International Journal of Food Nutrition and Safety, 2020, 11(1): 1-12
International Journal of Food Nutrition and Safety
Journal homepage:www.ModernScientificPress.com/Journals/IJFNS.aspx
ISSN: 2165-896X
Florida, USA

Article

Heavy Metal Analysis in Fruit and Vegetable Juices Available in Nigerian Community by Atomic Absorption Spectroscopy

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Article history: Received 9 October 2019, Revised 19 November 2019, Accepted 22 December 2019, Published 4 January 2020.

Abstract: Fruits and vegetables are considered as protective supplementary food as they contain significant quantities of minerals, vitamins, carbohydrate, essential amino acids and dietary fibers required for normal functioning of human metabolic processes. In this study metal analysis of four samples for both fresh and packaged fruit and vegetable juices of orange, pineapple, apple and tomato was carried out using AAS technique. The highest concentration of Fe and Cd was observed in fresh tomato juice, while the highest concentration of Cu is recorded in fresh pineapple juice. However Pb was not detected in all the samples which is good to know. The Cd levels observed in all the samples are below the permissible limit while the concentration of Fe and Cu are above the permissible limits imposed by WHO/FAO. The results of the sample analysis and their comparison with standard values showed that the concentrations of the essential heavy metals in the studied samples were above the standard limits imposed by joint WHO/FAO. This implies that considerable attention should be paid to the potential health risk of these metals, because they are essential only at appropriate concentrations.

Keywords: fruits, vegetables, juice, heavy metal, AAS analysis, digestion

1. Introduction

Consumption of fruits is essential for a diversified and nutritious diet. Sufficient consumption of fruits and vegetables significantly reduces the incidence of chronic diseases such as cancer, cardiovascular diseases and other aging related pathologies (Prakash *et al*., 2012). Fruits offer protection against free radical that damage lipids , proteins, and nucleic acids polyphenols, carotenoids (pro vitamin A), vitamin C and E present in fruits have antioxidants and free radical scavenging activities and play a significant role in the prevention of many diseases (Prakash *et al*.,2012)

A number of trace elements protect the cell from oxidative damage as these minerals are the cofactor of antioxidant enzymes, Zinc, copper, and manganese are necessary for superoxide dismutases in both cytosol and mitochondria. Iron is a component of catalase and hemeprotein which catalyses the decomposition of hydrogen peroxide (Machlin and Bendich, 1987).Small amount of micronutrients are required for good physical condition along with energy food and protein. Sodium, potassium, iron, calcium and many trace elements together with antioxidant vitamins and minerals are vital for the body. Fruits and vegetables, particularly leafy have noteworthy amounts of calcium, iron, and potassium (Jahan *et al*., 2011)

On the other hand, no one can guarantee for sure whether these foodstuffs are safe or not as these days rarely any food is free from food adulteration. Most of the adulterants that are intentionally added or are invisible or they are made indistinguishable by astutely camouflaging by means of the colour or texture. Food safety is essential to maintain nutrition, combat food/water borne diseases, maintain food quality and stop food adulterations, being rampant in Bangladesh. Nowadays some growers as well as traders in Bangladesh are commercially using some chemicals namely Ripen, Gold–plus, Profit etc., for the ripening of tomato , papaya, mango , and banana, directly to the fields and processing areas. These chemicals change nutritional properties of fruits and vegetables as well as lead serious health hazards to human beings like cancer, skin irritation, diarrhea, liver disease, kidney disease, gastro intestinal irritation with nausea, vomiting, diarrhea, cardiac abnormalities etc (Hakim *et al*., 2012). Children are at particular risk to the harmful side effects of food adulteration, which may lead to serious liver and kidney diseases including various forms of cancer and hepatitis (Per *et al.*, 2007).

Because of the richness in valuable nutrients and potential health benefits, fruits are readily consumed on a daily basis. The high consumption of these fruits may expose humans to heavy metals intoxication. Fruits contamination by heavy metals is a major aspect of health concerns (Ibrahim *et al.*, 2006). During plant growth, certain essential minerals for growth and development like magnesium, iron, manganese, zinc, copper, molybdenum and nickel are usually absorbed from the soil (Yang *et al.*, 2003). However plants can also accumulate other metals such as cadmium, chromium, silver, selenium, lead, mercury etc. which do not have any biological importance but rather have harmful effect to the body (Duffus, 2002). These heavy metals have the potentials of causing acute and chronic toxicity by various modes of action in humans (Sharma and Agrawal, 2005). Lead can affect the circulatory renal system which is characterized by mild glucosuria, aminoaciduria and hyperphosphaturia in the kidney

(Staudinger and Roth, 1998) and may impair the central nervous system (CNS) especially in children (Lidsky and Schneider, 2003).

Soil is an environmental and biochemical reaction system comprising of three important phases solid (i.e mineral particles, organic debries, plant roots), solution (i.e groundwater, rain water, biological excreta, products of biochemical reactions), and gas (i.e atmospheric products of biochemical reactions) which moves towards equilibrium with one another. Agricultural soil is the most important sink for heavy metals due to soils metal retention capacities. Lack of self-purification occurrence of eutrophication, the complex and fragile ecosystem make stagnant lake water more susceptible to pollutants than flowing water. Waste from house hold, polluted streams and refuse industrial disposal sites pollute underground water. Underground salt intrusion to coastal lands cause accumulation of excess salt in soil which interferes with the osmotic process and metabolisms.

Heavy metals are metallic element with high atomic weight and density. These include the transition metals some metalloids, lanthanides and actinides. Amounting to more than 20 metals generally exist in a positively charged form and can bind on to negatively charged organic molecules. Being metals ion, heavy metals cannot be degraded or destroyed, therefore their stability make them as the persistent toxic substances in environment. Heavy metals as the environmental contaminants can be found in the air, soil and water, which pose health hazard to the general public. Heavy metals are wide spread pollutants of great concern as they are non-degradable and thus persistent. These metals are used in various industries from which effluents are consequently discharged into the environment. Introduction of heavy metals in various forms into the environment can produce numerous modifications of microbial communities and affect their activities (Doelman *et al.*,1994; Hiroki,1994;Staezecka and Bendnatz, 1993). Common sources of heavy metal pollution include discharge from industries such as electroplating, plastics manufacturing, fertilizer producing plants and wastes left after mining and metallurgical processes (Zouboulis *et al.*, 2004). Although some heavy metals are essential trace elements, most can be at high concentrations toxic to all forms of life, including microbes, humans and animals. Heavy metals generally exert inhibitory action on microorganisms by blocking essential functional group, displacing essential metal ions or modifying the active conformations of biological molecules (Doelmann *et al.*,1994; Gadd and Griffiths 1978; Wood and Wang 1983). However at relatively low concentrations some heavy metals ions (e.g Co^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+}) are essential for microorganisms since they provide vital cofactors for metallo-proteins and enzymes (Eiland, 1981; Doelmann *et al.*, 1994). Publicity regarding the concentration of heavy metals in fruits and vegetables will create apprehension and fear in the public as to the presence of heavy metals in their daily food, keeping in mind the potential toxicity and persistent nature of heavy metals, and the frequent consumption of vegetables and fruits, it is necessary to analyze these food items to ensure the levels of these contaminants meet agreed international requirement (Radwan and Salama, 2006). The main aim of this work therefore, is to determine the concentrations of essential and toxic heavy metals in fruit and vegetable juices that are commonly consumed in Nigeria by using AAS technique.

2. Materials and Methods

2.1. Sample Collection

Packaged commercial fruits juices (consisting of pineapple, orange, apple) and tomato paste (pouch type) were purchased from local supermarket, while the fresh samples (pineapple, orange, apple and tomato) were purchased from local market in Aliero town, kebbi state of Nigeria and brought to chemistry department of Kebbi state university of science and technology, Aliero and preserved in refrigerator. The samples were numbered as follows:

Sample 1 is packaged pineapple juice

Sample 2 is packaged orange juice

Sample 3 is packaged apple juice

Sample 4 is packaged tomato paste

Sample 5 is fresh pineapple juice

Sample 6 is fresh orange juice

Sample 7 is fresh apple juice

Sample 8 is fresh tomato juice

2.2. Sample Preparation

The fruit and vegetable samples were washed with distilled water and peeled. The juice was extracted using a mortar and pestle and filtered using a sieve.

2.3. Digestion of Sample

Prior to quantization of analyte by the Atomic Absorption Spectroscopy it is usually necessary to destroy the organic matrix and bring the element into clear solution, for this reason the juice samples were first digested with acid washed thoroughly under a running tap water and peeled. The method of sample digestion by (AOAC, 2000) was used with a slight modification. 10ml of concentrated nitric acid (HNO_3) is added to 5ml of the fruit juices sample in a closed vessel (acid washed 250ml beaker covered with a watch glass) to avoid loss by volatilization of metals especially Pb and the solution is heated on a hot plate in a fume chamber until all the fumes were given off. Then the digested samples were allowed to cooled and acidified with 10ml of 1:1 mixture of $HCl:H_2O$. This was filtered into a 50ml volumetric flask and made up to the mark with deionised water. The digested samples were then transferred into plastic bottles and taken for analysis.

2.4. Metal Analysis of Sample

After the digestion process, the digests were taken to Energy Research Laboratory at Usman Dan-Fodio University, Sokoto State, Nigeria, for AAS metal analysis. Pb, Cu, Cd, and Fe are the metals that were analyzed in the samples. Atomic absorption spectroscopy measures the absorption of specific wavelength of radiation by neutral atoms which are present in the ground state and gets excited. The chemical compounds are dissociated into free atoms required for the atomic absorption measurements are produced by supplying enough thermal energy. Hollow cathode lamp helps in the generation of specific wavelength of radiation. The solution of the sample aspirated into the flame aligned in the light beam serves the purpose of atomic absorption spectroscopy.

$$\text{The level of heavy metal in the sample (mg / kg)} = \frac{\text{Conc.of metal from instrument X Vol.of digest}}{\text{mass of sample taken for digestion}}$$

2.5. Basic Principle of AAS

Atomic absorption spectroscopy (AAS) is a spectro-analytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution or directly in solid samples used in pharmacology, biophysics and toxicology research. The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law.

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law. The relationship between the spectroscopic phenomena is given by:

$$\underset{\text{(unexcited)}}{\textbf{Atom}} + \underset{\text{(photon)}}{h\nu} \underset{\text{emit}}{\overset{\text{absorb}}{\rightleftharpoons}} \underset{\text{(excited)}}{\textbf{Atom}}$$

There are five basic components of an atomic absorption instrument:

(1). The light source that emits the spectrum of the element of interest. (**2).** An "absorption cell" in which atoms of the sample are produced [flame, graphite furnace, Mercury/Hydride System Cell (MHS cell), Flow Injection Analysis System Cell or Flow Injection for Atomic Spectroscopy System Cell (FIAS cell), and Flow Injection Mercury System Cell (FIMS cell)]. (**3).** A monochromator for light dispersion. (**4).** A detector, which measures the light intensity and amplifies the signal. (**5).**A display that shows the reading after it has been processed by the instrument electronics

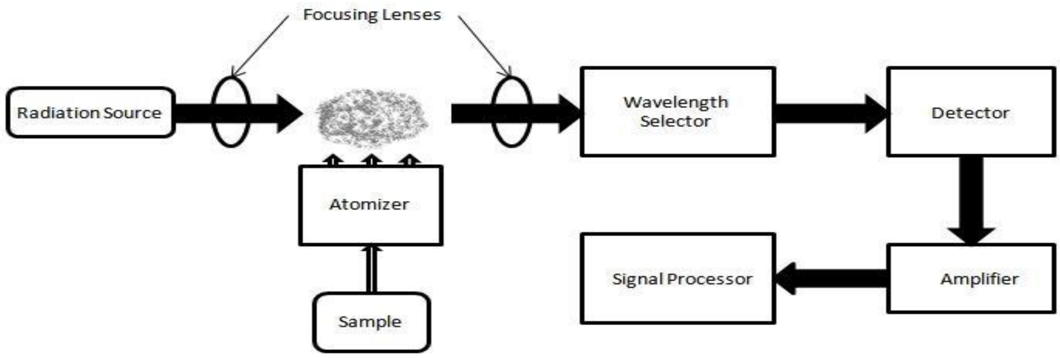

Figure 1: A.A.S. Block Diagram

In order to analyze a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and electrothermal (graphite tube) atomizers. The atoms should then be irradiated by optical radiation, and the radiation source could be an element-specific line radiation source or a continuum radiation source. The radiation then passes through a monochromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector.

There are two basic types of atomic absorption instruments: *single beam* and *double-beam.*

2.5.1. Single beam AAS

The light source (hollow cathode lamp or electrodeless discharge lamp) emits a spectrum specific to the element of which it is made, which is focused through the sample cell into the monochromator. The light source must be electronically modulated or mechanically chopped to differentiate between the light from the source and the emission from the sample cell. The monochromator disperses the light and the specific wavelength of light isolated passes to the detector, which is usually a photomultiplier tube. An electrical current is produced depending on the light intensity and processed by the instrument electronics. The electronics will measure the amount of light attenuation in the sample cell and convert those readings to the actual sample concentration. With single-beam systems, a short warm-up period is required to allow the source lamp to stabilize.

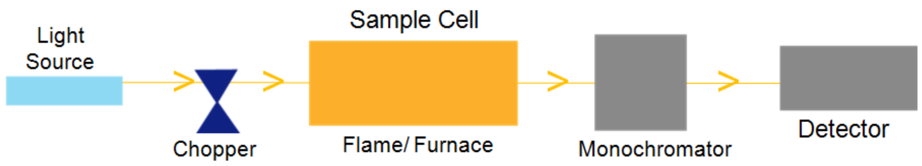

Figure 2: Diagram of a Single Beam AAS

2.5.2. Double beam AAS

The light from the source lamp is divided into a ***sample* beam**, which is focused through the sample cell, and a ***reference* beam**, which is directed around the sample cell. In a double-beam system, the readout represents the ratio of the sample and reference beams. Therefore, fluctuations in source intensity do not become fluctuations in instrument readout, and stability is enhanced. Generally, analyses can be performed immediately with no lamp warm-up required.

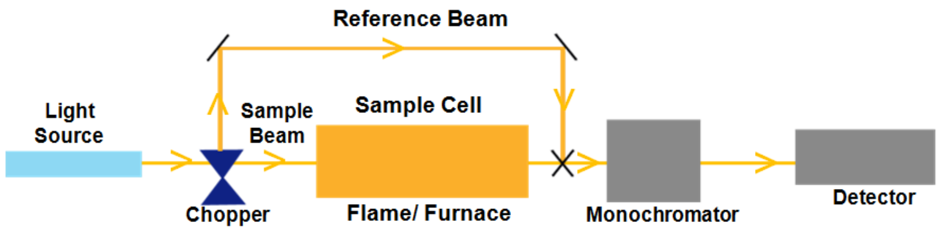

Figure 3: Diagram of a Double Beam AAS

The Instrument is usually calibrated by blank solution and finally metal content in the sample is analyzed. Four or five standard solutions of different concentrations are prepared. The absorbances of these standard solutions are measured and a calibration curve is prepared from these values. Then the absorbance of the sample solution is measured and then the concentration of the element from the calibration curve is determined. Each element is usually determined at a particular wavelength. Concentrations are usually in ppm = mg/L = µg/g. All the elements detectable or that can normally be measured by atomic absorption spectrometry include: Al, Sb, As, Ba, Be, Bi, B, Cd, Cs, Ca, Cr, Co, Cu, Dy, Er, Eu, Gd, Ga, Ge, Au, Hf, Ho, In, Ir, Fe, La, Pb, Li, Lu, Mg, Mn, Hg, Mo, Nd, Ni, Nb, Os, Pd, P, Pt, K, Pr, Re, Rh, Rb, Ru, Sm, Sc, Se, Si, Ag, Na, Sr, Ta, Te, Tb, Tl, Tm, Sn, Ti, W, U, V, Yb, Y, Zn, and Zr.

3. Results and Discussion

Table 1 shows the results of the heavy metal analysis for the juice samples. The concentration of Cadmium (Cd) in this study for sample 1, sample 2, sample 3, sample 4, sample 5, sample 6, sample 7, and sample 8 are, 0.055mg/kg, 0.045mg/kg, 0.047mg/kg, 0.047mg/kg, 0.071mg/kg, 0.079mg/kg, 0.075mg/kg, 0.085mg/kg respectively, which all are below the joint maximum accepted levels by WHO/FAO of (0.1mg/kg) as shown in table 2.

Cd is a non-essential element in food and natural waters and it accumulate principally in the kidneys and liver. The highest concentration of Cd was recorded in sample 8. Similar study were reported concerning heavy metals just like the present study (Sajib *et al.,* 2014) (Eneji *et al.*,2015) all conducted researches on heavy metals in fruit and fruit juices. The effects of cadmium on humans are nephrotoxicity, osteotoxicity, cardio vascular toxicity and effect on reproduction, development and genotoxicity. Kidney damage also occurs as a result of cadmium exposure. Occasional peaks in cadmium intake may cause a drastic increase in fractional absorption of cadmium. Ingestion of highly contaminated foodstuffs results in acute gastrointestinal effects in form of diarrhea and vomiting about 5% of ingested cadmium absorbed. The absorption of cadmium in foodstuffs may be of importance for the evaluation of the health hazards associated with areas of cadmium contamination or high cadmium intake. Cadmium is transported to the liver through the blood which form a protein complexes that are transported to the kidney and accumulated in the kidney where it damage filtering mechanisms and cause the excretion of essential protein and sugar from the body and chronic inhalation of high level of cadmium also causes fibrotic and emphysematous lung damage, but also has major effect in bones and kidney (Meulenbelt *et al.,* 2001)

Pb is not detected throughout the samples and therefore they have or pose no any harmful health effect to human life and are consumable. Similar studies were also reported (Sajib *et al.*, 2014) (Eneji *et al.*,2015) reported the concentration of Pb as 0.068 plus or minus 0.03mg for tamarind fruit and mango respectively. Lead is naturally occurring substance and can be found both in organic and inorganic forms; in organic forms it can affect the central nervous system (CNS), peripheral nervous system (PNS, and Hematopoietic, renal, cardiovascular and reproductive system. Organic toxicities tend to predominantly affect the CNS (Kaya *et al*., 2002).Under typical conditions Lead is absorbed and stored in several body compartments. Five to ten percent is found in blood. Most of which is located in erythrocytes, 80-90% is taken up in the bone and stored in the hydroxyl appetite crystals, where it easily exchange with blood (Maag et al., 2001)

Iron (Fe) is an essential element in man and plays a vital role in the formation of haemoglobin, oxygen and electron transport in human body. The concentration of iron (Fe) in this study were for sample 1, sample 2, sample 3, sample 4, sample 5, sample 6, sample 7, and sample 8 are, 19.561mg/kg, 18.864mg/kg, 18.901mg/kg, 43.453mg/kg, 28.956mg/kg, mg/kg, 31.672mg/kg, and 57.509mg/kg respectively and fall above the permissible levels of WHO/FAO.

Copper (Cu) is an essential trace element required for proper health in an appropriate limit. its high uptake in fruits can be harmful for human health and in the same way, lower uptake in human consumption can cause a number of symptoms like growth retardation, skin ailments, gastrointestinal disorders, etc from this study, its concentration are, 0.279mg/kg, 0.812mg/kg, 0.630mg/kg, 0.654mg/kg, 26.018mg/kg,1.03 mg/kg, 0.4mg/kg, and 0.921mg/kg for sample 1, sample 2, sample 3, sample 4, sample 5, sample6, sample 7, and sample 8 respectively and six of the analyzed samples were above the permissible limit of WHO/FAO except sample 7 which is below the maximum permissible limit.(Sobukola *et al.*, 2010) reported 0.015mg/kg, 0.002mg/kg, 0.003mg/kg and 0.009mg/kg for pineapple, orange, pawpaw and banana respectively. It work with ion to form red blood cells, and it also aids in the production of myelin collagen and melanin. Copper's antioxidant property allows it to get rid of the free radicals that can damages human cells, which could lead to premature aging and various health conditions (Hartmann *et al.*, 2009). Exposure to copper can lead to its accumulation in brain, liver, kidney and cornea leading to the classic of impairment and stigma of Wilson diseases and Indian childhood cirrhosis. Many of the heavy metals have been implicated as carcinogens in setting of chronic exposure (Khan *et al.*, 2008).

Table 1. The Mean Results of AAS Essential and Toxic Heavy Metal Analysis in the Juice Samples

Spice samples	Fe (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
JS1	19.561	0.279	0.055	Nd
JS2	18.864	0.821	0.045	Nd
JS3	18.901	0.630	0.047	Nd
JS4	43.453	0.654	0.047	Nd
JS5	28.956	26.018	0.071	Nd
JS6	35.269	1.030	0.079	Nd
JS7	31.672	0.400	0.075	Nd
JS8	57.509	0.921	0.085	Nd

Key: JS = Juice Sample, Nd = Not detected

In table 2 below, the maximum permissible limits of the four heavy metals as analyzed in this work are presented which are given by the joint FAO/WHO organizations.

Table 2. Maximum Permissible Limit (MPL) of heavy Metals in Juices by (WHO/FAO).

Metal	MPL (mg/kg)
Fe	0.80
Cu	0.05 – 0.5
Cd	0.10
Pb	0.2 (0.1 for oranges)

The heavy metal concentrations determined in the samples are also represented in Figure 4 as shown below together with the limits by the joint FAO/WHO.

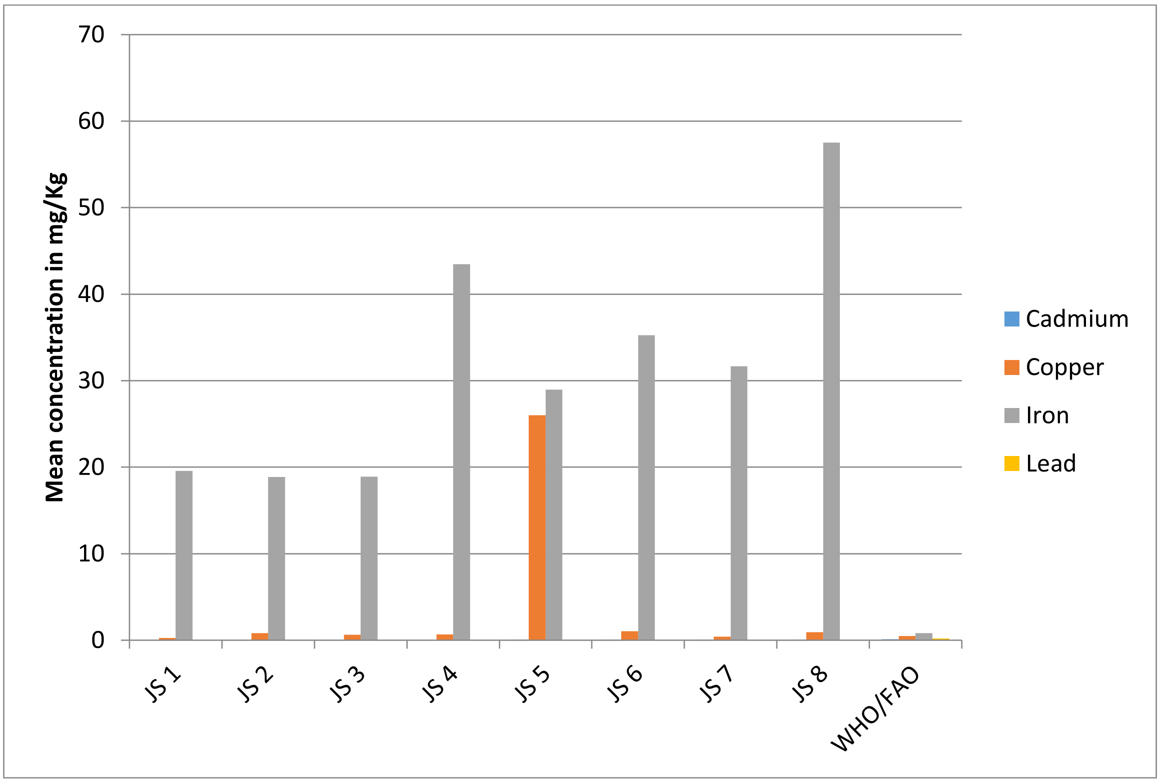

Figure 4. Mean concentrations of essential and toxic Heavy Metals in the juice samples in mg/kg.

Key: JS= Juice Sample

4. Conclusions

In conclusion, fruits and vegetable juices are largely consumed throughout the entire world as they contain micro nutrients which are essential for health. AAS has been successfully applied in the analysis of the juice samples. The fresh tomato juice had the highest concentration of Fe and Cd while the highest concentration of Cu was observed in fresh pineapple juice. All the analyzed samples had Cd levels which are below the limit of joint WHO/FAO. Pb was not detected in all the juice samples. Fe and Cu fall above the maximum limit by WHO/FAO. This implies that consumers should be cautious, because these metals Fe and Cu are only essential at appropriate concentrations.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors are very grateful to the staff in the Department of Pure and Applied Chemistry, Kebbi State University of Science and Technology, Aliero, for providing all the chemicals, glass ware apparatus and other facilities used in this work. Special thanks go to Dr A. Muhammad, Head of the Department of Chemistry, KSUST, Aliero for his help regarding the chemicals and materials in this work.

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