International Journal of Modern Analytical and Separation Sciences, 2020, 8(1): 1-13
Modern Scientific Press
International Journal of Modern Analytical and Separation Science
ISSN: 2167-7778
Journal homepage: www.ModernScientificPress.com/Journals/IJMAS.aspx
Florida, USA

Article

Heavy Metal Analysis in Spices Available in Nigerian Community by Atomic Absorption Spectroscopy

M. S. Zubairu [1]* and J. Zegge [2]

[1] *Department of Pure and Applied Chemistry, Kebbi State University of Science and Technology, Aliero, P.M.B. 1144, Birnin Kebbi, Kebbi State, Nigeria*

[2] *Department of Pure and Applied Chemistry, Kebbi State University of Science and Technology, Aliero, P.M.B. 1144, Birnin Kebbi, Kebbi State, Nigeria*

* Author to whom correspondence should be addressed; E-Mail: mszubair2015@gmail.com

Article history: Received 22 September 2019, Revised 16 February 2020, Accepted 20 March 2020, Published 16 April 2020.

Abstract: Spices are widely used in Nigeria for cooking as food condiments or taste enhancing substances, some of which are locally made by different tribal groups. They are also used in many parts of the world for cooking, processing of foods or other applications. In this study, an analysis of four heavy metals: Fe, Cu, Cd, and Pb was done by FAAS technique. Eight spice samples namely: maggi star, curry powder, thyme, mr. chef, royco, ajino-motto, indomie spice and fermented locust beans were analyzed for these heavy metals. The ajino-motto sample had the highest concentration of Fe and Cu while the highest concentration of Cd was observed in the fermented locust bean sample. All the analyzed samples had Cd levels that breached the international standard by WHO. Pb however, was not found in any of the samples which is good to know. Fe and Cu fall below the maximum permissible limits by WHO. Though, some of the values of Cd are slightly above WHO limit, it generally implies that consumers of these spices should be cautious.

Keywords: spices, heavy metal, AAS analysis, sample digestion

1. Introduction

A spice is a dried seed, fruit, root, bark, or vegetable substance primarily used for flavouring, coloring or preserving food. Many spices have antimicrobial properties. This may explain why spices are more commonly used in warmer climates, which have more infectious diseases, and why use of spices is especially prominent in meats, which is particularly susceptible to spoiling (Thomas, et al., 2012). Spices are dried parts of plants, which have been used as dietary components of food often to improve its color, aroma, palatability and acceptability. They consist of rhizomes, barks, leaves, fruits, seeds, and other parts of the plant. There have been many definitions for ‘spices’ which are often used interchangeably with ‘herbs’. Spices are defined as the aromatic parts of any plants that are used to add flavor to food. But herbs are the aromatic leaves of any plant that can add flavor to food .The origins of herbs are leaves of plants that have soft stems or are shrubs but not trees. In general, spices are defined as unleafy dried substances, whereas herbs are leafy un-dried substances (Kassa et al., 2015).

The addition of spices that may be contaminated with trace heavy metals to food as a habit may result in accumulation of these metals in human organs and lead to different health hazards. Contamination of plants and processed spices could occur through air, water and soil during cultivation an during industrial processing and packaging .The Nigerian culinary holds a wide and colorful array of spices which makes the country an important center for spices and herbs. In Nigeria, spices are not only used in culinary but also as galenicals in folk medicine for their putative health benefits. In times past, spices were used primarily for their organoleptic and preservative properties. However; recent studies on their medicinal and nutritional properties have opened new vistas in the fields of nutraceuticals and functional foods. A number of these studies have come up with exciting results; for(clove), and capsaicin (red pepper), were experimentally shown to control oxidative stress in cells due to their antioxidant properties and their capacity to block the production of oxygen radicals in aerobic metabolism and interfering with signal transduction pathways (Rubió, et al., 2010).

Many common spices have outstanding antimicrobial effects. On the other hand, the process of preparation and handling can make them a source of food poisoning [Sherman and billing, 1998]. Moreover, in the last three decades, mainly because of their medicinal values, the use of spices has increased markedly in most regions of the world. Several researches have shown that heavy metals could be present in spices and the addition of contaminated spices to food may result in accumulation of these metals in human organs. Heavy metals above the permissible levels affect human health and may result in illness to human foetus, abortion and preterm labor, and mental retardation to children. Adults also may experience high blood pressure, fatigue kidney and neurological disorder (Mubeen et al, 2009).

Heavy metals is the generic term for metallic elements having an atomic weight higher than 40.04 (the atomic mass of Ca) .Heavy metals enter the environment by natural and anthropogenic means. Such sources include: natural weathering of the earth’s crust, mining, soil erosion, industrial discharge, urban runoff, sewage effluents, pest or disease control agents applied to plants, air pollution fallout, and a number of others (Ming-Ho, 2005). According to a definition, a general term “heavy metals” applies to the group of metals and metalloids with atomic density is greater than 4g/cm^3, or is 5 times or more, greater than water density . In this group, lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), silver (Ag), arsenic (As), and the platinum group elements are included. There is also an alternative classification of metals which is based on their coordination chemistry. This classification categorizes heavy metals as class B metals that are non-essential trace elements and are highly toxic elements such as Hg, Ag, Pb, Cd and Ni (Ackova, 2018). The term "heavy metal" also refers to any metallic element that has a relative density greater than 4g/cm^3. Heavy metals include; Lead (Pb), Cadmium (Cd), Zinc (Zn), Mercury (Hg), Arsenic (As), Silver (Ag), Chromium (Cr), Copper (Cu), Iron (Fe) and the Platinum group elements. They are non-biodegradable and industrious natural contaminants which get saved on the surface and afterward retained into the tissues of vegetables. They accumulate in the food chain with risks to the health of animals and humans**.**

The use of spices in culinary predates recorded history and is said to have been an integral part of local dishes in South Asia and the Middle East as far back as 2000 BCE (Tapsell et al., 2006). The legendary Christopher Columbus' explorations in 1492 were in search of herbs and spices [Kaefer and Milner, 2011]. In Mesopotamia, the cradle of civilization where agriculture began, there is evidence that humans were using thyme for their health properties as early as 5000 BC and were growing garlic as early as 3000 BC (Singletary, 2016). Spices are often gathered from plants when they have stopped flowering. Spices are functional foods, these are foods that can be demonstrated to have a beneficial effect on certain target functions in the body beyond basic nutritional requirements. With the current emphasis on eating more healthy diets that are low in fat and salt, people are turning to various herbs and spices to flavor their food. The culinary herbs and spices that are used to enhance the flavor of vegetables, soups, stir-fry, and pasta dishes can be derived from the bark, buds, flowers, leaves, fruit, seeds, rhizome, or roots of plants (lobo et al., 2010).

Many work have been done on food spices, some of these include the study by Kassa *et al.*, 2015, on Validation of a method for determining heavy metals in some Ethiopian Spices by dry ashing using Atomic Absorption Spectroscopy. The optimal conditions for determination of Pb, Cd and Cr in spices sample by FAAS after dry mineralization 1g spice samples for 5hr at a temperature of 500 ºC followed by dissolution in 4 mL HNO in Fenugreek, Black cumin, garlic and 3ginger. Working linear ranges are given: for Cd, 0 -6mg/l, for Cr, 0.2-1mg/l and for Pb, 2-20 μ g/l. Found detection limits are0.2mg/l, 0.13mg/l and 0.02 μ g/l for Cd, Cr and Pb, respectively. Kudirat and Fumilayo, (2011), worked on Heavy metal levels in vegetables from selected markets in Lagos, Nigeria**.** Recently matured leafy vegetable (fluted-pumpkin) from ten (10) different markets were sampled, digested using 98% nitric acid (HNO_3) and analyzed with the aid of Atomic Absorption Spectrophotometer (AAS) to determine heavy metals. The mean concentration for each heavy metal in the samples gotten from each market were calculated and the comparison of these data was done amongst the ten markets, and compared with the permissible levels set by the FAO and WHO. Gaya and Ikechwuku, (2016), worked on heavy metal contamination of selected spices obtained from Nigeria. In their study, the levels of trace metals (Cd, Cr, Cu, Co, Fe, Mn, Ni, Mo, Pb, and Zn) in twenty two spices representing four spice groups (seeds, bulbs, leaves, fruit pods and rhizome) from a major market in Northern Nigeria were determined using atomic absorption spectroscopy, and assessed based on regulatory standards. Mohammed, (2016) worked on using stripping voltammetry to determine heavy metals in cooking spices used in Iraq. Monitoring heavy metals in herbs and spices is increasingly being reported from different parts of the world. In Iraq and the Middle East, there is limited information on the levels of heavy metals in these additives, although several spices are widely used in daily diets. In his study the concentrations of Cu, Zn, Fe, Mn, Cr, Ni, Co, Cd, Pb, and Hg in 32 kinds of natural spices traditionally consumed in Iraq were determined by stripping voltammetry. The highest concentration found (in mg kg^{-1} dry weight) was that of iron (32.44-1,147) followed by manganese (6.42-285.8), zinc (5.45-129.3), and copper (2.58-30.71) respectively.

Food spices are used as diet components often to improve colour, aroma, palatability and acceptability of food. Natural spices include ginger, garlic, onion and locust beans and processed spices include curry, thyme, nutmeg and beef spicy. Due to the significant amount of spices consumed, it is important to know the toxic metal contents in these spices. Hence, results obtained from this kind of study can serve as a guide or source of information to people consuming these spices, thereby providing vital information on the safety of these substances as related to health and nutrition. The main aim of this work therefore, is to screen for the presence of essential and toxic heavy metals in spices that are commonly consumed in Nigeria by using AAS technique.

2. Materials and Methods

2.1. Sample Collection

The spice samples used in this study were purchased from a super market and local market in Aliero town, Kebbi State, Nigeria. A total of eight samples were purchased which include the following maggi star, curry powder, thyme, mr. chef, royco, ajino-motto, indomie spice and fermented locust beans (called dawdawa in Hausa language). These samples include plant and chemical based spices. The samples were collected in clean plastic bags and taken to the laboratory for preparation. These food condiments represent some of the most widely used taste enhancers in Nigeria. The samples were labeled as SS1= Maggi star, SS2= Curry powder, SS3= Indomie spice, SS4= Mr. chef, SS5= Ajino-motto, SS6= Thyme, SS7= Fermented locust beans, and SS8= Royco.

2.2. Sample Preparation

Some of the samples such as locust beans (fermented) and thyme (packaged) which are plant based spices were grinded to fine particle size while the other spices were used as purchased.

2.3. Digestion of Sample

The method of sample digestion by Oladoye and Jegede, 2016, was adopted with a slight modification. 20mL of concentrated nitric acid (HNO_3) is added to 2g of the prepared sample in a closed vessel. The addition of the nitric acid is to remove organic material by decomposing them into carbon dioxide (CO_2); and also to convert the metals present into soluble forms according to the equation:

$$(CH_2)_n + 2HNO_3 \rightarrow nCO_2 + 2NO + 2H_2O$$

The mixture (in a beaker covered with a watch glass) was evaporated on a hot plate in a fume cupboard until the reddish-brown fumes disappears leaving white fumes. The digest was filtered and made up to 50 ml with deionized water and was then ready for AAS analysis.

2.4. Metal Analysis

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:

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

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

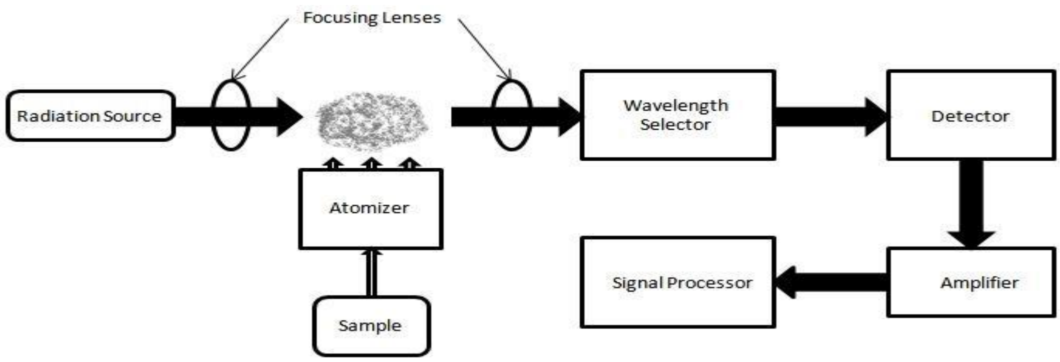

Figure 1: A.A.S. block diagram

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.

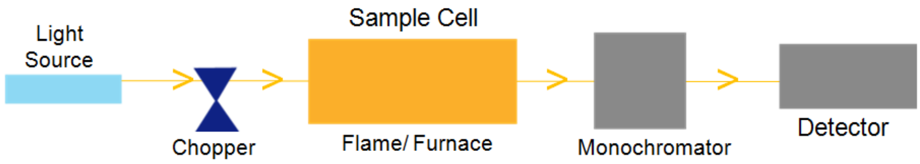

Figure 2: Diagram of a Single Beam AAS

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.

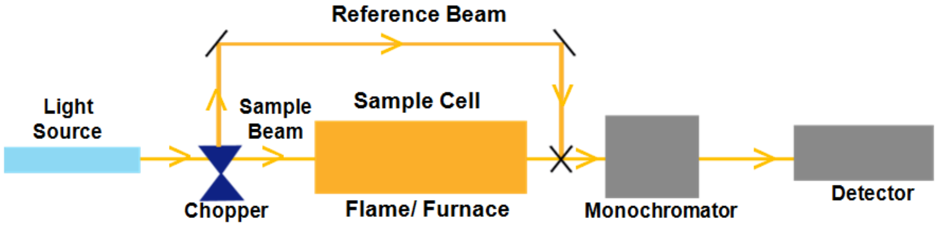

Figure 3: Diagram of a Double Beam AAS

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 mean results of essential and toxic heavy metal analysis of Fe, Cu, Cd, and Pb by AAS for the Spice Samples. The mean concentrations of Fe in the samples follow the order: SS5 > SS8 > SS1 > SS6 > SS3 > SS7 > SS2 > SS4. The order for Cu is: SS5 > SS8 > SS1 > SS7 > SS2 > SS6 > SS4 > SS3. The order for Cd is: SS7 > SS8 > SS5 > SS6 > SS4 > SS3 > SS1 > SS2 while Pb was not detected in any of the samples. Thus SS5 had the highest concentration of Fe and Cu while the highest concentration of Cd was observed in SS7. Pb however, was not found in any of the samples. The chart in Figure 4 represents these concentrations for all the four metals that were analyzed in the spice samples. In comparison with the maximum permissible limits by WHO, both Fe and Cu fall below these limits while Cd was above the limit set by WHO in all the samples.

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

Spice samples	Fe (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
SS1	30.645	0.9075	0.470	Nd
SS2	15.323	0.3625	0.423	Nd
SS3	25.140	0.1525	0.585	Nd
SS4	14.773	0.2125	0.598	Nd
SS5	38.078	1.4525	0.730	Nd
SS6	28.168	0.3025	0.670	Nd
SS7	24.130	0.8175	0.780	Nd
SS8	30.828	1.120	0.775	Nd

Key: SS = Spice Sample, Nd = Not detected

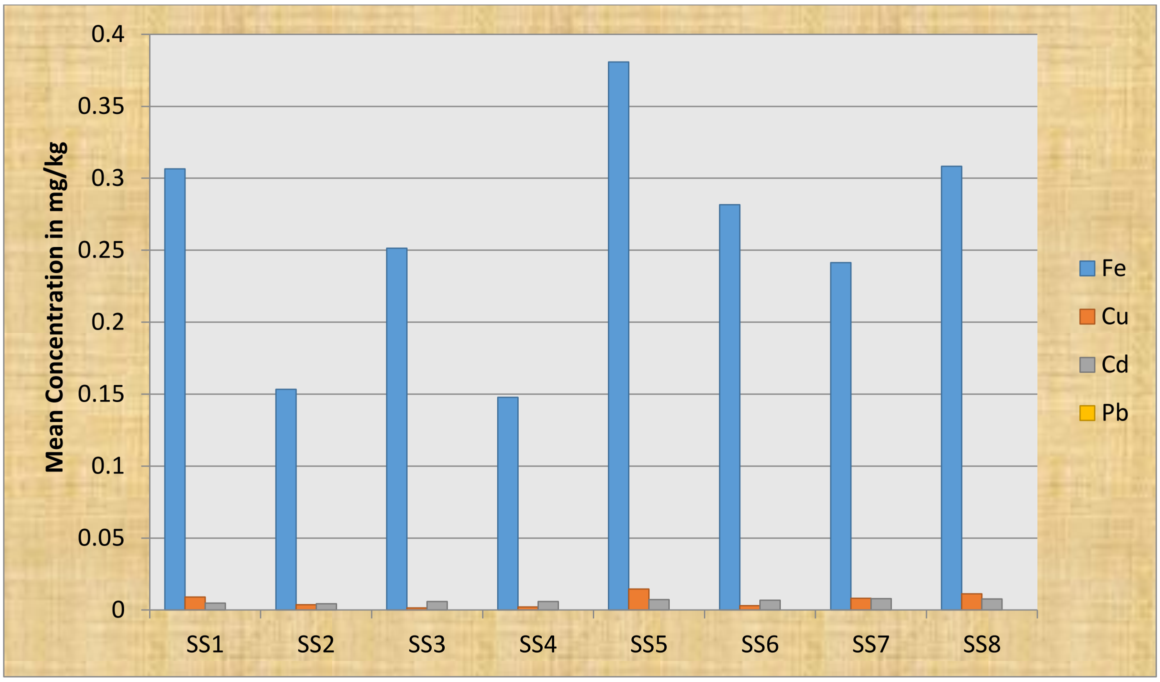

Figure 4: The Mean Concentration of Essential and Toxic Heavy Metals in the Spice Samples in mg/kg. **(**Key: SS = Spice Sample)

Table 2. Maximum Permissible Limit (MPL) of Heavy Metals in Spices by World Health Organization (WHO)

Metal	MPL (mg/kg)
Fe	300
Cu	40
Cd	0.3
Pb	5

Source: Bazargani-Gilani and Pajohi-Alamoti, 2017

In the context of nutrition, a mineral is a chemical element required as an essential nutrient by organisms to perform functions necessary for life. Minerals originate in the earth and cannot be made by living organisms. Plants get minerals from soil. Most of the minerals in a human diet come from eating plants and animals or from drinking water. As a group, *minerals* are one of the four groups of essential nutrients, the others of which are vitamins, essential fatty acids, and essential amino acids. Mineral nutrients are divided into two broad groups; major and trace elements. Major minerals represent 1 per cent of bodyweight and are required in amounts greater than 100 mg per day, while trace minerals make up less than 0.01 per cent of bodyweight and are essential in much smaller amounts. The five major minerals in the human body are calcium, phosphorus, potassium, sodium, and magnesium. All of the remaining elements in a human body are called "trace elements". The trace elements that have a specific biochemical function in the human body are sulfur, iron, chlorine, cobalt, copper, zinc, manganese, molybdenum, iodine and selenium. Some essential metals are involved in numerous biochemical processes and adequate intake of certain essential metals relates to the prevention of deficiency diseases. Iron (Fe) deficiency anemia for instance affects one third of the world population. On the other hand, excessive iron intake has been associated with an overall increase risk of colorectal cancer. Copper (Cu) and zinc (Zn) are essential metals which perform important biochemical functions and are necessary for maintaining health throughout life. Zn constitutes about 33 ppm of adult body weight and is essential as constituent of many enzymes involved in number of physiological functions, such as protein synthesis and energy metabolism. Zn deficiency, resulting from poor diet, alcoholism and malabsorption, causes dwarfism, hypogonadism and dermatitis, while toxicity of Zn due to excessive intake may lead to electrolyte imbalance, nausea, anemia and lethargy. Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. The criteria used, and whether metalloids are included, vary depending on the author and context. In metallurgy, for example, a heavy metal may be defined on the basis of density, whereas in physics the distinguishing criterion might be atomic number, while a chemist would likely be more concerned with chemical behaviour. More specific definitions have been published, but none of these have been widely accepted. A density of more than 5 g/cm^3 is sometimes quoted as a commonly used criterion. Some heavy metals are either essential nutrients (typically iron, cobalt, and zinc), or relatively harmless (such as ruthenium, silver, and indium), but can be toxic in larger amounts or certain forms. Other heavy metals, such as cadmium, mercury, and lead, are highly poisonous. Potential sources of heavy metal poisoning include mining, tailings, industrial wastes, agricultural runoff, occupational exposure, paints and treated timber. Hence, this study indicated that no toxic Pb was found in the samples which is good news, but Cd, however, was found to be present above the MPL set by WHO. Though, some of the values of Cd are slightly above WHO limit, it generally implies that consumers of these spices should be cautious.

4. Conclusions

In conclusion, it is clear that many people use spices in Nigeria and other places for cooking, processing of foods or other applications. AAS has been successfully applied in the analysis of the spice samples. The ajino-motto sample had the highest concentration of Fe and Cu while the highest concentration of Cd was observed in the fermented locust bean sample. All the analyzed samples had Cd level that breached the international standard by WHO, which is not good news. Pb however, was not found in any of the samples which is good to know. Fe and Cu fall below the maximum permissible limits by WHO. Though, some of the values of Cd are slightly above WHO limit, it generally implies that consumers of these spices should be cautious.

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.

References

[1] Akwasi Akomeah Agyekum, Felicia Akuamoa, Isaac Delali Kotton, Isacc Kwabena Asare, John Opoku Danquah, Daniel Armah. Evaluation of trace metal contents of three local spices on Accra market. *International journal of nutrition and food sciences*. 2015, 4(6): 681-687.

[2] Ata1 S., S. Tayyaband A. Rasool. Analysis Of Non-Volatile Toxic Heavy Metals (Cd, Pb, Cu,Cr And Zn) In ALLIUM SATIVUM (Garlic) And Soil Samples ,Collected From Different Locations Of Punjab, Pakistan By Atomic Absorption Spectroscopy. *Mediterranean Journal of Chemistry*, 2014, 2(5): 667-678

[3] Ajasa M.A, Bello O.M, Ogunwande A.I., Olawore, O.N. Heavy trace metals and macronutrient status in herbal plants of Nigeria. *Food Chemistry*, 2004, 85: 67-71.

[4] Ackova D.G. Heavy metals and their general toxicity on plants. *Plant Science Today*, 2018, 5(1):1418. doi:https://dx.doi.org/10.14719/pst.2018.5.1.355.

[5] Behnaz Bazargani-Gilani, Mohammadreza Pajohi-Alamoti. Evaluating, of Heavy Metal Contaminations in the Most Applicable Food Spices and Flavors in Hamedan Iran. *Arch. Hyg. Sci.* 2017, 6(3): 268-275

[6] Farhin Inam, Sujata Deo, Neha Narkhede, (2013). Analysis of minerals and heavy metals in some spices collected from local market. *IOSR Journal of Pharmacy and Biological Sciences*, 2013, 8: 40-43.

[7] GayaU. I and Ikechukwu S.A, (2016). Heavy metal contamination of selected spices obtained from Nigeria. *J. Appl. Sci. Environ. Manage*. 2016, 20 (3): 681-688.

[8] . Henrietta Aritetsoma Ogbunugafor, Chidozie Godwin Ugochukwu and Ada Evelyn Kyrian-Ogbonna. The role of spices in nutrition and health: a review of three popular spices used in Southern Nigeria. *Food Quality and Safety*, 2017, 1(3): 171-185

[9] Hifsa Mubeen, Ismat Naeem, Abida Taskeen and Zeb Saddiqe. Investigation of heavy metals in commercial spices brands, *New York Sciences journal*, 2009, 2(5).

[10] Krejpcio1 Z., E. Król1 and S. Sionkowski. Evaluation of Heavy Metals Contents in Spices and Herbs Available on the Polish Market. *J. of Environ. Stud*. 2006, 16(1): 97-100.

[11] Kaefer, C. M., Milner, J. A. *Herbs and spices in cancer prevention and treatment*. Chapter 17. In: Benzie, I. F. F., Wachtel-Galor, S. (eds.) Herbal Medicine: Biomolecular and Clinical Aspects. CRS Press/Taylorand Francis, Boca Raton, FL, 2011.

[12] Khlifi, R. & Hamza-Chaffai, A. Head and neck cancer due to heavy metal exposure via tobacco smoking and professional exposure: A review. *Toxicology & Applied Pharmacology*, 2010, 248: 71-88.

[13] Kassa Belay, Abi Tadesse, and Tesfahun Kebede. Validation of a Method for Determining Heavy Metals in Some Ethiopian Spices By Dry Ashing Using Atomic Absorption Spectroscopy. *International Journal of Progressive Sciences and Technologies (IJPSAT)*, 2015, 1(2): 28-33

[14] Ladipo Margaret Kudirat and Doherty Victoria Funmilayo. Heavy metal levels in vegetables from selected markets in Lagos, Nigeria. *African Journal of Food Science and Technology*, 2011, 2(1): 018-021.

[15] Logan, T.J., Goins, L.E., Lindsay, B.J. Field assessment of trace element uptake by vegetables from n-Viro soil. *Water and Environmental Research*, 1997, 69: 28-33.

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

[17] Mohammed Hashim Matloob, (2016). Using stripping voltammetry to determine heavy metals in cooking spices used in Iraq. *Pol. J. Environ. Stud.* 2016, 25(5): 2057-2070.

[18] Mubeen H, Naeem I, Taskeen A, Saddiqe Z. Investigations of heavy metals in commercial spices brands. *New York Science Journal*, 2009, 2: 155-200.

[19] Marian Asantewah Nkansah, Cosmos Opoku Amoako. Heavy metal content of some common spices available in markets in the Kumasi metropolis of Ghana. *American journal of scientific and industrial research*. 2010, 1(2): 158-163

[20] Patwardhan, B., Warude, D., Pushpangadan, P., Bhatt, N. Ayurveda and traditional Chinese medicine: a comparative overview. *Evidence Based Complement Alternative Medicine*, 2005, 2: 465-473.

[21] Peter Olusakin Oladoye and David Olaoluwa Jegede. Evaluation of Effects of Heavy Metal Contents of Some Common Spices Available in Odo-Ori Market, Iwo, Nigeria. *J Environ Anal Chem*, 2016, 3(1): 1-4

[22] Rubió, L., Motilva, M. J., Romero, M. P. Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles. *Critical Reviews in Food Science and Nutrition*, 2013, 53: 943-953.

[23] Singletary, K. Thyme: history, applications, and overview of potential health benefits. *Nutrition Today*, 2016, 51: 40-49.

[24] Sherman WP, Billing J. Antimicrobial functions of spices: why some like it hot. *Quarterly Review of Biology*, 1998, 73: 1-47.

[25] Tapsell, L. C., et al. Health benefits of herbs and spices: the past, the present, the future. *The Medical Journal of Australia*, 2006, 185: S4-S24.

[26] Thomas F, Daoust SP, Raymond M. Can we understand modern human without considering pathogens. *Evolutionary applications*, 2012, 5: 368-379.