner. Fruits contain food nutrients including vitamins, minerals, and flavonoids. Some times after starvation of serious diseases, doctors recommend to take some citrus fruits for vitamins, and minerals which recover weak health conditions by improving appetite quickly. Fruits as a class are valuable chiefly for their content of vitamins. Fruits also contain appreciable amount of essential minerals [11, 12].

1.3.1 Role of vitamin C in human health

Vitamin C is a water-soluble vitamin that is very unstable in air and would loose some of its activity when exposed to heat, moisture or agitation [13]. Because of its instability it is highly recommendable that it be acquired from fresh and natural sources, which are mainly plants. Fruits have been reported to be the best source of vitamin C. If people have to consume and acquire this essential vitamin from fruits, a detailed documentation of the contents in the various fruits is necessary, so as to serve as a guide to the consumers.

Vitamin C is essential for healthy teeth, gums and bones; helps heal wounds, scar tissue, and fractures; prevents scurvy; builds resistance to infection; gives strength to blood vessels; aids in the absorption of iron. It is required for the synthesis of collagen, the intercellular "cement" which holds tissues together [14].

Furthermore, vitamin C is an antioxidant. Antioxidants help to neutralize free radicals that are generated in the body and damage cell proteins, lipids and carbohydrates. Antioxidants prevent this damage. Ascorbate is an excellent

reducing agent. It readily undergoes one-electron oxidation processes to form the ascorbate radical (Asc•–) as an intermediate. Because Asc•– has its unpaired electron in a highly delocalized π-system, it is a relatively unreactive free radical (Figure 2). These properties make ascorbate a superior biological, donor antioxidant [15, 16].

The kinetics of these electron (hydrogen atom) transfer reactions is rapid. Thus, both thermodynamically and kinetically, ascorbate can be considered to be an excellent antioxidant. Although ascorbate itself forms a radical in this reaction, a potentially very damaging radical (X•) is replaced by the domesticated Asc•–. Asc•– does not react by an addition reaction with O_2 to form dangerous peroxyl radicals. Ascorbate (probably Asc^{2-}, and/or Asc•–) appears to produce very low levels of superoxide [17, 18]. But by removing $O^{2\bullet-}$, superoxide dismutase provides protection from this possibility [19]. The biological organism is protected from further free radical-mediated oxidations. In addition, Asc•– as well as the dehydroascorbic that is formed can be reduced back to ascorbate by enzyme systems. Thus, it is recycled.

$$2\ Asc^{\cdot-} + H+ \rightleftharpoons AscH^- + DHA$$

All oxidizing free radicals with greater reduction potentials, which include HO•, RO•, LOO•, GS•, urate, and even the tocopheroxyl radical (TO•), can be repaired by ascorbate. That is, $AscH^-$ reacts rapidly with these and similar oxidants making it an outstanding donor antioxidant. Therefore, we have:

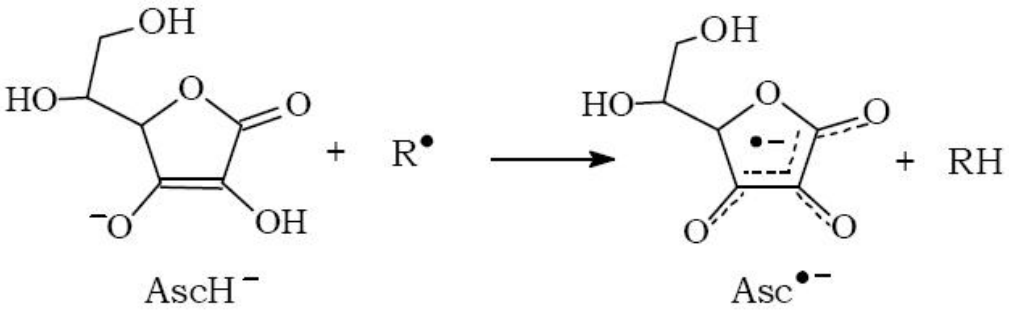

Figure 2. Reaction of $AscH^-$ with free radicals.

where R• is any of these oxidizing free radicals. $AscH^-$ donates a hydrogen atom ($H\cdot$ or H^+ + e-) to an oxidizing radical to produce the resonance-stabilized tricarbonyl ascorbate free radical [20].

When biological fluids or tissues are examined by electron paramagnetic resonance spectroscopy (EPR), Asc•– will most likely be observed [15, 21]. The ascorbate free radical is naturally detectable by EPR at low steady-state levels in biological samples. As oxidative stress increases in a system, the steady state Asc•– concentration increases. These findings are consistent with ascorbate's role as the terminal small-molecule antioxidant. It is proposed that ascorbate, i.e., the ascorbate free radical, which is naturally present in biological systems, can be used as a noninvasive indicator of oxidative stress [21].

1.3.2 Role of metals in fruits in human health

The importance of optimal intakes of essential mineral elements to maintain peak health is widely recognized. Inadequate intake of mineral elements has been a major nutritional problem in the environment [22]. Generally, too low or too high of a concentration of trace elements in human diet can affect quality of human life. Equally, industrial-based metallic contamination of the air, soil and water supplies can have dramatic effects on human well-being. This is why water, air, soil and foods are tested for metals concentration to ensure that they meet acceptable standards and should not pose a health hazard.

It has long been known that the proper functioning of life is critically dependent on trace elements in a number of different ways. Some metals *(e.g.*, Hg, Pb) and metalloids (As) are highly toxic whereas others *(e.g.*, Mo, Mn, Fe, Co, Cu, and Zn) considered essential, are needed for the accomplishment of life processes. A number of other elements *(*e.g., V, Cr, and Ni) are recognized as

being beneficial to life. The physiological role of Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Pb, and Cd are briefly described below one by one.

Sodium (Na)

Sodium which supplies the major positive ion (Na^+) in extracellular fluid is vital in fluid balance and conduction of nerve impulse. It also participates in nutrient absorption [23-25]. Sodium deficiency can cause muscle cramps [24, 25]. Excess intake of sodium is related to hypertension and some increase in calcium loss in the urine [24, 25].

Potassium (K)

Potassium is the third most abundant mineral found in human body next to P and Ca. It is a major electrolyte of intracellular fluid, and functions similarly to sodium in many cases [23-25]. Irregular heart beat, loss of appetite and muscle cramps are deficiency symptoms of potassium [24]. Some toxicity due to high consumption includes slowing down of heartbeat and kidney failure [24, 25].

Calcium (Ca)

Calcium forms a vital part of bone and tooth structure, and is also important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve-impulse transmission. It also participates in glycogen metabolism [23, 25, 26]. Inadequate intake of calcium increases the risk of osteoporosis (bone loss with no apparent cause) [24]. Excess intake of Ca may cause kidney stones and reduces mineral absorption in general [24, 25].

Magnesium (Mg)

Magnesium is important for nerve, lung and heart function. It is a co-factor for many coenzymes, and as well affects potassium and calcium metabolism [23-26]. It is also important in bone strength. Deficiency of magnesium is linked to high blood cholesterol, high blood pressure, pregnancy problems, weakness, muscle pain and poor heart function [23-25]. Excess intake of magnesium causes weakness in people with kidney failure [24].

Iron (Fe)

Iron is critical components of hemoglobin, myoglobin, and the cytochromes. It is a co-factor for some enzymes and also used for immune function [22-25]. Deficiency of iron results in anemia which is recognized by its symptoms such as low blood iron levels, small and pares red blood cells and low blood hemoglobin values [23-25, 27]. Iron deficiency can also decrease learning ability of children [23]. Iron toxicity usually results from a generic disorder called *hemochromatosis*. This disease causes over absorption and accumulation of iron, which can result in severe liver and heart damage [24].

Manganese (Mn)

Manganese activates several enzymes and needed for hemoglobin synthesis, urea formation, growth, reproduction, lactation and bone formation [23-26]. The manganese is associated with cartilages and bones formation [28]. Exposure to high level of manganese can cause both mental and emotional disturbances, along with increased slowness and clumsiness of body movements. Any brain injury due to the accumulation of manganese in brain is permanent. In animal studies, it has been indicated that manganese may also be a reproductive toxicant, especially to males, injuring the testis and causing impotence [29].

Copper (Cu)

Cu is required with iron for synthesis of hemoglobin. It works with many enzymes such as those involved in protein metabolism and hormone synthesis [23, 24, 26, 27]. Deficiency of copper causes anemia, low white blood cell count and poor growth [24, 28]. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's disease [24, 27, 30].

Zinc (Zn)

Zinc, essential trace element for human, has been reported since 1934 [31]. It is a co-factor for over 300 enzymes those involved in growth, immunity, alcohol metabolism, sexual development, reproduction and DNA and RNA synthesis [23-25, 28]. A zinc deficiency results in inadequate growth, loss of appetite, inadequate mental function, reduced sense of test and smell, fall in immune function, hair loss, and a persistent rash [23-25, 28]. Excessive dietary exposure of zinc can cause gastrointestinal distress, diarrhea, pancreatic damage and anemia [32], reduction of copper absorption and depressed immune function [24, 25].

Lead (Pb)

Lead is known as toxic element. The toxicity effect includes impairment of mental activity, reproductive and development problems, disorder in bone formation and renal function [27, 33].

Cadmium (Cd)

Cadmium has no known nutritional function, and is highly toxic to humans. The biochemical effects of cadmium include interference with enzymatic activity, damaging kidney, hypertension and anosmia (absence of smell) [27].

1.3.3 Physicochemical forms of metals in environmental samples

Metals can exist in a range of physicochemical forms in environmental samples, including hydrated metal ions, inorganic and organic complexes, and adsorbed on organic and inorganic colloidal particles. The toxicity of a metal ion varies with its physicochemical form, and the predominant reason for speciation studies is to measure the "toxic" fraction of the metal. Typically, the most toxic forms are the hydrated metal ions and labile complexes (i.e., dissociation can readily occur), and the least toxic forms are strongly bound metal complexes and metal adsorbed on colloidal particles (indeed, in unpolluted natural waters, metals are usually present in one of these latter two forms). Metal complexes with very lipophilic organic ligands can also be highly toxic due to the ability of these complexes to cross cell membranes (this group also includes organometallic alkyl compounds of lead, tin, and mercury). One simple example of speciation is the measurement of the concentrations of inorganic lead and organometallic lead compounds. The latter species are considerably more toxic than the former [34]. Therefore, the intake, accumulation, transport and storage of essential or toxic metals and metalloids are realized by complexation of the element ion by electron-pair donating biological ligands [35].

One of the most serious problems facing the world today is the contamination of the environment by inorganic, organic, and organometallic species. One area of particular interest is the detection of heavy metals and metalloids in environmental matrices. Ultimately, these toxic metals are incorporated into drinking water and various food chains. Since metals are biologically nondegradable, they tend to accumulate in various vital organs; therefore, even exposure to trace concentrations of various metal ions can lead to long-term toxic effects [34].

The preceding discussions indicate that the accurate evaluation of toxicity of metals can be achieved through speciation studies. However, the first step in speciation involves determination of total elemental concentration of samples as this provides preliminary information on the extent and probable effects of the metal on the environment. Since fruits can be important sources of essential and toxic metals, and nutrients, assessment of the composition of fruits before consumption is highly desirable to minimize health risk.

1.3.4 Factors affecting fruits nutrient compositions

Several authors have demonstrated that there are variations of nutrient composition among and within the existing species of fruits depending on the characteristics of the land, climate, cultivation conditions, stage of maturation, fertilization, applications of herbicides and composition of irrigation water [36-38]. *This is why the United Nations Organization for Food and Agriculture (FAO) has recommended that food composition charts should be prepared for food produced and consumed locally*. In response to such recommendations, several analytical techniques have been developed to assess the composition of foodstuff including fruits.

1.4 Objectives

Reliable data on the nutrient composition of foods are important in many areas of endeavour including health assessment, formulation of appropriate institutional and therapeutic diets, nutrition education, food and nutrition training, epidemiological research on relationships between diet and disease, plant breeding, nutrition labeling, food regulation and consumer protection as well as for a variety of applications in agriculture, trade, research, development and assistance [39].

A. General objective

The main objective of this project was to determine the extent of accumulation of essential nutrients, toxic metals and total ascorbic acid in fruits produced in Awara Melka and Nura Era Farms, Ethiopia.

B. Specific objectives

1. to develop suitable methods for the digestion of fruits including banana, grape, guava, mandarin, orange; and soils;
2. to determine the levels of metals (Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Pb, and Cd) in fruits, soils and waters using flame atomic absorption spectrometer (FAAS);
3. to determine total ascorbic acid and moisture contents in fruits;
4. to correlate the levels of metals in fruits with that of soil and irrigation water.

The essential metals Na, K, Ca, Mg, Fe, Cu, Zn, and Mn, and ascorbic acid were selected because of their important biological roles in the human body whereas Cd and Pb, being non-essential were included due to their toxic nature.

1.5 Analysis of Nutrients in Fruits

Metals may be determined by a variety of methods. Basic parameters that are important in establishing a method for analysis of foods are precision, accuracy, sensitivity, and suitability for quality control and ability to automate. Freedom from interferences is crucial because of the wide ranges of matrices that may be encountered [40].

1.5.1 Analysis of vitamin C in fruits

Many instrument-based analytical methods including high performance liquid chromatography (HPLC) [41, 42], polarography [43, 44], and photometric [45-47] methods have been reported in the literature for the analysis of vitamin C in fruits. However, photometric methods are particularly attractive because of their simplicity. Especially, the coupling of 2,4-dinitophenylhydrazine (DNPH) with ketonic groups of dehydroascorbic acid (DHAA) and diketogulonic acid has been the basis of many methods for the determination of total vitamin C contents. Adding trichloroacetic acid precipitates proteins present in the samples to avoid matrix interference during absorption measurements and; aliquots of filtrate are shaken with acid-washed charcoal (norit) or activated charcoal or bromine to clarify the solutions and to oxidize ascorbic acid to DHAA. The osazones thus formed during the 3 hours incubation at 37°C by the reaction of DNPH and DHAA gives a reddish colored product upon treatment with 85% H_2SO_4 which is measured photometrically [45, 48].

1.5.2 Analysis of metals in fruits

Elemental analysis of the majority of organic and inorganic matrices requires the partial or total dissolution of the sample prior to most instrumental analysis; though, techniques for analyzing solid samples are available in the literature [28, 49].

1.5.2.1 Sample decomposition

Routine digestion of biological samples is undertaken by dry ashing (mostly at 450-550°C) [27, 50-52], fusion [27, 50-52], and wet-digestion [27, 50-52]. There is no universal procedure for all types of samples. The most desirable features of such procedures are: (1) the ability to dissolve the sample completely (no insoluble residues); (2) reasonably quick and always safe; (3) no possible source of sample loss through volatility, adsorption on to the walls of the vessel; and (4) elimination of sample contamination from the reagents used in the dissolution process [27]. The decomposition serves several purposes such as (i) converting all the species in which an element X is present in such a way that it becomes present in one uniform and defined form like the elements (e.g., Hg) the hydride (As, Se), the cation (Cd, Co, Cu) or the anion (chromium), (ii) eliminating interfering substances from the matrix, and (iii) obtaining the element X in a homogeneous and easily accessible matrix [49].

Both wet and drying ashing procedures in open systems are slow and time consuming. In wet digestion, only a small sample must generally be used, because a relatively large volume of reagents must be employed, and contamination due to reagent impurities can be a problem. In dry ashing, relatively large samples can be treated if high-temperature ashing is employed. However, in high-temperature ashing, contamination caused by airborne substances and by component of the ashing apparatus may be a problem. In both dry and wet ashing in open systems, volatility loss of analytes may be a problem. Wet digestion in closed vessels can eliminate loss of volatile elements and increase the rate of digestion.

In recent years, microwave digestions in closed vessels have been developed as rapid and reproducible sample preparation methods for trace element analysis of a various environmental samples [49]. An extra advantage is the ease with which microwave dissolution can be automated in comparison to traditional flame, hot plate and furnace dissolution techniques.

1.5.2.2 Determination of concentration of metals

Digests obtained employing one of the techniques described in section 1.5.2.1 are generally suitable for the determination of metals using several instrumental methods. A lot of atomic spectroscopic based techniques including flame photometry for K, and Na and AES and/or FAAS for Ca, Cu, Fe, Mg, Mn and Zn, etc., have been used in the determination of metals in fruits [11, 37, 38, 52, 53].

The most common instrumental methods used for determination of metals in biological samples are flame atomic absorption spectroscopy [38, 53], inductively coupled emission spectroscopy [54], inductively coupled mass spectroscopy [55], and anodic stripping voltammetry [56]. Atomic absorption spectroscopy after wet digestion method for fruit analysis was found efficient for most metals determination [38, 53]. In practice FAAS is simple and easy to use for a wide range of elements [28]. Simultaneous multielement methodology is important to cut down time required for analysis. Thus ICP-AES is potentially attractive approach to analysis of trace metals nutrients and toxicants. Unfortunately in many cases the detection limits by this technique are not adequate to determine background level of these substances [28]. ICP-MS is an analytical technique capable of simultaneous multielement determinations at the ultratrace level. Other atomic spectroscopic techniques, such as flame atomic absorption, graphite furnace atomic absorption, and ICP-AES, are broadly capable of determining the same suite of elements, but ICP-MS is more versatile in terms of the range of elements that can be measured in any given sample without interference, and with respect to its speed, sensitivity and isotopic discriminatory capabilities.

2. EXPERIMENTAL

2.1. Equipments and Reagents

2.1.1 Equipments

Freeze dryer (Freeze dry-3, Labconco, Kentucky, U.S.A.) was used for drying fruits samples to constant mass. A digestion apparatus consisting of 100 mL round or flat bottom flask fitted with condenser and a hotplate was used for decomposing the sample matrix. A flame atomic absorption spectrometer (Buck Scientific Model 210VGP AAS, East Norwalk, U.S.A.), was used for measuring the concentrations of metals in fruit, soil and water samples.

S2000 Lightwave Spectrophotometer (Cambrige, UK) equipped with UV/VIS diode array detector was used for the absorbance measurements during the analysis of total ascorbic acid.

WTW Inolab pH/ION Level 2 pH meter was used for the measurement of the soil pH.

Thermo Orion Conductivity meter Model 145 was used for the measurement of electrical conductivity of soil samples.

2.1.2 Reagents

Deionized water was used throughout the experiments for all dilutions and rinsing purposes. The following reagents were used as received for the indicated purposes:

Fruit digestion: 70% HNO_3 (Spectrsol®, England), 70% $HClO_4$ (Aldrich, A.C.S. Reagent, Germany).

Soil digestion: 70% HNO_3 (Spectrsol, England), 37% HCl (Riedel-deHaen, Chem. Pure, Germany).

Metal standard curve preparation: Buck Scientfic Puro Graphic^{tm} calibration standard (East Norwalk, U.S.A.).

Vitamin C analysis: 98.5% Trichloroacetic acid (Hopkin and Williams, A.C.S Reagent, England), meta-phosphoric acid sticks (BDH, G.R, England), 2,4-DNPH (Schiapparelli, A.R, Italy), 95-97% H_2SO_4 (Merck, Germany), 99.5% thiourea (Merck, G.R, Germany), 99.5% Br_2 (Merck, A. R, Germany), 99.7% L-ascorbic acid standard (BDH, AnalaR®, England).

2.2 Sample Collection and Pretreatment

2.2.1 Cleaning sample containers

All polyethylene bottles used in analytical work were soaked in detergent for one day, rinsed with tap water, soaked in 10% nitric acid for two days, rinsed more than three times with deionized water, dried in air and kept in plastic bags until needed to avoid contamination from the surrounding [27].

2.2.2 Fruits sampling and pretreatment

The selection of fruit type among the available varieties was based on the largest productivity of the fruit. The largest citrus orchard (about 1000 ha) in Ethiopia is found in Nura Era Farm [9]. Unripe banana (*Musa Cavendish*) was collected in December 2004 and March 2005 from Awara Melka Farm and allowed to ripen in the laboratory according to the procedure followed by the farm (providing heat by covering the fruit with sacks and plastics). White grape, guava (unknown variety), mandarin (Orlando variety), and orange (Pineapple variety) of similar degree of maturity were collected randomly from different plants grown in Nura Era Farm in December 2004 and March 2005. All these fruits were fully matured and first grade according to the farms' criteria. The selection of the varieties for each fruit type among the available varieties was based largely on high consumption by the society [9]. The fruits except banana were placed in ice-box to keep them as fresh as possible with polyethylene sheet between each fruits to minimize diffusion through contact. The fruits

were transported to the laboratory within one day after collection. On the next day to remove surface contaminants, fruits were lightly scrubbed with a paintbrush in tap water, washed in deionized water containing few drops of detergent and a few milliliters of concentrated HNO_3, rinsed in deionized water and finally blotted dry with tissue paper [57]. Some of the fruits were kept at – 20 ^{0}C until analysis for ascorbic acid without peeling the fruits. From the remaining fruits, a single fruit was dissected into quarters along the equatorial plane [58] with plastic knife. A quarter or a representative sub-sample of each fruit of the same species was collected. Banana, guava, mandarin, and oranges were peeled using plastic knife. Seeds and large particles of cellular materials of grapes, mandarin, and oranges were thrown with the help of plastic knife to represent the consumed portion (e.g., without seeds and peel) [53, 59]. Seeds of guava were removed from edible part by passing through a fine plastic strainer. Seeds of grapes were removed with the help of plastic knife. These samples were homogenized with blender. Each homogenized sample was placed separately in weighed freeze-drying bottles. The masses were measured and the bottles were placed in the freeze dryer and samples were dried until constant masses have been obtained. The dry samples were stored in polyethylene bags until the time of analysis.

2.2.3 Soil sampling and pretreatment

Soil samples were collected from Awara Melka and Nura Era Farms only from lands (blocks) on which the selected fruits have been planted. Sampling points for soil samples were selected based on an imaginary zigzag line/pattern. At each selected point the soil was dug to 45 cm depth in V-shape hole. A 5 cm

slice of soil was cut from top to bottom and mixed in the hole using plastic spade. Stones, grass and other extraneous materials were removed. More than half kilogram of soil was collected from each point in plastic bags, and then brought to laboratory. About 500 g of soil from each gross sample was taken and thoroughly mixed in the laboratory. The soil sample was air dried in the laboratory, ground using mortar and pestle, sieved through 0.090 mm sieve and then stored in plastic bags until analysis.

2.2.4 Water sampling and pretreatment

Water samples were collected from Bulga and Awash Rivers that are used for irrigation by Awara Melka and Nura Era Farms, respectively. The samples were collected at inversion points and from the channels that feed the selected fruits. In all cases the water samples were taken by placing the mouth of polyethylene containers (nitric acid cleaned) approximately 2 cm below the surface of the water. The water samples were brought to the laboratory and then centrifuged to remove the suspended particles, filtered, and preserved with 2 mL of 10% HNO_3 per 100 mL of water and stored in a refrigerator without freezing until analysis [27].

2.3 Digestion of Fruits and Extraction of Metals in Soils

This study involved the analysis of organic species, (vitamin C), and inorganic species, i.e., metals. Thus, two types of sample preparation methods were applied to convert analytes to suitable forms.

2.3.1. Digestion of fruits for metal concentration determination

Different wet-digestion methods were tested to select optimum procedure. The optimization was based on production of clear solution, shorter digestion time and minimum reagent consumption. The optimal procedure was developed

with banana and orange samples. Reagents differing in composition and volume were used in an attempt to determine a method that decomposes the organic matrix completely. As can be seen from Table 3, method 1 was the best in terms of giving clear solution compared to all attempted procedures, and consumed minimum volume of reagents compared to method 4. Therefore, method 1 was selected for this particular study since it seemed to ensure complete destruction of organic materials in the samples. This procedure was adopted from that used by Clemson University with some modification [60].

Table 3. Digestion procedures tested for decomposing fruit samples.

Sample	Method	Amount of sample (g)	Reagents	Condition of digest	Time (min)	Remark
Banana, orange	1	0.25 g dried	4mL HNO_3 4 mL $HClO_4$	Clear and colorless	125	Optimum condition
Orange	2	0.25 g dried	5 mL HNO_3	Clear and yellow	Overnight and 145 min	
Banana, orange	3	0.25 g dried	4 mL HNO_3 2 mL H_2O_2	Clear but pale-yellow	130	Optional
Banana, orange	4	5 g wet	15 mL HNO_3 7 mL $HClO_4$	Suspension	160	

Briefly, the procedure was as follows: a 0.2500 g of dried and ground fruit sample was transferred into a 100 mL round or flat-bottomed flask and 4 mL HNO3 was added to it. The mixture was heated under reflux for 65 min, evaporated for 5 min and then cooled for 5 min. To the cooled solution, 4 mL of HClO4 was added and then the contents were further heated under reflux for

45 min. Finally, the resulting solution was evaporated to a volume of 1 to 2 mL and then cooled to room temperature. The cooled digest and its washings were drawn off and transferred in to a 25 mL volumetric flask with the help of a glass dropper and diluted to the mark with deionized water. Such digests were prepared in triplicates for each sample.

2.3.2 Extraction of soil samples

Conventional aqua regia digestion was used [61] using 100 mL glass Erlenmeyer flask. Well-mixed soil sample weighing 0.5000 g was digested with 12 mL of aqua regia on water bath for 3 h at 92 ^{0}C. The mixture was cooled, diluted with 20 mL of 2% (v/v with H_2O) nitric acid and transferred into a 100 mL volumetric flask after filtering through Whatman No.1 filter paper and diluted to 100 mL with deionized water.

2.4 Determination of Metals in Fruits, Soils and Water Samples

The concentrations of Ca, Mg, Fe, Mn, Cu, Zn, Pb and Cd in the digested fruit and soil samples, and pretreated water samples were determined using flame atomic absorption spectrometer equipped with deuterium background corrector. An appropriate dilution was done with 0.5% (w/w) $Sr(NO_3)_2$ to overcome ionic interference during the determination of Ca and Mg [11]. Optimum acetylene and air flow rates were chosen to obtain suitable flame conditions. Other conditions such as slit width, wavelength and lamp current were selected for each hollow-cathode lamp according to the manufacturer's recommendation. However the concentrations of Na and K were determined in emission mode of FAAS with optimum instrumental condition [11]. Metal concentrations were determined in the digested solutions using external calibration curves.

2.5 Extraction and Determination of Vitamin C

A homogenized sample (5.0000 g) was blended in glass pestle and mortar with 100 mL of 6% (w/v) trichloroacetic acid (TCA) solution for 5 min. The mixture was filtered through a Whatman No. 41 filter paper to remove the suspended solids. Two drops of a solution of bromine-water were added to the filtrate in a hood and the contents were shaken gently until the solution was slightly yellow. The solution was aerated until the dissolved bromine was expelled. To a 10 mL aliquot of oxidized extract, 10 mL of 2% (w/v) thiourea was added. A 4 mL aliquot of the resulting solution was pipetted into each of four test tubes (starting from this step the same treatment was used for calibration curve preparation). One test tube was set aside to serve as a blank. A 1 mL portion of 2% (w/v) 2,4-DNPH reagent was added to each of the remaining tubes. All the tubes were placed in a water-bath at 37 ± 5 ^{0}C for exactly 3 h. The tubes were removed from the water bath and placed in an ice-bath for 5 min. While the tubes were in ice-bath, 5.0 mL of 85% H_2SO_4 was added to the tubes drop wise from a burette. A 1.0 mL of 2% (w/v) 2,4-DNPH reagent was added to the blank with the tubes in the ice-bath. All tubes were shaken without removing from ice-bath to mix the contents. The test tubes were removed from the ice-bath and allowed to stand for 30 min. at room temperature. The absorbance of the blank and test sample was read at 515 nm after calibrating the instrument with matrix matched aqueous standards [45-47].

Calibration curve was prepared as follows; 100 mg ascorbic acid standard was dissolved in 100 mL 5% HPO_3 to prepare stock ascorbic acid standard solution containing 1 mg/mL. A 1.0 mL portion of stock ascorbic acid standard was transferred to a 50 mL volumetric flask; about 40 mL of 5% HPO_3 was added and shaken well. Finally, the solution was diluted to volume with 5% HPO_3 and mixed again to ensure homogeneity. The oxidized solution was transferred into a 250 mL Erlenmeyer flask and aerated to remove excess bromine. The solution became colorless when the bromine was expelled. To the clear solution in the Erlenmeyer flask, 0.5 g thiourea was added and shaken until it was dissolved to prepare ascorbic acid solution containing 20 µg of ascorbic acid per milliliter.

From this solution 0-2.5 mL were taken at 0.5 mL intervals and diluted each to 4 mL with 5% HPO_3 solution. Each of the standard ascorbic acid solutions were treated in the same manner as the samples beginning from transferring 4 mL of the samples into tubes. A separate blank was prepared for each tubes following similar procedure. A standard curve was prepared by plotting absorbance (A) as ordinate and concentration of ascorbic acid (µg/mL) in reaction mixture as abscissa [45-47].

2.6 Measurement of pH of Soils

A 10 g portion of air dried soil that passed through 0.09 mm stainless steel sieve was weighed in 100 ml beakers. 25 mL deionised water was added to get the 1:2.5 soil (g dry weight) /water (mL) suspension. Then the beaker was placed on an automatic stirrer and stirred for 30 minutes and finally the samples were removed from the automatic stirrer and stood for about 5 minute for the soil particles to sediment. Finally the electrodes were immersed into the soil/water suspension and the pH was measured on the upper part of the suspension [62].

2.7 Measurement of Electrical Conductivity

10 g portion of air dried soil that passed through 0.09 mm stainless steel sieve was weighed in 100 mL beaker. 50 mL of deionised water was added. Then the mixture was stirred using an automatic stirrer for 30 minute. Finally, the conductivity of each sample was measured from the upper part of the mixture after the suspension was settled. The instrument displays the temperature during this measurement automatically [62].

3. Results and Discussion

3.1 Moisture Content of Fruits

The study of fruit samples involved drying of samples followed by determination of specific analytes. Results from a food analysis are usually

expressed on a wet weight basis. Thus it is important that no loss of moisture occur until the sample is weighed. Subsequently the sample can be dried either conventionally or by freeze-drying. Freeze drying is favored by many workers [40]. Freeze drying ensures high flavor retention and minimal damage to product structure and nutritional value; permits fast and nearly complete dehydration [12].

In freeze drying (also called lyophilization) the moisture is removed from the fruit by sublimation. Therefore, no transfer of liquids occurs from the center of the mass to the surface. As drying proceeds, the ice layer gradually recedes toward the center, leaving vacant spaces formerly occupied by ice crystals .The process involves two basic steps: (1) the raw fruit is first frozen in the conventional manner followed by (2) drying to around 2% moisture in a vacuum chamber while still frozen [12].

The moisture contents (Table 4) of the fruits were determined by measuring the mass loss of the fruits using freeze-drying unit until constant mass was obtained.

As can be seen from Table 4 the moisture content is the lowest for banana and the highest for mandarin. These values are comparable with literature values [12, 64, 65].

Table 4. The moisture content of fruits (mean ± ts/√n, n =9, 95% confidence interval).

Fruit	Moisture (%) found in this study (Ethiopia)	Literature		Reference
		Moisture (%)	Country	
Banana	74.90 ± 0.20	76	Netherlands	12

Grape	82.50 ± 0.15	83	Netherlands	12
		85.3	Bangladesh	11
		81.5	Mexico	37
Guava	79.71 ± 0.36	81	Netherlands	12
Mandarin	88.35 ± 0.42	88	Netherlands	12
Orange	85.94 ± 0.26	87	Netherlands	12
		87.8	Bangladesh	11
		85.5	Mexico	37

3.2 Validation of Analytical Procedure

3.2.1 Accuracy of procedures in the determination of metals

Analytical results must be both reliable and comparable because they are often used as a piece of valuable information for a certain aim. Therefore, analysts are increasingly impelled to validate analytical procedures and to estimate uncertainty associated to the results these procedures provide. One of the constraints in method validation is the lack of references which have a high level of traceability, such as certified reference materials or reference methods of analysis. As a result, the analysts often have to resort to references with a lower level of traceability, such as spiked samples [66].

No appropriate certified reference materials were available in our laboratory to check the accuracy of results. Moreover, no reference analytical method applicable to our purpose was on hand. Thus, the validity of optimized digestion procedures for fruits, soils and total ascorbic acid were checked by carrying out spiking test and evaluating percent recovery. This involved spiking of fruit and soil samples with standard solutions of metals in the case of metal

determination and with ascorbic acid in the case of total ascorbic acid determination.

3.2.1.1 Recovery of metals in spiked fruit samples

To check the validity of the optimized digestion procedure for the determination of metals in fruit samples, spiking tests were conducted. To do this, a fixed volume of a mixture of metal standards was added to a mixture of 0.25 g of fruit and 4.0 mL HNO_3, digested for 65 min, evaporated for 5 min and then cooled for 5 min. To the cooled solution, 4 mL of HClO4 was added and then the contents were further heated under reflux for 45 min. Finally, the resulting solution was evaporated to a volume of 1 to 2 mL and then cooled to room temperature. The cooled digest and its washings were drawn off and transferred in to a 25 mL volumetric flask with the help of a glass dropper and diluted to the mark with deionized water. Such digests were prepared in triplicates. The concentrations of the metals were determined with FAAS using external calibration graph. The elements were divided into two groups to do recovery studies. Accordingly, the first set of standards contained a mixture of Na, Fe, Cu, Zn, Mn, Cd, and Pd whereas the second set contained K, Mg, and Ca. Likewise, digestion processes were also done separately for each set. As can be seen from Table 5, the recoveries are within acceptable range (100 ± 10) for all elements, except Mg, for which 89% recovery was obtained. These findings demonstrated suitability of the digestion procedure developed. Thus, all subsequent determinations of metals in samples were performed using this optimized digestion procedure.

Table 5. Recoveries of metals in fruit samples obtained by spiked with metal standards (mean ± ts/√n, n =3, 95% confidence interval).

Metal	Concentration (mg/L)		Recovery (%)
	Amount added	Amount found	
Na	0.20	0.18 ± 0.02	90.0 ± 13
K	2.00	1.83 ± 0.07	91.5 ± 3.45
Ca	1.00	0.92 ± 0.02	92.0 ± 2.17
Mg	1.00	0.89 ± 0.04	89.0 ± 4.4
Fe	0.20	0.19 ± 0.01	95.0 ± 4.5
Mn	0.20	0.19 ± 0.01	95.0 ± 3.5
Cu	0.20	0.21 ± 0.01	105.0 ± 2.5
Zn	0.20	0.18 ± 0.01	90.0 ± 4.0
Pb	0.20	0.22 ± 0.02	110.0 ± 10
Cd	0.20	0.22 ± 0.01	110.0 ± 3.0

3.2.1.2 Recovery of metals in spiked soil sample

Various digestion methods were used for extraction of metals from soils. The commonly used digestion procedures are aqua regia, aqua regia + HF [61], HNO_3-$HClO_4$, or HNO_3–H_2O_2 [67]. These digestions can be carried out using hot plate or microwave digestion unit. Complete dissolution of the soil matrix or "partial", digestion which leaves a residual fraction unaffected can be used depending on the objective of the analysis. When the availability of the elements to the plant is to be evaluated "partial" method is acceptable [67] because the availability of the metals to the plant is highly less than that can be extracted by partial digestion with strong acids. Therefore, partial digestion method was selected for this particular study.

To check the validity of the optimized digestion procedure for the determination of metals in soil samples, spiking tests were conducted. To do this, a fixed

volume of a mixture of metal standards was added to a mixture of 0.5000 g of soil and 12 mL aqua regia. The selected digestion procedure was carried out as it was done for the sample (section 2.3.2). Such digests were prepared in triplicates. The concentrations of the metals were determined with FAAS using external calibration graph. The elements were divided into two groups to do recovery studies. Accordingly, the first set of standards contained a mixture of Na, Cu, Zn, Cd, and Pd whereas the second set contained K, Ca, Mg, Fe and Mn. Likewise, digestion processes were also done separately for each set. The concentration given as amount added in Table 6 is the concentration in the final stage of the analysis; that is, after dilution to the 100 mL.

Table 6 shows the recoveries of metals in soil samples, which are obtained using the aqua regia digestion procedure. The results shown in Table 6 are good for most elements. The percentage recoveries of Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Pb, and Cd are 75, 77.5, 85.5, 87, 90.5, 91.5, 91, 95, 115, 95, and 95, respectively. However, the recoveries were not satisfactory for K and Na. Indeed, low recoveries are expected for Na and K, because these metals cannot be fully extracted from silicate lattice [61] using aqua regia. Moreover, the extraction method showed poor reproducibility for Na. For full recovery of these two metals hydrofluoric acid in combination with other strong acids is mostly used [61]. However, such study was not attempted, as it required special facilities.

Table 6. Recoveries of metals in soil samples obtained after carrying out spiking (mean ± $ts/\sqrt{n}$, n =3, 95% confidence interval).

Metal	Concentration mg/kg		Recovery (%)
	Amount added	Amount found	
Na	0.20	0.15 ± 0.01	75.0 ± 13
K	2.00	1.55 ± 0.03	77.5 ± 1.7
Ca	2.00	1.71 ± 0.01	85.5 ± 1.5
Mg	2.00	1.74 ± 0.06	87.0 ± 3.0
Fe	2.00	1.81 ± 0.01	90.5 ± 0.3
Mn	2.00	1.82 ± 0.01	91.0 ± 0.5
Cu	0.20	0.19 ± 0.01	95.0 ± 2.0
Zn	0.20	0.23 ± 0.02	115.0 ± 10
Pb	0.20	0.19 ± 0.01	95.0 ± 4.5
Cd	0.20	0.19 ± 0.01	95.0 ± 2.0

3.2.2 Assessment of accuracy of procedures for the determination of total ascorbic acid

To study the recovery for total ascorbic acid using the selected method 0.4 mL of of L-ascorbic acid (1mg/mL) standard was added to 5.000 g of wet fruit sample to give 0.8 μg ascorbic acid/mL in the final stage of the analysis. The mixture was extracted following the selected analytical procedure. Triplicates of analysis were done. This total ascorbic acid measured in spiked sample was 0.957 ± 0.002 μg/mL; whereas that in unspiked sample was 0.218 ± 0.001, which transpires to a percentage recovery of 92.4 ± 0.002. This confirms that the method is efficient and valid for the determination of total ascorbic acid in the fruits.

3.3 Method Detection Limits (MDL)

Defining detection limit in quantitative manner is a more complex matter. In essence, the requirement is to define the point at which a signal from an analyte is distinguishable and measurable above the background signals. This has to be done taking into account the inherent variability associated with the measurement. The standard deviation is extensively used to quantify this variation. If a good estimate of the standard deviation of the background is available, a point 2δ or 3δ above mean background can be used with confidence levels of 90% and 95.5%, respectively [69].

The detection limits of the metals for fruits and soil samples were calculated after analyzing six reagent blanks. The detection limits were obtained by multiplying the standard deviation of the reagent blanks by three. As can be seen in Table 7 the detection limits are above or equal to the instrument detection limit for both soil and fruit samples.

Table 7. Method detection limits of the soil and fruits (mg/kg dry weight) samples (mean, n =6).

Metal	DL instrument	MDL fruits	MDL soil
Na	0.002	0.003	0.04
K	0.01	0.01	0.03
Ca	0.01	0.02	0.02
Mg	0.001	0.001	0.002
Fe	0.03	0.03	0.04
Cu	0.02	0.02	0.02
Zn	0.005	0.02	0.03
Mn	0.01	0.01	0.02
Pb	0.1	0.1	0.1
Cd	0.005	0.01	0.01

3.4 Concentration of Metals in Fruits

Nutrient levels in foods are variable. In the case of fruits and vegetables, mineral levels can be affected by factors such as the variety of the produce item, time of harvest, ripeness, climate, soil conditions including fertilizer application, and storage and marketing conditions. As biological materials, fruits and vegetables are also subject to random variation in mineral content [70]. Results for each fruits are summarized in the following sections.

Banana: The pattern of concentrations of elements in banana is decreased as follows: K > Mg > Ca > Na > Fe > Zn > Mn > Cu. The concentrations of Pb and Cd were below the detection limit of the method. As can be seen from Table 8, banana can be good source of both major and trace elements that are essential for our body. Especially, banana is a good source of Mg (27.02 mg/100 g wet weight) and K (362 mg/100 g wet weight) compared to other selected fruits. The obtained concentrations are within the ranges of literature values. For instance the trace element intervals for banana reported in different literatures from 1968-1998 for Ca, Mg, Fe, Cu, Zn, Mn, Pb, and Cd (mg/100 g edible portion) were: 2.00-50.0, 19.0-44.0, 0.34-0.90, 0.07-0.40, 0.15-0.34, and 0.06-1.40, respectively [38]. In another literature, the values that were reported for banana (Cavendish variety) grown in Australia were 330, 5, 33, 0.3 and 0.2 mg/100 g edible portion for K, Ca, Mg, Fe, and Zn, respectively [71]. These values are similar to the results obtained in this study.

Grape: As indicated in Table 8, the trend in concentrations of metals in grape is K > Mg > Ca > Na > Fe > Mn > Cu> Zn. The average metal concentrations of grape are 1.37 (Na), 178 (K), 25.03 (Ca), 6.03 (Mg), 0.38 (Fe), 0.26 (Cu), 0.22 (Zn) and 0.34 (Mn) mg/100 g edible portion (Table 8). The obtained result is comparable with literature value [53]. As can be seen from Figure 3 copper content of grape is highest compared to other analyzed fruits.

Guava: The concentration pattern of the metals in guava is K > Ca > Mg > Na > Fe > Mn > Zn > Cu > Cd. The average concentrations of metals in guava are 1.56 (Na), 208 (K), 48.10 (Ca), 14.02 (Mg), 0.56 (Fe), 0.12 (Cu), 0.14 (Zn), 0.48 (Mn) and 0.02 (Cd) mg/100 g edible portion. These values are more or less comparable to what have been reported in U.S.A. [31]. 7.2 (Na), 229 (K), 17 (Ca), 0.64 (Fe), 0.84 (Cu) and 0.24 mg/100 g edible portion were reported for guava from Sidamo, Ethiopia [10]. 6 (Na), 211 (K), 32.00 (Ca), 3.00 (Fe), 0.37 (Cu), and 0.14 (Zn) mg /100 g edible portion were also reported for guava from Konso, Ethiopia [10]. These values are nearly similar to what have been found for the guava from Nura Era Farm except that amount of Ca is higher and amount of Na is lower compared to guava from Konso and Sidamo. In this study it was found that guava contains highest amount of Mn, Na and Fe compared other selected fruits (Figure 3).

Mandarin: The elemental concentration pattern is K > Ca > Mg > Na > Fe > Zn > Mn > Cu > Cd. Ca content of mandarin is about half of the amount in orange. The average concentrations of metals in mandarin are 138 (K), 28.64 (Ca), 8.38 (Mg), 1.08 (Na), 0.28 (Fe), 0.12 (Zn), 0.03 (Cu), 0.03 (Mn) and 0.013 (Cd).

Orange: The concentration pattern of the metals in orange is K > Ca > Mg > Na > Fe > Zn > Mn ≈ Cu > Cd. There is a similarity on trends of the concentrations of metals in mandarin and orange. The average concentrations of metals in orange are 122 (K), 55.36 (Ca), 7.94 (Mg), 1.47 (Na), 0.26 (Fe), 0.16 (Zn), 0.13 (Mn), 0.07 (Cu) and 0.03 (Cd) mg/100 g edible portion (Table 8). Ethiopian Health and Nutritional Research Institute reported 50 for Ca and 0.8 for Fe [72] which is nearly similar to the value obtained in this study. The obtained result is also comparable with literature values other in other reports [53]. Compared to other analyzed fruits, orange contains highest amount of Ca (Figure 3).

Generally, for most metals the values are comparable with what have been reported so far [10, 38, 53]. Pb was below the detection limit of the method in all fruits analyzed. This may be because of the fact that Pb forms relatively insoluble minerals in soils [73]. The pH of the soils (alkaline) of Awara Melka and Nura Era Farms (Table 9) also favors the insolubility therefore unavailability of lead. However, unlike Pb, Cd was detected in guava, orange and mandarin, but its amount was below the detection limit of the method for banana and grape. It has been reported that Cd, Cu and Zn were the main elements that plant could accumulate and pass up the food chain. Therefore, the detection of cadmium in the fruits is maybe because of the fact that cadmium ions are readily transferred from the soil to plants, which absorb the element and accumulate it to different degrees, depending on the species [74].

Table 8. Metal level of fruit samples collected from the farms (mean ± ts/√n, n =6, 95% confidence interval).

Fruit	Concentration of metals (mg /100 g edible portion)									
	Na	K	Ca	Mg	Fe	Cu	Zn	Mn	Pb	Cd
Banana	0.64 ±0.02	348± 2	7.46 ± 0.10	28.12 ± 0.1	0.46 ± 0.01	0.08 ± 0.01	0.23 ± 0.01	0.12 ± 0.01	<0.1*	< 0.01*
Grape	1.37 ±0.03	178± 3	25.03 ± 0.02	6.03 ± 0.01	0.38 ± 0.02	0.26 ± 0.01	0.22 ± 0.01	0.34 ± 0.02	<0.1*	< 0.01*
Guava	1.56 ±0.02	208± 5	48.10 ± 0.27	14.02 ± 0.22	0.56 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.48 ± 0.01	<0.1*	0.0200 ±0.0004
Orange	1.47 ±0.01	122± 2	55.36 ± 0.02	7.94 ± 0.01	0.26 ± 0.01	0.07 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	<0.1*	0.0300 ± 0.001
Mandarin	1.06 ±0.02	138± 2	28.64 ± 1.13	8.38 ± 0.26	0.25 ± 0.01	0.03 ±0.001	0.12 ± 0.01	0.03 ± 0.001	<0.1*	0.013 ± 0.001

* Method Detection Limit of the elements in mg/L.

(a) (b)

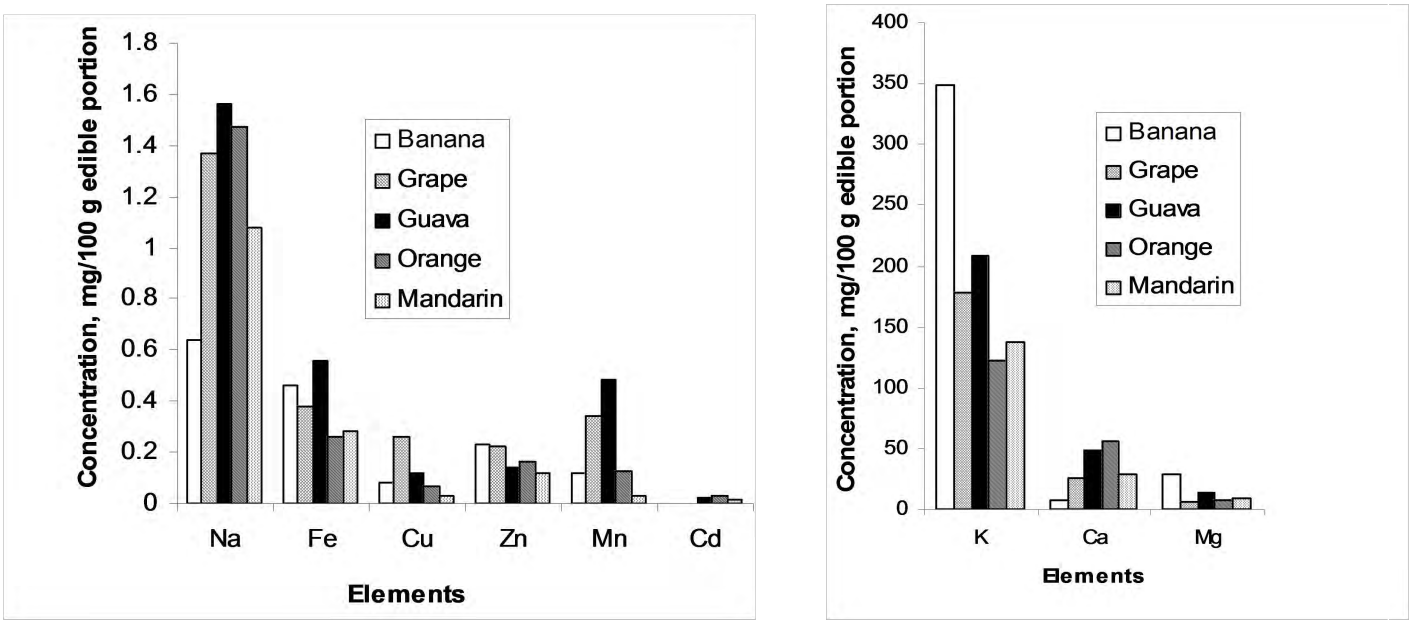

Figure 3. Average metal concentrations [(a) and (b)] in the fruits collected from Awara Melka and Nura Era Farms.

3.5 Soil Analysis

3.5.1 Some characteristics of soil

The impact of contaminated elemental uptake by plant roots is dependent upon many factors, including: the magnitude and chemical forms of trace element(s) present; soil pH, moisture, aeration, temperature, organic matter and phosphate content; the presence or absence of other competing ions; the plant species, rooting depth, age; and seasonal growth effects [27]. Most relevant six characteristics achievable with the scope of this study included soil pH, electrical conductivity (EC), organic matter (OM), soil texture, available phosphorus, and cation exchange capacity (CEC). These characteristics were determined and analytical results are given in Table 9. A brief account of the principles underlying the relevance of these parameters to the current study is given below.

Organic matter and pH: Organic Matter is the portion of the soil that includes microflora and microfauna (living and dead) and residual decomposition products of plant and animal tissue; any carbon assembly (exclusive of carbonates), large or small, dead or alive, inside soil space; generally consists primarily of humus. Organic matter of soil can influences soil properties like soil structure, the water holding capacity, nutrient contributions, biological activity, water and air infiltration rate and pesticide activity. Organic matter decomposition is also influenced by pH of the soil. Microbial activity is affected by pH which in turn influences the organic matter decomposition [75]. In the solid phase, metals can be bound mainly to organic matter and onto iron and manganese oxide surfaces [76, 77], but these adsorbents have different selectivity for metals.

The stability of organo-complexes is strongly influenced by the pH range. Generally at low pH, most metals are in the cationic form, but as pH increases, humate complexes are formed [76]. If the soil undergoes acidification, the solubility and activity of metals will be further enhanced [78]. Therefore soil pH is very important for most heavy metals, since metal availability is relatively low when pH is around 6.5 to 7. With the exception of Mo, Se, and As, the mobility of trace elements is reduced with increasing soil pH because of the precipitation as insoluble hydroxides, carbonates, and organic complexes [79].

Soil pH is usually measured potentiometrically in the supernatant suspension of a 1:1 or 1:2.5 soil (g dry weight) /water (mL) suspension using a pH meter.\

As can be seen from Table 9 the pH values of the soil samples range from 8.00 to 8.23 which shows the soils of Awara Melka and Nura Era Farms are slightly alkaline. Therefore, the availability of the trace elements is low in soils of Awara Melka and Nura Era Farms if only pH is considered. But availability of elements depend on other factors it is difficult to give generalize that the

availability of those elements in the soil are high. However one can say that the pH of the soil may contribute for the decrease of availability of the elements. Comparatively, the pH of orange and mandarin land is high compared to other lands (Table 9).

As can be seen from Table 9, the organic contents of soil samples are low (1.52 to 2.84%). These values show that the soils of Awara Melka and Nura Era Farms are mineral soil. Information of organic content of soil does not provide a farmer much quantitative information that is helpful in managing soils for crop production. Instead, the information is generally evaluated on a relative or comparative basis. For instance soils with a higher organic matter will have a higher cation exchange capacity and higher water holding capacity than soil with a lower organic matter.

Salinity and Electrical conductivity (EC): Soil salinity and electrical conductivity can also affect metal availability. Higher salinity may cause higher metal availability to plants [76]. Plant uptake differs depending on the metal source. When metals are added to soils as soluble salts, a linear response is expected; in other words, as the concentration of metal increases in the soil, there is an increase in the metal concentration in the plants [76].

Conductivity is a numerical expression of the ability of a solution to carry an electric current. This ability depends on the presence of ions, on their total concentration, and on the temperature of the measurement [63]. The commonly used ratio of combination of soil to water for conductivity measurement purpose are a soil (g dry weight)/water (mL) mixture of 1:1, 1:5 and 1:10.

It was found that the electrical conductivity of soil from Awara Melka and Nura Era Farms ranges from 174 to 319 μScm^{-1} which shows the EC of the soils are low. The results, therefore, show that amount of ions in the soil are less.

Cation Exchange Capacity (CEC): Soils are composed of a mixture of sand, silt, clay and organic matter. Both the clay and organic matter particles have a net negative charge. Thus, these negatively-charged soil particles will attract and hold positively-charged particles, much like the opposite poles of a magnet attract each other. Cations held on the clay and organic matter particles in soils can be replaced by other cations; thus, they are *exchangeable*. The total number of cations a soil can hold--or its total negative charge--is the soil's cation exchange capacity. This indicates the CEC of soil is highly influenced by clay content and organic matter of soil. Soil with higher clay content and organic matter has higher CEC. The CEC is directly related to the soils capacity of adsorbing heavy metals. The greater the CEC values, the more exchange sites on soil minerals will be available for metal retention [80].

Soils with a high CEC tend to more fertile and are capable of providing more nutrients to crops. A high CEC value (> 25) is a good indicator that a soil has a high clay and/ or organic matter content and can hold a lot of cations. A soil with a low CEC value (<5) is a good indication that a soil is sandy with little or no organic matter that cannot hold many cations.

As can be seen from Table 9, the CEC of the soil samples range from 36.65 to 47.2 meq/100 g dry soil. The CEC of grape land is lowest compared to other lands. This is confirmed by what have been obtained, that is, soil from grape land contains lowest percentage of organic matter and clay content (Table 9). CEC of soil from guava land is high (47.20 meq/100 g dry soil) compared to soil samples from other lands. This may be the cause for higher accumulation of Na, Fe and Mn in guava (Figure 3).

Available phosphorus: High phosphorus concentrations in the soil decrease Zn availability, as do elevated soil concentrations of Fe, Cu and Ca [74]. Phosphorus is strongly bound to particular soil minerals including aluminum and iron oxides.

Table 9. Some characteristics of the soils collected from the Awara Melka and Nura Era farms [a]. Soil pH (H_2O) was measured at 25°C (1:2.5) whereas EC at 21°C (1:5); n = 2 in both cases.

Farm	Lands from which soil sample collected	pH	EC (μScm^{-1})	OM (%)	Clay and texture (%)	Available P (mg/kg)	CEC (meq/ 100 g)
Awara Melka	Banana land	8.11	185 ± 2	1.55	40.32 (Clay loam)	6.08	45.23
Nura Era	Orange, mandarin land	7.87	319 ± 3	2.68	45.24 (Clay)	3.68	45.88
	Grape land	8.23	174 ± 2	1.52	37.78 (Clay loam)	4.48	36.65
	Guava land	8.00	184 ± 4	2.84	45.42 (Clay)	4.00	47.20

[a]Samples were analyzed for the above characteristics, except for EC and pH, at Agricultural Research Center, Debre Zeit, Ethiopia.

Texture, organic matter, available phosphorus and CEC were analyzed by Boyucous hydrometer, Walkely and Black, Olsen, and Neutral Ammonium acetate method respectively.

Generally, as can be seen from Table 9, the soils collected from Awara Melka and Nura Era Farms are similar in most of the soil characteristics. The soil samples from both farms are slightly alkaline.

3.5.2 Concentrations of metals in the soils

As indicated in section 3.5.1 the uptake of metal elements by the roots depend on many factors. Beside elemental uptake and toxicity are closely related. Toxicity of one element will affect the uptake and toxicity of another element. Moreover, excessive elemental uptake will induce toxicity or deficiency effects on both the plant and plant-soil microorganisms. For example, the addition of high phosphate fertilizers or sewage sludges to surface soils reduces the biological activity of mycorrhizal fungi which in turn imbalances the movement of nutrients from the soil solution to the plant [27].

Amount of metals in the soils collected from each lands on which fruits were planted are shown in Table 10. The concentrations of the selected metals vary from farms to farm and lands to land on which fruits are grown.

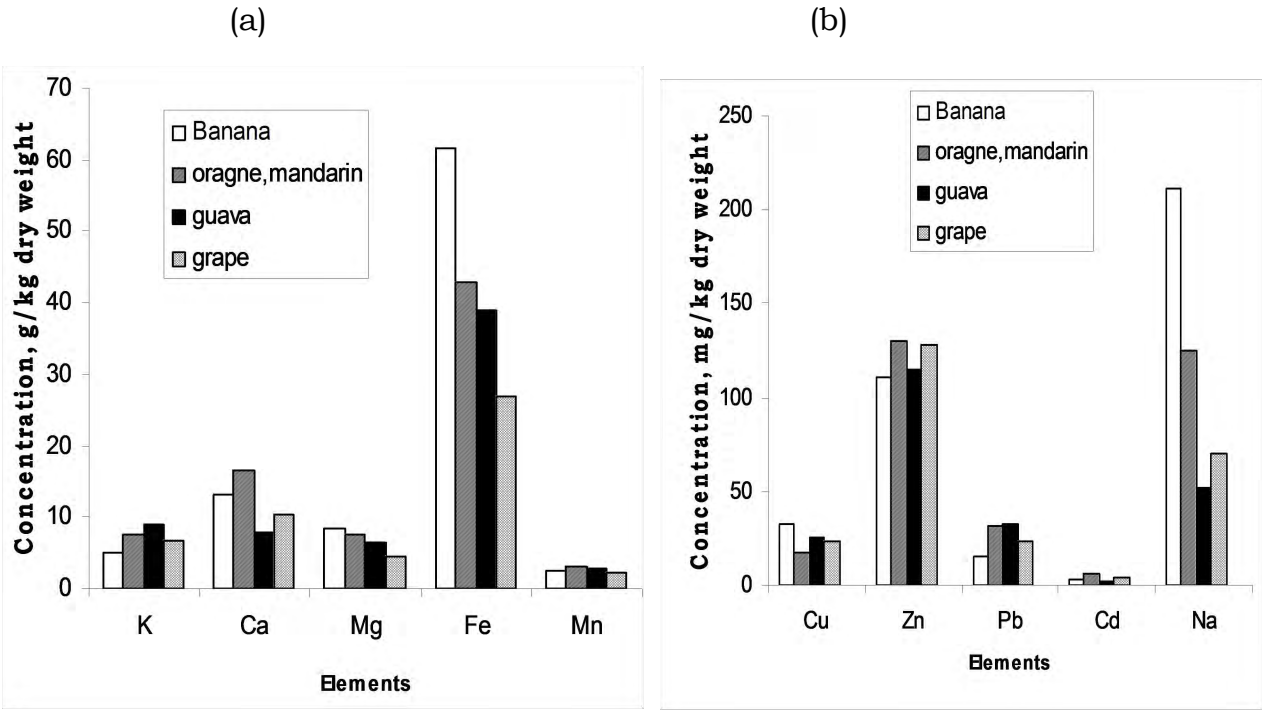

Figure 4. Average metal concentrations [(a) and (b)] in the soils with respect the lands on which fruits are planted.

Table 10. Average metal concentration in soils from Awara Melka and Nura Era Farms, (mean ± ts/√n, n =6, 95% confidence interval).

Farm	Lands from which soil sample collected	Concentration of metals in soils									
		g/kg dry soil					mg/kg dry soil				
		K	**Ca**	**Mg**	**Fe**	**Mn**	**Cu**	**Zn**	**Pb**	**Cd**	**Na**
Awara Melka	Banana land	5.02 ± 0.05	13.24 ± 0.21	8.41 ± 0.07	61.57 ± 0.18	2.44 ± 0.06	32.86 ±0.47	111.26 ± 1.35	15.3 ±0.60	3.54 ±0.16	211.0 ± 2.8
Nura Era	Orange, mandarin land	7.50 ± 0.17	16.53 ± 0.16	7.69 ± 0.30	42.78 ±1.51	2.95 ± 0.09	17.28 ± 0.87	129.89 ± 1.33	31.32 ± 1.68	6.23 ±0.40	125.2 ± 5.1
	Grape land	9.07 ± 0.08	7.91 ± 0.12	6.39 ± 0.07	38.82 ±0.08	2.94 ± 0.11	25.60 ± 0.93	114.41 ± 2.27	32.16 ± 0.90	2.54 ±0.20	51.8 ± 3.3
	Guava land	6.68 ± 0.11	10.43 ± 0.26	4.62 ± 0.22	26.83 ± 0.20	2.30 ± 0.08	23.05 ± 1.12	127.81 ± 1.65	22.87 ± 0.48	3.69 ±0.13	70.2 ± 3.3
Concentration ranges reported for surface soil [27, 81]		0.2-2.5% [82]	0.01-3.9% [82]	0.01-1.6 % [82]	0.5% - 10%	0.007-2.0	5-80	17-125	1.5-80	0.01-2.5	Trace - 1.5% [82]

For this study the soil sample were categorized according to lands /blocks on which the fruits are grown. For instance the soil that was taken from blocks on which banana is grown (named in this case banana land) was analyzed separately. The results were given separately for each land of fruit. Soils from orange and mandarin land were pooled together because they are adjacent to each other and further more, application of fertilizers, insecticides, pesticides and irrigation programs are almost the same [9].

Na, K, Ca and Mg

As can be seen from Figure 4 and Table 10, sodium is higher in Awara Melka (banana land) soil compared to soils from Nura Era Farms (other lands of fruits). In all cases, the values are in the ranges reported for surface soil (Table 10).

On the otherhand, the average concentrations of K in soils from banana, orange and mandarin, guava, and grape lands are 5.02, 7.5, 9.07, and 6.68 g/kg dry soil, respectively. These values are nearly similar to each other (Figure 4). However, average concentration of Ca is different from land to land; highest in orange and mandarin land (16.53 g/kg dry weight) and lowest in guava land (7.07 g/kg dry weight).

The average concentrations of Mg in the soils are almost equal even though the least amount was found in grape land. All are in the ranges reported for surface soil (Table 10).

Generally, the above metals are very abundant in nature in soils compared to trace elements. Therefore, their variation could be also large

from lands to lands. The use of fertilizers could be also a source for these metals.

Fe, Mn, Cu, Zn, Pb, and Cd

The average concentration (g/kg dry soil) of Fe in the soil samples from banana land (Awara Melka), orange and mandarin, guava and grape land are 61.57, 42.28, 38.82 and 26.83, respectively (Table 10). Figure 4 and Table 10 shows that there is largest concentration of Fe in Awara Melka Farm (banana land) compared to Nura Era Farm. Eventhough the values are in the range that has been reported for surface soil, it is above the maximum allowable level (1500 ppm) [83].

The average concentrations of Mn in the soil samples are also above the allowable value (2000 ppm) [84]. As can be seen from Table 10, the average concentrations of Mn range from 2.301 to 2.95 g/kg dry weight. These values indicate there is much amount of Mn in soils of Awara Melka and Nural Era Farms.

The average concentration of copper in the sampled soils range from 23.05 (grape land) to 32.86 (banana land) mg /kg dry soil. The values are in range reported for surface soil (5-80 ppm). The concentrations of Cu in the sampled soils are also below maximum allowable level (135 ppm) [84].

The average concentration of Zn in soil samples is almost equal on orange and mandarin land (129.89 mg/kg dry soil) and grape land (127.81 mg/kg dry soil). Similarly, average concentrations of Zn in banana and guava land are 111.26 and 114.41 mg/kg dry soil, respectively. In all cases the amount of Zn in the soil samples are below the maximum allowable level (300 ppm) [84].

The average concentration of Cd in soil samples are above is the maximum allowable level (3 ppm) [87] except for soils from grape land. The most probable source of Cd is phosphates fertilizers and may be pesticides.

The average concentrations of Pb in soil samples are found high in Nura Era Farm compared to that of Awara Melka Farm. The values are in the ranges that are reported for surface soil [27, 81]. All the values are below the maximum allowable level (100 ppm) [81].

Generally the concentrations of Cu, Zn, and Pb in soil samples are in the ranges that have been reported for surface soils. To some extent the concentration of Cd, and to most extent the concentration of Mn and Fe are above the ranges reported for surface soils [27, 81]. As can be seen from Figure 4 and Table 10 concentration of Cu in soil from Awara Melka is higher compared to that of Nura Era Farm soil samples. The reverse is true for Zn. Concentrations of Pb, is higher at Nura Era Farm compared to that of Awara Melka. Cd is higher in lands on which orange and mandarin are planted. Relatively Zn is higher in soil from Nura Era Farm.

3.6 Concentration of Metals in the Water

As can be seen from Table 11, the concentrations of metals in the irrigation water collected from the farm channels are generally higher than waters collected from the rivers from which the irrigation water is taken. This can be from the soil, fertilizers added to the soil, pesticides.

Awash River contains higher amounts of Fe, Cu, Zn, Mn, and Cd than Bulga River. Similar pattern was obtained for these metals in irrigation water from channels. From agricultural point of view, the maximum concentration limits of metals in irrigation waters are (µg/L): 17 (Cu), 2000 (Zn), 200 (Mn), 65 (Pb) and 10 (Cd) [85]. The concentrations of Zn, Mn, Pb, and Cd in the waters from the rivers are below the maximum limit. But Cu, in the river water is slightly higher than the maximum recommended limit.

Table 11. Average metal concentration and their ranges in water samples collected from Rivers and Channels used to irrigate Awara Melka and Nura Era Farms (mean ± ts/√n, n =4, 95% confidence interval).

Farm	River/ irrigation channels		Concentration of metals									
			mg /L				µg/L					
			Na	K	Ca	Mg	Fe	Cu	Zn	Mn	Pb	Cd
Awara Melka	Bulga River		20.84 ± 0.23	13.26 ± 0.36	28.00 ± 0.10	11.81 ± 0.11	38 ± 1	23.5 ± 1	26 ± 1	68 ± 1	<0.1*	4.3 ± 1
	Channels	Mean	28.31 ± 0.32	15.14 ± 0.42	38.40 ± 0.10	12.53 ± 0.60	36 ± 4	46 ± 5	22 ± 2	80 ± 4	<0.1*	7.5 ± 0.3
		Range	26.90 - 28.99	11.12 - 24.25	22.2 - 34.44	8.25 - 17.37	32 - 37	43 - 48	<1-37	62 - 96	<0.1*	7.3 - 7.8
Nura Era	Awash River		14.92 ± 0.21	13.23 ± 0.58	26.87 ± 0.11	6.67 ± 0.05	142 ± 12	37 ± 2	92 ± 2	106 ± 2	<0.1*	<0.1
	Channels	Mean	16.31 ± 0.54	15.23 ± 0.60	28.57 ± 0.38	6.82 ± 0.23	352 ± 11	44 ± 5	61 ± 5	90 ± 6	<0.1*	7.0 ± 0.4
		Range	12.79 - 18.84	12.65 - 19.66	22.02 - 33.14	5.83 - 7.97	124 - 709	11 - 66	12 - 184	53 - 125	<0.1*	<0.1 - 9.1

* Method Detection Limit of the elements in mg/L.

3.7 Correlations among Levels of Metals in Fruits, Soils and Irrigation Waters

It is well known that plants take up not only essential elements necessary for their normal development but also other ones if these are present in growth medium in a sufficiently mobile form. For instance it has been found that increasing the mineral elements in growth media of apricot resulted in a highly pronounced concentration increase in tissue for Zn, Pb, Mn, and Cd while a limited increase or even a decrease for Fe, and Cu, was observed [86].

The relationship among the content of elements in soil, their concentrations in plant tissues, and growth media is a complex phenomena. In most cases minerals accumulate in the upper layer of the soil where they are integrated in the complex equilibrium system of precipitates, organometal complexes, adsorbed and exchangeable forms of free ions in solutions. Only free aquated metal ions are available to plants, and this fraction depends on pH organic matter content, etc, which influence the forms and the mobility of the elements.

3.8 Total Ascorbic Acid in the Fruits Obtained from the Selected Farms

The total ascorbic acid contents in the fruits are shown in Table 12. The concentrations of ascorbic acid in the fruits have been calculated from the external calibration curve (R^2 =0.9994) obtained with standard solution (Figure 5).

From Table 12, the ascorbic acid content ranges from 5.07 (grape) to 117.1 mg ascorbic acid/100 g edible portion (guava). This indicates that guava is rich in ascorbic acid contents. This has been also shown by many literature reports [11, 87]. Orange is also shown to be a good source compared to the analyzed fruits analyzed.

Table 12. Average concentrations of total ascorbic acid in the fruits (mean ± ts/√n, n =6, 95% confidence interval).

No.	Fruit	Concentration (mg AA/100 g edible portion)			
		Found in this study (Ethiopia)	Literature values	Country	Reference
1	Banana	9.03 ± 0.31	9	-	88
2	Grape	5.07 ± 0.01	23	Bangladesh	11
			10	-	88
3	Guava	117.1 ± 6.3	400	South African	87
			100	-	88
4	Mandarin	19.31 ± 0.73	30	-	88
5	Orange	28.19 ± 1.05	62	Bangladesh	11
			50	-	88

It is pointed out that the tropical guava, *Psidium guajava*, is regarded as an excellent source of vitamin C, but there is greater variation in vitamin C level amongst the various cultivated varieties. For example, the variety “Donaldan” has 372 mg/100 g flesh, but variety, ‘Supreme’ has only 44mg/100 g edible portion. Some South African pink guava cultivars grown for the canning industry are said to have an astonishing 400 mg/100 g edible portion [87]. In another literature 9, 10, 100, 30, and 50 mg vitamin C /100 g edible portion were also reported for banana, grape, guava, mandarin, and orange, respectively [88]. The origin of this variation can be due to difference in the variety of the fruits, climate, maturity at which the fruits collected, handling during collection, storage and methods used for the analysis.

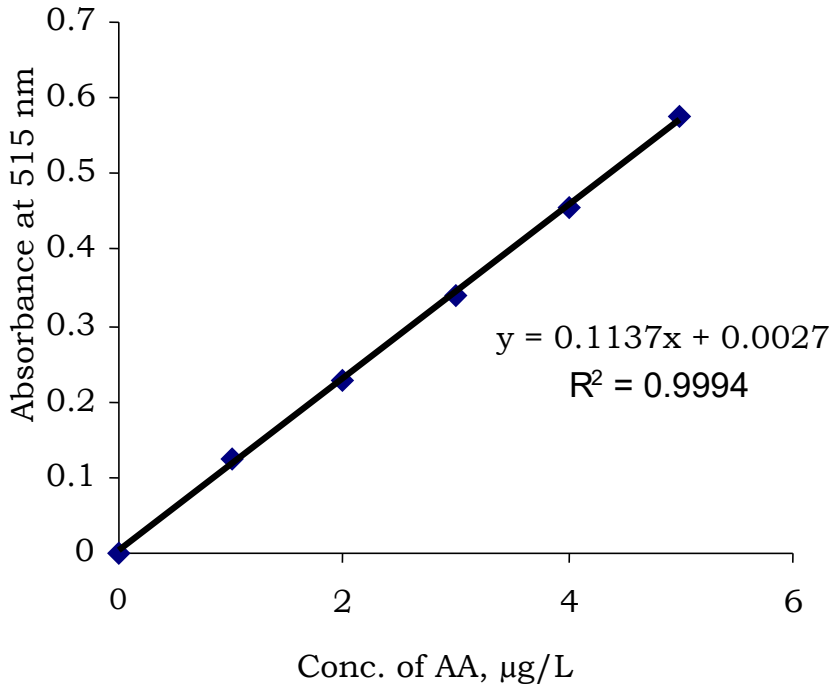

Figure 5. Working curve for vitamin C

4. Conclusions and Recommendations

4.1 Conclusions

In this study, fruits including banana, grape, guava, mandarin, and orange were analyzed for their content of Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Pb, and Cd. It is found that banana (Cavendish variety) contains the highest amounts of Mg and K compared to other fruits. These elements are very important for human being and required in much amount in daily diet. Therefore, banana can be a nice source Mg and K.

Total ascorbic acid was also determined in the selected fruits. The analysis showed that guava is rich in total ascorbic acid content. Mandarin and orange also contain appreciable amount of total ascorbic acid. The results generally indicate that the consumption of these fruits can satisfy daily intake of the society to some extent.

The optimized wet digestion method for fruit analysis was found efficient for all analyzed metals as evaluated from good recovery values. The optimized method also consumes less amount of reagent which leads to reduced blank values.

The aqua regia digestion method for soils also gives good recovery for trace metals. This indicates method is efficient for especially for trace elements extraction from soil matrix.

The recovery test for total ascorbic acid determination using 2,4-dinitrophenylhydrazine showed good percentage recovery, which confirms that the selected procedure is efficient enough for determination of total ascorbic acid in fruits.

The presence of cadmium in some fruits indicates that the soil is to some extent polluted with this element. This is confirmed from the amounts found in the soil samples which are above the maximum allowable limit. Water could be also the source of cadmium that contributes to the accumulation of this metal in soil and fruits.

4.2 Recommendations

It recommended that continuous monitoring of the water, soil and plants for heavy metal accumulation is necessary because trace elements are not biotransformed and have a tendency to accumulate on the soil surface which increase availability for uptake by plants. Attempts have to be made to know the source of toxic elements especially cadmium and lead since they have to be kept as small as possible. The use of organic manure instead of industrial fertilizers may minimize the accumulation of toxic metals in fruits as well as in soils.

Since there is lack of compositional data for fruits in Ethiopia, nation wide metal analysis program appears highly desirable. Especially peoples who need vitamin C can include guava in their daily diet since it was found it has much amount of vitamin C.

This study only includes few fruits grown in the country therefore further research have to be done on others.

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