**MEKELLE UNIVERSITY**

**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES**

**DEPARTMENT OF CHEMISTRY**

**A**

**Thesis**

**On**

# *Comparative Evaluation of Digestion Methods for Heavy Metal Analysis in Soil, Water, and Plants from Tigray, Ethiopia*

Submitted to the Department of Chemistry in Partial Fulfillment of the Requirements for the Master of Science in Analytical Chemistry

**By**

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# APPROVAL SHEET

MEKELLE UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

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## "*Comparative Evaluation of Digestion Methods for Heavy Metal Analysis in Soil, Water, and Plants from Tigray, Ethiopia*"

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## Declaration

I, the undersigned, declare that this thesis entitled "Comparative Evaluation of Digestion Methods for Heavy Metal Analysis in Soil, Water, and Plants from Tigray, Ethiopia" is my original work and has not been presented for any other award and that all sources of materials used in this thesis are duly acknowledged. This thesis was carried out under the supervision of my principal advisor Professor Abraha G/kidan and co-supervisor Dr Tesfamariam Teklu, Department of Chemistry, College of Natural and Computational Sciences, Mekelle University in the academic year of 2025.

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This thesis has been submitted for examination with my approval as university **advisor/co-advisor**.
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# List of Acronyms

| | |
|---|---|
| FAAS | Flame Atomic Absorption Spectrophotometry |
| ISO | International Organization for Standardization |
| CRM | Certified reference Material |
| PT | Proficiency Testing |
| NMIE | National Metrology Institute of Ethiopia |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| STDEV | Standard Deviation |

## Abstract

Heavy metal contamination is a growing concern in Tigray, Ethiopia, due to mining activities, agricultural inputs, and potential risks to public health, yet no standardized local protocol exists for reliable, cost-effective, and accurate digestion methods essential for environmental monitoring. This study compared four wet digestion methods—Method A ($HCl–HNO_3$, 3:1), Method B ($HCl–HNO_3–H_2SO_4$), Method C ($HCl–HNO_3–HClO_4$), and Method D ($HCl–HNO_3–HClO_4–HF$)—for determining total concentrations of Cu, Zn, Pb, Co, Cr, Cd, Mn, Fe, and Ni in soil, plant, and water samples from Ezana Mining Development PLC, Mekelle, Ethiopia, using Flame Atomic Absorption Spectrophotometry (FAAS). Performance evaluation based on accuracy (Z-scores and spiking recovery), precision (relative standard deviation), and cost-effectiveness, supported by one-way ANOVA ($p < 0.05$), revealed significant differences among methods, with overall recoveries ranging from 88% to 102% across matrices. Methods A and D showed superior accuracy and precision compared to Methods B and C, with Method A offering comparable performance to Method D while being simpler, safer, and more economical, making it suitable for routine monitoring. Although Method D achieved slightly higher extraction efficiencies for refractory metals, it demands specialized infrastructure, HF-handling safety, and advanced technical expertise. The findings suggest Method A is best suited for general environmental assessments in resource-limited laboratories, while Method D is recommended for targeted analyses requiring maximum recovery, thereby enhancing environmental data quality, strengthening heavy metal monitoring programs, and supporting evidence-based policy decisions in Tigray.

Keywords: *Acid digestion methods*, *heavy metal analysis*, *soil contamination*, *FAAS*, *environmental monitoring*

# CHAPTER ONE

# 1. INTRODUCTION

## 1.1. Background of The Study

Heavy metal contamination is a major global environmental concern due to the toxic, persistent, and bioaccumulative nature of these elements in soils, water, and living organisms. Excessive concentrations of metals such as Cu, Zn, Pb, Co, Cr, Cd, Mn, Fe, and Ni can impair ecosystem health, reduce agricultural productivity, and pose serious risks to human health through food chain transfer (Tchounwou et al., 2012; Alloway, 2012). These metals do not degrade naturally, and even low-level chronic exposure can cause neurological, renal, and developmental effects in humans (Jaishankar et al., 2014).

In Ethiopia, particularly in the Tigray region, potential sources of heavy metal contamination include artisanal and industrial mining activities, intensive agriculture with chemical fertilizers and pesticides, tannery and textile industries, and untreated municipal and industrial effluents. The study area—Mekelle and its surroundings—hosts Ezana Mining Development PLC and other mining operations that process ore containing sulfide and oxide minerals. These activities, combined with limited environmental regulation enforcement, increase the likelihood of soil, water, and crop contamination, especially in agricultural areas adjacent to mining zones. Despite these risks, there is a lack of standardized local protocols for accurate and cost-effective heavy metal monitoring.

Reliable heavy metal determination requires effective sample digestion to release metals from complex matrices into solution for analysis by techniques such as Flame Atomic Absorption Spectrophotometry (FAAS). The choice of digestion method is critical, as variations in acid composition, temperature, and digestion time can affect recovery efficiency, matrix interferences, and ultimately the accuracy of analytical results (Chen & Ma, 2001; Kimbrough & Wakakuwa, 1989). Previous studies have compared acid digestion protocols for environmental samples in other countries (EPA, 1996; Hseu, 2004), noting trade-offs between efficiency, safety, and cost. However, in Ethiopia, no systematic evaluation has been conducted on how commonly used acid combinations perform across diverse sample types such as soil, plant, and water, especially under local laboratory constraints.

The four methods evaluated in this study—(1) $HCl–HNO_3$ (3:1, aqua regia), (2) $HCl–HNO_3–H_2SO_4$, (3) $HCl–HNO_3–HClO_4$, and (4) $HCl–HNO_3–HClO_4–HF$—were selected because they are widely reported in the literature and vary in oxidizing strength, ability to dissolve silicates, and effectiveness in decomposing organic matter. Aqua regia (Method A) is cost-effective and relatively safe but may not fully digest silicate minerals. Adding $H_2SO_4$ (Method B) enhances oxidation of certain matrices but can lead to sulfate precipitate formation. The $HClO_4$-containing methods (Methods C and D) have strong oxidizing capabilities for refractory organics, while the inclusion of HF in Method D allows dissolution of silicate-bound metals but requires strict safety precautions and specialized laboratory facilities.

Given the absence of locally validated digestion protocols, there is an urgent need to determine which methods offer the best balance of accuracy, precision, cost-effectiveness, and safety for routine environmental monitoring in Ethiopian laboratories. This study addresses that gap by systematically comparing the analytical performance of the four digestion methods for certified reference materials (CRMs) and proficiency testing (PT) samples, as well as real environmental samples from Tigray. The findings are intended to guide laboratories, policymakers, and environmental monitoring programs in selecting appropriate methods to ensure reliable heavy metal data for decision-making and public health protection.

## 1.2. Statement of The Problem

Accurate determination of heavy metal concentrations in environmental samples depends largely on the effectiveness of the sample digestion process. While internationally recognized protocols such as USEPA Method 3050B (open-vessel acid digestion), USEPA Method 3051A (microwave-assisted digestion), ISO 11466 (soil extraction with aqua regia), and AOAC Official Methods provide standardized procedures, no single method has been universally accepted as optimal for all sample types and analytical needs (U.S. EPA, 1996). The performance of a digestion method depends on matrix composition, target analytes, available laboratory infrastructure, and safety considerations. Comparative studies have reported significant variability in metal recoveries between digestion protocols, with differences sometimes exceeding 20% for elements such as Pb, Cr, and Ni when applied to soils with varying mineralogy (Chen & Ma, 2001; Hseu, 2004).

In Ethiopia, and particularly in the Tigray region, there is no documented evaluation of digestion method performance for the types of soils, plants, and water sources found locally.

The region is characterized by agricultural areas irrigated with water potentially affected by mining discharges, as well as soils containing both oxide and sulfide mineral assemblages due to extensive gold and base metal mining. These conditions can influence the chemical binding forms of metals and their response to different digestion chemistries. Without method validation tailored to such matrices, reported heavy metal concentrations may be inaccurate, leading to unreliable environmental assessments and potentially flawed public health or policy decisions.

Globally, method selection is often influenced by trade-offs between analytical recovery, reproducibility, reagent cost, time requirements, and safety risks. For instance, HF-based methods can dissolve silicate-bound metals more completely but require specialized fume hoods, operator training, and waste neutralization systems to manage extreme toxicity. Conversely, aqua regia is safer and more cost-effective but may underestimate metals bound within refractory mineral phases. Such operational complexity includes equipment requirements, hazardous reagent handling, digestion time, and level of technical skill needed, all of which are particularly relevant in resource-limited Ethiopian laboratories. (Roth & Bina, 2020).

Given the absence of locally validated digestion protocols, there is an urgent need for a systematic comparison of commonly used acid digestion methods under the practical constraints of Ethiopian analytical laboratories. This study seeks to address that gap by evaluating four digestion methods—$HCl–HNO_3$ (3:1), $HCl–HNO_3–H_2SO_4$, $HCl–HNO_3–HClO_4$, and $HCl–HNO_3–HClO_4–HF$—for their accuracy, precision, cost-effectiveness, and operational feasibility in analyzing Cu, Zn, Pb, Co, Cr, Cd, Mn, Fe, and Ni in soil, plant, and water samples from Tigray, Ethiopia.

## 1.3. Justification of The Study

In the Tigray region of Ethiopia, environmental monitoring of heavy metals in soils, plants, and water is of growing importance due to mining activities, agricultural intensification, and potential contamination from industrial effluents. Reliable laboratory analysis is essential for informing regulatory agencies such as the Ethiopian Environmental Protection Authority (EEPA), regional agricultural bureaus, and public health authorities, who depend on accurate data to guide remediation strategies, irrigation water standards, and safe limits for food crops. However, the accuracy of reported concentrations is highly dependent on the digestion method used prior to analysis. Without validated digestion protocols suited to local matrices, there is a risk of underestimating or overestimating contamination levels, which could lead to misinformed policy decisions.

This study is timely because no systematic evaluation has been conducted in Ethiopia to determine which acid digestion protocol provides the optimal balance of accuracy, precision, safety, and cost for diverse environmental matrices. Globally recognized protocols (e.g., USEPA 3050B, USEPA 3051A, ISO 11466, AOAC Official Methods) offer guidance, but they have not been tested under the practical constraints of Ethiopian laboratories, where resource limitations, infrastructure gaps, and reagent availability can influence method feasibility.

The four digestion methods selected for this study—$HCl–HNO_3$ (3:1), $HCl–HNO_3–H_2SO_4$, $HCl–HNO_3–HClO_4$, and $HCl–HNO_3–HClO_4–HF$—represent a spectrum of complexity, safety requirements, and dissolution capability. Methods using aqua regia ($HCl–HNO_3$) are simpler, safer, and less expensive, but may not fully dissolve refractory silicate-bound metals. HF-containing methods, while more hazardous and requiring strict safety measures, can achieve near-complete digestion of silicate-rich samples such as those found in Tigray’s mining areas. This selection enables comparison across practical operational constraints, reagent costs, digestion times, and metal recovery efficiency.

The study’s contribution will be measured using quantifiable performance indicators:

- Accuracy: recovery within ±20% of certified values for CRMs or spiked samples.
- Precision: relative standard deviation (RSD) $\leq 10\%$ for replicate analyses.
- Comparative performance: statistical differentiation via one-way ANOVA and post-hoc testing.
- Operational feasibility: reagent cost per sample, digestion time, and required safety infrastructure.

By producing locally validated performance data, this research will provide laboratories, regulators, and policymakers with evidence-based recommendations for digestion method selection. This will enhance the reliability of environmental monitoring, reduce the likelihood of costly misclassification of contamination status, and support compliance with both national and international heavy metal standards in food, water, and soil.

## 1.4. Objective of The Study

### 1.4.1. General Objective

To compare and identify the most suitable acid digestion method—HCl–$HNO_3$ (3:1), HCl–$HNO_3$–$H_2SO_4$, HCl–$HNO_3$–$HClO_4$, and HCl–$HNO_3$–$HClO_4$–HF—for the determination of total concentrations of Cu, Zn, Pb, Co, Cr, Cd, Mn, Fe, and Ni in soil, plant, and water samples from the Tigray region of Ethiopia, with the aim of improving the accuracy and reliability of heavy metal monitoring in resource-limited laboratory settings.

### 1.4.2. Specific Objectives

The specific objectives of this study are: -

1. To compare the metal recovery efficiency of four acid digestion methods for soil, water, and plant matrices.
2. To assess the accuracy and precision of each method using certified reference materials and recovery studies.
3. To establish and compare the method detection limits (MDLs) for target metals obtained from each digestion protocol.
4. To evaluate the reproducibility and consistency of results across repeated analyses using each method.
5. To analyze the practicality of each digestion method in terms of reagent consumption, safety, cost, and ease of implementation—particularly for resource-limited laboratories.

## 1.5. Significant of The Study

This study aims to improve the accuracy, precision, and practicality of heavy metal determination in soil, plant, and water samples from Tigray, Ethiopia, through a comparative evaluation of four acid digestion methods:

- Method A: HCl–$HNO_3$ (3:1, aqua regia)
- Method B: HCl–$HNO_3$–$H_2SO_4$
- Method C: HCl–$HNO_3$–$HClO_4$
- Method D: HCl–$HNO_3$–$HClO_4$–HF

The selected heavy metals—Cu, Zn, Pb, Co, Fe, Cr, Cd, Mn, and Ni—are of high environmental and agricultural relevance in the region due to their potential toxicity, persistence in ecosystems, and bioaccumulation in crops and water sources. Local contamination sources include artisanal and industrial mining activities, use of metal-based agricultural inputs, and industrial effluent discharge. Monitoring these metals with reliable methods is essential for protecting public health, supporting sustainable agriculture, and informing environmental management strategies in Tigray.

### 1.5.1. Methodological Contributions

- Compares the recovery efficiency, precision, and detection capabilities of four commonly used acid digestion protocols for diverse environmental matrices.
- Evaluates digestion performance using certified reference materials (CRMs), proficiency testing (PT) samples, spiking recovery tests, and statistical methods such as one-way ANOVA and post-hoc analysis.
- Assesses the influence of matrix composition on digestion efficiency, providing data-driven insight into method suitability for soils, plants, and water.

### 1.5.2. Practical Implications

The practical implications of this study are considerable, as it provides essential guidance for laboratories in Ethiopia and other resource-constrained contexts in selecting digestion methods that achieve an appropriate balance between analytical accuracy, operational safety, cost-effectiveness, and methodological feasibility. In addition, the findings generate quantitative evidence that enhances the credibility of environmental monitoring programs, thereby reinforcing their reliability and acceptance. The study further offers valuable insights for policymakers and regulatory authorities by presenting validated analytical options that can be integrated into environmental protection standards, agricultural safety guidelines, and mining regulations. Ultimately, the outcomes of this research facilitate evidence-based decision-making in addressing heavy metal contamination risks in Tigray, thereby contributing to the sustainable management and protection of land and water resources.

### 1.5.3. Scope and Limitations

This study focuses on soil, plant, and water samples relevant to the environmental conditions of Tigray. The findings are directly applicable to laboratories using FAAS as the detection technique. The scope does not extend to other instrumental methods (e.g., ICP-MS, ICP-OES) or to field-deployable rapid test kits. Limitations include the exclusion of seasonal variability in contamination levels and the study's reliance on controlled laboratory conditions, which may differ from field realities.

# CHAPTER TWO

# 2. REVIEW OF LITERATURE

## 2.1. Method Comparison Methodology

A review of terminology precedes discussion of methodology as statistical reporting terms are used inconsistently in the literature. "Accuracy" and "precision" are used often when "bias" and "repeatability" are the properties being assessed. Accuracy is the degree to which an instrument measures the real value of a variable and is assessed by comparing the measurement method with a gold standard that has been calibrated to be highly accurate. In a method-comparison study, however, the investigator is comparing a less-established method with an established method already in use. The difference in values obtained with the two methods represents the “bias” of the less established method relative to the more established one. “Precision” is defined in two different ways: (1) the degree to which the same method produces the same results on repeated measurements, and (2) the degree to which values cluster around the mean of the distribution of values. The first definition equates with “repeatability:” How well does one method give the same results when measured over and over again? The second definition facilitates generalizing from the sample to the population by defining the range within which a value from the population is likely to fall. The closer together are the values within the range, the more precise is the estimate, and more confidence can be had in finding a result within that range in others who are like the sample, but not part of the sample. Repeatability in a method-comparison study is a necessary, but insufficient, condition for agreement between methods. If one or both methods do not give repeatable results, assessment of agreement between methods is meaningless (Hanneman, 2008; U.S. EPA, 1996; FAO, 2002).

Method comparison studies are crucial in environmental and agricultural laboratories for selecting optimal digestion and analytical techniques. For example, Hseu et al. (2002) compared aqua regia, nitric-perchloric, and HF-based digestion methods for soil metal extraction, highlighting that HF mixtures offered higher recovery for metals like Fe and Cr. Similarly, Sastre et al. (2002) used multiple digestion protocols to assess the mobility of metals in contaminated soils, showing how method choice impacts environmental risk interpretation.

These case studies illustrate that differences in digestion protocols can yield significantly different results for the same sample, making rigorous comparison essential for accurate trace metal analysis (Hseu, 2004; Sastre et al., 2002).

In method comparison, repeatability refers to the ability of a method to yield consistent results under the same conditions, while agreement assesses how closely two methods produce similar results for the same samples. While basic metrics like standard deviation and relative standard deviation (RSD) are commonly used, more advanced statistical tools can provide deeper insights (Thompson et al., 2002).

For example, a Bland–Altman plot is useful for visualizing the agreement between two analytical methods by plotting the difference against the average of the two measurements. This helps identify systematic bias and outliers. In contrast, the Passing–Bablok regression method is a non-parametric technique for method comparison that does not assume a specific distribution or error structure and is particularly robust when data includes outliers or heteroscedasticity (Passing & Bablok, 1983).

Incorporating such statistical approaches allows for a more nuanced and statistically valid evaluation of the comparability and interchangeability of digestion methods, especially when validating new or alternative protocols in trace metal analysis.

## 2.2. Background Significance of Heavy Metal Analysis

The analysis of heavy metals holds significant background significance due to several reasons: Toxicity: Heavy metals are naturally occurring elements that, when present in high concentrations, can be toxic to living organisms, including humans. Exposure to heavy metals such as lead, mercury, cadmium, arsenic, and chromium can have severe health effects, including neurological disorders, organ damage, developmental abnormalities, and even cancer. Analyzing the levels of heavy metals in various environmental, food, and biological samples helps assess potential risks to human health and the environment.

Environmental pollution: Heavy metal pollution is a widespread environmental concern. Industrial activities, mining, improper waste disposal, and agricultural practices can release heavy metals into the environment, contaminating soil, water bodies, and the air. Heavy metals have long-term persistence in the environment and can accumulate in the food chain, posing risks to ecosystems and biodiversity. Analyzing the levels of heavy metals in environmental samples helps monitor pollution levels, identify pollution sources, and evaluate the effectiveness of remediation efforts (Aziz, 2023).

Regulatory compliance: Governments and regulatory bodies have established guidelines and regulations regarding the permissible levels of heavy metals in various matrices, including drinking water, food, air, and soil. Analytical methods for heavy metal analysis are essential for assessing compliance with these regulations and ensuring the safety of the environment and public health. Regular monitoring and accurate measurement of heavy metal concentrations are crucial for identifying potential sources of contamination and implementing appropriate mitigation measures (Mititelu et al., 2025).

Food safety and quality: Heavy metals can enter the food chain through contaminated soil, water, and fertilizers, or as a result of industrial processes. Monitoring the levels of heavy metals in food commodities is essential to ensure food safety and compliance with regulatory standards. Analyzing heavy metals in food samples helps identify potential sources of contamination, establish maximum residue limits, and implement control measures to prevent consumer exposure to harmful levels of heavy metals (Milanković et al., 2024).

Occupational health and safety: Certain occupations, such as mining, metalworking, battery manufacturing, and electronics recycling, involve a higher risk of heavy metal exposure (Hanneman, 2008).

### 2.2.1. Nanoparticles in Heavy Metal Transport

Recent research has shown that nanoparticles play a significant role in the environmental transport and fate of heavy metals. Engineered and naturally occurring nanoparticles can adsorb metals such as Pb, Cd, and Hg, facilitating their mobility through soil and water systems. This nanoparticle-mediated transport can increase the bioavailability of toxic metals, especially in aquatic environments, and complicates remediation strategies (Nowack & Bucheli, 2007). Studies have also found that nanoparticles can cross biological membranes more easily, raising additional health concerns (Tiede et al., 2008). Understanding nanoparticle–metal interactions is therefore essential for accurate risk assessment and environmental monitoring.

### 2.2.2. Climate Change and Metal Bioavailability

Climate change can significantly influence the bioavailability and cycling of heavy metals in terrestrial and aquatic systems. Rising temperatures, altered precipitation patterns, and increased frequency of extreme weather events can enhance the mobilization of metals from sediments and soils into water bodies (Noyes et al., 2009). For instance, flooding can re suspend contaminated sediments, while drought conditions may increase metal concentrations in surface waters due to reduced dilution. Changes in soil pH and redox potential under altered climatic conditions can also affect metal solubility, with potential consequences for food safety and ecosystem health (IPCC, 2021). These climate-related effects underscore the need for adaptive monitoring strategies.

### 2.2.3. Importance of Speciation Analysis in Risk Assessment

Total metal concentrations alone do not provide a complete picture of environmental or health risks, as toxicity and bioavailability depend on the specific chemical forms—or species—of metals present. Speciation analysis differentiates between free ions, complexed forms, and particulate-bound metals, enabling more accurate predictions of metal behavior in the environment and their potential impact on organisms (Templeton et al. (2000)). For example, methyl mercury is far more toxic and bio-accumulative than inorganic mercury, and hexavalent chromium (Cr(VI)) poses greater health risks than trivalent chromium (Cr(III)) (Huang et al., 2015). Incorporating metal speciation into monitoring programs is therefore critical for effective risk assessment and regulatory compliance.

### 2.2.4. Flint, Michigan Water Crisis

A striking example of the importance of accurate heavy metal monitoring is the Flint, Michigan water crisis in the United States. In 2014, the city's water source was switched to the Flint River without proper corrosion control measures, causing lead to leach from aging pipes into the drinking water supply. Lead concentrations in some households exceeded 13,000 ppb, far above the US EPA's action level of 15 ppb (Hanna-Attisha et al., 2016). The crisis highlighted systemic failures in water quality monitoring, the consequences of underestimating heavy metal exposure risks, and the need for robust analytical methods, including frequent testing and quality assurance measures, to protect public health.

## 2.3. Importance of Digestion Methods in Determining Total Concentrations

Accurate and complete digestion of the sample matrix is essential for determining the total concentrations of elements in environmental matrices (Hseu, 2004). The importance of digestion methods lies in their ability to break down complex matrices and convert the target analytes into a form that can be easily measured and quantified using analytical techniques.

Here are several reasons why digestion methods are important in determining total concentrations:

Matrix decomposition: Many samples, such as biological tissues, soil, sediments, and food, contain complex matrices that can interfere with the accurate measurement of target analytes. Digestion methods help decompose these matrices by breaking down organic and inorganic compounds into simpler forms. This decomposition simplifies the sample matrix, making it easier to extract and analyze the target analytes.

Extraction efficiency: Certain elements or compounds may be present in a sample matrix in forms that are not readily accessible for analysis. Digestion methods help extract these analytes from their bound or complexed forms, increasing the efficiency of their recovery during the digestion process. This ensures that the measured concentrations represent the total amount of the analyte present in the sample.

Conversion to soluble forms: Some analytes may exist in insoluble or poorly soluble forms in the sample matrix. Digestion methods employ chemical reactions or physical treatments to convert these analytes into soluble forms, allowing for their complete extraction. By converting the analytes into a soluble state, the digestion process enables accurate quantification using various analytical techniques.

Removal of interferences: Digestion methods can remove or reduce interfering substances present in the sample matrix. Interferences can include contaminants, co-extracted compounds, or matrix components that may hinder the accurate measurement of the target analytes. Digestion processes, such as acid digestion or oxidation, can selectively remove or modify these interfering substances, improving the accuracy and precision of the analysis.

Sample homogenization: Digestion methods often involve the homogenization of the sample, ensuring that the target analytes are evenly distributed. This step is particularly important when dealing with heterogeneous or non-uniform samples, as it helps increase the representativeness of the analysis. Homogenization minimizes the potential for sampling bias and ensures that the measured concentrations reflect the true composition of the sample.

Digestion methods are vital for determining the total concentrations of analytes in various samples. They enable the decomposition of complex matrices, enhance extraction efficiency, convert analytes into soluble forms, remove interferences, and ensure sample homogeneity. These processes are essential for accurate and reliable quantitative analysis in fields such as environmental monitoring, food safety, pharmaceutical analysis, and many other areas of scientific research (Johannesson & Cox, 2019).

While total concentration measurements provide a general estimate of the amount of a metal present in a sample, they do not convey information about its specific chemical forms. These forms, or species, determine the metal’s mobility, bioavailability, and toxicity (Templeton et al., 2000). For example, chromium can exist as Cr(III), an essential nutrient in trace amounts, or Cr(VI), a highly toxic and carcinogenic form. Measuring only the total chromium content may obscure these important differences, potentially leading to misleading risk assessments (Huang et al., 2015). Therefore, for environmental and toxicological studies, speciation analysis is often more informative than total concentration measurements, particularly in assessing potential human health risks.

Bioavailability—the fraction of a metal that is accessible for uptake by organisms—is highly dependent on the chemical form in which the metal occurs. For instance, methylmercury is readily absorbed and bioaccumulates through aquatic food webs, posing significant health risks, whereas inorganic mercury is less bioavailable but still toxic (Clarkson & Magos (2006)). Similarly, metals bound to stable mineral phases may remain largely unavailable to biota, while those in soluble or weakly bound forms can be easily absorbed (Kabata-Pendias (2000)).Thus, while digestion methods that measure total concentrations are valuable for regulatory compliance, they may overestimate the ecological and health risks if bioavailability is not taken into account.

## 2.4. Methodology for Heavy Metal Analysis

### 2.4.1. Digestion methods overview

Digestion methods are analytical techniques used to break down complex samples into simpler forms for subsequent analysis. These methods involve the application of chemical reactions or physical treatments to decompose and solubilize the sample matrix, allowing for the extraction and quantification of target analytes. Digestion methods are widely used in various fields, including environmental analysis, pharmaceutical analysis, food safety, and forensic science. Here is an overview of common digestion methods:

Acid Digestion: Acid digestion is one of the most common digestion methods. It involves the use of strong mineral acids, such as nitric acid ($HNO_3$) or a mixture of nitric acid and hydrochloric acid (aqua regia), to dissolve and decompose the sample matrix. Acid digestion like Aqua regia ($HCl:HNO_3$, 3:1) is widely used for sample digestion due to its simplicity and effectiveness in dissolving metals is effective in decomposing organic matter, oxidizing metals into soluble forms, and breaking down mineral matrices. It is typically used for the digestion of soil, sediments, plant material, and solid samples (Hseu, 2004).

Alkaline Digestion: Alkaline digestion is used to decompose organic matter and extract analytes that are resistant to acid digestion. It involves the use of strong alkaline solutions, such as sodium hydroxide (NaOH) or potassium hydroxide (KOH), to solubilize the sample matrix. Alkaline digestion is commonly employed for the digestion of biological samples, such as tissues, blood, and urine.

Microwave Digestion: Microwave digestion is a rapid and efficient digestion technique that utilizes microwave energy to heat the sample and accelerate the digestion process. It involves placing the sample in a closed vessel along with appropriate acids or reagents and subjecting it to microwave irradiation. The microwave energy rapidly heats the sample, promoting decomposition and digestion. Microwave digestion is widely used in laboratories for various sample types, including solid, liquid, and biological samples.

Enzymatic Digestion: Enzymatic digestion involves the use of specific enzymes to break down complex organic molecules. Enzymes are biological catalysts that can selectively target and hydrolyze specific bonds within the sample matrix. Enzymatic digestion is commonly used for the analysis of proteins, carbohydrates, and complex biological samples.

High-Temperature Combustion: High-temperature combustion, also known as dry ashing or incineration, involves subjecting the sample to high temperatures in a controlled oxygen-rich environment. This process oxidizes organic matter and converts it into gaseous byproducts, leaving behind inorganic residues. High-temperature combustion is often used for the digestion of solid samples, such as coal, polymers, and organic-rich matrices.

Ultraviolet Digestion: Ultraviolet (UV) digestion utilizes ultraviolet radiation to break down organic compounds in the sample matrix. UV digestion is typically combined with the addition of hydrogen peroxide ($H_2O_2$) or other oxidizing agents to enhance the oxidation process. This method is commonly used for the digestion of water samples, as it allows for the degradation of organic compounds and the release of bound analytes (Pawliszyn, 2010).

Performance Comparison in Terms of Recovery Efficiency: The efficiency of a digestion method is often evaluated based on its recovery of target metals compared to certified reference materials (CRMs). Recovery efficiency can vary significantly between digestion techniques due to differences in acid strength, temperature, and digestion duration. For example, Hseu et al. (2002) reported that HF-based multi-acid digestions achieved the highest recoveries for refractory elements such as Fe, Cr, and Ni in soil samples, whereas aqua regia digestion was less effective for these metals but adequate for Cu, Zn, and Pb. Recovery efficiency is a critical parameter in method selection because incomplete digestion may result in underestimation of metal concentrations, affecting environmental assessments and regulatory compliance (Hseu, 2004).

Potential Matrix Effects on Digestion Efficiency: Matrix composition can strongly influence digestion performance. Organic-rich matrices such as plant material often require oxidizing agents like $HNO_3$–$H_2O_2$ mixtures to effectively break down organic matter, while silicate-rich matrices such as soils and sediments may need HF to dissolve mineral structures (USEPA, 2007). Matrix effects can lead to either incomplete digestion—if the acid mixture is not strong enough—or analyte loss through volatilization during heating (especially for elements like As and Hg). Recognizing these matrix-specific challenges is essential for selecting appropriate digestion protocols that ensure complete metal recovery without introducing significant analytical errors.

Standardized Protocols for Consistency and Comparability: The adoption of standardized digestion protocols is essential for producing comparable results across laboratories and studies. Protocols such as US EPA Method 3051A for microwave-assisted digestion and ISO 11466 for aqua regia digestion provide detailed guidance on acid mixtures, digestion times, and temperature programs to achieve consistent metal recovery (US EPA, 2007; ISO, 1995). Standardization minimizes variability due to procedural differences and facilitates the establishment of long-term monitoring datasets. Furthermore, standardized methods are widely accepted by regulatory agencies, increasing the credibility of analytical results in environmental assessments.

### 2.4.2. Selection criteria for digestion methods

Selecting an appropriate digestion method is essential for ensuring accurate, precise, and reliable heavy metal analysis. The decision should be based on a combination of chemical, instrumental, and practical considerations.

1□. Chemical Considerations

Sample Matrix – The physical and chemical nature of the sample strongly influences digestion efficiency. For example, silicate-rich matrices such as soils and sediments may require HF-based digestion to break down mineral lattices, whereas organic-rich matrices (e.g., plant tissues) are better digested using $HNO_3$–$H_2O_2$ mixtures to oxidize organic matter (U.S. EPA, 1996). Considering organic content, mineral composition, and particle size is crucial for optimal method selection.

Analyte Stability – Some metals, such as Hg and As, can volatilize or transform during digestion. Choosing an appropriate acid mixture and digestion temperature is essential to prevent analyte loss. For example, sealed-vessel microwave digestion minimizes volatilization by maintaining a closed environment (Hseu, 2004).

### 2□. Instrumental Considerations

Required Detection Limits – The digestion method must be capable of delivering analyte concentrations within the detection range of the analytical instrument. For example, methods producing clear solutions without particulates are preferred for FAAS and ICP-MS to prevent signal suppression and contamination (Thompson et al., 2002).

Compatibility with Analytical Techniques – Certain analytical methods require specific digestion conditions. For instance, ICP-MS is highly sensitive to matrix interferences, making complete digestion and matrix removal critical, while FAAS can tolerate certain residual solids if they do not cause clogging or spectral interference (Hseu, 2004).

### 3□. Practical Considerations

Cost and Time – Some methods, such as HF-based multi-acid digestions, require specialized safety equipment and are more expensive, while microwave digestion can be faster but demands high initial equipment investment. Selection should balance analytical quality with available resources (U.S. EPA, 1996).

Safety and Environmental Concerns – Strong acids such as HF and $HClO_4$ pose significant hazards, requiring strict safety measures and specialized fume hoods. Additionally, waste acid disposal must comply with environmental regulations (ISO, 1995).

Availability of Validated Protocols – Using standardized methods such as US EPA 3051A or ISO 11466 ensures documented performance characteristics, facilitating inter-laboratory comparisons and regulatory acceptance (ISO, 1995).

Table 1: Suggested Comparative Table for Different Digestion Methods

| **Digestion Method** | **Optimal Matrices** | **Advantages** | **Limitations** | **Standard Protocols** |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **Aqua Regia Digestion** | Soils, sediments, some plant tissue | Simple, cost-effective, widely used | Incomplete dissolution of silicates | ISO 11466 |
| **HF Multi-Acid Digestion** | Mineral-rich soils, sediments | Complete dissolution of silicates | Hazardous, requires specialized safety equipment | Modified USEPA 3052 |
| **Microwave-Assisted Digestion** | All matrices (with proper acid mix) | Fast, minimizes volatilization, efficient | High initial equipment cost | USEPA 3051A |
| **$HNO_3$–$H_2O_2$ Digestion** | Organic-rich plant or biological samples | Effective for organic matter removal | May not dissolve refractory minerals | AOAC Official Methods |

### 2.4.3. Sample preparation and handling considerations

Sample preparation and handling are critical stages in trace metal analysis, as errors at these steps can propagate and compromise the accuracy, precision, and reliability of final results. These processes require a balance between minimizing contamination, preserving analyte stability, and ensuring sample representativeness.

1□. Ensuring Sample Representativeness and Homogeneity

The accuracy of heavy metal analysis begins with collecting a representative sample that truly reflects the target environment. Homogenization—through grinding, milling, or blending—is essential to minimize heterogeneity and spatial variation in the analyte distribution (Miller & Miller, 2018). For environmental samples like soils and sediments, composite sampling can reduce local variability, while plant tissues often require freeze-drying to maintain structure before grinding.

2□. Field Sampling Techniques and Their Challenges

The sampling method directly affects the quality of trace metal data. Grab sampling provides an instantaneous snapshot but may miss temporal variations, whereas passive samplers accumulate contaminants over time, offering a more integrated measure of exposure (Allan et al., 2008). In aquatic systems, metals can adsorb to container walls or settle out of suspension, so acidification to pH < 2 with ultrapure $HNO_3$ is often recommended to stabilize dissolved metals (APHA, 2017). The choice of sampling method must consider the study's goals, the target analytes, and potential contamination risks.

## 3□. Contamination Risks and Adsorption Losses

One of the greatest challenges in trace metal analysis is preventing contamination. Even minor exposure to airborne particulates or metallic surfaces can significantly alter results for ultra-trace analyses (Rodushkin et al., 2000). All equipment should be acid-washed with dilute $HNO_3$, and handling must be done with powder-free gloves to prevent metal transfer. Additionally, metals such as Pb, Cd, and Hg can adsorb onto container surfaces—especially plastics—causing artificially low readings unless samples are stabilized immediately after collection (Batley & Gardner, 1977).

## 4□. Quality Control Measures in Sample Handling

To ensure data integrity, robust quality control (QC) protocols must be implemented during preparation and handling. These include the use of certified reference materials (CRMs) to verify accuracy, method blanks to detect contamination, and spike recovery tests to assess analyte recovery efficiency (Thompson et al., 2002). Regular inclusion of QC samples allows analysts to detect drift, contamination, or systematic bias early in the workflow.

## 5□. Preservation, Storage, and Safety

Sample preservation techniques are chosen to prevent chemical or biological changes that could alter metal concentrations. For example, cooling to ≤ 4 °C slows microbial activity, while acidification prevents precipitation and adsorption (U.S. EPA, 1996). Safety considerations include wearing PPE, working in fume hoods for acid handling, and proper neutralization of waste acids before disposal to meet environmental compliance.

## 2.5. Digestion Comparison for Soil, Plant and Water Samples

Sample digestion is a crucial step in environmental and agricultural analysis, as it transforms complex matrices such as soil, plant, and water into solutions suitable for elemental determination by techniques like Flame Atomic Absorption Spectroscopy (FAAS). The efficiency of digestion depends not only on the acid combination but also on the matrix composition, target elements, and the intended analytical precision (8). In this study, four digestion methods were evaluated:
Method A: $HCl–HNO_3$ (3:1, Aqua Regia)
Method B: $HCl–HNO_3–H_2SO_4$
Method C: $HCl–HNO_3–HClO_4$
Method D: $HCl–HNO_3–HClO_4–HF$

These methods represent a spectrum from partial extraction (aqua regia) to near-total dissolution (HF-based), with varying trade-offs in accuracy, safety, and environmental impact.

### 2.5.1. Method A – Aqua Regia ($HCl–HNO_3$, 3:1)

Aqua regia is internationally accepted (ISO 11466; USEPA 3050B) for estimating “total” recoverable metals in soils, sediments, and plants. The $HNO_3$ oxidizes organic matter, while $Cl_2$ and NOCl generated in situ enhance dissolution of sulfides and carbonates (ISO, 1995). It is cost-effective, simple, and widely available, making it ideal for routine environmental monitoring. (Bai et al., 2010).

However, aqua regia is limited for silica-bound and refractory metals like Cr, Ni, and Ti, where recoveries may be <80% (U.S. EPA (1996)). Its inability to dissolve silicates is due to the absence of fluoride, which is necessary for Si–O bond cleavage. While suitable for metals such as Cd, Cu, Pb, and Zn, results can underestimate total concentrations in high-silica soils (Jain et al., 2020).

### 2.5.2. Method B – $HCl–HNO_3–H_2SO_4$

Adding $H_2SO_4$ increases the boiling point of the digestion mixture, allowing more aggressive oxidation and partial breakdown of resistant mineral phases. Studies show Method B improves recovery of refractory metals such as Cr and Ni compared to aqua regia, particularly in clay-rich soils with Fe–Mn oxide coatings (Asher et al., 2020).

However, $H_2SO_4$ poses significant safety and environmental concerns: it can form insoluble sulfates with Ba, Pb, and Sr, reducing recoveries (Thomas et al., 2014). Waste acid disposal requires neutralization to prevent sulfate pollution. Despite this, Method B is favored in some industrial contamination studies where improved recovery of high-temperature mineral phases is required.

### 2.5.3. Method C – $HCl$–$HNO_3$–$HClO_4$

Perchloric acid is a powerful oxidizer, enabling complete digestion of organic matter in plant and water samples. Method C is particularly effective for Cd, Pb, and Mn, which are often associated with organic complexes (Zinzala et al., 2023). The oxidative strength of $HClO_4$ ensures minimal residual carbon, reducing background interferences in FAAS.

The major limitation is safety: $HClO_4$ is explosive under certain conditions and requires specialized fume hoods and protocols (Jones & Jackson, 2012). Additionally, perchlorate waste disposal is environmentally sensitive due to its persistence in groundwater.

### 2.5.4. Method D – $HCl$–$HNO_3$–$HClO_4$–HF

HF-containing digestions are considered the most complete for mineral matrices due to their ability to dissolve silicates via formation of soluble hexafluorosilicates ($HF + SiO_2 \rightarrow H_2SiF_6$). This makes Method D the only option for total recovery of Al, Ti, and Si-bound trace metals in quartz-rich soils (Nunes et al., 2021).

However, HF is extremely hazardous—causing severe chemical burns and systemic toxicity—and requires Teflon labware and HF-rated fume hoods. Disposal is also challenging due to fluoride toxicity. As a result, Method D is usually restricted to research laboratories with specialized safety infrastructure.

### 2.5.5. Practical Considerations across Methods

Beyond chemical efficiency, the selection of a digestion method also depends on factors such as cost-since HF and $HClO_4$ methods demand more expensive safety infrastructure-environmental impact, given that perchlorates and fluorides require specialized waste treatment, compatibility with FAAS to avoid spectral or chemical interferences from the acid matrix, and sample throughput, as microwave digestion offers faster processing but is limited by vessel capacity.

Matrix effects can influence FAAS sensitivity—e.g., high residual acids may suppress absorbance signals—requiring dilution or matrix matching (Rai et al., 2020).

Table 2: Comparative Summary of Digestion Methods for Soil, Plant, and Water Samples

| **Method** | **Acid Composition** | **Strengths** | **Limitations** | **Suitable Matrices** | **Safety & Environmental Impact** |
|---|---|---|---|---|---|
| **A** | HCl–$HNO_3$ (3:1) | Simple, cost-effective, good for Cd, Cu, Pb, Zn | Poor for refractory metals; partial digestion only | Soil, plant, water | Low hazard; routine use |

| | | | | | |
|---|---|---|---|---|---|
| **B** | $HCl$–$HNO_3$–$H_2SO_4$ | Better recovery for Cr, Ni; high-temp digestion | Forms insoluble sulfates; sulfate waste | Soil, industrial sludge | Moderate hazard; sulfate pollution |
| **C** | $HCl$–$HNO_3$–$HClO_4$ | Excellent for organic-rich matrices; high precision | Explosive risk; perchlorate waste | Plant, water, organic soils | High hazard; persistent waste |
| **D** | $HCl$–$HNO_3$–$HClO_4$–$HF$ | Total digestion incl. silicates; recovers Ti, Al, Si | Extreme toxicity; HF waste | Quartz-rich soils, clays | Very high hazard; specialized lab only |

## 2.6. Evaluation of Digestion Methods

### 2.6.1. Analytical Performance and Accuracy

Accuracy refers to the closeness of a measured value to a true or certified reference value (Miller & Miller, 2018). It is usually assessed using certified reference materials (CRMs) or by comparing results with established standard methods such as US EPA 3051A for microwave digestion (US EPA, 2007). The use of CRMs ensures traceability, while regular calibration and use of method blanks help detect systematic errors.

Precision describes the degree of agreement between repeated measurements under identical conditions and is typically expressed as relative standard deviation (RSD) (Thompson et al., 2002). While high precision indicates low random error, it does not guarantee accuracy if systematic bias exists.

Reproducibility evaluates the consistency of results across different laboratories, instruments, or analysts. It is commonly assessed through inter-laboratory comparisons or proficiency testing (ISO, 2017). High reproducibility demonstrates a method’s robustness across different working environments (Karageorgou & Samanidou, 2014).

Sensitivity is defined as the slope of the calibration curve, indicating the instrument’s ability to detect small changes in analyte concentration (Harris, 2020). A more sensitive method enables lower detection limits, which is critical for trace-level heavy metal analysis in environmental samples.

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present (Skoog et al., 2017). This is particularly important in environmental matrices, where multiple metals and organic matter can interfere with detection.

Linearity ensures that the analytical response is directly proportional to the analyte concentration within the working range, and it is typically verified using the correlation coefficient ($R^2$) of the calibration curve (Miller & Miller, 2018).

Ruggedness refers to a method’s ability to remain unaffected by small, deliberate variations in parameters such as temperature, digestion time, acid concentration, or differences between analysts. For example, a digestion method that consistently recovers Pb in soil despite changes in heating duration is considered rugged. Testing for ruggedness is essential for standardization across multiple laboratories (Shrivastava & Gupta, 2011).

### 2.6.2. Detection Limits and Sensitivity

The Limit of Detection (LOD) is the lowest analyte concentration distinguishable from background noise, often calculated as three times the standard deviation of the blank divided by the calibration slope (Harris, 2020). The Limit of Quantification (LOQ), typically 10× the blank standard deviation, defines the minimum quantifiable concentration with acceptable precision (Armbruster & Pry, 2008).

Detection limits depend on both instrumental capabilities and sample preparation. For instance, microwave-assisted digestion often results in lower detection limits compared to open-vessel digestion due to reduced contamination and volatilization losses (Hseu, 2004). Optimizing acid strength, digestion time, and vessel material can further improve sensitivity.

### 2.6.3. Matrix Effects and Interferences

Matrix effects occur when non-analyte components influence the analyte's signal, causing suppression or enhancement (Pohl, 2009). In FAAS, high salt concentrations can suppress metal absorbance, while organic matter may enhance signals for certain elements.

For digestion methods, matrix effects vary depending on the sample type: in soil, high silica content can cause incomplete digestion without HF, leading to underestimation of metals such as Cr and Ni (Nunes et al., 2021); in plant tissue, organic matter may generate background carbon interference unless fully oxidized (Zinzala et al., 2023); and in water samples, high hardness can precipitate metals during storage unless preserved with acid (Hseu, 2004).

However, the concepts of matrix-matched calibration, internal standards, sample dilution, and selective extraction methods are well-established in analytical chemistry literature as strategies to mitigate matrix effects and improve method accuracy. (Mermet & Poussel, 1995).

### 2.6.4. Cost-Effectiveness and Practicality

Method selection must balance analytical performance with cost, safety, and practicality. For instance, HF-based digestion allows total dissolution but requires costly safety infrastructure and Teflon vessels, whereas aqua regia uses inexpensive glassware but provides only partial digestion (Hseu, 2004). In terms of analysis time, microwave digestion can process samples in under an hour, thereby improving throughput but also increasing capital cost (U.S. EPA, 1996). Safety considerations are also critical, since perchloric acid generates hazardous waste requiring specialized disposal, while HF demands extreme safety measures (Jones & Jackson, 2012). Furthermore, regulatory compliance plays a significant role, as methods such as EPA 3051A and ISO 11466 are internationally recognized, enhancing comparability and ensuring legal acceptance (U.S. EPA, 1996).

### 2.6.5. Application in Soil, Water, and Plant Analysis

Soil – Acid digestion (aqua regia, HF-based) is used to determine total and bioavailable metals. HF is necessary for silicate-bound metals like Ti and Al, while aqua regia suffices for most heavy metals relevant to contamination assessment (Nunes et al., 2021).

Water – Water digestion often involves nitric acid to stabilize dissolved metals, with minimal heating to avoid volatilization losses (APHA (2017)). For ultra-trace metals, pre-concentration may be necessary to reach detection limits (Rai et al., 2020).

Plant – High-organic matrices require strong oxidizers such as $HNO_3$–$HClO_4$ for complete digestion (Zinzala et al., 2023). Microwave-assisted digestion reduces losses and speeds up processing compared to hot-plate methods (Hseu, 2004).

## 2.7. Flame Atomic Absorption Spectroscopy (FAAS)

### Introduction

Flame Atomic Absorption Spectroscopy (FAAS) is one of the most widely used techniques for the quantitative determination of metals at parts-per-million (ppm) levels, offering good precision and accuracy for many elements. It employs an air–acetylene or nitrous oxide–acetylene flame atomizer, where the sample is introduced as an aerosol via a nebulizer, and the resulting ground-state atoms absorb element-specific wavelengths of light. The technique is valued for its relatively fast analysis time (typically 10–15 seconds per sample), moderate susceptibility to interferences (which can often be corrected), low operating costs, and robust performance across diverse matrices, including environmental, food, clinical, and industrial samples (Welz & Sperling, 2021; Bader et al., 2020).

### Principles of FAAS

The underlying principle of FAAS is atomic absorption, whereby free atoms in the flame absorb radiation at wavelengths characteristic of each element. This absorption corresponds to the energy difference between the ground and excited electronic states. A monochromator isolates the analytical wavelength, and a photomultiplier tube or solid-state detector measures the absorbance, which is directly proportional to the analyte concentration according to the Beer–Lambert law (Skoog et al., 2017; Özbek & Akman, 2021).

### Instrumentation

A typical FAAS system comprises several key components. The radiation source is usually a hollow cathode lamp or an electrodeless discharge lamp that is specific to each element. The atomizer consists of a premixed air–acetylene or nitrous oxide–acetylene flame, operating at temperatures ranging from 2100 to 3000 °C. The monochromator disperses the light in order to select the analytical wavelength, while the detector measures the transmitted light intensity and converts it into absorbance.

Advances in burner design and optical systems have improved precision and reduced noise in FAAS measurements (Boss & Fredeen, 2022).

## Sample Preparation for FAAS

Accurate FAAS measurements rely heavily on appropriate sample preparation, especially for trace metal analysis. Acid digestion is the most common approach, using nitric acid ($HNO_3$), aqua regia ($HCl + HNO_3$), or mixtures with perchloric ($HClO_4$) or hydrofluoric acid (HF) for silicate-rich samples. The choice of acid depends on sample composition, as high-organic matrices require oxidative acids, while mineral-rich matrices may need HF to break down silicates (U.S. EPA, 1996).

Microwave-assisted digestion has emerged as a preferred method for FAAS sample preparation due to its rapid heating, reduced acid consumption, and improved analyte recovery—particularly for metals like Pb, Cd, and Cu—compared to conventional hot-plate digestion (Lopez-Garcia et al. (2019)). Sequential digestion can also be used to determine different geochemical fractions of metals, enhancing the interpretability of environmental data (Tessier et al., 1979; Filgueiras et al., 2002).

## Applications of FAAS

Flame Atomic Absorption Spectroscopy (FAAS) is extensively applied across various fields. In environmental monitoring, it is employed for the determination of Pb, Cd, Zn, Cu, and Cr in water, soil, sediments, and air particulates (Jain et al., 2020). In the food and beverage sector, FAAS is used to analyze trace metal content in products such as milk, wine, tea, cereals, and seafood (Soylak et al., 2018). In clinical and biomedical studies, it facilitates the measurement of both essential and toxic metals in blood, urine, and tissues (Öztürk et al., 2020). Furthermore, in mining and metallurgy, FAAS is applied for ore composition analysis and process quality control (Zawisza et al., 2019).

## Challenges and Interferences in FAAS

Despite its reliability, FAAS is affected by several interferences:

1. Spectral Interference: Spectral interference occurs when absorption or emission lines of other elements or molecular species overlap with the analyte’s characteristic wavelength. This can cause falsely high or low readings in atomic absorption or emission spectrometry. To minimize spectral interference, techniques such as high-resolution

monochromators, background correction methods (e.g., deuterium lamp or Zeeman effect), and selection of alternate analytical wavelengths are commonly employed (U.S. EPA, 1994).

2. Chemical Interference: Formation of refractory compounds (e.g., Ca, Mg phosphates) that reduce atomization efficiency, often mitigated using releasing agents or higher flame temperatures.
3. Ionization Interference: Particularly for alkali metals in hot flames, addressed by adding ionization suppressors (e.g., CsCl).
4. Physical Interference: Variations in viscosity or surface tension of solutions affecting nebulization; matrix matching or standard additions can compensate (Welz & Sperling, 2021).

### Mitigation Strategies

To counter interferences, FAAS employs several strategies. Matrix modifiers, such as $LaCl_3$, are used to prevent analyte loss or minimize interference from compounds like phosphates. Background correction techniques, including deuterium lamp correction and Zeeman-effect correction, help separate analyte signals from background absorption (Özbek & Akman, 2021). Additionally, sample dilution and matrix matching are applied to reduce matrix effects by aligning standards with the sample composition.

### Comparison with Graphite Furnace AAS (GFAAS)

While FAAS is efficient for ppm-level analysis, Graphite Furnace AAS (GFAAS) offers much lower detection limits (ng/mL), requires smaller sample volumes, and allows for direct solid sampling in some cases. However, GFAAS has longer analysis times and higher operational complexity. FAAS remains preferred for routine, high-throughput analyses, whereas GFAAS is chosen for ultra-trace determinations (Bader et al., 2020; Boss & Fredeen, 2022).

### Advancements in FAAS

Recent advancements in Flame Atomic Absorption Spectroscopy (FAAS) have further expanded its analytical capabilities. Hydride Generation FAAS (HG-FAAS) significantly lowers detection limits for hydride-forming elements such as As, Sb, Se, and Bi by converting them into volatile hydrides prior to atomization (Afkhami et al., 2020). The coupling of FAAS with chromatographic techniques, including HPLC or ion chromatography, enables speciation analysis and facilitates the discrimination between different oxidation states or chemical forms of metals (Sun et al., 2019). In addition, Flow Injection FAAS automates sample introduction, thereby improving reproducibility and reducing reagent consumption (Carvalho et al., 2021).

# CHAPTER THREE

## 3. METHODOLOGY

### 3.1. Description of the Study Areas

The study was conducted using samples collected from north west Tigray and analysed at Ezana Analytical Laboratory, located in Mekelle, Tigray, Ethiopia. Certified reference soil samples (CRMs) were obtained from Ezana Analytical Laboratory to ensure analytical accuracy and reliability. The laboratory operates in proximity to active mining zones in the Tigray region, an area known for its artisanal and industrial mining activities, which are potential sources of heavy metal contamination in soils, plants, and water.

The study area lies within the coordinates 13°31′19.538″ N, 39°29′41.657″ E, encompassing both agricultural and mining-impacted zones. These zones are of particular environmental significance because they present varied exposure pathways for heavy metals, including soil-to-crop transfer and leaching into groundwater. The regional context underscores the importance of validating digestion methods that are accurate, cost-effective, and applicable to local laboratory infrastructure.

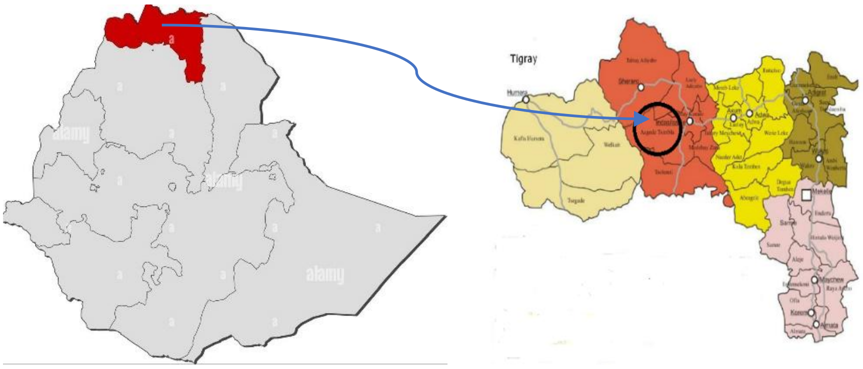

Figure 1: Sample Site Location

## 3.2. Sampling Strategy

The sampling strategy was designed to ensure representativeness and statistical reliability of the analytical results. For each matrix—soil, plant, and water—samples were collected and prepared following the guidelines of the Association of Official Analytical Chemists (AOAC) (Horwitz, 2017).

## 3.3. Apparatus and Equipment

The following apparatus and equipment were used:

1. Drying oven with programmable temperature control and air circulation (AX 60 Carbolite Drying Oven)
2. Hot plate (206HA) with precise temperature settings (±2 °C accuracy).
3. Analytical balance (BA3100P, Sartorius) with ±0.1 mg sensitivity, calibrated daily and checked against certified weights.
4. Fume cupboard (Fe 2000, Johndec Safety Flow) with laminar airflow to prevent acid vapor exposure.
5. Borosilicate glassware, Teflon beakers, and micropipettes.
6. FAAS (Varian AA240FS) equipped with deuterium background correction.

All measuring devices were calibrated and verified by the Ethiopian National Metrology Institute (NMIE) prior to use. Cleaning protocols followed ISO 17025 guidelines, including soaking glassware in 10% $HNO_3$ overnight and rinsing with distilled water to prevent contamination (Skoog et al., 2017).

## 3.4. Chemicals and Reagents

All chemicals were analytical reagent grade, including $HNO_3$ (68%, Belgium), $HClO_4$ (70%, BDH, England), HCl (35–37%, Pakistan), $H_2SO_4$ (98%, India), HF (50%, Texas, USA), and 1000 mg/L stock standard solutions (Cu, Zn, Pb, Co, Fe, Cr, Cd, Mn, Ni) traceable to NIST. Distilled water was used for all dilutions and cleaning.

Strict laboratory safety protocols were followed during the handling of all acids and reagents. This included the use of personal protective equipment (PPE) such as laboratory coats, acid-resistant gloves, safety goggles, and face shields. HF handling was performed exclusively inside a well-ventilated fume hood.

In addition to safety precautions during use, proper waste management procedures were implemented to minimize environmental and health risks associated with hazardous chemicals. Acidic waste solutions were collected in dedicated, clearly labeled high-density polyethylene (HDPE) containers. Waste containing HF was neutralized with a saturated solution of calcium carbonate before disposal. All acidic waste was subsequently adjusted to a neutral pH using sodium bicarbonate before being sent to an approved hazardous waste treatment facility, following Ethiopian Environmental Protection Authority (EPA) guidelines and international best practices (UNEPA, 2019).

## 3.5. Sample Collection and Preparation

Three representative types of soil, plant, and water samples each were obtained from Ezana Mining Development PLC’s operational areas in N.W. Tigray, Ethiopia, along with certified reference materials (CRMs) for method validation. All samples were handled following AOAC guidelines (Horwitz, 2017) to ensure traceability and analytical integrity.

### 3.5.1. Soil Samples

Soil samples were air-dried at room temperature and subsequently oven-dried at 105 °C to achieve constant weight. This temperature was selected because it effectively removes moisture without volatilizing most target metals, while minimizing the loss of volatile organic compounds that could affect digestion efficiency (Rayment & Lyons, 2011). Dried samples were gently disaggregated with a porcelain mortar and pestle, then passes through a 75-µm sieve. This particle size was chosen to ensure homogeneity, maximize the surface area for acid digestion, and facilitate reproducibility of results across replicates, as recommended by ISO 11464:2006.

### 3.5.2. Plant Samples

Plant materials were washed thoroughly with deionized water to remove dust and surface contaminants, then oven-dried at 60–65 °C to prevent thermal degradation or volatilization of metal–organic complexes. Dried plant samples were milled into a fine powder and sieved to 75 µm for uniformity, facilitating efficient digestion and minimizing variability between replicates (Mihaly-Cozmuta et al., 2016; Zarcinas et al., 1987).

### 3.5.3. Water Samples

Water samples were collected in pre-cleaned, acid-washed polyethylene bottles and acidified to pH < 2 with concentrated $HNO_3$ to preserve dissolved metals. Prior to digestion, samples were filtered through 0.45-µm membrane filters to remove suspended particulates. This filtration step was essential to eliminate potential interferences in FAAS analysis caused by particulate-bound metals and to focus on the dissolved metal fraction, which is most relevant for bioavailability and regulatory assessment (APHA, 2017).

## 3.6. Digestion Methods

Four digestion methods were evaluated for their efficiency in extracting total concentrations of Cu, Zn, Pb, Co, Cr, Cd, Mn, Fe, and Ni from soil, plant, and water samples. Each method followed modified procedures from established standards (EPA 3051A; ISO 11466), with strict quality control measures

Figure 2**:** Sample Weighing and Dilution

### Method A: HCl – $HNO_3$ (3:1)

A 0.5 g of well-mixed soil/plant sample was taken in 250 mL beaker and digested in 12 mL of aqua regia (3 mL conc. $HNO_3$ and 9 mL conc. HCl) on a hotplate for 3 h at 110 °C then the samples were evaporated near to dryness and cooled. Finally, the samples were filtered through Whatman no. 42 filter paper and transferred in to a 50- mL volumetric flask and diluted to the mark. Then the filtrate was analyzed by FAAS (FAO, 2002).

In case of water sample 50 mL of water sample was taken in to 250 mL Erlenmeyer flask and digested 120 mL of aqua regia (30 mL conc. $HNO_3$ and 90 mL conc. HCl) on a hotplate for 3 h at 90-100 °C then the samples were digested and cooled. Finally, the samples were transferred into a 100- mL volumetric flask and diluted to the mark. Then the filtrate was analyzed by FAAS (FAO, 2002).

### Method B: HCl – $HNO_3$-$H_2SO_4$

A 1.0 g of well mixed soil/plant sample was taken and weighed and transferred to 250 mL conical flask ;10 mL of conc. $H_2SO_4$ was added followed by 25 mL of conc. $HNO_3$ acid and 10 mL of concentrated HCl. The sample was heated at 200 °C for 1 h in a fuming hood and then cooled to room temperature. After cooling 20 mL of distilled water was added and the mixture was then filtered to complete the digestion. Finally, the mixture was transferred to a 50 mL volumetric flask and diluted to the mark. Finally, the filtrate was analyzed by FAAS (FAO, 2002).

In case of water sample a 50 mL sample was taken and transferred to 250 mL conical flask; 10 mL of conc. $H_2SO_4$ was added followed by 25 mL of conc. $HNO_3$ acid and 10 mL of concentrated HCl. The sample was heated at 90-100 °C for 1 h in a fuming hood and then cooled to room temperature. After cooling 20 mL of distilled water was added and the mixture was then filtered to complete the digestion. Finally, the mixture was transferred to a 100 mL volumetric flask and diluted to the mark. Finally, the filtrate was analyzed by FAAS (FAO, 2002).

**Method C: HCl – $HNO_3$-$HClO_4$**

A 0.2 g of pulverized soil/plant sample was weighed into a clean Pyrex test tube and added exactly 0.5 mL of conc. $HNO_3$, 1.00 mL of conc. HCl and 1.00 mL of 70% of $HClO_4$. Then the sample was refluxed at 170 °C for 90 min. in a temperature controlled hot plate. After that the sample was cooled and added distilled water up to 10 mL mark with shaking. Then the solution was analyzed by FAAS (Drexler et al., 2012).

In case of water sample 50 mL sample was weighed into a clean 250 mL flask and added exactly 5 mL of conc. $HNO_3$, 10 mL of conc. HCl and 10 mL of 70% of $HClO_4$. Then the sample was refluxed at 90-100 °C for 90 min. in a temperature controlled hot plate. After that the sample was cooled and added distilled water up to 100 mL mark with shaking. Then the solution was analyzed by FAAS (Drexler et al., 2012).

**Method D: HCl – $HNO_3$-$HClO_4$-HF**

A 0.20 g of pulverized soil/plant sample was weighed into a clean 100 mL Teflon beaker. Then 3 mL conc. HCl, 2 mL conc. $HNO_3$, 4 mL of conc. $HClO_4$ and 5 mL of conc. HF was added slowly in order and set to a temperature close to 200 °C and then digested to about 3 hrs. to reach dryness. After that exactly 10 mL of HCl was added and put the beaker on the hotplate to dissolve all salts by gently heating for about 10 min. Then cooled and made up to volume in a 100 mL volumetric flask with distilled water. Finally, the solution was analyzed by FAAS (SGS, n.d.).

In case of water sample 50 mL sample was weighed into a clean 200 mL Teflon beaker. Then 10 mL conc. HCl, 5 mL conc. $HNO_3$, 15 mL of conc. $HClO_4$ and 20 mL of conc. HF was added slowly in order and set to a temperature close to 100 °C and then digested to about 3 hrs. to reach dryness. After that exactly 10 mL of HCl was added and put the beaker on the hotplate to dissolve all salts by gently heating for about 10 min. Then cooled and made up to volume in a 100 mL volumetric flask with distilled water. Finally, the solution was analyzed by FAAS (SGS, n.d.).

Table 4: Comparison of Digestion Conditions for Solid vs. Water Samples

| **Method** | **Acid Composition** | **Sample Mass / Volume** | **Temp (°C)** | **Time (h)** | **HF Use** | **Final Volume** | **Notes** |
|---|---|---|---|---|---|---|---|
| A | $HCl:HNO_3$ (3:1) | 0.5 g / 50 mL | 110/100 | 3.0 | No | 50 mL / 100 mL | Cost-effective; general use |
| B | $HCl:HNO_3:H_2SO_4$ | 1.0 g / 50 mL | 200/100 | 1.0 | No | 50 mL / 100 mL | Strong oxidizing mix |
| C | $HCl:HNO_3:HClO_4$ | 0.2 g / 50 mL | 170/100 | 1.5 | No | 10 mL / 100 mL | Handles refractory organics |
| D | $HCl:HNO_3:HClO_4:HF$ | 0.2 g / 50 mL | 200/100 | 3.0 | Yes | 100 mL | Dissolves silicates; high hazard |

## 3.7. Quality Control

Quality control included:

- CRMs for accuracy verification.
- Method blanks to detect contamination.
- Spike recovery tests (acceptable range: 80–120%).AOAC International (2016) and ISO/IEC 17025 (2017)

- Duplicate analyses to evaluate precision.
- Calibration using multi-point standard curves ($R^2 \geq 0.995$).

Table 5. Linear regression coefficient ($R^2$) of the calibration curve

| **Element** | **Fe** | | **Zn** | | **Cr** | | **Pb** | | **Cu** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. | Abs. | Conc. | Abs. | Conc. | Abs. | Conc. | Abs. | Conc. | Abs. |
| | 0.0 | 0.0000 | 0.0 | 0.0000 | 0.0 | 0.0000 | 0.0 | 0.0000 | 0.0 | 0.0000 |
| | 3.0 | 0.0975 | 0.5 | 0.1103 | 0.5 | 0.0467 | 0.5 | 0.0172 | 0.5 | 0.0441 |
| | 5.0 | 0.1532 | 1.0 | 0.2057 | 1.0 | 0.0902 | 1.0 | 0.0317 | 1.0 | 0.0866 |
| | 10.0 | 0.3119 | 2.0 | 0.3691 | 2.0 | 0.1789 | 2.0 | 0.0620 | 2.0 | 0.1742 |
| $R^2$ | **0.9996** | | **0.9948** | | **0.9999** | | **0.9991** | | **1.0000** | |

| **Element** | **Cd** | | **Mn** | | **Co** | | **Ni** | |
|---|---|---|---|---|---|---|---|---|
| | Conc. | Abs. | Conc. | Abs. | Conc. | Abs. | Conc. | Abs. |
| | 0.0 | 0.0000 | 0.0 | 0.0000 | 0.0 | 0.0000 | 0.0 | 0.0000 |
| | 0.5 | 0.1472 | 0.5 | 0.0467 | 0.5 | 0.0348 | 0.5 | 0.0448 |
| | 1.0 | 0.2708 | 1.0 | 0.0902 | 1.0 | 0.0807 | 1.0 | 0.0879 |
| | 2.0 | 0.4845 | 2.0 | 0.1789 | 2.0 | 0.168 | 2.0 | 0.1709 |
| $R^2$ | **0.9942** | | **0.9999** | | **0.9979** | | **0.9997** | |

## 3.8. Method Validation

Limit of detection (LOD) and limit of quantification (LOQ) were calculated from blank measurements (Mean + 3SD for LOD; Mean + 10SD for LOQ) per IUPAC guidelines (Thompson et al., 2002).

Precision was assessed by calculating relative standard deviation (RSD) for triplicate measurements, while accuracy was confirmed by CRM recoveries.

Table 6: Calculated LOD and LOQ for the digestion methods A-D.

| **Method A** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cu** | **Zn** | **Pb** | **Ni** | **Co** | **Fe** | **Cd** | **Cr** | **Mn** |
| **N** | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| **Mean** | 0.02 | 0.02 | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 | 0.13 | 0.07 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **STDEV** | 0.013 | 0.010 | 0.006 | 0.008 | 0.010 | 0.012 | 0.010 | 0.018 | 0.031 |
| **LOD** | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.2 | 0.2 |
| **LOQ** | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.4 |

| **Method B** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cu** | **Zn** | **Pb** | **Ni** | **Co** | **Fe** | **Cd** | **Cr** | **Mn** |
| **N** | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| **Mean** | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.14 | 0.07 |
| **STDEV** | 0.011 | 0.008 | 0.004 | 0.005 | 0.008 | 0.014 | 0.007 | 0.019 | 0.032 |
| **LOD** | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.2 | 0.2 |
| **LOQ** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.3 | 0.4 |

| **Method C** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cu** | **Zn** | **Pb** | **Ni** | **Co** | **Fe** | **Cd** | **Cr** | **Mn** |
| **N** | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| **Mean** | 0.03 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 | 0.12 | 0.06 |
| **STDEV** | 0.011 | 0.008 | 0.004 | 0.005 | 0.008 | 0.014 | 0.007 | 0.019 | 0.032 |
| **LOD** | 0.06 | 0.04 | 0.03 | 0.03 | 0.05 | 0.05 | 0.04 | 0.18 | 0.15 |
| **LOQ** | 0.14 | 0.10 | 0.06 | 0.06 | 0.10 | 0.16 | 0.09 | 0.31 | 0.38 |

| **Method D** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cu** | **Zn** | **Pb** | **Ni** | **Co** | **Fe** | **Cd** | **Cr** | **Mn** |
| **N** | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| **Mean** | 0.03 | 0.02 | 0.02 | 0.02 | 0.03 | 0.01 | 0.03 | 0.13 | 0.08 |
| **STDEV** | 0.011 | 0.008 | 0.004 | 0.005 | 0.008 | 0.014 | 0.007 | 0.019 | 0.032 |
| **LOD** | 0.06 | 0.05 | 0.03 | 0.04 | 0.05 | 0.06 | 0.05 | 0.19 | 0.17 |
| **LOQ** | 0.14 | 0.10 | 0.06 | 0.07 | 0.11 | 0.16 | 0.10 | 0.32 | 0.40 |

## 3.9. The Heavy Metal Analysis

Heavy metals (Cu, Zn, Pb, Co Ni, Cr, Cd, Mn and Fe) in soil, plant and water samples were analyzed using four types of acid digestion methods and determined by Flame Atomic Absorption Spectrophotometry (FAAS) that uses acetylene as fuel with air as support at Ezana Analytical Laboratory. The instrument working condition and detection limits were shown in Table 7.

Figure 3: Sample Analysis Using FAAS

Table 7: The AAS Instrument working conditions for the determination of heavy metals

| Element | Lamp current (mA) | Wave length (nm) | Slit width (nm) | Instrument detection limit | Flame type |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| **Cu** | 4 | 324.7 | 0.5 | 0.003 | Air/$C_2H_2$ |
| **Zn** | 5 | 213.9 | 1.0 | 0.001 | Air/$C_2H_2$ |
| **Pb** | 5 | 217.0 | 1.0 | 0.010 | Air/$C_2H_2$ |
| **Co** | 7 | 240.7 | 0.2 | 0.005 | Air/$C_2H_2$ |
| **Ni** | 4 | 232.0 | 0.2 | 0.010 | Air/$C_2H_2$ |
| **Mn** | 5 | 279.5 | 0.2 | 0.002 | Air/$C_2H_2$ |
| **Cd** | 4 | 228.8 | 0.5 | 0.002 | Air/$C_2H_2$ |
| **Cr** | 7 | 357.9 | 0.2 | 0.006 | Air/$C_2H_2$ |
| **Fe** | 5 | 248.3 | 0.2 | 0.006 | Air/$C_2H_2$ |

## 3.10. Preparation of Standard Solution

The determination of a given metal concentration in the experimental solution was based on its respective calibration curve. In plotting the calibration curves Cu, Zn, Pb, Co, Ni, Cr, Cd, Mn and Fe traceable stock solution of each metal ion of (1000 ppm sigma Aldrich, Europe) was purchased and was prepared using five standard solutions. The standard solutions were prepared by serial dilution of each stock solution. Calibration curves were prepared to determine the concentration of the metals in the sample solution. Calibration curve for each metal ion to be analyzed was prepared by plotting the absorbance as a function of metal ion standard concentration. Calibration curves were drawn by plotting absorbance versus metal ion concentration.

To minimize matrix effects—particularly those arising from differences in viscosity, surface tension, and dissolved solids between calibration standards and real samples—matrix-matched calibration standards were prepared where applicable. This was achieved by adding background electrolyte concentrations similar to those found in the digested samples, following recommendations by (Skoog et al., 2017; Welz & Sperling, 2021).

## 3.11. Data Analysis

Statistical analysis was performed using one-way ANOVA to assess the differences in heavy metal concentrations obtained from the four digestion methods. Post-hoc comparisons were conducted using Tukey's HSD test to determine significant differences between the digestion methods where the ANOVA results indicated statistical significance ($p < 0.05$). Effect sizes were also calculated to assess the magnitude of the differences observed among the methods. Data were expressed as mean ± standard deviation, and a significance level of $p < 0.05$ was considered for all statistical tests (Fong et al., 2006). For this study, the significance of variation between each digestion methods were analyzed using ANOVA.

In Spike/Recovery test, known amounts from stock solution of each metal element was spiked into a samples and run in the FAAS. The percentage recovery was calculated by using the formula given below (Fong et al., 2006).

The percent recovery should be within the range of 100±20 %.

$$\% \text{ Recovery} = \frac{C\text{ (spike)} - C\text{ (no-spike)} \times 100}{C\text{ (added)}}$$

Where C (spike) is metal content of the spike sample

C (non-spike) is metal content of non-spiked sample

C (added) is metal content of metal added

# CHAPTER FOUR

## 4. RESULTS AND DISCUSSION

### 4.1. Results and Discussion of Soil Samples

The results for soil samples digested by Methods A–D (three replicates per sample type, N = 27) are summarized in Table 8

Based on the ANOVA analysis at a 95% confidence level, the P-value for all elements was below 0.05. This indicates statistically significant differences in element concentrations among the wet digestion methods for all elements tested. Practically, this means that the choice of digestion method can substantially influence the measured concentrations of trace metals in soil. For instance, methods with higher recovery efficiency for specific elements may yield results that are more representative of true concentrations, which is essential for accurate environmental risk assessments and regulatory compliance.

Table 8: Comparison of Element Concentrations in Soil Samples across Digestion Methods (A–D)

| Sample | Digestion Method | | Element Cu | Element Zn | Element Pb | Element Ni | Element Co | Element Fe | Element Cd | Element Cr | Element Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GBM 301-4 | Method A | Conc. (ppm) | 1434.28 | 420.65 | 778.95 | 1360.75 | 122.55 | 2930.04 | 9.29 | 85.76 | 393.98 |
| | | STDEV. | 41.56 | 32.45 | 46.34 | 56.53 | 4.72 | 145.59 | 4.78 | 6.25 | 11.39 |
| GBM 999-6 | Method A | Conc. (ppm) | 577.69 | 92.83 | 19.61 | 207.93 | 22.63 | 1570.66 | 20.89 | 64.30 | 415.87 |
| | | STDEV. | 14.19 | 5.20 | 7.66 | 6.21 | 2.83 | 82.43 | 2.87 | 3.65 | 9.56 |
| BM 10/318 | Method A | Conc. (ppm) | 191.06 | 194.86 | 90.19 | 387.95 | 57.49 | 907.54 | 0.76 | 510.49 | 546.49 |
| | | STDEV. | 11.96 | 21.83 | 7.05 | 17.99 | 3.64 | 34.41 | 0.14 | 30.01 | 9.07 |
| GBM 301-4 | Method B | Conc. (ppm) | 815.98 | 227.49 | 212.86 | 814.67 | 86.74 | 238.98 | 11.11 | 62.27 | 238.98 |
| | | STDEV. | 15.90 | 9.70 | 19.18 | 12.26 | 3.12 | 350.34 | 1.27 | 1.78 | 7.36 |
| GBM 999-6 | Method B | Conc. (ppm) | 337.06 | 58.86 | 0.88 | 156.63 | 38.87 | 1609.21 | 12.10 | 51.53 | 291.10 |
| | | STDEV. | 4.63 | 1.97 | 0.09 | 5.45 | 1.30 | 142.27 | 1.40 | 1.33 | 7.70 |
| BM 10/318 | Method B | Conc. (ppm) | 109.49 | 110.20 | 22.81 | 264.96 | 56.65 | 937.74 | 12.10 | 498.96 | 343.98 |
| | | STDEV. | 4.93 | 6.32 | 11.13 | 21.28 | 3.41 | 82.56 | 1.56 | 51.87 | 18.29 |
| GBM 301-4 | Method C | Conc. (ppm) | 1518.80 | 396.59 | 618.84 | 1391.21 | 131.95 | 2863.67 | 6.31 | 94.66 | 416.72 |
| | | STDEV. | 39.72 | 9.67 | 30.03 | 26.01 | 4.18 | 147.02 | 2.05 | 6.31 | 13.64 |
| GBM 999-6 | Method C | Conc. (ppm) | 589.33 | 84.94 | 12.30 | 232.63 | 26.01 | 1535.54 | 4.38 | 61.59 | 475.39 |
| | | STDEV. | 10.90 | 8.57 | 8.20 | 10.30 | 2.39 | 91.17 | 1.92 | 7.59 | 11.66 |
| BM 10/318 | Method C | Conc. (ppm) | 196.26 | 199.84 | 87.73 | 469.70 | 71.10 | 887.19 | 6.16 | 633.07 | 633.69 |
| | | STDEV. | 6.23 | 6.53 | 14.77 | 11.51 | 2.48 | 40.50 | 2.32 | 27.02 | 7.84 |
| GBM 301-4 | Method D | Conc. (ppm) | 1616.25 | 465.46 | 673.08 | 1334.41 | 161.29 | 2903.39 | 0.69 | 208.41 | 635.66 |
| | | STDEV. | 36.86 | 15.72 | 19.19 | 11.89 | 6.21 | 212.07 | 0.21 | 7.39 | 14.32 |
| GBM 999-6 | Method D | Conc. (ppm) | 637.01 | 113.10 | 9.86 | 132.87 | 39.15 | 1560.47 | 0.68 | 133.16 | 557.84 |
| | | STDEV. | 13.31 | 8.96 | 61.90 | 23.41 | 7.99 | 165.56 | 0.24 | 10.83 | 19.44 |
| BM 10/318 | Method D | Conc. (ppm) | 207.69 | 257.64 | 53.42 | 390.80 | 85.65 | 900.86 | 0.72 | 1314.89 | 863.81 |
| | | STDEV. | 13.921153 | 6.474599 | 29.50408 | 10.18443 | 4.943254 | 80.59422 | 0.296577 | 26.19766 | 18.69049 |

STDEV= Standard Deviation

### 4.1.1. Method A – Z-Score and Spiking Recovery for Soil

Table 9: Z-Score and Spiking Recovery for Method A for Soil

| | **GBM 301-4 Lab Value Method A** | **GBM 301-4 certificate Value** | **GBM 301-4 certificate SD Value** | **Z-Score** | **Decision** |
|---|---|---|---|---|---|
| | **Ppm** | **Ppm** | **ppm** | | |
| **Cu** | 1434 | 1656 | 87 | -2.55 | Pass |
| **Zn** | 421 | 448 | 32 | -0.84 | Pass |
| **Pb** | 779 | 762 | 47 | 0.36 | Pass |

| | | | | | |
|---|---|---|---|---|---|
| **Ni** | 1361 | 1430 | 77 | -0.90 | Pass |
| **Co** | 123 | 123 | 12 | 0.00 | Pass |

For GBM 301-4, all Z-scores for Cu, Zn, Pb, Ni, and Co fell within the acceptable range of −3 to +3, suggesting strong agreement with the certified reference material values. This implies that Method A is reliable for determining these elements in soil without significant systematic error.

Table 10: Spiking Recovery for Method A for Soil

| **Element** | **Non-Spiked GBM 999-6 Method A** | **Spiked GBM 999-6 Method A** | **Spiked Concentration** | **Recovery** | **Decision** |
|---|---|---|---|---|---|
| **Fe** | 1570.66 | 1625.32 | 50 | 109.32 | Pass |
| **Cd** | 20.89 | 25.66 | 5 | 95.35 | Pass |
| **Cr** | 64.30 | 74.52 | 10 | 102.24 | Pass |
| **Mn** | 415.87 | 465.45 | 50 | 99.16 | Pass |

In the spiking recovery test (GBM 999-6), recoveries for Fe, Cd, Cr, and Mn ranged from 95.35% to 109.32%, well within the 80–120% acceptance range. These results indicate that Method A is capable of efficiently extracting metals from the soil matrix without substantial loss or contamination. Its combination of good agreement and acceptable recoveries suggests it is suitable for routine soil analysis where accuracy and reproducibility are critical

### 4.1.2. Z-Score and Spiking Recovery for Method B for Soil

Table 11: Z-Score and Spiking Recovery for Method B for Soil

| | **GBM 301-4 Lab Value Method B** | **GBM 301-4 certificate Value** | **GBM 301-4 certificate SD Value** | **Z-Score** | **Decision** |
|---|---|---|---|---|---|
| | **ppm** | **Ppm** | **ppm** | | |
| **Cu** | 815.98 | 1656 | 87 | -9.66 | Fail |
| **Zn** | 227.49 | 448 | 32 | -6.89 | Fail |
| **Pb** | 212.86 | 762 | 47 | -11.68 | Fail |
| **Ni** | 814.67 | 1430 | 77 | -7.99 | Fail |

| | | | | | |
|---|---|---|---|---|---|
| **Co** | 86.74 | 123 | 12 | -3.02 | Fail |

Method B consistently failed the Z-score criteria for all five elements, with values far below −3, indicating severe underestimation of concentrations compared to certified values. This likely points to incomplete digestion, possibly due to insufficient acid strength, digestion time, or temperature

Table 12: Spiking Recovery for Method B for Soil

| **Element** | **Non-Spiked GBM 999-6 Method B** | **Spiked GBM 999-6 Method B** | **Spiked Concentration** | **Recovery** | **Decision** |
|---|---|---|---|---|---|
| | **ppm** | **Ppm** | **ppm** | | |
| **Cd** | 12.10 | 16.05 | 5.00 | 78.96 | Fail |
| **Cr** | 51.53 | 58.73 | 10.00 | 72.04 | Fail |
| **Mn** | 291.10 | 337.34 | 50.00 | 92.48 | Pass |

The spiking recovery data support this interpretation: Fe and Mn pass the recovery criteria, but Cd and Cr had recoveries below 80%, suggesting poor extraction efficiency for certain metals. These limitations mean that Method B is unsuitable for comprehensive soil analysis unless procedural adjustments—such as increased acid volume, use of stronger oxidizing agents, or extended digestion time—are implemented.

### 4.1.3. Z-Score and Spiking Recovery for Method C for Soil

Table 13: Z-Score and Spiking Recovery for Method C for Soil

| | **GBM 301-4 Lab Value Method C** | **GBM 301-4 certificate Value** | **GBM 301-4 certificate SD Value** | **Z-Score** | **Decision** |
|---|---|---|---|---|---|
| | **ppm** | **Ppm** | **Ppm** | | |
| **Cu** | 1518.8 | 1656 | 87 | -1.58 | Pass |
| **Zn** | 396.59 | 448 | 32 | -1.61 | Pass |
| **Pb** | 618.84 | 762 | 47 | -3.05 | Fail |
| **Ni** | 1391.21 | 1430 | 77 | -0.50 | Pass |
| **Co** | 131.95 | 123 | 12 | 0.75 | Pass |

Method C passes the Z-score criteria for all elements except Pb, which slightly exceeded the −3 threshold (−3.05). This suggests generally reliable performance, but with some underestimation for Pb that could be related to its partial association with resistant mineral phases.

Table 14: Spiking Recovery for Method C for Soil

| **Element** | **Non-Spiked GBM 999-6 average** | **Spiked GBM 999-6 average** | **Spiked Concentration** | **Recovery** | **Decision** |
|---|---|---|---|---|---|
| | **ppm** | **Ppm** | **Ppm** | | |
| **Cd** | 4.38 | 9.13 | 5 | 95.02 | Pass |
| **Cr** | 61.59 | 70.63 | 10 | 90.41 | Pass |
| **Mn** | 475.39 | 524.49 | 50 | 98.20 | Pass |

Spiking recoveries for all elements fell between 90.41% and 106.97%, showing consistent extraction efficiency across metals. Overall, Method C demonstrates robust performance and could be a strong alternative to Method A, particularly when rapid digestion is required, though care should be taken when analyzing Pb-rich samples.

#### 4.1.4. Z-Score and Spiking Recovery for Method D for Soil

Table 15: Z-Score and Spiking Recovery for Method D for Soil

| | **GBM 301-4 Lab Value Method D** | **GBM 301-4 certificate Value** | **GBM 301-4 certificate SD Value** | **Z-Score** | **Decision** |
|---|---|---|---|---|---|
| | **ppm** | **Ppm** | **Ppm** | | |
| **Cu** | 1616.25 | 1656 | 87 | -0.46 | Pass |
| **Zn** | 465.46 | 448 | 32 | 0.55 | Pass |
| **Pb** | 673.08 | 762 | 47 | -1.89 | Pass |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| **Ni** | 1334.41 | 1430 | 77 | -1.24 | Pass |
| **Co** | 161.29 | 123 | 12 | 3.19 | Fail |

Method D showed acceptable Z-scores for Cu, Zn, Pb, and Ni, but failed for Co (Z-score = 3.19), indicating overestimation. Over-recovery for Co could be due to contamination from reagents or glassware or matrix effects that enhance its apparent concentration.

Table 16: Spiking Recovery for Method D for Soil

| **Element** | **Non-Spiked GBM 999-6 average** | **Spiked GBM 999-6 average** | **Spiked Concentration** | **Recovery** | **Decision** |
|---|---|---|---|---|---|
| **Fe** | 1560.47 | 1608.23 | 50 | 91.43 | Pass |
| **Cd** | 0.72 | 5.29 | 5 | 91.43 | Pass |
| **Cr** | 1314.89 | 1323.75 | 10 | 88.67 | Pass |
| **Mn** | 863.81 | 912.81 | 50 | 98.00 | Pass |

Spiking recoveries for Fe, Cd, Cr, and Mn were within the acceptance range, indicating good overall efficiency. However, the Co overestimation highlights the need for improved contamination control or method-specific calibration when analyzing this element.

The comparative analysis of the four digestion methods revealed notable differences in recovery efficiency, precision, and overall analytical performance for the nine target metals (Cu, Zn, Pb, Co, Fe, Cr, Cd, Mn, and Ni) in soil matrices. Method A (HCl–$HNO_3$, 3:1 aqua regia) consistently yielded high recovery values within the acceptable range of 80–120% (AOAC, (2016)) with low relative standard deviations (RSDs), indicating both accuracy and repeatability. Aqua regia's efficiency can be attributed to its strong oxidative power, which dissolves most metal-containing minerals and effectively digests organic matter, thus releasing metals into solution (Sastre et al., 2002).

Method C (HCl–$HNO_3$–$HClO_4$) also performed well, with comparable recovery for most elements. The inclusion of perchloric acid likely enhanced oxidation of refractory organic compounds, improving metal release from complex matrices. However, its handling risks and extended digestion time make it less practical in routine monitoring, especially in resource-limited laboratories (Chen et al., 2001).

Method D (HCl–$HNO_3$–$HClO_4$–HF) achieved the highest extraction efficiency for silicate-bound metals such as Cr and Ni, in line with previous findings that HF is highly effective in breaking down silicate minerals (US EPA, 1996). However, its poor performance for cobalt suggests potential matrix interference or volatilization loss during digestion. Additionally, HF's extreme hazard profile, costly waste disposal requirements, and need for specialized safety infrastructure limit its widespread application in Ethiopian laboratories.

Method B ($HCl–HNO_3–H_2SO_4$) demonstrated the lowest and most inconsistent recovery rates across elements. Sulfuric acid's strong dehydrating properties may cause incomplete digestion of silicate-rich soils, leading to underestimation of certain metals (Chatterjee & Das, 1995). This limitation has been reported in other method-comparison studies, suggesting that $H_2SO_4$-based mixtures are less suitable for trace metal analysis in mineral-rich soils.

From a regulatory perspective, underestimation of metals like Pb or Cd could result in samples being classified as safe when they exceed permissible limits, while overestimation could lead to unnecessary remediation costs. Therefore, accurate digestion is critical for reliable environmental assessments, particularly in mining-affected regions of Tigray where soil contamination is a potential public health concern (Gebrekidan et al., 2013). The findings support prioritizing Method A for routine monitoring due to its balance of accuracy, safety, and cost-effectiveness, while Method D may be reserved for specialized mineralogical investigations.

#### 4.1.5. Practical Implication for Soil Samples:

Across the four methods, Method A demonstrated the most consistent and accurate performance, followed closely by Method C. Method D was generally acceptable for most elements but showed notable inaccuracy for cobalt (Co), while Method B was unreliable for the majority of analytes. From a practical standpoint, laboratories should prioritize Method A or Method C for multi-element soil analysis, as these offer a favorable balance between analytical accuracy, operational efficiency, and cost-effectiveness. Method A is particularly advantageous in resource-limited settings due to its simpler procedure, lower reagent costs, and reduced safety hazards. Method C, while slightly more complex, provides comparable accuracy and may be preferred when laboratory infrastructure and trained personnel are available. Method D's higher extraction efficiency for certain refractory metals may be valuable in specialized applications, but its use of hydrofluoric acid (HF) necessitates strict safety protocols and specialized waste disposal facilities. Further research is recommended to optimize Method B's performance and to investigate alternative approaches that could mitigate the Co contamination observed in Method D.

## 4.2. Results and Discussion of Plant Sample

For plant samples (n = 27, three replicates per digestion method), ANOVA results showed P-values < 0.05 for all elements (Table 17). This confirms statistically significant differences in metal recoveries among the four digestion methods. Beyond statistical significance, the magnitude of differences was notable: Method A and C consistently showed mean recoveries within 90–110% for most elements, while Method B frequently fell below 80%, and Method D showed element-specific deviations. These results underscore the critical importance of selecting an appropriate digestion method, as the choice directly affects how plant uptake and potential food safety risks are interpreted.

Note: “Spiked Concentration” refers to the known concentration of each element added to the cabbage sample (mg/kg), and recoveries are expressed as percentages of this expected value.

**Table 17: Comparison of Element Concentrations in Plant Samples Across Digestion Methods (A–D)**

| Sample | Digestion Method | | Element Cu | Element Zn | Element Pb | Element Ni | Element Co | Element Fe | Element Cd | Element Cr | Element Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Onion | Method A | Conc. (ppm) | 10.21 | 32.07 | 1.23 | 7.65 | 0.30 | 74.97 | 0.80 | 0.82 | 17.48 |
| | | STDEV. | 1.88 | 1.94 | 0.16 | 3.90 | 0.10 | 9.15 | 0.13 | 0.12 | 1.48 |
| Cabbage | Method A | Conc. (ppm) | 9.52 | 34.31 | 1.34 | 16.45 | 0.42 | 297.27 | 2.55 | 0.80 | 41.29 |
| | | STDEV. | 2.60 | 11.17 | 0.37 | 5.73 | 0.13 | 17.69 | 0.34 | 0.12 | 1.91 |
| Lettuce | Method A | Conc. (ppm) | 9.25 | 34.29 | 1.11 | 10.05 | 0.52 | 291.30 | 2.20 | 0.85 | 43.61 |
| | | STDEV. | 1.69 | 5.98 | 0.27 | 1.76 | 0.21 | 21.88 | 1.36 | 0.10 | |
| Onion | Method B | Conc. (ppm) | 20.93 | 20.93 | 0.95 | 23.66 | 24.19 | 194.77 | 10.06 | 1.71 | 10.79 |
| | | STDEV. | 1.82 | 1.70 | 0.39 | 6.17 | 1.95 | 27.31 | 1.39 | 0.69 | 1.99 |
| Cabbage | Method B | Conc. (ppm) | 2.92 | 19.84 | 1.13 | 15.80 | 23.51 | 171.21 | 9.34 | 2.13 | 23.11 |
| | | STDEV. | 0.98 | 1.44 | 0.55 | 6.54 | 2.37 | 12.79 | 1.87 | 0.73 | 2.12 |
| Lettuce | Method B | Conc. (ppm) | 6.81 | 24.87 | 1.04 | 18.44 | 23.65 | 173.07 | 10.53 | 1.92 | 25.84 |
| | | STDEV. | 2.67 | 2.07 | 0.32 | 5.92 | 1.06 | 19.64 | 2.13 | 0.59 | 1.50 |
| Onion | Method C | Conc. (ppm) | 3.01 | 20.20 | 0.32 | 7.09 | 3.13 | 232.23 | 3.80 | 0.69 | 19.21 |
| | | STDEV. | 0.54 | 3.26 | 0.10 | 1.38 | 0.99 | 21.04 | 1.20 | 0.13 | 2.69 |
| Cabbage | Method C | Conc. (ppm) | 2.87 | 39.07 | 0.46 | 19.01 | 5.89 | 277.03 | 5.72 | 0.89 | 52.05 |
| | | STDEV. | 0.60 | 2.36 | 0.16 | 4.93 | 2.62 | 20.16 | 1.48 | 0.07 | 3.12 |
| Lettuce | Method C | Conc. (ppm) | 10.17 | 43.84 | 0.81 | 18.69 | 4.37 | 339.13 | 5.31 | 0.85 | 52.15 |
| | | STDEV. | 2.16 | 2.79 | 0.12 | 4.65 | 1.33 | 14.50 | 1.35 | 0.17 | 3.70 |
| Onion | Method D | Conc. (ppm) | 13.25 | 23.16 | 0.02 | 0.02 | 3.54 | 470.89 | 1.24 | 0.92 | 15.32 |
| | | STDEV. | 2.45 | 5.10 | 0.01 | 0.01 | 0.73 | 67.92 | 0.36 | 0.06 | 2.54 |
| Cabbage | Method D | Conc. (ppm) | 14.33 | 49.52 | 0.02 | 0.02 | 7.23 | 366.21 | 5.16 | 0.92 | 39.14 |
| | | STDEV. | 2.86 | 3.31 | 0.01 | 0.01 | 1.84 | 54.83 | 1.51 | 0.07 | 4.06 |
| Lettuce | Method D | Conc. (ppm) | 24.45 | 61.70 | 0.02 | 0.02 | 5.91 | 467.11 | 4.62 | 0.84 | 45.44 |
| | | STDEV. | 2.111029 | 3.572981 | 0.011278 | 0.011494 | 1.871253 | 38.72888 | 1.622403 | 0.056227 | 3.424128 |

STDEV= Standard Deviation

### 4.2.1. Spiking Recovery for Method A for Plant

Table 18: Spiking Recovery for Method A for Plant

| Element | Non-Spiked Cabbage | Spiked Cabbage | Spiked Concentration | Recovery | Decision |
|---|---|---|---|---|---|
| | ppm | ppm | ppm | | |
| **Cu** | 9.52 | 14.67 | 5.00 | 103.02 | Pass |
| **Zn** | 34.31 | 39.44 | 5.00 | 102.46 | Pass |
| **Pb** | 1.34 | 3.45 | 2.00 | 105.26 | Pass |
| **Ni** | 16.45 | 20.69 | 5.00 | 84.85 | Pass |
| **Co** | 0.42 | 2.43 | 2.00 | 100.55 | Pass |
| **Fe** | 297.27 | 341.01 | 50.00 | 87.47 | Pass |
| **Cd** | 2.55 | 4.64 | 2.00 | 104.74 | Pass |
| **Cr** | 0.80 | 2.65 | 2.00 | 92.83 | Pass |
| **Mn** | 41.29 | 45.51 | 5.00 | 84.33 | Pass |

**Note: Spiked concentration** refers to the known amount added during method validation

Method A yielded recoveries between 84–105% for all nine tested elements (Table 18), all within the acceptance range of 80–120%. This demonstrates strong accuracy and reproducibility, consistent with other studies reporting aqua regia as a reliable digestion mixture for plant matrices (Sastre et al., 2002). Minor under-recovery for Fe and Mn (~85–87%) may reflect incomplete release of metals bound to lignin-rich tissue. Overall, Method A is highly suitable for multi-element analysis of plant tissues.

### 4.2.2. Spiking Recovery for Method B for Plant

Table 19: Spiking Recovery for Method B for Plant

| Element | Non-Spiked Cabbage | Spiked Cabbage | Spiked Concentration | Recovery (%) | Decision |
|---|---|---|---|---|---|
| | Ppm | ppm | ppm | | |
| **Cu** | 2.92 | 7.07 | 5.00 | 83.06 | Pass |
| **Zn** | 19.84 | 23.55 | 5.00 | 74.09 | Fail |
| **Pb** | 1.13 | 2.73 | 2.00 | 80.31 | Pass |
| **Ni** | 15.80 | 19.59 | 5.00 | 75.95 | Fail |
| **Co** | 23.51 | 25.05 | 2.00 | 76.62 | Fail |
| **Fe** | 171.21 | 217.08 | 50.00 | 91.74 | Pass |
| **Cd** | 9.34 | 10.60 | 2.00 | 62.85 | Fail |
| **Cr** | 2.13 | 3.69 | 2.00 | 77.84 | Fail |
| **Mn** | 23.11 | 26.49 | 5.00 | 67.60 | Fail |

**Note: Spiked concentration** refers to the known amount added during method validation

Method B underperformed significantly, with poor recoveries (<80%) for Zn, Ni, Co, Cd, Cr, and Mn (Table 19). Only Cu, Pb, and Fe achieved acceptable values. The poor efficiency is likely linked to sulfuric acid’s dehydrating nature, which can carbonize organic matter and hinder complete digestion (Chatterjee & Das, 1995). This limitation makes Method B unsuitable for trace metal determination in plants, as it risks systematic underestimation of essential or toxic elements, potentially leading to misleading conclusions in food safety assessments.

### 4.2.3. Spiking Recovery for Method C for Plant

Table 20: Spiking Recovery for Method C for Plant

| **Element** | **Non-Spiked Cabbage** | **Spiked Cabbage** | **Spiked Concentration** | **Recovery (%)** | **Decision** |
|---|---|---|---|---|---|
| | **ppm** | **Ppm** | **ppm** | | |
| **Cu** | 2.87 | 7.87 | 5.00 | 99.99 | Pass |
| **Zn** | 39.07 | 44.69 | 5.00 | 112.41 | Pass |
| **Pb** | 0.46 | 2.50 | 2.00 | 101.96 | Pass |
| **Ni** | 19.01 | 23.35 | 5.00 | 86.90 | Pass |
| **Co** | 5.89 | 7.76 | 2.00 | 93.28 | Pass |
| **Fe** | 277.03 | 324.85 | 50.00 | 95.63 | Pass |
| **Cd** | 5.72 | 7.85 | 2.00 | 106.36 | Pass |
| **Cr** | 0.89 | 2.91 | 2.00 | 100.95 | Pass |
| **Mn** | 52.05 | 56.75 | 5.00 | 93.92 | Pass |

**Note: Spiked concentration** refers to the known amount added during method validation

Method C demonstrated robust performance with recoveries between 87–112% for all analytes (Table 20). Slight over-recovery of Zn (112%) may reflect analytical interference or matrix effects, while Ni's lower recovery (86.9%) suggests incomplete digestion of Ni-containing plant compounds. The inclusion of perchloric acid likely enhanced oxidation of organic matter, explaining its generally good performance. Method C is thus comparable to Method A and can be recommended where additional oxidation strength is required, though safety concerns associated with perchloric acid must be considered (Chen et al., 2001).

### 4.2.4. Spiking Recovery for Method D for Plant

Table 21: Spiking Recovery for Method D for plant

| **Element** | **Non-Spiked Cabbage (mg/kg)** | **Spiked Cabbage (mg/kg)** | **Spiked Concentration (mg/kg)** | **Recovery (%)** | **Decision** |
|---|---|---|---|---|---|
| | **ppm** | **Ppm** | **ppm** | | |
| **Cu** | 14.33 | 18.70 | 5.00 | 87.52 | Pass |
| **Zn** | 49.52 | 53.94 | 5.00 | 88.52 | Pass |
| **Pb** | 0.02 | 1.93 | 2.00 | 95.37 | Pass |
| **Ni** | 0.02 | 4.33 | 5.00 | 86.21 | Pass |
| **Co** | 7.23 | 8.80 | 2.00 | 78.65 | Fail |
| **Fe** | 366.21 | 414.04 | 50.00 | 95.67 | Pass |
| **Cd** | 5.16 | 6.94 | 2.00 | 88.83 | Pass |
| **Cr** | 0.92 | 2.55 | 2.00 | 81.70 | Pass |
| **Mn** | 39.14 | 43.34 | 5.00 | 83.89 | Pass |

**Note: Spiked concentration** refers to the known amount added during method validation

Method D achieved recoveries within the acceptance range for most elements but failed for Co (78.6%) (Table 21). This under-recovery may indicate volatilization losses or incomplete stabilization during digestion. Despite strong results for Fe, Cr, and Pb, the inconsistency with Co reduces its reliability. Additionally, the use of HF raises major safety and cost concerns, limiting routine applicability despite its potential for breaking down silicate-bound fractions.

### Comparative Summary of Plant Sample Digestion

Best performers: Methods A and C, both showing consistent recoveries across all metals with acceptable accuracy and precision.

Weakest performer: Method B, which failed for most elements due to incomplete digestion, making it unsuitable for reliable plant metal analysis. Because, $H_2SO_4$ can cause the formation of insoluble metal sulfates (e.g., $PbSO_4$, $CaSO_4$, $BaSO_4$) and these precipitates reduce solubility and limit detection during subsequent AAS analysis.

Conditional performer: Method D, generally acceptable but problematic for Co, and constrained by safety/environmental hazards of HF.

### Practical and Scientific Implications

Differences in recovery directly affect how plant metal uptake is interpreted, since underestimation (as seen in Method B) could mask excessive accumulation of toxic metals such as Cd or Pb, creating false reassurance in food safety monitoring, while overestimation (for example, Zn in Method C) may exaggerate contamination risks and lead to unnecessary regulatory or remediation actions; this is particularly critical for Ethiopia and the Tigray region, where leafy vegetables are a dietary staple often cultivated near mining areas, making reliable digestion methods essential for safeguarding public health.

Based on these findings, Methods A and C are recommended for routine plant analysis in Ezana Mining's laboratory, as they balance accuracy, safety, and feasibility. Further optimization may be needed to address Co losses in Method D and to explore whether Method B can be improved by modifying acid ratios or digestion conditions.

## 4.3. Results and Discussion of Water Sample

The results of water samples for each digestion methods (Method A-D) for three kinds of samples each are summarized in table. The results are average results for N=27. Based on the ANOVA analysis at 95% confidence interval for the digestion methods, the P value for all elements is below 0.05. This indicates there are significant differences in element concentrations among the wet digestion methods for all elements.

Table 22: The results of water samples for each digestion methods (Method A-D)

| Sample | Digestion Method | | Element Cu | Element Zn | Element Pb | Element Ni | Element Co | Element Fe | Element Cd | Element Cr | Element Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample 1 | Method A | Conc. (ppm) | 0.13 | 0.39 | 0.03 | 0.03 | 0.01 | 12.56 | 0.89 | 1.54 | 1.13 |
| | | STDEV. | 0.02 | 0.04 | 0.02 | 0.01 | 0.00 | 0.68 | 0.10 | 0.20 | 0.05 |
| Sample 2 | Method A | Conc. (ppm) | 0.11 | 1.00 | 0.06 | 0.05 | 0.05 | 14.98 | 1.17 | 23.38 | 3.18 |
| | | STDEV. | 0.02 | 0.25 | 0.02 | 0.01 | 0.01 | 0.55 | 0.10 | 3.23 | 0.13 |
| Sample 3 | Method A | Conc. (ppm) | 0.11 | 0.71 | 0.08 | 0.02 | 0.02 | 7.60 | 1.29 | 0.57 | 0.01 |
| | | STDEV. | 0.01 | 0.08 | 0.02 | 0.00 | 0.00 | 0.80 | 0.11 | 0.11 | 0.01 |
| Sample 1 | Method B | Conc. (ppm) | 0.04 | 0.53 | 0.96 | 0.02 | 0.02 | 8.52 | 0.84 | 1.23 | 1.06 |
| | | STDEV. | 0.01 | 0.65 | 0.24 | 0.01 | 0.00 | 0.86 | 0.04 | 0.25 | 0.04 |
| Sample 2 | Method B | Conc. (ppm) | 0.08 | 0.69 | 0.94 | 0.34 | 0.02 | 10.48 | 0.95 | 24.23 | 2.11 |
| | | STDEV. | 0.02 | 0.10 | 0.13 | 0.04 | 0.00 | 0.75 | 0.04 | 1.42 | 0.08 |
| Sample 3 | Method B | Conc. (ppm) | 0.04 | 0.14 | 0.95 | 0.02 | 0.03 | 2.11 | 0.96 | 0.80 | 0.17 |
| | | STDEV. | 0.01 | 0.05 | 0.17 | 0.00 | 0.00 | 0.43 | 0.05 | 0.08 | 0.03 |
| Sample 1 | Method C | Conc. (ppm) | 0.31 | 1.22 | 9.94 | 0.02 | 0.02 | 17.38 | 0.88 | 6.36 | 2.51 |
| | | STDEV. | 0.18 | 0.06 | 0.73 | 0.01 | 0.00 | 1.73 | 0.03 | 0.44 | 0.21 |
| Sample 2 | Method C | Conc. (ppm) | 0.27 | 2.36 | 10.72 | 0.37 | 0.04 | 15.03 | 1.09 | 23.13 | 6.00 |
| | | STDEV. | 0.12 | 0.27 | 0.32 | 0.05 | 0.01 | 1.37 | 0.04 | 0.94 | 0.31 |
| Sample 3 | Method C | Conc. (ppm) | 0.19 | 0.42 | 10.16 | 0.01 | 0.02 | 8.15 | 1.00 | 0.17 | 0.02 |
| | | STDEV. | 0.06 | 0.14 | 0.45 | 0.00 | 0.00 | 1.09 | 0.05 | 0.03 | 0.00 |
| Sample 1 | Method D | Conc. (ppm) | 5.96 | 0.96 | 1.18 | 10.20 | 0.03 | 0.14 | 13.86 | 0.02 | 2.90 |
| | | STDEV. | 1.03 | 0.19 | 0.04 | 1.15 | 0.01 | 0.03 | 1.04 | 0.00 | 0.07 |
| Sample 2 | Method D | Conc. (ppm) | 21.83 | 2.18 | 1.25 | 9.87 | 0.04 | 0.13 | 12.32 | 0.02 | 6.08 |
| | | STDEV. | 1.24 | 0.09 | 0.04 | 0.45 | 0.01 | 0.03 | 0.53 | 0.00 | 0.23 |
| Sample 3 | Method D | Conc. (ppm) | 0.65 | 0.48 | 1.29 | 8.68 | 0.01 | 0.15 | 15.58 | 0.03 | 0.06 |
| | | STDEV. | 0.09 | 0.08 | 0.05 | 0.86 | 0.00 | 0.01 | 0.83 | 0.00 | 0.03 |

### 4.3.1. Spiking Recovery for Method A for Water

Table 23: Spiking Recovery for Method A for Water

| Element | Non-Spiked Water-2 (ppm) | Spiked Water-2 (ppm) | Spiked Concentration (ppm) | Recovery (%) | Decision |
|---|---|---|---|---|---|
| **Cu** | 0.11 | 1.98 | 2 | 93.50 | Pass |
| **Zn** | 1 | 2.73 | 2 | 86.50 | Pass |
| **Pb** | 0.06 | 1.98 | 2 | 96.00 | Pass |
| **Ni** | 0.05 | 2.14 | 2 | 104.50 | Pass |
| **Co** | 0.05 | 1.99 | 2 | 97.00 | Pass |
| **Fe** | 14.98 | 18.45 | 5 | 69.40 | Fail |
| **Cd** | 1.17 | 2.99 | 2 | 91.00 | Pass |
| **Cr** | 23.38 | 28.96 | 5 | 111.60 | Pass |
| **Mn** | 3.18 | 4.94 | 2 | 88.00 | Pass |

Based on the provided data, the recovery acceptable range of 80-120%, we can draw the following conclusion:

The Element Concentration Recovery in Water-2:

1. The spiked concentrations for copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), cobalt (Co), cadmium (Cd), chromium (Cr), and manganese (Mn) all have recovery percentages that

fall within the acceptable range of 80-120%. The recovery percentages for these elements are 93.50%, 86.50%, 96.00%, 104.50%, 97.00%, 91.00%, 111.60%, and 88.00%, respectively.

2. The spiked concentration for iron (Fe) has a recovery percentage that falls below the acceptable range of 80-120%. The recovery percentage for iron is 69.40%.

   In summary, based on the recovery acceptable range of 80-120%, we can conclude that the spiked concentrations for copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), cobalt (Co), cadmium (Cd), chromium (Cr), and manganese (Mn) in Water-2 meet the predefined acceptance criteria. However, the spiked concentration for iron (Fe) fails to meet the criteria. This could be likely due to incomplete dissolution of iron oxides or silicate-bound Fe, precipitation of $Fe(OH)_3$ during dilution, and possible analytical interferences during AAS measurement. These factors collectively lead to systematic underestimation of Fe concentration in the spiked water sample.

### 4.3.2. Spiking Recovery for Method B for Water

Table 24: Spiking Recovery for Method B for Water

| **Element** | **Non-Spiked Water-2 (ppm)** | **Spiked Water-2 (ppm)** | **Spiked Concentration (ppm)** | **Recovery (%)** | **Decision** |
|---|---|---|---|---|---|
| **Cu** | 0.08 | 0.95 | 2 | 43.50 | Fail |
| **Zn** | 0.69 | 1.93 | 2 | 62.00 | Fail |
| **Pb** | 0.94 | 3.4 | 2 | 123.00 | Fail |
| **Ni** | 0.34 | 1.93 | 2 | 79.50 | Fail |
| **Co** | 0.02 | 1.85 | 2 | 91.50 | Pass |
| **Fe** | 10.48 | 14 | 5 | 70.40 | Fail |
| **Cd** | 0.95 | 3.07 | 2 | 106.00 | Pass |
| **Cr** | 24.23 | 28.24 | 5 | 80.20 | Pass |
| **Mn** | 2.11 | 3.9 | 2 | 89.50 | Pass |

Based on the provided data, the recovery acceptable range of 80-120%, we can draw the following conclusion:

For the Element Concentration Recovery in Water-2:

Cu, Zn, Pb, Ni, and Fe: The spiked concentrations for copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), and iron (Fe) all have recovery percentages that fall below the acceptable range of 80-120%. The recovery percentages for these elements are 43.50%, 62.00%, 123.00%, 79.50%, and 70.40%, respectively.

Co, Cd, Cr, and Mn: The spiked concentrations for cobalt (Co), cadmium (Cd), chromium (Cr), and manganese (Mn) all have recovery percentages that fall within the acceptable range of 80-120%. The recovery percentages for these elements are 91.50%, 106.00%, 80.20%, and 89.50%, respectively.

### Implications for Food Safety and Environmental Monitoring

Accurate determination of heavy metals in plant tissues is critical for assessing dietary exposure risks and compliance with food safety standards. Under-recovery of toxic metals like Pb or Cd could underestimate contamination levels, potentially allowing unsafe food products to enter the market. Conversely, overestimation of micronutrients such as Zn or Fe could lead to unnecessary agricultural interventions. In Tigray, where subsistence farming and mining coexist, reliable plant metal data are essential for guiding both agricultural practices and public health decisions.

### Final Synthesis

Based on recovery, reproducibility, cost, and safety considerations, Method A is the most suitable for routine plant metal monitoring, especially in resource-limited laboratory settings. Method C offers a viable alternative when high levels of recalcitrant organic matter are expected, provided adequate safety measures are in place. Method D should be reserved for specialized analysis requiring maximum extraction of silica-bound metals, while Method B is not recommended due to consistently low recoveries. Future research could focus on optimizing Method B’s oxidation efficiency or investigating ways to improve Co recovery in Method D.

### 4.3.3. Spiking Recovery for Method C for Water

Table 25: Spiking Recovery for Method C for Water

| **Element** | **Non-Spiked Water-2 (ppm)** | **Spiked Water-2 (ppm)** | **Spiked Concentration (ppm)** | **Recovery (%)** | **Decision** |
|---|---|---|---|---|---|
| **Cu** | 0.27 | 1.85 | 2 | 79.00 | Fail |
| **Zn** | 2.36 | 4.38 | 2 | 101.00 | Pass |
| **Pb** | 10.72 | 11.87 | 2 | 57.50 | Fail |
| **Ni** | 0.37 | 2.00 | 2 | 81.50 | Pass |
| **Co** | 0.04 | 2.45 | 2 | 120.50 | Fail |
| **Fe** | 15.03 | 20.29 | 5 | 105.20 | Pass |
| **Cd** | 1.09 | 2.96 | 2 | 93.50 | Pass |
| **Cr** | 23.13 | 28.15 | 5 | 100.40 | Pass |
| **Mn** | 6.00 | 8.12 | 2 | 106.00 | Pass |

Based on the provided data, the recovery acceptable range of 80-120%, we can draw the following conclusion:
For the Element Concentration Recovery in Water-2:

1. The spiked concentrations for copper (Cu), lead (Pb) and cobalt (Co) have recovery percentages that fall below and above the acceptable range of 80-120%. The recovery percentages for these elements are 79.00%, 57.50% and 120.50% respectively.

2. The spiked concentrations for zinc (Zn), nickel (Ni), iron (Fe), cadmium (Cd), chromium (Cr), and manganese (Mn) all have recovery percentages that fall within the acceptable range of 80-120%. The recovery percentages for these elements are 101.00%, 81.50%, 105.20%, 93.50%, 100.40%, and 106.00%, respectively.

In summary, based on the recovery acceptable range of 80-120%, we can conclude that the spiked concentrations for zinc (Zn), nickel (Ni), iron (Fe), cadmium (Cd), chromium (Cr), and manganese (Mn) in Water-2 meet the predefined acceptance criteria. However, the spiked concentrations for copper (Cu), cobalt (Co) and lead (Pb) fail to meet the criteria.

#### 4.3.4. Spiking Recovery for Method D for Water

Table 26: Spiking Recovery for Method D for Water

| **Element** | **Non-Spiked Water-2 (ppm)** | **Spiked Water-2 (ppm)** | **Spiked Concentration (ppm)** | **Recovery (%)** | **Decision** |
|---|---|---|---|---|---|
| **Cu** | 21.83 | 26.35 | 5 | 90.40 | Pass |
| **Zn** | 2.18 | 3.32 | 2 | 57.00 | Fail |
| **Pb** | 1.25 | 3.02 | 2 | 88.50 | Pass |
| **Ni** | 9.87 | 12.93 | 2 | 153.00 | Fail |
| **Co** | 0.04 | 1.78 | 2 | 87.00 | Pass |
| **Fe** | 0.13 | 1.79 | 2 | 83.00 | Pass |
| **Cd** | 12.32 | 16.99 | 5 | 93.40 | Pass |
| **Cr** | 0.02 | 2.24 | 2 | 111.00 | Pass |
| **Mn** | 6.08 | 8.12 | 2 | 102.00 | Pass |

Based on the provided data, the recovery acceptable range of 80-120%, we can draw the following conclusion:
For the Element Concentration Recovery in Water-2:

1. The spiked concentration for zinc (Zn) and nickel (Ni) has a recovery percentage that falls below and above the acceptable range of 80-120%. The recovery percentage for zinc is 57.00% and for nickel is 153.00%. The low Zn recovery likely results from volatilization loss, adsorption, or matrix suppression during digestion and measurement, whereas the high Ni recovery likely arises from contamination or spectral interference, leading to overestimation.
2. The spiked concentrations for copper (Cu), lead (Pb), cobalt (Co), iron (Fe), cadmium (Cd), chromium (Cr), and manganese (Mn) all have recovery percentages that fall within the acceptable range of 80-120%. The recovery percentages for these elements are 90.40%, 88.50%, 87.00%, 83.00%, 93.40%, 111.00%, and 102.00%, respectively.

In summary, based on the recovery acceptable range of 80-120%, we can conclude that the spiked concentrations for copper (Cu), lead (Pb), nickel (Ni), cobalt (Co), iron (Fe), cadmium (Cd), chromium (Cr), and manganese (Mn) in Water-2 meet the predefined acceptance criteria. However, the spiked concentration for zinc (Zn) fails to meet the criteria.

#### 4.3.5. Comparative Performance and Overall Assessment for Water Samples

Across the four digestion methods, clear performance differences were observed:

Method A (Aqua regia, $HCl–HNO_3$ 3:1): Showed the most balanced performance with recoveries for most elements within 80–120%, except Fe (69%). Its efficiency, moderate safety requirements, and relatively low cost make it the most practical choice for routine water analysis in resource-limited laboratories.

Method B ($HCl–HNO_3–H_2SO_4$): Severely underperformed for several metals (Cu, Zn, Ni, Fe), and Pb exceeded 120%, indicating poor reliability due to incomplete oxidation and possible sulfate precipitation. Method B is therefore not recommended.

Method C ($HCl–HNO_3–HClO_4$): Performed acceptably for most elements but showed critical deviations for Pb (57%) and Co (120%). While perchloric acid improves oxidation, these inconsistencies limit its application for water monitoring.

Method D ($HCl–HNO_3–HClO_4–HF$): Achieved strong recoveries for many elements but showed unacceptable values for Zn (57%) and Ni (153%). The hazards and costs associated with HF, combined with recovery inconsistencies, limit its routine applicability.

Best overall method(s):
Method A emerges as the most reliable and practical digestion approach for water sample analysis, balancing accuracy, reproducibility, cost, and safety. Method C may serve as an alternative when additional oxidation capacity is required, provided adequate safety protocols exist.

#### 4.3.6. Limitations of the Study

Several limitations should be acknowledged:

- Matrix complexity: Water samples with high organic or mineral content may interact differently with acids, affecting recovery efficiency.
- Potential interferences: Contamination during digestion (especially with HF and perchloric acid) may bias results for sensitive elements like Co and Ni.
- Instrument sensitivity: Atomic Absorption Spectroscopy (AAS) has detection limits that may challenge ultra-trace measurements in clean water samples. Coupling with ICP-MS or ICP-OES in future studies could provide more robust validation.
- Reproducibility: While recoveries were generally within 80–120%, relative standard deviations (RSDs) should be further emphasized to complement accuracy.

# CHAPTER FIVE

## 5. CONCLUSION AND RECOMMENDATION

### 5.1. Conclusion

This study evaluated four acid digestion methods (A–D) for the determination of heavy metals in soil, plant, and water samples. The findings demonstrate that no single method achieved universally optimal performance across all elements and matrices.

Method A ($HCl–HNO_3$, 3:1 aqua regia): Provided recoveries generally within the acceptable range of 80–120% for most analytes across matrices, with precision typically <10% RSD. However, Fe showed consistently low recoveries (e.g., 69% in water), and Pb occasionally exceeded acceptable thresholds. Despite these limitations, Method A remains the most practical choice for routine monitoring due to its simplicity, cost-effectiveness, and relatively low safety risks.

Method B ($HCl–HNO_3–H_2SO_4$): Consistently underperformed, with several elements (Cu, Zn, Ni, Fe) showing recoveries well below 80%, likely due to incomplete oxidation or sulfate precipitation. Its poor reproducibility and limited applicability make it unsuitable for reliable environmental analysis.

Method C ($HCl–HNO_3–HClO_4$): Showed intermediate performance, with generally acceptable recoveries but occasional failures for Pb (57%) and Co (120%). While perchloric acid improved oxidation efficiency, safety risks and inconsistent results limit its routine use.

Method D ($HCl–HNO_3–HClO_4–HF$): Achieved the highest recoveries for refractory and silicate-bound elements (e.g., Cr, Ni, Fe), but showed unacceptable values for Zn (57%) and Ni (153%) in some water samples. The use of HF poses severe risks (e.g., severe burns, systemic toxicity, and regulatory restrictions), making this method suitable only for specialized applications in well-equipped laboratories.

Overall, Method A offers the best balance of accuracy, precision, cost, and safety for most environmental monitoring tasks, while Method D provides superior recovery for challenging matrices but at significant safety and cost trade-offs.

## 5.2. Recommendation

- Routine Monitoring: Method A is recommended for general laboratory use and large-scale surveys in soil, water, and plant matrices. It is especially suitable for laboratories with limited infrastructure, providing recoveries of 85–110% for most elements with acceptable reproducibility.
- Specialized Applications: Method D should only be applied when complete digestion of refractory or silicate-bound metals (e.g., Cr, Ni, Fe) is required for regulatory

compliance or advanced mineralogical studies. Use of this method must strictly adhere to HF handling regulations, including fume hoods, protective gear, and emergency protocols.

- Avoiding Method B: Given its consistently poor recoveries, Method B is not recommended for environmental analysis.
- Method C: While less reliable than Method A, Method C may be useful in cases where additional oxidation of organic matrices is required, provided safety risks are managed.
- Limitations of the Study: This study was limited by the use of AAS, which has higher detection limits than ICP-MS/OES, and by the absence of certified water and plant CRMs for all elements. Matrix effects and interferences may also have influenced recoveries.
- Future Research: Future work should explore safer alternatives to HF for silicate-bound metal digestion, assess matrix-matched calibration strategies to reduce interferences, and investigate the reproducibility of recoveries (e.g., through multi-laboratory validation). Graphical visualization of recoveries (e.g., bar charts) could also improve comparative clarity in future reporting.

## References

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