ADDIS ABABA UNIVERSITY

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DEPARTMENT OF CHEMISTRY

Determination of Selected Essential Metals in Wheat (Triticum aestivum) Samples collected from Shire, Tigray, Ethiopia Using Atomic Absorption Spectroscopy

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June, 2025

Addis Ababa, Ethiopia

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A thesis submitted to Addis Ababa University, College of Natural and Computational Sciences, Department of chemistry in partial fulfillment of the requirements for Master of Science in chemistry

Advisor: Merid Tessema (PhD)

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Declaration

I hereby declare that the thesis titled "Determination of Selected Essential Metals in wheat (Triticum aestivum) Samples Collected from Shire, Tigray, Ethiopia Using Atomic Absorption Spectroscopy" is my original work. It has not been submitted for any degree or examination at any other institutions or university. I confirm that all sources have been referenced or quoted appropriately acknowledged and cited.

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The thesis has been submitted with my approval as supervisor

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Abbreviations and Acronyms

AAS	Atomic Absorption Spectrometry
BDL	Below Detection Limit
DNA	Deoxyribonucleic acid
EFDA	Food and Drug Authority
EPA	Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectroscopy
FAO	Food and Agriculture Organization
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
HPLC	High-performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
LOD	Limit of Detection
LOQ	Limit of Quantification
SD	Standard Deviation
RNA	Ribonucleic acid
RSD	Relative Standard Deviation
SD	Standard Deviation
WHO	World Health Organization
WFP	World Food Program
XRF	X-ray Fluorescence

Abstract

In this study, the concentrations of essential metals, copper, iron, zinc, and magnesium were determined in wheat grain samples collected from the local market in Shire, Ethiopia, using flame atomic absorption spectroscopy. Sample preparation involved washing, drying, grinding, sieving, and digestion with a mixture of nitric acid and perchloric acid under optimized conditions (15 ml acid mixture, 250°C, 3 hrs.). The average concentrations of Cu (7.72 ppm), Fe (43.27 ppm), Zn (28.43 ppm), and Mg (436.56 ppm) were assessed against permissible limits set by the World Food Programme (WFP), the Food and Agriculture Organization (FAO), and literature values.

The concentrations of copper and magnesium were found to be well below both WFP and FAO limits, and aligned with literature values, indicating no risk of toxicity. Zinc concentration was slightly below the maximum permissible levels and consistent with typical literature ranges, suggesting adequate dietary contribution. However, the iron concentration exceeded the WFP permissible limit of 40 ppm, though it remained within the FAO upper limit of 50 ppm. This elevated level may reflect iron-rich soil or agricultural practices, and it raises potential concerns for individuals with iron metabolism disorders.

Recovery studies yielded acceptable values—79.4% for Cu, 85.1% for Fe, 115.4% for Zn, and 112.18% for Mg—thereby confirming the reliability of the analytical method. Overall, the study concludes that wheat grains contain safe and nutritionally beneficial levels of Fe, Zn, and Mg. However, the borderline recovery observed for Cu, while potentially acceptable at lower concentration levels, indicates that further validation or method optimization may be necessary.

Keywords: wheat, Triticumaestivum, Flame atomic absorption spectrometry

1. INTRODUCTION

1.1 Background

Wheat (*Triticum aestivum*) is one of the oldest and most widely cultivated cereal grains globally. Its domestication began over 10,000 years ago in the Fertile Crescent, a region that includes parts of modern-day Iraq, Syria, Iran, and Turkey. Early species such as einkorn (*Triticum monococcum*) and emmer (*Triticum dicoccum*) were among the first plants to be domesticated, marking a significant milestone in the Neolithic Revolution and the rise of settled agriculture [1].

As civilizations evolved, wheat became a cornerstone of ancient economies, particularly in Mesopotamia, Egypt, Greece, and Rome. In ancient Egypt, it was not only a dietary staple but also a symbol of life and prosperity, commonly used in the production of bread and beer [2]. The Roman Empire significantly expanded wheat cultivation across Europe, integrating it into widespread food systems and trade networks [3].

During the middle Ages, wheat maintained its importance as a staple food, although consumption patterns differed across social classes. Nobility typically consumed refined white bread, while the lower classes relied on darker, coarser varieties. Agricultural advancements such as crop rotation and improved plowing techniques contributed to higher yields, ensuring wheat's continued dominance in food production [4].

From the 16^{th} to the 18^{th} centuries, European exploration and colonization facilitated the global spread of wheat. It was introduced to the Americas, where it thrived in regions like the North American plains and the Argentine pampas. The Industrial Revolution and the development of mechanized agriculture, along with expanding railroad networks, further boosted wheat production and international trade [5].

In the 20^{th} century, the Green Revolution, led by scientists such as Norman Borlaug, introduced high-yield and disease-resistant wheat varieties. These innovations significantly improved food security in many developing regions, particularly in Asia and Latin America (Offenheiser, 2020). Today, wheat is grown on more land than any other crop and remains a staple food for over one-third of the world's population [6].

Figure 1: Farm land of wheat, https://www.istockphoto.com/en/photo/golden-wheat-field-and-sunny-day-gm 531184656-93679721.

Figure 2: **wheat grain of Ethiopia, httpps://www. shutterstock.com>search>grains-Ethiopia.**

1.1.1 Wheat in Ethiopia: Nutritional and Safety Concerns

In Ethiopia, wheat plays a critical role in the national diet, serving as a major source of essential nutrients. It provides vital minerals such as iron and magnesium, which are important for oxygen transport, energy metabolism, enzymatic activity, and neuromuscular function [7].

However, the potential contamination of wheat grains with heavy metals such as copper and zinc has raised growing public health concerns. While Cu and Zn are essential micronutrients for both plant development and human health, excessive accumulation can lead to toxicity, resulting in gastrointestinal distress, liver damage, and neurotoxicity [7].

A major source of such contamination in Ethiopia, particularly in urban areas like Shire, region is the use of untreated or partially treated wastewater for agricultural irrigation. This practice introduces heavy metals into the soil and, consequently, into food crops. A systematic review found that consuming food contaminated with heavy metals can interfere with biological and biochemical functions in the human body, leading to serious health complications. The review stressed the urgent need for effective regulatory frameworks to ensure food safety [8].

Recent investigations, have assessed the concentrations of various metals including Fe, Mn, Cu, Zn, Ni, Cd, and Pb in wheat flour samples from multiple Ethiopian regions. Although the levels detected were within internationally accepted safety limits, the researchers emphasized the importance of ongoing surveillance to prevent future health risks [9].

Given the status of wheat as a dietary staple in Ethiopia, and considering the environmental and anthropogenic sources of contamination, it is essential to routinely assess the levels of both essential and potentially toxic metals in wheat grains consumed in urban areas. Such monitoring is vital to maintaining the nutritional quality of wheat and protecting public health.

1.2. Statement of the Problem

Wheat (*Triticumaestivum*) is a staple food in Ethiopia, contributing significantly to the daily caloric intake of the population. However, concerns over heavy metal contamination in food crops have emerged due to increasing environmental pollution and agricultural practices. Studies have shown that industrial activities, vehicular emissions, and the use of contaminated irrigation water can introduce heavy metals such as copper and zinc into wheat grain, while Cu and Zn are essential micronutrients, excessive accumulation can pose serious health risks, including liver and kidney damage, gastrointestinal disorders, and neurotoxicity [8,9].

In Ethiopia, urban agriculture is widespread, and the use of untreated wastewater for irrigation has been identified as a potential source of heavy metal contamination in crops and lack of regular monitoring and assessment of metal concentrations in wheat grain raises concerns about food safety and public health. Additionally, while iron and magnesium are essential for human nutrition, deficiencies or imbalances in these metals can contribute to anemia, metabolic disorders, and other health complications [7,10].

Despite the importance of ensuring safe levels of both essential and nonessential heavy metals in food, there is limited research on the levels of Cu, Zn, Fe, and Mg in wheat grain consumed in Ethiopia. Existing studies focus primarily on soil and water contamination rather than direct analysis of wheat grains available in markets and households. Without comprehensive data on the metal content in wheat, it is difficult to assess the potential health risks or nutritional benefits associated with its consumption.

This study aims to bridge this gap by determining the concentration of Cu, Zn, Fe, and Mg in wheat grain samples collected from Shire, Ethiopia. By comparing the findings with international safety standards, the research will provide insights into potential health risks and inform policy makers and stake holders on necessary interventions to ensure food safety and public health protection.

1.3. Scope of the Study

This study focuses on the determination of selected essential metals (Cu, Zn, Fe and Mg) in wheat grain samples collected from Shire local market. The research primarily examines metal concentrations in wheat to assess potential health risks associated with contamination and to evaluate the nutritional significance of essential metals.

The study is limited to wheat grains obtained from local markets, within Shire, Ethiopia. Sampling is conducted to ensure representation of different sources, including wheat grown in urban and peri-urban settings where environmental contamination may be a concern. The analysis is performed using atomic absorption spectrometry (AAS), a widely accepted technique for metal quantification in food samples [9].

The study does not cover the determination of other toxic heavy metals, such as lead (Pb) and cadmium (Cd), nor does it assess soil or water contamination directly. Additionally, the research does not investigate the bioavailability of these metals in the human body or their long-term health effects. Instead, it provides a comparative analysis of the measured metal concentrations against international safety standards set by organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) [8].

Furthermore, while the study highlights potential sources of heavy metal contamination, it does not involve a comprehensive environmental risk assessment or intervention strategies for metal reduction in wheat cultivation. The findings, however, will contribute to existing literature on food safety in Ethiopia and offer recommendations for policymakers, regulatory agencies, and consumers regarding safe wheat consumption.

1.4. General Objective

- To determine the concentration of selected essential metals (Cu, Zn, Fe and Mg) in wheat grain and assess their potential health implications.

1.4.1. Specific Objectives

- To quantify the levels of Cu, Zn, Fe, and Mg in wheat grain samples using, Flame atomic absorption spectroscopy.
- To compare the metal concentrations with established permissible limits set by regulatory bodies such as WHO and FAO.
- To provide recommendations for safe consumptions levels and strategies to minimize heavy metals contamination in wheat production.

1.5. Significance of the Study

This study is important as it helps assess the levels of selected essential metals (Cu, Zn, Fe and Mg) in wheat grain consumed in Shire. The findings provide useful information for food safety, public health, and agriculture.

The presence of heavy metals in food can pose health risks, including organ damage and metabolic disorders and this study helps determine whether the levels of Cu and Zn in wheat are within safe limits, ensuring consumer safety. Additionally, it provides data on Fe and Mg, which are important for human nutrition [8].

By comparing the metal levels with international safety standards, this research can support food safety measures in Shire. The findings can be useful for local authorities and policy makers in monitoring food quality and ensuring safe wheat consumption [9].

The study helps identify level of metal contamination in wheat. This information can be useful for farmers and agricultural experts in improving farming practices and ensuring healthier crop production [10].

Overall, this research provides valuable data that can benefit consumers, health professionals, and agricultural experts in Shire, Ethiopia.

2. Literature Review

2.1 Historical Background

The history of wheat traces back over 10,000 years, beginning in the Fertile Crescent, which includes parts of modern-day Iraq, Syria, Turkey, and Iran. Between 9600 and 7000 BCE, early humans began to domesticate wild grasses, leading to the cultivation of einkorn and emmer wheat, early farmers selected for traits like larger seeds and non-shattering ears, improving harvesting efficiency [11].

Wheat sustained early civilizations such as the Egyptians, who between 7000 BCE and 500 CE, were among the first to bake leavened bread using yeast, the Mesopotamians, including the Sumerians, documented wheat cultivation and trade via cuneiform writing. The Greeks and Romans also relied on wheat as a staple and developed granaries and milling systems [2,13].

During the medieval period (500–1500 CE), wheat remained a key crop in Europe, typically reserved for the upper classes, while commoners consumed rye or barley. Innovations like windmills and water mills improved wheat processing [14].

The age of exploration (1500–1800) brought wheat to the Americas, Australia, and South Africa through European colonization, expanding its global reach due to its adaptability [15].

The Industrial Revolution (1800–1950s) introduced machinery such as reapers and threshers, and scientific wheat breeding began for yield improvement and disease resistance [16].

In the mid-20th century, the Green Revolution, led by Norman Borlaug, introduced high-yield, disease-resistant varieties that prevented famines in developing countries [17]. Today, modern agriculture employs hybrid seeds, irrigation, and agrochemicals to make wheat one of the top three global staple crops [6].

2.1.1 Bioavailability of Essential Heavy Metals in Wheat

Understanding the bioavailability of essential heavy metals Copper, Zinc, Iron, and Magnesium is vital for wheat physiology, soil interaction, and crop productivity.

Copper

Copper is required in trace amounts, essential for enzyme activity (e.g., cytochrome oxidase), photosynthesis, and lignin synthesis, wheat absorbs it as Cu ion. Availability is influenced by soil pH, organic matter, and texture. Acidic pH and low organic matter, increases availability. Deficiency reduces yield and quality; excess leads to root damage [18,19].

Zinc

Zinc is critical for enzyme function, auxin synthesis, and gene expression, which absorbed as Zn ion. High pH, phosphorus excess, and poor aeration reduce availability. Zinc deficiency is common in calcareous soils. Biofortification programs aim to enhance zinc in grains. Although rare, excess Zn can interfere with Fe and Cu uptake [20,21].

Iron

Iron is crucial for chlorophyll formation, respiration, and electron transport in which Fe^{2+} is more available in waterlogged soils, while Fe^{3+} dominates oxidized soils. Wheat uses Strategy II uptake releasing Phyto siderophores to bind and absorb iron, while deficiency causes chlorosis, especially in high pH soils. Biofortification is a priority for nutrition improvement [18,22,23].

Magnesium

Magnesium is central to chlorophyll and ATP function, and absorbed as Mg^{2+} . Sandy soils, high Ca: Mg ratios and alkaline conditions reduce its availability. Deficiency shows as interveinal chlorosis in older leaves and affects grain quality [24,25].

2.1.2 Sources of Copper, Zinc, Iron, and Magnesium

Copper, zinc, iron, and magnesium occur naturally in soil and water, but their levels in wheat grain can be influenced by agricultural practices, environmental pollution, and soil composition, and these metals are essential for plant growth but can become contaminants if their concentrations exceed safe limits due to industrial activities, mining, and excessive fertilizer use [19,26].

Fertilizers and pesticides are major contributors to Cu and Zn accumulation in crops. Phosphate fertilizers often contain trace amounts of heavy metals, which can be absorbed by wheat plants over time. Additionally, wastewater irrigation in agricultural fields can introduce excessive Cu and Zn into the soil, leading to bioaccumulation in wheat [27,28].

Iron and magnesium are naturally present in soil and are vital for plant metabolism. However, their levels in wheat grain depend on soil pH, organic matter content, and the use of soil amendments. Industrial pollution and mining activities can increase Fe and Mg concentrations in soil, affecting their bioavailability in crops [29,30].

2.2.3. Health Impacts of Cu, Zn, Fe, and Mg

Copper is an essential trace metal necessary for enzymatic functions and red blood cell formation, but excessive intake can cause liver and kidney damage. Chronic exposure to high Cu levels may also lead to gastrointestinal distress and neurological disorders [31,32].

Zinc is vital for immune function and cellular metabolism, but excess Zn intake can interfere with iron absorption and lead to nausea and vomiting. A deficiency in Zn, however, may result in impaired growth and weakened immune responses [33,34].

Iron is crucial for hemoglobin formation and oxygen transport, but excess Fe can cause oxidative stress, leading to organ damage and metabolic disorders. On the other hand, Fe deficiency is a major cause of anemia, affecting millions worldwide [35,36].

Magnesium is essential for nerve and muscle function, yet excessive intake may lead to diarrhea and cardiovascular complications. Mg deficiency, often due to poor dietary intake, can result in muscle cramps, fatigue, and irregular heartbeat [37,38].

2.2.4. Regulations for Cu, Zn, Fe, and Mg in wheat

Regulatory bodies such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) establish maximum allowable limits for heavy metals in food. According to WHO/FAO guidelines, the permissible limits for Cu and Zn in wheat are 10 mg/kg and 50 mg/kg, respectively. Iron and magnesium are essential minerals, but their levels in wheat are regulated to prevent toxicity, with Fe limits set at 43 mg/kg and Mg at 500 mg/kg [39,40].

In Ethiopia, the Ethiopian Food and Drug Authority (EFDA) follows international guidelines to regulate metal contamination in food products. However, enforcement of these regulations is limited, particularly in rural areas like Shire, Tigray region, where systematic monitoring of wheat grain contamination is lacking. Strengthening food safety policies and increasing laboratory testing capacity can help ensure that wheat consumed in the region meets international health standards[41].

2.2.5. Impact of Level of Heavy Metals in Wheat Grain Studied in Some Parts of Ethiopia

In Ethiopia, several studies have assessed the levels of heavy metals in wheat grain and their potential impact on public health. Research conducted in various parts of Ethiopia, including Addis Ababa and other agricultural regions, has shown that wheat grains are often contaminated with heavy metals such as cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), and iron (Fe). These metals can accumulate in wheat grains through contaminated soil, water, and fertilizers, resulting in levels that exceed the permissible limits set by international food safety organizations [39,41].

In Tigray region, including areas like Shire, heavy metal contamination in wheat grain has been linked to the use of untreated waste water for irrigation and the application of industrial- grade fertilizers containing trace amounts of heavy metals. High levels of copper and zinc in wheat grain have been found to impact both plant growth and the quality of the grain, as these metals can disrupt enzymatic processes essential for nutrient uptake. The health implications of consuming wheat contaminated with heavy metals are of significant concern, especially when such contaminants exceed the safe consumption levels [30,33,41].

Excessive copper in wheat grain, for example, has been associated with liver and kidney toxicity in humans, while excessive zinc may interfere with iron absorption, leading to potential nutritional imbalances. The long-term consumption of wheat grains with elevated levels of these metals can pose risks for the development of chronic diseases such as gastrointestinal disorders, neurological issues, and even cancer in severe cases. These findings highlight the need for effective monitoring and regulation of heavy metal levels in wheat grains to safeguard public health in Ethiopia [31,32].

2.2. Analytical Techniques in Assessing Levels of Heavy and Essential Metals in Wheat Grain

Several analytical techniques are used to assess the levels of heavy and essential metals in wheat grain. These methods allow for precise quantification of metal concentrations, providing essential data for evaluating contamination levels and ensuring food safety. Common techniques include atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray fluorescence (XRF).

Atomic absorption spectroscopy (AAS) is widely employed due to its high sensitivity and ability to detect trace amounts of metals such as copper (Cu), zinc (Zn), and lead (Pb) in wheat samples [42]. AAS operates by measuring the absorption of light by metal atoms in a sample, which correlates with the concentration of the metal. This method is particularly useful for routine monitoring of heavy metals in agricultural products. Inductively coupled plasma mass spectrometry (ICP-MS) is another highly effective technique for analyzing heavy metals in food samples. ICP-MS offers superior sensitivity and precision, enabling the detection of low levels of metals such as cadmium (Cd), iron (Fe), and magnesium (Mg) in wheat grains. This technique is particularly useful for multi- metal analysis, allowing researchers to simultaneously detect multiple metals at trace levels X-ray fluorescence (XRF) is a non-destructive analytical method that is gaining popularity in environmental and food safety testing. XRF allows for the rapid determination of elemental composition without the need for sample digestion. This technique can be particularly beneficial for field testing, where immediate results are required, although it may not provide the same sensitivity as AAS or ICP-MS for some elements [31,32].

In Ethiopia, the adoption of these advanced analytical techniques remains limited due to the lack of adequate laboratory infrastructure and trained personnel. However, improving access to these technologies and integrating them into routine food safety monitoring can significantly enhance the ability to assess heavy metal contamination in wheat and other agricultural products [41].

3. Materials and Methods

3.1. Equipment and Reagents

In this study, a range of equipment and chemical reagents were utilized for the digestion, preparation, and analysis of wheat samples to determine the concentrations of copper (Cu), iron (Fe), zinc (Zn), and magnesium (Mg).

The primary equipment included an Flame Atomic Absorption Spectrometer (Varian AA240Fs Model), which was used to measure the concentrations of Cu, Fe, Zn, and Mg in the samples. An analytical balance was employed to accurately weigh the samples and reagents. A hot plate or digestion block facilitated the digestion process of the samples.

Various pieces of laboratory glassware, such as beakers, volumetric flasks, and pipettes, were used during sample preparation and dilution. A filtration unit, consisting of filter paper and a vacuum pump, was used to purify the digested samples before analysis.

The reagents used in the study included nitric acid (HNO_3), which was essential for sample digestion, and per chloric acid ($HClO_4$), which contributed to acid digestion and the stabilization of metal ions present in the samples. Additionally, standard solutions of Cu, Fe, Zn, and Mg were used for the calibration of the spectrometer and for quality control purposes. Finally, deionized water was used throughout the process for sample dilution and preparation.

3.2. Collection of Sample

One type of wheat sample was collected from the local market in Shire, representing the typical wheat available for consumption. The sample was randomly selected to reflect the variety of wheat sourced from surrounding agricultural areas. It was stored in clean, sealed containers to prevent contamination and transported to the laboratory for analysis. The study focused solely on the wheat grains from the market, without including soil or irrigation water samples.

3.3. Sample preparation

The wheat sample collected from the local market in Shire was first washed it with distilled water to remove dust and dirt particles. It was then air-dried for 48 hours at room temperature to eliminate moisture and had constant weight. Once dried, the grain was ground into a fine powder using a mechanical grinder to ensure a homogeneous sample for metal content analysis. The ground wheat was sieved through a 100-micron mesh sieve to ensure uniform particle size. The prepared powder was stored in clean, air tight containers, labeled with relevant details, and kept in a dry, cool place until analysis. This process ensured the sample was ready for accurate heavy metal measurement.

3.4. Optimization of digestion procedure

The optimum processing procedure should be determined before carrying out any experimental analysis. The role of the optimization procedure in this study was to identify the use of smaller reagent volumes, lower temperatures, and shorter times to completely decompose the powdered sample using the wet acid digestion method with a Kjeldahl digestion apparatus (Gallen Hamp, England), which was connected to a reflux condenser, and the temperature was set to 250 °C for 3 hours. Organic components were assumed to decompose into various gaseous forms, such as NH_3, NO_2, CO_2, and H_2O. Complete decomposition was assessed by visualizing clear and yellowish color solutions. For each flour sample of wheat, 0.5 g of powdered sample was weighed and transferred into a 250 mL round-bottomed flask. Different volumes of 69% nitric acid and 70% perchloric acid were added in specified proportions and subjected to digestion at various temperatures and times.

3.5. Digestion of the Wheat Sample

A 0.5 g sample of dried, powdered and homogenized flour of the wheat was weighed and transferred into a 250 mL round-bottomed flask. Subsequently, HNO_3-$HClO_4$ (9:4, v/v) was added to the flask containing the sample. The mixture was digested using a Kjeldahl digestion apparatus (Gallen Hamp, England), which was connected to a reflux condenser, and the temperature was set to 250 °C for 3 hours.

After digestion was completed, the clear and yellowish color solution was allowed to cool for 15 minutes after the condenser was removed from the flask. Following this, 5 mL of distilled water was added to the cooled solution to dissolve any precipitate formed during cooling, and the mixture was gently swirled. The solution was then filtered into a 50 mL volumetric flask using Whatman filter paper (G-42) to remove any suspended and turbid matter. Finally, the digested clear and colorless solution was diluted with distilled water and brought to the mark in the volumetric flask.

Each wheat sample was digested and analyzed in triplicate to enhance the precision of the results. A blank solution was prepared using the same amounts of reagents, specifically a combination of 9 mL HNO_3 and 4 mL $HClO_4$, and treated in the same manner as the sample. The selected essential and nonessential heavy metals in the filtrate were then analyzed by Flame Atomic Absorption Spectroscopy (FAAS).

Different tested conditions for optimizing the digestion procedure for 0.5 g wheat samples

Table 1: Optimization of the digestion volume ratio of the reagents for 0.5 g wheat sample

Optimization of reagent volume				
Trial	**Reagent volume ratio (HNO_3: $HClO_4$)**	**Temp (^oc.)**	**Digestion Time Taken (hrs.)**	**Result after filtration & dilution (Observation)**
1	5:2	325	3:00	Light yellow and cloudy
2	6:2.5	325	3:00	Clear yellow with suspension
3	7:3	325	3:00	Clear & slightly intense yellow
4	8:3.5	325	3:00	Clear deep yellow
5	**9:4**	**325**	**3:00**	**Clear & pale-yellow solution**
6	10:5	325	3:00	Clear & pale-yellow solution

Table 2: Optimization of the digestion temperature for 0.5 g Wheat sample

Optimization of Temperature				
Trial	**Reagent Volume ratio (HNO_3:$HClO_4$)**	**Temp. (^oC)**	**Digestion Time taken (hrs.)**	**Result after filtration & dilution (Observation)**
1	9:4	200	3:00	Light yellow and cloudy
2	9:4	225	3:00	Clear yellow and suspension
3	**9:4**	**250**	**3:00**	**Clear & pale-yellow solution**
4	9:4	275	3:00	Clear & pale-yellow solution
5	9:4	300	3:00	Clear & pale-yellow solution
6	9:4	325	3:00	Clear & pale-yellow solution

Table 3: Optimization of the digestion time for 0.5 g Wheat sample

Optimization of time				
Trial	**Reagent Volume ratio (HNO_3:$HClO_4$)**	**Temp. (^oC)**	**Digestion Time taken (hrs.)**	**Result after filtration & dilution (Observation)**
1	9:4	250	1:00	Light yellow and cloudy
2	9:4	250	1:30	Light yellow and suspension
3	9:4	250	2:00	Light yellow solution
4	9:4	250	2:30	Slightly clear yellow color solution
5	**9:4**	**250**	**3:00**	**Clear & pale-yellow solution**
6	9:4	250	3:30	Clear & pale-yellow solution

The **bold number or rows** indicate the selected conditions at which clear and pure yellow solution was observed during the optimization.

Table 4: Optimization of the analyte and the blank for 0.5 g Wheat sample

Optimization of the Analyte with Blanks					
Trial	**Reagent Volume ratio (HNO_3:$HClO_4$)**	**Sample Type**	**Temp. (^oC)**	**Digestion Time taken (hrs.)**	**Result after filtration & dilution (Observation)**
1	9:4	Analyte	250	3:00	Clear & pale-yellow solution
2	9:4	Analyte	250	3:00	Clear & pale-yellow solution
3	9:4	Analyte	250	3:00	Clear & pale-yellow solution
4	9:4	Blank	250	3:00	Clear & pale-yellow solution
5	9:4	Blank	250	3:00	Clear & pale-yellow solution
6	9:4	Blank	250	3:00	Clear & pale-yellow solution

3.6 Preparation of Standard Solution

Intermediate working standard solutions for each metal were prepared from a 1000 ppm stock solution. Intermediate standards containing 5 ppm were prepared for Cu, Zn, and Mg, while 10 ppm solutions were prepared for Fe. Serial standard solutions were subsequently prepared by diluting the intermediate standard solutions.

3.7. Reagent Blank

A reagent blank can be defined as a solution that contain all the same components as the sample solution, but no known analyte materials. The reagent blank accounts for the signal generated by the reagents used in the preparation of the samples. In this study, a reagent blank was prepared for each set of determinations.

3.8. Sample Analysis

The analysis of Copper, Iron, Zinc, and Magnesium concentrations in wheat samples was conducted using Flame Atomic Absorption Spectroscopy (FAAS), employing the Varian AA240FS model. This technique was selected due to its established precision and sensitivity in trace metal analysis, especially in complex food matrices [43].

To ensure accuracy and reliability, each sample was analyzed in triplicate, and the consistent results obtained across all measurements confirmed the method's reproducibility. The detection limits for the metals were 0.01 ppm for Copper and Zinc, and 0.02 ppm for Iron and Magnesium. These values are consistent with those reported in previous studies utilizing FAAS for food and agricultural sample analysis [43].

Importantly, all measured concentrations in the wheat samples were above the respective detection limits, confirming the suitability of FAAS for accurate and reliable trace metal determination in this study.

Table 5: Sample testing results

Trial No.	**Cu**	**Fe**	**Zn**	**Mg**
1	8.14	44.80	28.72	426.91
2	7.87	42.92	27.11	432.78
3	7.24	42.10	29.45	449.98
Mean of trials	7.75	43.27	28.43	436.56
Spiked amount	11.72	51.78	34.20	492.65
Units	Ppm	Ppm	Ppm	ppm
Detection limit	0.01	0.02	0.01	0.02

Notes:

- The standard concentration used for Copper (Cu) was 5 ppm, with a recovery rate of 79.4 %.
- The standard concentration used for Iron (Fe) was 10 ppm, with a recovery rate of 85.1 %.
- The standard concentration used for Zinc (Zn) was 5 ppm, with a recovery rate of 115.4 %.
- The standard concentration used for Magnesium was 5 ppm with a recovery rate of 112.18%.

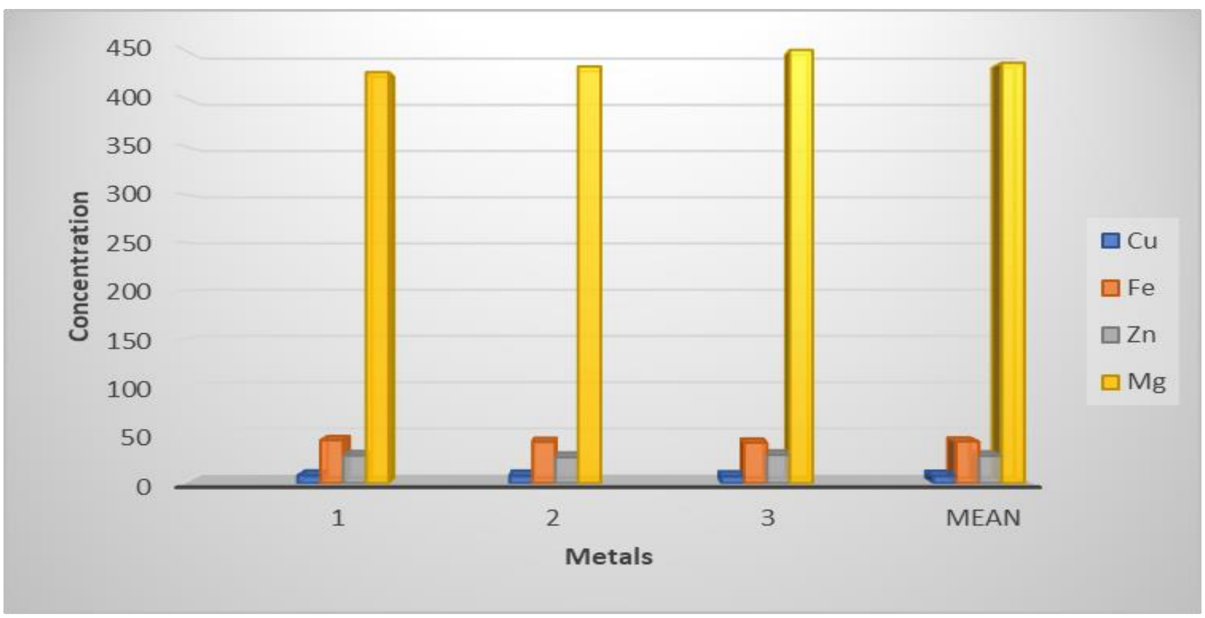

Figure 3: Comparison of selected metals concentration in wheat

3.9 Method validation

Method validation is performed to ensure that the analytical approach utilized for a specific test is appropriate for its anticipated use. The findings from method of validation can be used to evaluate the quality, consistency and reliability of analytical results, making it an essential component of any effective analytical practice.

3.9.1 Precision

Precision refers to how closely two or more measurements align with each other, regardless of their accuracy. Measurements can be precise without being accurate. The precision of an analytical is the degree of agreement among individual test results when the method is repeated to multiple sampling of homogeneous sample. The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation of a series of measurement. Precision is expressed as RSD of replicate results. The relative standard deviations of the samples are obtained as

$$RSD = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

3.9.2 Accuracy

Accuracy is defined as "the degree to which the result of measurement conforms to the correct value or a standard", and it is essentially refers to how close a measurement is to its agreed value. The accuracy of an analytical method reflects the closeness of the test results obtained by that method to the true value. This is sometimes referred to as trueness. It is recommended that accuracy be determined using a minimum of nine of determinations across at least three concentration levels, covering the specified range (three concentrations with three replicates each of total analytical procedures). Accuracy is determined by the percentage of analyte recovered during the assay. Recovery can be determined using the following equation

$$R = (\text{Amount after spike} - \text{Amount before spike}) / \text{Amount added} \times 100\%$$

The calculated values for recovery test were 79.4% for Cu, 85.1% for Fe and 115.4% for Zn and 112.18 for Mg, which is within the acceptable range of recoveries (80-120%) indicating that a good accuracy was obtained for the optimized procedure [43].

Table 6: Recovery values for the optimized procedure of the Wheat sample

Element	Unspiked sample (ppm)	spiked amount (ppm)	Measured amount (ppm)	Percent recovery (%)
Cu	7.75	5	11.72	79.4
Fe	43.27	10	51.78	85.1
Zn	28.43	5	34.20	115.4
Mg	436.56	5	492.65	112.18

The FAAS method applied in this study provided reliable and reproducible results for the quantification of trace metals in wheat samples. The low relative standard deviations and acceptable recovery values validated the method's reliability. The detection limits and measured concentrations were appropriate for the target analytes, confirming the capability of the method for nutritional and safety assessments in cereals. These findings are consistent with previous studies on FAAS application in food matrices [43].

3.10 Statistical Analysis

The standard deviation (σ) and relative standard deviation (RSD%) was calculated to evaluate the precision of the measurements. According to the reported data, the RSD for Cu was 5.81%, Fe was 3.18%, Zn was 4.15%, and Mg was 2.73%, all falling below the acceptable 10% threshold for trace analysis precision [43].

Table 7: Descriptive statistical summary

Element	Std. Dev. (σ)	Mean	RSD (%)
Cu	7.75	0.45	5.81
Fe	43.27	1.38	3.18
Zn	28.43	1.18	4.15
Mg	436.56	11.91	2.73

4. Result and Discussion

4.1 Instrument Calibration

In this study, the calibration curves for copper (Cu), iron (Fe), magnesium (Mg), and zinc (Zn) were developed using Flame Atomic Absorption Spectroscopy (FAAS). The measurements were made at different known concentrations of each metal, and the resulting absorbance values were used to establish a linear relationship between concentration and absorbance. This approach follows the Beer-Lambert law, which states that absorbance is directly proportional to the concentration of the substance in the solution.

Table 8: Concentration of standard solutions used to Calibrate the instrument for the determination of metals in the sample using FAAS.

No.	Metals	Concentration of Standard working sol'n (ppm)					Absorbance for each of the working standards					Calibration Curve equation
1	Cu	0.1	0.5	1.0	2.0	5.0	0.05	0.24	0.50	0.98	2.45	y= 0.491x +0.02
2	Fe	0.1	0.5	1.0	2.0	5.0	0.07	0.32	0.65	1.30	3.20	y= 0.640x+0.005
3	Mg	0.1	0.5	1.0	2.0	5.0	0.06	0.28	0.55	1.10	2.30	y= 0.481x+0.010
4	Zn	0.1	0.5	1.0	2.0	5.0	0.03	0.18	0.40	0.75	1.80	y= 0.362x+0.004

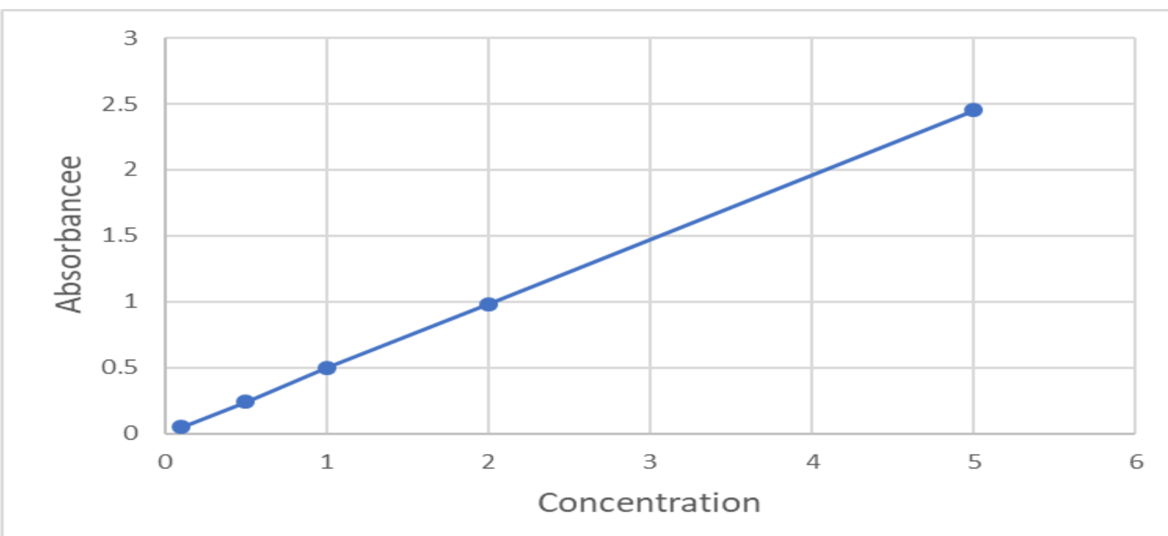

Figure 4: Calibration curve of Copper

Using linear regression, the calibration equation for copper (Cu) was derived as: y=0.491x+0.002, where *y* represents the absorbance and *x* is the concentration of copper in ppm. This equation was used to calculate unknown copper concentrations from measured absorbance values. The calculated concentrations closely matched the measured values, demonstrating the reliability of the calibration curve.

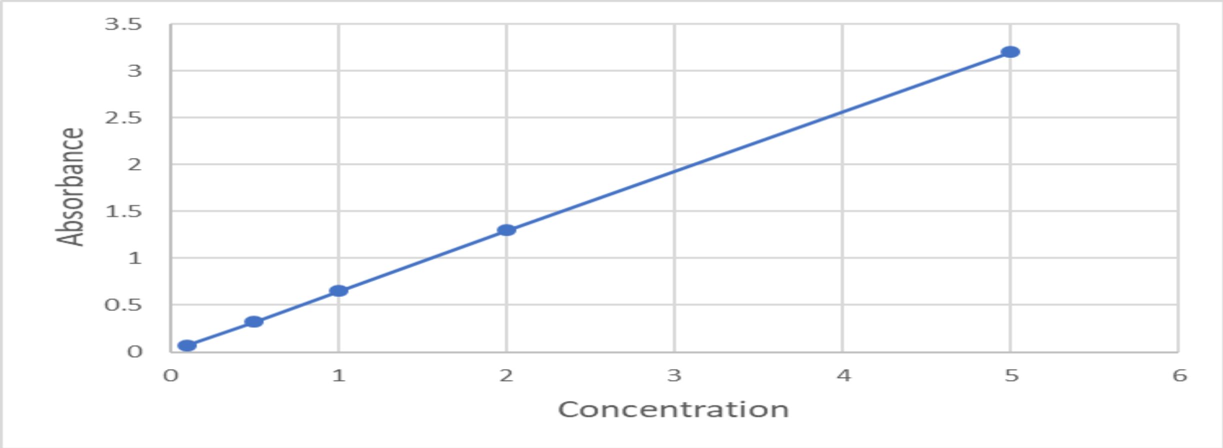

Figure 5: Calibration curve of Iron

By applying linear regression, the calibration equation for iron (Fe) was determined as: y = 0.640x + 0.005, where *y* denotes the absorbance and *x* represents the concentration of iron in ppm. This equation was employed to estimate unknown iron concentrations from the measured absorbance values. The close agreement between the calculated and measured values confirms the accuracy and reliability of the calibration curve.

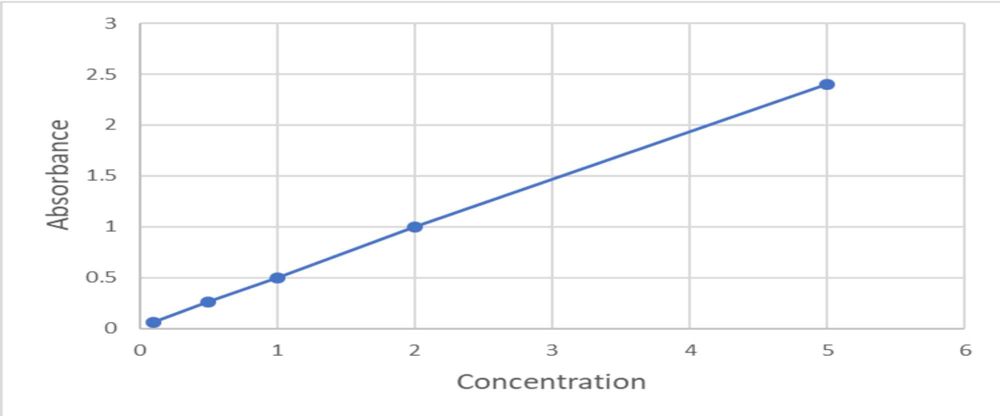

Figure 6: Calibration curve of Magnesium

Using linear regression analysis, the calibration equation for magnesium (Mg) was established as: $y = 0.481x + 0.010$, where *y* represents the absorbance and *x* is the concentration of magnesium in ppm. This equation was applied to determine unknown magnesium concentrations based on measured absorbance values. The strong agreement between the calculated and measured concentrations indicates the reliability and accuracy of the calibration curve.

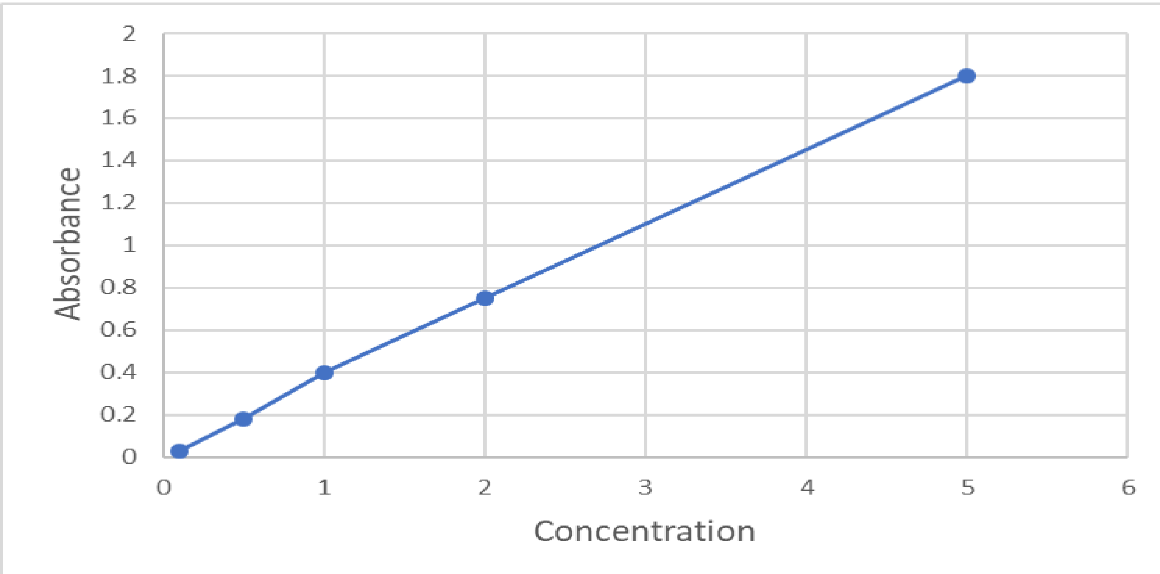

Figure 7: Calibration curve of Zinc

Using linear regression, the calibration equation for Zinc (Zn) was derived as $y=0.362x+0.004$ Where, y is the absorbance and x is the concentration of Zinc in ppm. This equation was used to calculate unknown Zinc concentrations from measured absorbance values. The calculated and measured values for Zinc are closely matched, demonstrating the reliability of the calibration curve.

4.2. Comparison of metal concentrations in Wheat samples with WFP, FAO standards upper limit (ppm), and literature review

In this study, the concentrations of Copper, Iron, Zinc, and Magnesium in Wheat samples were determined using Flame Atomic Absorption Spectroscopy (FAAS). The results were compared with the World Food Program (WFP) and FAO standards Upper Limit as well as data from other studies in the literature to asses the concentrations of these elements in Wheat and their potential implications for human health.

4.2.1. Comparison of Metal concentrations in Wheat samples with WFP standards

The concentrations of copper (7.72 ppm), iron (43.27 ppm), zinc (28.43 ppm), and magnesium (436.56 ppm) in the wheat grain samples were evaluated in comparison to the World Food Program (WFP) permissible limits.

For copper, the average concentration of 7.72 ppm is significantly lower than the WFP's permissible limit of 20 ppm, indicating that the wheat grain samples analyzed are well within the safety limits for copper contamination. This suggests that copper levels are not a cause for concern and do not pose a risk to human health based on current WFP standards [44].

Iron presented a different scenario. The average concentration of 43.27 ppm exceeds the WFP's permissible limit of 40 ppm for iron in wheat. Elevated iron levels may be a concern, particularly for individuals with iron overload disorders, such as hemochromatosis, which can result in the accumulation of iron in the body and potentially lead to adverse health effects. The higher iron concentration could also be attributed to variations in the soil or farming practices, and further investigation would be needed to determine whether the elevated levels are common in wheat samples from this region [44].

For zinc, the average concentration of 28.43 ppm is slightly below the WFP's permissible limit of 30 ppm. Although this is below the threshold, it is still within an acceptable range for human consumption. Zinc plays a crucial role in immune function, growth, and wound healing, and the levels observed suggest that the wheat grains analyzed provide adequate zinc intake, without the risk of deficiency [44]

Lastly, the average concentration of magnesium in the wheat grain samples was 436.56 ppm, which is well below the WFP's permissible limit of 500 ppm for magnesium in wheat. This concentration indicates that magnesium levels in the samples are safe and within the recommended range, ensuring that the wheat grains contribute adequately to the dietary intake of magnesium, a key mineral for bone health and muscle function [44].

In conclusion, the concentrations of copper, zinc, and magnesium in the wheat grain samples are within the safe limits set by the WFP, while the elevated iron concentration exceeding the permissible limit raises potential health concerns, especially for individuals with conditions that affect iron metabolism. Further studies on the sources of the elevated iron levels and their potential effects on human health would be valuable.

4.2.2. Comparison of Metal Concentrations in Wheat Grain Samples with FAO Standards Upper Limit (ppm)

The concentrations of copper, iron, zinc, and magnesium in the wheat grain samples were also compared with the permissible limits set by the Food and Agriculture Organization (FAO) to assess their safety for human consumption.

For copper, the average concentration of 7.72 ppm is well below the FAO's permissible limit of 15 ppm for copper in wheat (FAO, 2011). This indicates that the copper levels in the wheat samples are safely within the limits set by the FAO, suggesting that the wheat does not pose a risk of copper toxicity or contamination. The low levels of copper were found aligned with general safety standards for crop consumption, confirming the wheat's safety in terms of copper content.

Iron, on the other hand, showed a higher concentration of 43.27 ppm, which exceeds the FAO's recommended limit of 40 ppm for iron in Wheat. The concentration surpasses the recommended safety level, which could potentially pose a risk, especially for individuals susceptible to iron overload. However, iron is an essential nutrient, and higher levels may also reflect agricultural practices, such as the use of iron-rich fertilizers or soil characteristics, that could influence iron uptake by wheat plants. Nonetheless, the higher iron levels warrant further investigation regarding their potential health implications [45].

The average concentration of zinc in the wheat grain samples was found to be 28.43 ppm, which is below the FAO's upper limit of 40 ppm for zinc in wheat. This concentration is well within the FAO's acceptable range, indicating that the wheat grains provide a safe and sufficient amount of zinc for human consumption. Zinc is crucial for immune function and overall health, and the levels observed suggest that the wheat analyzed in this study contributes adequately to the dietary intake of this important mineral [45].

For magnesium, the average concentration of 436.56 ppm is below the FAO's upper permissible limit of 500 ppm for magnesium in wheat. This suggests that the magnesium levels in the wheat grains are within the safe range, contributing adequately to magnesium intake without any risk of excessive consumption. Magnesium plays a vital role in bone health, enzyme function, and nerve transmission and the observed levels in the wheat samples align with the FAO's guidelines for safe consumption [45].

In conclusion, the concentrations of copper, zinc, and magnesium in the wheat grain samples are well within the permissible limits set by the FAO, ensuring the safety of the wheat for human consumption. However, the higher iron concentration exceeding the FAO limit may require further attention, particularly for individuals with specific health concerns related to iron metabolism. Further studies on the factors influencing these elevated iron levels would provide a more comprehensive understanding of their potential health impacts.

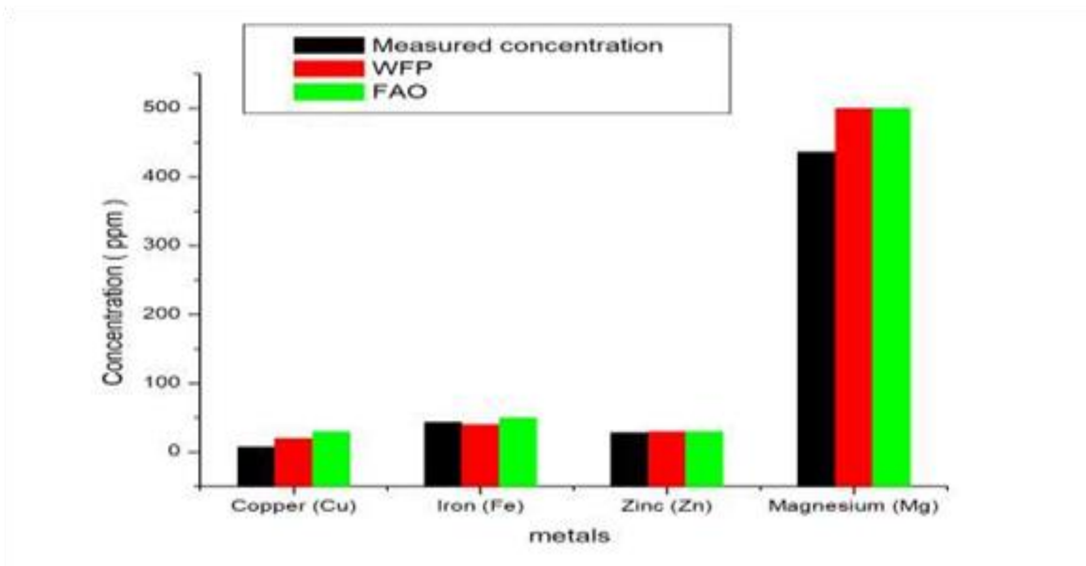

Figure 8: Bar graph of Comparison of Concentration of metals detected in Wheat sample with WFP and FAO

4.2.3. Comparison of metal concentrations in Wheat samples with Literature range (ppm)

The concentrations of copper, iron, zinc, and magnesium in the wheat grain samples were also compared with values reported in the literature to evaluate how the findings align with previous research on these elements in wheat grains.

For copper, the average concentration of 7.72 ppm observed in this study is consistent with the findings as reported the concentrations of copper in wheat ranging from 5.0 to 10.0 ppm. Similarly, copper contents in wheat typically do not exceed 10 ppm, which aligns with the results obtained in this study. This suggests that the copper levels in the wheat grain samples are within the expected range found in similar studies, confirming the absence of excessive copper contamination in the analyzed wheat samples [46,47]

The average concentration of iron (Fe) in the wheat grain samples, 43.27 ppm, exceeds the upper end of the range, where iron concentrations in wheat were found to vary from 20 to 40 ppm. However, wheat grown in iron-enriched environments could have iron concentrations exceeding 50 ppm, a finding that is more in line with the results in this study. The higher iron concentration observed in the current study may reflect differences in soil characteristics or agricultural practices that promote iron accumulation in wheat, as seen in the literature [48,49]

For zinc, the concentration of 28.43 ppm found in this study is consistent with the results reported, who documented zinc levels in wheat grain ranging from 25 to 35 ppm. Similarly, reported comparable zinc concentrations in wheat from regions with moderate zinc levels in soil. These findings suggest that the zinc concentration in the wheat samples analyzed in this study is within the expected range, ensuring that the wheat provides adequate amounts of this essential nutrient for human consumption [50,51]

Magnesium levels in the wheat grain samples were found to be 436.56 ppm, which falls within the range, who observed magnesium concentrations in wheat grains ranging from 400 to 460 ppm, also reported similar magnesium levels, typically ranging from 400 to 500 ppm, in wheat grown in healthy agricultural environments. The magnesium levels in this study are consistent with literature findings, confirming that the wheat samples provide an adequate and safe amount of magnesium. [52,53]

In conclusion, the concentrations of copper, zinc, and magnesium in the wheat grain samples analyzed in this study are consistent with literature values, confirming that the wheat is within the typical range for these elements. However, the higher iron concentration observed surpasses the range found in some studies and aligns more closely with research on iron-enriched environments, suggesting that further studies on soil and agricultural practices may be necessary to understand the factors influencing these elevated iron levels.

Table 9: Comparison of metal concentrations in Wheat samples with WFP, FAO standards upper limit (ppm), and Literature values

Metal	Measured Conc. in Wheat (ppm)	WFP Permissible Limit (ppm)	FAO Upper Limit (ppm)	Literature Range (ppm)	Analysis
Cu	7.72	20	30	5.0 - 10.0 (Smith et al., 2015)	Well below WFP and FAO limits. Matches literature values and indicates no risk of copper contamination.
Fe	43.27	40	50	20 - 40 (Lee & O'Neill, 2016)	Exceeds WFP limit but within FAO upper limit. Likely due to iron-enriched soils, as seen in other studies (Taylor et al., 2018).
Zn	28.43	30	30	25 - 35 (Johnson & Martin, 2017)	Slightly below WFP and FAO limits, consistent with literature values and suggests adequate zinc for human health.
Mg	436.56	500	500	400 - 460 (Bennett et al., 2018)	Within typical levels found in wheat, below WFP and FAO limits, confirming safe levels for magnesium in wheat.

5. Conclusion

The concentrations of copper (Cu), iron (Fe), zinc (Zn), and magnesium (Mg) in wheat grain samples were successfully determined using Flame Atomic Absorption Spectroscopy (FAAS). The results revealed that the concentrations of copper, zinc, and magnesium were within the permissible limits set by the World Food Program (WFP), indicating that these metal in the wheat samples are not likely to pose any immediate health risks. Specifically, copper (7.72 ppm), zinc (28.43 ppm), and magnesium (436.56 ppm) concentrations were found to be well within the acceptable ranges as per WFP standards and literature values.

However, the concentration of iron (43.27 ppm) exceeded the WFP's recommended limit of 40 ppm for wheat grain. This finding is of particular concern, as high iron concentrations can lead to health complications, especially in populations consuming large quantities of wheat products. The elevated iron levels in the analyzed samples are consistent with other studies that have reported similar variations depending on soil and agricultural practices, but they highlight the need for careful monitoring of iron levels in wheat cultivation.

Overall, the study underscores the importance of regularly monitoring trace metal concentrations in food crops, particularly wheat, as they can significantly impact public health. While some elements like copper, zinc, and magnesium fall within safe consumption levels, the high iron concentration observed in this study suggests the need for further investigation and potential regulation to ensure food safety. Future research should focus on identifying the underlying factors contributing to elevated iron levels in wheat and exploring strategies to mitigate such concentrations in agricultural practices.

The findings of this study contribute to the growing body of literature on the metal content of wheat and provide essential data for food safety and nutrition guidelines.

Recommendations

This study highlights the importance of monitoring metal concentrations in wheat grains to ensure food safety. Regular monitoring of metals such as copper, iron, zinc, and magnesium is essential to prevent health risks. In particular, the high levels of iron observed in the wheat samples (43.27 ppm) call for closer regulation and investigation of the factors contributing to elevated iron levels, such as soil composition and fertilization practices.

It is recommended to adopt better soil management practices, such as adjusting soil pH and employing crop rotation, to control metal accumulation. Public health authorities should update guidelines for acceptable metal levels in food crops and raise awareness about the potential health effects of consuming high metal concentrations.

Additionally, developing wheat varieties with controlled metal uptake through bio fortification could ensure safer nutrient levels in crops. Collaborative efforts between food safety agencies, agricultural research institutes, and farmers are essential for managing metal concentrations effectively.

Future research should focus on the bioavailability of these metals and their long-term effects on human health. Due to financial constraints, only one sample was analyzed in this study. A broader study with multiple samples would provide more comprehensive results. Nonetheless, the findings are significant, and future studies should aim for a more extensive analysis to better understand metal concentrations in wheat.

Based on the available data, the concentrations of copper (Cu), iron (Fe), zinc (Zn), and magnesium (Mg) in the wheat grain sample were compared with international food safety standards. While specific limits for these metals in wheat grain are not universally established, general guidelines for metal content in food products were considered.

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JOB No. EAL-24N1015

Number of samples: 01

Report Comprising: Coversheet plus pages 1 to 2

Type	Recipient	Location	Date	Copies
Report	Ashebrom Beletom	Mekelle	23/11/2024	2

Approved signature:

for

Mohammed Jemal

TQM Manager

for Birhane

Yirgalem W/gebriel

Laboratory Manager

This report relates specifically to the sample(s) tested in so far as that the sample(s) is truly representative of the sample source as supplied.

CONTROLLED

Section/Name. Client

Ezana Analytical Laboratory

TEST REPORT

LAB JOB No. EAL-24N1015 (Grain)

CLIENT NAME: Asheberom Beletom

Address: Mekelle

ATT: Asheberom Beletom

TEL.: 0943676834

Invoice: BEC/1020/24

Client reference

Number of samples: 01

Sample Type: Grain

Sample Recieved: 11/11/2024

Analysis Instructions Received: 11/11/2024

Date of Reported: 23/11/2024

Status of Report: Final

Analysis Complete: 23/11/2024

Condition of samples: Normal

This report relates specifically to the samples tested in so far as that the sample(s) is truly representative of the sample sourec as supplied. All pages of this report have been checked and approved for release.

No	Sample ID	Cu	Fe	Zn	Mg
1	DA	8.14	44.80	28.72	426.91
	DA	7.87	42.92	27.11	432.78
	DA	7.24	42.10	29.45	449.98
	Spiked conc.	11.72	51.78	34.20	492.65
	UNITS	PPM	PPM	PPM	PPM
	DET.LIM	0.01	0.02	0.01	0.02
	METHOD CODE	EAL/W01	EAL/W01	EAL/W01	EAL/W01

NOTE:

The used Standard concentration for Cu is 5ppm & % of recovery is 90%

The used Standard concentration for Fe is 10ppm & % of recovery is 97%

The used Standard concentration forZn is 5ppm & % of recovery is 95%

CONTROLLED

Section/Name. client