

DEBRE BERHAN UNIVERSITY
COLLEGE OF NATURAL AND COMPUTAIONL SCIENCE
POSTGRADUATE PROGRAM
DEPARTMENT OF CHEMISTRY



DETERMINATION OF SELECTED TOXIC (Cd, Pb) AND ESSENTIAL (Zn, Cu) METALS IN RICE CULTIVATED IN ACHEFER, WESTGOJJAM, ETHIOPIA USING ICP-OES

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FEBRUARY, 2021

DEBRE BERHAN, ETHIOPIA

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A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY
POSTGRADUATE PROGRAM FOR THE PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (M SC.) IN
CHEMISTRY

FEBRUARY, 2021
DEBRE BERHAN, ETHIOPIA

DECLARATION

I, the undersigned, hereby declare that this thesis is my original work and all sources of materials used for the thesis have been acknowledged.

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Date: _____

Thesis Approval Sheet

This thesis which is entitled “**DETERMINATION OF TOXIC (Cd, Pb) AND ESSENTIAL (Zn, Cu) METALS IN RICE CULTIVATED IN NORTH ACHEFER, WESTGOJJAM, ETHIOPIA USING ICP-OES**”, by Yekoye Belete Fetene has been accepted as a thesis for the partial fulfillment of the degree of Master of Science in Chemistry.

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List of Abbreviations and acronyms

AAS	Atomic absorption spectroscopy
AFS	Atomic fluorescence spectroscopy
ANOVA	Analysis of variance
CDM	Conventional wet acid digestion method
FAAS	Flame atomic absorption spectrometry
FAO	Food and agricultural organization
IARC	International Agency for Research on Cancer
ICP-MS	Inductively coupled plasma mass spectroscopy
ICP-OES	Inductively coupled plasma optical emission spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
PMTDI	Provisional Maximum Tolerable Daily Intake
RSD	Relative standard deviation
SD	standard deviation
WHO	World health organization

ACKNOWLEDGMENT

Primarily, I thank GOD who enabled me everything to be possible throughout my study. All things are possible by the will of GOD. Next, my special and profound gratitude goes to my advisor Dr. Hulugirgesh Degefu for her unlimited advice, closer help, valuable idea, suggestion, inspired guidance throughout my work, and devoted her leisure time in reading and correcting my paper. It is difficult to express in words about her work as an advisor. I will forever remain grateful to her. I am also thankful to the ministry of education (Amhara National Regional State Education Bureau) for giving me this sponsorship. My thanks also go to Debre Birhan university and Bahir Dar university chemistry department laboratory for allows me to use laboratory apparatus, instruments and chemicals during my experimental work. My deepest gratitude also goes to my father Belete Fetene who always sacrificed in all the matter and dream to see my success and well-being and also for his endless effort and contribution. I am also grateful to my mother Yeswdeg Belay who has done her level best to see my success. Again I want to express my gratitude to my wife Bilen Lakacew and brothers and sisters and friends Tsegaye Birhanu (Msc), Zeleke Manaye (Msc) Messele, Yilkal, Masiresha, Muluken and others for their unreserved help and support. Last but not least, I express my gratefulness for all who directly or indirectly contributed to the successful completion of the study and the write up of the thesis.

Abstract

Rice is the most important staple food crop for more than half of the world's population. In present study the rice samples were collected from Esetumite kebele farmers and Fogera rice samples from Bahir Dar market .Acid digestion methods were applied to digest the collected samples under fume hood on a hot plate. The concentration of Cu, Zn, Pb and Cd were observed and analyzed by using inductively coupled plasma optical emission spectrometry (ICP-OES). The measurements for metals copper, zinc, cadmium and lead in the present study of rice samples resulted in mean± SD values. Sample results from Esetumite kebele were determined in 0.0317± 0.0020, 0.2613± 0.0810, 0.0007± 0.0006 and 0.0093± 0.0049 as well as Fogera were 0.0453± 0.0064, 0.3333± 0.0483, 0.0217± 0.0133 and 0.0007± 0.0006 respectively. The determined concentrations of Zn, Cu and Pb in Fogera were greater than Esetumtie. But Cd in both sample collected sites had comparable results. The reasons for concentration difference of metals in rice might be the difference in geographical location, soil properties and genetic variation of the rice grains. According to the results obtained by the present study, the concentrations levels of elements in both sampling sites were included within the limits of the accepted values set by FAO/WHO.

Keywords: - Rice, Toxic metals, essential metals, ICP-OES, wet-acid digestion

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the Study

Rice is one of the most commonly consumed cereals in the world next to wheat and maize. It is part of the staple diet of the world population; and it is considered as the most important source of nutrients for billions of people around the world [1]. The Asian rice, *Oryza sativa* and Africa rice *Oryza glaberrima* are the two most cultivated species [4]. Rice is also the most rapidly growing source of food in Africa, and is of significant importance to food security and food self-sufficiency in an increasing number of low- income food deficit countries. Therefore, improving the productivity of rice systems would contribute to hunger eradication, poverty alleviation, national food security and economic development [2].

Rice was introduced in Ethiopia during 1970s and has been cultivated in small pockets of the country. Rice is among the most important cereals and stem crops grown in different parts of Ethiopia as food crop. It is reported that the potential rice production area in Ethiopia is estimated to be about thirty million hectares [3, 5]. Even though rice is not a traditional staple food in Ethiopia, the knowledge of its mineral nutrition is of particular interest [4].

Although little information is available on the levels of some essential and non-essential elements present in literature, several studies have been carried out on determination of the level of toxic metals in rice, using different methods such as ICP and FAAS employing dry ashing and wet digestion techniques[7].

Due to the occurrence of the industrialization and urbanization without environmental care, toxic elements such as lead (Pb) and cadmium (Cd), coming mainly from mining, industrial processes, pesticides, chemical fertilizers, and atmospheric deposition, have become a major source of environmental contamination [3].

Toxic elements are considered highly hazardous to human health and they may cause acute or chronic poisoning. This chronic poisoning exposure leads to induction of pathological changes and damage in organs and central nervous system, further leading to lower intelligence quotient mainly in children. Cadmium is highly toxic to the kidneys and this metallic element is

considered as carcinogenic. Besides, cadmium may cause bone mineralization, osteoporosis being a critical effect resulting from this element exposure. Cadmium is also considered as carcinogenic, and the majority of its chronic exposure reports are focused on skin problems [8]. Because of the high soil mobility and availability of the total cadmium and lead derived from the human activities and natural sources, there is a general concern about their phytotoxicity and risks to organisms, as they are rapidly able to spread out at different levels in the food [9, 10].



Figure: 1 Farmers visiting Esetumite rice

1.2 Statement of the problem

Rice is one of the most commonly used food items; therefore, evaluating its quality in terms of heavy metal pollution is more important. Over the years, different studies have showed chemical contamination of food in the country is very serious and heavy metals are potential pollutants. Toxic elements are considered highly hazardous to human health and they may cause acute or chronic poisoning. Chronic exposure to lead has been associated with the induction of pathological changes and damage in organs and central nervous system, leading to lower intelligence quotient mainly in children. However, it is better to detect the level of toxic metals (Pb, Cd) and essential metals (Zn, Cu) in such food items since toxic metals like Cd, Pb are not recommended to take excessively. If the levels of these metals are elevated, then they can show negative effects. The most cultivated cereal in North Achefer Woreda is rice and most of the people feeds rice. Therefore, I initiate to study, to determine the concentration level of toxic and essential metals in rice samples.

1.2 Objectives of the Study

1.2.1 General objective

- The general objective of this thesis was to determine the level of selected toxic and essential metals from rice cultivated in Esetumite kebele, North Achefer.

1.2.2 Specific objectives

The specific objectives of the study are:

- To evaluate the concentration level of toxic (Cd, Pb) and essential (Zn, Cu) metals in rice sample from Esetumite kebele, North Achefer.
- To compare the concentration level of toxic and essential metals present in rice samples which is observed by using ICP-OES and the result by using other techniques
- To compare the concentration level of toxic and essential metals with international tolerable limit this is accepted by FAO/WHO.

1.3 Significance of the Study

It would give some information about the determination of the selected essential metals zinc and copper and toxic metals lead and cadmium in rice samples locally produced in Esetumite, Gojjam Ethiopia which is used to indicate the concentration level of toxic and essential metals in the samples under study. The findings of this study will also provide adequate information on the concentration of toxic and essential metals in rice samples. Furthermore, the findings of the study will help to ensure health dietary safety of individual utilizing this rice in daily activity as a source of food.

1.4 Scope of the Study

The study focuses only on the analysis of two selected essential metals copper and zinc and two non-essential metals cadmium and lead in rice samples locally cultivated in Esetumite kebele, Gojjam available and Fogera rice samples brought from Bahir Dar markets ICP-OES. However, due to scarcity of budget the study was not continuing all essential and non-essential metals in all rice samples cultivated in Ethiopia and available in all Ethiopian markets.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Origin and Distribution of Rice

Archaeological and historical evidence points to the foothills of the Himalayas in the North and hills in the North-east of India to the mountain ranges of South-east Asia and South-west China as the primary center of origin of *Oryza sativa*, and the delta of river Niger in Africa for that of *O. glaberrima*, the African rice [16]. There are about 25 species of *Oryza*. Of these only two species are cultivated, these are *Oryza sativa* Linus and *Oryza glaberrima*. The former is originated from North Eastern India to Southern China but has spread to all parts of the world. The latter is still confined to its original home land, West Africa [17]. Rice was introduced in Ethiopia during 1970s and has been cultivated in small pockets of the country [18].

2.2 Uses of Rice as Food and Economic importance

The nutritional value of rice per 100 g has 1510 kJ energy, 79 g carbohydrates, 0.6 g fat, 7 g protein, 0.4 g vitamin B₆ and 12 g importance of rice as a food security crop, source of income and employment opportunity due to its relative high productivity as compared to other cereals is recognized by farmers as well as private investors who frequently request for improved varieties for different ecosystems [19, 20]. At the Achefer plain, rice plays an important role in relaxing the problem of food-insecurity of the farming community. Rice is used in the preparation of local foods such as injera, dabbo, genffo, kinchie, shorba and local beverages like tella and katikalla either alone or mixed with other cereal grains such as teff, millet, wheat, barley, sorghum and maize [21].

According to (Onwueme and Sinha, 1991) rice can also be used in the manufacture of cosmetics and textiles. Beer and wine are also made from rice. Rice is the most important crop to millions of small farmers who grow it on millions of hectares throughout the region, and to the many landless workers who derive income from working on these farmers. The performance of marketing of rice has impact on the income of producers, processors, traders and consumers too.

2.2.1 Non-essential Heavy Metals in Plants

Heavy metals are elements with a high relative atomic mass (>20). They are metals with no known role in plant and animal metabolism. These include Co, Cr, Fe, Cd, Pb, Hg, Zn, Ni, Mn, Ag, etc. [22]. Metals normally occurring in nature are not harmful, because they are only present in very small amounts. However, if the levels of these metals are elevated, then they can show negative effects [23].

2.2.2 Chemistry of Lead and Cadmium

Lead (Pb)

Lead (Pb) has an atomic number of 82 and atomic mass of 207. It is the heaviest non-radioactive metal that naturally occurs in substantial amounts in the earth's crust. Lead has two oxidation states Pb^{2+} and Pb^{4+} . Pb^{2+} dominates environmental chemistry. There is a great similarity in the ionic sizes of Pb^{2+} and Ca^{2+} such that Pb^{2+} may proxy for Ca^{2+} [24]. It is a bright and silvery metal with a very slight shade of blue in a dry atmosphere. It has a density of 11.34 g/cm^3 , melting point of $327.46 \text{ }^\circ\text{C}$ and a boiling point of $1749 \text{ }^\circ\text{C}$.

Cadmium (Cd)

Cadmium has an atomic number 48 and relative atomic mass of 112.40. It is a silvery grey solid in nature at 298 k. It has a density of 8.65 g/cm^3 , melting point of $320.9 \text{ }^\circ\text{C}$ and a boiling point of $767 \text{ }^\circ\text{C}$ at 100 kPa [24].

2.3 Sources of Lead and Cadmium

Inputs of heavy metals to agricultural soils can occur from a variety of sources. These include the application of bio solids, fertilizers, fungicides, livestock manure, wastewater sludge, pesticides, automobile exhaust, metal sheets, paints, storage batteries, pigment and chemicals, alloys, cables, irrigation water and from atmospheric deposition [24]. Some of the concerns about accumulation of heavy metals in agricultural soils stem from their possible negative impacts on soil fertility and in some case their potential to accumulate in the human chain [26]. Some metals like cadmium and lead are good indicators of contamination in soils because they appear in gasoline, car components, lubricants, industrial emissions, alloys, cables, irrigation water, metal sheets and paints [25].

2.3.1 Source of Lead

Lead is one of the most dangerous naturally occurring toxic heavy metals because it is a ubiquitous metal which presents everywhere including homes, soil, work place, foods and water. The main sources of pollution of natural water by lead are lead pipes, effluents of automobiles, batteries, metal sheets or paints [28]. According to (Madyiwa, 2006) Pb is a mineral found deep within the earth and mined together with silver deposits. It exists in nature as sulphate (PbSO_4), carbonates (PbCO_3) and sulphide (PbS), which constitute the principal ore of Pb, known as galena. It is present in tap water primarily from household plumbing systems containing lead in pipes, solder, fittings or service connections to homes. This makes domestic waste a major source of lead. Lead may enter the atmosphere during mining, smelting, refining, manufacturing processes and by the use of lead containing products.

The automobile exhaust containing leaded gas and particulate lead. Road side plants contain high concentration of lead in their tissue and this has a sub lethal effect on the health of animals. There are other sources such as storage batteries, building construction and cable coating. Under acidic soil condition, there is desorption of lead into solution making it available for uptake by plants or vegetable [29].

2.3.2 Source of Cadmium

Though cadmium is a naturally occurring element, it is rarely found as a pure metal in nature. It is mostly associated with oxygen, chlorides, sulphate, and sulphide. Cadmium enrichment also occurs due to the application of sewage sludge, manure and limes. Presently the main sources of Cd are; Cd rich phosphate fertilizers, fungicides, pesticides, irrigation of water, atmospheric events, sewage sludge, industrial and mining wastes [30]. One of the factors which significantly influence cadmium concentrations in soils and its uptake into plant tissues is pH. When soil pH decreases, cadmium is desorbed from organic and clay particles and ends up in soil solution and become more mobile [31].

According to Madyiwa the main sources of Cd in the environment are:

- 1) Air emission from zinc, lead and copper smelters and industries involved in manufacturing alloys, Paints, batteries and plastics
- 2) Wastewater from mining
- 3) Agricultural use of sludge and fertilizers containing Cd

- 4) Burning of fossil fuels and
- 5) Deterioration of galvanized materials and Cd-plated containers.

2.3.3 Toxicity of lead and cadmium

Heavy metals like lead and cadmium don't have any useful biological role and they are considered among the most important contaminants of the environment and are highly toxic even at relatively low concentration [32].

The toxicity of these metals has two main aspects: (a) the fact that they have no known metabolic function, but when present in the body they disrupt normal cellular processes, leading to toxicity in a number of organs; (b) the potential, particularly cadmium and lead, to accumulate in biological tissues, a process known as bioaccumulation. This occurs because the metal, once taken up into the body, is stored in particular organs, for example the liver or the kidney, and is excreted at a slow rate compared with its uptake. This process of bioaccumulation of metals occurs in all animals, including food animals such as fish and cattle as well as humans. It is therefore necessary to control the levels of these toxic metals in foodstuffs in order to protect human health [33].

2.3.4 Lead Metal Contamination and Toxicity to Human Beings

Short-term exposure to high levels of lead can cause brain damage, paralysis (lead palsy), anemia and gastrointestinal symptoms. Longer-term exposure can cause damage to the kidneys, brain, red blood cell, reproductive and immune systems in addition to effects on the nervous system [33]. The most critical effect of low-level lead exposure is on intellectual development in young children and it crosses the placental barrier and accumulates in the fetus. Excessive lead accumulation in children is known to cause hyperactivity, a reduced intelligence and anti-social behavior. Traditional sources of lead poisoning such as paint flakes and home dirt are usually ingested by small children at an age when iron deficiency is very common. Consumption of food containing lead is the major source of exposure for the general population [32, 33].

An estimated 99% of the Pb that enters the adult human body and 33% that enters a child's body are excreted in about two (2) weeks. As a result of this, Pb poisoning is of much concern in children because they are susceptible to developmental delays secondary to Pb toxicity. There is substantial evidence that a high Pb level in the environment could affect blood Pb

level, intelligence and behavior. Pb poisoning in young children may cause permanent damage to the central nervous system and reduces intellectual capabilities. It also causes high blood pressure and hypertension in adults [34].

2.4 Cadmium Metal Contamination and Toxicity to Human Beings

The principal toxic effect of cadmium is its toxicity to the kidney, although it has also been associated with lung damage and skeletal changes in occupationally exposed populations. Cadmium poisoning is known to cause liver damage, kidney failure, and pulmonary diseases. Cadmium also appears to be a contributing factor in high blood pressure, bone diseases, anemia, pulmonary fibrosis, prostates cancer, lung cancer and yellow discoloration of the front teeth near gum line. The mechanism of cadmium poisoning may involve substitution insertion of cadmium in place of zinc in enzymes [35].

Acute doses (10-30 mg/kg per day) of Cd can cause severe gastrointestinal irritation, vomiting, diarrhea and excessive salivation, and doses of 25 mg/kg body weight can cause death [36]. The excessive content of a metal like Cd in food is associated with etiology of a number of diseases, especially with cardiovascular, kidney, nervous as well as bone diseases [37]. For instance, in Japan, Cd contamination of rice led to renal impairment and bone disease in an exposed population. It is necessary to decrease toxic heavy metal accumulation in cereals for food production, particularly in rice, which is one of the most frequently consumed cereals worldwide. Cd increases in content with age and is estimated to peak at 40 mg in the human body at 50 years of age [34].

Copper (Cu)

Copper is a naturally-occurring metallic element that occurs in soil at an average concentration of about 50 parts per million (ppm). It is present in all animals and plants and is an essential nutrient for humans and animals in small amounts [38]. The major sources of environmental copper releases include the mining, smelting and refining of copper, industries producing products from copper such as wire, pipes and sheet metal, and fossil fuel combustion. Water pipes are often made of copper and bath fixtures may be made from brass and bronze alloys that contain copper. The principal source of copper in drinking water results from the leaching of copper from pipes and bath fixtures due to acidic water. Blue-green stains left in bath fixtures are a sign of the presence of copper in water. Other releases of copper to the

environment include agricultural use against plant diseases and treatments applied to water bodies to eliminate algae. Health Effects Absorption/Metabolism Studies investigating oral absorption of copper have found the percentage absorbed ranging from 24-60 percent. Factors affecting the amount absorbed include the amount of copper in the diet and competition with other metals found in food such as iron and zinc. It is malleable, ductile and an extremely good conductor of both heat and electricity. Copper belongs to IB group of periodic system of elements, which is characterized by electronic configuration with one unpaired element [39].

Zinc (Zn)

Zinc occurs naturally in air, water and soil, but zinc concentrations are unnaturally due to addition of zinc through human activities. Most zinc is added during industrial activities, such as mining, coal and waste combustion and steel processing. Some soils are heavily contaminated with zinc, and these are to be found in areas where zinc has to be mined or refined, or sewage sludge from industrial areas has been used as fertilizer usually found in nature as the sulfide, it is often associated with sulfides of other metals, especially lead, copper, cadmium and iron [40]. It is essential and beneficial element in human metabolism. Zinc is considered to be relatively non-toxic, without enough zinc in the diet, people may experience loss of appetite, decrease sense of taste and smell, decrease immune function, slow wound healing, and skin sores. Zinc shortages can even cause birth defects [41]. The toxicity of zinc compounds to aquatic animals depends on several environmental factors. Particularly hardness, dissolved oxygen and temperature. Both an increase in temperature and a reduction in dissolved oxygen increase the toxicity of zinc. Toxicities of zinc in nutrient solutions have been demonstrated a number of plants [42].

2.4.1 Review of Some Methods used for Metal Analysis in Rice

Metals may be determined satisfactorily by a variety of methods, with the choice often depending on the precision and sensitivity required. Several spectrometry techniques have been used for heavy and trace elements determination in rice or rice products. The different techniques so far reported for the determination of metals in rice or rice products are: Atomic Absorption Spectrometry (AAS) [25, 26]. Hollow fiber solid phase micro extraction combined with differential pulse anodic stripping voltammetry (DPASV), Instrumental Neutron Activation Analysis (INAA) and Induced Coupled Plasma Spectrometry (ICPS) [26],

inductively coupled plasma atomic emission spectrometry (ICP-AES) [44] and Inductively coupled plasma- mass spectrometry (ICP-MS) [45].

2.4.2 Sample digestion methods

Wet Acid method

This method involves the destruction of organic matter through decomposition of the sample by an acid or mixture of acids, which is carried out in open vessels, in tubes, on hot plate or in aluminum heating block or in closed vessels at elevated pressure with thermal or microwave heating. The applicability of wet digestion technique is strictly dependent on the type of food: carbohydrates are easily mineralized with nitric acid at 180°C, while fats, proteins, and amino acids cause incomplete digestion due to the relatively low oxidation potential of nitric acid at 200°C. These materials require the addition of HClO₄ and/or HNO₃ with all the problems related to their use at high temperature and pressure [44, 46].

Wet digestion with HNO₃ and HClO₄ acids is recommended because of better recovery. The type of acid used in the preparation procedure can have important consequences in the measurement step. It is commonly known that in all atomic spectrometric techniques HNO₃ is the most desirable reagent. In spite of occasionally observed signal suppression in its presence, no severe analytical problems are encountered in practice with HNO₃ at concentrations up to 10% sometimes higher, in all atomic spectrometric techniques as long as its concentration is similar in calibration and sample solutions [46].

Principles of ICP-OES

The analysis of rice samples by ICP-OES requires Decision to make regarding sample preparation, wavelength selection and preparation of standard solution and consideration of potential interferences. Once the samples and standards are prepared, the hardware is set up properly and the computer is programmed, the analysis can be started. The analyst usually starts by introducing the first standard solution to the plasma and pressing a key on the computer. Assuming everything is found to be working properly, the analyst continues by introducing further standards (if used) and a blank solution to complete the calibration of the instrument [53]. To generate plasma, first argon gas is supplied to the torch coil and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. When plasma energy is given to the sample from outside, the

component elements (atoms) are excited. When the excited atoms return to the low energy position, emission rays are released and the emission rays that correspond to the photon wavelength are measured. The type of element is determined based on the position of the photon rays. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube. In OES, the intensity of the light emitted at specific wavelengths is measured and used to determine the concentrations of the elements of interest [53]. The advantages of OES over other spectroscopic techniques is that high temperature sources used in OES can populate a large number of different energy levels for several different elements at the same time. All of the excited atoms and ions can then emit their characteristic radiation at nearly the same time. This brings in the flexibility to choose from several different emission wavelengths for an element and the ability to measure emission from several different elements simultaneously [53].

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of Study Area

North Achefer is found in Amhara Regional state. It is one of the Woredas in West Gojjam zone located at a distance of 545 kilometers northwest of Addis Ababa, 45 kilometers southeast of Bahir Dar-the capital of the regional state. In terms of relative location, the Woreda is bounded in the Northeast by Lake Tan, in the northwest by Alefa Woreda of South Gonder, in the east by Bahir Dar Zuriya, Mecha to the Southeast and South Achefer to southwest. Astronomically, North Achefer lies between 11°30'00" to 11°50'00" North Latitude and from 36° 40'00" to 37°10'00" East longitude (CSA, 2007).

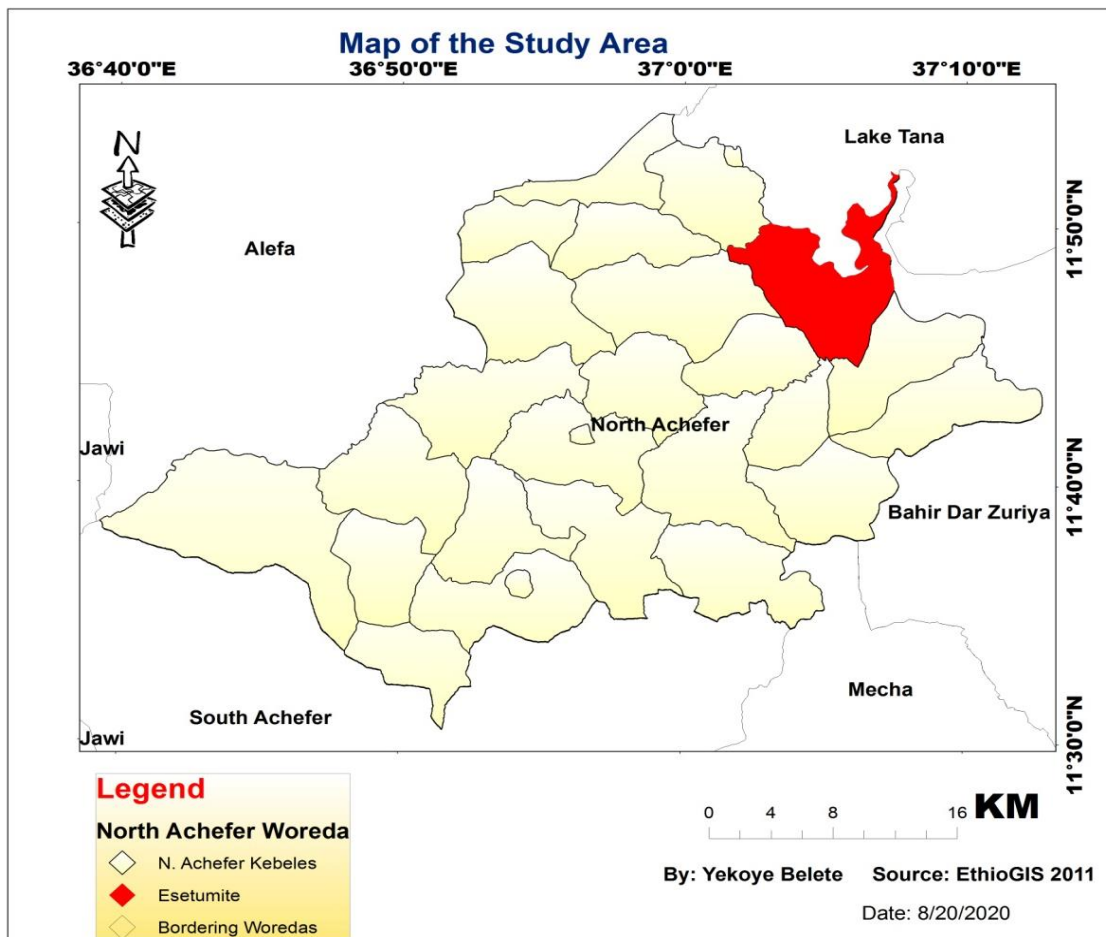


Figure 3.1: Map of North Achefer Woreda.

3.2 Sample Collection

Rice samples were collected from three farmers of Esetumite kebele (0.5Kg from each farmer) and Fogera rice samples from three merchants bought from Bahir Dar market randomly. The husk and other light and heavy contaminants were removed from the grain by siftings, winnowing and sieving to ensure that it is free from chaffs, dust and other impurities. The sample were washed in a plastic bag by shaking up and down with tap water until all dusts removed and then repeated three times with distilled water. Then, to have constant mass, the washed rice sample is oven dried at 85⁰C for 3 hours prior to digestion. The dried rice grain was powdered using mortar and pestle until it feels smooth [1].

3.3 Cleaning the apparatus

The polyethylene sampling bottle, reagent bottles, volumetric flask, measuring cylinder, beakers, porcelain mortar and pestle and any other materials and apparatus were washed regularly with tap water and detergent, then soaked with diluted HNO₃(10%). After washing, all the apparatus were rinsed with in distilled water.

3.4 Equipment and Apparatus

Analytical balance (ADAM Nimbus), ICP-OES (PerkinElmer Optima 8000), desiccators (VAKUUM), oven (US Binder), oven (DAIHAN Scientific), refrigerator (Long ford Engineering Company), and water bath (China Hh. S Series), Borosilicate volumetric flasks (50 ml and 100 ml), measuring cylinders, pipettes, porcelain crucible, Filter paper Whatmann No. 1 and funnel were used to carried out the analysis.

3.4.1 Apparatus

Electronic beam balance (Nimbus, NBL-254i, and UK) for mass measurement, whatmann No.1 filter paper for filtering the solution, fume hood for digestion of samples, laboratory Para film for covering the volumetric flasks containing the solution and microwave oven for drying the sample were used. The digested samples were kept in the refrigerator (LEC, Refrigerator, PLC, England) prior to characterization. Besides this, the researcher was used laboratory glassware, volumetric flasks, beakers, hot plate (Witeg, MSH-20D), digestion flasks, plastic bottles, reagent bottles and thermometer during the laboratory work.

Inductively coupled plasma optical emission spectrometer (ICP-OES) was applied for the determination of toxic and essential metals in the rice samples.

3.4.2 Chemicals and Reagents

All the chemicals and reagents used for the laboratory work were analytical grade. Distilled water was used throughout the laboratory work for sample preparation, dilution and rinsing each apparatus before working with it. Nitric acid, HNO_3 (69%) and per chloric acid HClO_4 (70%) were used for sample digestion. Stock standard solutions containing 1000 mg/L of the metals zinc, copper, cadmium and lead were prepared from $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Pb}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ certified standard solutions respectively. Standard solutions of 1000 mg/L and intermediate solutions of 10 mg/L of each metal were prepared to plot the calibration curves for the metals. Working solutions were prepared from the standard solutions (1000 mg/L) of the metals by appropriate dilution of the intermediate solution (10 mg/L).

3.5 Instrumentation

A PerkinElmer optimum **8000** inductively coupled plasma emission spectrometry (ICP-OES) equipped with argon plasma was used for the determination of their respective metals in rice samples. Before analysis of the samples the instrument was optimized to give maximum signal strength by adjusting the parameters such as RF power, plasma argon flow rate, nebulizer argon flow rate and pump flow rate (Table : 3.1).

Table: 3. 1: The level of the studied metals in rice samples was determined using ICP-OES under the following operational parameters or conditions.

Parameter	Conditions
RF power	1500 Watts
Plasma argon flow rate	12 (L/min)
Nebulizer argon flow rate	0.8 (L/min)
Auxiliary argon flow rate	1.0 (L/min)
Pump flow rate	2.0 (ml/min)

3.6 Standard solution preparation

Standard solutions are solutions that contain a known and accurate amount (concentration) of a substance or element. These solutions are commonly used to determine the concentration of a substance whose concentration is unknown. Because these solutions contain accurate concentrations of a chemical component, they will increase confidence regarding the determination of substances with unknown concentrations.

Therefore, in this study the working solutions were prepared by first preparing intermediate standard solution (10mg/L) from stock standard solution (1000mg/L). To prepare 10 mg/L intermediate standard solution, 1ml stock solution was pipated into 100ml calibrated flask and finally diluted with deionized water up to the level and the solution was mixed thoroughly. The working standards prepared were 0.05mg/L, 1.05mg/L, 2.05mg/L, 3.05mg/L, 4.05mg/L, 5.05mg/L to each metal.

Table: 3.2 Series of working standard concentrations for the determination of metals in rice using ICP-OES.

S. No.	Metals	Concentration of standards(mg/L)
1	Cu	0.05, 1.05, 2.05, 3.05, 4.05, 5.05
2	Pb	0.05, 1.05, 2.05, 3.05, 4.05, 5.05
3	Zn	0.05, 1.05, 2.05, 3.05, 4.05, 5.05
4	Cd	0.05, 1.05, 2.05, 3.05, 4.05, 5.05

S. No. = sample number

3.7 Standard solution preparation for Calibration Curve

Calibration curves were prepared to improve the accuracy of the instrument and to determine the concentration of the metals in the sample solution. The calibration curves were drawn for Cu, Zn, Cd and Pb using linear regression analysis of the concentrations of the standard solutions versus intensity values. Six series of working standard solutions of metals were prepared by diluting the intermediate standard solution (10 mg/L) with deionized water. The blank and standards were run in ICP-OES and six point of calibration curve were established. Each standard solution was measured three times and the mean was plotted. The correlation coefficients of calibration curves are given in the table below. The correlation coefficient of more than 0.9999 showed that there is strong linear relationship between concentrations and

intensity. Each of the sample solutions were aspirated into ICP-OES instrument and the intensity values of the metals was recorded. Triplicate determinations were carried out on each rice sample. Concentrations of each metal were determined by interpolation of their intensity with the calibration curve.

3.8 Sample Digestion and preparation of analyte for ICP-OES

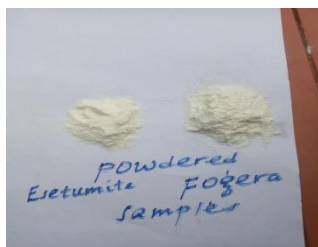
All glassware's were washed cleaned before use with deionized water periodically: soaked in nitric acid, then rinsed in deionized water and air dried. The glass ware kept in a clean place to avoid contamination.

The rice sample (1.5 kg) collected from Esetumite kebele, North Achefer Woreda then it was dried using the microwave oven for about 3 hours at 85⁰C and then grinded by mortar. 20g of rice sample were taken. From the mass of this sample, 0.5g triplicate samples were measured and then kept for digestion.

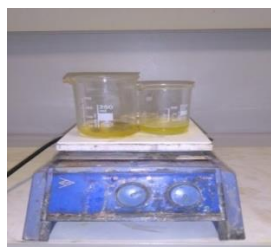
Acid digestion methods were applied to digest the collected samples. A mixture of 6 ml of HNO₃, and 4 ml of HClO₄ (3:2) were used to digest and heated at 80°C for 2:30 hour until clear and color less solution was appeared [1]. The mixture was cooled for 10 minutes. Then, each replicate samples were transferred into 50ml round bottom flask using a funnel and what man No.1 filter papers. Next are going to be diluted to 25 mL distilled water and kept in the refrigerator till analysis [2]. Digestion of reagent blanks were also performed in parallel with the rice samples keeping all the digestion parameters the same [1].



A) Rice samples



B) Rice powder



C) beginning of digestion



D) digestion after 1hr



E) Digestion after 2:30 hrs. was clear and colorless

Figure: 3.2 Sample solutions at different stages of digestion

3.8.1 Calibration Curve, toxic metal analysis, and statistical data analysis

Working standard solutions of Cu, Zn, Pb and Cd were prepared by serial dilution of 1000 mg/L. The solutions were subjected to ICP-OES (Optima 8000, Perkin Elmer) determination. Calibration curves were plotted for each element as a function of intensity versus concentration. The plotted calibration curves were subsequently utilized to determine the concentration of each toxic and essential metal in the rice samples.

3.8.2 Analysis of Metal contents of Digests

ICP-OES was used to determine the concentration of digested samples after treatment through different conditions of the parameters. The analysis was done at working conditions of the ICP-OES for copper, zinc, lead and cadmium.



Figure: 3.3 shows ICP-OES used for the determination of heavy metals (Zn, Cu, Cd &Pb) from digested rice samples

3.9 Data analysis

Data was analyzed using Microsoft Excel and Tukeys one way analysis of variance (ANOVA). All statistical analysis was done by the statistical package for social science (spss.20) program. The data were expressed in term of descriptive statistics while the figures were presented with mean values as (Mean \pm SD). A p-value less than 0.05 were considered as significant.

Details of the procedures followed during the experiment are summarized in figure below.

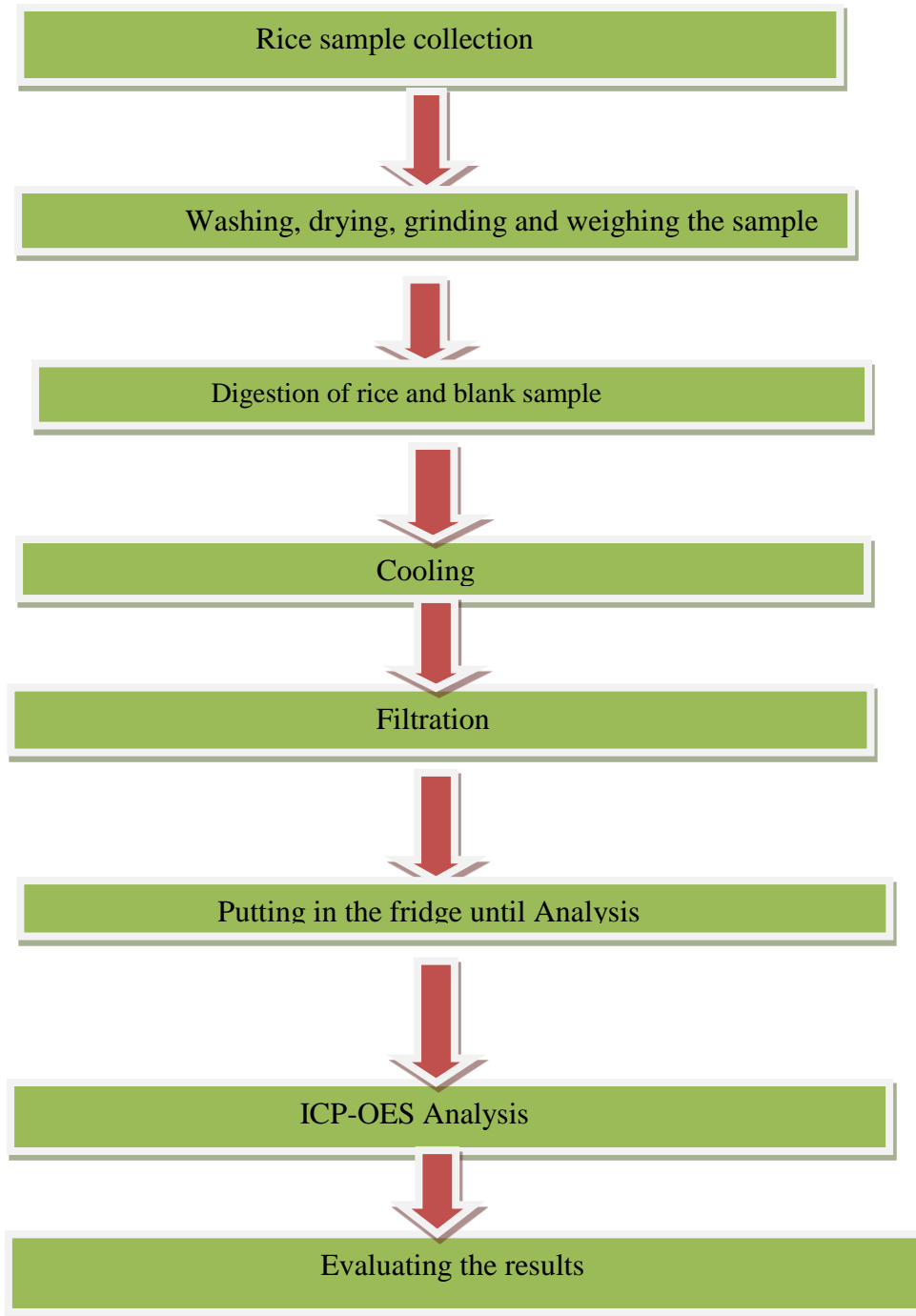


Figure: 3.4 Flow chart of the general Experimental procedure

3.9.1 Method Validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. The parameters: accuracy, precision, specificity, limit of detection and limit of quantification are used for method validation. In this study, precision of the results was evaluated by standard deviation of the results of the six measurements, triplicate digestion and triplicate readings.

3.9.2 Procedure for recovery of metal analysis

The recovery of metals was studied by spiking known amounts of standard solution to the selected sample. The amounts added were (5-7%) of the sample concentration, so that they would not cause significant deviation from the amounts naturally found in the rice. This means there is a very small change in the concentration the rice sample so that the calibration curve does not change. Accordingly, 0.0248 mg/L of Cu and 0.0115 mg/L of Pb were added to 1 mL of rice samples and digested with the same procedure of hot plate apparatus for rice samples. After diluting the spiked samples to 50 mL with deionized water, they were analyzed with the same procedure followed for the analysis of rice samples [2]. Then the recovery was calculated as:

$$\text{Percentage recovery} = \frac{X_s - X}{X_{\text{added}}} * 100 \text{-----6}$$

Where; X_s - mean result of spiked samples, X - Mean result of un-spiked samples, X_{added} - Amount of known analyte (standard) added.

3.9.3 Precision

Precision is probable term to express the extent of errors in a given analytical results. Analytical results must be evaluated to decide on the best values to report and to establish the probable limits of errors of these values. The analyst will be concerned with the question of precision (repeatability of results), that is, the agreement between a set of results for the same quantity. In this study, the precision of the results was evaluated by standard deviation of the results of two samples and triplicate readings for each sample giving a total of six measurements for given rice sample with in a day.

On the other hand, the accuracy and validity of the measurements were determined by analyzing samples [2].

3.9.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) are two important performance characteristics in method validation. In general, the LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test [2].

Therefore, from the calibration curves of each heavy metal, the LOD & LOQ were calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (m) at levels according to equation 1&2. The instrument detection limit (IDL) is obtained from the instrument manual [2].

$$\text{LOD} = 3 * \text{SD}_{bl} \dots\dots\dots 1$$

$$\text{LOQ} = 10 * \text{SD}_{bl} \dots\dots\dots 2$$

Table: 3.3 LOD, LOQ and IDL for the determination of heavy metals in rice samples

Heavy metal	Calibration equation	LOD (mg/L)	LOQ(mg/L)
Cu	$y = 918378x - 21416$	0.000000030	0.00000009
Zn	$y = 168070x - 1252.5$	0.000001404	0.00000425
Cd	$y = 485522x - 10506$	0.000000004	0.00000001
Pb	$y = 37949x - 814.67$	0.000000975	0.00000295

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1 Calibration Curve and the determination Metal Concentrations

The quality of data resulted from ICP-OES measurements are affected by sample digestion, calibration and standard solution preparation procedures. In this study, the instrument was calibrated by using six series of working standard solutions prepared from intermediate solutions for each metal under determination [2]. The table below shows the working range of standard solutions and the value of correlation coefficients of calibration curves for each toxic and essential metal as well as the linearity of data on the calibration for Copper, Zinc, Cadmium and Lead are seen in figure 4.1, 4.2, 4.3 & 4.4 respectively.

Table 4.1: show series working standards and correlation coefficient of the calibration curve.

S.No	metal	Concentration of Working standard solutions(mg/L)	Correlation coefficient of the calibration curve
1	Zn	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.9998
2	Cu	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.9999
3	Cd	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.9997
4	Pb	0.05, 1.05, 2.05, 3.05, 4.05, 5.05	0.9999

The obtained squared correlation coefficient (R^2) values of calibration curves ranged between 0.9997 and 0.9999. These high square correlation coefficient values demonstrate that there is a linear correlation between the intensity and metal concentrations [2].

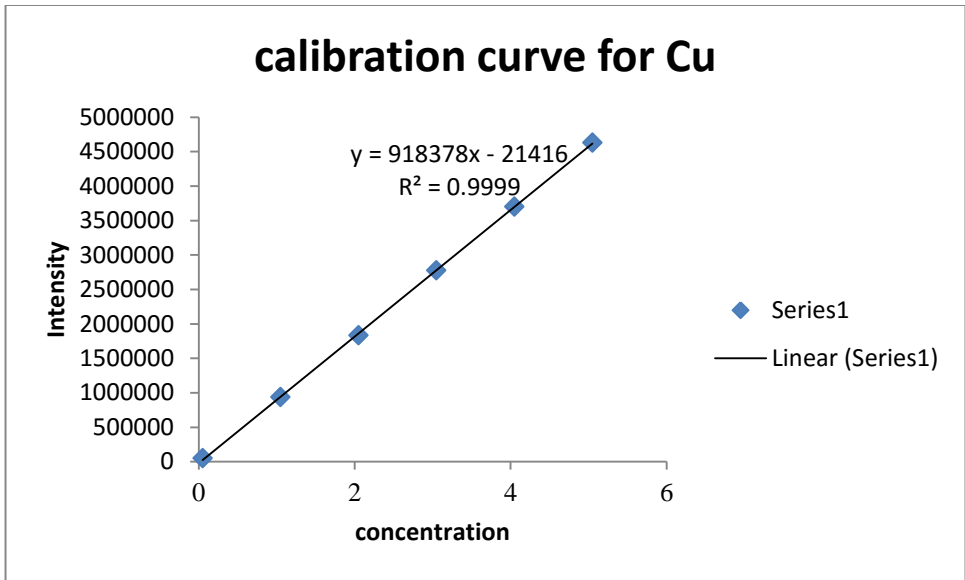


Fig. 4.1 calibration curve for copper

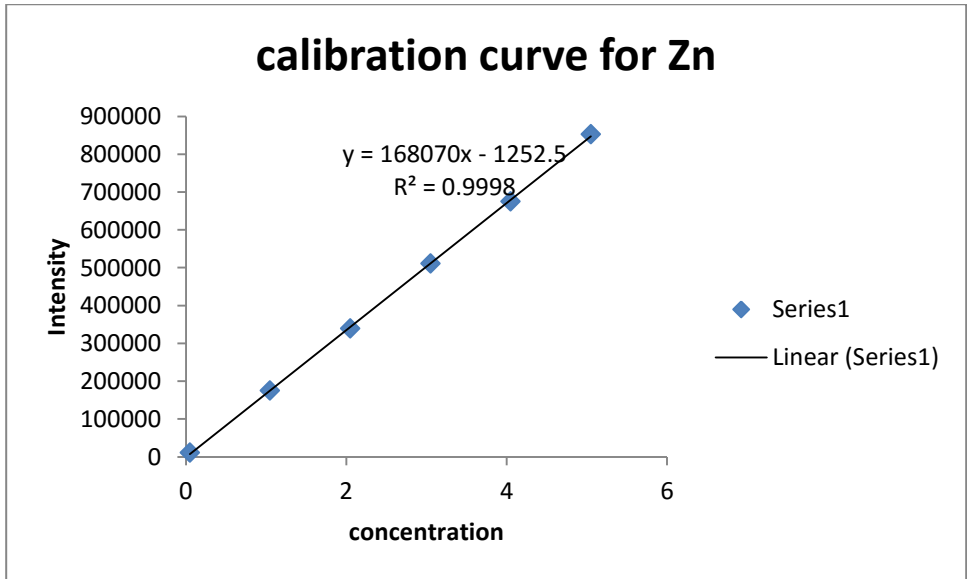


Fig.4.2 calibration curve for zinc

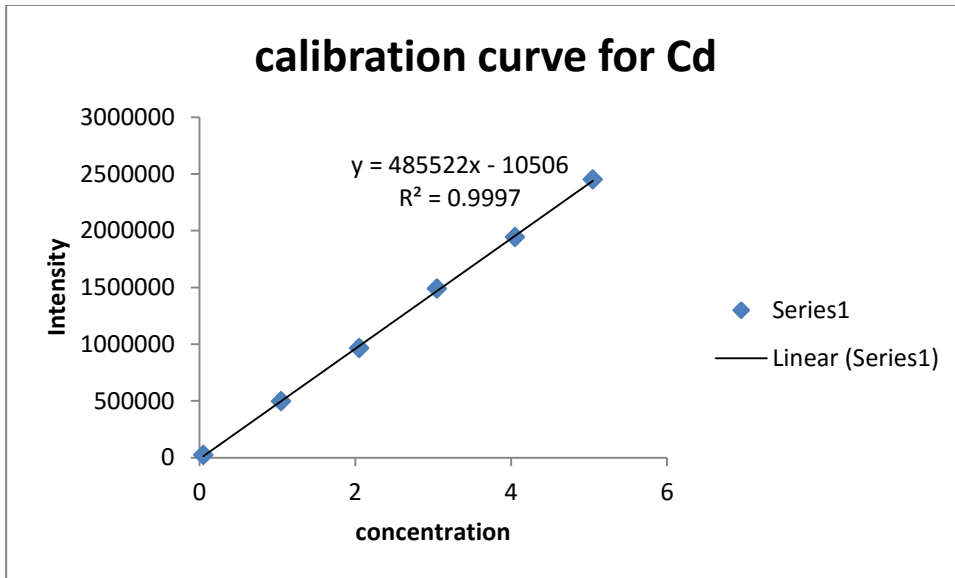


Fig.4.3 calibration curve for cadmium

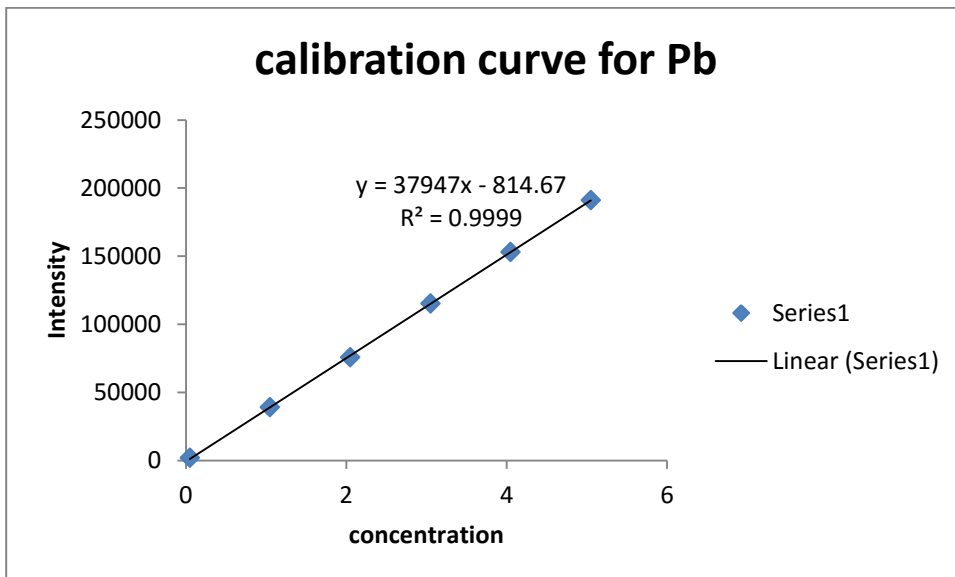


Fig.4.4 calibration curve for lead

4.1 Evaluation of Results

4.1.1 Precision of the Measurements

When we see the measurements for essential and toxic metals copper, zinc, cadmium and lead in the present study of rice samples, the resulted SD values for samples from Esetumite were 0.0317 ± 0.0020 , 0.2613 ± 0.0810 , 0.0007 ± 0.0006 and 0.0093 ± 0.0049 as well as Fogera were 0.0453 ± 0.0064 , 0.3333 ± 0.0483 , 0.0007 ± 0.0006 and 0.0217 ± 0.0133 respectively. It indicates that the measured data for toxic and essential metal determination in rice samples was highly precise.

4.1.2 Mean concentration of Heavy metals in rice sample

The average heavy metal concentrations in the rice sample from Esetumite kebele represented by Esetumite (E) and Fogera rice sample represented by (F) from Bahir Dar market are presented in table: 4.2 below.

Table: 4.2 mean concentration of Cu, Zn, Cd & Pb (mean \pm SD, n=3) in rice samples from Esetumite kebele and percussed Fogera rice from Bahir Dar market

Sl. No.	Heavy metal	Average concentrations in mg/L (n=3), (mean \pm SD)				
		E	F	Total	Min	Max
1	Cu	0.0317 ± 0.0020	0.0453 ± 0.0064	0.038500 ± 0.0086	0.0300	0.0500
2	Zn	0.2613 ± 0.0810	0.3333 ± 0.0483	0.297333 ± 0.0715	0.1810	0.3860
3	Cd	0.0007 ± 0.0006	0.0007 ± 0.0006	0.000667 ± 0.0005	0.0000	0.0010
4	Pb	0.0093 ± 0.0049	0.0217 ± 0.0133	0.015500 ± 0.0112	0.0060	0.0370

- Means within a raw are significantly different ($p \leq 0.05$), ND= Not detected ($p \leq 0.05$)

4.1.3 The limit of detection, limit of quantification and percentage Recovery

In the following table the LOD, LOQ and IDL (instrument's detection limit) are given. The LOD values were calculated by using an equation, $LOD = 3 SD$, where SD is for standard deviation of the blank in the calibration curve of each toxic and essential metal. Similarly, LOQ values were calculated by the equation, $LOQ = 10 SD$.

S.no	Metal	Amount spiked	Concentration in sample (mg/l) n=3	Concentration in spiked sample (mg/L)	Recovery (R %)
1	Cu	1	1.123	0.021	110.2
2	Zn	1	1.123	0.099	102.4
3	Cd	1	1.093	0.001	109.2
4	Pb	1	1.017	0.005	101.2

Percentage recovery of the spiked concentrations for metals zinc, copper, cadmium and lead in rice samples was calculated. Percentage recovery of the spiked metals ranged from 101.2% to 110.2%. The percentage recovery range value between (80% - 120%) indicates that the method applied to determine toxic metals was valid [2].

Table 4.3: Instrument detection limit, method detection limit and method quantification limit

Heavy metals	Analyte wavelength	IDL (mg/L)	LOD (mg/L)	LOQ (mg/L)	% Recovery
Cu	327.293	0.0097	0.846	2.807	110.2
Zn	206.200	0.0059	0.077	0.256	102.4
Cd	228.802	0.0027	0.055	0.184	109.2
Pb	220.353	0.0420	0.108	0.363	101.2

As we can see from the table above, the LOD and LOQ values for each toxic and essential metal are greater than the IDL values indicating that the precision of the selected analytical method was good [2].

4.2 Concentration of toxic and essential metals in rice samples

The concentration level of toxic (Cd, Pb) and essential (Zn, Cu) metals in rice were determined by ICP-OES. Triplicate solutions of each sample were measured. The concentration levels were calculated using the mean of each value with standardization of the instrument by using the standard solutions and blank reagent. Toxic and essential metal concentration levels were based on the samples' dry weight and the mean results are the average values of the three replicates. The results indicate that the mean concentration of zinc and copper in rice samples was greater than that of their mean concentration of lead and cadmium samples. The concentration level of each toxic and essential metal ($\text{mean} \pm \text{SD value}$), in the rice sample is given in table 4.4 below. Concentrations in mg/Kg of each toxic metal in the digested samples were also calculated using the following expression.

$$[M] = \frac{C}{W} \times V$$

Where [M]=total metal concentration (in mg/Kg)

C=concentration of the metal in the digested samples (in mg/L)

W=weight/mass/ of the digested sample

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

Table 4.4: Concentration levels ($\text{mean} \pm \text{SD}$) of toxic and essential metals determined in rice samples

Sample	Concentration (mg/Kg)			
	Zn	Cu	Cd	Pb
Esetumite Rice (E)	26.1±0.2	3.17±0.02	0.1±0.01	0.9±0.07
Fogera Rice (F)	33.3± 0.09	4.5±0.01	0.1±0.01	2.1±0.1

4.3 Comparison of the average toxic and essential metal concentrations in rice samples

From the rice sample, essential metal like zinc and copper were present. On the other hand, cadmium and lead were found in the rice sample to contain toxic and essential metals.

Rice samples: From both the determined toxic metals, lead was found in the greatest concentration level. Its concentration is 2.1 mg/Kg. The least determined toxic metal was cadmium whose concentration is 0.1mg/Kg. The concentration level of trace metals in rice was decreased in the order of Zn>Cu>Pb>Cd.

From both the determined essential metal, zinc was found in the greatest concentration level. Its concentration is 33.3 mg/Kg from Esetumite. The least determined essential metal was copper whose concentration is 3.17 mg/Kg from Fogera. Of the toxic metals, in the rice sample, the concentration of cadmium was found to be relatively higher than any other toxic metal determined. Its concentration is somewhat varied another sample.

In general, the concentration level of toxic and essential metals in rice samples in Esetumite kebele is relatively higher different and its amount is significant. This high concentration of toxic and essential metals in such types of food items is expected because of the high flooded area used by the farmers.

Research reports indicate that Pb contamination in rice may be associated with atmospheric emission of lead gasoline, human activities such as mining, manufacturing and fossil fuel burning has resulted in the accumulation of lead and its compound in the environment, including air, water and soil. The industrial revolution gave rise to an increase for lead in the environment and even bigger increase occurred when lead gasoline was introduced. Farm inputs like fertilizers, livestock manure, pesticides and fungicides are used in the production of rice and all these might contain cadmium and lead metals for rice plant uptake on the farms. However, if the levels of these metals are elevated, then they can show negative effects. Therefore, in order to be aware about the levels of metals in food, especially in rice, their analysis is mandatory.

Rice can accumulate cadmium naturally from the soil. As a result, its concentration could vary significantly in rice sample. According to the FAO/WHO (2007) data, the level of Cadmium for rice ranges from ND–0.2 mg/Kg [34]. The FAO/WHO also recommends the maximum of 0.2 mg/Kg Cd in rice sample. As compared to this, the present study showed lower level of

cadmium in rice sample, it should be noted that the high intake of cadmium could lead to distortion in calcium and the formation of kidney stones. It can also affect the skeletal and respiratory system [51, 52]. Copper and zinc were detected heavy metal in rice sample.

4.4 Comparison between the average toxic and essential metal concentration of this study with that of literature and JECFA values

There are some reports from different countries on the determination of toxic and essential metals in rice sample. It is important to compare the result obtained from the analysis of toxic and essential metals in this study with the values determined in other literatures JECFA's guideline values. The comparative study of toxic and essential metals determined in this study and the reported values of some other literatures is given in table 4.4 below.

The concentration level of Pb in rice samples tested were in the value of 0.9 ± 0.07 mg/Kg of Esetumite rice and 2.1 ± 0.1 mg/Kg of Fogera rice. This concentration range of Pb agrees with the literature value reported in India and Pakistan of rice samples. However, its contents were found to be above the provisional maximum tolerable daily intake limits of JECFA. Higher concentration of Pb may lead to hematologic, immunologic, neurotoxin, pulmonary toxic, nephrotoxic, hepatotoxic, and carcinogenic effects. Nielson and Flyvholm (1984) reported that higher intakes of Pb in diet could result in its accumulation in the lungs, which may lead to bronchial hemorrhage, while other symptoms include nausea, weakness, and dizziness [53].

Pb was detected in rice sample with a concentration level of 0.9 ± 0.07 mg/Kg of Esetumite rice and 2.1 ± 0.1 mg/Kg Fogera rice. This concentration level of Pb is below the concentration levels reported by other literatures listed in the table 4.4 and above the provisional maximum tolerable daily intake limits of JECFA. Accumulation of Pb in body tissues may cause acute toxicity by causing anemia, headache, fatigue, and peripheral nervous disorders [9, 13]. Therefore, it is advised to minimize the excessive intake of food items that can contain Pb like rice.

Excess concentration of lead causes damages the nervous system and causes brain disorder. Excessive lead also causes blood disorders in mammals. Regarding to the toxicity aspect; the level of cadmium in the analyzed rice samples from this study was in the range of the reported literature values. It was also above the permissible limits set by JECFA. For rice samples, the concentration of Cd was found to be in the range of 0.1 ± 0.001 mg/Kg. This indicates that the rice samples under study are high for cadmium toxicity.

The IARC has classified cadmium as a human carcinogen and linked it with increased risk of cancer [31, 32]. Cadmium can accumulate in the kidney and liver and may cause prostate cancer and other carcinogenic effects. Short-term and long-term exposure to high levels of Cd in food items may affect human health and cause problems such as nausea, vomiting, diarrhea, muscle cramps, salivation, sensory disturbances, liver injury convulsions, shocks, and kidney-related problems [51]. Hence, it is a necessary to control and monitor the concentration level of cadmium while consuming rice.

In general, the findings of this study indicate that our body can accumulate toxic metal like (Cd, Pb) and essential metal like (Zn, Cu) when we consume food items like rice. This higher concentration of toxic and essential metals in rice sample may be due to the higher flooded area, vehicle, and factories and so on.

Table 4.5: Comparison between the average essential and toxic metal concentration of samples of this study with that of literature and JECFA`s values.

Sample	Toxic and essential metal concentration (mg/Kg)				Country	Reference
	Zn	Cu	Cd	Pb		
Ri E	26.1±0.2	3.17±0.02	0.1 ± 0.01	0.9± 0.07		This
ce F	33.3±0.09	4.5 ± 0.01	0.1 ± .01	2.1 ± 0.1	Ethiopia	study
	17.706±0.001	0.778-1.018	<0.02-1.78	0.135-1.53	Saudi Arabia	[25]
Differe						
nt rice	14.956-15.45	0.835-1.035	ND	1.0-22.73	Nigeria	[30]
sample	51.4-51.8	13.7- 16.3	0.52 – 0.56	3.1-3.5	NERICA	[42]
	10.3-16	2.7-4.9	0.02-0.178	0.135-1.53	Saudi	[25]
	17.1-24.4	1.27-1.53	0.34 mg/kg	3.3 mg/kg	Ethiopia	[20]
PMTD	16 – 20.4	2.8 – 10	0.2- 0.3	0.3- 3.8	JECFA	[35]
I						

CHAPTER FIVE

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Rice is a staple food crop for more than half of the world's population. In this study, the rice samples were collected from Esetumite kebele, North Achefer, west Gojjam and Fogera rice samples from Bahir Dar markets were subjected to their toxic and essential metal content. The analysis was done after digesting the samples using acid digestion method. Determination of the concentration level of selected toxic and essential metals was performed by using ICP-OES. The results showed that the understudy toxic and essential metals the concentrations of Zn are generally higher than the FAO/WHO limit. While Cu, Pb and Cd shows that comparable results. The concentration of Zn was found in the range value of 33.3 ± 0.09 mg/Kg – 26.1 ± 0.2 mg/Kg for rice and another essential metal determined was Cu whose concentration value of 4.5 ± 0.01 mg/Kg – 3.17 ± 0.02 mg/Kg. The toxic metal determined was Pb whose concentration value is in the range of 2.1 ± 0.1 mg/Kg – 0.9 ± 0.07 mg/Kg in rice sample. The concentration value Cd in both samples is 0.1 ± 0.01 mg/Kg is also in the range of its values reported by other literatures as well as the JECFA provisional maximum tolerable daily intake limits.

It should be stressed at this point that a number of additional samples need to be analyzed before any general conclusion regarding the lead and cadmium residue in rice sample can be drawn. In addition, further studies on toxic metals and even the essential one is required not only on rice sample for quality assessment but also various characteristic properties of soil, like soil pH, total Cd total Pb, soil organic matter level, soil salinity, soil oxides, and cation exchange capacity (CEC) and identify bioaccumulation of heavy metals in relation to specific variety of rice and geographical location.

In addition, there has to be assessment on levels of lead and cadmium on the imported fertilizer since a number of countries have established limits on the content of lead and cadmium in phosphate fertilizers. The aim of these restrictions is generally to limit the supply of lead and cadmium to agricultural soils.

5.1 Recommendation

Based on the outcomes from this study, the following recommendations are being suggested;

- Awareness must be created by relevant organization and authority on the concentration of metals about rice sample.
- Continuous research work should be carried out by relevant organization and individual experts especially in the levels of toxic metals like Pb and Cd in the rice grains.
- Further studies should be conducted with those metals and other metals to investigate rice sample usability for the society.
- Further studies should be carried out to conduct exposure assessment on the dietary levels of these heavy metals to provide consumer advice.
- Further studies should be conducted to investigate the levels of heavy metals in the soils of rice farms to ascertain the correlation between soil and grain concentration.
- Environmental protection managers and other relevant stakeholders should use the findings obtained to regulate the rate of agrochemical application and formulate policies to safeguard the safety of rice produced in the environment.

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