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DEPARTMENT OF CHEMISTRY

DETERMINATION OF THE LEVELS OF SELECTED ESSENTIAL AND NON-ESSEN TIAL METALS IN UNPROCESSED AND PROCESSEDED TOMATOES FROM COM MERCIAL MARKET OF JIMMA CITY

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DETERMINATION OF THE LEVELS OF SLECTED ESSENTIAL AND NON-ESSENTIAL METALS IN UNPROCESSED AND PROCESSED TOMATOES FROM COMMERCIALMARKET OF JIMMA CITY

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APPROVAL SHET

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This is to certify that the thesis prepared by Demisse Bekele, entitled**: ''Determination of the Levels of selected essential and non-essential metals in the unprocessed and processed Tomatoes from commercial market of Jimma City**'' and submitted in partial fulfillments of requirement for Degree of Master of Science in chemistry complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

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List of Abbreviation

- AAS ___________ Atomic absorption spectrometry.
- ATSDR ________ Agency for toxic substances and disease registry.
- AT ____________ Average time.
- CAC ___________ Coldex alimenterius commission.
- CR _____________Contact rate.
- DOE ___________ ORO-department of energy oak ridge operations.
- EPA ____________ Environmental protection agency.
- ED _____________ Exposure duration.
- EFD ____________ Exposure frequency and duration.
- FAO ____________ Food and agricultural organization.
- EFSA ___________ European food safety authority.
- FFQ ____________ Food frequency questionnaires.
- IDL ____________ Instrumental detection limit.
- JEFCA __________ Joint world health organization expert food committee additives.
- LOD ____________ Limit of detection.
- LOQ ____________ Limit of quantification.
- MCL ____________ Maximum contaminant limit.
- Mn -SOD ________ Mitochondrial or Mn dependent superoxide dismutase.
- ND _____________ Not detected.
- PTW ____________ Provisional tolerable weekly intake.
- RSD ____________ Relative standard deviation.
- USDA ___________United states department of agriculture.
- WHO ___________ World health organization

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Abstract

Tomato is one of the most important edible nutritional vegetable and accumulates essential and non-essential metals. Tomatoes have, undoubtedly, assumed the status of a food with functional properties, considering the epidemiological evidence of reducing the risk of certain types of cancers. In this study, the concentration of essential and non-essential metals in unprocessed and processed tomato samples was evaluated. After sample preparation, and digestion the levels of those metals in tomatoes were determined by atomic absorption spectrometry (AAS).

The experimental results of each tomatoes, the mean concentration of essential and non-essential metals (mg/g) in unprocessed tomato sample exhibited decreased tend in the order of k, (20.23 ± 0.28) > Ca, (2.02 ± 0.01) > Cd, (0.072 ± 0.001) > Pb, (ND), while mean concentration for the pr ocessed tomato samples follow the decreasing order of K, (21.32 ± 0.42) > Ca, (1.92 ± 0.01) > Cd, (0.098 ± 0.001) > Pb, (ND), slightly a significant difference was observed between the mean concentrations of all metals in tomato. This indicates that the tomato sample contain the higher level of some selected essential metals than non-essential metals in unprocessed and processed tomatoes samples, and in the precision test, %RSD (relative standard deviation) for selected essential and non-essential metals in unprocessed and Processed tomato sample was in the range between 1.4% to 6.9% and 1.9% to 10%, for both tomato samples in commercial market of Jimma city. Therefore, the content of essential and non-essential metals such as Ca, K, Cd and Pb in unprocessed and processed tomato samples was below the permissible value of WHO/FAO.

Keywords: Essential and non-essential metals, tomatoes, Atomic Absorption Spectrometry.

1. INTRODUCTION

1.1. Background of the study

A tomato plant, the species that originated in the South American, Andes and it's used as a food originated in Mexico and was spread throughout the world by Spanish conquistadors [1]. Tomat o has one of the most widely consumed fresh vegetables in the world especially in Ethiopia, and an important source of healthy constituents of the human diet. The word tomato is derived from the Aztecan "meaning the swelling fruit". Despite the unique flavor characteristics of tomatoes, which make them extremely valuable in cooking and their recognized beneficial role in the diet, the quality of tomato was traditionally only considered in connection to external appearances. A s it happened with other highly requested crops. Breeding programs of tomatoes focused their efforts on developing new varieties with higher yields and stress resistance, with better uniformi ty in fruit size, brighter color, and prolonged shelf life [2].

In the human diet, it has an important source of micronutrients, antioxidants, and secondary metabolites such as vitamins C and E, b-carotene, lycopene, flavonoids, organic acids, phenolic, and chlorophyll. Tomato fruits are usually used in salads, cooked in sauces, soup, and meat fish dishes. Tomato consumption has been found to reduce the risks of cardiovascular disease and certain types of cancer, such as prostate, lung, and stomach [3].

Tomatoes constitute essential components of the diet, by contributing protein, vitamins, iron, calcium, and other nutrients which are usually in short supply. However, intakes of selective essential and non-essential metals that contaminate tomatoes may pose a risk to human health. Selective essential and non-essential metals contamination of the food item was one of the most important aspects of food quality assurance. Dietary exposure to heavy metals, namely cadmium (Cd), and lead (Pb), had been identified as a risk to human health through the consumption of vegetable crops. Toxic Heavy metals were given special attention throughout the globe due to their toxic and mutagenic effects even at very low concentrations [4, 5].

Exposing of tomatoes with selective essential (K, Ca) and non-essential (Cd, Pb) metals may be due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, the harvesting process [5] storage, and/or at the point of sale and/or market. Or selective essential and non-essential metals may enter the human body through inhalation of dust, consumption of contaminated drinking water, direct ingestion of soil and consumption of food plants grown in metal contaminated soil [6].

Lead and cadmium were among the most abundant heavy metals and are particularly toxic. The excessive content of these metals in food was associated with the etiology of several diseases, especially cardiovascular, kidney, nervous as well as bone diseases. Other metals such as potassium and calcium were essential for important biochemical and physiological functions and necessary for maintaining health throughout life, but if these metals have excessive concentration above the WHO value, which cause diseases [7].

Human beings were encouraged to consume more tomatoes and fruits which were beneficial for health. Publicity regarding the high level of heavy metals in the environment has created apprehension and fear in the public as to the presence of heavy metal residues in their daily food. Keeping in the potential toxicity, persistent nature, cumulative behavior as well as the consumption of vegetables and fruits, there was necessary to test and analyzed these food items to ensure the levels of these contaminants meet agree with international requirements [5].

Several studies were reported on essential and non-essential metals levels in unprocessed and processed tomato samples in different parts of the world using different techniques [8]. Some studies were carried out in Ethiopia on the levels of essential and non-essential metals [9].

However, to the best of our knowledge, there was no reported in the literature on the levels of essential and non-essential metals in processed and unprocessed tomatoes in commercial market of Jimma city. Therefore, intake of essential and non-essential metal contaminated tomatoes ma y pose a risk to human health. So, the present work is to focus biomonitoring contamination of e ssential and non-essential metals in processed and unprocessed tomato to establish some recom mendations on human diet food stuff in order to assure a significant improvement in tomato safety.

1.2. Statement of the Problem

Even though there are some reports about the metallic contents of Tomatoes from many countries in the world, no detail investigations were made as major and, toxic minerals (elements) for processed and unprocessed Tomatoes cultivated in Ethiopia. Therefore the objective of this study was to study the levels of essential and non-essential metals in processed and unprocessed Tomatoes.

The present research has been worked to answer the following equations.

- What were the levels of selected essential (K, Ca) and non-essential (Cd, Pb) metals in unprocessed and processed tomatoes?

- Do the levels of selected essential and non-essential metals in unprocessed and proces sed tomatoes are comparable?

1.3. Significance of the study

The major significance of this study is to provide information about the level of essential and non-essential metals in processed and unprocessed tomato from the commercial market of Jimma city. This study is believed to give a clue for further studies in Tomato fruit cultivated in different parts of the country and processed tomato from Ethiopia and from other countries. Also it will give information for the concerned institutions and organizations to take some necessary actions for the wellbeing of the society.

1.4. Objective of the study

1.4.1. General objective

- To analyze the levels of selected essential (K, Ca) and non-essential (Cd, Pb) metals is u nprocessed and processed tomatoes from a commercial market of Jimma city.

1.4.2. Specific objectives

- To determine the levels of selected essential (K, Ca) and non-essential (Cd, Pb) metals in unprocessed and processed tomatoes sample collected from a commercial market of Jim ma city.
- To compare the levels of essential and non-essential metals in processed and unprocesse d tomatoes.
- To identify and compare the levels of essential and non-essential metals in processed and unprocessed tomatoes with national and international recommended level.

2. LITERATURE REVIEW

2.1. Tomato

Vegetables is any part of a plant that was consumed by humans as a food as a part of a savory meal. It normally excludes other food derived from the plant. And cereal grains, but includes seeds such as pulses [10]. Tomato is the edible berry of the plant (Solanum lycopersicom) and nutritional vegetable plant parts including stem and stalk, root, tubers, bulbs, leaves, flowers, and fruits; usually includes sea weed and sweet corn. According to FAO 126 million tons of tomatoes were produced in the world. Tomatoes are important not only because of the large amount consumed, but also because of their healthy aspects and nutrition. May or may not include pulses or mushrooms; generally consumed raw or cooked with the main dish in a mixed dish as an appetizer or salad [11].

2.2. Packaging of food

Packaging was essential for the processing, manufacturing, storage, marketing, transportation, and handling of food. The genesis of modern food packaging began during the Industrial revolution when new manufacturing processes and materials were developed. At that time, large numbers of rural agricultural workers began migrating towards the cities where they sought employment. At the same time, household items began to be mass-produced, new shops opened, and the initiation of modernized techniques and developments led to an increased demand for packaging. This translated in to demands for barrels, boxes, kegs, baskets and bags to transport many of the mass produced goods to the cities, and thus the modern role of packaging began initially, metal cans were made for tobacco packaging because of its high barrier to moisture and gases [12].

Packaging serves three primary functions: protection, utility and communication. The most obvious function of a package is to contain the contents that it holds in order to protect the food quality and prevent contamination from moisture, gases, microorganisms, and dust, as well as provides stability from shock, vibrations, and other modes of physical stresses. The package acts as a barrier to many of these potential contaminants thus prolonging the shelf-life of the food [13].

2.3. Compounds in tomatoes

Tomato fruits are fleshy berries, that when ripened, the color can be red, pink, orange, yellow, or colorless. Most western cultures are familiar with the red pigmentation of the tomato fruit. This is caused by lycopene. Yellow is seen because of the carotenoid pigments present. When the fruit is matured, the locales are filled with a gelatinous material surrounding the seeds [14]. The composition of the fruit is 90% water, sugars, and acids. During ripening, glucose and fructose increase while the malic acid content decreases. The sucrose and citric acid contents generally remain constant throughout the development of the fruit. The pH is typically around 4.3 to 4.7, however, when processed the pH is lowered to 4.5 to prevent the growth of Clostridium botulinum. Tomatoes are generally grown in various soil types from sandy to fine clays. They grow in temperatures that are frost-free or warm (above 16°C). To successfully grow the tomato crop, it 16 is treated with nitrogenous fertilizers. During this process, the NO_3^- is mixed with NH_4^+ at a higher proportion to obtain optimal plant growth. The nitrogen fertilizer is added before planting and then phosphorous and potassium are added after planting the tomato seeds. Many steps are done to ensure the complete growth and optimal quality of the tomato fruit [15].

2.4. The Essential Metals under study and their benefits

Essential metals are metals that are important for the life of living things. Metal accumulation in plants depends on the plant species, types of soil, environment, and agricultural practice. Some of the trace element deficiencies in plants can cause nutrient deficiencies in the animals that graze those plants. The following were some of the essential elements under the study and their roles [16].

The metals require in living organisms are called essential elements such as K, Mg, Ca, Mn, Fe, Co, Cu, Se, and Zn and they are very important for growth and health, on the other hand, some non-essential toxic metals like Cd, Cr, Hg, and Pb are toxic. Studies conducted originally, on humans and animals show that optimal intake of elements such as sodium, potassium, Magnesium, Calcium, manganese, copper, zinc, and iodine could reduce individual risk factors, including those related to cardiovascular diseases. It was believed that the great majority of these trace elements act as key components of essential enzyme systems or other proteins. For instance, the hemoglobin, which performs vital biochemical, functions. All trace elements or metals are toxic if consumed at sufficiently high levels for long enough periods [17].

2.5. Effects of toxic heavy metals

Heavy metals form a major global threat as they are present widely in the earth's crust, water, air, and food [18]. According to [19], human activities such as mining, industrialization, and the use of agricultural pesticides coupled with natural activities have led to increased levels of heavy metal pollution in the ecosystem. Human health risk associated with heavy metal intake has been widely studied since it creates potential barriers for international trade. Whiles studies have shown that crops and vegetables grown in heavy metal contaminated soils have high heavy metal concentrations than those grown in uncontaminated soils. There has also been significant leaching of metals from machinery and equipment used for processing crops and vegetables [20].

Heavy metals exert either beneficial or harmful effects depending upon their chemical properties and concentrations [21]. Noted that excess consumption of arsenic and cadmium which are non-essential trace elements resulted in several skin lesions, renal dysfunction, cardiovascular diseases, and various cancers, even at very low doses, another study in the Van region of Eastern Turkey [22].

Related the high prevalence of upper gastrointestinal cancer in rats to high levels of Co, Cd, Mn, Ni, and Cu present in fruits and vegetables in that region. The presence of heavy metals in food and animal feed poses severe risks and can lead to toxicological effects as a result of long-term exposure. Excessive exposure to elements such as cadmium, lead, arsenic, chromium, and mercury is toxic for plants, animals, and human beings [23-24], reported high levels of cadmium, arsenic, mercury, and copper in food samples suggesting possible leaching from the utensils as well as machinery. Local machinery for grinding vegetables and cereals is manufactured using scrap metal from diverse sources including industrial machinery and vehicle parts [25].

2.6. Some essential and non-essential metals

2.6.1. Cadmium

Cadmium is a metal from group II B that has an atomic weight of 112.41with specific gravity of 8.65. Cadmium is a soft, malleable, ductile, bluish-white divalent metal and is highly carcinogenic for living beings. The Joint FAO/WHO has recommended the PTWI as 0.007 mg/kg/BW for cadmium [26].

The EPA maximum contaminant level for cadmium in drinking water is 0.005 mg/L whereas the WHO adopted the provisional guideline of 0.003 mg/L [26]. The Codex General Standard for Contaminants and Toxins in food and feed maximum levels of cadmium in fruiting vegetables is 0.05mg/kg. In general, for non-smokers and non- occupationally exposed workers, food products account for most of the human exposure burden to cadmium [27].

In food, only inorganic cadmium salts are present. Organic cadmium compounds are very unstable. In contrast to lead and mercury ions, cadmium ions are readily absorbed by the plant and are equally distributed over the plant. Cadmium is taken up through the roots of plants to edible leaves, fruits, and seeds. During the growth of grains such as wheat and rice, cadmium is taken from the soil is concentrated in the core of the kernel. Cadmium also accumulates in animal milk and fatty tissues [28]. Therefore, people are exposed to cadmium when consuming plants and animals based foods. Seafood, such as mollusks and crustaceans, can also be a source of cadmium [29].

And [30], both reported in their respective studies that Cadmium compounds are extremely toxic for plants, animals and human beings having been found widely distributed in air, soil, water, plants, and finally in animal tissues [31], reported that cadmium did not have a single physiological function within the human body, thus diverting attention to the study of its bio hazardous potential. Once cadmium is absorbed it accumulates in the body throughout life can adversely affect the number of metabolic processes in an animal body. Kidney, bone, and pulmonary damages are attributable to cadmium intoxication [32, 33].

Cadmium is primarily toxic to the kidney, especially to the proximal tubular cells where it accumulates over time and may cause renal dysfunction. Cadmium can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. After prolonged and/or high exposure the tubular damage may progress to decreased glomerular filtration rate, and eventually to renal failure [34]. In animals, cadmium toxicity affects organs such as the liver, lung, testis, and hematopoietic systems [35].

2.6.2. Lead

The current annual world production of lead is approximately 5.4 million tons and continues to rise [36] resulting in intensive pollution of the environment with this metal [37]. Reported that lead has been mined for centuries. Lead as a toxicologically relevant element has been brought into the environment by man in extreme amounts, despite its low geochemical mobility, and has been distributed worldwide [38]. Lead amounts in deep Ocean waters are about 0.01-0.02 μg/L, but in surface ocean waters is 0.3 μg/L [37].

Lead still has several important uses in the present day; from sheets for roofing to screens for X-rays and radioactive emissions. Like many other contaminants, lead is ubiquitous and can be found occurring as metallic lead, inorganic ions, and salts [39]. Lead has no essential function in man. Food is one of the major sources of lead exposure; the others are air (mainly lead dust originating from petrol) and drinking water. Plant food may be contaminated with lead through its uptake from ambient air and soil; animals may then ingest the lead-contaminated vegetation. In humans, lead ingestion may arise from eating lead-contaminated vegetation or animal foods. Another source of ingestion is through the use of lead-containing vessels or lead-based pottery glazes [40].

In humans, about 20 to 50% of inhaled, and 5 to 15% of ingested inorganic lead is absorbed. In contrast, about 80% of inhaled organic lead is absorbed, and ingested organic Pb is absorbed readily. Once in the bloodstream, lead is primarily distributed among blood, soft tissue, and mineralizing tissue [40].

The bones and teeth of adults contain more than 95% of the total body burden of lead. Children are particularly sensitive to this metal because of its more rapid growth rate and metabolism, with critical effects on the developing nervous system [41]. The Joint FAO/ World Health Organization Expert Committee on Food Additives (JEFCA) established a provisional tolerable weekly intake (PTWI) for the lead as 0.025 mg/kg/BW [42]. The WHO provisional guideline of 0.01 mg/L has been adopted as the standard for drinking water [43].

2.6.3. Potassium

Potassium is a very significant body mineral, important to both cellular and electrical functions. It is one of the main blood minerals electrolytes. It has been found that an average human body weighing 70 kg contains 0.25 kg of potassium where its deficiency result in various neurological dysfunctions, influences the vascular volume, and led an increase in blood pressure. Potassium metal electro chemical cells are widely utilized to examine potassium insertion materials for non-aqueous potassium-ion batteries. However, large polarization during K plating–stripping and unstable rest potential are found at the potassium electrodes, which lead to an underestimation of the electrochemical performance of insertion materials. In this study, the electrochemical behavior of K-metal electrodes is systematically investigated. Electrolyte salts, solvents, and additives influence the polarization of K metals [44].

2.6.4. Calcium

In the human body calcium has different roles, such as building and strengthening the bones and teeth, cell signaling, muscle contraction, blood clotting, transmitting of the nerve impulses, and regulating the heart's rhythm. 99% of the calcium in the human body is stored in bones and teeth. The remaining one percent is found in the blood and cellular and extracellular fluid. The body gets calcium by pulling it from the bones when blood levels of calcium drop too low, usually when quite a long time passes since having taken calcium with the meal. Calcium can also be found in soybean, dark green leafy vegetables, spinach, dried beans, and legumes, calcium fortified juice in addition to milk powder and dairy products are a convenient source of calcium for many people. The Calcium taken up from the soil is trans located to the leaves but very little goes from the leaves to the fruit [45].

2.6.5. Magnesium

Magnesium ions regulate over 300 biochemical reactions in the body. Magnesium produces energy in our bodies. It exists on an average of 0.042 kg in 70 Kg human body. Its deficiency results in a permanent state of muscles contraction in our body and we could not adjust the levels of cholesterol produced and released into the bloodstream. Mg play important role in chlorophyll components, cell wall and membrane integrity, enhancing pollen germination and growth, the effect of calcium in prolonging activators of enzyme [46].

2.6.6. Chromium

The most common forms of chromium are chromium VI and chromium III. Cr (III) is an essential element required for normal sugar and fat metabolism. It is effective in the management of diabetes and it is a cofactor with insulin. Cr (III) and its compounds are not considered a health hazard. Chromium III is an important component of a balanced human and animal diet and its deficiency causes disturbance to the glucose and lipid metabolism in humans while chromium VI is carcinogenic. Alteration of genetic material, Lung cancer and death [47].

2.7. Ready-to-eat foods at the retail level

Ready-to-eat foods refer to food that is in the form that is edible without cooking, washing, or heating by the consumer and that is reasonably expected to be eaten in that form [48]. Vegetables such as ready-to-eat blended tomatoes are considered ready-to-eat foods. This category of foods is considered high risk since food workers, food contact surfaces, etc. can transmit pathogens on to the food [49].

Conducted a survey of Listeria monocyotogenes in ready-to-eat foods from retail markets and indicated higher frequencies of Listeria monocyotogenes in-store packaged meats than manufacturer packaged products is reported that street vending in poverty-stricken areas will have limited resources to train their staff on safe handling practices and guarantee the safest food supply to consumers **[**50].

2.8. Atomic Absorption Spectroscopy

Atomic Absorption Spectrometry (AAS), Inductive Coupled Plasma Mass Spectroscopy (ICP – MS) and X-Ray Florescence spectroscopy (XRFS) etc, was a technique for measuring quantities of chemical elements present in different samples by measuring the absorbed radiation by the chemical element of interested. This is done by reading the spectra produce when the sample was excited by radiation. Atomic absorption methods measure the amount of energy in the form of photons of light that were absorbed by the sample. 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. The energy required for an electron to leave an atom is known as ionization energy and is specific to each chemical element [51].

3. Methods and Materials

3.1. Study site

The study was conducted in Jimma city, Jimma zone, Oromia state Ethiopia. It is located 345k m from Addis Ababa in south west Ethiopia. It is available at latitude and longitude of $7^0$40'N3 6' 50'E and altitude, of about 173 m above sea level, it lies in the climate zone locally known.

3.2. Reagents and Instruments

3.2.1. Reagents and Chemicals:

Nitric acid (HNO_3) and Hydrogen peroxide (H_2O_2) were used as extraction solvent. Nitrates of Cd, Pb, K, and Ca were to prepare stock solutions (1000 ppm, in 2% HNO_3) of the metal ions. Deionized water was used as diluents for preparing desired working standard the metal ion solutions and to wash the apparatuses.

3.2.2. Apparatus and Instruments:

Apparatus like digestion flasks, volumetric flasks, measuring cylinders and pipettes, separating funnels and filter papers were used. Instruments such as, Digital analytical balance and, Atomic absorption spectrometry (Model SM- M107F or JPG 280 FS AAS)), were used in this study.

3.2.3. Cleaning apparatus:

Apparatus such as conical flasks, volumetric flasks, pipettes, measuring cylinders were thoroughly washed with distilled water; then soaked in 10 % (v/v) HNO_3 solution for 24 hours followed by rinsed several times with deionized water. Finally the apparatus were dried in a hot air oven and kept in a clean placed to avoid contamination until analysis began.

3.3. Sample collection and preparation

3.3.1. Tomato samples collection

1000 g of unprocessed tomato and 600 g of two packed metal cans of processed tomato samples would be collected from commercial market of Jimma city. The unprocessed tomato would be washed thoroughly with tap water and then again washed with distilled water for the removal of any soil particles. These samples are stored in polythene plastic bags separately.

3.3.2. Sample preparation

The unprocessed tomato sample was washed with tap water followed by deionized water to eliminate soil particles and dust. The samples were chopped by scissors in to small pieces and packed in aluminum foil. The tomatoes samples would be placed in acid washed clean porcelain crucibles and oven-dry at 85 °C for 48 hours in an oven. The dried samples were ground and homogenized in to a fine powder with a grinding device and the powder store in polythene bags separately for further chemical analysis.

3.3.3. Optimization of digestion

In this study, sample digestion was carried out using wet digestion for both tomato samples, to prepare a clear colorless sample solution suitable for the analysis. Used for the digestion contains nitric acid, hydrochloric acid, and hydrogen peroxide. Before the samples were digested for analysis the parameters, acid ratio, temperature, and duration of time for complete digestion were carefully optimized. Optimum procedure of sample dissolution was required to give at most resul t with minimum reagents, time, temperature, simple procedure and in an environment friendly m anner. Nitric acid and hydrogen peroxide mixture was used for tomatoes sample decomposition a nd digestion. Digestion conditions for the samples using HNO_3 and H_2O_2 were assessed by varyi ng volume of acids, digestion temperature and digestion time.

Optimization procedure of the digestion conditions for processed and unprocessed samples of tomato powder are presented in Table 3.The digested samples were cooled at room temperature, and were diluted, filtered, and make up to 250 mL each in a measuring flask using deionized water. The best optimum condition, which gives a clear colorless solution, with minimum solvent volume, low temperature and time were selected for further analysis by FAAS. Digestion of a reagent blank was also performing in parallel keeping all digestion parameters the same.

3.4. Analysis of tomato samples for metal levels

For the determination of metals in processed and unprocessed tomato samples, different series of working standard solutions were prepared from the intermediate standard solutions of their respective metals, which were prepared by diluting the stock standard solutions of the metals with deionized water. Optimum acetylene and air flow rates were chosen to obtain suitable flame conditions. Other conditions such as lamp current, wave length, slit and flame were selected for each hallow cathode lamp according to the manufacturer’s recommendation. Different point calibrations were established by introducing the prepared working standard solutions in flame atomic absorption spectrophotometer. Immediately after calibration of the instrument, the reagent blank and followed a minimum of three readings were taken for each sample solution and reagent blank solution by the sample solutions were introduced into the atomic absorption spectrophotometer, and the mean value of the concentration signal was used for subsequent calculations. The operating parameters for FAAS employed for each analyte are given in Table 1.

Table 1: Instrumental operating conditions

S. No	Element	Wave Length(nm)	Slit width (nm)	Lamp Current (mA)
1	Ca	422.7	0.5	10
2	K	766.5	1.0	5
3	Cd	228.9	0.7	4
4	Pd	283.2	0.7	5

3.5. Standard and working solution preparation

Metal standard solutions for calibration were prepared from an intermediate standard solution containing 10 mg/g which was prepared from standard stock solution that contaminated 1000 mg /g for each of the metals. Secondary standards for instrument calibration were prepared by dilute ing with de-ionized water to obtain five working standards for each metals as follows; Ca, (0.2 mg/g, 0.4 mg/g, 0.8 mg/g, 1.6 mg/g, 1.8 mg/g), K, (0.2 mg/g, 0.4 mg/g, 0.8 mg/g, 1.6 mg/g 1.8 mg/g), Cd, (0.01 mg/g, 0.02 mg/g, 0.04 mg/g, 0.08 mg/g, 0.1mg/g) and Pb, (0.01 mg/g, 0.02 mg/g, 0.04 mg/g , 0.08 mg/g 0.1 mg/g).

3.6. Method performance and method validation

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

3.7. Precision

The precision of an analytical procedure has usually expressed as the variance, relative standard deviation and percentage relative standard deviation of a series of measurements. In this study, the precision of the results was evaluated by percentage relative standard deviation of the results of three samples and triplicate readings for each sample giving nine measurements for a given bulk sample.

$$RSD = SD/X*100$$

Where; RSD - Relative standard deviation, SD - standard deviation, X - mean

3.8. Accuracy

The performance of the methods was evaluated by employing spiking experiments in which the concentration of the analyte is already known. For this purpose standard solution of 1000 mg/g was used and intermediate standards were prepared. Thus, spiking was done by adding known concentration of each metal in 1 g of powdered samples. The spiked and non-spiked of processed and unprocessed samples were digested and analyzed in similar conditions using optimized procedure for sample analysis. Then, the percentage recoveries of the analytes were calculated by the use of the equation:

$$(\%R) = \frac{\text{Concentration of spiked} - \text{Concentration of un spiked}}{\text{Concentration of added}} \times 100 \text{ -------------- } (1)$$

Where: %R - Percent Recovered

3.9. Method detection limit

Method detection limit is defined as the minimum concentration of analyte that can be identified, measured and reported with 99% [52]. Method detection limit for heavy metal may vary with wavelength selected and the spectrometer configuration and operation conditions.

Method detection limit for both tomato sample were determined using reagent water blank with HNO_3:H_2O_2 that was digested in the same condition as the sample. In this study, after digestion of three blank solutions, seven readings have taken for each blank and the standard deviations were calculated.

The method detection limit of each metal has obtained by multiplying the standard deviation of the reagent blank by three and the mean concentration of the of blank was added [53].

$$LOD = 3 \times SD_b + mean \text{ ------------------------------ } (2)$$

Where SD_b --standard deviation of the blank reading

3.10. Limit of quantification (LOQ)

Is the lowest concentration level at which measurement is quantitatively meaningful, the LOQ w as calculated by multiplying the standard deviation of the reagent blank by ten and the mean con centration of the of blank was added [54].

$$LOQ = 10 \times SD_b + mean \text{ ------------------------------ } (3)$$

3. 11. Statistical analysis

Descriptive statistical analysis including mean and standard deviation of each essential and non-essential metal were calculated for each tomato samples, and were presented as mean ± SD of triplicate measurements. Mean and SD of each sample were used to compute the calculated t-value, to see if there are significance difference between the samples and analyzed metals at 95 % confidence level ($p > 0.05$).

4. RESULTS AND DISCUSSIONS.

4.1. Instrumental calibration

Calibration curves were prepared to determine the concentration of metals in the sample solution. The calibration graphs and correlation coefficients of each of the elements were determined by plotting working standards concentration versus their corresponding absorbance. The working st andard solutions and the correlation coefficient of the calibration curve for each of the metals are presented in table 2 below. The obtained calibration were exhibited a good correlation coefficient (r^2) ranging from 0.995 – 0.997 [55]. The performance characteristics of the utilized method are shown in the table 2.

Table 2: Calibration curve Regression equation and correlation coefficient.

Metals	Standard solution (mg/g)	Regression equation (y = mx+ b)	Correlation coefficient
Ca	0.2, 0.4, 0.8, 1.6, 1.8	Y = 0.0023x + 0.000	0.996
K	0.2, 0.4, 0.8, 1.6, 1.8	Y = 0.0025x + $3E^{-06}$	0.997
Cd	0.01, 0.02, 0.04, 0.08, 0.1	Y= 0.151x - $9E^{-05}$	0.995
Pb	0.01, 0.02, 0.04, 0.08, 0.1	Y = 0.1215x + 0.0002	0.997

Therefore, the calibration curve was showed that there was linearity between the instrument response and prepared concentration which indicating the best working condition of the instrument. The calibration curves are given in Appendix.

4.2. Optimization of digestion result

Two different HNO_3: H_2O_2 rations yielded clear and colorless solution during volume optimizati on for the digestion of 1 g of each tomato samples in table 3. But trail 1 was taken as optimum co ndition of volume reagent for fixing other parameters of temperature, and time. The obtained opti mum temperatures are 210 ℃ and 240 ℃ at which clear and colorless digestion solution were obt ained as indicated in Table 3. Therefore, the selected optimized conditions for the digestion of tomato samples were 6 mL HNO_3, 2 mL H_2O_2, 210 ℃ of temperature and 150 min of digestion time.

Table 3: Optimization of procedures for the samples

S. No	Weight of powder tomatoes sample (g)	Volume of Reagent (mL)		Maximum Temperature (°C)	Time (min)	Physical appearance of digest
		HNO_3	H_2O_2			
1	1	6	2	210	150	Clear and colorless
2	1	6	3	240	150	Clear and colorless
3	1	8	4	240	120	Clear but yellowish

4.3. Limit of detection and Limit of quantification

Method detection limit and quantification for both tomato samples were determined using reagent water blank with HNO_3:H_2O_2 that was digested in the same condition as the sample. In this study, after digestion of three blank solutions, seven readings have taken for each blank and the standard deviations were calculated for a 99% confidence level was taken for each analyzed metals. The result of LOD, LOQ and IDL are presented in table 4.

Table 4: IDL, LOD, and LOQ for determination of metals in tomato samples (mg/g)

Metals	Ca	K	Cd	Pb
IDL	0.001	0.007	0.0027	0.007
LOD	0.12	0.17	0.007	0.0083
LOQ	0.36	0.295	0.022	0.025

4.4. Precision

The reproducibility of the analytical procedure was checked by carrying out a triplicate analysis and calculating the relative standard deviation (%RSD) for each metal. The precision of the unprocessed and processed tomato results was evaluated from % RSD of the samples and triplicate readings for each sample making total of four measurements for a given sample.

In the precision test, %RSD for selected essential and non-essential metals in unprocessed tomato sample was in the range between, 1.4% to 6.9%, and for processed tomato sample was in range between 1.9% to 10%, in table 7. In this study % RSD was below 10% [56] the mean which indicated that the analytical method used is precise and reliable. Therefore, the results obtained by this method are in good agreement or accepted with each other.

4.5. Recovery test.

The efficiency of the optimized procedure was checked by adding known concentration of each metal in 1 g sample of tomato samples. 1 mg/g of Ca and K, 1.05 mg/g Cd and Pb from stock sol ution (1000 mg/g) were taken and spiked at once in to 250 mL of round bottom flask with the sa me digestion process as followed.

Then the samples were digested with the optimized procedures for both tomato samples. After di luting the digested samples with deionized water, they were analyzed by the same procedure foll owed for the analysis of tomato samples. As used for both tomato samples triplicate spiked samp les were recorded.

The recovery values for the unprocessed tomato sample were in the range of 84.6% to 96% in table 5, while for the processed tomato sample were in the range of 87% to 108%, in table 6. All values are lie in the acceptable ranges. Therefore, the optimized procedures for the essential and non-essential metal analysis of both tomato samples were validated and the proposed method de monstrated its good accuracy and precision.

Table 5: Recovery test for the optimized procedure of Unprocessed Tomato samples

Metal	Con. Of Metal in the Un-spiked Sample (Mean Val ue) (mg/g)	Amount adde d (mg/g)	Con. of Metal in the spiked Sample (Mean value) (mg/g)	Percent Recovered (%R) for unprocessed Tomato
Ca	2.02 ± 0.01	1	2.962 ± 0.002	91
K	20.23 ± 0.28	1	21.05 ± 0.032	86
Pb	0.001 ± 0.0001	1.05	0.097 ± 0.0003	96
Cd	0.072 ± 0.001	1.05	0.08 ± 0.0002	84.6

Table 6: Recovery test for the optimized procedure of Processed Tomato samples

Metal	Con. of Metal in the Un-spiked Sample (Mean Value) (mg/g)	Amount added (mg/g)	Con. of Metal in the spiked Sample (Mean value) (mg/g)	Percent Recovered (%R) for processed Tomato
Ca	1.92 ± 0.01	1	2.42 ± 0.003	104.6
K	21.32 ± 0.42	1	22.06 ± 0.043	87
Pb	0.002 ± 0.0003	1.05	0.098 ± 0.007	108
Cd	0.098 ± 0.001	1.05	0.678 ± 0.008	103.6

4.6. Level of selected essential and non-essential metals in unprocessed and processed tomatoes samples.

The unprocessed and processed tomato samples collected in the study area was digested and analyzed for some essential and non-essential metals. The results obtained revealed that all the metals analyzed were detected and there was variation in the values obtained in unprocessed and processed tomatoes, respectively. The metal concentrations was obtained in the samples are presented inTable 7 respectively. The concentrations of four metals (Ca, K, Cd, and Pb) in unprocessed and processed tomato samples were determined by AAS. The average concentration of the metals was expressed as mean ± standard deviation (SD) in Table 7.The results are presented as average of the determination of triplicate recording for each of the four sample solution.

Therefore, the level of concentration of the selected essential and non-essential metal such as k, Ca, Cd, and Pb was determined in the samples of tomato are reported for each metal as the mean of four measurements along with the corresponding total standard deviation and percent relative standard deviation for the given sample. All the values obtained for the metal contents in analyzed tomato sample were expressed in (mg/g) on dry weight the results in (Table 7), indicated that in the tomato sample some essential and non-essential metals were detected and the mean concentration of four metals are shown above the detection limit.

Table 7: Level of selective essential and non-essential metals (Ca, K, Pd, &Cd) in unprocessed and processed tomato samples was given by (mean ± SD N =3) and %RSD.

Tomato Sample	Unprocessed				Processed			
Metal Analyzed	K	Ca	Cd	Pb	K	Ca	Cd	Pb
Mean ±SD (mg/g)	20.23 ± 0.28	2.02 ± 0.01	0.072 ± 0.001	ND	21.32 ± 0.42	1.92 ± 0.01	0.098 ± 0.001	ND
%RSD	1.4	6.4	6.9	ND	1.9	3.6	10	ND

The concentration of metals determined in tomato samples revealed the fact that potassium was present in higher concentration than the other essential and non-essential metals in both samples, in the present study while leads concentration was not detected in the unprocessed and processed tomato samples as shown in Table 7.

The results showed that the mean concentration of metals (mg/g) in unprocessed tomato sam ples sequence followed was k (20.23 ± 0.28) > Ca (2.02 ± 0.01) > Cd (0.072 ± 0.001) > Pb (ND), while mean concentration for the processed tomato samples follow the decreasing order, K (2 1.32 ± 0.42) > Ca (1.92 ± 0.01) > Cd (0.098 ± 0.001) > Pb (ND). This indicates that the tomato fruits from commercial market of Jimma city contain the higher level of essential metals (Ca and K) than non-essential metals (Cd and Pb).

When we compared unprocessed and processed tomato samples, contain silitly the same conc entration of essential metal potassium, but both samples contain silitly different concentration of essential metal calcium and non-essential metal cadmium was obtained in tomato sample. Ho wever these two samples, Processed and unprocessed tomato samples contain more concentrat ion of essential metal (K) and less concentration of (Ca) metal while, contain more concentration non-essential metal (Cd), and the concentration of (Pb) is not detected. It is because the tomato sucks essential and non-essential metal found in the soil with the minerals needed for its growth. Therefore contaminations of the tomato fruits were an indication that the soil is polluting the vegetable and due to variation in sources and processes of contaminati ons that could attribute to metals contamination to take place during pre-harvest and post-harvest process.

4.7. Statistical evaluation.

All the descriptive statistical procedures were conducted using Excel software and t-test analysis was performed in triplicates. Results were expressed by mean ± SD. The means and standard deviations of the essential and non-essential metals levels in the processed and unprocessed tomato samples were calculated. Various descriptive statistical procedures (mean, standard deviation, percent relative standard deviation (% RSD), calculation of slope (m) and linear regression) were utilized in this study. In addition to that t-test was also used to identify statistical difference between the two mean concentration of the metal in processed and unprocessed tomatoes from commercial market of Jimma city respectively. Therefore, using t-test statistical analyses, the results were made to verify whether there were significant differences in concentration of the essential and non-essential metals in the processed and unproc essed tomato samples.

As indicated in Table 9 from the appendices, there was a statistical significant difference in the concentration of potassium in the processed and the unprocessed tomato; $t = 3.648$, $p = 0.022$. This shows that the two groups were not similar in the concentration of potassium between processed and unprocessed tomato, since p-value is less than 0.05. But, there was no a statistical significant difference in the concentration of calcium and cadmium in the processed and unprocessed tomato; $t = -1.223$, $p = 0.289$ for Ca, and $t = 2.214$, $p = 0.091$ for Cd (two-tailed). This shows that the two groups were similar in concentration of calcium and cadmium between processed and unprocessed tomato, since the p-value is greater than 0.05. Therefore, the result indicated that there was statistical significant difference obtained ($p < 0.05$) for K, but there is no a statistical significant differences were obtained ($p > 0.05$) at 95% confidence levels for Ca and Cd metals in unprocessed and processed tomato.

For the present study, the significance of variation within tomato sample unprocessed and processed has been studied using t-test. At 95% confidence level, for the tomato samples significant difference, $P < 0.05$ was observed for K metals and there was no significance of variation observed within tomato sample unprocessed and processed tomatoes for Ca and Cd metal. $p > 0.05$

4.8. Comparison of essential and non-essential metals levels in unprocessed and processed tomatoes with literature values

Many researchers have reported the levels of essential and non-essential metals in tomato. The report indicated that; the comparison of the obtained essential and non-essential metal contents with results published in existing literature varies widely among different countries in table 7. Levels of essential (Ca and K) and non-essential (Cd and Pb) metals found in this study were lower than those obtained in several countries, but some essential metal (K) and non-essential metals (Cd and Pb) were with exception of Nigeria, and Egypt. That difference was due to low standards of environmental protection and contaminated soils and water [62]

Table 8: Comparison of essential and non-essential metals levels in processed and unprocessed tomatoes with literature values.

Country	Metals	Concentration	Reference
Turkey	K	15.84±0.24 (mg/kg)	[56, 57]
Turkey	Ca	10.98±0.16 (mg/kg)	[56, 58]
Greece	K	30-35 (mg/kg)	
Turkey	Cd	5.12±0.28 (mg/kg)	[58]
Libya	Cd	0.250±0.025 (mg/kg)	[59]
Turkey	Pb	4.31-5.51 (µg/g)	[60]
Turkey	Pb	0.17-0.40 (µg/g)	[60]
Nigeria	Cd	0.01 (mg/kg)	
Nigeria	Pb	2.96-3.92 (mg/kg)	[61].
Egyptian	Pb	0.26 (µg/kg)	
Egyptian	Cd	0.01 (µg/kg	
Jimma city	K	20.775 (mg/g)	
Jimma city	Ca	1.97 (mg/g)	
Jimma city	Pb	0.085 (mg/g)	

5. CONCLUSION AND RECOMMENDATION.

5.1. Conclusion.

Determination of heavy metals concentration in vegetables and food products is important for health risk assessment during food consumption. The metals such as calcium, potassium, lead an d cadmium were analyzed by using atomic absorption spectrometry in the processed and unproc essed tomato samples around the commercial market of Jimma city, Ethiopia. The optimum procedure selected for digestion process produced good recovery results ranged from 87% up to 108% for processed, while 84.6 up to 96% for unprocessed tomato with %RSD below 10% which shows the efficiency of method used. The concentration of K was found in the higher conc entrations in both tomato samples compared to other metals analyzed. However, the levels of the concentration of K, Ca, Cd, and Pb metals in processed and unprocessed tomato samples was col lected from commercial market of Jimma city were below the permissible level set by FAO/WH O specification. Therefore concentration results of all metals between samples were also compared using Excel software and T-test to be proved statistically. The results of T-test indicated that there was statistical significantly different in the levels of K metals between the two samples each, but there was no statistical significantly different in levels of Ca and Cd metals in the processed and unprocessed tomato samples.

5.2. Recommendation

Based the finding and the conclusion drawn above, the following recommendations are for warded.

- ❖ Monitoring of the levels of some essential and non-essential metals in tomato should be encouraged.
- ❖ To minimize the impact of essential and non-essential metals in tomato on the heath of user, tomato should be thoroughly washed before consumption washing can remove a significant amount of aerial contamination from the tomato surface.
- ❖ Processed and unprocessed tomatoes should not be cultivated in farms and fields near by urban areas which have heavy vehicle movements and irrigated with questionable water quality which could be sources of heavy metals contamination.
- ❖ Tomatoes should be properly covered before transportation from the farm to the market, to avoid contamination of essential and non-essential metals.

6. REFERENCES.

1. Etem, O.; IbrahimI, O.; Zeliha, Goksel, D.; Memduh, S. Determination of heavy metal concentrations in Tomato (*Lycopersiconesculentum Miller*) grown in different station types. Rom anian *Biotech. Let.*, **2012**, 17, 6962 – 6974.
2. Ranjanm A.; Ichihashi, Y.; Sinha, NR. The tomato genome: implications for plant breeding, genomics and evolution. *Genome biology*, **2012**, 13, 1.
3. Zoran, SI, NikolasK, Ljubomir, S.; Dragoljub, B.; Natasa, M. Heavy metals and nitrate contents in tomato fruit grown in organic and conventional production systems. P*oland Journal of Envr. Stu.*, **2014**, 23, 2027-2032.
4. G.; Brhane, K.; Belay, K.; Gebremedhin, T.; Abraha, T.; Aleymayehu, T.; Mezegebe and M.; His, *ISSN*, 2351-8014, Vol, 10, No. 1 Oct **2014**, pp. 246-254.
5. R. k.; Sharma, M.; Agrawa land F.; M.; Marshal, Heavy metal in vegetable collected from production and market sites of a tropical urban area of India, *J, Food and Chem. Toxico*, 47, 2009, 583-591.
6. M. A.; Radwan and A. K.; Salama, Market basket survey for some heavy metals in Egyptian fruits and vegetables, *Food and Che, Toxicol*, **2006**, 44, 1273 -1278.
7. S.R.; Khan, Farooq, S.; Shahbaz, M. A.; Khan, and M.; Sadique, Health risk assessment of heavy metals for population via consumption of vegetables. *J. World App, Scie*, 6, 1, **2009**, 1602-1606.
8. Fernanda, C. B.; Geysa B. B.; Isa, S.B.; Leonardo, S. G. T.; Maria, G. A. K. Determination of trace element concentrations in tomato samples at different stages of maturation by ICP O-ES and ICP-MS following micro wave-assisted digestion. *chemical*, **2013**, 109**,**145-149.
9. Kelly, D. S,; Bateman, A. S. Comparison of mineral concentrations in commercially grown organic and conventional crops tomatoes (Lycopersicon esculentum and lettuces Lactuca sativa). *Journal of experimental Food Chemistry*, **2010**, 119, 738-745.
10. Ozcan, M. Determination of mineral contents of Turkish herbal tea (*Salviaaucheri Var. concerns*) at different infusion periods. *J. Med. Food*, **2005,** 8: 110-112.
11. J. SHI, M.; LE MAGUER, Lycopene in tomatoes: chemical and physical properties affected by food processing. *Crit Re. Biotechnol*, **2000**, 20 (4), 293-334.

12. Soroka, W. Fundamentals of Packaging Technology, 3rd edition. *Inst of Packaging Professio nals* **2002.**

13. Robertson G. Food Packaging: Principles and Practice, *Third Edition, 3rd edn. CRC Press* **2012.**

14. Yamaguchi M. World Vegetables: *Principles, production and Nutritive Values*, **2012**.

15. Peet, M. Irrigation and fertilization. In: Heuvelink E, (ed) tomatoes. CABI, *Wallingford*, **2005**, pp, 171–198

16. Frieden E. The chemical elements of life. *Sci, Am*, **1972**, 227, 52-60

17. Frieden E. New perspectives on the essential trace elements. *J.Chem Educ*; **1985**, 62, 917-23.

18. Matthew, M. M. R.; Henke and A.; Atwood, Effectiveness of commercial metal chelators wi-th new insights for the future in chelate design. *J.Hazar. Mater*., **2002**. 92(2), 129 142

19. Srikanth, R. A. M.; Rao, C. H. S.; Kumar and A.; Khanum, Lead, cadmium, nickel, and zinc contamination of ground water around Hussain Sagar Lake, Hyderabad, India. Bull. *Environ. Contam. Toxicol*. **2004,** 50(1), 138-143.

20. Dowdy, RH.; Larson, WE. *The availability of sludge borne metals to vegetable crops quality* **1995,** 4, 278-282.

21. Calderon, J,; Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, et al. Exposure to arsenic and lead and neuropsychological development in Mexican children. *Environ*. **2000** Res. (Sec. A) 85:69-76

22. Türkdogan, M. K.; FevziK, KazimK, Ilyas, T. and Ismail U. Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. *Environmental and Pharmacology*, **2002,** 13, 175–179.

23. Llobet, J.,M., G.; Falco, C.; Casas, A.; Teixido and J. L.; Domingo, Concentrations of arsen-ic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain, J.; Agric, *Food Chem*., **2003,** 51:838 - 842.

24. Tomlins, K.; & Johnson, P.N. Developing food safety strategies and procedure through redu ction of food hazards in street-vended foods to improve food security for consumers, street food vendors, and in put suppliers. *Crop Post Harvest Programme (CPHP) Project Funded by the DFID,* **2004,** R 8270

25. KwofieS, Chandler H.D. Potential health effects of locally manufactured corn- mill grindi nding plates, *JSci-Tech*, **2006**, 26(2), p. 137-147.

26. JEF**C, A.** Evaluation of certain food additives and contaminants (*Sixty-firs report of the Joint FAO/WHO. Expert Committee on Food Additives*). WHO Technical Report Series, **2004,** No. 922.

27. Extox Net. Cadmium contamination of food, Available from http: //ace. orst. edu/ Info/ *exto xnet/ faqs* /*food on/ cadmium, htm,* **2003.**

28. Figueroa, E. Are more restrictive food cadmium standards justifiable health safety measure s or opportunistic barriers to trade? An answer from economics and public health. *Science of the Total Environment*, **2008,** 389, 1-9

29. Castro-González, M. I.; Méndez-Armenta, M. Heavy metals: implications associated with fish consumption. *Environmental and Pharmacology,* **2008,** 26, 263–271.

30. Zhang, I.; & Wong, M. H. Environmental mercury contamination in China: sources and impacts. *Environment International,* **2007,** 33, 108-121

31. Bernard **A**. Cadmium and its adverse effects on human health, *Indian Journal of Medical Research,* **2008,** 128, 557–564.

32. Nordberg, G. F. Biomarkers of exposure, effects, and susceptibility in humans and theira pplicationin studies of interactions among metals in China. *Toxicology Letters*, **2007,** 192, 45-49.

33. Godt, J. F.; Scheidig, C. Grosse-Siestrup, V.; Esche, P.; Brandenburg, A.; Reich and A.; Groneberg, The toxicity of cadmium and resulting hazards for human health. *J. Occup.Med . Toxicol*, **2006,** 1, 22-23**.**

34. EFSA**,** European Food Safety Authority. Scientific Opinion of the Panel on contaminants in the, food Chain on are quest from the European Commission on cadmium in food. *EFSAJ,* **2009b**, 980.

35. Kocak, M. E.; Akc, The effects of chronic cadmium toxicity on the hemostatic system. *P athophy, Haem, Thromb*, **2006,** 35, 411-416.

36. Howard, H. Human health and heavy metals exposure, In Life Support: *The Environment and Human Health, McCally, M. (ed.). MIT Press,* **2002** pp: 1-13.

37. Dunham, C.L. Biologic Effect of Atmospheric Pollutants. Lead, airborne lead in perspective *National Academy of Sciences*. *Washington DC*, **1972,** pp. 213.

38. Oehlenschläger, J. Identifying heavy metals in fish In Safety and Quality issues in fish processing, Bremner, H. A. (Ed), pp. 95-113*, Wood head Publishing Limited*, **2002***,* 978-1845, 69 -019-9, Cambridge.

39**.** Harrison, N. Inorganic contaminants in food, In Food Chemical Safety Contaminants, Wats on, D.H. (Ed.), pp. 148-168, Ltd, first Edition, *Wood head Publishing ISBN*, **2001,** 1-85573-462-1, Cambridge.

40. Ming, Ho. Y. Environmental Toxicology: Biological and Health Effects of Pollutants, Chap. 12, *CRC Press LLC*, ISBN, 1-56670-670-2, 2nd Edition, *Boca Raton, USA*, **2005.**

41. ATSDR, Toxicological profile for lead US Department of Health and Human Services, public Health Service, *Agency for Toxic Substances and Disease Registry, Atlanta,* **2007b,** GA. 63.

42. WHO, Evaluation of certain food additives and contaminants. Sixty-first report of the Joint FAO/WHO, Expert, Committee on. *Food Additives, WHO, Technical Report Series* **2004a, 9**, 22.

43. USDA, Food Safety and Inspection Service. *Revised action plan for control of Listeria mono cytogenes for the prevention of food borne listeriosis,* **2002**

44. Recent Advances in Understanding Potassium Metal Anodes. *Journal, the Electrochemical Society* **2022,** *169* (3), 030510.

45. Kadir, S. A. Fruit quality at harvest of Jonathan apple treated with foliar applied calcium chloride. *Journal of Plant Nutrition,* **2004**, 27, 1991-2006.

46. Morarad, P. A.; Pujos, A. growth and mineral composition. *Journal Plant maturation,* 19, 115-127.

47. Jacques Guerin, Toxicity and Health Effects of Chromium (*All Oxidation States*) **2004,** L16 08_C06, pp, 213.

48. Gombas, D. E.; Chen, Y.; Clavero, R. S. and Scott, V. N: Survey of Listeria monocytogenes in ready-to-eat foods. *Journal of Food Protection* **2003,** 66, (4), 559-569.

49. Lopez, F. F.; Cabrera, C.; Lorenzo, M. L. and Lopez, M.C. Aluminum contents of drinking waters, fruit juices, and soft drinks: contribution to dietary intake. *Sci. Total Environ,* **2002,** 292, 205-213

50. Lemma, D. Tomatoes research experiences and production prospects. *Research Report, N EARO, Ethiopia,* **2002**, 5.

51. Maria, B: Sample digestion methods for determination of traces of precious metals by spectr-ometric techniques, *Anal. Sci,* **2002,** 18, 737- 750.

52. "*Methods for Chemical Analysis of Municipal and Industrial Wastewater*," EPA-600/4-82-057, July **1982**, EMSL, Cincinnati, OH 45268

53. Luis, G.; Hernandez, c.; Gonzalez-Weller, D.; Gutierrez, A.; Revert, C.; Hardisson, A. Trace element and toxic metals in intensively produced tomatoes (*lycopersicom esculentum) Nutr-. ation of Hospital.* **2012**, 27 (5), 1605-1609.

54. Gebre, A,; Singh, B. C. Level of essential and non-essential metals in gesho (*Rhamnus Prio-ides) cultivated in Ethiopia. Bulletion of chemical society of Ethiopia*, **2011**, 26, 329-242

55. Almeida A. M.; Castel-Branco, M. M,; Falcao, A.C. Linear regression for calibration lines revisited: Weighting schemes for bio analytical methods. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*. **2002**, 774(2), 215-222

56. Morard, P.; Pujos, A.; Bernadac, A.; Bertoni, G, Effect of temporary calic deficiency toma-to growth and mineral nutrition. *Journal of Plant Nutrition*, *19*, *Analytical Chemistry***. 2014,** 4, 1, 202-216

57. Gundersen, V.; McCall, D.; Bechmann, I. E: Comparison of major and trace element concentrations in Danish Green house tomatoes (*Lycopersiconesculentum*cv. Aromata, F1) cultivated in different substrates. *Journal of Food Chemistry, 49*, **2001**, pp, 3808–3815. DOI: 10.1021/ 0103774.

58. Gore, A.; Chandravanshi, B. S. T. W. Godeto, Metalin g reen coffee beans from the major coffee-growing region of Ethiopia. *Chemistry International*, **2017**, 3, 359-369.

59. Lugwisha, E. H.; Othman, C. O. Heavy metal Levels in soil, Tomatoes and Selected Veg-eetables from Morogoro Region, Tanzania. *Journal of Environmental Monitoring and analysis,* **2016**, 4(3), 82-88.

60. So, S.; Abdel G. Concentrations of heavy metals in water, sediment, and mollusk Gas tropod, Lanistes carinatus form Lake Manzala, Egypt. *Egyptian Aquatic Journal Research*, **2018**, 44,77-82.

61. Josu, T. M.; Hector, M.; Ziortza, G.; Alberto, D.; Juan, M. M. Uptake of metals by tomato pl-ants (*Solanum Lycopersicum*) and distribution inside the plant, *Journal of Food Composition* , **2017**, 59, 161-169.

62. Manan, A.; YingHui, C.; Xinxin, G.; Xing-Min, Z. Micro wave-assisted digestion followed by ICP-MS for determination of trace metals in atmospheric and Lake Ecosystem. *Journal of Environmental*, **2017**, 55, 1-10

7. APPENDICES

Table 9: Independent t-test for the K, Ca, and Cd concentration of Processed and Unprocessed tomatoes

Metals	Group	N	Mean	SD	T- test	P- value
K	Processed tomato	3	21.32	0.42	3.648	0.22
	Unprocessed tomato	3	20.23	0.28		
Ca	Processed tomato	3	1.92	0.01	-1.223	0.289
	Unprocessed tomato	3	2.02	0.01		
Cd	Processed tomato	3	0.098	0.001	2.214	0.091
	Unprocessed tomato	3	0.072	0.001		

7.1. CALIBRATION CURVE OF FOUR METALS

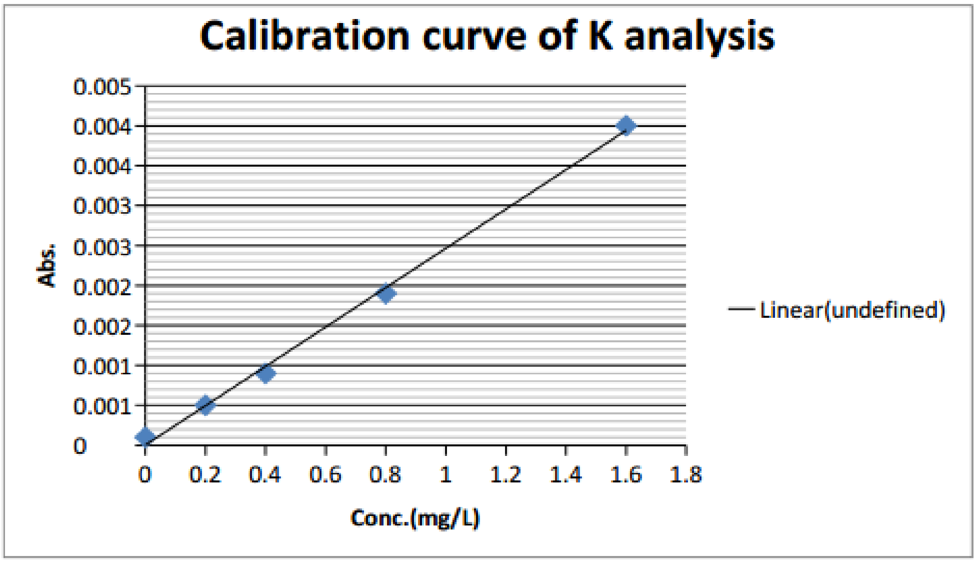

Figure 1: Calibration curve of K.

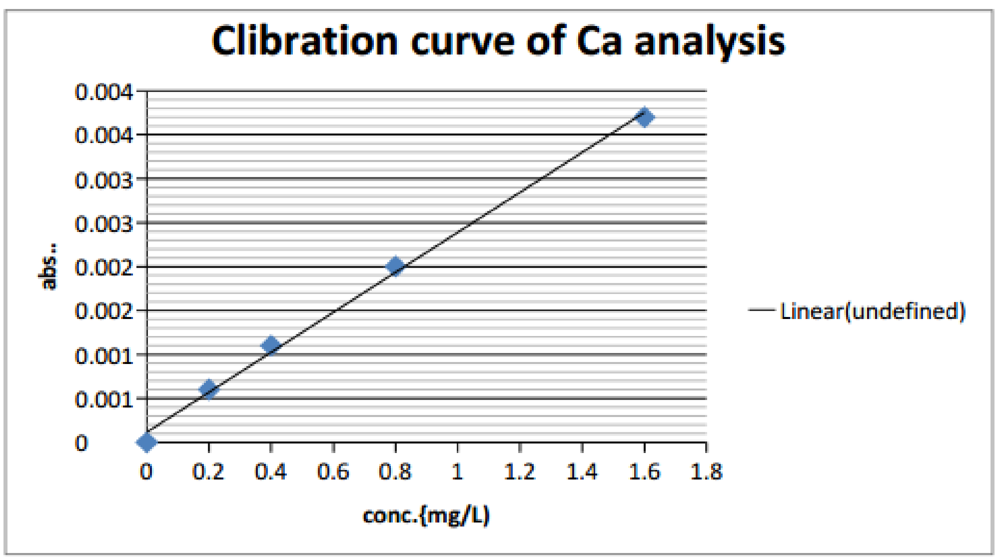

Figure 2: Calibration curve of Ca

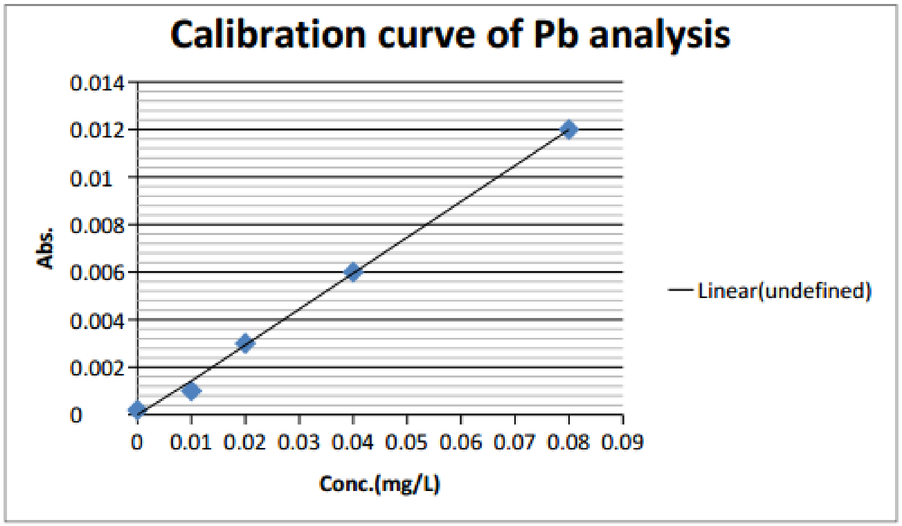

Figure 3: Calibration curve of Pb

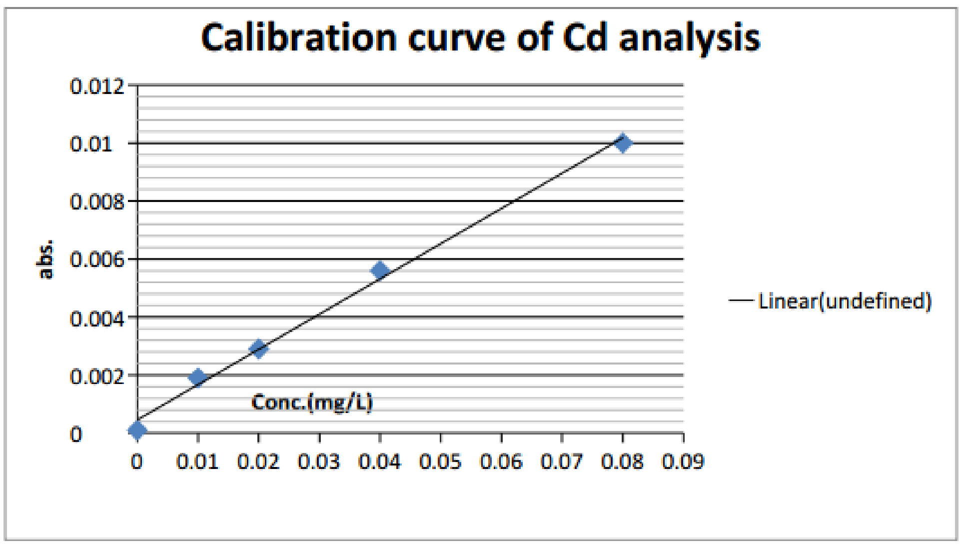

Figure 4; Calibration curve of Cd