COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

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DETERMINATION OF SELECTED HEAVY METALS IN FRUITS OF *CASIMIROA EDULIS* (WHITE SAPOTE) BY USING FLAME ATOMIC ABSORPTION SPECTROSCOPY (FAAS)

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DECLARATION

I, the undersigned, declare that this research study is my original work under the supervision of my advisor in the college of Natural and Computational sciences, Department of Chemistry, Addis Ababa University and it has not been submitted in full or in part to in this or any other University. All sources of ideas and materials used for the project work are honestly acknowledged.

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This thesis has been submitted for examination with my approval as university advisor.

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Place and date of submission: Department of chemistry, Addis Ababa University.

September 2024

ADDIS ABABA UNVERSITY

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ABBREVIATIONS

AAS	Atomic absorption spectrometry
BDL	Below detection limit
ESADDI	Estimate safe and adequate daily dietary intake
EDL	Electrodeless discharge lamp
FAAS	Flame atomic absorption spectrometry
FAO	Food and Agriculture Organization
GTF	Glucose tolerance factor
GFAA	Graphite flame atomic absorption
HCL	Hallow cathode lamp
LOD	Limit of detection
MMED	Mass median equivalent diameter
MWADO	Merehabete Wereda Agricultural Development office
MS	Matrix spike
PMT	Photo multiplier tube
RO	Reverse osmosis
WHO	World Health Organization

ABSTRACT

The purpose of this study was the determination of selected heavy metals in fruits of *casimiroa edulis* samples that were randomly collected from three kebele of Merehabete wereda that is Kofna Sibewasha, Geb Zomoy and Alem Ketema 03 kebele. 0.5 g of oven dried sample was digested by mixing 2ml of 69% HNO_3 and 1 ml of 70% $HClO_4$ at a temperature of 240°C for 2 hrs. The content of heavy metals (Cr, Ni Cu, Cd, Zn and Pb) in fruits of *casimiroa edulis* was determined using FAAS and obtained concentrations of these metals were compared with limit values set by WHO. The mean content of the selected heavy metals were determined from triplicate samples of fruits of *casimiroa edulis*, which were grown in three areas of Merehabete wereda. In this study the mean concentrations of selected heavy metals obtained in fruits of *casimiroa edulis* sample in mg/l were; Ni (BDL), Cd (0.01833±0.00338), Cu (BDL), Zn (0.21205±0.07279), Cr (BDL) and Pb (0.34883±0.0619).The mean concentration of heavy metals obtained in fruits *casimiroa edulis* were in the order of Pb > Zn > Cd and Cr, Cu and Ni were BDL. The concentrations of all metals (Cr, Ni Cu, Cd, and Zn) were below the guide line set by WHO. However, the concentrations of lead was higher the guide line set by WHO. Because of this they were not safe in terms of health risk. The level of some analyzed metals in this study is lower than the concentration of metals reported in different literatures with other similar family fruits.

1. INTRODUCTION

1.1. BACKGROUND

Heavy metals are generally refers to as those metals have a defined density more than 5 g/cm^3 and adversely affect the environment and living organisms[1, 2]. These heavy metals are quite essential for different cellular, metabolic, biochemical, physiological and hormonal functioning in humans however, if the limitation exceeds its leads to the cause of severe hazardous effects in health [3].

The presence of heavy metals in different food constituents were serious health impact, depending on their relative levels. High concentration of heavy metals in humans have been associated with chronic and acute health problems such as cancer diseases, depression, hepatic, gastrointestinal and renal failure, osteoporosis, tubular and glomerular dysfunctions, femoral pain, skeletal deformations, and low intelligent quotients in children [4- 6]. Humans can potentially be exposed to heavy metals poisoning through various routes, including the consumption of heavy metals contaminated foods, industrial and environmental pollution, or occupational exposure [4, 7].

Feeding of foods having high concentration of heavy metals may lead to the disruption of biological and biochemical processes in the human body [8]. These disorders are characterized by gastrointestinal disorder, stomatitis, diarrhea, hemoglobinuria, paralysis, vomiting, convulsions, and depression [9]. In addition to this, consumption of food and vegetation contaminated with heavy metals can seriously deplete some essential nutrients in the body causing a decrease in immunological defenses, intrauterine growth retardation, impaired psycho-social behavior, disabilities associated with malnutrition, and a high prevalence of upper gastrointestinal cancer [10]. Similarly, heavy metals have the ability to disrupt metabolic activity and genetic makeup, or to affect embryonic or fetal development [11]. For example, cadmium and mercury can injury the kidney and cause symptom of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumors and hepatic dysfunction [12]. Lead cause renal failure and liver damage. Some other metals (e.g. chromium, zinc and copper), cause nephritis, anural and extensive lesion in kidney. Therefore, the problem of food contamination (including fish) by toxic metals is receiving global attention.

The content of essential elements in plants affected by the characteristics of the soil and the ability of plants to selectively accumulate some metals .Additional sources of heavy metals for plants are: rainfall in atmospheric polluted areas, traffic density, and use oil for treatment of plant to complete the Maturation of some crops or to give crops attractive to costumers observation [12].

Removal of heavy metals from polluted /contaminated/ samples has been analysis by different methods. The method includes ion-exchange, reverse osmosis (RO), chemical precipitation, hydride generation, electrolysis, cold vapor atomic absorption and sorption. Most techniques are timely, costly, chemical and energy consuming. But, most of these procedures are inefficient for metals traces [13].

Many different instrumental methods have been used to determine the metal concentration of foods (fruits). These methods which on spectroscopic studies of atoms or of elementary ions with ultraviolet and visible radiation; can be performed only in a gaseous medium in which the individual atoms / ions/ are separate from one another. Some of the commonly used methods for the determination of the concentration of heavy metals in fruits of *casimiroa edulis* are: The Atomic absorption Spectrometry; includes: Flame Atomic Absorption Spectrometry, Graphite Furnace Atomic Absorption Spectroscopy, Inductively Coupled Plasma-Mass Spectrometry and Inductively Coupled Plasma-Absorption Emission Spectrometry and Inductively Coupled Plasma-Optical Emission Spectrometry [14].

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environment samples **[15].** Flame atomic absorption spectroscopy is one of the precise methods for elemental analysis giving concentration in mg/L (ppm) levels. The method is relatively simple, rapid, and applicable to a large number of environmental samples including, but not limited to, ground water, aqueous samples, extracts, industrial wastes, soils, sludge's, sediments, and similar wastes.

1.2. Statement of the problem.

Sufficient consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies [16]. Fruits mainly protect against free radicals that affect lipids, proteins, and nucleic acids. Polyphenols, carotenoids, vitamin A, C and E present in fruits have antioxidant and free radical scavenging activities and play a significant

role in the prevention of many diseases [16]. The determination of heavy metals in different types of biological, environmental, and food samples has drawn significant attention due to several reasons with the most important one being the nutritional and toxic effects of these elements or their compounds [17].

Casimiroa fruits are edible and having nutritional food value, which provides the minerals like sodium, potassium, magnesium, and iron, calcium, phosphorous and rich in vitamins A and C and it also possesses a high content of carbohydrates. The tree fruit planted for multi-purpose such as fruit for foods and tree for recreation, fence and wood. Hence the purpose of this thesis was to determine the concentration of selected heavy metals in fruits of *casimiroa edulis* sample. The contents of heavy metals (Ni, Cr, Cd, Cu, Pb and Zn) in fruits of *casimiroa edulis* sample was determined using flame atomic absorption spectrometry.

1.3. Objectives

1.3.1. General objective

To determine the concentration of selected heavy metals (Ni, Zn, Cr, Cu, Cd, and Pb) in fruits of *casimiroa edulis* (white sapote).

1.3.2. Specific objective

To develop suitable digestion method of fruits of *casimiroa edulis* sample.

To determine the level of heavy metals, (Ni, Zn, Cr, Cd, Cu, Pb) in fruits of *casimiroa edulis* by using FAAS.

To compare the level of heavy metal determined in fruits of *casimiroa edulis* with other similar fruits reported in literature.

To compare the concentration of heavy metal in this study with those set in guide lines.

1.4. Significance of the study.

This work focused on the determination of the level of heavy metals like Zn, Ni, Cu, Cr, Pb and Cd in the fruits of *casimiroa edulis* which were collected from three different areas of Merehabete and concentrations in the samples was determined by using Flame Atomic Absorption Spectrometry (FAAS).

To estimate the reliability of the test experiments of the samples measurements were conducted in triplicate and the mean and standard deviation of the result was taken.

To give information to the society about advantage and disadvantage of feeding the

casimiroa edulis fruits. Finally, it could be a gate for researchers and producers to make further investigations and to invest on this resource.

1.5. Structure of the Study

This thesis is composed of five chapters. The first chapter is background information that consists of the research problem, the objectives, significance and scope of the study. Chapter two provides a review of related literature relevant to the subject under study. The third chapter is about the various methods and methodologies applied in the research. In this chapter the experimental process, such as sampling procedure, methods of data collection and analyses are explained. Chapter four is give information about the observation (results), discussion and the analysis of the experimental data. Chapter five presents the summary and the recommendations of the study.

2. LITERATURE REVIEW

2.1. Description of Casimiroa (*casimiroa edulis*)

Casimiroa edulis belongs to the kingdom: Plantae, division: Tracheophyta, Class: Mognoliopsida, order: Sapindales, Family: Rutaceae, genus**:** Casimiroa and species**:** Casmiroa [18].The white sapote, scientific name: *Casimiroa endulis* also called casimiroa and Mexican apple and known as *Cochitzapotl* in the Nahuatl language (meaning "sleep-sapote") [19].

The plant is commonly adapted to tropical and sub-tropical environment and is an evergreen plant because of its deep rooted system which enables it to tap water and minerals from deep ground table water resources [20]. Casimiroa (*casimiroa edulis*) is a climatic fruit with potential for commercialization due to its organoleptical quality and usually found growing naturally at elevations between 600 and 2,700 m [18]. The plant is known to be easily managed and established with a minimum agricultural practices and it is also known for its high biomass production [18, 20].

Mature *casimiroa edulis* trees have length of from 5–16 m (16–52 ft) tall and are evergreen. The leaves are arrange in palmately compound with three to five leaflets, the leaflets 6–13 cm long and 2.5–5 cm broad with an entire margin, and the leaf petiole 10–15 cm long [21].

The fruit is an ovoid drupe, 5–10 cm in diameter, a thin inedible skin turning from green to yellow when matured, and an edible pulp, which can range in flavor from bland to banana-like to peach to pear to vanilla *flan* [21].The fruit can be creamy-white in green-skin varieties or a beige-yellow in yellow-skin varieties and has a smooth texture similar to ripe avocado which contains from one to five seeds that are said to have narcotic properties [21].

2.1.1. Nutritional Composition and Comparison with Other Commonly Utilized Fruits

In the past 40 years, experiments carried out on the white sapote's seeds have identified many pharmacologically active compounds, including: *N*-methyl histamine,

N, N-dimethylhistamine, and histamine. It contains 2, 5, 6-trimethoxyflavone, 2, 5, 6, 6-tetramethoxyflavone (zapotin), and 5-hydroxy-2, 6, 7-trimethoxyflavone (zapotinin) [22, 23].

Casimiroa fruits having nutritional food value, that provides the minerals like sodium, potassium, magnesium, iron, calcium, phosphorous etc and rich in vitamins A and C (180 and 800 mg/kg, wet weight respectively) and it also possesses a high content of carbohydrates (160 g/kg) [18]. It provides fibers which prevent constipation. The fruits are higher energy source than apple, Banana, Mango, Guava which is also high amount of ash than the apple. The ash content of the fruit indicates the good mineral source and very important for the metabolism. Calcium content of 9.9 mg/100 g of white sapote was reported which is higher than banana and apple and equals to mango. The phosphorous content of 20.4 mg/100 gm in casimiroa fruit which is higher than apple, mango and almost all equals to banana and its Iron content (0.33 mg/100 g) was grater in casimiroa fruit than the apple, Mango, Banana and Guava and vitamins, Thiamine content is greater in the casimiroa fruit than Apple, Banana and Mango [18]. Riboflavin content of 0.043 mg/100 g casimiroa fruit was is higher than the riboflavin content of apple, Mango and Guava fruits and Niacin content is higher than the apple [18]. In addition to its source of food; it is known to have medicinal properties. They are immune to many diseases and often used in different formulation of Folk- medicine [18].

2.1.2. Distribution of *Casimiroa Edulis*

The fruit is native to the Mexican and the Central American highlands [18, 24]. Casimiroa is the family of Rutaceae trees found in the tropical and subtropical areas of Central America and Mexico, the Caribbean, the Mediterranean region, India, Southeast Asia, South Africa, Australia, and New Zealand. The best-known species is *Casimiroa edulis* [25, 26].This species is popularly known as “zapoteblanco”, “matasano”, “cochitz´apotl”, “abache” and “zapotedormil´on”which are also called white sapote in English [18].

Casimiroa fruits are locally known in Ethiopia as Amba in Afan Oromo and casimire in Amharic languages. Casimiroa (*Casimiroa edulis*) is found in different parts of the country (Ethiopia): mostly in Hawasa, Yirgachefe, Dilla, Wolayta, Arbaminch, Dire Dawa, Konso, Benishangul gumuze, Hassosa, Gambela, Tigray, Amhara and Oromia regions are the most plantation areas of white sapote fruits [18].

2.2. Heavy Metals

Heavy metals are metallic chemicals that have a relatively high density that are toxic, persistent and hazardous to human health at low concentrations [27].These include

mercury (Hg), lead (Pb), copper (Cu), cadmium (Cd), arsenic (As), chromium (Cr), thallium (TI), manganese (Mn), zinc (Zn), and nickel (Ni) [28]. Some of these metals (Fe, Mn, Cu, and Zn) are essential for metabolism in their lower concentrations [29]. As, Cd, Cr, Co, Pb, Ni, and Zn are the most common heavy metals potentially hazardous to human health [30]. However, cadmium and lead have more significant side effects on human health since they are easily accessible through the food chain [31, 32]. Their multiple industrial, domestic, agricultural, medical and technological importance have led to their large distribution in the environment; raising concerns over their serious effects on human health and the environment. The toxicity effect of heavy metals depends on different factors such as the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals.

2.2.1. Selected heavy metals

A. Nickel

Nickel is the 24th most abundant element in the Earth's crust, comprising about an average concentration of about 75 μg/g or 3% of the composition of the earth and the 5th most abundant element by weight next to iron, oxygen, magnesium and silicon. It is a member of the transition series elements and belongs to group VIII B of the periodic table along with iron, cobalt, palladium, platinum and five other elements. Nickel is a naturally occurring element in various mineral forms such as; a component of silicate, sulfide or arsenide ores and it also naturally present in soil, water, air and biological materials. It is resistant to corrosion by air, water and alkali, but, dissolves completely in dilute oxidizing acids. In nature nickel is a mixture of five stable isotopes and nineteen unstable isotopes. Although it has different oxidation states, the commonest oxidation state under environmental conditions is nickel in the +2 valence state while other valences (-1, +1, +3, and +4) are also encountered, though less frequently [33, 34].

The exposure to nickel is most serious harmful health effects, such as chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus, have occurred in people who have breathed dust containing certain nickel compounds while working in nickel refineries or nickel processing industry. The levels of nickel in work places were higher than usual (background) levels in the environment. Lung and nasal sinus cancers occurred in workers who were exposed to more than 10 mg

nickel/m^3 as nickel compounds that were hard to dissolve (such as nickel sub sulfide). Exposure to high level of nickel compounds that easily soluble in water may also result in cancer than nickel compounds that are less soluble are present, or when other chemicals that can produce cancer are present. The International Agency for Research on Cancer (IARC) has determined that some nickel compounds are carcinogenic to humans and that metallic nickel may possibly be carcinogenic to humans [34]. The EPA has determined those nickel refinery dust and nickel sub sulfides are human carcinogens [34].

B. Copper

Copper is the earliest metal human used. The average abundance of copper in the earth crust is 55 mg/kg. In nature, copper mainly exists in the form of sulfide and oxide ores and its distribution is very wide. The content of normal copper in soil is 2-200 mg/kg and the average is 22 mg/kg [35].The average daily intake of copper in the US is about 1 mg Cu with the primary source being the diet. The bioavailability of copper from the diet is from 65-70% depending on different factors including chemical form, interaction with other metals, and dietary components of the food intake. The concentration copper in serum reached up to approximately 1.5 mg/L in healthy persons. Gastrointestinal symptoms occur at whole blood concentrations near 3 mg Cu/L [36].

Copper is essential element for energy production in the cells which also takes place in nerve conduction, connective tissue, the cardiovascular system and the immune system. Copper is highly related to estrogen metabolism, needed for women's fertility and to maintain pregnancy. Normal Values of Cu in Serum = 12 - 26 µmol/L and Urine = 0.05 - 0.55 µmol/L [37].

Copper is crucial element for plant nutrients; however it is toxic at high concentrations. Copper is required for constituting enzymes catalyzing redox reactions, and is involved in photosynthetic functions [38]. Excess copper induces high levels of reactive oxygen species (ROS) and affects the photo system in photosynthesis [39], which subsequently reduces the yield or quality of crops. The toxic effects of copper are observed in different crops. When the soil’s copper level is over 300 mg/kg, the rice grain yields a decrease of about 50% [40].

C. Zinc

Zinc is a bluish-white metallic element (atomic number 30, atomic weight 65.4), which makes up about 0.02% of the earth's crust and is the twenty-third most abundant element. Among a transitional element in the periodic table, zinc have certain chemical properties in nature that make it especially useful and important in biological systems. As an example zinc is able to constitute strong, but readily exchangeable and flexible, complexes with organic molecules, thereby enabling it to modify the three-dimensional structure arrangement of nucleic acids, specific proteins, and cellular membranes and affect the catalytic properties of many enzyme systems and intracellular signaling. Zinc is associated with more than 50 known metalloenzymes, which have a diverse range of functions, including the synthesis of nucleic acids and specific proteins, such as hormones and their receptors [41, 42]. Zinc is found in foods such as meat, liver, kidney, fish, chicken and cereals, eaten alongside vegetables to enhance zinc absorption [43].

Zinc toxicity due to acute or chronic ingestion of high quantities of zinc supplements can also occur and lead to impaired immune response, hypocupremia, microcytosis, and neutropenia and consumption of excess zinc can cause ataxia, lethargy [43]. High level zinc intake leads to chronic effects such as nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches. Inhalation of 150–450 mg of zinc per day have been associated with chronic effects such as low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipoproteins [44].

Zinc deficiency influences many organ systems, like the integumentary, gastrointestinal, central nervous system, immune, skeletal, and reproductive systems. Deficiency of zinc causes in dysfunction of both humoral and cell-mediated immunity and increases the susceptibility to infection. Disturbances in nucleic acid metabolism and protein synthesis may profile for some symptoms of zinc deficiency. Relatively high intake of dietary nitrogen secondary to zinc deficiency can cause anorexia and impaired taste. Zinc affects function of growth hormones, gonadotrophins, sex hormones, prolactin, thyroid, corticosteroids and insulin. Growth retardation occurs because of disruption of function of insulin like growth factor which mediates the cellular effects of growth hormone which also delayed sexual maturation and impotence as well as hypogonadism and hypospermia can

occupy [45]. Increased synthesis of prostaglandin especially PGE-2 which occurs in zinc deficiency may result in diarrhea, alopecia, acro-orificial skin lesions, and glossitis and nail dystrophy may also be manifested. Mental changes in the form of apathy, depression and change in behavior have also been noted [45]. Delayed healing of wounds, burns and decubitus ulcers also occur [45]. Eye lesions including photophobia and lack of dark adaptation, conjunctivitis, corneal opacities, macular degeneration and night blindness have been documented [46]. The optimal therapeutic dosage that is required to reverse the symptoms of zinc deficiency is still unclear; however, the pharmacologic zinc dose should be adapted to the actual requirements to avoid negative side effects on immune functions. In dermatitis caused by low dietary zinc, treatment with elemental zinc supplementation at the dose of 0.5-1 mg/kg/day is recommended [47].

D. Chromium

Chromium has different oxidation states from -2 to +6 valence. Among those oxidation states 0 (elemental metal), +3 (trivalent), and +6 (hexavalent) are the most stable. Chromium in chromites ore is in trivalent state but the industrial processes produce the elemental metal in hexavalent state of chromium. The main health effects of chromium are associated to the oxidation state of the metal at the time of exposure. The most biologically significant compounds of chromium's are trivalent (Cr [III]) and hexavalent (Cr [VI]) compounds. Cr (III) is an important dietary mineral in low doses. Cr (VI) compounds are carcinogenic which Cr is generally considered 1,000 times more toxic than Cr (III) [48 - 50].

Cr (III) is needed to potentiate insulin for normal glucose metabolism. Important for insulin and improve in hypoglycemic patients following chromium supplementation. Another effect of chromium supplementation that could be a result of its potentiation of insulin sensitivity is the redistribution of body fat, protein and water [48]. Estimated Safe and Adequate Daily Dietary Intake (ESADDI) per day about 10-200 µg/day different age lower this dose caused problem in insulin system and over dose caused Weakened immune systems, Kidney and liver damage, alteration of genetic material, lung cancer and death [51].

Cr (III) is mainly found in fresh foods including drinking water. Dietary sources rich in Cr (III) include: breads, cereals, fish, fresh vegetables, meats, and spices. Additional important sources of Cr (III) are mineral supplements, brewer's yeast, and

beer. On the average, adults in the United States take in an estimated 60-80 micrograms of Cr (III) per day in food [52]. Hence, many people's diets may not provide enough Cr (III). The biologically active form of Cr (III) organic complex sometimes referred to as glucose tolerance factor (GTF) that is believed to function by facilitating the interaction of insulin with its cellular receptor sites. Studies have shown that the Cr (III) supplementation in deficient and marginally deficient subjects can result in the rapid reversal of many of the symptoms of chromium-deficiency [53, 54].

E. Cadmium (Cd)

Cadmium, a heavy metal, causes toxicity in humans in very small amounts when consumed. Cadmium occurs in trace concentrations in agricultural soils. Contamination of agricultural soils with Cd is derived from sources, such as phosphatic fertilizers manufactured from rock phosphates high in Cd and by the application of sewage sludge and pesticides at higher extent and gypsum to lesser extent. Zinc smelters in the vicinity of agricultural soils can also be significant contributors to soil contamination with Cd. Food crops grown on contaminated soils may take up substantial amounts of Cd and this could result in Cd entering the food chain of animals and humans when consumed [55]. There is now general concern that under certain conditions the Cd content of plants may be raised and thus become hazardous to man. The sources of soil Cd are varied. Cadmium is added to soils in very small amounts in phosphate fertilizers. Along with other heavy metals, it is also present in sewage sludge. Levels from about 10 to as much as 1500 ppm Cd have been observed in the dry matter of sewage sludge, which is being used more and more on agricultural land [56]. Normal Cd levels in plant material are in the range of 0.1 - 1.0 ppm, but, the roots of several species can take up large quantities of Cd from the soil in the form of solution, the movement of Cd through the plant is restricted. Cadmium appears to be held in the roots on exchange sites, and can be replaced by Ca^{2+}, Mn^{2+}, 29 and Zn^{2+}. As Ca^{2+} is normally the dominant cation in soil solution it may substantially affect the uptake of Cd from the roots to the tops is particularly depressed by phosphate [56]. The basic cause of toxicity probably lies in the much higher affinity of Cd for thiol groupings (SH) in enzymes and other proteins. The presence of Cd therefore disturbs enzyme activity [56]. Most recently, interest in Cd has been directed at progressive accumulation in biological systems at

low levels at which Cd generally occurs environmentally [57]. Toxic effects in man have been observed from the regular consumption of plants in excess of 3 ppm [56]. Continued exposure to small amounts of Cd leads to accumulation, in human and animal liver and kidney tissues resulting in damage and malfunction of these organs [57]. It disturbs the metabolism of Ca and P and cause bone disease, which is very painful, and causes excessive demineralization and embrittlement of the skeleton [56].

F. Lead

Lead poisoning may be a medical condition that happens once individuals area unit exposed to steer compounds through inhalation, swallowing, and rarely, through the skin. Lead may be a colorless, tasteless, and odorless metal which will be found in dirt, dust, toys, dishes, and furnishings. Unwellness typically happens from perennial exposure to tiny amounts of lead [58].

Lead may be a harmful environmental substance that has high cytotoxic effects to several body organs. Even supposing Pb will be absorbed from the skin; it's largely absorbed from metabolism and biological process systems. Pb exposure will induce medicine, metabolism, urinary, and vas disorders thanks to immune modulation, oxidative, and inflammatory mechanisms. Moreover, Pb may disturb the balance of the oxidant–antioxidant system and induce inflammatory responses in varied organs. Exposure to Pb will turn out alteration in physiological functions of the body and is related to several diseases [58-60]. Pb is extremely cytotoxic that has adverse effects on the medicine, biological, and psychological feature functions within the bodies. The international level-of-concern for Pb poisoning is 10 μg/l within the blood [61, 62]. Adulteration of controlled substance with Pb has been thought of as a threat to human health in recent years [63].

2.2.2. Environmental Effects of Heavy Metals

Environmental pollution is one of the most unsafe occurrences resulting from human activities and natural events. Example; metal mining, processing and smelting are common human activities with negative impact on the environment contributing to environmental pollution. High concentrations of heavy metals and metalloids accumulate at mine sites following the extraction of elements for economical value. This results to contamination of environments with metals like As, Cd, Cr, Pb, Ni, Sb and Zn [64]

The metals can pose a serious health implication to all living things in general and humans in particular if accumulated in elevated concentration above body requirements [65, 66]. Heavy metals, in general, are not biodegradable, have long biological half-lives, and have the potential for accumulation in different body organs, leading to unwanted side effects [67- 69]. Heavy metals including- Lead, Cadmium, Zinc, Mercury, Arsenic, Silver, Chromium, Copper and Iron are largely found in soil and water sources as environmental toxicity. These pollutants on deposition on the earth surface accumulated into the fruits tissues [67].

The growth of small and medium scale industries (mainly working on production of brews, fabrics, chemicals, floriculture and tanneries) are growing in a fastest rate and are generally established around urban and sub-urban areas and sideways of rivers [69-71]. The waste water being released from these businesses are reportedly encompasses increase levels of toxic metals including cadmium, arsenic, mercury, copper and lead [69, 72]. These toxic metals have been long regarded as serious environmental contaminants even at smaller concentration because of their detrimental effect to public health [73- 75].

2.2.3. Adverse Health Effects of Heavy Metals

Heavy metal pollution is widely spread globally due to the rapid pace of urbanization, land use changes, and industrialization, especially in developing countries with extremely high populations. This has caused emerging issues of food security because of the increasing risk of contamination of food by pesticides, heavy metals, and/or toxins [76, 77]. Heavy metals have been implicated in causing human disorders such as nervous, renal, their carcinogenic, mutagenic disorders among others [78].

Some heavy metals are important to the human biological process; however it depends upon their dosage intake leads to unexpected serious effects on health and the physiological system. Dissolved toxic metals through different forms as soil pollutants, water pollutant and air pollutants entering into food chain and finally reaching in humans, these are leading to many damage to the cellular system and leading to cancer nonessential heavy metals (As, Cd, Cr) are major cancer- causing agents [79].

2.3. Atomic Absorption Spectrophotometers

Atomic absorption spectrometry (AAS) is one of the most often used techniques for the quantitative determination of heavy metals in environmental materials at trace and ultra-trace levels. This is done by reading the spectra produced when the sample is excited by radiation. The amount of energy absorbed by the sample in the form of photons of light is measured by Atomic absorption techniques. A detector measures the wavelengths of light transmitted by the sample and compares them to the wavelengths which originally passed through the sample. A signal processor then integrates the changes in wavelength absorbed which appear in the readout as peaks of energy absorption at discrete wavelengths. Every atom has its own distinct pattern of wavelength at which it will absorb energy, due to the unique configuration of electrons in its outer shell. This enables the qualitative analysis of a sample **[80].**

AAS is an optical atomic spectrometric technique based on the measurement of the specific absorption originating from free non-ionized atoms in the gas phase. Different types of atomizer are used in the flame and the graphite furnace types being to transfer the analyte to free atoms mostly used. Typical detection limits of flame atomic absorption spectrometry (FAAS) are of the order of 1-100 ppb, making it a perfect tool for the determination of minor and trace elements, at least for contaminated samples. Graphite furnace atomic absorption spectrometry (GFAAS), offering detection limits which are about a factor of 20–200 times lower than for FAAS, is the standard method for many trace elements, especially for background values, and for unpolluted samples, such as fresh water and biological materials. AAS in its conventional configuration is a single-element technique, which has to be used in a sequential mode when more than one element has to be determined. However, there are commercial instruments available that can be used for the determination of 6–8 elements simultaneously **[81].**

Atomic absorption spectrometry has many applications in different areas of chemistry such as clinical analysis of metals in biological fluids and tissues such as whole blood, liver hair, plasma urine, saliva, brain tissue, muscle tissue. AAS can be used to in qualitative and quantitative analysis.

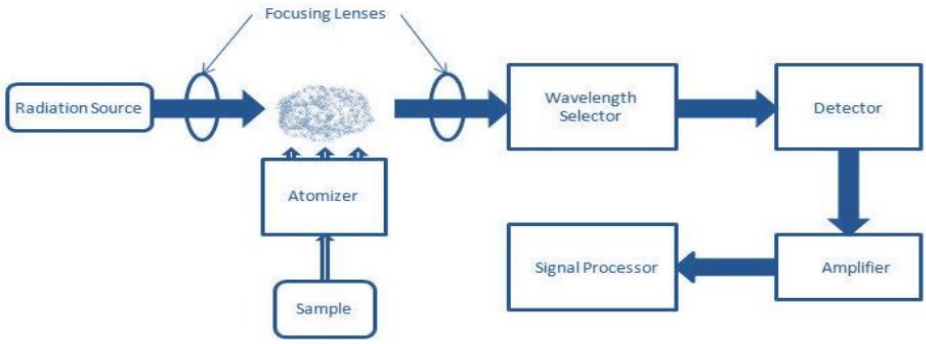


Figure 1: Block diagram of Atomic absorption spectrometer

2.3.1. Basic principles

The selectivity in AAS is very more applicable; due to each element has a specific set of energy levels which gives rise to very narrow absorption lines. Hence, the selection of the monochromatic is important to obtain a linear calibration curve (Beers' Law), the bandwidth of the absorbing species must be wider than that of the source of light; and it is difficult to compare with ordinary monochromators. The monochromator is the most useful part of an AA spectrometer because it is used to separate the thousands of lines generated by elements in a sample [82].

Without a good monochromatic, detection limits are severely compromised. A monochromator is used to select the specific wavelength of light that absorbed by the sample and to exclude other wavelengths. The selection of the specific wavelength of light allow for the determination of the specified element of interest in the presence of other elements. The light selected by the monochromator is directed to a detector, typically a photomultiplier tube, whose function is to convert the light signal into an electrical signal proportional to the light intensity. The challenge of requiring the bandwidth of the absorbing species to be broader than that of the light source is solved with radiation sources with very narrow lines [82].

2.3.2. Instrumentation

In order to analyze a sample in FAAS the atoms of the analyte must be atomized. The main atomizers commonly used are flames and electro thermal graphite tube atomizers. The atoms should then be irradiated by optical radiation, and a continuum radiation source could be an element-specific line radiation source or a continuum radiation source. The radiation then passes through a mono-chromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector [82].

A. Atomizers

Atomization is the process of making available atoms for absorbance measurement. Atomic absorption analysis is dependent on making a supply of free analyte atoms in the ground state and exposing this atom population to light of the characteristic of specific wavelength for that element. As with other petrochemical techniques, AAS is used to determine element concentrations, usually in liquid form. AAS is best suited to the analysis of elements in aqueous solutions of a dissolved or diluted sample, or samples diluted with other solvents such as organic solvents. Since the development of AAS a number of variety atomizer methods have been developed. The three major divisions of atomizers are flames, graphite furnaces and vapor generation.

B. Flame atomizers

The flame atomization process used in atomic absorption convert the sample solution into free atoms in the optical path via consecutive stages. The main purpose of the sample introduction system is to create an aerosol of the sample in the fuel mixture. This needs the production of an aerosol with a sufficient amount of small droplets and to introduce a portion of the sample in the flame without experiencing complexity such as nebulizer or burner blockage.

The main crisis of atomic absorption using flame atomization is that the atomization system is a relatively limit of sampling device. Only a small fraction (about 10 %) of the sample aspirated through the atomization system reaches the flame. In moreover, the analyte is diluted with an excess volume of gas, which carries the aerosol into the flame. the ground state formation of atoms is governed by different variables such as the flame temperature, interactions between flame gases, matrix metals and analyte, chemical interferences and the amount to which the sample molecular species are dissociated. The free metal atoms are only reaching in the light path for a short period of time–typically 10-40 seconds. The velocity of the flame gases affect the residence time. This limits the minimum useful concentration at which measurements can be made by flame AAS. This is generally around the low part per million levels [82].

2.3.3. Graphite furnace atomic absorption spectroscopy

Measurement principle the GFAA and flame AAS is the same. The distinctions between these two techniques are the method the sample is introduced into the

instrument. The analysis of GFAA is used an electro thermal graphite furnace. The sample is heated stepwise until to 3000ºC to dry. The advantage of the graphite furnace is that the detection limit is about two times of magnitude better than AAS. The analysis of variety of species of a given sample is important because different oxidation states of the same element may present different toxicities and, resulting, different risks. Therefore, sequential extraction procedures for the separation and further analysis of a species have been developed for many metals [82].

Atoms absorb light at high specific wavelengths, it is necessary to use a narrow-line source which emits the narrow-line spectra of the interest of elements. Narrow-line sources give high intensity and do atomic absorption a specific analytical technique. The main sources used for atomic absorption are the hollow cathode lamp (HCL) and the electrode less discharge lamp (EDL) [82].

The hollow cathode lamp is a good, bright, stable line supply for many elements. However, for a few volatile elements, where low intensity and short lamp time life disturbance, EDLs are available. EDLs are generally high intense than hollow cathode lamps and, therefore, could provide higher exactness and lower detection limits for a few elements. Hollow cathode lamps are available for greater than 60 elements.

The hollow cathode lamp (HCL) uses a cathode fabricated from the element of interest with small internal pressure of a noble gas. A low electrical current (~ ten mA) is obligatory in such the simplest way that the metal is excited and emits some spectral lines characteristic of that element [82].

Electrodeless discharged lamps (EDL) contain a small quantity of the analyte as a metal or a salt in a quartz bulb together with an inert gas, typically argon gas, at low pressure. Electrode-less discharge lamps provide high intensity (10-100 times) and narrow emission lines which lead to higher signal -to -noise ratio over the lines obtained using hollow cathode lamps [83].

Types of light sources in Atomic Absorption Spectroscopy covered the essential features of the two commonly used light sources hollow cathode lamps and electrode - less discharge lamps. The benefits of electrode- less discharge lamps are realized especially when analyzing volatile elements like As, Sb, Bi, Cd, Hg, Rb, Sn, Te, etc. Sputtering of metal atoms and their adsorption on cathode lamp side walls and windows begins to influence the useful life of the lamps. On the other hand

electrode-less discharge lamp because of the high emission intensities overcome the problem easily and provide lower detection limits. Electrode-less discharge lamp with all their benefits are not as popular as hollow cathode lamps and are used mainly for analysis of about 15 volatile elements. The reasons are mainly higher cost and difficulty in operation in comparison to hollow cathode lamps [83].

B. Wave length selectors

Wavelength selectors limit the radiation absorbed by a sample to an explicit wavelength or a slender band of wavelengths. Sensitivity of associate degree AAS is improved once the bandwidths or a slender and detectability is improved once transmission is high. There are many forms of wavelength selectors. A number of these are filters, grating monochromators, and prism monochromators.

Filters are wavelength selectors that enable slender bandwidths of radiation to submit to. They will be divided into four main categories: absorption filters, cut-off filters, interference filters, and interference wedges.

Grating monochromators are set inside compartments of some AAS instruments and are liable for manufacturing slender bands of radiation. There are 5 parts found in most grating monochromators: associate degree entrance slit, a collimating lens or mirror, a mirrored image grating, a focusing component, associate degreed an exit slit [83].

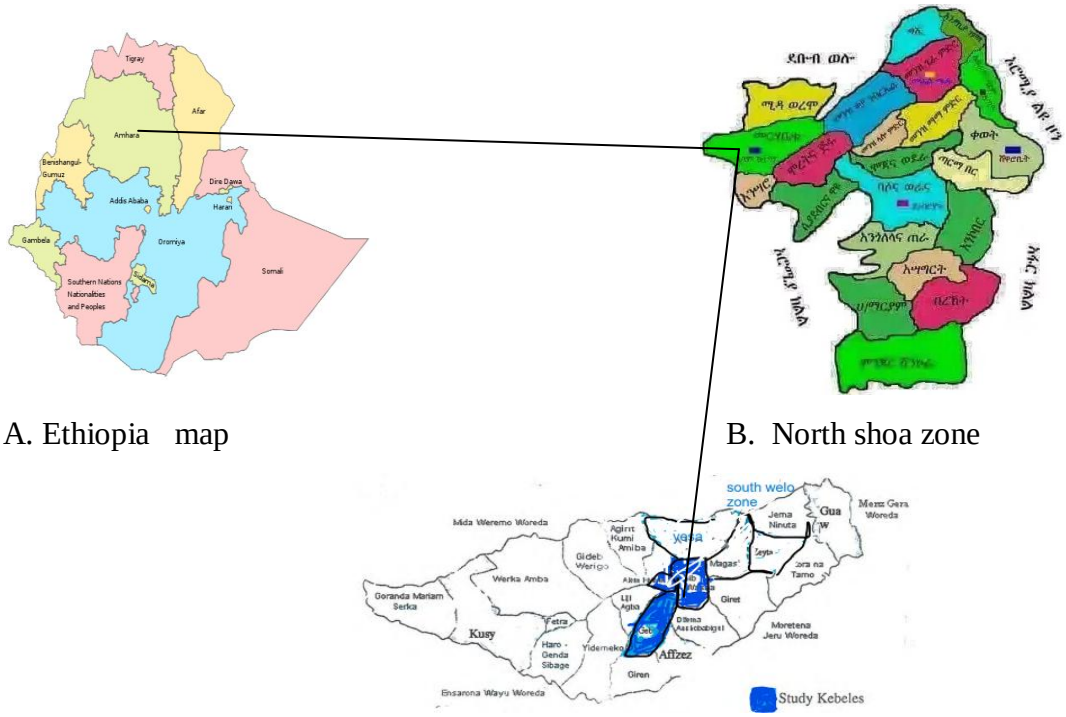
C. Detectors

A detector will be a mechanical, chemical, or electrical device that measures the modification of a variable in its surroundings. In Atomic Absorption qualitative analysis, the quantity of radiation that passes through a sample is measured and quantitatively delineate by transmission. As lightweight passes through a sample, power is attenuated because it is absorbed by the analyte within the sample. Transmission, T, is that the quantitative relation of the supply radiation's power exiting the sample, (P), to the supply radiation's power coming into the sample, (P0) [83].

3. EXPERMENTAL

3.1. Description of the study area

Merehabete is one of the wereda in the Amhara Region of Ethiopia. The administrative center for Merehabete wereda is Alem Ketema town; which is, 180 km far from Northwest of Addis Ababa (National capital city), 589 km far from west of Bahir Dar city the regional capital city of the Amhara region and 142 km, away from Debreberhan, the capital city of North Shoa zone, to the west. A part of the north Shoa Zone, which is bordered on the south by Ensaro, on the west by the Oromia Region, on the north by Mida Woremo, on the east by Menz Keya Gebreal, and on the southeast by Moret ena Jiru. The Jamma River defines this woreda's southern and eastern boundaries, and its tributary also defines its western and northern. The elevation ranges the wereda from 1300 to 3200 meters. The agro ecological zone of the wereda is classified in to three traditionally categorized groups: - Woyna-Dega, which accounts for 70 percent of the area coverage, Dega, which covers 6 percent of the wereda and Kola, covers 24 percent of the wereda area (MWADO). The average rainfall of the wereda ranges between 700-1200 mm per annual. The temperature of the wereda varies from place to place due to altitudinal effect. The mean annual range of temperature is higher for kebele or places along lower altitude and gorges of the two rivers Jema and Wenichite. However, the average temperature of the Wereda ranges from 14.4℃ to 23℃ (MWADO). Casimiroa edulis is grown in woina dega and kola parts of the wereda.



A. Ethiopia map B. North shoa zone

C. Merehabete Wereda

Figure 2: Map (geographical location) of the study area

3.2. Apparatus and Instruments

In this thesis different types of apparatus and instruments were used. They are oven (J. P.SELECTA, s. a), analytical balance (SCIENTECH, ZSA, 120), filter paper no. 42, funnels, digestion apparatus (Gollenhamp Kjeldahl apparatus), and flame atomic absorption spectrometer (Analytikajena Model ZEEnit 700P#150Z71025 Tech).

3.3. Chemicals and Reagents

The chemicals and reagents in this work were high purity of analytical grade. Chemicals used for digestion of fruit samples and blanks were 69% HNO_3 and 70% $HClO_4$. Standard solutions of the selected metals (Ni, Cr, Cu, Zn Pb and Cd) were prepared from 1000 mg/l stock solution of Certified Reference Materials from Europe laboratory. Distilled water and deionized water were used for rinsing and preparation of solution (samples) and standard solution respectively.

3.4. Sample collection and preparations

Six fruits of casimiroa edulis were collected from three different sampling areas of Merehabete wereda (Kofna Sibewasha kebele, Gebzomoy kebele and Alem Ketema 03 kebele) in plastic bags. The samples were transported to the laboratory in one day for preparation. The sample was washed thoroughly using tap water, and distilled water to remove soil and dirt's from the outer surface of the samples. The outer

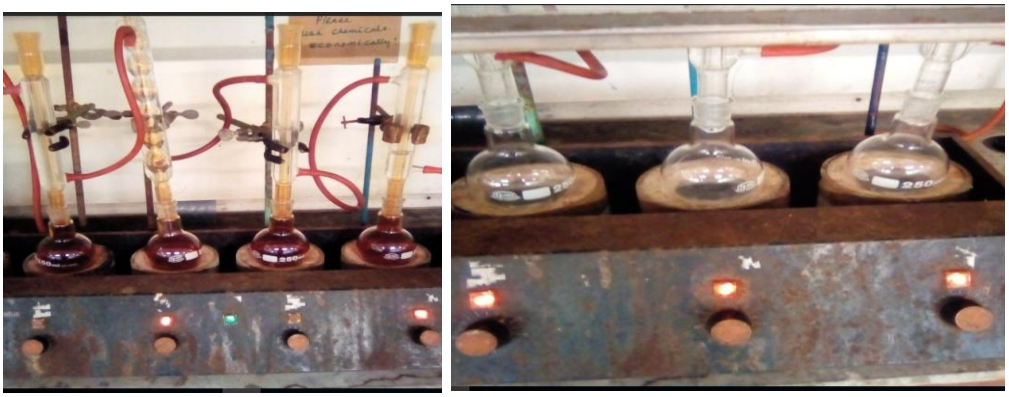
surface of casimiroa fruit were peeled with a knife and then the inner parts of the samples were cut in to smaller pieces with a knife and separate the flesh (edible) parts from the seed, the samples (edible) were dried for one week at room temperature and finally, they were dried in an oven at 105^{o}C for 24 hrs. The samples were ground using pestle and mortar, and then sieve the powder, mixed together to get a representative sample and stored in polyethylene bottled flask for digestion.

a. Fruits c. Flesh d. Seed

Figure 3: Sample of fruits of *casimiroa edulis*

3.5. Digestion of fruits of casimiroa edulis

To obtain a clear sample solution for the analysis using flame atomic absorption spectrometry, different digestion procedures for the plant samples (fruits *of casimiroa edulis*) were assessed using concentrated HNO_3 with $HClO_4$ acid mixtures by varying the volume of the acid mixture, digestion time, and temperature of the method. Optimized procedure was chosen based on usage of lesser reagent volume, shorter digestion time, and reasonably mild temperature for obtaining clear and colorless solutions of the resulting digests. Among the different digestion procedures tested, digesting a fruit sample weighing 0.5 g with 3 ml of 2:1 (v/v) mixtures of concentrated HNO_3 and concentrated $HClO_4$ heated at 240^{o}C for 2 hrs on a Kjeldahl digestion apparatus was chosen. The solutions were cold fifteen minute on the apparatus, and five minute at room temperature. Finally, the mixture was diluted with 10 ml distilled water then filtered to remove any suspended and turbid matter using filter paper no. 42 in 50 ml volumetric flask, shacked and diluted to the label mark with distilled water. The digestion was made in triplicates for each of the fruit samples collected from the three sampling sites. Digestion of a reagent blank was performed along with the fruit keeping all digestion parameters the same. All the digested and diluted samples were stored in room temperature until analysis.



a, b.

Figure 4: Digestion of a) *casimiroa edulis samples* and b) blank

3.6. Operating condition

In this study sample digestion apparatus (Gollenhamp Kjeldahl) and Flame Atomic Absorption Spectrometry instruments were used. The digestion apparatus was optimized the ratio of acid volume 2:1 at temperature of 24O^{o}C for 2 hrs. The operating condition for Flame Atomic Absorption Spectrometer is given in the Table 1

Table 1: FAAS operating conditions for determination of metals

Element	Wavelength (nm)	Lamp current (mA)	Slit width (nm)	Types of flame	Photo multiplier tube (PMT)(v)
Cu	324	1.5	1.2	Air-acetylene	243
Ni	232	7	0.2	Air-acetylene	309
Zn	213	2	0.5	Air-acetylene	391
Cd	228	2	1.2	Air-acetylene	253
Cr	357	2	0.5	Air-acetylene	303
Pb	283	2	1.2	Air-acetylene	233

3.7. Stock solution and Working standards

For the analysis of metals (Ni, Cd, Cu, Zn, Cr and Pb), the standard containing the stock solution (1000 mg/l) for each metal was prepared from well known European laboratory. These solutions were used to prepare intermediate standards (10 mg/l) and different working standards. Intermediate and working

standards were prepared from stock solution by serial dilution with deionized water. After establishing the performance standards**,** the instrument was adjusted to achieve a good relationship between absorbance and concentration, which is used to determine the concentration of the unknown sample.

3.8. Method Performance and Method Validation

Method validation is the process of indicating that analytical procedures are suitable for their planned purpose and that they support the identity, quality, purity, and accuracy of the tested sample and accuracy, precision and detection limit of the method.

Method performance and method validation is evaluated by matrix spike. A "good" matrix spike result increases our confidence in both accuracy and validity of the analyte test results. Spike (MS) is generated a Matrix by adding a known amount of analyte to a sample, testing the spiked sample, and determining if we have recovered the amount that we added. The two parts of the sample are prepared for testing. In the "matrix spike" portion, we add a known amount of standard (increase the concentration by a known quality). When to test the sample and the spiked sample, the result of the spiked sample must greater than the result of tested sample. If the analytical process does not work well for our sample, the result of the spike ma trix will be higher or lower than expected. A modern solution is a standard chosen to prepare a modern matrix; the concentration of the analyte in the spike solution is generally much higher than the concentration found in the unspiked sample [84].

3.8.1. Precision

Precision is closeness of the agreement between individual analyses, the more precise the results. A particular importance of the uncertainty in measuring the signal and the ease of handling samples reproducibly. The major signal for a total analysis method can be measured with a higher Precision than the corresponding signal for concentration method [85]

3.8.2. Accuracy

Accuracy is a measure of how closely the result of an experiment mean values agrees with the expected result. The difference between the calculated result and the true value is usually divided by the true and reported as percent relative error;

$$\%\text{error} = \frac{(\text{expected result} - \text{obtianed value})100}{\text{expected resut}}$$

Analytical methods may be divided into three divisions based on the amount of their relative errors. When an experimental result is within 1% of the correct result; the analytical method is highly accurate. Methods resulting in relative errors between 1% and 5% are moderately accurate, but methods of low accuracy produce relative errors greater than 5%. In general, total analysis methods produce results of high accuracy, and concentration methods range from higher to low accuracy [85].

3.8.3. Method Validation/ Recovery Test/

Method validation is the way of providing that analytical technique is acceptable for its intended purpose. Due to the absence of certified reference material for the fruit samples in our laboratory, the validity of the optimized digestion procedure was assured by spiking the samples with a standard of known concentration of the metals in the sample. Then percent recovery % is calculated by the formula:

$$\%\text{Recovery} = \frac{(Spiked\ amount\ value - \text{unspiked amount value})100}{\text{known added amount}}$$

4. RESULTS AND DISCUSSION

4.1. Optimization of the digestion procedure

The optimum working procedure should be determined before carrying out any experimental activities in digestion of samples. Optimized procedure was chosen based on usage of lesser amount of reagent volume, short digestion time, and reasonably mild temperature for obtaining clear colorless solutions of the resulting digested sample. The Optimization parameters for digestion procedure were 2:1 volume ratio of acids at 240^{o}C and for 2 hrs.

Table 2: Digestion results for 0.5 g fruit at different volume and constant temperature and time.

Volume ratio (ml) (HNO_3 to $HClO_4$)	Temperature (^{o}C)	Time (hrs)	Observation of experiment
3:3	240	3	yellow brown color
4:1	240	3	Clear colorless solution
3:2	240	3	Slightly yellow brown color
3:1	240	3	Clear colorless solution
2:2	240	3	Clear colorless solution
2:1	**240**	**3**	**Clear colorless solution**
1:1	240	3	Yellow

From table 2 observed that optimization results the digestion of fruit has the ratio of 4:1, 3:1, 2:2 and 2:1 volume of HNO_3 to $HClO_4$ at 24^{o}C for 3 hrs indicated the clear colorless solution. Among these volumes which give clear colorless solution 2:1 Volume ratios of acid is the minimum amount of volume to produce colorless solution. Therefore, 2:1 volume of HNO_3 to $HClO_4$ is an optimum volume.

Table 3: Digestion results for 0.5 g fruit at different temperature and constant volume and time.

Volume ratio of acids (HNO_3 to $HClO_4$)	Temperature (^oC)	Time (hrs)	Observation of experiment
2:1	**240**	**3**	**Clear colorless solution**
2:1	180	3	colorless but not clear
2:1	150	3	Colorless but not clear.
2:1	120	3	Colorless but not clear.
2:1	90	3	Colorless but not clear.
2:1	60	3	Yellow wish color
2:1	30	3	Yellow color

From table 3 observed 0.5 g of sample optimized at constant volume and time to produce colorless solution at 240^{o}C. Therefore optimum temperature is 240^{o}C.

Table 4: Digestion results for 0.5 g fruit at different time and constant temperature and volume.

Volume ratio of acids	Temperature (^oC)	Time (hrs)	Observation of experiment
2:1	240	3:00	Clear colorless solution
2:1	240	2:30	Clear colorless solution
2:1	**240**	**2:00**	**Clear colorless solution**
2:1	240	1:45	Colorless but not clear
2:1	240	1:30	Colorless but not clear
2:1	240	1:15	Colorless but not clear
2:1	240	1:00	Yellow brown color
2:1	240	0:45	Slightly yellow color

From table 4 observed the optimization results the 2:1 volume ratio of HNO_3 to $HClO_4$ and at 240^{o}C temperature the digestion obtained at time of 2 hrs gave clear colorless solution. From table 2-4 the digestion volume, temperature and time selected for digestion was 2:1, 240^{o}C and 2hrs respectively.

4.2. Calibration Curve of Standards

Flame Atomic Absorption Spectrometer (Analytikajena Model ZEEnit 700P#150Z71025 Tech) was used to determine the concentration of metals (Ni, Cd, Zn, Cu, Pb and Cr) in fruit of casimiroa sample. The qualities of the result obtained for analysis of heavy metals using FAAS are affected by calibration and standard solution preparation procedures. Calibration curves of the selected heavy metals were prepared to determine the concentration of metals in the sample solution. The calibration curves were calibrated using a series of working standards which were prepared from their intermediate solutions (10 mg/l). The stock solution that is 1000 mg/l of each metal was taken and 10 mg/l was prepared as an intermediate for preparing different concentration working standards. The working standards were prepared according to the sensitivity of each lamp in the AAS instrument. By using working standards the curve was calibrated with good correlation coefficient. After making sure the standard calibration curve was properly calibrated, the concentration of metals in each sample was measured and the calibration of each metal was drawn and presented in Figure 5 to 10.

Table: 5. Concentration of standard solutions used to calibrate the standard calibration curve and their corresponding correlation coefficients for the determination of metals

Metals	stock solution (mg/l)	intermediate solution (mg/l)	standard solution series (mg/l)	Correlation coefficient(R^2)
Cr	1000	10	0.25, 0.5, 0.75, 1	0.9963
Ni	1000	10	0.25, 0.5, 0.75, 1	0.999
Zn	1000	10	0.25, 0.5, 0.75, 1	0.9971
Cu	1000	10	0.25, 0.5, 0.75, 1	0.9956
Cd	1000	10	0.25, 0.5, 0.75, 1	0.9958
Pb	1000	10	0.25, 0.5, 0.75, 1	0.9993

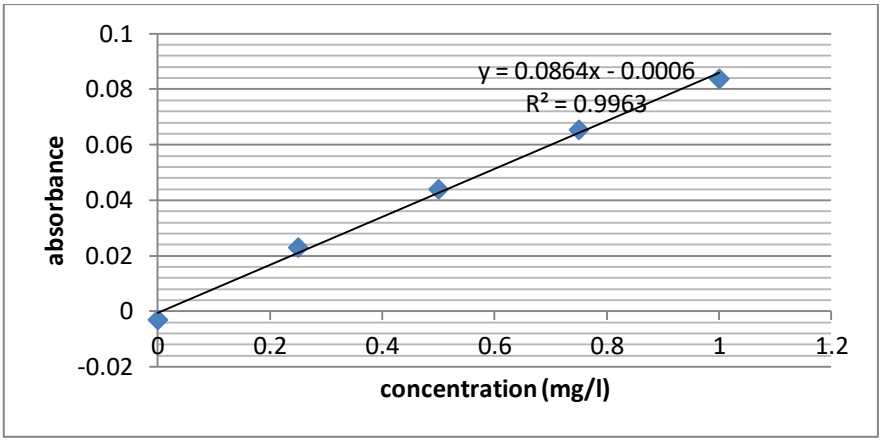


Figure 5: Calibration graph of chromium

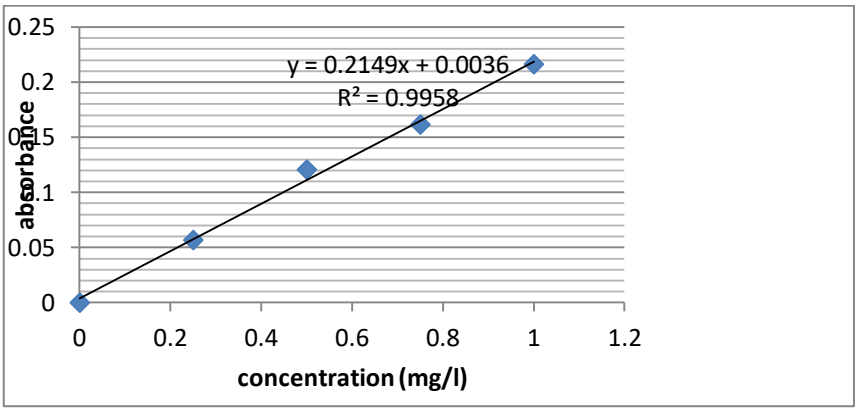


Figure 6: Calibration graph of cadmium

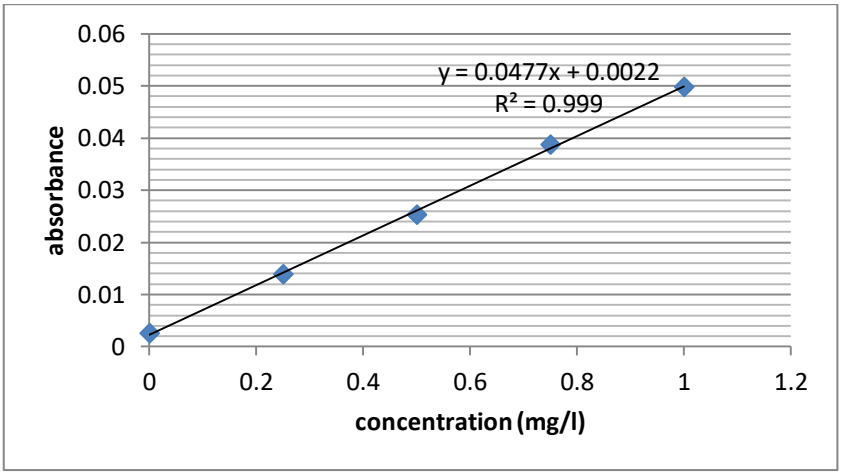


Figure 7: Calibration graph of nickel

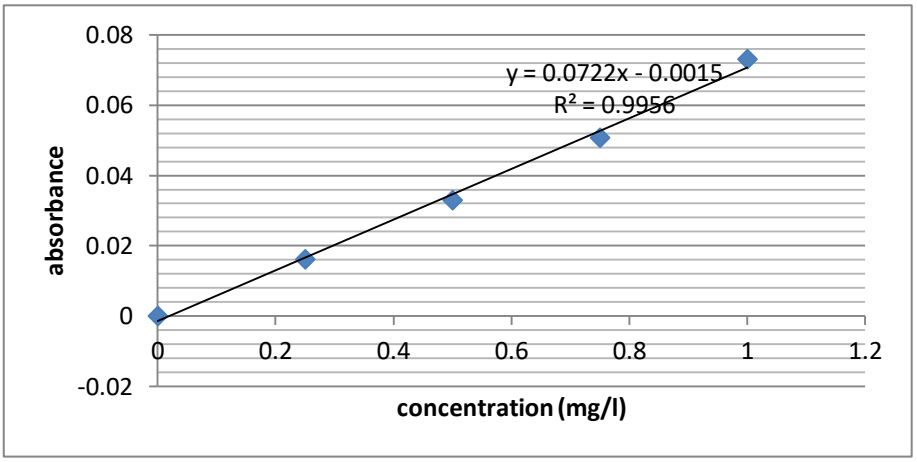


Figure 8: Calibration graph of Copper

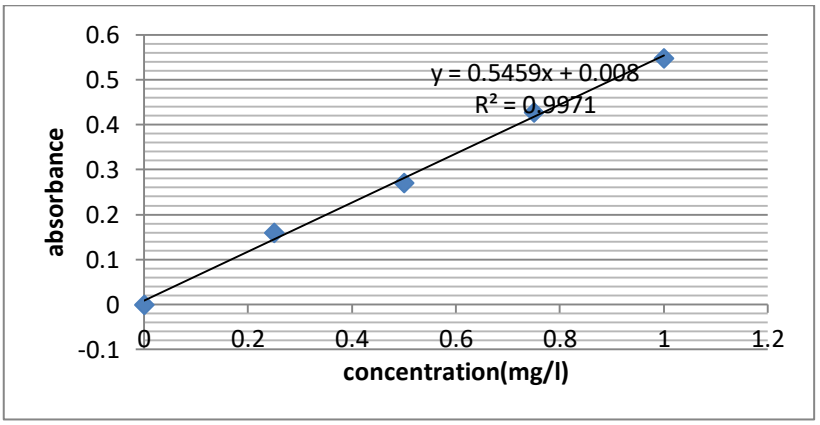


Figure 9: Calibration graph of Zinc

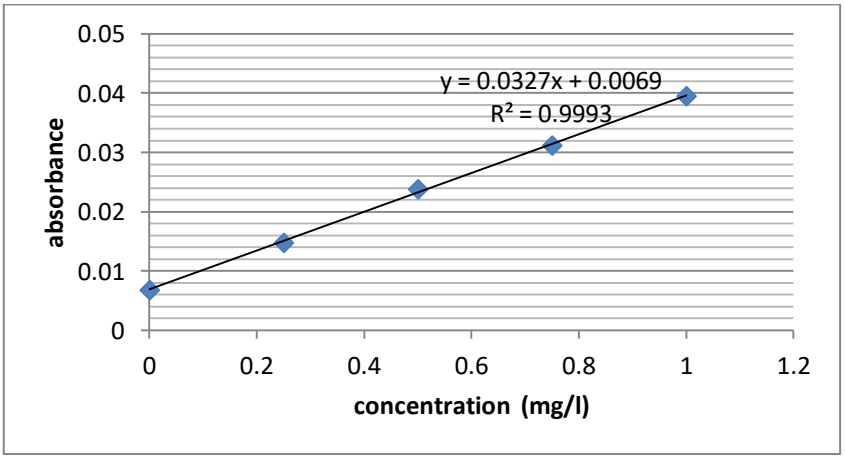


Figure 10: Calibration graph of lead

4.3. Method Detection Limit

The identical methods used to digest the Fruits of *Casimiroa Edulis* samples were applied to three replicate blank samples. The concentration of each metal (Cr, Ni, Cd, Cu, Zn, and Pb) in the blank was measured by FAAS. The method detection limit (LOD) was calculated using the standard deviations (SD) of the three duplicate blanks. The method detection limit (LOD) was determined by three times standard deviations dividing by slope of the curve (LOD = 3SD/S).

Table 6: Method Detection Limit of the metals in fruits of *casimiroa edulis*

Metals	Equation of curve	SD	LOD(mg/l)
Cr	Y=0.08565x -0.0006	0.001957	0.068546
Cd	Y=0.2163x +0.0036	0.001278	0.01772
Ni	Y=0.0474x+0.0022	0.000453	0.02867
Cu	Y=0.072x-0.001	0.0004272	0.0178
Zn	Y=0.5455x -0.008	0.026531	0.1459
Pb	Y=0.0325x+ 0.0069	0.0005525	0.051

4.4. Recovery Test

Recovery test is one of the most commonly used techniques utilized for validation of the analytical results and evaluation how far the method is acceptable for its intended purpose [86]. It studies involve the addition of a known of analyte to a sample and then determining what percent of the amount added is detected. The validity of the method was evaluated by spiking samples with standards of known concentrations and calculating percentage recoveries. The sample should be run in triplicate and the results taken as averaged. The recovered amount and percent of analyte are calculated by the formula as follow:

$$\%R = \left(\frac{\text{spiked sample result} - \text{unspiked sample result}}{\text{known spike added concentration}}\right) x\ 100\%$$

The percentage recoveries of metals are given below in table 7 within the acceptable range of 80-120% expected. So FAAS method was a good validation of analytical procedure.

Table 7: Recovery values of metals for the analyzed Casimiroa Edulis Fruit sample.

Mean ± SD					Percent recovery (%)
Metals	Un-spiked samples (mg/l)	Added amount (mg/l)	Spiked amount (mg/l)	Recovered amount (mg/l)	
Ni	BDL	Not added	Not spiked	-	-
Cu	BDL	Not added	Not spiked	-	-
Cr	BDL	Not added	Not spiked	-	-
Zn	0.21205 ± 0.07279	0.04	0.25161 ± 0.07349	0.03956 ± 0.0007	98.9±1.75
Cd	0.01833 ± 0.00338	0.04	0.05495 ± 0.00678	0.03662 ± 0.0034	91.55±8.5
Pb	0.34883 ± 0.0619	0.064	0.410907 ± 0.05601	0.062077 ± 0.00589	97.0±9.2

4.4. Determination of Heavy Metals in Fruits of *Casimiroa Edulis*

The concentration of six selective heavy metals (Ni, Cd, Cu, Cr, Pb and Zn) in the digested fruit of *Casimiroa Edulis* sample was analyzed by using FAAS. The mean ± SD level of the analyzed metals obtained are given in table 8. The result obtained from FAAS was in mg/l, but it was converted into mg/kg by using the following mathematical equation for the comparison with the guideline set by WHO/FAO.

$$\text{Concentration (mg/kg)} = \frac{\text{concentration}\left(\frac{\text{mg}}{\text{l}}\right) \text{X V}}{w}$$

Where, W is the weight in gram of sample, and v the volume of solution digested in milliliter.

Table: 8 Mean concentrations of heavy metals in fruits *of Casimiroa Edulis* sample

Metals	Ni	Cd	Cu	Cr	Zn	Pb
fruit (mg/l)	BDL	0.01833±0.00338	BDL	BDL	0.21205±0.07279	0.34883±0.0619
Fruit (mg/kg)	BDL	0.11	BDL	BDL	1.2723	2.09298

Among the analyzed selected heavy metals given in table 8 the level of chromium, copper and Nickel were below detection limit (LOD) in fruits of *Casimiroa Edulis* samples taken from Merehabete wereda, while the other selected heavy metals cadmium, lead, and zinc were detected in fruit of Casimiroa *Edulis* sample.

4.5. Comparison of Metals in fruits of *casimiroa edulis* with the guideline set by WHO.

Metals obtained from the different sources are released into the environment and tend to bioaccumulations in plants, organisms and even biomagnified in the food chain where human beings are highly exposed. So, if fruits of casimiroa *edulis* polluted with heavy metals. Metals will be transferred to animals and humans immediately and it affects human health when the concentration of heavy metals is above maximum limit. But the concentration of metals detected in fruit of *casimiroa edulis* is lower than the WHO maximum allowable concentrations except lead.

Table 9: Recommended concentration of heavy metals in fruits by WHO (mg/kg).

Metals	Cr	Ni	Cu	Zn	Cd	Pb	Reference
Sample	BDL	BDL	BDL	1.2723	0.11	2.09298	This study
WHO	1.2	1.0	2.0	1.5	O.2	0.5	[86]

The concentration of cadmium in the analyzed fruits of *casimiroa edulis* sample was (0.11) mg/kg. As shown in table 8 and 9, the concentration of cadmium in fruits of *casimiroa edulis* was relatively lower than the maximum allowable concentration (0.2 mg/kg) of cadmium given by WHO.

The concentration of lead in the analyzed fruits of *casimiroa edulis* sample was (2.09298) mg/kg which is higher than the maximum allowable concentration (0.5 mg/kg) of lead given by WHO. The concentration of zinc in the analyzed fruits of

casimiroa edulis sample was (1.2723) mg/kg which is lower compared to the level of maximum allowable Zn concentration (1.5 mg/kg) given by WHO.

Chromium, copper and nickel were not determined by flame atomic absorption spectrometric method because the level of chromium, copper and nickel in fruits of *casimiroa edulis* sample was below the detection limit of the instrument.

4.6. Comparison of Metals in *Casimiroa Edulis* with similar fruits reported in the Literature

Table10: Comparison of Metals in fruits of *Casimiroa Edulis* with similar fruits. (mg/kg)

Fruit	Element						Country	Place	Reference
	Zn	Cu	Ni	Cr	Cd	Pb			
Sapota	31.96	4.74	14.06	104.7	4.06	38.06	India	Karnataka	[87]
Grapes (*Citrus paradisic malfide*)	17.40	BDL	44.42	28.57	3.63	57.142	India	Karnataka	[87]
	0.10± 0.01	0.03± 0.00		0.002 ±0.00			Spain		[88]
	9	-		0.8		-	Pakistan	-	[89]
Lemon (*citrus Limon),*	22.19	6.76	37.94	227.5	9.09.	58.70	India	Karnataka	[87]
	4.2						Pakistan		[89]
Orange (citrus sinensis)	22.39	12.78	33.2	61.25	15.39	70.44	India	Karnataka	[87]
	0.98	0.28	0.25	0.12	BDL	0.18	Ethiopia	Ambo	90
Casimiroa endulis fruits	1.2723	BDL	BDL	BDL	0.11	2.09298	Ethiopia	Merehabete	In this study

The mean concentration of zinc in the analyzed fruits of *casimiroa edulis* in this study was lower than those reported in India with sapota, Grape fruit (*Citrus*

paradisic malfide), Lemon (*citrus Limon*), and Orange (*citrus sinensis*), and reported in Pakistan with Grape fruit (Citrus paradisic malfide), and Lemon (*citrus Limon*), [87, 89]. But the concentration of zinc was higher than reported in Spain with Grape fruit (*Citrus paradisic malfide*), and orange reported in Ethiopia [88, 90].

The level of nickel, chromium and copper in this study was below detection limit. However, in other literature as shown in Table 10 was in Spain for grape, in India for sapota, orange, lemon and grape and in Ethiopia for orange determined [87-90].

The mean concentration of cadmium in the analyzed fruits of *casimiroa edulis* sample in this study was 0.1308 as shown in table 10. The mean concentration of cadmium in the analyzed fruits of *casimiroa edulis* in this study was lower than those reported in India with Grape fruit (*Citrus paradisic malfide*), Lemon (*citrus Limon*), sapota and Orange (*citrus sinensis*) [87]. But the level of cadmium in the reported literature in Spain, and Pakistan was not analyzed in those reported literature for Grape fruit (*Citrus paradisic malfide*), Lemon (*citrus Limon*), and Orange (*citrus sinensis*) [88, 89].

The mean concentration of lead analyzed in fruits of *casimiroa edulis* sample in this study was lower than those reported in India with Greap fruit (*Citrus paradisic malfide*), Lemon (*citrus limon*), sapota and Orange (*citrus sinensis*) [87]. But the level of lead analyzed in this sample was lower than Orange (*citrus sinensis*) analyzed in the literature reported in Ethiopia [90].

5. CONCULUSSION AND RECOMMENDATION

5.1. Conclusion.

The purpose of this study was the determination of some selected heavy metals (Ni, Cd, Cu, Cr, Pb and Zn) in fruits of *casimiroa edulis* sample using FAAS. The result showed that the concentration of Pb analyzed in fruits of *casimiroa edulis* sample was relatively higher than the other analyzed heavy metals in the sample. But the level of Cr, Cu and Ni in the analyzed sample was below detection limit. The concentrations of the two metals (Cd, and Zn) in the analyzed sample were intermediate between Pb and Cr or Ni or Cu. The level of most analyzed metals in this study was lower than the maximum allowable concentration set by WHO except lead. So, fruits of *casimiroa edulis* taken from Merehabete are not safe for consumption as food due to higher concentration of lead.

5.2. Recommendations

This study was conducted in a very short period of time from the beginning of the November to the end of December; especially the sample was collected at beginning of November only at end of maturation of fruit. Because of this it was difficult to cover more sample site and collect larger sample size from all areas of Merehabete wereda and samples during all seasons to have taken more representative sample for the study. Hence, it is recommended for other researchers to take more time and large number of sample to address the limitation of this study.

This work also did not study the level of heavy metals of the soil. The levels of heavy metals in the soil have its own effect on the concentration of metals in the fruits of *casimiroa edulis*. So, it is recommended for other researchers to study level of heavy metals in the soil.

Types of fertilizers, insecticides, herbicides, water for plantation, and compost and weather condition of the growing area of casimiroa edulis also have effects on the level of heavy metals in fruits of *casimiroa edulis*. Therefore it is recommended for researchers to investigate all these factors.

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