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Original Research Article

Review on Levels of Some Selected Heavy Metals in Commercially Available Rice in Ethiopia

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Abstract: The objective of this seminar is to present the levels of metals in commercially available imported and Ethiopian rice. The levels of thirteen metals (Ca, Mg, K, Na, Fe, Mn, Zn, Cu, Co, Ni, Cr, Cd and Pb) were determined in six varieties of raw rice collected from Addis Ababa supermarket, Fogera town and Amahara Regional Agricultural Research Institute and in one selected cooked rice by flame atomic absorption spectrometry after digesting the powdered rice samples with HNO_3, $HClO_4$ and H_2O_2 mixture. The levels of metals found in the imported and Ethiopian rice, respectively, were in the ranges (mg/kg): Ca 75.8- 630, 205-427; Mg 90.6-150, 99.5-2250; K 1680-2150, 1100-3020; Na 70.6-78.6, 26.7-80.9; Fe 48.9-117, 41.3-113; Mn 4.1-15.5, 3.7-16.6; Zn 16.4-25.7, 15.6-140; Cu 2.7-4.9, 3.3-15; Co 12.6- 14.6, 8.8-10.4; Ni 2.5-75.1, 41.5-69.7; Cr 2.2-3.12, 2.32-4.82; Cd Comparison between levels of metals in the imported and Ethiopian rice showed significant differences for most of the metals. The results indicated that Ethiopian rice is comparatively rich in essential metals than imported one. A statistical analysis of variance (ANOVA) at 95% confidence level for metal determination indicated significant difference between the means of each variety of samples. Comparison between levels of metals in cooked and raw rice showed that the difference in the level is not significant.

Keywords: Heavy metals, Rice, Ethiopian rice, imported rice.

1. INTRODUCTION

1.1 Background of the Study

Food is an essential part of human diet consumed to provide energy, maintain life and stimulate growth. It sustains, strengthens and provides cure and resistance to diseases by repairing and building of tissues in the body [1]. Metals are the most predominant and dangerous contaminants of food. The uptake of heavy metals by crops and animals from the environment varies with species, and the local environmental levels also play a role in the resulting heavy metal content in raw agricultural commodities [2]. Trace elements are potential environmental contaminations with the capability of causing human health problems if present to excess in the food chain [3].

Heavy metals like iron (Fe), tin (Sn), copper (Cu), manganese (Mn) and vanadium (V) occur naturally in the environment and could serve as plant nutrients depending on their concentrations. Mercury (Hg), lead (Pb), cadmium (Cd), silver (Ag), chromium (Cr) and Arsenic(As) are indirectly distributed as a result of human activities could be very toxic even at low concentrations [4]. For instance, cadmium is highly toxic to the kidneys and this metallic element is considered as carcinogenic. Besides, cadmium may cause bone mineralization, Arsenic (As) is also considered as carcinogenic and chronic exposure to as can because skin problems like pigmentation and keratosis. Lead can affect the nervous system, the reproductive system, and the heart and blood system.

Rice (*Oryza sativa L.)* is widely consumed staple food in the daily meals for a large part of the world human population. It ranks second in the worldwide production of cereals[5]. Rice (*Oryza sativa*

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L.) is an important grain crop that feeds over 50% world population, and accumulates cadmium (Cd) more easily than other crops. Plants acquire the necessary nutrients, such as N, P, and K, from the environment. However, they may also accumulate unnecessary and toxic metals, such as Pb, as, and Cd. Several plants have the ability to accumulate high metal concentrations. Toxic metals such as As, Cd, and Pb can be taken up from cereal crops and transferred to their grains [6]. Some studies have indicated excessive amount of heavy metals, especially for Cd, Cr, and Pb in rice grains from different countries [7] Rice is one of the components of a healthy diet and nutrition and as the food ingredients substance most used by a large. portion of society [8]. The Asian rice, *Oryza sativa* and African rice *Oryza glaberrima* are the two most cultivated species. In Asia alone; more than 2 billion people obtain 60-70 per cent calories from rice and its products [9]. It is also the most rapidly growing source of food in Africa, and is significant importance for food security and food self-sufficiency in an increasing number of low-income food deficit countries [8**]**. There is the potential contamination of these products with heavy metals in Asian countries due to weather and cultivation conditions [7**]**. Therefore, it is important to evaluate the quality of rice in terms of its heavy metals contents.

1.2 OBJECTIVE

1.2.1 General Objective

The objective of this study was to quantify the amounts of heavy metals present in commercially available rice in Ethiopia.

1.2.2 Specific Objective

The study specifically intends to:

- To determine the levels of essential and non-essential metals (Cu, Cd, Pb, Fe ,As and Zn in commercially available imported rice and Ethiopian rice.
- To compare the level of metals in raw rice and cooked rice.
- To compare the levels of metals in commercially available imported and Ethiopian rice with data obtained from other countries.

2. LITERATURE REVIEW

2.1 Rice

Rice especially white rice, *Oryza sativa* is the staple in the diet of various people including Chinese, Japanese, Korean and the other Asian countries [10]. In the Philippines, rice is the main food eaten three times a day; with fish and seafood supplying the principal source of protein.

Rice (*Oryza Sativa*) is a plant of the grass family, which provides the bulk of the human diet throughout Asia i.e. feeds more than half the world's human population. It is the world's third largest crop, behind maize and wheat [11]. The determination of trace elements in rice is very important because it is consumed in the order of magnitude of tens of kilograms per year per person by millions of people all over the world [11]. Rice is considered a complex matrix because it contains carbohydrates, mainly starch, water, proteins (18 essential amino acids), fiber, vitamins (B1, B2, B3), and minerals [12].

2.2 Essential and Non-Essential Metals

Some elements such as (K, Mg, Ca, Mn, Fe, Co, Cu, Se and Zn) are beneficial for living organisms are called essential elements. They are very important for the growth and healthy of living things. Elements like Cd, Cr, Hg, As, and Pb are non-essential and toxic [13].

2.3 Heavy metals

The term heavy metals refer to any metallic element that has a relatively high density and is toxic or poisonous at low concentration [14]. Heavy Metals are a general collective term, which applies to the group of metals and metalloids with atomic density greater than 5 g/cm^3, or more, greater than water and also heavy metals are defined as those elements with a specific density at least five times the specific gravity of water [15]. Heavy metals include Cd, Cu, Pb, Zn, Hg, As, Ag, Cr, Fe and Platinum group elements, Copper and Zinc are essential trace elements for living organisms at low concentration (<10 mg/L). However, they become toxic at high concentration (>10 mg/L).

Heavy metals are pollutants that are classified as toxic to the environment, even at minute concentrations. Although different sources of heavy metals exist, metals also occur naturally in the environment as ores and few other complexes; hence, several metals play useful roles in the environment, particularly in plant growth [16]. Heavy metals such as Pb ,Hg ,As, Cu, and Cd are substantially toxic elements known for their ability to bio-accumulate to the human body resulting in multi-organ disruption [17]. Recently heavy metal has attracted a great deal of attention worldwide and become main pollutant in areas with high anthropogenic pressure (18). Heavy metal is ubiquitous and persistent in environment, non-biodegradable and easily exerts toxic effects to human compared to other pollutants such as pesticides or organic pollutants [18].

2.4. Heavy Metals in Rice

Heavy metals and trace elements have been traced through bio-monitoring where detection of the deposition, accumulation and distribution of trace metals in ecosystems through the use of different types of vegetation have been successfully monitored. These trace elements are now sourced in the atmosphere, in the soil, plants and food substances. However, the basic source of trace metals to the body is through food since food is the main source where the body gets its nutrition [19].

Plants absorb heavy metals from the soil, the surface 25 cm depth zone of soil is the most affected by such pollutants resulting from anthropogenic activities. Heavy metals accumulate in this soil layer due to the relatively high organic matter content [20].

2.5 Source of Heavy Metal Pollution in Rice

Soils are also the main source of heavy metals in plants, where they become the main source of heavy metals in plant-derived foods [21]. Heavy metals that accumulate and migrate in soil not only influence plant growth and yield, but also have the potential to accumulate in the human body through the food chain, thus posing a serious threat to human health [21]. Heavy metals such as arsenic, cadmium, and mercury, are of primary concern in soil and food contamination, particularly in rice cropping system, because of their toxicity. Among heavy metals, Hg, Pb, and Cd had been categorized as highly toxic [22]. Lead and cadmium even at low concentrations can be very harmful when ingested over a long time [22]. Point source pollution of paddies with arsenic has occurred in both USA and SE Asia. These point sources can be considered in four categories: pesticide, base and precious metal mining and processing, ground water contamination and municipal solid wastes. Heavy metals enter the surroundings by natural means and through human activities. Various sources of heavy metals include soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops, and many others [23].

Wastewater is known to contribute significantly to the heavy metal contents of soils; hence disposal of sewage and industrial waste into agricultural lands leads to contamination of crops including rice grown on that land. This is because these effluents that are considered a rich source of organic matter and other nutrients also have high levels of heavy metals such as iron, manganese, copper, zinc, lead, cadmium, nickel and cobalt. Most of the heavy metals are extremely toxic because of their solubility in water [24]. Plants grown on a land polluted with municipal, domestic or a land polluted with municipal, domestic or industrial wastes can absorb heavy metals in the form of mobile ions present in the soil through their roots or through foliar absorption.Rice can absorb metals from soil as well as from deposits on the parts of the rice exposed to the air from polluted environments. Emission of heavy metals from the industries and vehicles may be deposited on the rice surfaces during their production, transport, harvesting and marketing

2.6 Role of Heavy Metals in Human Health

2.6.1 Chromium

Chromium is a naturally occurring lustrous metallic element found in rocks, soil, volcanic dust and gases and also in animals and plants [25]. At low concentrations, Cr is involved in natural human lipid and protein metabolism, so that very small amounts are needed for normal human life functions. Much of the daily intake of chromium, typically about 100 µg, is from foods such as grains, fruits and vegetables, potatoes, seafood, mushrooms and egg yolk [26].

Chromium occurs in the environment primarily in two valence states, trivalent chromium (Cr III) and hexavalent chromium (Cr VI). Exposure may occur from natural or industrial sources of chromium. Chromium III is much less toxic than chromium (VI). The respiratory tract is also the major target organ for chromium (III) toxicity, similar to chromium (VI). Chromium (III) is an essential element in humans. The body can detoxify some amount of chromium (VI) to chromium (III) [27].

The hexavalent form is a known toxin, mutagen and carcinogen. Cr (VI) is a strong oxidant in the form of chromates and di-chromates, it penetrates biological membranes and reacts with cell contents, proteins or nucleic acids, while being reduced to Cr (III) [28] The metal chromium is used mainly for making steel and other alloys. Chromium compounds, in either the chromium (III) or chromium (VI) forms, are used for chrome plating, the manufacture of dyes and pigments, leather and wood preservation, and treatment of cooling tower water. Smaller amounts are used in drilling muds, textiles, and toner for copying machine [28].

2.6.2 Lead

Lead is a bluish-grey-coloured heavy metal with low melting point. It can be moulded easily into any shape and forms alloys with other metals [29]. Lead is a naturally occurring toxic metal found in the Earth's crust. It has many uses, including in the manufacture of lead-acid batteries for motor vehicles and energy storage, in pigments and paints, solder, ammunition, ceramic glazes, jewellery, toys and also in some cosmetics and traditional medicines [30] Lead has no biological function in the body. It accumulates in the body and affects practically all organ systems. Lead exposure can cause chronic and debilitating health impacts in all age groups [31].

2.6.3 Mercury

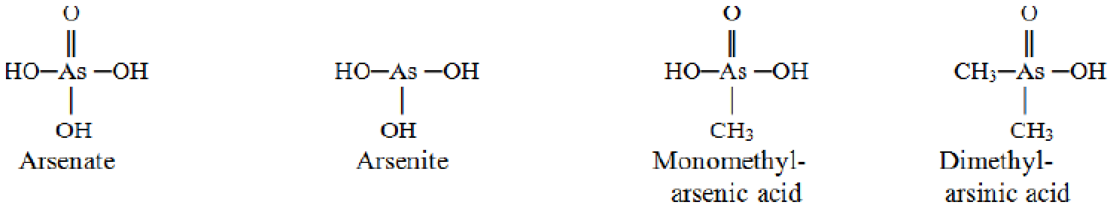
The metallic mercury is a naturally occurring metal which is a shiny silver-white, odourless liquid and becomes colourless and odourless gas when heated. Mercury is very toxic and exceedingly bio-accumulative. Its presence adversely affects the marine environment and hence many studies are directed towards the distribution of mercury in water environment. Major sources of mercury pollution include anthropogenic activities such as agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial waste water. Mercury toxicity is caused, mainly, by the fact that it enters the living organism, and reacts with different enzymes inhibiting the catalysis of basic metabolic reactions. The general population may be exposed to mercury compounds through inhalation of ambient air; consumption of contaminated food, water or soil; and/or dermal exposure to substances containing mercury [32].

Minamata disease is a disease of the central nervous system, caused by the consumption of fish and shellfish contaminated with methyl mercury compounds discharged into the environment as factory waste etc. and then accumulating in the marine life. There have also been cases of Congenital Minamata disease, in which victims were born with a condition resembling cerebral palsy. This form of the disease is methyl mercury poisoning of the fetus via the placenta, caused when the mother consumes contaminated seafood during pregnancy. Minamata disease is not an infectious disease transferred by air or food, neither is it genetically inherited.

The symptoms of Minamata disease include sensory disorders in the distal portion of the four extremities (loss of sensation in the hands and feet), ataxia (difficulty coordinating movement of hands and feet), concentric constriction of the visual field (narrowing of the field of vision), hearing impairment, disequilibrium (impairment of faculties for maintaining balance), speech impediments (speech becomes slurred and unclear), tremors (trembling of the hands and feet), and disorder of the ocular movement (eye movement becomes erratic). In very severe cases, victims fal1 into a state of madness, lose consciousness, and may even die [33].

2 6.4 Arsenic

Arsenic (As) is a metalloid and is found in the environment both from natural occurrence and from anthropogenic activity. Arsenic is considered to be one of the most important toxic elements because of its potential risk to human health Arsenic exist in two forms in nature. These are inorganic and organic forms. The organic arsenic is an arsenic compound that contains carbon, including (CH3As (OH)2) and (CH3)2AsOH). Inorganic arsenic is an arsenic compound that does not contain carbon. $H_2AsO_3^-$ and $HASO_4^{2-}$ are the inorganic arsenic compounds typically found in rice. Inorganic arsenic is considered the significant toxic form of arsenic in rice. Sources of arsenic in the environment can be natural or anthropogenic, since this element occurs in trace amounts in most rocks as well as in soil, water, and atmospheric dust [34]. The most common arsenic species are presented in figure one.



Arsenate

Arsenite

Monomethyl-arsenic acid

Dimethyl-arsinic acid

Figure 1: The common arsenic species

2.6.5 Cadmium

Cadmium is naturally present in the environment: in air, soils, sediments and even in unpolluted seawater. Cadmium is emitted to air by mines, metal smelters and industries using cadmium compounds for alloys, batteries, pigments and in plastics, although many countries have stringent controls in place on such emissions [35].

Cadmium is taken up through the roots of plants to edible leaves, fruits and seeds. During the growth of grains such as wheat and rice, cadmium taken from the soil is concentrated in the core of the kernel. Cadmium also accumulates in animal milk and fatty tissues. Therefore, people are exposed to cadmium when consuming plant- and animal-based foods. Seafood, such as molluscs and crustaceans, can be also a source of cadmium.

Cadmium and cadmium compounds are known human carcinogens. Smokers get exposed to significantly higher cadmium levels than non-smokers. Severe damage to the lungs may occur through breathing high levels of cadmium. Ingesting very high levels severely irritates the stomach, leading to vomiting and diarrhoea. Long-term exposure to lower levels leads to build up in the kidneys and possible kidney disease, lung damage, and fragile bones [36].

Itai-itai disease is the most severe form of chronic Cd poisoning caused by prolonged oral Cd ingestion. It developed in numerous inhabitants of the Jinzu River basin in Toyama Prefecture, Japan, an area most severely polluted by Cd that originated from zinc mine located upstream. The main target organ of Cd toxicity in itai-itai disease is the kidney, where injury is manifested by tubular and glomerular dysfunction. Renal dysfunction causes an insufficiency of active vitamin D, followed by bone injury consisting of a combination of osteomalacia and osteoporosis [37].

2.6.6 Zinc

In the periodic table of the elements, zinc found in group IIB together with the two toxic metals cadmium and mercury. Compared to several other metals with similar chemical properties, zinc is relatively harmless. Only exposure to high doses has toxic effect. There are three major routes of entry for zinc into the human body: by inhalation, through the skin, or by ingestion. Each exposure type affects specific parts of the body. The most widely known effect of inhaling zinc-containing smoke is the so-called metal fume fever (MFF), which is mainly caused by inhalation of zinc oxide.

2.6.7 Importance of Zinc for human being

Zinc is essential for body's good immune system, hormone secretion, mental well-being fetal growth, normal body development Zinc also performs a vital biological role in maintenance of biomembranes and is also considered essential for DNA replication, transcription and translation. Other important roles attributed to zinc include maintenance of adequate immune function and brain development [38].

2.6.8 Copper

Copper is an essential trace mineral that is vitally important for physical and mental health. But due to wide occurrence of copper in our food, hot water pipe, nutritional deficiencies table and birth control pills increase chance of copper toxicity. Copper is not poisonous in its metallic state but some of its salts are poisonous. Copper is a powerful inhibitor of enzymes. It is needed for a body for a number of enzymes but sometimes copper salts are poisonous for human organ system. Copper toxicity is increasingly becoming common these days. It is a condition in which an increase in the copper retention in the kidney occurs. Copper first start depositing in the liver and disrupts the livers to ability detoxify elevated copper level in the blood thus adversely affect the nervous system, reproductive system, adrenal function, connective tissue, learning ability of new born baby, etc. [39].

2.7 Sample Decomposition Techniques

Sample decomposition is useful for converting all the species in which a given element is present form eliminating interfering substances from the matrix and obtaining the element in a homogeneous and easily accessible matrix. The choice of decomposition techniques should take into account the objective of the final determination and factors such as the matrix composition, the elemental contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory [40]. Different decomposition methods could be classified into dry ashing, wet digestion and microwave digestion.

2.7.1 Dry -Ashing Techniques

Dry oxidation or ashing eliminates or minimize the effect of organic materials in mineral element determination in the materials. It involves ignition of organic compounds in air at atmospheric pressure and at relatively elevated temperatures (450-550 ^{0}C) in a muffle furnace. For tissues high in carbohydrate and oils the ashing aids may be required to achieve complete decomposition of organic matter. Water and other volatile materials are vaporized and organic substances are burned in the presence of air. The resulting ash residue is dissolved in an appropriate acid.

2.7.2 Wet-Digestion Techniques

Wet digestion methods involve the use of both heat and mineral acid/s. Acids that have been used in this procedure include H_2SO_4, HNO_3 and $HClO_4$. either in combination or alone [41]. Hydrogen peroxide is also used to enhance the reaction speed and to ensure complete digestion.Wet digestion has the advantage of being effective on both inorganic and organic materials. It often destroys or removes the sample matrix, thus helping to reduce or eliminate some types of interference.

2.7.3 Microwave-Assisted Digestion

Microwave-assisted digestion are the much shorter reaction time needed, direct heating of samples and reagents, reduced need for aggressive reagents, minimal contamination and lack of loss volatile elements. Microwave-assisted digestion in closed vessels under pressure has gained popularity as a simple and fast dissolution technique that minimizes acid consumption, the risk of sample contamination, and loss of volatile elements. One of its limitations is the time required for cooling before the vessels can be opened, which may take hours, depending on the type of equipment used. The main advantages of focused microwave radiation are safety, versatility, control of microwave energy released to the sample, and the possibility for programmed addition of solutions during the digestion.

Advantages and disadvantages of separation techniques shown in table below.

Table -1: Advantages and Disadvantages of the common separation techniques

Name	Advantage	Disadvantage
Dry ashing	Safe, few reagents are required, many samples can be analyzed simultaneously not labor intensive, and ash can be analyzed for specific mineral content	Long time required(12-24) hours loss of volatile minerals at high temperature, use high temperature muffle furnaces
Wet digestion	Little loss of volatile minerals Easy to operate	Labour intensive consumes a lot of acids requires special cupboard if low sample throughput
Microwave assisted acid digestion	Allows shorter reaction time increased sample throughput better precision	Required time for cooling before the vessels can be opened

2.8 Flame Atomic Absorption Spectrometry

FAAS in one of the most extensively used techniques for discriminating various elements with significant precision and accuracy. The main advantages of FAAS with atomization in a widespread air acetylene flame are low operational costs and reasonably good analytical performance [42]. Aqueous external standard solutions can be used for calibration. Otherwise, multiple linear regressions can be applied to minimize the effects of chemical interferences in the flame.

Advantage	Disadvantage
Easy to use	Moderate detection limit
Very fast	Element limitation
Lowest capital cost	1-10 element determination
Relatively few interference	No screening ability
Very compact instrument	-
Good performance	-
Robust interference	-

2.9 Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

This technique is essentially the same as flame AA, except the flame is replaced by a small, electrically heated graphite tube, or cuvette, which is heated to a temperature up to 3000°C to generate the cloud of atoms [43].

Advantage	Disadvantage
Very good detection	Slower analysis time
Limit Small sample size	Chemical interference
Moderate price	Element limitation
Very compact instrument	Limited dynamic range
Few spectral interference	

Table -2: Comparison of GFAAS and FAAS

	GFAAS	FAAS
Advantage	Solutions, slurries and solid samples can be analyzed. Much efficient atomization greater sensitivity Smaller quantities of sample (typically 5 – 50 µL) Provides a reducing environment for easily oxidized elements	Inexpensive (equipment, day-to-day running) High sample throughput Easy to use High precision
Disadvantage	Expensive Low precision Low sample throughput Requires high level of operator skill	Only solutions can be analyzed Relatively large sample quantities required (1 – 2 mL) Less sensitivity (compared to graphite furnace

2.1.0 Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, or ICP)

ICP-AES, often referred to simply as ICP, is a multi-element analysis technique that uses an inductively coupled plasma source to dissociate the sample into its constituent atoms or ions, exciting them to a level where they emit light of a characteristic wavelength. A detector measures the intensity of the emitted light, and calculates the concentration of that particular element in the sample. When undergoing ICP analysis, the sample experiences temperatures as high as 10,000°C, where even the most refractory elements are atomized with high efficiency. As a result, detection limits for these elements can be orders of magnitude lower with ICP than with FAAS techniques, typically at the 1-10 parts-per-billion level [44].

Strengths	Limitation
• Easy to use	Moderate to low detection limit (but often much better than FAAS)
• Multi-element	Spectral interference is possible
• High productivity	Some element limitation
• Very economical for many samples and/or elements	
• Few chemical interferences	
• Robust interface	

Strengths	**Limitation**
• Excellent screening abilities	
High total dissolved solids	
Solid and organic samples	

2.1.1 Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

ICP-MS is a multi-element technique that also uses an ICP plasma source to dissociate the sample into its constituent atoms or ions. However, in this case, the ions themselves are detected, rather than the light that they emit. The ions are extracted from the plasma and passed into the mass spectrometer, where they are separated based on their atomic mass-to-charge ratio by a quadrupole or magnetic sector analyser. The high number of ions produced, combined with very low backgrounds, provides the best detection limits available for most elements, normally in the parts-per trillion ranges. It is important to remember that detection limits can be no better than lab cleanliness allows.

Advantage	**Disadvantage**
Multi-element	Higher initial capital cost
High productivity	Some spectral interferences, but well define
Wide dynamic range	
Very economical for many samples and/or elements	
Excellent detection limits	Some method development skill required
Easily interpreted spectra	
Fast semi quantitative screening	

3. MATERIALS AND METHODS

3.1 Chemicals and Reagents

Reagents that were used in the analysis were all analytical grade. (69-72%) HNO_3 (Spectrosol, BDH, England), 70% $HClO_4$ (Aldrich, A.C.S. Reagent, Germany) and H_2O_2 (30%) (BDH Chemicals, England) were used for digestion of rice samples. Lanthanum nitrate hydrate (98%, Aldrich, Muwaukee, USA) was used to avoid refractory interference (for releasing calcium and magnesium) from their phosphates. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Ca, Mg, K, Na, Fe, Mn, Zn, Cu, Co, Ni, Cr, Cd and Pb (Buck Scientific Puro-Graphic^{tm}) were used for preparation of calibration standards and in the spiking experiments.

3.2 Apparatus and Instruments

The dried sample was ground to obtain the Flame atomic absorption spectrometer (Buck Scientifc Model 210VGP AAS, East Norwalk, USA) equipped with deuterium arc background correctors and hollow cathode lamps with air-acetylene flame was used for the determination of the analyte metals in raw and cooked rice samples. Round bottom flasks with ground glass joint (100 mL) fitted with reflux condenser were employed in digesting the sample on Kjeldahl heating apparatus (Gallenkamp, England) Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA), micropipettes (Dragonmed, 1-10 µL, 100-1000 µL, Shangai, China) were used during measuring different quantities of volumes of sample solution, acid reagents and metal standard solutions.

3.3 Sampling and Pre-Treatment

Sample sites were selected according to the areas in which rice is available to the users. Samples were collected from different sites. Basmati, Jasmine, Royal and Ethiopian white rice were collected from supermarkets in Addis Ababa. For Ethiopian red rice Fogera was selected to collect the sample the last NERICA (New Rice for Africa) were collected from Amhara Regional Agricultural Research Institute (ARARI), BahirDar, Ethiopia. 1 kg of each sample was collected from three subsample sites and mixed to form 3 kg bulk sample. 5 L tap water was collected from Addis Ababa in clean plastic polyethylene container (Jerican) for cooking the rice and for the determination of metal levels. 500 mL of tap water sample were filtered and preserved by adding 2% (v/v) nitric acid and kept in a refrigerator for metal determination.

All the raw rice samples were washed with tap water followed by deionised water to avoid any dust materials on the grain and dried until constant weight. The dried rice sample was then ground using blender in the laboratory and sieved through a 0.457 mm sieve to remove large particles. Rice was cooked using absorption and excess method. To assess the effect of cooking on metal level. About 135 g of Ethiopian white rice sample was measured then cooked in tap water by absorption method which is most commonly practice in Ethiopia. After the cooking process was completed, the cooked rice was allowed drying up at constant weigh. The dried sample was ground to obtain required particle size.

3.4 Wet digestion of Rice

For digestion purpose, 0.5 g of powdered and homogenized samples were weighed and transferred in to a 100 mL round bottom flask. To this, 2 mL concentrated HNO_3 (69-72%), 1 mL of $HClO_4$ (70%) and 0.5 mL of H_2O_2 (36 %) were added. The mixture was then digested on Kjeldahl digestion apparatus

(Gallenkamp, England) fitting the flask to a reflux condenser by setting the temperature at 120 °C for 30 min followed by 210 °C for 120 min until a clear solution was obtained following the optimized digestion procedure. After a total of 2:20 h, the digested solutions were allowed to cool for 30 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution, 2-5 mL portions of deionized water were added and gently swirled to reduce dissolution of the filter paper by digest residue. The cooled digested samples were filtered into a 50 mL standard volumetric flask with a Whitman filter paper (110 mm) to remove any suspended or turbid matter. Subsequent rinsing of the filtrate with 5 mL deionized water was followed until the volume reached the mark. At this point, the solution was clear and colourless. To each sample 1% ‘matrix modifier’ lanthanum nitrate hydrate were added so that lanthanum may bind the phosphate and liberate calcium and magnesium in case large phosphate exist in the sample. For each rice samples, triplicate digestions were carried out. Blank solutions were also digested accordingly in triplicate. The digested and diluted sample solutions were then kept in refrigerator until analysis. This digestion process was applied for the metal determination in the raw and cooked rice samples.

3.5 Figures of Merit

The analytical wavelengths, the correlation coefficients, and the correlation equations of the calibration curves for the determination of metals in rice samples by FAAS are given in Table 3. The correlation coefficients of all the calibration curves were > 0.999 and these correlation coefficients showed that there was very good correlation (relationship) between concentration and absorbance. The method detection limits were calculated as the concentrations that give signals equal to three times the pooled standard deviations of the six blanks are given in Table 3. The method detection limits are low enough to detect the metals at trace levels.

Table-3: Analytical parameters for the determination of metals in rice samples by FAAS

Metals	Wave length	Method detection limit(mg/kg	Correlation coefficient	Equation of calibration curve
Ca	422.7	0.7	0.993	$Y=-2.09* 10^{-3} +0.00167x$
Mg	285.2	0’03	0.999	$Y=3.44*10^{-3}+0.10344x$
K	766,5	0.07	0.9998	$Y= -1.18*10^{-4}+0.0084x$
Na	589	0.05	1.000	$Y= 9.59*10^{-4}+1.07957x$
Fe	248.3	0.3	0.9995	$Y= -3.65*10^{-3}+0.00157x$
Mn	279.5	0.03	0.9999	$Y=652*10^{-1}+0.0238x$
Zn	213.9	0.2	0.9997	$Y=-647*10^{-4}+0.11418x$
Cu	324.8	0.04	0.9999	$Y=6.32*10^{-4}+0.11418$
Co	240.7	0.2	0.9999	$Y=-1.38*10^{-4}+0.00913x$
Ni	232	0.1	0.9997	$Y=-1.38*10^{-3-}+0.00913x$
Cd	228.9	0.3	0.9999	$Y=-1.50*10^{-3}+0.05516x$
Pb	283.2	1.8	0.9999	$Y= -1.02*10^{-4}$+0’00259x

3.6 Method Validation for Metal Determination

The spiked samples were prepared by adding a small known quantity of metal standard solutions. The method validation of metal analysis was established by spiking experiments. Spiking procedure for raw rice was carried out as follow: from the stock solution 1000 mg/L 25 µL of Mg, 24 µL of Ca, 145 µL of K, and from 100 mg/L stock solution 44 µL of Cu solution were added to round bottomed flask (100 mL) containing 0.5 g rice sample. In the second round bottomed flask (100 mL), from 100 mg/L solution 108 µL of Ni, 111 µL of Na, 25 µL of Pb and from 10 mg/L solution 13 µL of Cd were added. In the third round bottomed flask (100 mL), from 100 mg/L solution 19 µL of Cr, 22 µL of Co, 115 µL of Fe, 15 µL of Mn and 73 µL of Zn solution were added. Similarly, spiking in the cooked rice, from 1000 mg/L stock standard solution 24 µL of Ca , 41 µL of K, 28 µL of Mg and from 100 mg/L stock solution 70 µL of Na were added in to 0.5 g sample present round bottomed flask (100 mL). 10 µL of Cu, 11 µL of Co, 22 µL of Ni, 20 µL of Fe, 16 µL of Cd and 10 µL of Cr from 100 mg/L stock solution added in to another round bottomed flask. 12 µL of Pb, 10 µL of Mn and 27 µL of Zn from 100 mg/L stock solution added into the third round bottomed flask. Then the samples were digested with the optimized procedures. After diluting the digested samples to 50 mL with distilled deionized water, they were analysed by the same procedure followed for the analysis of rice sample. As used for original samples triplicate spiked samples were prepared and triplicate readings were recorded.

4. RESULT AND DISCUSSION

4.1. Recovery Results of Metal Determination

The percentage recovery for raw and cooked rice samples were obtained with in the acceptable range (100 ± 10%) for all the metals except for Co and Pb in raw rice sample, for which recovery of 87% and 88%, respectively, were obtained. Similarly, for cooked rice sample, Co and Pb recoveries obtained were 89% and 86%, respectively. The lower recovery for the above elements may be attributed to the matrix analyte interaction which might be high and that is why their recovery values decreased.

4.2. Level of Metals in Raw Rice Samples

The levels of metals in raw rice samples are given in Table 4. From the whole rice analysed for metal level determined, K was the highest in concentration followed by Mg while Cd was the lowest in concentration in the samples. This is because of metals such as K and Mg are mobile in to plant tissue [45]. As shown in Table 4, except in Royal and Jasmine variety of rice samples in all other rice's toxic metals such as Cd and Pb was detected.

Table 4: Average level (mean ± SD, n = 9, mg/ kg dry weight) of metals in rice samples

Metals	Basmite rice	Jasimine rice	Royal rice	Ethiopian white rice	Ethiopian red rice	NERICA New rice for Africa
Ca	75.8±7.5	79.6±6.5	630±27	210±2	205±6	427±3
Mg	135±6	90±6	150±4	99.5±0.7	971±444	2.2550±202
K	1.830±88	1.680±87	2.15±100	1,100±103	3.02±273	2.2550±173
Na	73.8±3.1	78.6±1.3	70.6±1.3	74.6±0.7	26.7±1.4	80.9±4
Fe	49.5±3.9	48.9±4.8	117±1.8	108±8	113±10	41.3±1.7
Mn	4.7±0.4	4.11±0.2	15±0.8	3.7±0.3	16.6±0.5	16.1±0.8
Zn	25.7±2.1	20.9±0.3	16.4±0.4	51.6±0.2	16.7±0,2	140±9
Cu	3±0.2	2.7±0.09	4.9±0.3	15±1.3	3.3±0.02	3.3±0.02
Co	12.6±1.1	14.6±1.2	12.7±1.2	8.8±0.7	8.9±0.7	10.4±0.7
Ni	75.1±1.7	2.5±0.1	10.5±0.9	69.7±1.5	41.5±2.3	ND
Cr	3.12±0,5	2.2±0.5	ND	4.82±0.09	2.32±0.04	3.9±0.2
Cd	0.34±0.01	ND	ND	0.54±0.02	0.45±0.01	2.54±0.2
Pb	5.3±0.7	4.2±0.4	2.1±0.2	3.3±0.2	0.8±0.07	3.8±0.3

ND: Not detected, concentration of the tested metal was below the method detection limit

Lead and cadmium cause accumulation and in the long term cause an insufficiency in different tissues and organs. The use of contaminated water in the rice fields causes an increase in the Pb and Cd content of the grains of rice and the consumption of this rice causes it to enter the body [46]. Metals detected were found in Basmati rice in the order of *K > Mg > Ca > Ni > Na > Fe > Zn > Co > Pb > Mn > Cr > Cu > Cd*. The results showed that the Basmati sample contains the highest level of K with identified concentration 1,830 mg/kg followed by magnesium with a value of 137 mg/kg. The lowest concentration was found for toxic metal Cd which was 0.34 mg/kg. In Jasmine, Ethiopian red and NERICA rice samples of the metals the highest level was K (1,680, 3,020, 2,550) mg/kg followed by Mg (90.6, 970, 2,250) mg/kg and Ca (79.6, 205, 427) mg/kg. The lowest concentration in Ethiopian red rice and NERICA rice was Cd and the value obtained as 0.45 mg/kg and 2.54 mg/kg, respectively. However, the lowest concentration in Jasmine rice was Cr with the value of 2.2 mg/kg and Cd was not detected that it gives value below the method detection limit. Similarly in NARICA rice Ni was below the method detection limit. The highest level of metal in Royal and Ethiopian white rice was K followed by Ca with the value of (2,150, 1,100) mg/kg for K and (630, 210) mg/kg for Ca, respectively. The lowest concentration found in Royal rice was Pb with 2.1 mg/kg. In this rice type, Cr and Cd was not detected they were below the method detection limit. Cadmium provides the lowest concentration in Ethiopian white rice (0.54 mg/kg).

4.3 Level of Metals in Cooked Rice and Tap Water Used for Cooking

Uptake of heavy metals by plants from soil and contamination of food by heavy metals during harvesting, transportation, storage, marketing and processing stages are major sources of heavy metals in foods [47]. All selected thirteen metals were detected by the same procedure as the raw rice. As shown in Table 3, the level of metals in decreasing order in cooked rice observed as K > Ca > Mg > Fe > Na > Ni >Zn > Cu > Co > Mn > Cr > Pb > Cd. However, the trend in raw rice were K > Ca > Fe > Mg > Na > Ni > Zn > Cu > Co > Cr > Mn > Pb > Cd. The trend in raw and cooked rice is not the same. The order of Mg and Fe and Mn and Cr in raw rice is interchanged in cooked rice. Moreover, the determination indicates that the level of the metal shows some increment except in K, Co and Cr. In general the difference between raw and cooked rice is not significant. In tap water used to cook rice for their metal determination, metals which are major, trace and toxic (Ca, Mg, K, Na, Fe, Mn, Zn, Cu, Co, Ni, Cr, Cd and Pb) were also determined in it. Frequent use of heavy metal contaminated water in the agricultural fields leads to soil pollution and gradually enriched the soil with heavy metals [8]. The investigation of this study provides results in Table 5 which indicate the highest level of metals in tap water was Mg followed by Ca and Fe. Cd provides the lowest concentration in tape water.

Table 5: Average concentration (mean ± SD, n = 9 of metals in cooked rice sample and tap water

Metals	Ethiopian white rice	Tape water
Ca	240±20	33.5±2,8
Mg	130±4	29±2.8
K	1.010±90	1.5± 0.6
Na	84.3±4.7	12.3±0.8
Mn	111±3	14.8±0.2
Fe	4.2±0.3	1.22±0.01
Zn	54.5±4.9	6.5±0.5
Cu	16.3±1.1	2.2±0.08
Co	7,7±0,7	1.47±0.02
Ni	70.1±3.3	1.5±0.08
Cr	4.06±0.4	0.7±0.04
Cd	0.7±0.04	5±0. 106
Pb	4.05±0.4	0.7±0.04

4.4 Comparison of Metals Level in Imported and Ethiopian Rice

From the general view as shown in table four most of metals range in imported rice included in the range of Ethiopian rice. The difference observed in the range of the metals between imported and Ethiopian rice indicates mainly that the species difference may causes such a difference. Since rice imported from abroad belonged to the species *Oryza sativa* but rice from Ethiopia belonged to species *Oryza glaberrima*. The difference might be due to genetic make-up, the variety rice plant, fertilizer used, soil type and quality of water used for plantation.

4.5 Comparison of Metals Levels in this Study with Literature Values

As the comparison in the showed most of the values reported in the literature are comparable with the present study Ca, and Mg level in the literature are higher than present study.

Fe level in the present study was higher than reported in the literature [49] Zn [50]; Ni ; and Co are relatively lower than the present study. However, Na in the literature is higher than the present study. Cr is higher to some extent than result of the present study [51]. Toxic metals Pb and Cd are higher in rice varieties in the present study than reported in the literature. This implies that the variety and the environmental condition are also the reason for the difference.

Table 6: Comparison of metals levels of the rice sample with values reported in literature

Metals	Rice variety	concentration	Origin	References
Ca	Basmati rice Basmati rice Jasmine rice Black sweat rice Ethiopian white rice Imported rice	800 500 200 1,200 196-427 76-630	United state India United state China Ethiopia Thailand and Pakistan	Zhang 2009 Zhang 2008 Zhang, 2009 Zhang,2009 this study this study
Mg	Basmati rice Basmati rice Jasmine rice Black sweat rice Ethiopian white rice Imported rice	10,000 2000 900 8000 99.5-2.249 90.5-149.5	United state India United state China Ethiopia Thailand and Pakistan	Zhang 2009 Zhang 2008 Zhang, 2009 Zhang,2009 this study this study
Na	Basmati rice Basmati rice Jasmine rice Black sweat rice Ethiopian white rice Imported rice	1600 1400 1200 2200 27.1-81 70-80	United state India United state China Ethiopia Thailand and Pakistan	Zhang 2009 Zhang 2008 Zhang, 2009 Zhang,2009 this study this study
K	Basmati rice Basmati rice Jasmine rice Black sweat rice Ethiopian rice Imported rice	10,000 11000 12000 3000 1,101-3.029 1679-2145	United state India United state China Ethiopia Pakistan and Thailand	Zhang *et al.*, 2009 Zhang *et al.*, 2009 Zhang *et al.*, 2009 Zhang *et al.*, 2009 this study this study

Metals	**Rice variety**	**concentration**	**Origin**	**References**
Fe	Super Basmati rice	3.16	Pakistan	Mehdi et al ,2003
	Shane Basmati rice	3.35	Pakistan	Mehdi *et al.*, 2003
	Ethiopian rice	41-113	Ethiopia	this study
	Imported rice	49-117	Thailand and Pakistan	thist study
Mn	Super Basmati rice	1.33	Pakistan	Mehdi *et al.*, 2003
	Shane Basmati rice	1.36	Pakistan	Mehdi *et al.*, 2003
	Sabin rice	1.53	Malaysia	Yap *et al.*, 2009
	Ethiopian white rice	4.17	Ethiopia	this study
	Imported rice	4-15.5	Thailand and pakistan	this study
Zn	Sabin rice	0.69	Malaysia	Yap *et al.*, 2009
	Super Basmati rice	1.55	Pakistan	Mehdi *et al.*, 2003
	Shane Basmati rice	1.6	Pakistan	Mehdi *et al.*, 2003
	Twain rice	13.1	Taiwan	Fu *et al.*, 2003
	Ethiopian white rice	17-140	Ethiopia	this study
	Imported rice	15-21	Pakistan and Thailand	this study
Cu	Saban rice	0.312	Malaysia	Yap *et al.*, 2009
	Taiwan rice	2.22	Tawan	Un *et al.*, 2004
	Super Basmati rice	0.93	Pakistan	Mehdi *et al.*, 2003
	Shane rice	1.08	Pakistan	Mehdi *et al.*, 2003
	Ethiopian rice	3.3-15	Ethiopia	this study
	Imported rice	2.7-5	Thailand and Pakistan	thist study
Co	Taizhou rice	0.45	Taizhou	Fu *et al.*, 2005
	Black sweat rice	0.29	China	Fu *et al.*, 2005
	Ethiopian rice	9-78.5	Ethiopia	this study
	Imported rice	13-15	Pakistan and Thailand	this study
Ni	Taiwan rice	0.29	Taiwan	Un *et al.*, 2004
	Super Basmati rice	0,13	Pakistan	Mehdi *et al.*, 2003
	Shane Basmati rice	0.09	Pakistan	Mehdi *et al.*, 2003
	Ethiopian rice	42-70	Ethiopia	this study
	Imported rice	25-75	Thailand and Pakistan	this study
Cr	Saban	1.34	Malaysia	Yap *et al.*, 2009
	Taiwan rice	0.1	Taiwan	Un *et al.*, 2004
	Ethiopian rice	2.3-4.5	Ethiopia	this study
	Imported rice	2.2-3.12	Pakistan and Thailand	this study
Cd	Saban	0.18	Malaysia	Yap *et al.*, 2009
	Taiwan	0.01	Taiwan	Un *et al.*, 2004
	Super Basmati rice	0.16	Pakistan	Mehdi *et al.*, 2003
	Shane Basmati rice	0.13	Pakistan	Mehdi *et al.*, 2003
	Iranian rice	0.41	Iran	Mehdi *et al.*, 2007
	Ethiopian rice	0.25-0.45	Ethiopia	this study
	Imported rice	< 0.34	Thailand and Pakistan	this study
Pb	Saban	ND	Malaysia	Yap *et al.*, 2009
	Taiwan	0.01	Taiwan	Un *et al.*, 2004
	Super Basmati rice	2.95	Pakistan	Mehdi *et al.*, 2003
	Shane Basmati rice	2.85	Pakistan	Mehdi *et al.*, 2003
	Ethiopian rice	0.8-3.5	Ethiopia	this study
	Imported rice	2.1-5.3	Thailand and Pakistan	this study

4.6 Analysis of Variance

Variations in the mean levels of metals between the samples were tested whether it was from a random error or treatment. ANOVA use the F statistic to compare whether the difference between sample means are significant or not. The result showed that for all metals, at the 95% confident level, the means were significantly different ($p < 0.05$). The source for this significant difference between sample means may be the difference in mineral contents of soil, pH of soil, pesticides and insecticides used during cultivation.

5. CONCLUSION

The levels of metals (Ca, Mg, K, Na, Fe, Mn, Zn, Cu, Co, Ni, Cr, Cd, and Pb) in three imported and three Ethiopian varieties of commercially available rice were determined. The wet digestion method and the determination of selected metals at trace levels in rice by flame atomic absorption method were found to be efficient precise and accurate. The efficiency of sample preparation and instrument were tested by assessing standard deviation and conducting recovery experiments. K was highly accumulated in rice samples and toxic metal Cd was found to be the lowest. Comparison between levels of metals in the imported and Ethiopian rice showed significant differences for most of the metals. Analysis of variance showed that there was significant difference at 95% confidence level in the means of metal levels in the six rice samples. Comparison between levels of metals in cooked and raw rice showed that the difference in the level is not significant.

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