DETERMINATION OF LEVELS OF SOME HEAVY METALS (Zn, Fe, Cu, Cr Cd AND Pb) IN THREE LOCALLY AVAILABLE COMMERCIAL BRANDS OF BOTTLED ORANGE JUICE OBTAINED FROM SUPERMARKETS IN HARAR ANDDIRE DAWA TOWNS, ETHIOPIA

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ABSTRACT

In the present work , the contents of some heavy metals including Zn, Cu, Fe, Cr, Cd and Pb in three Brands of Orange Juice samples obtained from Local supermarkets found in Harar and Dire Dawa Towns were analysed by FAAS after digestion of all samples with optimum amounts of mixtures of concentrated HNO_3 and 30%H_2O_2 solutions. The mean (±SD) concentrations of Zn, Cu, Fe, Cr, Cd and Pb in the analyzed samples were found to be , 2.761±0.056, 2.680±0.061 ,1.730±0.017 , 0.346±0.025 , 0.241±0.031 and 0.463±0.034 ppm, respectively. The values were compared with the WHO Recommended Dietary Allowance (RDA) as well as with values reported in the available literature for the same metals on similar studies heavy metal contents of fruit juice. The concentrations of some of the heavy metals found in orange juice were above the safe limit recommended by WHO. The recoveries of metals were in the range 86% - 100%. And the relative standard deviations for most metals in the samples were less than 10%. A statistical analysis of variance (ANOVA) at 95% confidence level was used to test whether the variation between the mineral content of three sample means were significant or not.

***Key Words**: Orange juice, FAAS, Heavy Metals, Dietary Intake, Toxicity of Heavy Metals*

1. INTRODUCTION

1.1. Back ground of the Study

Fruit juices are becoming important part of the modern diet in many communities. They are nutritious beverages and play significant part in a healthy diet because they offer good taste and a variety of nutrients found naturally in fruits**.** Juices are available in their natural concentrations or in processed forms. Juice is prepared by mechanically squeezing of fresh fruits or extracted by water. Juices are fat-free nutrients dense beverages and are rich in vitamins, minerals and naturally occurring phyto nutrients that contribute to a good health. For example, orange juice is rich in vitamins, an excellent source of bio-available antioxidant photochemical [6] and significantly improves blood lipid profiles in peoples affected by hyper-cholestrolemia [11]. Fruits juices found to promote detoxification in the human body ([3].

The constituents of processed juice are mainly water, sugar, preservatives, colors and fruit pulp. The most commonly used preservatives are benzoic acid, ascorbic acid or sulphur dioxide; natural colours such as anthocyanin’s and betanin are used. Acid is an essential universal constituents of juice and the most commonly used acid is citric acid. Most fruit juices contain sufficient nutrients that could support microbial growth. Several factors encourage, prevent or limit the growth of microorganisms in Juices; the most important are pH, hygienic practice and storage temperature and concentration of the preservative. Storage of products at refrigerator temperature or below is not always best for the maintenance desirable quality of some fruits. Water used for juice preparation can be a major source of microbial contaminants such as total coli forms, faecal coli forms, faecal streptococci, etc. Environmental conditions for mites may also make the fruits unsafe and these, may have a role in the spread of Salmonella Shigella, Vibrio, Escherichia coli and other and cause diseases as well as fruit spoilage [4].

Spoil yeasts such as saccharomyces cerevisiae, Candida lipolytica and Zygosaccharomyces spp. can tolerate acidic environments. Juices should also tolerate acidic environments. It should also be noted that changes in pH could transform a food into one which can support the growth of pathogens [23].

Juices are the perfect fast foods for today's eat-on the run lifestyle. They contain all the goodness of the- whole product in a condensed form. 100% juices are convenient way for adults and children to get a part of their recommended 4.5 or more cups of fruits and vegetables each day. The 2005 Dietary Guidelines for Americans recommended consumption of several cups per day of fruits and vegetables, and acknowledge the vegetable allowance. According to the new USDA My Pyramid food guidance program, there are portion sizes and recommended amounts of 100% juices for children and adults, depending on one's age, gender, and level of physical activity [26]. 100% fruit juices are nutritious beverages that have been enjoyed by adults and children for decades .

In the present era of industrialization and development, one concern should be the health of the future generation. Children are the most vulnerable age group to any kind of contamination in the food chain. Majority of research confirms that micro nutrients are involved in numerous biochemical processes [13], and an adequate intake of certain micronutrients relates to the prevention of deficiency diseases. Fruits and hence fruit juices and vegetables are valuable sources of minerals. Diets high in fruits and vegetables are also linked to decreased risk of diseases (diabetes, cancer, etc.) and their consumption should be encouraged [22].

Evaluation of micronutrients and essential trace elements levels of fruits and vegetables is a growing trend in nutritional studies throughout the world. Trace elements do not provide any calorie but they play an important role in the metabolic regulations of body. Increased fruit and vegetable consumption can improve the mineral regulation and reduce cardiovascular diseases and certain cancer risks. There is always a concern about dietary balance in the contents of micro nutrients and essential trace elements, particularly daily foods and beverages as some heavy metals are toxic and dangerous to our health beyond their permeable levels. Since fruits and vegetables cannot be obtained always freshly from their sources (farms) by users, getting them in packed (bottled or canned) form may be the only option. However, there are always associated problems of contamination or spoilage possibly due to faults during harvesting, transporting or industrial processing mostly lack of attention for contaminants that may be picked up from the surroundings (farms, transport, vehicles, industrial plants etc.) carried with through to the end and reach the consumers. Heavy metals, even in trace amounts are likely to be the source of

contamination and therefore, monitoring and assessing their levels at every stages of our food supply chain is of paramount importance. Research conducted in this regard is a serious drawback in most developing countries like Ethiopia. In other words, these countries are putting little efforts in promoting the kind of quality control research on food products thereby to safe guard the community from dangerous contaminants that cause public health at risk. In the absence of a system of quality control and lack of feedbacks on regular basis about the safety of food items (mainly processed and packed) foods and beverages in our country particularly in our locality, it is worthwhile to initiate a research investigation in this important area.

The present study intended to address this issue. This study was therefore directed to the determination of levels of some heavy metals (Zn, Cu, Fe, Cr, Cd, and Pb) in three locally available commercial brands of bottled orange juice obtained from different super markets in Harar and DireDawa towns using Atomic Absorption spectrometry.

1.2 .Objectives

General Objective

- To determine the levels of some heavy metal (Zn, Cu , Fe, Cr, Cd, and Pb) contents of bottled orange juice of three commercial brands locally available in Harar and Dire Dawa towns by using flame atomic absorption spectroscopy .

Specific Objectives

- ✓ To determine the levels of heavy metals (Zn, Cu , Fe, Cr, Cd, and Pb) in locally available brands of bottled Orange juice by flame atomic absorption technique
  - ✓ To develop the optimum working procedure for analysis of contents of each of metals in the sample orange juice products;
  - ✓ To compare the concentrations of the Zinc, Iron, Chromium, cadmium, lead and copper determined in the sampled bottled juices with of the recommended dietary standards and daily intake reference values of food substances.

2. MATERIALS AND METHODS

2.1. Experimental site

Most of experiments were conducted at Haramaya University in General Chemistry Post Graduate Research Laboratory and AAS reading was performed in Soil Science Laboratory.

2.2. Apparatus and Equipment

The materials used in this study include: Digital analytical balance (Meter Toledo model AG 204, Switzerland) to weigh orange juice samples collected from super markets a refrigerator to keep orange juice samples until analysis; micropipettes (1-10ml, 100-1000ml) and different size beakers were used for measuring volume of reagents and standards; BUCK SCIENTIFIC MODEL 210 Atomic Absorption Spectrophotometer equipped with deuterium background corrector for analysis of Zinc, Iron, Chromium, cadmium, lead and copper using air acetylene flame.

2.3 Reagents and Chemicals

Reagents and chemicals used during the laboratory session were all analytical grade 99% $Cucl_2$, 99.99% Cdcl.21/2 H_2O, 95.5%Zn $(NO_3)_2$, 99% Fe $(NO_3)_2$, 95%Cr $(CH_3COO)_2$ and 99.5% Pb $(NO_3)_2$ were used to prepare calibration standards of the respective metals for the analysis of the samples. Distilled and deionised water were used throughout the experiment. All glassware and plastic containers before and after use, were washed with-distilled water, detergent then soaked in nitric acid solution, rinsed several times in distilled de-ionized water and dried in air. All items were kept in a clean place to avoid contamination

2.4. Methods and Procedures

2.4.1. Sample Collection

Depending on the availability, three locally available commercial brands of orange juice samples were collected from different supermarkets located in Harar and Dire Dawa Towns, and transported to Haramaya University Chemistry Post Graduate Research Laboratory for the investigations

2.4.2. Dry Ashing

The dry ashing method was used in the present study for AAS. The orange juice was homogenously mixed before samples were taken for analysis. 100 mL of sample was measured into a platinum crucible and carefully evaporated on a hot plate till dryness. The crucible with the test sample was then placed in an oven and heated a temperature of 500ºC for 8 hours to get sample ash. The crucible containing ash was put in desiccators for cooling and then about 5 mL of nitric acid was added ensuring that all the ash came into contact with the acid and the resultant solution was heated on a hot plate until the ash dissolved. After adding extra 10 mL nitric acid and stirring it was then filtered into a 50 ml volumetric flask.

2.4.3. Sample Digestion and Preparation of Analyte Solution for AAS

Prior to quantification of the analyates by Atomic Absorption Spectroscopy it is usually necessary to destroy the organic matrix and bring the element in to clear solution. To this effect all the orange juice samples, xtra/manufactured from Lebanon, twist/Egypt manufactured and Mirinda /manufactured from Moha PLC were first digested with acid mixture for the organic matrix in the samples to be destroyed completely and a clear solution was obtained. In the present study .all of the homogenized samples were treated a mixture of with 5ml of HNo_3 and 2ml H_2O_2 in mineralizing heated at 110℃ for 1h20min. After cooling, resulting digest was diluted to a volume of 10 ml with distilled deionised water and kept until analysis.

2.4.4. Preparation of Standard Solution

A stock solution of each metal ion containing 2.117g $Cucl_2$, 2.032g Cdcl.21/2 H_2O, 2.896g Zn $(NO_3)_2$, 3.220g $_{Fe}$ $(NO_3)_2$, 3.154g Cr $(CH_3COO)_2$ and 2.165g Pb $(NO_3)_2$ in 1000 mg/L was prepared by dissolving calculated amount of a soluble salt of the respective metal. Intermediate standard solutions (10 mg/L) were preparing from the stock solution in 100 ml volumetric flasks. This way then appropriate working standard solutions were prepared for Zinc, Iron, Chromium, Cadmium, Lead and Copper metal ions by serial dilution of the intermediate solutions using deionised water. Each set of serial dilute solutions was aspirated one after the other into the atomic absorption spectrometer and their respective absorbance was recorded. A calibration curve was plotted with four points for each metal ion standard solution using absorbance versus concentration (mg/L) values. Immediately after calibration using the standard solutions, all the analyte sample solutions were aspirated into the AAS instrument and direct readings of the metal concentrations were recorded. Three replicate determinations were carried out on each sample and the mean values of concentrations for the respective metal ions.

2.4.5. Method Detection Limit

Method detection limit is the smallest mass of analyte that can be distinguished from statistical fluctuations in the blank, which usually corresponds to the standard deviation of the blank solution times a constant. The limit of detection is most commonly defined as the amount of analyte that gives a signal equal to three times the standard deviation of a blank [2].

The method detection limit for each metal ion was estimated by digesting three analytical blanks with the optimized procedure used for digesting orange juice samples. Triplicate analyses of three blank samples for each of the six elements were performed and the standard deviation of the three blank reagents was calculated. The detection limits were obtained by multiplying the standard deviation of the reagent blank (S blank) by three ($MDL = 3 \times S_{blank}$, $n = 3$).

2.4.6. Precision and Accuracy

The errors in analytical results are most often expressed using precision and accuracy. The precision of an analytical procedure expresses the closeness of agreement between a set of results where as accuracy expresses the closeness of measurements to the true value. The precision of an analytical procedure is usually expressed as the variance, relative standard deviation and percentage relative standard deviation of a series of measurements [15].
In this study, the precision of the results were evaluated by percentage relative standard deviation of the results of three samples (n=3) and triplicate readings for six metals calculated and reported in Table7.

2.4.7. Method Validation

In this work, the method validation was established by spiking experiments. The spiked samples were prepared by adding a small known quantity of metal standard solutions. For spiking orange juice sample, 300 µL of 1000 mg/L Fe, 500µL of 1000 mg/L Cu, 250 µL of 1000 mg/L Zn, 20 µL of 1000 mg/L Cr, 200 µL of 1000 mg/L Cd, and 20 µL of 1000mg/L Pb standard solutions were added to round bottomed flask (50 mL) containing 0.5 g orange juice sample. The digested spiked samples were finally analyzed for their respective metals using FAAS. Then the percentage recovery of the analyte was calculated by:

$$\%\text{Recovery} = \frac{CM \in spiked\ sample - CM\ non\ spiked\ sample}{CM\ added\ for\ spiking} \text{x}100$$

Where, CM = concentration of metal of interest

2.5. Statistical Analysis

Analysis of variance (ANOVA) is a powerful statistical technique which can be used for the separation and estimation of the different causes of variation of more than one means obtained from different experiments. For the present study, the significance of variation between samples was analyzed using one-way ANOVA. This was made possible through detail calculations using MicrosoftExcel2007Software.

3. RESULTS AND DISCUSSION

3.1. Optimization of the Digestion Procedure

The basic requirements for sample preparation for analysis were to get an optimum condition for digestion. The optimum condition is the one which consumed minimum reagent volume, minimum digestion time, clarity of digests, and ease of simplicity [21].

In this study, to get a clear sample solution suitable for the analysis using AAS, different orange juice brands digestion procedures were optimized using HNO_3 and H_2O_2 mixtures by varying volume of acid mixtures, digestion time and digestion temperature. From various trials of the optimization, the acid mixture containing 5 mL of HNO_3 (69-70%) and 2 ml of H_2O_2 (30%), digestion time of 1:20 h and digestion temperature of 110 °C was found to be the optimal conditions for the orange juice samples analyzed in this study The optimum condition was selected based on clarity of digests, minimum reagent volume consumption, minimum digestion time, simplicity and minimum temperature applied for complete digestion of sample.

Table 1: Different conditions tested for optimization of digestion procedure for orange juice sample

No	Volume of reagent		Temp (°C)	Digested Time (h)	Observation
	HNO_3(mL)	H_2O_2(mL)			
1	5	1	110	1:20	Light yellow with suspension
2	4	1.5	110	1:20	Light yellow without suspension
3	5	2	110	1:20	Clear and colourless
4	5	2	50	1:20	Light yellow
5	5	2	75	1:20	Clear yellow
6	5	2	110	1:20	Clear and colourless
7	5	2	110	40’	Light yellow with suspension
8	5	2	110	1h	Light yellow without suspension
9	5	2	110	1:20	Clear and colourless

3.2. Calibration of Instruments

In this study, atomic absorption spectroscopic standard solutions containing 1000 mg/L were used for preparing intermediate standard solutions (10 mg/L) in 100 mL volumetric flasks. As indicated in Table 3, appropriate working standards were prepared for each of the metal ions by serial dilution of the intermediate solutions using deionised water. Each of the sets of serial dilutions was then aspirated one after the other into the Atomic Absorption Spectrometry and its absorbance recorded. Calibration curves were plotted with four points for each standard using absorbance against concentrations (mg/l) and the calibration curves obtained as such are given in appendix 2 and table 3. Immediately after calibration using the standard solutions, the sample solutions were aspirated into the AAS instrument and direct readings of the metal concentrations were recorded. Three replicate determinations were carried out on each sample. The same analytical procedure was employed in the determination of elements in each of the three digested blanks.

Table 2: The Operating Conditions of AAS employed for each Analyte

Metals	Concentration of intermediate standard (mg/L)	Concentration of working standard(ppm)	Correlation coefficient of calibration curve
Fe	10	0.5, 1.5 ,2.5 ,3	0.9981
Zn	10	0.5, 0.8 , 1 , 3.5	0.9961
Cu	10	1 , 2.5 ,3 , 4	0.99556
Cr	10	0.5 ,1.5 ,2 , 3	0.9981
Cd	10	0.5, 1 ,1.5 ,2	0.9985
Pb	10	0.5, 1.5 ,2 ,2.5	0.9938

3.3. Method Detection Limit

In the present study, method detection limit for each metal was estimated by digesting three analytical blanks with the optimized procedure orange juice samples. Triplicate analyses of three blank samples for six metals were performed and the standard deviation of the three blank reagents was calculated. The detection limits were obtained by multiplying the standard deviation of the reagent blank (S blank) by three (MDL = 3 xS blank, n = 3). As shown in Table 4, the method detection limit of each element is above the instrument detection limit.

Table 3: Standard Conditions of the AAS Used in Determination of Different Elements.

metal	Wavelength (nm)	IDL (mg/L)	MDL(mg/L	Slit width (nm)	Lamp current (mA)	Energy(ev)
Fe	248.3	0.030	0.037	0.2	7.0	2.546
Cu	324.7	0.020	0.181	0.7	1.5	3.970
Cd	228.9	0.005	0.104	0.7	2.0	3.07
Zn	213.9	0.005	0.059	0.7	2.0	3.047
Cr	357.9	0.050	0.099	0.7	2.0	3.750
Pb	217	0.100	0.101	0.7	0.3	3.16

3.4. Method of Validation

The efficiency of the optimized procedure is checked by various methods. These are certified standard reference material analysing and spiking sample with known concentration of the analyte

In this work, the method validation was established by spiking samples. The spiked samples were prepared by adding a small known quantity of metal standard solutions. The recovery tests of the total analytical procedures were carried out for the metals analysed in the selected samples by spiking by small amounts of metal standards. An acceptable recovery of 86%-100% was obtained.

Table 4: Recovery Test

Metals	Concentration of spike (µg/L)	Concentration added(µg/L)	Concentration of un spike (µg/L)	Recovery (%)
Fe	20224.60±1.53	300	1730 ±0.02	97.696±1.12

Cu	3154±0.58	500	2679 ±0.06	94.957±0.21
Zn	2975±0.13	250	2760 ±0.06	85.970±1.06
Cr	366±1.00	20	346 ±0.03	90.125±9.75
C d	434±0.33	200	241 ±0.31	96.588±0.08
P b	489±1.00	20	470 ±0.03	90.165±5.17

3.5. Precision and Accuracy

The precision of an analytical procedure expresses the closeness or agreement between a replicate measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions (repeatability or reproducibility). The common terms used to measure variability is the coefficient of variation (CV) or relative standard deviation (RSD). Which may also be expressed as a percentage and it is a parameter of choice for expressing precision in analytical sciences [17].

In this study, the precision of the results were evaluated by the relative standard deviation of the results of triplicate measurement. Triplicate measurements of each sample (n=9) were used for the analysis of trace metals in orange juice samples. Values of relative standard deviations (% RSD) are less than 10% for most of the mean concentrations of metals except Cd. Percent Relative standard deviation values for studied metals are summarized in Table7.

3.6. Determination of Heavy Metals Contents of Samples of Orange Juices

Table 6: Concentration and Standard Deviation of Heavy Metals

Metals	Concentration(x ±SD)(mg/L)			AVERAGE	RSD%
	Sample				
	L	E	M		
Fe	1.650±0.017	1.549±0.021	1.991±0.012	1.730±0.017	0.983
Cu	2.993±0.034	2.626±0.020	2.420±0.130	2.680±0.061	2.276
Zn	2.613±0.060	2.493±0.047	3.044±0.061	2.716±0.056	2.062
Cr	0.313±0.022	0.397±0.034	0.329±0.020	0.346±0.025	7.225
Cd	0.163±0.031	0.291±0.040	0.268±0.021	0.241±0.031	12.863
Pb	0.557±0.055	0.441±0.025	0.391±0.021	0.463±0.034	7.343

Key:

- L= xtra /juice manufactured in Lebanon
- E= twist/ juice manufactured Egypt
- M= Mrinda/juice manufactured by Moha PLC

The concentrations of heavy metals and the SD of measurement, Fe, Cu, Zn, Cr,Cd, and Pb, in analyzed commercial orange juices samples are presented in Table 6. The results of elemental concentration of the three brands of orange juice samples collected from Harar and Driedawa Town super markets given as calculated average concentrations of the corresponding metal ions of (Fe, Cu, Zn, Cr, Cd and Pb) are presented in Table 6. The variations in the metal contents in the sample of the three orange juice brands discussed below for each metal.

Iron

The highest concentration of Iron (1.979-2.003 mg/L) in orange juice was recorded in brand M, while the lowest concentration range of (1.528-1.570 mg/L) was recorded in the sample branded as E. The mean concentrations of iron in the three sample brands which were obtained between 1.713-1.747 mg/L in this study are higher than those reported in previous studies in (different countries) as indicated in Table 8. The maximum iron concentration obtained for the orange juice brand coded as M was (1.979-2.003 mg/L) , and this value is well above the safe limit recommended by WHO as 0.3 mgL^{-1} [24]. However, this concentration falls within the 10-50 mg per day of iron requirement for human body depending on age, sex, and iron bioavailability [7].

Zinc

Zn is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms. Nevertheless, higher concentrations of Zn can be toxic to the organism [19]. The highest concentration of zinc in the range of (2.982 -3.105 mg/L) in orange juice was recorded in the sample M. while the lowest concentration in the range of (2.446-2.540mg/L) was recorded in the sample branded as E. The average concentration of zinc in three orange juice brands (2.660-2.772 mg/L) sample for higher than those studied in different countries shown by table8. The maximum concentration of zinc determined was 2.983 -3.105 mg/L in orange juice which is fall in the 10 – 75 mg/L limit set by WHO which is also not a threat to public health [24] *and the concentration of Zn in the range of 0.063-2.830 mg/L in* orange juice lower than in the present study [12].

Copper

The highest concentration of copper 2.959 -3.027 mg/L in orange juice was recorded in sample L. the lowest concentration of 2.290-2.550 mg/ L was also recorded in sample for M. the mean concentration of copper (2.619-2.74 mg/L) in the present study were higher than those studied in different countries shown by Table8. The maximum concentration of copper determined was for orange samples which is above the safe limit set by WHO i.e. 3 mg.L-1 [24]. A daily dietary intake of 2 to 3 mg of copper is recommended for human adults [9]. And the concentration of Cu in the range of 0.047-1.750 mg/L in orange juice lower than in the present study [12].

Chromium

Cr is an essential micronutrient for animals and plants and is considered as a biological and pollution significant element [10]. The average concentration of Cr is 0.321-0.371 mg/L. The concentration Cr in sample E is 0.363-0.431mg/L while Cr in sample M and L are 0.309-0.349 mg/L and 0.291-0.335 mg/L respectively.

The average concentration of chromium higher than determined by different countries like Spain, Brazil, Australia and Saudi Arabia listed in Table 8.

Cadmium

Cd is highly toxic and responsible for several cases of poisoning through food. Small quantities of Cd cause adverse changes in the arteries of human kidney. It replaces Zn biochemically and causes high blood pressures, kidney damage, and so forth [18].

When dealing with the determination of concentration in Cd, the highest concentration is observed in E (0.251-0.331mg/L) and lowest in sample L (0.132-0.194mg/L) while the concentration of sample M is 0.247-0.289 mg/L. the average concentration of Cd (0.210-0.272 mg/L) in the three samples in this study higher than the average concentration of orange juice in Nigeria in table and the concentration of Cd in the range of 0.004-0.040 mg/L in orange juice lower than in the present study [12].

Lead

The average concentration of lead in orange sample is (0.429 -0.497 mg/L). The highest concentration of lead in sample L (0.502 -0.632mg/L) and the lowest concentration (0.370-0.412mg/L) in sample M. the average concentration lead lower than the highest range concentration in Nigeria in table 8. The maximum concentration of lead detected orange juice Which is far above the safe limit of 0.01 mg.L-1 recommended by [24].

Over all, present study shows that the levels of heavy metals studied are generally above save limits and except pb compared well with levels in orange juices from the parts of the world.

Table 7: Comparison of the Concentration of heavy metals in Orange Juices with the Published Values

Country	Concentration (µg/L)						Reference
	Zn	Cd	Fe	Cu	Cr	Pb	
Spain					5.75		[8]
USA	242-480		450-641	239-460			[20]
USA	400			400			[5]
Brazil	842			271			[16]
Brazil	511		760	309	11.5		[14]
Brazil	731.5			422	2.4		[16]
Malaysia			630				[14]
China			500	370.8			[14]
Australia	350.2		623.3	422	2.4		[22]
Libya	535-863	Nd	443-663	255-459		Nd	[26]
Ethiopia	2704-2817	210-272	1713-1747	2618-2740	321-371	436-045	Present study

3.7. RDI of Minerals According to Age and Gender

The Recommended Daily Intake, RDI of metals is related directly to age, and gender. The requirements for babies, toddlers, children, adolescents, and elderly vary with gender and country due to soil type. These requirements are continually being reviewed in the light of more research that is undertaken by food regulating bodies such as Food Standards Australia and New Zealand, FSANZ, United Stated of America, Food and Drug Administration, FDA, and European Authorities to name three such groups. The work done by these bodies includes all food groups in addition to vitamins, minerals: cereals, fat, protein, carbohydrates, sugars and so on, as well as research on different age groups in particular locations in many countries, to assist in maintaining and improving the health of the various groups and the population in general. [1] www.intechopen.com Atomic Absorption Spectroscopy

Table 8: Trace Minerals Recommended Daily Intake, RDI

Chromium	120 µg
Copper	2 mg

Iron	15 mg
Zinc	15 mg

The Table (8) above represents RDI values recommended by experts and agencies for a normal adult population. http:// lenntech.com/recommended-daly-intake.htm

Table 9: Comparison of Daily Intakes of Metals from 0.0274 l of FRUIT Juice by Saudi Population with the Recommended Values

Metals	Amounts	Recommended/permission value mg/day or g/day
Zn	25.25µg	11mg (male) 8 mg(female)
Fe	10.20 µg	8mg(male)
Cu	14.11 µg	2-3mg
Cr	0.59 µg	2.5-5mg

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Now day a daily consumption of juice in Ethiopia presumed to be information regarding the higher but exact value of daily consumption of orange juice in the country could not be access able for technical reasons. The same could not be provided as far as the research area for the present work is concerned

3.8. ANOVA Analysis

In order to check whether there is significant difference or not in the metal concentration of the three different orange juice brands, one–way ANOVA was applied. The result of the analysis is described in the subsequent paragraphs and ANOVA output is attached at appendix B. There is a significant difference ($p < 0.05$) in mean concentrations of Cu, Zn, Pb, Cd, Cr and Fe at 95% confidence level between three samples of orange juice.

ANOVA use the F statistic to compare whether the difference between sample means are significant or not. If the calculated value of F (the ratio of SD between samples to SD within samples) is greater than Fcritical (the value obtained from Table 10 at specified confidence level and degree of freedom), the differences in sample means are significant. In this study, orange juice samples were collected from super markets and the metal level of each sample was analysed by FAAS. During the processes of sample preparation and analysis a number of random errors may be introduced in each aliquot and in each replicate measurement. The variation in sample mean of the analyte was tested by using ANOVA, whether the source for variation was from experimental procedure or heterogeneity among the samples. As it can be shown from the Table 10, there exist statistically significant differences at 95 % confidence level in mean concentration of six heavy metals.

Table 10: Analysis of Variance (ANOVA) Between and Within Orange Juices Sample

metals	Comparisons	Df	F_{CAL}	Fcr	Observation
Cu	Between groups	2	40.809	5.143	Significant difference b/n
	Within the groups	6			sample measurements
Cd	Between groups	2	8.848	5.143	Significant difference b/n
	Within the groups	6			sample measurements
Zn	Between groups	2	77.91	5.143	Significant difference b/n
	Within the groups	6			sample measurements
Cr	Between the groups	2	8.848	5.143	Significant difference b/n
	Within the groups	6			sample measurements
Fe	Between groups	2	553.308	5.143	significant d /ce b/n
	Within the groups	6			measurements
Pb	Between groups	2	15.713	5.143	significant d /ce b/n
	Within the groups	6			measurements

4. SUMMARY, CONCLUSION AND RECOMMENDATION

4.1. Summary and Conclusion

Heavy metals, even in trace amounts are likely to be the source of contamination and therefore, monitoring and assessing their levels at every stages of our food supply chain is of paramount importance. Research conducted in this regard is a serious drawback in most developing countries like Ethiopia. In other words, these countries are putting little efforts in promoting the kind of quality control research on food products thereby to safe guard the community from dangerous contaminants that cause public health at risk. In the absence of a system of quality control and lack of feedbacks on regular basis about the safety of food items (mainly processed and packed) foods and beverages in our country particularly in our locality, it is worthwhile to initiate a research investigation in this important area.

In this work, a study of the levels of some heavy metals in three commercial brands of orange juices obtained from supermarkets in Harar and Dire Dawa Towns. The concentration of heavy metals (Fe, Zn, Cu, Cr, Cd and Pb) has been analyzed by Flame Atomic Absorption Spectrometry. The dry ashing methods were used in this study. A recovery study of the analytical procedure was carried out by spiking the analyzed samples with varied amounts of standard solutions of the metals. The ranges recoveries obtained were 86-100% and are in a good agreement with the expected values. This indicated that the analyzed samples contain appropriate levels of these particular metals and procedures for determining them were reliable. Calibration curves were plotted with four points for Fe, Pb, Cu, Cr, Cd and Zn metal standards using absorbance against concentrations (mg/L) and the calibration curves obtained. Concentrations of the studied metals between in and within samples were also compared using one-way ANOVA. The results of ANOVA analysis indicated that there are significant differences ($p< 0.05$) in all metals. The results obtained in the present study the concentration of metals are also compared with WHO guidelines and other review literatures. Generally the highest content of Zn was found in orange juices, whereas Cd concentration was the lowest in orange juice. In the present work most concentration of trace metals analysed orange juices samples exceeded WHO safe limit and therefore may affect public health.

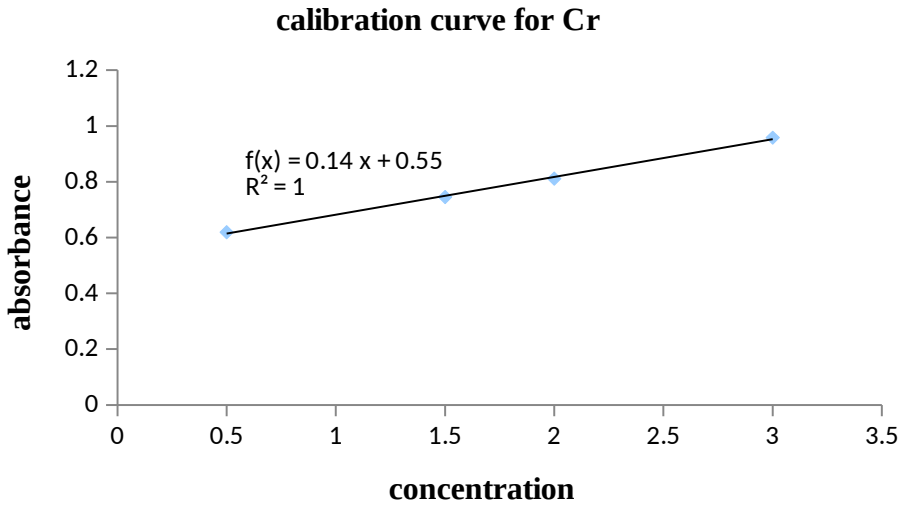
4.2. Recommendation

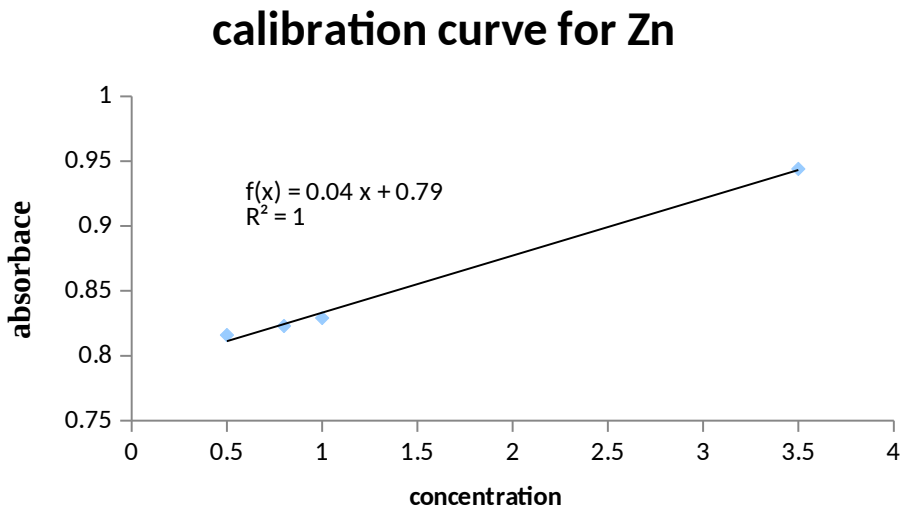
The following recommendations are made as a result of the outcome the present study.

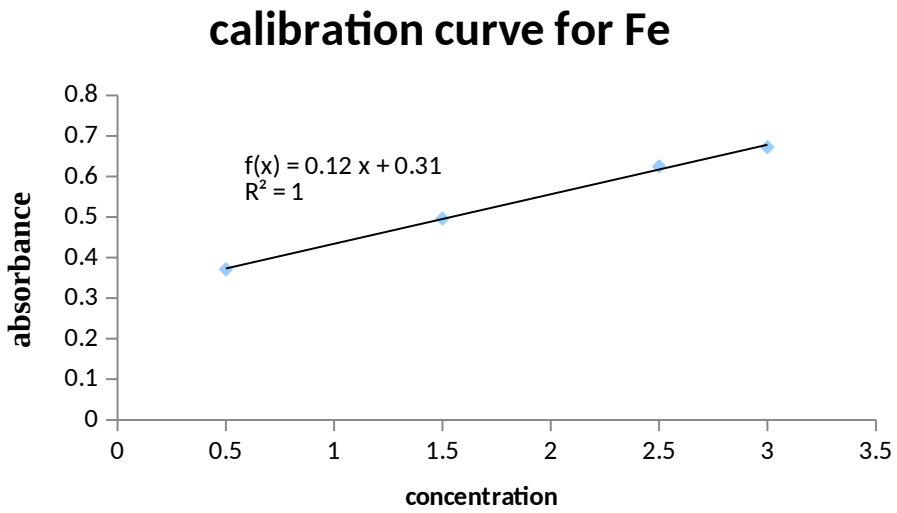
- The concentration of heavy metals in limited in three brands and the scope study limited only Dire Dawa and Harar however other parts part of Ethiopia that are not covered by this research should be determined.
- Monitoring of the levels of heavy metals in commercially available orange juice should be encouraged.
- Orange juice producing factories should be checked regularly the level of heavy metals to safe public health.
- Further investigations are needed to other heavy metals and different brands of orange juices.

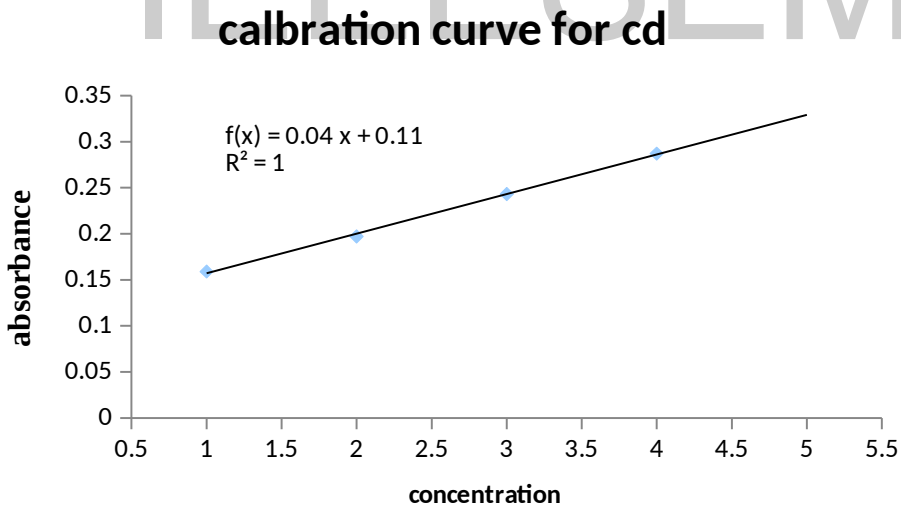
5. APPENDIXES

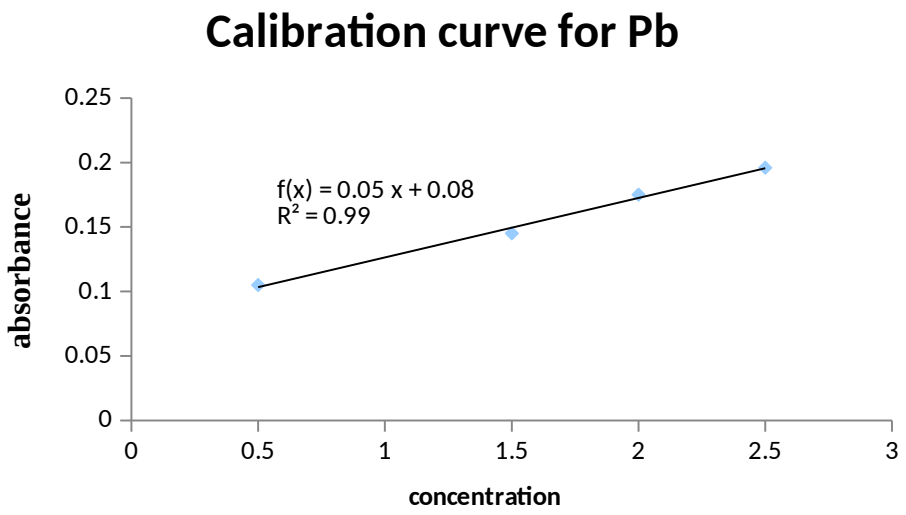
APPENDIX A 1 CALIBRATION CURVE

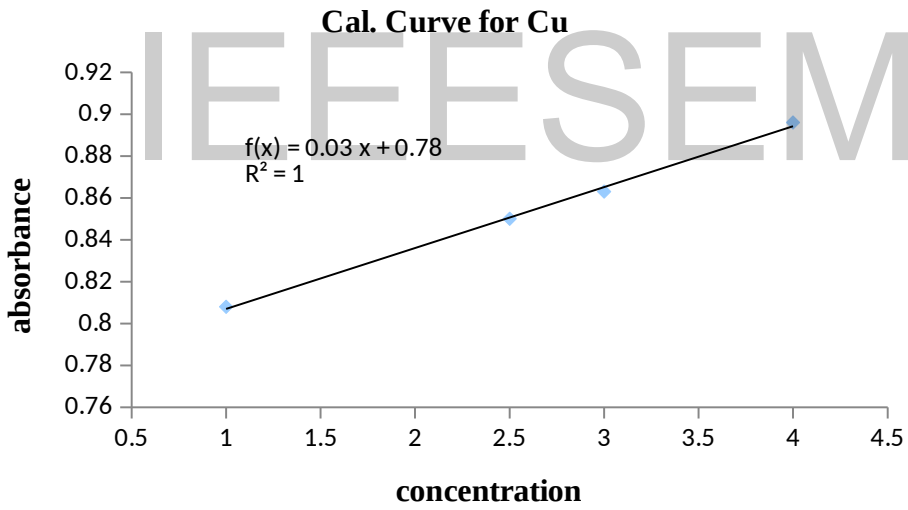












APPENDIX A 2 ANOVA

Anova: Zn

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	7.84	2.613333	0.003636
Column 2	3	7.479	2.493	0.002239
Column 3	3	9.132	3.044	0.003819

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.503555	2	0.251777	77.91483	5.09659E-05	5.143253
Within Groups	0.019389	6	0.003231			
Total	0.522944	8				

Anova: Cr

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	0.939	0.313	0.000481
Column 2	3	1.19	0.396667	0.001137
Column 3	3	0.987	0.329	0.000388

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.011835	2	0.005917	8.848148	0.016234	5.143253
Within Groups	0.004013	6	0.000669			
Total	0.015848	8				

An ova: Cu

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	8.979	2.993	0.001156
Column 2	3	7.879	2.626333	0.000385
Column 3	3	7.261	2.420333	0.017014

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.504828	2	0.252414	40.80917	0.000321	5.143253
Within Groups	0.037111	6	0.006185			
Total	0.541939	8				

An ova: Pb

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	1.67	0.556667	0.003065
Column 2	3	1.324	0.441333	0.000645
Column 3	3	1.173	0.391	0.000421

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.043281	2	0.021641	15.71303	0.004132	5.143253
Within Groups	0.008263	6	0.001377			

	0.05154					
Total	4	8				

An ova: Cd

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	0.939	0.313	0.000481
Column 2	3	1.19	0.396667	0.001137
Column 3	3	0.987	0.329	0.000388

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.011835	2	0.005917	8.848148	0.016234	5.143253
Within Groups	0.004013	6	0.000669			
Total	0.015848	8				

Fe

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	4.951	1.650333	0.000277
Column 2	3	4.647	1.549	0.000444

Fe

Column 3	3	5.972	1.990667	0.000149

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.321165	2	0.160582	553.3082	1.57E-07	5.143253
Within Groups	0.001741	6	0.00029			
Total	0.322906	8				

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