LEVELS OF IRON, ZINC, CADMIUM AND LEAD METALS IN SELECTED FRESH AND CANNED TOMATO PASTE PRODUCED IN ETHIOPIA

MSc GRADUATE PROJECT

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Levels of Iron, Zinc, Cadmium and Lead Metals in Selected Fresh and Canned Tomato Paste Produced in Ethiopia

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POSTGRADUATE PROGRAM DIRECTORATE

I hereby certify that I have read and evaluated this Graduate Project entitled "Levels of Iron, Zinc, Cadmium and Lead metals in selected fresh and canned tomato paste produced in Ethiopia" prepared under my guidance by Jemaneh Megersa. I recommend that it be submitted as fulfilling the Graduate Project requirement.

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As a member of the Board of Examiners of the MSc Graduate Project Open Defense Examination, I certify that I have read and evaluated the Graduate Project prepared by Jemaneh Megersa and examined the candidate. I recommend that the Graduate Project be accepted as fulfilling the Graduate Project requirements for the degree of Master of Science in Analytical Chemistry.

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BIOGRAPHICAL SKELCH

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ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
ETFRUT	Ethiopian Fruit and Vegetable Marketing Enterprise
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agriculture Organization
WHO	World Health Organization

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ABSTRACT

In the present study, levels of selected heavy metals including Iron, Zinc, Cadmium, and Lead in fresh and canned tomato paste from two Ethiopian Tomato Processing Factories were determined using Atomic absorption spectrometry. The performance of the acid digestion procedure used in this study was evaluated utilizing recovery test and recoveries in the range of 93.29-99.33% were obtaind. In the fresh tomato, concentrations of Fe, Zn, Cd and Pb were found to be in the ranges of 3.01-3.23, 2.44-2.59, 2.19-2.63, and 10.42-14.99 mg/kg, respectively. Similarly, in the canned tomato paste, concentrations of Fe, Zn, Cd and Pb were found to be in the ranges of 3.28-3.48, 2.97-3.25, 2.58-2.80, and 12.33-13.85 mg/kg, respectively. The values obtained in this study were compared with international standards and it was observed that, the concentrations of Iron and Zinc in both fresh and canned tomato samples were not beyond the acceptable limits of these metals set by World Health Organization (WHO). However, the concentrations of Cadmium and Lead were found to be above the recommended limits for each metal. Based on the findings of the present study, the sources responsible for the observed high concentrations of Cadmium and Lead need to be given due consideration so as to protect the consumers from adverse health effects by the metals.

KEYWORDS: Canned Tomato, Fresh Tomato, Heavy metals

1. INTRODUCTION

1.1. Background

Tomato is one of the most important vegetables for the food industry. Consumption of its product is large and widely included in human diet. The commercial tomato belongs to the genus *Lycopersicon*. It is a relatively small genus within the large and diverse botanic family Solanaceae. The genus is currently thought to consist of the cultivated tomato a species most frequently referred to as, *Lycopersicon esculentum* (Attia *et al.,* 2000). The tomato is grown worldwide for its edible fruits with varying fruit types (FAO, 2001). Tomatoes contribute to a healthy well balanced diet. They are rich in vitamins, minerals and proteins (Attia *et al.,* 2000). Tomatoes contain lycopene, one of the most powerful natural antioxidants which have been found to help prevent prostate cancer. Tomatoes and tomato products are used as ingredients in many traditional dishes, because of the compatibility with other food ingredients due to the concentration and availability of several nutrients in these products and to their wide spread consumption by humans all over the world (OECD, 2008).

Metals like Iron, copper; Zinc, cobalt and manganese are essential metals since they play an important role in biological systems; whereas mercury, lead, cadmium, etc are non-essential metals which can be toxic even in trace amounts. The essential metals can also have harmful effects when their intakes exceed the recommended quantities significantly (Soylak *et al.,* 2004). However, heavy metals are one of range of contaminants that can be found on the surface and in the tissue of fresh tomatoes as well as processed tomato products. The term heavy metals refer to any metallic element that has relatively high density and is toxic or poisonous at low concentrations. As a result heavy metal accumulation in tomato and processes tomato products may pose a direct threat to human health (Turkdogan *et al.,* 2006).

Tomato processing is in its infancy stage in Ethiopia. At present, many private commercial farms and small-holding farmers are producing these tomatoes both for domestic and export markets. Currently there are three privately owned tomato processing plants in the country, Namely, Gonder food processing factory located in North Gondar Zone of Amhara Regional State , Melge Wendo food processing factory located in Sidama Zone of Southern, Nations, Nationalities

Peoples' Regional State and the other one is Merti processing factory located in Arsi zone Oromia National Regional State. The daily processing capacities of the factories range from 1250 to 8000 cans (EFDRE, 2015). These processing plants are dependent on their farmland, most of which is irrigated, for the supply of raw materials (Dendena, 2005).

Different techniques can be used in the determination of metals in fresh tomato and tomato paste. Among them, atomic absorption spectrometry (AAS) based procedures, for instance flame atomic absorbation spectrometry (FAAS) (Turker and Yuksel, 1997) is the most frequently used. Because of rapidity and reproducible determination at trace to ultra-trace concentrations an electranalytical techniques such as differential pulse polarography (DPP) have been used for the measurement of elements (such as Cd, Pb,Cu and Zn) in low levels (Kocak, 2007) while cyclic voltammetry has been applied when the elements are present at higher levels (Davies and Hobson,1981).

Several authors have proposed acid digestion, using different reagents, for the pre-treatment of fresh tomato and tomato paste sample. David *et al.* (2008) proposed an acid digestion procedure in a MWS-2 Berghof mineralization and digestion system with HNO_3 (65%) in order to analyze tomato paste samples. Turker and Yukel (1997) have studied the effectiveness of various acid digestion procedures for tomato paste samples in Kjeldahl digestion flask; they reached the conclusion that the wet ashing procedure involving 15 mL of HNO_3 and 2 mLof H_2O_2 (30%) was the most suitable for digestion of tomato paste. Waheed *et al.* (2003) in a study of selected essential and non-essential metals in various canned and raw food stuffs consumed in Pakistan, proposed an acid digestion procedure in an open beaker with 50 mL of HNO_3 (70%) under controlled heating. Heavy metals have recently received the attention of researchers all over the world, mainly due to their harmful effects on plant and human beings. The toxic effects of heavy metals in different crops may differ significantly (Leon *et al.,* 2002).

The author present the research due to tomato and tomato products are widely used as food in all the country and the availability of tomatos as a food are no more considered about heavy metals it contain either the negative effect or not. Hence, it has become necessary to know about the levels of heavy metals in fresh and canned tomato by comparing with each other and with the World Health organization to address the main risk factors it follows for other researcher.

Both plants use fertilizer and agricultural chemicals for the production of fresh tomatoes. In this study, the levels of Iron, Zinc, Cadmium and Lead in fresh tomato and in tomato canned paste and also the relative content of fresh tomato and canned tomato paste can be reported.

Taking all this in to account, the levels of essential and toxic metals in canned tomato paste produced by commercial canning plants in Ethiopia is studied. However, evaluation of Iron, Zinc, Cadmium and Lead in a fresh tomato and canned tomato paste with relative contents and their concentration is not included. The aim of this work is to investigate the levels of Iron, Zinc, Cadmium and Lead metals in fresh tomato and canned tomato paste, which are available in local markets and consumed as a part of food.

1.2. Objectives

The general objective of this study is to determine the levels of Iron, Zinc, Cadmium and Lead metals in fresh and canned tomato paste produced by commercial canning plants in Ethiopia.

The specific objectives of this study is to:

- To determine the concentration of Iron, Zinc, Cadmium and Lead metals in samples of fresh and canned tomato pastes.

2. LITERATURE REVIEW

2.1. Tomatoes

Tomatoes are grown for their excellent source of many nutrients and secondary metabolites that are important for human health: mineral matter, vitamins C and E, flavonoids, organic acids, phenolics and chlorophyll. Tomatoes also consist of β-carotene and lycopene, which related epidemiologically to a lower incidence of cardiovascular disease and of prostate, gastrointestinal and epithelial cell cancer (Rao *et al.,* 2007). Heavy metal and nutritive contents of tomatoes depend on growing conditions. Compared to crops grown using conventional and organic methods, organic tomatoes contained more salicylic acid but less vitamin C and lycopene. Organic tomatoes had higher Cd and Pb levels but a lower Cu content. Organic fruits had slightly higher protein content than conventionally cultivated fruits but the difference was minimal and consequently the significance was poor (Rossi *et al.,* 2008). The concentration of heavy metals in tomato edible and non edible parts is directly associated with their concentration in the soil. However the level significantly differs depending on the plant species and also on the genotypes within the same species. (Kabata and Pendias, 2001). Many other factors like climate soil pH, organic matter content, atmospheric deposition, nature of soil, degree of plant maturity may be responsible for the bio-accumulation of heavy metals (Naser *et al.,* 2011).

2.2. Composition of Whole Tomato

The tomato fruit comprises skin, pericarp and locular contents. The locular cavities are filled with jelly-like parenchyma cells that surround the seeds. The cell walls are composed of α-cellulose, pectins, hemicelluloses and some protein (Macrae, 1993). Tomato-based products consist mainly of disintegrated cells of the pericarp suspended in a clear serum. Of the 5 to 10% dry matter in the whole ripe fruit, about 75% is soluble (Hewitt and Garvey, 1987). Such a wide variation in the dry matter content is due to tomato variety, the nature of the soil in which the tomato is grown, and the amount of rainfall during the growing and harvesting season (Gould, 1983).

Nearly half the total dry matter consists of the reducing sugars glucose and fructose, about 10% is organic acids, principally citric and malic acids, about 1% is skin and seeds, with the remainder being alcohol insoluble solids (cellulose, pectins, hemicelluloses and proteins), minerals (mainly potassium), pigments, vitamins and lipids (Petro-Turza, 1987). The organic acid content is responsible for a pH between 4.2 and 4.6. Glutamic acid is the principal amino acid found in tomato (Lamb, 1977). Lycopene is a red plant pigment found in tomatoes, apricots, guavas, watermelons, papayas, pink grape fruits and rosehips, with tomatoes being the largest contributor to the dietary intake of humans. Lycopene is not an essential nutrient for humans, but is commonly found in the diet, mainly from dishes prepared with tomato sauce. When absorbed from the stomach, lycopene is transported in the blood by various lipoproteins and accumulates in the liver, adrenal glands and testes. (Chalabi *et al.,* 2004)

According to Radwan and Salama (2006) who carried out similar studies in Egyptain fruits and vegetables, they reported that heavy metals in tomatoes consist of Pb, Cd, Cu and Zn were 0.26 ± 0.09 mg/kg, 0.01 ± 0.00 mg/kg, 1.83 ± 0.01 mg/kg and 7.69 ± 0.91 mg/kg.

2.3. Heavy Metals

According to Zaidi *et al.* (2005) heavy metals are the major contaminants of food supply. The heavy metal levels in the plants are hardly affected by pH and organic matter content of the soil. The main source of heavy metal pollutants is the use of various agrochemicals, fertilizers and pesticides (Yang *et al.,* 2005). Some of fertilizers and pesticides contain heavy metals such as Cd, Hg, Pb, and Zn (Pendias, 1992). Food contamination by heavy metals has become a burning issue in recent years because of their potential accumulation in bio-systems through contaminated water, food and soil. Although, heavy metals normally occurring in nature may not be harmful, since they are only present in very small amounts, elevated levels of these metals can show negative effects. The subject of heavy metals is receiving increasing scrutiny in food industry due to increasing incidents of contamination in agriculture and seafood sources (Al-Thagafi *et al*., 2014). Some of the heavy metals like zinc, manganese, nickel, copper, and iron act as micronutrients at lower concentrations but become toxic at higher concentrations. Bioavailability of lead, cadmium, copper and zinc in the human gastrointestinal tract from edible parts of vegetables were also assessed earlier. The levels of heavy metals in analyzed vegetables lower than the amounts recorded in the soils in which the crops were grown because only limited quantities of metals are absorbed into the upper parts of the plants by roots that act as a barrier to the translocation of metals within the plant (David *et al.,* 2008).

2.3.1. Iron (Fe)

Iron is a necessary trace element found in nearly all living organisms and is an essential part of hemoglobin. Iron plays many key roles in biological systems, including oxygen transport (hemoglobin and myoglobin), respiration and energy metabolism (cytochromes and iron-sulfure proteins), destruction of hydrogen peroxides (hydrogen peroxidase and catalase) and DNA synthesis (ribonucleotidereductase). The deficiency of iron is one of the leading risk factors for disability and death worldwide. It results in anemia which is recognized by its symptom such as low blood Iron level, small red blood cells and low blood hemoglobin values (Underwood, 1977).

2.3.2. Zinc (Zn)

Zinc is one of the important metals for normal growth and development in human beings. Deficiency of zinc can result from inadequate dietary intake and results in impaired absorption, excessive excretion defects in zinc metabolism (Colak *et al.,* 2005). Maximum zinc tolerance for human health has been established for edible part of crops is 20 mg/kg by WHO maximum permitted level for zinc in vegetables is 100 mg/kg (WHO, 2007). The symptoms that an acute Orel zinc dose may include: tachycardia, vascular shock, dyspeptic nausea, vomiting, diarrhea, pancreatic and damage of hepatic parenchyma (Salgueiro *et al.,* 2000).

Heavy metals may be present in canned tomato paste through uptake by plants from contaminated soil, from polluted water or from applied agrochemicals. Harvested fruits may also became contaminated during canning process or via teaching from the metal containers into the canned produced during storage (Nincevic *et al.,* 2009). Heavy metals may also contaminate processed tomato paste through the addition of preservatives, stabilizers and synthetic coloring agents (Oduaoza, 1992). Other sources of these metals are natural occurrence such as earthquake, volcanic eruption and agricultural activities (pesticides, herbicides etc) are contributing factors

(Ademoroti,1994). Copper and zinc are the key components of many enzymes and vital elements for humans, animals, plants and microorganisms. However, as for all essential elements, their excess or deficiency can result in the emergency of chronic or acute disorders. Therefore, the correct and precise determination of maximum level of metals in foods is very important. Hence, the need to determine and/or monitor the levels of essential and toxic heavy metals in domestic processed fruits and vegetables such as tomato paste become essential due to their beneficial or damaging effects. Moreover, it has become necessary to know about the concentration of essential and toxic heavy metals in canned tomato paste to ensure the quality of the product and to comply with quality and specification standards developed by the codex committee on processed fruits and vegetables (Reilly, 2002).

2.3.3. Cadmium (Cd)

Cadmium is present at low levels in most foods, with commodities such as cereals, fruit, vegetables, meat and fish making the largest contribution to dietary exposure, given the fact that they are also the foodstuffs consumed in largest amounts. Highest levels of cadmium are found in the offal (kidney and liver) of mammals and in mussels, oysters and scallops. Certain wild mushrooms may also contain high levels, as can rice grown in certain geological areas where the soil is rich in cadmium. These foodstuffs are however minor contributors to overall intake of cadmium.The principal toxic effect of cadmium is its toxicity to the kidney, although it has also been associated with lung damage and skeletal changes in occupationally exposed populations. Cadmium is relatively poorly absorbed into the body, but once absorbed is slowly excreted, like other metals, and accumulates in the kidney causing renal damage. The kidney of food animals is a major source of cadmium in the diet although lower levels are found in many foods (Rose *et al.,* 2001).

2.3.4. Lead (Pb)

As indicated, lead contamination of food arises as a result of environmental emissions, such as mining and the now diminished use of leaded petrol. Levels of lead in fruit and vegetables generally are stringently regulated. The toxic effects of lead have been on people exposed to lead in the course of their work. Short-term exposure to high levels of lead can cause brain damage, paralysis (lead palsy), anaemia and gastrointestinal symptoms. Longer-term exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system. The most critical effect of low-level lead exposure is on intellectual development in young children and, like mercury, lead crosses the placental barrier and accumulates in the fetus. Infants and young children are more vulnerable than adults to the toxic effects of lead, and they also absorb lead more readily. Even short-tem, low-level exposures of young children to lead is considered to have an effect on neurobehavioural development. Consumption of food containing lead is the major source of exposure for the general population (Rose *et al.,* 2001).

2.4. Tomato Production

2.4.1. Washing, Sorting and Trimming

In modern plants, tomatoes are washed in water tanks agitated with compressed air, followed by rinsing with high-pressure water sprays to remove spray residues, microorganisms, dirt, mold, fruity fly(eggs) and larvae adhering to the fruit. The wash water is chlorinated to 5 to 10 ppm to maintain sterility. Unfit whole fruit is picked out and discarded, while partly defective fruit is trimmed by hand. Sorters and trimmers remove off-color fruit or parts. They also trim rotten areas,mold portions, insect damage and sunscald (Gould, 1983). This is the final control point for ensuring a low mold count in the final product.

2.4.2. Breaking

In the process described by Moresi and Liverotti (1982) the washed tomatoes are chopped into small pieces by a rotary comb chopper, and the chopped tomatoes are pumped into a heat exchanger and preheated to either 60^{o}C for a ‘cold break’ or preheated to 90 to 95 ^{o}C and held for 1 to 2 min for a ‘hot break’. A ‘hot break’ juice has a high consistency due to better extraction of pectic substances and due to retention of pectin by denaturation of the enzymes that would have caused its breakdown. A ‘cold break’ juice has a low consistency due to the activity of pectolytic enzymes and gives greater serum separation (Shomer *et al.,* 1984).

2.4.3. Apparatus and Instruments

The heated tomato pulp is passed through two (or three) juice extractors to remove the skin and seeds, and to squeeze the juice out of the remaining pulp Juice extractors may be either of the screw type or paddle type; a screw-type extractor uses an expanding helical screw to subject the pulp to increasing pressure against a screen, whereas a paddle type extractor beats the pulp against a screen (Goose and Binstead, 1973). Two juice extractors of screen size 1.5 and 0.4mm, respectively. In the terminology of the tomato processor the first juice extractor is known as a 'pulper' and the second juice extractor is known as a 'finisher'. The yield of tomato juice from the juice extraction stage to be 95.0% . A further screw press may be added to extract more juice from the residue leaving the juice extractors (Moresi and Liverotti, 1982)

2.4.4. Concentration

Tomato juice is concentrated by evaporation under partial vacuum either in a batch or continuous process. In the traditional batch process, the evaporation may be entirely carried out in steam-jacketed vacuum pans (known as 'boules') fitted with agitators, or the juice may be preconcentrated in a tubular evaporator to about 12% solids before transfer to the boules. Continuous processes tend to produce a more consistent paste than batch processes (Goose and Binstead, 1973). Evaporation at low pressure reduces the boiling point of the juice so that the resulting paste retains most of its color and flavor (Gould, 1983).

2.4.5. Pasteurization

Continuous pasteurization of tomato paste at 90 to 92 ^{o}C, before it is canned, prevents subsequent spoilage by lactobacilli (Moresi and Liverotti, 1982). A gear pump transfers viscous tomato paste, a tubular heat exchanger for pasteurization, and a recirculation tube. The recirculation tube return the hot paste to the receiving tank if flow through the filling nozzles is restricted, to prevent 'burn-on' fouling of the heat exchanger and loss of product quality (Goose and Binstead, 1973).

2.4.6. Filling, Closing and Cooling

The pasteurized paste is automatically hot-filled into lacquered tin cans that have been pre-sterilized with steam (Moresi and liverotti, 1982). The cans are immediately seamed, inverted to sterilize the lids, and held for about 3 min prior to cooling. Air cooling simply requires that the cans be stacked in rows with air spaces in between to allow the passage of an air current (Goose and Binstead,1973).Water cooling involves agitating the cans for about 2 hours under a spay of atomized water that has been chlorinated to 15 ppm residual chlorine (Gould, 1983).

3. MATERIALS AND METHODS

3.1. Experimental Site

The study was conducted in the Chemistry Department Research Laboratory of Haramaya University. The major experimental works include sample preparation, digestion and elemental determination.

3.2. Sampe Collection

In this study two different brands of commercially available tomato paste and fresh tomato samples were used. The two types of tomato paste samples and fresh tomato samples considered in the present study were collected from Upper Awash Agro industry enterprise Merti processing plant, located at Wondo Genet and Sodere areas. Fresh tomato samples were obtained from Merti Agro Processing Share Company farm located at the two tomato processing factories in Wondo Genet and Sodere areas which make use of the raw tomato for canned tomato paste production. The tomato paste samples of 410 g of Merti were purchased from ETFRUT mini supermarket.

3.3. Chemicals and Reagents

Nitric acid (69-72%) and hydrogen peroxide (30%) were used for digestion of samples. Hydrated iron(II)nitrate ($Fe(NO_3)_2.H_2O$), Hydrated zinc nitrate ($ZnNO_3.6H_2O$), Cadmium nitrate ($Cd(NO_3)_2$) and Lead nitrate $Pb(NO_3)_2$ were used for preparation of stock standard solution of the metals Iron, Zinc, Cadmium and Lead. Deionized water was used throughout this work.

3.4. Apparatus and Instruments

Analytical digital balance was used to weigh fresh tomato and tomato paste samples. Micropipettes were used for measuring different amounts of acid mixtures and standard solutions. A 100 mL round bottom flask was used to keep the sample for digestion. A Kjeldahl digestion apparatus was used to heat the sample. A 50 mL volumetric flask was used to dilute digestion sample solutions and for preparation of standard solutions. A refrigerator was used to keep the digested samples until analysis. A flame atomic absorption spectrophotometer was used for determination of the concentration of metals in the sample solution using air-acetylene flame. A single element hollow cathode lamp of each element of interest was used as radiation sources. An electric blender was used for homogenization of the sample.

3.5. Methods and Procedures

3.5.1. Sample Preparation /Digestion Method/

The collected fresh raw tomatoes were first washed with tap water and rinsed with distilled water three times to remove surface pollutants. The washed samples were put into jars and dipped in boiling water for 60 seconds. Then dipped in cold water to remove cores. Any bruise or discolored portions were piked out The fresh tomatoes were added to beaker and the portions were crushed with a wooden mallet. Some juice and the contents were transferred to an electric blender for homogenization.

For the preparation of bulk samples from tomato pastes, two cans of each brand were opened with a can opener and the contents were transferred to an electric blender for homogenization. About 1g fresh tomato sample and 1g tomato paste sample were taken from the bulk samples and weighed in a plastic cup. The weighed samples were placed in a 100 mL round bottom flask. 4mL Nitric acid (69-72 %) was added to a round bottom flask containing samples and allowed to stand for 10 min. Then the round bottom flask containing samples were fitted with a condenser and heated in a Kjeldahl heating apparatus by setting the temperature first at 120^{o}C for 10 min, then at 270^{o}C for 50 min and continued heating at a temperature of 300^{o}C for 1 h. After a total of 2 h digestion, the sample was cooled for 10 min.and then 2mL of hydrogen peroxide (30 %) was added continued heating at a temperature of 300^{o}C for 1:30 h. After a total of 3:30 h digestion, the digested sample was allowed to cool for 10 min. The content of the flask was rinsed into a 50 mL volumetric flask and the volume was made up to the mark with deionized water. Fresh tomato and each tomato paste sample was digested in triplicate and hence a total of twelve digests were prepared for the fresh tomato and tomato paste samples. All the digested samples were stored in refrigerator, until the levels of all the metals in the sample solutions were determined by FAAS (Turker and Yuksel, 1997).

3.5.2. Preparation of Standard Solution

Four series of working standard solutions were prepared from the 10 mg/L intermediate standard solution of the respective metals, which were prepared by diluting the stock standard solutions of the metals with deionized water. The stock solution was prepared and the flask was shacked to ensure the proper mixing of the solution. Iron standard solution was prepared by dissolving 3.550 g of hydtated iron(II)nitrate ($Fe(NO_3)_2.H_2O$) in distilled water in 1 L volumetric flask upto the mark to obtain 1000 ppm. Zinc standard solution was prepared by dissolving 4.547 g of hydtated zinc nitrate ($Zn(NO_3)_2.6H_2O$) in distilled water in 1 L volumetric flask upto the mark to obtain 1000 ppm. Cadmium standard solution was prepared by dissolving 2.103 g of cadmium nitrate ($Cd(NO_3)_2$) in distilled water in 1 L volumetric flask upto the mark to obtain 1000 ppm. Lead standard solution was prepared by dissolving 2.165 g of lead nitrate ($Pb(NO_3)_2$) in distilled water in 1 L volumetric flask upto the mark to obtain 1000 ppm. Commercially available standard solutions for AAS were used for all metal standards. Working Standard solution was prepared for Flame analysis from dilute standard solutions with 0.1 M HNO_3 to a range of standards that covers the concentration of the elements to be determined. Five point calibrations were prepared for the prepared working standard solution using flame atomic absorption spectrophotometer (Chemiasoft, 2011). The atomic absorption spectrophotometer working conditions are given in table 1.

Table 1. Instrument operating condition for the determination of heavy metals in fresh and canned tomato paste sample by AAS.

Elements	**Wavelength (nm)**	**Current lamp (mA)**	**Slit width (nm)**	**Flame type**
Fe	248.3	7.0	0.2	Air-aceytlene
Zn	213.9	5.0	0.2	Air-acetylene
Cd	228.9	2.0	0.7	Air-acetylene
Pb	217.0	3.0	0.7	Air-acetylene

3.6. Calibration of the Instrument

Calibration curves were drawn to determine the concentration of metals in the sample solution. The calibration graphs and coefficient of determination of each element were determined by plotting absorbance versus concentration of the corresponding working standard. These curves were used to determine the concentration of metals in the sample solution (Matusiewicz and Kopras, 2009). The working standard solutions and the coefficient of determination of the curve for each of the metals are shown in table 2.

Table 2. Concentration of the standard solution used to establish calibration graph and their corresponding coefficient of determination.

Element	Concentration of standard (mg/L)	coefficient of determination	Equation of the line
Fe	2,4,6,8,10	0.987	Y= 0.001X + 0.001
Zn	2,4,6,8,10	0.986	Y= 0.001X + 0.006
Cd	2,4,6,8,10	0.982	Y= 0.002X + 0.005
Pb	2,4,6,8,10	0.945	Y= 0.001X

3.7. Analysis of Standards and Sample Solutions

Three readings were taken for each sample solution and reagent blank solution and the mean value of the concentration signal was used for subsequent calculations. Then the concentration (mg/Kg) of metals in the aliquot digestion was calculated using the following equation (Keran *et al.,* 2012).

$$C = (A/W)V$$

Where,

C = total metal concentration (mg/Kg)

V = final volume of the digestion sample solution (mL)

A = mg/mL of metal in digestion sample

W = weight of digestion sample (mg)

3.8. Detection Limit

Detection limit is the smallest concentration of analyte that was distinguished from statistical fluctuation in a blank, which usually corresponds to the standards of the blank solution times a constant. The detection limit is most commonly defined as the mass of analyte that gives a signal equal to three times the standard deviation of the blank. Three reagent blank (HNO_3, H_2O_2) samples were digested following the same procedure as the samples and each of the samples were determined for the element iron, zinc, cadmium and lead by the atomic absorption spectrophotometer. The standard deviation for each element was calculated from the three reagent blank measurements to determine detection limit (Butcher and Sneddon ,1998).

3.9. Recovery Test

To investigate the properness of the method employed in the present study, recovery test were performed for iron, zinc,cadmium and lead metals in selected samples by spiking the samples with known concentration of each metal separately. Accordingly, known amounts from stock solution (1000 mg/L) of each metal element was spiked on each 4 g tomatoe sample. The spiked and non-spiked samples were digested in parallel and concentration of each metal was determined in triplicate samples (experimental replicate) by taking three reading (instrumental replicate) for each metal. As used for original samples triplicate spiked samples were prepared and triplicate reading recorded (Gazso , 2001). The percentage recovery was then calculated using the following formula.

$$\textbf{Recovery (\%)} = \mathbf{(A\text{-}B)/C \times 100\%}$$

Where ,

A = mean result of spiked samples

B = mean result of unspiked samples

C = amount of added analyte.

3.10. Data Analysis

All the samples analyses in this study were carried out in triplicate and the results were reported as mean ± standard deviation. Statistical analyses were carried out using computer software. ANOVA was used for comparing mean concentration of heavy metals between the fresh and canned tomato paste.

4. RESULTS AND DISCUSSION

4.1. Method Validation

4.1.1. Recovery Test

As shown in table 3, the results of percentage recoveries for the studied metals were found to be in the range of 94.08-99.33 %. The results obtained revealed that the sample digestion procedure was reliable to be applied and was used for the digestion of the different tomato samples.

Table 3. Recovery tests for the method used for digestion of the tomato samples

Metal	Sample	Mean Unspiked Sample(mg/L)	Amont added (mg/L)	Mean Spiked Sample (mg/L)	(%) Recovery
Fe	Sodere fresh	3.01 ± 0.45	4	6.88 ± 0.60	96.87±3.94
	Sodere canned	3.28 ± 0.03	4	7.21 ± 0.08	98.24±1.47
	Wondo fresh	3.23 ± 0.28	4	7.14 ± 0.43	97.91±3.84
	Wondo canned	3.48 ± 0.36	4	7.40 ± 0.49	98.17±3.33
Zn	Sodere fresh	2.44 ± 0.30	4	6.20 ± 0.39	94.08±2.33
	Sodere canned	2.97± 0.15	4	6.85 ± 0.24	97.94±2.42
	Wondo fresh	2.59 ± 0.34	4	6.43 ± 0.39	96.01±1.41
	Wondo canned	3.25 ± 0.41	4	7.22 ± 0.49	99.33±2.03
Cd	Sodere fresh	2.19 ± 0.04	4	5.97 ± 0.11	94.66±1.83
	Sodere canned	2.58 ± 0.06	4	6.31 ± 0.14	93.29±2.07
	Wondo fresh	2.63 ± 0.08	4	6.46 ± 0.18	95.94±2.54
	Wondo canned	2.80 ± 0.14	4	6.66 ± 0.27	96.63±3.39
Pb	Sodere fresh	10.42 ± 0.93	4	14.42 ± 1.06	95.66±3.34
	Sodere canned	12.33 ± 0.53	4	16.12 ± 0.15	94.94±3.00
	Wondo fresh	14.99 ± 0.93	4	18.93 ± 1.06	98.53±3.39
	Wondo canned	13.85 ± 0.00	4	17.77 ± 0.08	98.09±2.23

4.1.2. Detection Limit

After digestion of each blank sample, several readings were taken by the instrument and the corresponding values of standared deviation were calculated. The method detection limit of each element was obtained by multiplying the standard deviation obtained above by three and the corresponding value for each element is presented in table 4.

Table 4. Instrument and method detection limits for the metals considered in the present study.

Metals	Instrument detection limit (mg/L)	Method detection limit for tomato sample (mg/L)
Fe	0.03	0.08
Zn	0.005	0.01
Cd	0.005	0.006
Pb	0.01	0.1

4.2. Concentration of Iron, Zinc, Cadmium and Lead in Fresh and Canned Tomato Paste

The tomato samples, both fresh and canned which were obtained from the two areas Sodere and Wondo, were analyzed for the levels of four selected heavy metals. In general, the results obtained in the present study revealed that, the concentrations of Iron and Zinc did not exceed the acceptable values of World Health Organization (WHO). However, the Cadmium and Lead levels in this work were found to be higher than the limit set by WHO. The concentration of each metal in both the fresh and canned tomato pastes are presented in table 5.

Table 5. The levels of metals concentration (mg/Kg) in fresh and canned tomato paste sample with their safe limit.

Element	Sodere Fresh	Sodere canned	Wondo fresh	Wondo canned	FAO/WHO Safe limit
Fe	3.01 ± 0.45^{a}	3.28 ± 0.03^{a}	3.23 ± 0.28^{a}	3.48 ± 0.36^{a}	425.5
Zn	2.44 ± 0.30^{a}	2.97 ± 0.15^{a}	2.59 ± 0.34^{a}	3.25 ± 0.41^{a}	99.4
Cd	2.19 ± 0.04^{a}	2.58 ± 0.06^{b}	2.63 ± 0.08^{b}	2.80 ± 0.14^{c}	0.2
Pb	10.42 ± 0.93^{a}	12.33 ± 0.53^{b}	14.99 ± 0.93^{c}	13.85 ± 0.00^{d}	0.3

Data in the same raw with similar superscript letter have no significant difference.

4.3. Comparison of Heavy Metal Concentrations in the Tomato Samples

Iron (Fe) is an essential element for human being and plays a very important role in the formation of haemoglobin which is needed for oxygen and electron transport in human body (Kalagbor and Diri, 2014). One-way ANOVA revealed that the concentrations of iron found in all the four samples were not significantly different ($p > 0.05$) from each other as indicated in Table 5 and Figure 1. The FAO/WHO (2007) maximum limit for Iron is 425.5 mg/Kg and the concentration of Iron determined in this study was much lower than the recommended limit.

Zinc is essential to all organisms and has an important role in metabolism, growth, development and general well being. It is an essential co-factor for a large number of enzymes in the body. Zinc deficiency leads to coronary heart diseases and various metabolic disorders (Saraf and Samant, 2013). Deficiency of zinc can also result from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in zinc metabolism (Ozturk *et al.,* 2011). The content of Zn reported in this study is generally lower than the permissible levels set by FAO/WHO (2007). One-way ANOVA revealed that there was no significant difference ($p > 0.05$) in levels of Zn in the tomatoes.

Cadmium is a highly toxic non-essential heavy metal and it does not have a role in biological process in living organisms. Thus even in low concentration, cadmium could be harmful to living organisms (Ambedkar and Muniyan, 2012). Cd poisoning in man could lead to anaemia, renal damage, bone disorder and cancer of the lungs (Edward *et al.,* 2013). The maximum concentration of cadmium was found in Wondo canned and the minimum concentration in the Sodere fresh tomato sample (Table 5 and Figure 1). Cadmium was found to be beyond the safe limit set by FAO/WHO (2007). One-way ANOVA revealed that there was no significant difference ($p > 0.05$) in levels of Cd in the tomatoe samples of Sodere canned and Wondo fresh. However, there was a significant differernce between the remaining samples.

There was a variation between the concentrations of lead found in the tomatoe samples considered in the present study. The maximum concentration of lead was found in Wondo canned and the minimum concentration was found in Sodere fresh tomato sample (Table 5 and Figure 1). The Pb contents of the tomatoes in this study were found to be higher when compared to the FAO/WHO (2007) safe limit of tomatoes. Furthermore, One-way ANOVA revealed that there were statistically significant differences ($P < 0.05$) between the levels of lead in all fresh and canned tomatoes. The levels of Lead obtained in the present study indicate a potential health hazard to consumers. Lead toxicity is known to cause musculo-skeletal, renal, ocular, neurological, immunological, reproductive and developmental effects (Ambedkar and Muniyan, 2012). As a result, the present study might be a clue for further research.

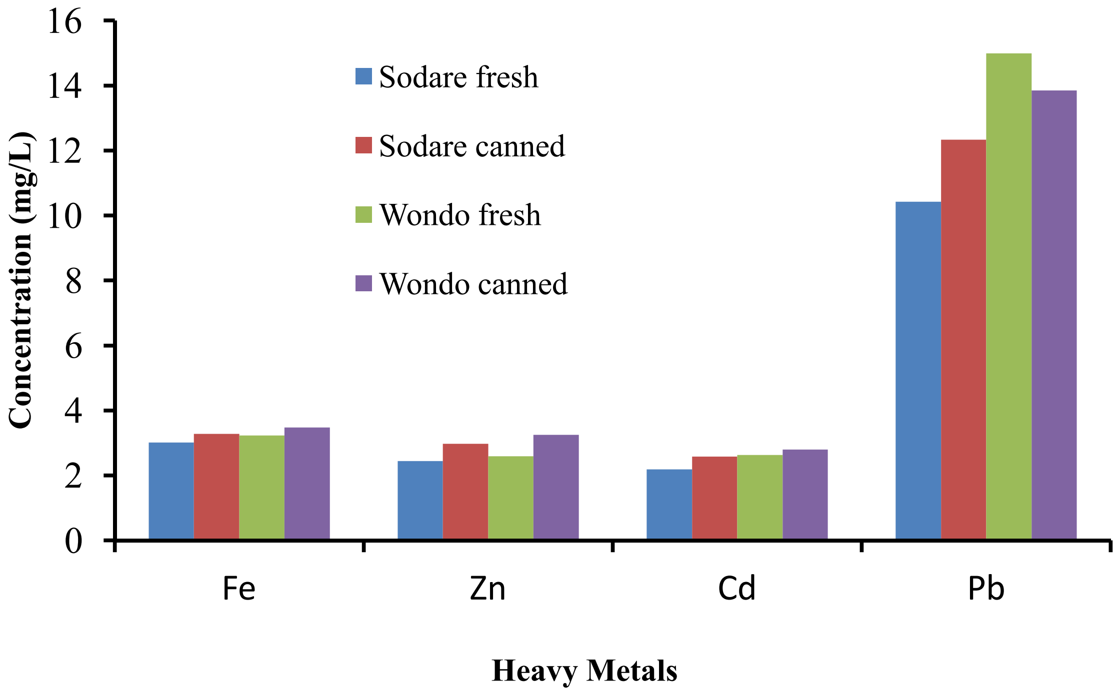

Figure 1. Comparison of metal concentrations obtained in fresh and canned tomato paste

4.4. Comparison of Metal Concentrations Obtained in Tomato Samples with other Similar Studies

Levels of Iron, Zinc, Cadmium and Lead metals are potential environmental contaminates with the capability of causing human health problems if present at any concentration level (Cd and Pb) or in excess (Fe and Zn) in the food we eat. The heavy metal concentrations obtained in the present study were compared with similar studies reported in the literature and are presented in table 6.

Iron concentration in canned tomato paste was studied and reported in the literature. Studies from different countries including Uysal *et al*. (2001) (Istanbul, Turkey); Makki and Ziarati. (2014) (Tehran, Iran) and Osma et al. (2012) (Istanbul, Turkey) reported higher concentration of Iron than this study. Similarly, concentration of iron in fresh tamato reported by Ali and Al-Qahtani, (2012) (Meka, Saudi Arbia) and Hashmi *et al*. (2007) (Karachi, Pakistan) were higher than the concentration observed in the present study. On the othe hand, the concentrations of Fe obtained in tomato fruits from different markets of Kano State, Nigeria (Akan *et al*., 2009) were very low when compared with the present findings.

Similarly, levels of Zinc in canned tomato pastes reported by Uysal *et al*. (2001) (Istanbul, Turkey); Makki and Ziarati. (2014) (Tehran, Iran) and Al-Thagafi *et al*.(2014-) in United State were higher than the concentration obtained in this study. The concentration of zinc reported by Osma *et al*. (2012) (Istanbul, Turkey) in canned tomato paste was comparable with the findings of the present study. On the other hands studies by Ali and Al-Qahtani, (2012) (Meka, Saudi Arbia); Hellen and Othman (2016); Hashmi *et al*. (2007) (Karachi, Pakistan); Demirezen and Aksoy (2006) (Kayseri, Turkey) and Akan *et al*.(2009) (Kano, Nigeria) reported concentration of Zinc lower than the present study.

The concentration of Cadmium obtained in the present study was also compared with other studies. In this regard, the report by Uysal *et al*. (2001) (Istanbul, Turkey) was found to exceed the concentration obtained in the present study. On the other hand, studies by (Hellen and Othman (2016); Al-Chaarai *et al.* (2009); Bhutto *et al.*(2010) and Demirezen and Aksoy (2006) (Kayseri, Turkey) reported lower concentration of Cd in fresh tomatoes than what was obtained in this study.

The concentration of lead reported in a study by Al-Thagafi *et al*.(2014) in United State was comparable with the value obtained for the canned tomato paste obtained in this study. On the other hand, Al-Thagafi *et al*. (2014) (Roma, Italy); Makki and Ziarati. (2014) (Tehran, Iran); Uysal *et al*.(2001) (Istanbul, Turkey) and Osma *et al*. (2012) (Istanbul, Turkey) reported lower concentration of lead in canned tomato paste than the present study. Similarly, Demirezen and Aksoy (2006) (Kayseri, Turkey); Akan *et al.* (2009) (Kano, Nigeria); Ali and Al-Qahtani (2012) (Kano, Nigeria); (Bhutto *et al.*(2010) and Al-Chaarai *et al.*(2009) reported lower lead levels in fresh tomato than the present study.

Table 6. Levels of metals in tomatoes previously published results from other parts of the world.

Tomato Type	Country	Fe (mg/Kg)	Zn (mg/Kg)	Cd (mg/Kg)	Pb (mg/Kg)	Reference
Canned	Turkey	64.40	39.19	5.12	5.31	Uysal *et al.,* 2001
Tomato Paste	Italy	-	18.60	1,00	5.4	Al-Thagafi *et al.,* 2014
	United State	-	32.00	1.60	13.80	Al-Thagafi *et al.,* 2014
	Iran	7.05297 ± 1.03	17.3112 ± 1.42	0.0419 ±0.45	0.0932 ±0.11	Makki and Ziarati, 2014
	Turkey	19.16-64.53	1.36-3.07	0.17-0.40	4.31-5.51	Osma *et al*., 2012
	Ethiopia (Sodere)	3.26-3.33	2.77-3.15	2.50-2.66	11.57-12.71	This study
	Ethiopia (Wondo)	3.00-3.86	2.8-3.80	2.66-3.03	13.85-13.85	This study
Fresh Tomato	Saudi Arabia	196.1 ± 1.20	22.44 ± 0.88	1.67 ± 0.09	3.32 ± 0.02	Ali and Al-Qahtani, 2012
	Tanzania	-	3.64	0.043	-	Hellen and Othman, 2016
	Pakistan	30.2	4.4	-	-	Hashmi *et al.,* 2007
	Lebanon	-	-	0.438	0.080	Al-Chaarai *et al.,* 2009
	Pakistan	-	-	0.071	0.009	Bhutto *et al.,*2010
	Poland	28	9.1	-	-	Bosiacki and Tyksinski, 2009
	Turkey	-	3.56-5.9	0.24-0.97	3-10.7	Demirezen and Aksoy, 2006
	Nigeria	-	8.93± 1.8	-	-	Abdullahi *et al*., 2008
	Nigeria	0.34-1	0.2-6.9	3-7	2.-4.55	Akan *et al.,* 2009
	India	-	41.46	-	3.55	Srinivas *et al*., 2009
	Ethiopia (Sodere)	2.53-3.60	2.06-2.80	2.16-2.25	9.28-11.57	This study
	Ethiopia (Wondo)	2.9-3.60	2.65-2.98	2.58-2.75	13.85-16.14	This study

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary and Conclusions

Consuming tomatoes contaminated with heavy metals has different detrimental effects on human health, therefore, monitoring contamination of heavy metals from different sources enables to take the proper measure and to avoid unnecessary exposures of the consumers. In the present study, the levels of selected heavy metals in fresh and canned tomatoe paste samples obtained from two different areas were studied. The results obtained revealed that concentrations of the heavy metals Fe, Zn, Cd and Pd in fresh as well as canned tomato paste showed some variation depending on their source. The concentrations of heavy metals in all samples determined were in the sequence Pb> Fe > Zn > Cd. Generally, the levels of Cadmium and Lead metals obtained in all tomatoes were not within the acceptable range and this might cause problem to the health of the consumers.

5.2. Recommendations

Based on the findings of the present study, the following recommendations are forwarded.

- Further studies are required on soil samples from the tomato farms, water used for irrigation of the farm lands, and the tomato processing materials is the possible sources of transfer of Cadmium and Lead to tomatoes.
- Similar studies on the concentration of heavy metals in fresh tomato samples from other parts of Ethiopia and other canned tomato paste factories are recommended.
- Other highly sensitive instruments like Inductively coupled plasma mass spectrometer (ICP-MS) and Graphite Furnace Atomic Absorption (GFAAS) could be used to see the levels of heavy metals in fresh and canned tomato paste.

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7. APPENDICES

7.1. List of Tables in the Appendix

Appendix Table 1. One way ANOVA for the concentration of Iron in tomato samples

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Sodere fresh	Sodere Canned	-.28000	.32263	.411	-1.0240	.4640
	Wondo fresh	-.23000	.32263	.496	-.9740	.5140
	Wondo Canned	-.48333	.32263	.172	-1.2273	.2606
Sodere Canned	Sodere fresh	.28000	.32263	.411	-.4640	1.0240
	Wondo fresh	.05000	.32263	.881	-.6940	.7940
	Wondo Canned	-.20333	.32263	.546	-.9473	.5406
Wondo fresh	Sodere fresh	.23000	.32263	.496	-.5140	.9740
	Sodere Canned	-.05000	.32263	.881	-.7940	.6940
	Wondo Canned	-.25333	.32263	.455	-.9973	.4906
Wondo Canned	Sodere fresh	.48333	.32263	.172	-.2606	1.2273
	Sodere Canned	.20333	.32263	.546	-.5406	.9473
	Wondo fresh	.25333	.32263	.455	-.4906	.9973

Appendix Table 2. One way ANOVA for the concentration of Zinc in tomato samples

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Sodere fresh	Sodere Canned	-.20000	.34058	.573	-.9854	.5854
	Wondo fresh	-.15333	.34058	.665	-.9387	.6320
	Wondo Canned	-.47667	.34058	.199	-1.2620	.3087
Sodere Canned	Sodere fresh	.20000	.34058	.573	-.5854	.9854
	Wondo fresh	.04667	.34058	.894	-.7387	.8320
	Wondo Canned	-.27667	.34058	.440	-1.0620	.5087
Wondo fresh	Sodere fresh	.15333	.34058	.665	-.6320	.9387
	Sodere Canned	-.04667	.34058	.894	-.8320	.7387
	Wondo Canned	-.32333	.34058	.370	-1.1087	.4620
Wondo Canned	Sodere fresh	.47667	.34058	.199	-.3087	1.2620
	Sodere Canned	.27667	.34058	.440	-.5087	1.0620
	Wondo fresh	.32333	.34058	.370	-.4620	1.1087

Appendix Table 3. Oneway ANOVA for the concentration of Cadmium in tomato samples

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Sodere fresh	Sodere Canned	-.39000*	.09107	.003	-.6000	-.1800
	Wondo fresh	-.44667*	.09107	.001	-.6567	-.2367
	Wondo Canned	-.61333*	.09107	.000	-.8233	-.4033
Sodere Canned	Sodere fresh	.39000*	.09107	.003	.1800	.6000
	Wondo fresh	-.05667	.09107	.551	-.2667	.1533
	Wondo Canned	-.22333*	.09107	.040	-.4333	-.0133
Wondo fresh	Sodere fresh	.44667*	.09107	.001	.2367	.6567
	Sodere Canned	.05667	.09107	.551	-.1533	.2667
	Wondo Canned	-.16667	.09107	.105	-.3767	.0433
Wondo Canned	Sodere fresh	.61333*	.09107	.000	.4033	.8233
	Sodere Canned	.22333*	.09107	.040	.0133	.4333
	Wondo fresh	.16667	.09107	.105	-.0433	.3767

*. The mean difference is significant at the 0.05 level.

Appendix Table 4. One way ANOVA for the concentration of Lead in tomato samples

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Sodere fresh	Sodere Canned	-1.90667*	.71359	.028	-3.5522	-.2611
	Wondo fresh	-4.57333*	.71359	.000	-6.2189	-2.9278
	Wondo Canned	-3.42667*	.71359	.001	-5.0722	-1.7811
Sodere Canned	Sodere fresh	1.90667*	.71359	.028	.2611	3.5522
	Wondo fresh	-2.66667*	.71359	.006	-4.3122	-1.0211
	Wondo Canned	-1.52000	.71359	.066	-3.1655	.1255
Wondo fresh	Sodere fresh	4.57333*	.71359	.000	2.9278	6.2189
	Sodere Canned	2.66667*	.71359	.006	1.0211	4.3122
	Wondo Canned	1.14667	.71359	.147	-.4989	2.7922
Wondo Canned	Sodere fresh	3.42667*	.71359	.001	1.7811	5.0722
	Sodere Canned	1.52000	.71359	.066	-.1255	3.1655
	Wondo fresh	-1.14667	.71359	.147	-2.7922	.4989

*. The mean difference is significant at the 0.05 level.

7.2. Appendix Figure

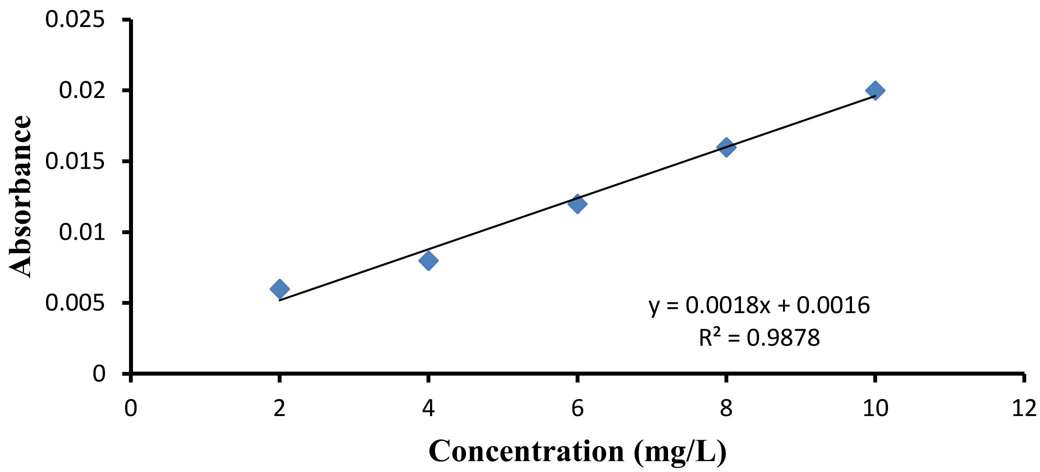

Figure 1. Calibration graph of the standard solution used for the determination of Iron

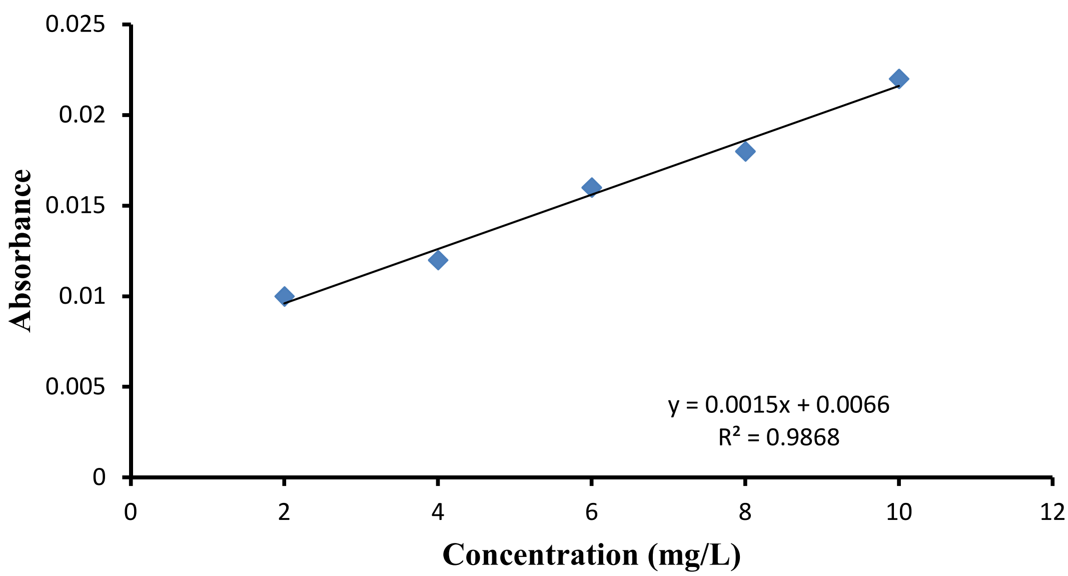

Figure 2. Calibration graph of the standard solution used for the determination of Zinc

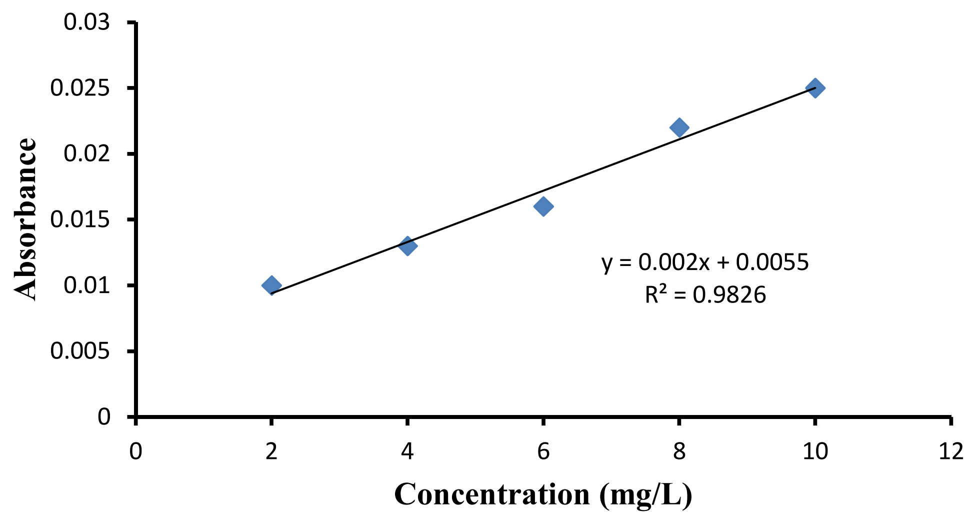

Figure 3. Calibration graph of the standard solution used for the determination of Cadmium

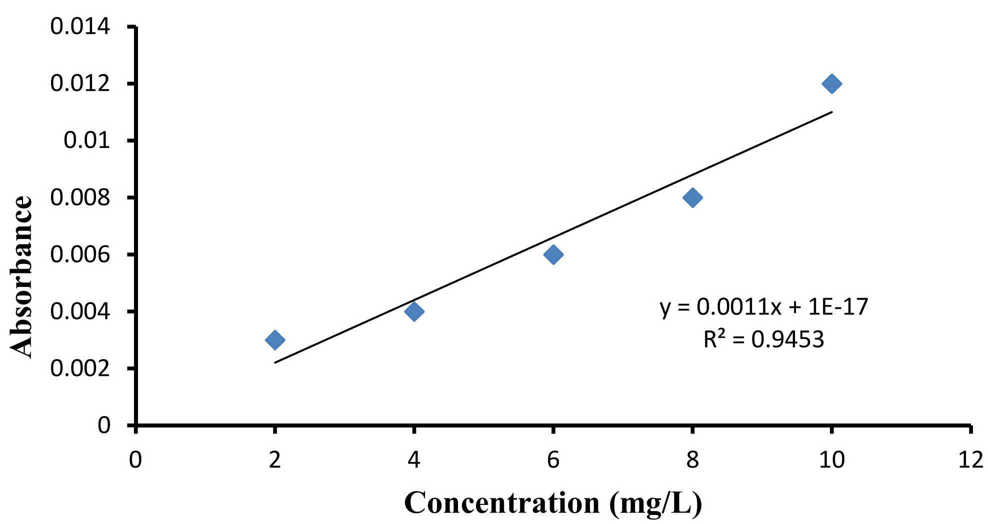

Figure 4. Calibration graph of the standard solution used for the determination of Lead