



**MEKELLE UNIVERSITY**  
**COLLEGE OF VETERINARY SCIENCES**  
**DEPARTMENT OF VETERINARY PUBLIC HEALTH AND FOOD SAFETY**

**Investigation of Physicochemical Characteristics and Heavy Metal Contamination  
in Honey from Selected Districts of the Tigray Region, Ethiopia**

**BY**  
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**A Thesis Submitted to the College of Veterinary Science for the Department of  
Public Health Food Safety and Zoonoses, in Partial Fulfillment of the requirements  
for the Degree of Master of Science**

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December, 2024  
Mekelle, Ethiopia

## **DECLARATION**

This is to certify that this thesis entitled “Investigation of Physicochemical Characteristics and Heavy Metal Contamination in Honey from Selected Districts of the Tigray Region, Ethiopia” submitted in partial fulfillment of the requirements for the award of the degree of Master in Veterinary Public Health and Food Safety, by Mr Hagos Tsegay, is an authentic work carried out by him under close follow-up by his principal advisors. The matter embodied in this thesis has not been submitted earlier for an award of any degree or diploma or publication.

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## **ACKNOWLEDGEMENTS**

The research support was provided by NORAD project in Mekelle University, Tigray, Ethiopia, project registry **RADO/EXTERNAL/SS0107/2024** and I would like to extend my sincere gratitude to my advisors (Dr. Tesfay Hailu and Dr. Etsay Kebede) for their unwavering support, guidance, and valuable contributions throughout from the research proposal until the final paper. Their mentorship has been instrumental in shaping my ideas and enhancing the quality of my work. I would like to thank Ezana Analytical Laboratory P.L.C and Holeta Bee Research Center (especially Teferi Damtew) for allowing me to conduct the laboratory analysis and for their valuable technical support. I also thank Dr. Enqubaher, Dr Yohannes Hagos, and Dr. Mebrahtu Berhe for their constructive review and feedbacks. Additionally, I am grateful to my family (especially my wife, my Dad and Mom and my lovey brothers) and friends, especially Dr Zemikael, Fissiha Beyene, Aregawi G/kidan, Dr Tesfay, Abadi G/medhin and all my classmates (Dr Haiay, Dr Mebrino and Sami Dann) for their full support and encouragement. The last but not least my special thanks goes to the Collage and all teachers of my department, love and respect to all who I don't mention you yet.

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## **LIST OF ABBREVIATIONS**

AOAC	Association of Official Analytical Collaboration
CAC	Codex Alimentarius Commission
CSA	Central Statistical Agency
EC	Electrical Conductivity
EU	European Union
FAAS	Flame Atomic Absorption Spectrometry
FA	Free Acidity
HMF	Hydroxy Methyl Furfural
H <sub>2</sub> O <sub>2</sub>	Hydrogen per Oxide
IDL	Instrument Detection Limit
IHC	International Honey Commission
LOQ	Limit of Quantification
MC	Moisture Content
MDL	Method Detection Limit
ND	No Detection
QSAE	Quality and Standard Authority of Ethiopia
REST	Relief Society of Tigray
TRARDO	Tigray Rural Agricultural Development Organization
UK	United Kingdom
USA	United State of America
USD	United State Dollar

## **ABSTRACT**

This study was conducted to assess the physicochemical properties and heavy metal concentrations in honey sourced from selected districts in the Tigray Region of Ethiopia. A cross-sectional study was conducted from January 2024 to December 2024, in which representative pooled honey samples (500 grams per district) were collected from six purposively selected districts. The physicochemical parameters: hydroxymethyl furfural (HMF) levels, free acidity, color, moisture content, electrical conductivity, ash content, and pH were analyzed, while concentrations of metals were also determined using flame atomic absorption spectrometry (FAAS). The findings revealed moisture content ranging from 16.8% to 20.5%, electrical conductivity between 0.215 and 0.645 mS/cm, pH values from 4.44 to 5.505, free acidity levels between 8.5 and 23 meq/kg, ash content from 0.045% to 0.29%, and HMF concentrations ranging from 0.8 to 15.95 mg/kg. The physicochemical characteristics of the honey complied with European, national, and Codex Alimentarius standards, confirming its quality and safety for human consumption. The validity of the analytical methods was confirmed by recovery rates for heavy metals ranging from 95.5% to 105% in spiking experiments. The highest concentration of heavy metals was found for iron (Fe), which ranged from 6.58 to 25.36 mg/kg, followed by manganese (Mn) at 0.06–2.74 mg/kg, zinc (Zn) at 0.01–1.90 mg/kg, and copper (Cu) at 0.04–0.19 mg/kg; lead (Pb) and chromium (Cr) were not detected in any samples, indicating the absence of these potentially toxic heavy metals in the honey analyzed. Overall, these findings are suggesting a good quality of honey production in the study area. However, regular monitoring of both physicochemical properties and heavy metal levels in honey is crucial for ensuring consumer safety and maintaining market standards, alongside further research into honey quality and safety practices.

**Keywords:** Heavy metals, Honey; Physicochemical properties, Tigray

# 1. CHAPTER I

## 1.1. INTRODUCTION (Background and Justifications)

Apiculture is one of the most innovative honey businesses and a substantial and quickly expanding worldwide honey production component of the agricultural subsector with a crucial role in guaranteeing ecological, economic, and nutritional security (Gebreselassie and Bekele 2012). Honey is a natural sweet substance produced by honey bees from plant nectar. Bees collect this nectar, combine it with their secretions, transform it, and then deposit, dehydrate, store, and leave it in the honeycomb to ripen and mature (Codex, 2001). A viscous liquid, freshly extracted honey has a density of 1.5 g/cm<sup>3</sup>, higher than that of water (1 g/cm<sup>3</sup> at 4°C). It also has a low surface tension, a strong hygroscopic character, a relatively low conductivity heat, and various colors (Adugna *et al.*, 2020). Honey must not taste strange, ferment, or be boiled to the point where it destroys its natural enzymes or includes substances that could harm human health (Kružik *et al.*, 2017). Codex (2001) asserts that although honey's flavor and aroma may vary, they are both derived from the plant.

The main components of honey are water (15–17%) and sugars (80–85%), while Fructose and glucose, honey's two main sugars, are responsible for the majority of its nutritional and physical qualities. Other sugars, such as disaccharides, tri-saccharides, and oligosaccharides, are present in trace amounts in honey along with minerals, free amino acids, flavonoids, vitamins, enzymes, and phenolic and organic acids. Honey is said to include important minerals such as potassium, magnesium, calcium, manganese, phosphorus, copper, iron, silver, barium, cadmium, lithium, zinc, sodium, and cobalt (Kadri *et al.*, 2017). Ethiopia has the largest bee population in Africa, with over 10 million colonies. The remaining bees are thought to be in the wild, while an estimated 5 to 7.5 million of these are hived (Bihonegn & Begna, 2021). This puts Ethiopia in 10th place and makes it the biggest honey producer in Africa. The most significant primary product of beekeeping, both economically and quantitatively, is honey. About 80% of the honey produced is used to make "tej," a local beverage that is created in urban areas or in farmers' houses and is also referred to as honey wine or honey beer. A very tiny amount

is exported, mostly to Saudi Arabia and Yemen. In Tigray, beekeeping is primarily a traditional practice with a long history that parallels the history of agriculture. The majority of the time, beekeeping has remained conventional and unprofitable. As a result, the yield of honey and other products from hives has remained consistent over the years. Modern hives did not exceed 45 kg annually, while traditional hives did not exceed 7 kg annually (REST, 2004). Geographical and environmental factors like climate, soil type, floral origin, honey maturity, bee species, beekeeping practices for extracting and removing honey, and processing and storage conditions all affect the composition and physicochemical characteristics of honey (Kayode and Oyeyemi, 2014). Bee forages are the primary elements of certain plant species, which determine the exact variance in composition (Gulfranz *et al.*, 2010). The country's export revenue as well as local prices and preferences are impacted, either directly or indirectly, by this compositional diversity. There is a direct correlation between the availability, diversity, and flowering pattern of bee food and the deforestation and extinction of endangered plant species in the Tigray region. This has consequently had a detrimental impact on the region's honeybee product production and productivity (Girma *et al.*, 2008). According to the review by (Grtzer *et al.*, 2021) the Tigray National Regional State produced 4,941,200q of honey from 331,407 hives, or roughly 8.43% of the nation's total honey production. In Tigray, honey production is a significant economic activity, deeply intertwined with the cultural and ecological landscape. But the quality and safety of the honey is questionable.

As per international standards; honey should fulfill certain quality standards and be safe for human consumption. The physicochemical parameters and levels of heavy metals in honey are crucial indicators of honey quality and safety. However, poor beekeeping practices and post-harvest handling in Tigray influence honey's quality. Increasing environmental pollution resulting from different activities raises concerns about the potential contamination of honey with heavy metals affecting honey safety. Mekelle, the capital city of the Tigray region in Ethiopia, is one of the country's rapidly developing and urbanizing cities. It is also one of the few industrial zones in Ethiopia. According to a study by Samuel, (2017) on the consequences of dust from the Mesebo cement industry near Mekelle, heavy metal contamination is present in the soil. Fitsum's research (Gebreyohannes *et al.*, 2015) revealed that the Elala River, which flows through Mekele

city, has heavy metal contamination in its water. Heavy and hazardous metals can contaminate the air, water, and land through sewage, emissions, and effluents from factories and enterprises. These substances both trace and heavy, have the potential to exceed safe limits and cause health problems. In addition to affecting honey's marketability, these pollutants, even in tiny concentrations, can have serious negative health effects on users, including neurogenic, carcinogenic, cardiovascular, and gastrointestinal effects. Few researchers have examined the physicochemical and mineral contents of Ethiopian honey from different regions (Belay *et al.*, 2013; Nigussie *et al.*, 2012; Liben *et al.*, 2018; Teka, 2018; Yohannes *et al.*, 2018).

Many districts from the moisture stress and moist areas of the region including Mekelle, Enderta Atsbi, Wukro, Degua Tembien, and Kola Tembien districts are famous for qualitative and quantitative honey production and are also known for their denser honey bee population density due to their high natural vegetation coverage (TRARDO, 2023). Among the districts of the region, Atsbi district (Eastern Zone), Degua Tembien district (Central Zone) are high land and Enderta (south-eastern zone), Wukro (eastern zone), and Mekelle (capital city of the region) are moderate temperate area districts and Kola Tembien (central zone) is low land. Both selected districts are potential honey producers per annum because the areas are endowed with different natural vegetation on which the bees forage to make honey.

As there is not enough research evidence, which can give us information about quality standards and the safety levels of honey in the Tigray Region; hence, investigating the physicochemical parameters and determining the potential heavy metal contaminants in honey could be critical. Therefore, the present study is conducted with an objective to further investigate the levels of selected heavy metals (copper, Iron, Manganese, chromium, zinc, and lead) and some physicochemical properties (moisture content, electrical conductivity, ash content, HMF, color, pH and free acidity) of honeys and comparing the obtained results with national and international standards in honey samples collected from six districts (Atsbi, Wukro, Degua Tembien, Kola Tembien, Enderta, and Mekelle) in the Tigray Region, Ethiopia.

## 1.2. Literature Review

### 1.2.1. Definition and Benefits of Honey

Codex Alimentary states that honey is a sweet substance that honey bees produce naturally from plant nectar, plant secretions, or plant-sucking insect excretions on living plant parts, as per Codex Stan (2001). The bees collect this material, transform it by combining it with specific substances, deposit it, dehydrate it, store it, and then leave it in the honeycomb to ripen and mature. Due to its broad use and protective health effects, honey is a highly prized bee product. Food, cosmetics, and pharmaceuticals are just a few of the industries that use it as a vital element because of its distinct physicochemical, chemical, and biological characteristics. The growing usage of honey in creative products as research into its potential continues to confirm its significance on a worldwide scale (Ízol, 2021). The demand for honey is occasionally increasing worldwide because bees produce honey, an edible nutritional food that helps our bodies function better physically and mentally. It can be consumed by people of all ages, from young children to elderly adults, as well as by patients and health professionals.

Honey is also used as a flavoring and sweetener in food and beverages and in the detergent and cosmetics industries. Among the four types of honey production practices traditional backyard beekeeping, traditional forest honey production, traditional beekeeping, and improved beekeeping. Beekeeping is one of the economic sectors that generate income for millions of Ethiopian small-scale farmers. It is thought that honey has antibacterial qualities that contribute to its positive benefits because of its high osmolality, low pH, abundance of hydrogen peroxide ( $H_2O_2$ ), and phytochemicals such as methylglyoxal. Honey's main antimicrobial ingredient is hydrogen peroxide. Honey's hydrogen peroxide content is determined by the proportions of catalase from flower pollen and glucose oxidase made by bees (Sebsib and Yibrah, 2018). Both non-peroxide components and hydrogen peroxide, which is generated by glucose oxidase in conventional honey, are thought to contribute to honey's antibacterial properties. Honey has been found to have the ability to eliminate microorganisms due to its phytochemical composition, high osmotic impact, and high concentration of acidic hydrogen peroxide. Honey has several medicinal uses, such as the treatment of diabetes, skin infections,

wounds, cancer, and bladder infections. Additionally, it has beneficial effects on the digestive system. Rich in high carbohydrate foods, honey also includes a variety of minor nutrients like vitamins, minerals, and proteins that provide human diets more nutritional value. Furthermore, honey has additional health benefits due to the presence of many bee products, such as Royal Jelly, Propolis, Beeswax, and Bee Pollen (Biruk, 2015). The bees use the honey they create as a store to feed their colonies when blooms are in short supply or the weather is bad. In northern temperate areas, for example, very few plants bloom from October to March on a winter basis. They rely on their honey stores to stay alive during this period, when it may be too cold for bees to leave the nest (Wahid *et al.*, 2022). Tropical bees must withstand flowerless spells and severe weather that hinders their ability to forage.

#### 1.2.2. Honey Production Globally and in Ethiopia

Bees and beekeeping are crucial either directly or indirectly to the livelihoods of every nation on Earth. The world produces more than 1.3 million tons of honey each year. The leading honey producing countries are Russia, China, the United States, and Mexico, with annual outputs of 19,333 tons, 161 tons, 75 tons, and 67 tons respectively. Developing countries account for over 47 percent of the world's honey production (Teklu and Dinku, 2016). Ethiopia is a significant player in honey production, contributing 21.7% of Africa's total and approximately 2.5% of the world's supply. With an annual output exceeding 43,373 metric tons of crude honey, the country represents 23.5% of Africa's honey production and 2.35% globally (Sebsib and Yibrah 2018). Ethiopia, the world's most populous nation, boasts a rich history of beekeeping (Samuel, 2017).

Ethiopia generally experiences two main honey harvesting seasons: the primary season, occurring from October to November, and the secondary season, taking place from April to June. In addition to these major periods, various smaller harvesting times are influenced by the types of flowering plants and rainfall patterns found in different agroecologies (Samuel, 2017). Approximately two million agricultural households in Ethiopia engage in the production and sale of honey. Additionally, a significant number of individuals are involved in the honey industry, contributing to its production and

marketing. For many citizens, honey production and sales provide employment opportunities as well as avenues for self-employment (Fenet and Alemayehu, 2016).

A promising nonfarm activity for rural households is beekeeping and honey production. It impacts household incomes and the national economy both directly and indirectly. Beekeeping is considered significant as a means for smallholder farmers to sustain their livelihoods (Fenet and Alemayehu 2016). This business venture has the potential to create a range of economic benefits, including revenue from honey sales and its byproducts. Additionally, the sector contributes roughly USD 1.6 million to the national economy (Biruk, 2015). The Tigray region features various agroecological zones that facilitate apiculture and the growth of diverse bee flora, similar to other areas in Ethiopia. Approximately 15% of Ethiopia's total honey production is sourced from Tigray. The region experiences two distinct harvest seasons, categorized into northern and southern honey production areas: the first season, known as the prime season, runs from September to December, while the second season occurs from May to June (Alemseged and Yildiz, 2008). Even though, deforestation in Tigray and the loss of endangered plant species have significantly impacted honeybee production and products in the region, as well as the diversity and availability of bee feed and flowering patterns.

### 1.2.3. Beehive Technology for Quality Honey Production

Beekeeping is the practice of humans maintaining colonies of honey bees, usually in hives. Beekeepers around the world utilize a variety of bee hives to produce honey. According to MORAD (2008), these hives fall into one of two categories: modern or traditional. Beekeeping involves people maintaining honey bee colonies, typically in hives. To get the benefits of moveable frame hives (no need to break combs, standardization, manageability, and quick honey harvest) without the drawback of expensive manufacturing, low-technology hives were created. The hive's container can be made from whatever materials are available in the area, just like traditional hives. Low-tech beehives can be kept close to home and, with careful construction and transportation, can be relocated between crops as they bloom one after the other (GDS, 2009). Because the combs on modern hives can be removed and then put back in, the beekeeper may check the colony's health without endangering it. Additionally, honeycombs can be

harvested from the hive without disrupting brood-containing combs. Because of this, the colony remains unharmed and the bees can keep collecting honey to replace that which has been collected, guaranteeing that high-quality honey free of pollen or brood contamination can be harvested. Honey should be processed as soon as possible after being removed from the hive.

Processing honey is a sticky process that requires patience and time to get the best results. At every stage of processing, careful precautions must be taken to prevent contamination by ants and flying insects. It is crucial to keep in mind that honey is a food and must be handled hygienically, that all equipment must be completely clean, and that honey is hygroscopic, meaning that it will absorb moisture. Additionally, honey ferments if it contains too much water (Caleb, 2017).

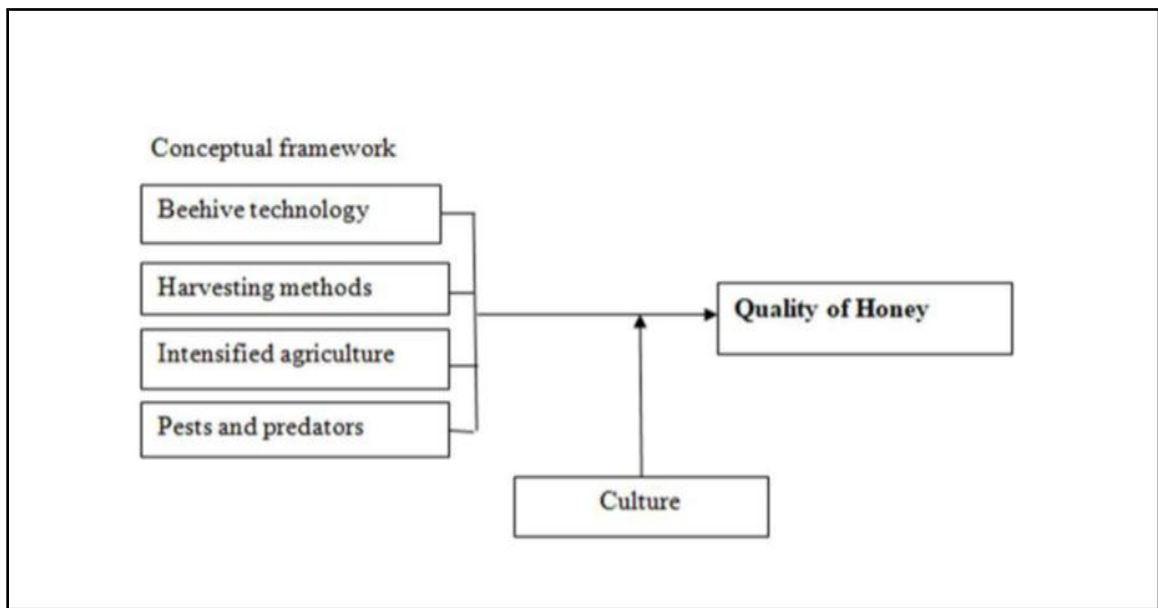


Figure 1. Conceptual framework potential contributors of honey quality (Caleb, 2017)

#### 1.2.4. Honey Quality

##### Physicochemical Characteristics and the Factors Affecting

Glucose and fructose make up the majority of the complex variety of carbohydrates found in honey; the presence of additional sugars varies depending on the floral source. Additionally, it has trace amounts of minerals, vitamins, enzymes, phenolic compounds, organic acids, lactones, amino acids, pollen, wax, colors, and volatile components. These component contents are the most crucial honey quality parameters and reveal some significant deterministic honey quality attributes (Sahinler and Gul, 2004). Geographical and environmental factors have a significant impact on the composition and quality of honey (Jones *et al.*, 2011). They rely on the honey's maturity, the manufacturing process, the climate, the conditions of treatment and storage, and the sources of nectar honey (Was *et al.*, 2011). Likewise, the kind of hive that is utilized for storage has a significant impact on the honey's quality.

The primary source of honey's chemical composition is the vegetation from which it is harvested; however, storage, harvesting conditions, and the environment can also have an impact. Numerous factors, including flower type, location and habitat, season, beekeeping operations, climate, soil composition, nectar supply, and honey processing and storage methods, can affect the quality and content of honey. Variations in the qualities of honey can also result from these causes (Vorlova and Prida, 2002). The kind of flower from which honey is produced has a significant impact on its characteristics, including its color, moisture content, pH, mineral content, electrical conductivity, and acidity. The manufacturing procedure used to produce honey affects other elements, including the purity and concentration of HMF (Vorlova and Pridal, 2002). Honey's maturation, stability, and quality changes over time can all be determined by its acidity level (Dimins *et al.*, 2006). The color of honey can range from nearly colorless to dark brown. Its consistency can range from partially to fully crystalline, fluid, to viscous.

Honey quality might be lowered by treating it carelessly. Among the most important factors affecting quality are high temperatures, storage duration, and moisture content exceeding 21%. Their effects include fermentation, elevated hydroxyl methyl furfural (HMF) levels, microbial growth, taste alterations, enzymatic activity loss, and darkening

(Moguel *et al.*, 2005). Honey must be attractive to consumers to command a higher price, and it must never contain ingredients in amounts that could threaten human health. Above important, having sufficient information about the quality status of honey attracts buyers and inspires local investors to participate in honey gathering and processing. The quality indicators that define each honey variety are the physicochemical characteristics of natural honeys, which include ash, moisture content, electric conductivity, pH and free acidity, hydroxyl methyl furfural, apparent reducing sugar, and sucrose content (Juszczak *et al.*, 2009). These parameters have been rigorously defined.

#### Physical Characteristics

The chemical composition of honey affects its physical characteristics, including crystallization, viscosity, hygroscopicity, optical properties, surface tension, color, pH, moisture content, and electrical conductivity. The physical characteristics of honey can vary depending on its extraction method, processing techniques, packaging practices, and preservation methods (Ajibola, 2015). The viscosity of honey is influenced by its composition, which comprises various components. As a thick liquid food, the viscosity of freshly extracted honey can fluctuate depending on its water content (Ajibola, 2015). The amount of water absorbed by honey is determined by the relative humidity of the surrounding air. Additionally, honey's high sugar content affects its capacity to attract moisture. Consequently, this process may lead to an increase in the water content of the surface layer of honey, potentially impacting its quality during fermentation and storage.

Honey appears foamy because of its high viscosity and surface tension (Ajibola *et al.*, 2012). Liquid honey comes in various colors: clear, yellow, amber, dark amber, or black. The color of honey can be influenced by several factors including its origin, age, and storage conditions. The clarity of the honey can be determined by the amount of suspended particles such as pollen. Honey exhibits different hues like bright yellow or sunflower, reddish undertones or chestnut, greenish tones or honeydew, and gray or eucalyptus. Heat can influence honey's color, crystallization, flavor, and aroma. As noted by Ajibola *et al.* (2012), authentic honey darkens when subjected to heat. The low electrical conductivity of honey is influenced by its protein, organic acid, and mineral

contents. Consequently, electrical conductivity is a widely used criterion for assessing honey quality and can accurately identify the plant source of a honey sample.

#### Chemical Composition

The composition of honey varies based on its floral source. Although natural honey contains over 300 bioactive substances, the bulk of its composition consists of water and carbohydrates. Specifically, fructose and glucose account for 95-99% of the dry matter, while fructo-oligosaccharides constitute approximately 4-5%. In addition to fructose (38%) and glucose (31%), other sugars identified include oligosaccharides such as laminaribiose, nigerose, sucrose, turanose, isomaltose, kojibiose, gentiobiose, and maltose (Ajibola *et al.*, 2012). Honey is primarily composed of sugars, with 34% glucose, 40.5% fructose, and 1.9% sucrose. Additionally, it contains 5.5% of other carbohydrates. Water is the second most critical component in honey, comprising a range of 15-23%. The water content influences various characteristics of honey such as viscosity, specific weight, maturity, flavor, crystallization, and specific gravity.

This variation depends on multiple factors including the type and strength of the bee colony, humidity and air temperature within the hive, processing and storage conditions, as well as the botanical origin of the honey. Honey is hygroscopic; thus, its water content can fluctuate based on ambient air humidity during storage. Since water content significantly impacts both stability and microbial resistance during storage, it plays a vital role in determining honey's quality and shelf life (Vahčić and Matković, 2009). Higher water content increases the risk of fermentation. Additionally, a chemical compound known as HMF is produced when simple sugars such as fructose break down spontaneously during honey storage. Honey forms much more rapidly when heated (Bradbear, 2009). It contains several types of acids, including amino acids (0.05–0.1%) and organic acids (0.57%, with a range from 0.17% to 1.17%). Additionally, honey has various free amino acids such as lysine, proline, and tryptophan. Proline constitutes 50-85% of the amino acids in honey and is frequently used as an indicator of its maturity level (Bogdanov *et al.*, 2004). The pH level of honey generally falls between 3.4 and 6.1, with an average value around 3.9.

## Effect of adulteration on honey physicochemical properties

Recently, the rising market value and prices of certain foods have intensified the issue of food adulteration. Among these items, honey stands out as one of the most commonly counterfeited foods (Valanou *et al.*, 2020). Honey fraud encompasses various practices, including mislabeling of its botanical or geographical origin, direct adulteration with sugar syrups, indirect adulteration through feeding bees' sugar during the collection period, and resin filtration (EU, 2018). A review by Damto (2021) has examined the prevalent methods of honey adulteration in Ethiopia that have led to quality degradation and impacted both domestic and international honey markets. Consequently, regular monitoring and assessment of the key properties of honey including moisture content, sucrose and reducing sugars, pH value, electrical conductivity, ash content, free acidity, diastase activity, and hydroxymethyl furfural (HMF) against established standard quality parameters are essential for preserving the natural quality and origin of honey. The honey color is one of its most variable features. Color is a significant parameter for quality degradation during honey storage and is influenced by moisture content as well as the storage temperature. Color analysis indicates that adulterated honey tends to be brighter, while pure honey exhibits a more reddish hue (Naila *et al.*, 2018). The alteration in color during adulteration depends on the substances involved. For example, Gemeda & Negera (2017) reported that when honey is adulterated with arid, its color changes to yellow.

When sugar is added by boiling it together with water, the resulting honey appears light white; mixing equal amounts of sugar with honey yields a red solid. Additionally, directly adding sugar to white honey results in an intensified white hue, and when molasses is used through heating, the altered product resembles black coffee in color according to their study conducted in the Oromia region. Additionally, according to (Ambaw & Teklehaimanot, 2018), the color of the adulterated honey adulterate in the Arsi zone sample is yellow, yellow-brown, and brown based on physical observation. These common adulterants include sugar, ripened bananas, wheat flowers, potatoes, maize flowers, pollen, empty combs, melted candy, molasses, and hot water. Phenolic chemicals, pollen, and minerals can all affect the color of honey. The primary criterion for assessing honey's storage quality and quality is its moisture content.

The humidity in the tropics may cause honey to contain more water. Honey's water content and fermentation are strongly associated. There is a greater chance of fermentation occurring in honey samples with higher water content. The refractive index, water activity, and moisture content of honey can all be changed by adulteration. Pure honey and adulterated honey differ in this feature, and the adulterant agent is the cause of this variation.

A higher moisture level in honey is a sign that it has been adulterated. The more adulterants added to pure honey, the faster the output voltage increases and the refractive index decreases. High water content honey may be unripe or a combination of honey and water, so it's important to check for adulteration (Gebremariam & Brhane, 2014). When compared to samples of adulterated honey, pure honey had the lowest moisture content since adulterant components raise the moisture level of honey. Food products with a water activity of less than 0.60 can be regarded as microbiologically stable. Water activity is a proportionate unit of the free water in food products. Currently, water activity is seen to be a more useful metric for honey than moisture content. The moisture content and water activity have a linear relationship, claim Oroian *et al.* (2018). Fructose and hydrolyzed inulin syrup were used to adulterate honey samples in a study. The results showed that water activity rose proportionately as the amount of the adulteration agent grew, indicating that water activity is rising in tandem with the adulteration agent percentage. Along with this, adding water to the honey increases its moisture content and water activity, which causes it to ferment and degrade more quickly.

A food's composition becomes irregular or certain component ratios are altered when any amount of any ingredient or substances is added. When it comes to identifying and categorizing the kinds and amounts of possible adulterants in honey, HMF, ash, free acidity, diastase activity, sucrose, and electrical conductivity are crucial (Amiry *et al.*, 2017). According to research by Ribeiro *et al.* (2014), the ash level of pure blossom honey (0.15%) increased gradually as the quantity of adulterant grew. They also noted that the ash content increased in all contaminated samples. Gebremariam & Brhane (2014) found that when commercial sugar is added, the pH value rises but the free acidity falls. When the proportion of the adulterant was raised, Ribeiro *et al.* (2014) found that

the pH values of the honey tainted with corn syrup increased noticeably. As compared to pure honey, a study by Ribeiro *et al.* (2014) found that adding water to honey lowered its sweetness and sugar concentration. Natural honey has a higher concentration of proteins, organic acids, and mineral salts than commercial sugars (Gebremariam & Brhane, 2014). The study conducted by Oroian *et al.* (2018) found that samples containing fructose had lower electrical conductivity than the original ones since fructose reduces conductivity, whereas samples containing hydrolyzed insulin syrup had higher electrical conductivity due to the acidic nature of the syrup.

According to some research, one of the most effective ways to identify honey adulteration is by electrical conductivity. Another reliable sign that honey has been adulterated by adding invert sugar is hydroxyl methyl furfural. According to Gebremariam & Brhane's (2014) study, which addressed the problem of determining the degree of adulteration in specific food products, including honey, honey samples gathered from local markets had higher moisture and hydroxyl methyl furfural (HMF) contents than the recommended level, suggesting some degree of heat treatment and adulteration. However, all pure honey samples that were directly purchased from beekeepers and household farmers meet all national and international standards and are of high quality. In other words, adulteration raises the moisture and HMF levels.

#### Influence of agrochemicals on honey quality

Because concentrations of specific metals and other compounds could be tested in hive products, mostly pollen but also honey, honeybees were employed to monitor environmental contamination in Canada, the United States, the United Kingdom, and Italy (MP. & JP. 2007). Pesticides, herbicides, and fungicides are sprayed on crops, but they get to the bees through pollen, nectar, and the soil, water, or air (Oliver, 2012). This happens when bees are present on the blooms when the poison is applied, and they promptly perish. Other pesticides let the bees go back to their homes, where they eventually perish. Unlike the earlier ones, these varieties are easier to recognize. However, certain pesticides harm young, immature honey bees but do not affect adult honey bees (Evans & Schwarz, 2011). According to Oliver (2012), pesticide spraying has

historically been the cause of some bee deaths as well as ongoing honey contamination that results in the production of honey of poor quality.

#### 1.2.5. Heavy Metal Contamination of Honey

Heavy metal pollution is found in soil and water supplies in many parts of the world, especially in and near cities and industrial areas. Plants that thrive in polluted soil frequently take up large amounts of heavy metals, which build up in their tissues. In addition to decreasing plant life and productivity, this contamination exposes pollinators and herbivores that eat these plants to potentially harmful metal concentrations. The elemental makeup of flowers, which differs according to their botanical and geographic origin, has a significant impact on the metal concentration in various kinds of honey. Therefore, the crops that surround the bee hives also affect the honey's unique composition and potential pollutants (Aliferis *et al.*, 2010). The amount of harmful metals in honey varies depending on its biological and geographic origin, and its mineral and toxic metal content has been employed as a quality indicator (Citak *et al.*, 2010).

Honey is known to accumulate trace metals, including hazardous metals like cadmium, nickel, and lead, as well as other critical elements like zinc, copper, manganese, and chromium that are necessary for human growth and health up to a certain point (Hernández *et al.*, 2005; Pohl, 2009). According to Ashenef (2014), chronic toxicity can still result from consuming excessive amounts of these substances. Since honey bees can easily fly up to 4 km in all directions from their apiary, giving them access to an area of about 50 km<sup>2</sup>, it is a helpful bio-monitoring mechanism for information about the environment where the bees live. Therefore, the quantity of heavy metals in honey reflects their abundance in the entire environment, as the bees come into touch with soil, water, and air. As a result, honey has been identified as a biological marker of pollution in the environment (Silici *et al.*, 2008).

Numerous studies have been conducted utilizing various analytical techniques to examine the mineral and heavy metal levels of honey, These include Flame Atomic Absorption Spectrophotometry (FAAS), particle-induced X-ray emission (PIXE), total reflection X-ray fluorescence (TXRF), capillary zone electrophoresis, inductively coupled plasma-optical emission and mass spectrometry (ICP-AES and ICP-MS) (Aghamirlou *et al.*,

2015; Kılıç *et al.*, 2019; Tuzen *et al.*, 2007), ion chromatography, and isotope dilution inductively coupled plasma mass spectrometry with direct injection nebulization (ID-ICP-MS) (Altun *et al.*, 2017). Solayman *et al.* (2016) conducted a comprehensive analysis of the analytical techniques used, as well as the values revealed for the metal content and physicochemical properties of honey worldwide.

#### Sources of Heavy Metal Contamination in Honey

Heavy metal contamination of soil and water reservoirs is found in many parts of the world, particularly in and near urbanized and industrialized areas (Gebreyohannes *et al.*, 2015). Environmental triggers, such as soil deposits, water contamination, and air pollution, can introduce metals like lead, cadmium, and chromium into honey (Tibebe *et al.*, 2022). In agricultural areas, industrial pollution and pesticide use are frequent sources of contamination. Furthermore, because minerals enter plants by their roots, travel through the nectar, and then enter the honey that is generated, the kind and concentration of minerals and heavy metals in honey are significantly influenced by the composition of the soil as well as the different kinds of flower plants.

Plants that may acquire metals from the soil or irrigation water provide nectar for bees to consume. It is possible to carry trace metals to hives and concentrate them in honey. However, the quantities of trace elements in honey can also be influenced by many anthropogenic activities that contaminate the air, water, and soil, such as neighboring industries, waste dumps, traffic related pollution, and chemical-intensive agriculture (Wahid *et al.*, 2022). Through the nectar, pollen, or honeydew of melliferous plants that grow on contaminated soils and absorb contaminated water, several contaminants can enter honey from polluted forage regions. The quality of honey can be altered by bees themselves bringing pollutants from the surroundings into their hives. Additionally, trace contaminants may contaminate honey when it is being collected, processed, sweetened, and stored. Toxic metals in honey can be detrimental to human health and a sign of environmental contamination (Wahid *et al.*, 2022).

#### Health Risks Associated with Heavy Metals in Honey

To preserve food quality and guarantee that people are protected from food-related health risks, food safety is a crucial public health concern. Food chains are the primary pathway

by which heavy metals enter the body (Sobhanardakani & Kianpour, 2016). Bees can use their sensory organs to identify some toxins, but they are unable to detect some toxins. Assessing the degree of damage that metal contaminated resources provide to bee populations and, consequently, to humans is made easier by looking into whether honey bees will readily consume them (Saxena *et al.*, 2010).

These metals may be hazardous to human health if they build up to toxic levels. Ethiopia's varied environment makes it a good place to host bee colonies (Adagaba, 2007). High food exposure levels of heavy metals can have harmful health effects, including toxicity to organs like the kidneys, liver, and lungs. The most significant sources of pollutants in food, soil, and water are heavy metals (Pirhadi *et al.*, 2022). Toxic heavy metals can lead to a number of illnesses, including cancer, anemia and hemoglobin biosynthesis issues, gastrointestinal bleeding, inflammation, and heart, kidney, lung, and gastrointestinal failure. It is well known that cadmium (Cd) can accumulate and cause respiratory and kidney harm. Even minute amounts of lead (Pb) exposure can result in neurological and developmental problems, particularly in young children. Most people agree that lead is one of the most dangerous pollutants in the environment. Chromium (Cr) Small amounts are necessary, but excessive levels are harmful; and linked to cancer and other systemic toxicities.

These days, it is known that heavy metals contribute to environmental contamination and have detrimental effects on human health (Malakootian *et al.*, 2011; Pirhadi *et al.*, 2022). Heavy metals in particular can be absorbed by plants and enter the food chain. Heavy metals are prone to build up in living things' tissues due to their lengthy half-life (Hegazi and El-Kay, 2010; Raja *et al.*, 2016). The ingestion of polluted water, pollen, and nectar, the inhalation of particles while in flight, and the attachment of particles to their hairy bodies as they move across plant and soil surfaces in search of food are additional ways that bees can absorb heavy metals (Hegazi & El-Kay, 2010).

Neurological problems, malignancies, nutrient deficiencies, obesity, cardiovascular and respiratory conditions, liver, kidney, and brain damage, allergies, asthma, endocrine disorders, and chronic viral infections are all generally caused by heavy metals. reduced immune system, gene damage, premature aging, skin diseases, loss of memory and

appetite, osteoporosis, anemia, fatigue, headaches, dizziness, enzyme failure, and in severe cases, death (Khaneghah *et al.*, 2020; Negahdari *et al.*, 2021). One way to measure environmental pollution, including heavy metal pollution, is by using honey as an indicator. For users, heavy metals in honey can be harmful, poisonous, and carcinogenic. Honey is one of the key food products that is more likely to be contaminated with heavy metals when pollutants are released into the environment in excess and permanently. To reduce hazards, international health agencies set acceptable metal levels in food items. These guidelines for honey quality monitoring guarantee consumer safety and control export quality (Negahdari *et al.*, 2021).

#### Benefits of Heavy Metals

Copper (Cu) is an essential trace element crucial for the health of all living organisms. It plays a key role in enhancing bone strength, maturing red and white blood cells, transporting iron, metabolizing cholesterol and glucose, contracting heart muscles, and supporting brain development. Furthermore, Cu plays a significant role in oxygen transport, bone development, and protein synthesis, serving as a crucial component of numerous enzymes (Soylak *et al.*, 2005). Chromium (Cr) supplementation helps normalize blood sugar levels. Elevated blood sugar, known as hyperglycemia, shows a positive response to chromium supplementation (Cheng *et al.*, 2004). In addition to normalizing glucose and insulin levels, supplemental chromium clearly reduces harmful total cholesterol and triglycerides. Zinc (Zn) is crucial and vital, particularly during puberty, pregnancy, and menopause. It is involved in most metabolic pathways in humans and plays a significant role in numerous enzymes that are essential for the immune system, wound healing, and reproduction (Malakootian *et al.*, 2011). Lead (Pb) is recognized for raising blood pressure and the risk of cardiovascular disease in adults, as well as for hindering cognitive development and intellectual performance in children (Malakootian *et al.* 2011).

#### 1.2.6 Honey Quality and Safety in Tigray

For both domestic and foreign markets, honey quality is a fundamental criterion that allows for the achievement of competitive premium prices while maintaining human health. The current global honey market trend is more demanding in terms of quality (Se *et al.*, 2019). The major factors influencing honey quality are weather, honey handling,

honey's nectar source, and chemical makeup. Heavy metal levels and physicochemical characteristics are important for quality and safety management. The total honey production estimate in Tigray for 2014 is approximately 1,433,920 kilograms, with the majority being sourced from traditional hives (CSA, 2014/15). Honey specifications outlined by the Codex Alimentarius Commission (2001) and Ethiopian Standard (2005) stipulate physicochemical criteria for honey characterization. These criteria include water content, ash, pH, electrical conductivity, hydroxymethyl furfuraldehyde (HMF), glucose, fructose, sucrose, and diastase activity. Research indicates that the average content of minerals, moisture, acidity, inverted sugar, pH, sucrose, specific gravity, and water-insoluble solids in traditional hive honey samples from the Tigray region were 22%, 18.25%, 29.89 meq kg<sup>-1</sup>, 70.95%, 4.13, 2.37%, 1.41%, and 0.07% respectively. In contrast, the corresponding values for honey samples from modern hive production systems in the same region were found to be 0.18%, 18.60%, 29.12 meq kg<sup>-1</sup>, 71.42%, 4.04, 2.71%, 1.40%, and 0.03% respectively (Gebreegziabher *et al.*, 2013). The honey market is not uniform; it features a bulk market characterized by low-priced honey of fair to good quality with notable differences in quality (Beyene and Phillips, 2007).

In Tigray, the demand for honey is growing steadily. Consumers are becoming more aware of foods that offer health benefits, including honey and other bee products. A persistent concern for both consumers and producers is sanitary quality; thus, hygienic standards must be upheld during harvesting and extraction processes. Consequently, it is essential to implement quality control measures and ensure the safety of honey and bee products through the analysis of chemical, physical, and safety aspects, such as detecting heavy metals (Gebreyohannes *et al.*, 2015). In recent years, research regarding the determination of mineral content in honey and other physicochemical parameters has been increasing in Tigray and has emerged as a significant issue. Since honey has therapeutic qualities and may be used as a nutritional supplement, it's critical to assess its quality based on its physicochemical characteristics and level of heavy metals. Honey must be free of excessive trace metals and polluting substances in order to be useful. According to Gebryohannes *et al.* (2015), every heavy metal that is found in honey in excess of the permitted level by pollution standards poses a risk to human health due to the potential adverse effects of the contaminants.

## **2. CHAPTER II: MATERIAL AND METHODS**

### **2.1. Description of the Study Areas**

Categories of agro-ecologies in the Tigray regions of districts include: (i) highland, characterized by areas over 2300 meters above sea level; (ii) midland, situated between 1500 and 2300 meters above sea level; and (iii) lowland, comprising areas below 1500 meters above sea level (Meles *et al.*, 2021). Based on this classification, the six study areas are listed below. Mekelle, the regional capital city of the Tigray region, is positioned between 13°24'30" to 13°36'52" N latitude and 39°25'30" to 39°38'33" E longitude. It has an average altitude of 2,200 meters above sea level (Kibrom, 2005). Rainfall in Mekelle is variable, averaging about 600 mm annually, with over 70% occurring between July and August, followed by a lengthy dry season (Kibrom, 2005).

The other five districts include Atsbi (Latitude N13°52'48", Longitude E39°44'24", Elevation: 2,600 meters), Dogua Tembien (Latitude N13°39'E39°10'; elevation ranges from 2,200 to 2,800 meters), Kola Tembien (Latitude N13°37'E39°00'; elevation below 1,500 meters), Enderta (Geographically located between latitudes of 13° - 14° north and longitudes of 39° - 40°30' east; elevation ranges from 1,800 to 2,300 meters), and Wukro (Latitude N13°49'48"; Longitude E39°36'0"; elevation is approximately 1,995 meters) (Kibrom, 2005).

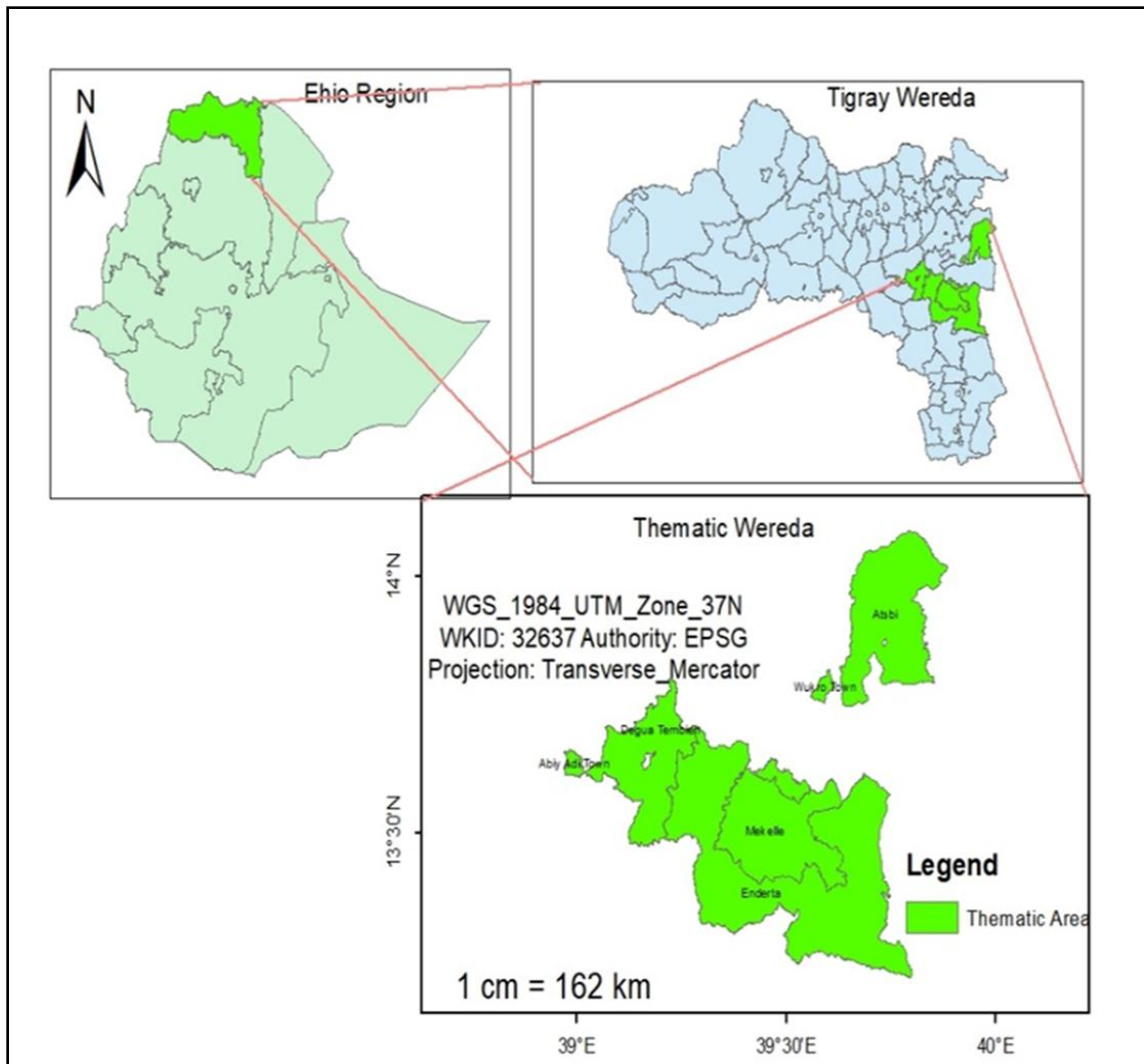


Figure 2. Map of the Study area indicating the sampling sites (ESRI, 2015)

## 2.2. Study Population

The study was focus on honey that are obtained from honey producers having more than five modern honey bee hives

## 2.3. Study Design and Sampling Methods

Across-sectional study was conducted from January 2024 to December 2024 and six districts were selected purposively considering the potential of bee keeping and geo-climatic conditions. Four kebelles were considered from each district and 20 honey producers from these four kebelles. Honey producers with more than five modern beehives were selected to ensure consistent and reliable honey production practices,

representative of the targeted production scale in the region. This threshold was chosen to minimize variability associated with small-scale or traditional methods.

#### **2.4. Sampling Procedures**

Sampling and pooling of honey were conducted in alignment with Codex Alimentarius standards (Codex Stan 12-1981) and International Honey Commission guidelines (IHC, 2009). These methods ensure representativeness and minimize variability across districts while maintaining sample integrity for physicochemical and heavy metal analysis (AOAC, 1999). A purposive sampling method was utilized across six districts ecology (Enderta, DeguaTembien, kolaTembien, Wukro, Atsbi and Mekelle town) in the Tigray region, focusing on areas with significant apiculture potential, Agro-ecology based and urbanization. Four kebelles were selected from each district based on their geographic and ecological diversity. Five honey producers, each with more than five modern beehives, were chosen from each kebele, resulting in a total of 20 producers per district. Each producer contributed 500 g of honey, which was pooled into a composite sample for the district to ensure representativeness while minimizing variability among individual contributions.

Honey samples were initially collected individually from each of the 20 producers in every district. Following collection, 25 g of honey from each producer was combined within their respective kebele to create a composite kebele sample. These kebele-level composite samples were then mixed to form a single representative district level composite sample of 500 g. This stepwise pooling ensured that each kebele's contribution was proportionally represented while preserving the integrity and diversity of the samples. Total of 3000 g of honey from six district honey samples were collected, each with 500 g weight. The honey samples were collected from areas of potential beekeeping sites. Fresh honey samples were collected directly from the honey producers during the harvesting season in October 2024. All the samples were collected in clean and closed glass bottles in order to protect them from contaminations. The samples were given their own label, sealed and before being analyzed, all the honey samples were kept at room temperature and protected from light.

## **2.5. Sample preparation**

Honey samples from all the six Districts were heated to 65°C in accordance with the Codex Alimentarius guidelines and International Honey Commission recommendations. This temperature was chosen to reduce viscosity, dissolve crystallized sugars, and ensure sample homogeneity without significantly altering the natural composition of honey. It prevents the degradation of thermolabile compounds such as enzymes and limits the formation of hydroxymethylfurfural (HMF), ensuring reproducibility and reliability of analytical results (Codex Alimentarius, 2001; AOAC (1999; IHC, 2009).

## **2.6. Determination of Physicochemical Parameters and Heavy Metals in Honey**

### **2.6.1. Chemicals and Reagents**

Concentrated nitric acid (70%) and hydrogen peroxide (30%) were utilized to digest honey samples because of their potent oxidizing properties, which effectively dismantle the organic matrix and solubilize heavy metals for FAAS analysis and Stock solutions containing 1000 mg/L of various heavy and trace metals (Fe, Cu, Cr, Mn, Zn, and Pb) were used to create calibration curves, standardize the methods, and prepare spiked samples. Sodium hydroxide (0.1 M) was employed to neutralize acidic compounds for free acidity determination, while Carrez Solutions I and II clarified the samples by precipitating proteins and colloidal materials during HMF analysis. Sodium bisulfite (0.2%) was used to correct spectrophotometric interferences, and deionized water guaranteed the purity of all prepared solutions while rinsing equipment to eliminate contamination (Codex Alimentarius, 2001; IHC, 2009). All chemicals and reagents in the lab were of analytical grade and obtained from reliable sources.

### **2.6.2. Apparatus and Instruments**

A digital analytical balance (D-72336 Bvange, Germany) was used to weigh the honey samples. Pipettes and micropipette syringes were used to measure accurate volumes of acids and standard solutions. Test tubes were used to hold the digested samples on the auto sampler, while borosilicate volumetric flasks were used for diluting the samples and preparing standard solutions. Filter funnels and filter papers were employed to filter the digested solutions vortex mixer for homogenizing honey samples. A refrigerator was used to store the samples and digested solutions until analysis. A fume hood was used for

heating the samples during digestion. The absorption spectra of heavy and trace metals were recorded using Atomic Absorption 240 Flammable Spectrometer. Apparatus used to analysis physicochemical parameters includes water bath, Abbérefractometer (Model: Bellingham RFM 330, Ser. No.016468, made in the UK) to determine moisture content, Conductivity meter (Model: SCHOTT handy lab LF11,Germany) to determine electrical conductivity, Electric furnace, adjustable to 600°C and hotplate (ash content), pH meter (Model: Elmetron CPI-501, Poland) to determine pH, Spectrophotometer operating in a wavelength range including 284 and 336 nm (HMF), Automatic titration for free acidity determination (titratable with sodium hydroxide) and optical comparators Pfund index grading for determination of honey color with a Pfund chromometer (Hanna Instruments, Padova, Italy).

## **2.7. Physicochemical and Heavy Metals Analysis in Honey Samples**

### **2.7.1. Determination of Physicochemical Parameters**

To determine the physicochemical parameters, the honey samples were analyzed according to AOAC (1999), (Codex Alimentarius commission, 2001), (EU, 2002), (IHC, 2009), and Ethiopian Standard (2005) to determine moisture content, HMF, free acidity, pH, electrical conductivity, ash content and color. Double replicates were used for each honey sample.

#### **Moisture content**

Homogenize the prepared sample and put in a flask. Close the flask and place in a water bath at 50°C ( $\pm 0.2$ ) until all the sugar crystals are dissolved. Cool the solution to room temperature and stir again. Ensure that the prism of the refractometer is clean and dry. Directly after homogenization, cover the surface of the prism evenly with the sample. After 2 minutes (Abbe refractometer) read the refractive index. Measure each honey twice and take the average value. Read the corresponding moisture content from the table (Codex Alimentarius Standard, 2001).

#### **pH**

The pH of the sample was measured by pH meter (Model: Elmetron CPI-501, Poland), which was calibrated with standard buffer solutions of pH 4.0 and 7.0. Approximately 10

g of honey was mixed with 75 mL of distilled water in 150 mL beaker. The pH was recorded while stirring the solution (IHC, 2009).

#### Free Acidity

Free acidity was determined by mixing 10 g of honey with 75 mL of distilled water and then the mixture was titrated with 0.1 M NaOH solution until pH value became 8.3. Free acidity in milliequivalents of acids/kg of honey was calculated using AOAC 1990 official method 962.19 as follows, Free acidity (meq/kg) = volume of 0.10 M NaOH (mL) x 10, where, 10 indicates the dilution factor of honey sample during analysis (IHC, 2009).

#### Ash content

About 5 g of each honey sample was weighed out into previously weighed porcelain crucible. Organic matter was charred by igniting the sample on a hot plate in the fume cupboard. The crucible was then placed in the muffle furnace and heated at 600°C for 6 h. After complete ignition to constant weight, the sample was cooled in a desiccator and weighed immediately (AOAC, 1999). The percent ash was calculated as:  $\text{Ash (\%)} = \frac{W1 - W2}{W0} \times 100$

Where: W0 = weight of honey sample taken (g), W1 = weight of crucible + ash (g) and W2 = weight of crucible (g).

#### Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) was determined using a spectrophotometric method as per the International Honey Commission guidelines (IHC, 2009). Accurately weigh approximately 5g of honey into a 50 ml beaker. Dissolve the sample in approximately 25 ml of water and transfer quantitatively into a 50 ml volumetric flask. Add 0.5 ml of Carrez solution I (dissolve 15 g of potassium hexacyanoferrate (II),  $K_4Fe(CN)_6 \cdot 3H_2O$  in water and make up to 100 ml), and mix. Add 0.5 ml of Carrez solution II (dilute 30 g of zinc acetate,  $Zn(CH_3COO)_2 \cdot 2H_2O$  and make up to 100 ml), mix and make up to the mark with water (a drop of ethanol may be added to suppress foam). Filter through paper; rejecting the first 10 ml of the filtrate. Pipette 5.0 ml in each of two 2 test tubes (18 x 150 mm). Add 5.0 ml of water to one of the test tubes and mix well (the sample solution).

Add 5.0 ml of sodium bisulphite solution 0.2% to the second test tube and mix well (the reference solution). The use of Carrez solutions (I and II) clarified the honey samples by precipitating proteins and colloidal materials, ensuring a clear filtrate for accurate absorbance readings. The absorbance of the sample solution was measured at 284 nm to determine HMF concentration, with 336 nm as a reference wavelength to correct for background interference. This dual-wavelength approach enhances specificity and accuracy, aligning with established international standards (Codex Alimentarius, 2001; IHC, 2009). Determine the absorbance of the sample solution against the reference solution at 284 and 336 nm in 10 mm quartz cells within one hour. The calculation is as follow,

$$\text{HMF in mg/kg} = (A_{284} - A_{336}) \times 149.7 \times 5 \times D/W \text{ (equation 1),}$$

Where: -  $A_{284}$  = absorbance at 284 nm,  $A_{336}$  = absorbance at 336 nm, 149.7 is constant, 5 = theoretical nominal sample weight, D = dilution factor, in case dilution is necessary and W = Weight in g of the honey sample.

#### Electrical Conductivity

Conductivity meter (Model: SCHOTT handy lab LF11, Germany) was used to determine electrical conductivity of the sample. About 20 g of sample was mixed with 100 mL of distilled water in a 150 mL beaker and the mixture was stirred for about 1 h (Adenekan *et al.*, 2010). The instrument was calibrated using 0.01M potassium chloride (KCl), which has an electrical conductivity of 1413 mS/cm at 25°C.

#### Color

The undiluted honey samples were filtered with Whatman filter paper and 2 cm<sup>3</sup> of each filtrate was measured directly with a Pfund chromometer (Hanna Instruments, Padova, Italy) (Guo *et al.*, 2019) to determine the colour in millimeter (mm) Pfund scale. The colour of honey were classified based on the Pfund scale readings: average scale reading  $\leq 8$  mm Pfund: water white; 8 mm Pfund < reading  $\leq 16$  mm Pfund: extra white; 16 mm Pfund < reading  $\leq 34$  mm Pfund: white; 34 mm Pfund < reading  $\leq 50$  mm Pfund: extra light amber; 50 mm Pfund < reading  $\leq 85$  mm Pfund: light amber (Guo *et al.*, 2019).

### 2.7.2. Optimization of the working procedure of heavy metals and detection using FAAS

To create a clear and colorless sample solution suitable for analysis, different digestion methods for honey samples were tested using a mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. The evaluation focused on varying the reagent volumes, digestion times, and temperatures. The optimal method was selected based on the clarity of the digested sample, efficiency in digestion time, lower temperature requirements, minimal reagent usage, and overall simplicity. Each procedure, with adjustments in reagent volume, time, and temperature, was tested in three trials to ensure accuracy. The heavy metal content was analyzed using Flame Atomic Absorption Spectrometry (FAAS), a method recognized for its precision and effectiveness in food safety analysis. Although FAAS is less sensitive than ICP-MS, it was chosen due to resource limitations and its proven reliability in detecting trace metals in honey.

### 2.7.3. Digestion method for heavy metals detection

Sample pretreatment is often required to break down the organic matrix and extract metal ions from inorganic complexes. In this work, we used a wet (acid) digestion method (Bogale, *et al.*, 2019). To reduce contamination, all glassware was first cleaned with 10% HNO<sub>3</sub>, rinsed thoroughly with distilled water, and dried in an oven before use. Precisely 5 grams of each honey sample were weighed and placed into beakers. Next, 30 mL of 70% HNO<sub>3</sub> and 30 mL of 30% H<sub>2</sub>O<sub>2</sub> were added to each sample. The mixture was gently stirred and heated under a fume hood at 270°C for 180 minutes. During heating, the organic components evaporated as red gases, which gradually shifted to yellow and then to white. Once the samples had turned dry and white, they were allowed to cool to room temperature. The solution was then filtered using filter paper, transferred to a volumetric flask, and diluted to 50 mL with distilled water and 0.5% HNO<sub>3</sub>. This digestion process resulted in a clear, colorless solution. To account for any potential contamination, a blank digestion was also carried out under identical conditions, ensuring accurate corrections for any blank effects. Subsequently, all digests will be refrigerated until analysis using flame atomic absorption spectrometry, maintaining sample integrity before analysis. This rigorous approach will ensure precise determination of metal concentrations in the honey samples.

#### 2.7.4. Method validations

Method validation ensures that the analytical procedure used for a specific test is reliable. The results from this validation process are essential for assessing the quality, reliability, and consistency of the analysis. To validate the analytical method, the following parameters were evaluated: recovery test, method detection limit, precision and accuracy (Ravisankar. *et al.*, 2015).

Recovery test: The recovery test of this work was obtained from spiking experiments as

$$\% \text{ Of Recovery} = \frac{\text{conc. after spike} - \text{conc. before spike}}{\text{Conc. Of added sample}} \times 100\%$$

Recovery values of the method within 95.5-105% range are acceptable (Bogale, *et al.*, 2019).

Method detection limit: The method detection limit (MDL) refers to the lowest concentration of an analyte that can be detected with 99% confidence that the concentration is greater than zero, although it may not be precisely quantified. The MDL was calculated by multiplying the standard deviation of the blank concentration (SD) by a factor of 3. The MDL for each element was determined based on procedures outlined in previous studies (Bogale, *et al.*, 2019).  $MDL = 3 \times SD$

#### 2.8. Statistical analysis

All concentration measurements of heavy and trace metals were performed in triplicate, and the results were reported as the mean  $\pm$  standard deviation (SD). Results of physicochemical parameters were double analyzed. The data for minimum, maximum, standard deviation, coefficient of variation, and least significant difference values for the measured concentrations were organized into tables. Analysis of variance (ANOVA) was conducted using SAS software version 9.1.3 to determine if there were significant differences in metal concentrations across the honey samples. Additionally, Pearson's correlation among the physicochemical parameters and heavy metal concentrations in the honey samples was calculated using SPSS software version 20

### 3. CHAPTER III: RESULTS

#### 3.1. Determination of Physicochemical Parameters

The physicochemical results of the samples are presented in Table 1 as mean  $\pm$  standard deviation of duplicate analyses. Highest Moisture content was observed in Enderta (20.5%) and the list was reported in Atsbi (16.8%), followed by Free acidity which ranges from 8.5 meq/kg to 19.5 meq/kg, pH (4.44 to 5.505), HMF (0.8mg/kg to 15.95mg/kg), electrical conductivity (0.215 to 0.645 mS/cm) and ash content (0.045 to 0.29%) and colour of the honey was found to be from white to extra light amber.

Table 1. Physicochemical properties of honey samples (mean  $\pm$  standard deviation (n=2)).

Parameters	Location of Honey Sample from honey producers (n=6)						Significance (p-value)
	DT	AT	WU	ET	ME	KT	
MC (%)	18.4 $\pm$ 0.283	16.8 $\pm$ 0.283	19.6 $\pm$ 0.283	20.5 $\pm$ 0.141	19.7 $\pm$ 0.141	18.6 $\pm$ 0.000	0.000**
pH	4.44 $\pm$ 0.283	4.605 $\pm$ 0.035	4.675 $\pm$ 0.247	4.455 $\pm$ 0.021	4.75 $\pm$ 0.014	5.505 $\pm$ 0.205	0.0016*
HMF (mg/kg)	11.35 $\pm$ 1.060	8.15 $\pm$ 3.465	4.1 $\pm$ 1.980	0.8 $\pm$ 0.141	13.9 $\pm$ 8.485	15.95 $\pm$ 4.313	0.0680 <sup>ns</sup>
Ash content (%)	0.065 $\pm$ 0.007	0.275 $\pm$ 0.007	0.29 $\pm$ 0.014	0.235 $\pm$ 0.035	0.26 $\pm$ 0.028	0.045 $\pm$ 0.021	0.0001*
EC (mS/cm)	0.25 $\pm$ 0.141	0.62 $\pm$ 0.141	0.645 $\pm$ 0.021	0.55 $\pm$ 0.056	0.595 $\pm$ 0.049	0.215 $\pm$ 0.035	0.0001*
FA (meq/kg)	19.5 $\pm$ 0.707	9.5 $\pm$ 0.707	10.5 $\pm$ 0.707	23 $\pm$ 2.828	16 $\pm$ 2.828	8.5 $\pm$ 0.707	0.0007*
Color	Extra light amber(50mm)	White(8mm)	White(8mm)	Extra light amber(50mm)	Extra light amber(50mm)	Extra light amber(50mm)	Pfund (8mm to 50mm)

\*\* Significant at (p<0.001); \* significant at (p<0.05). ns No significant at (p>0.05).

The results in table 2 shows that obtained results for the physicochemical analysis of the six honey samples collected from the study areas were compared with both national and international standards and it was within the acceptable level.

Table 2. Comparison of parameters with national and international standards

Parameters	Study area results						Standards		
	DT	AT	WU	ET	ME	KT	QSAE	EU	CAC
MC (%)	18.4±0.283	16.8±0.283	19.6±0.283	20.5±0.141	19.7±0.141	18.6±0.000	≤ 21	≤ 21	≤21
pH	4.44±0.283	4.605±0.035	4.675±0.247	4.455±0.021	4.75±0.014	5.505±0.205	3.4–6.1	-	3.2-4.5
HMF (mg/kg)	11.35±1.060	8.15±3.465	4.1±1.980	0.8±0.141	13.9±8.485	15.95±4.313	≤40	≤40	≤60
Ash (%)	0.065±0.007	0.275±0.007	0.29±0.014	0.235±0.035	0.26±0.028	0.045±0.021	≤ 0.6	≤0.6	0.25-1
EC (mS/cm)	0.25±0.141	0.62±0.141	0.645±0.021	0.55±0.056	0.595±0.049	0.215±0.035	-	-	0.8
FA (meq/kg)	19.5±0.707	9.5±0.707	10.5±0.707	23±2.828	16±2.828	8.5±0.707	≤ 40	≤ 40	≤ 50

CAC = Codex Alimentarius Commission; mS/cm=milli Siemes/centimeter; EU = European Union; mg/kg =milligram/ kilogram; meq = mill equivalent; QSAE = Quality and Standards Authority of Ethiopia; (MC) Moisture content, (FA) Free acidity; (HMF) hydroxyl methyl furfur aldehyde, and (EC) Electrical conductivity; DT refers to (Degua Tembien), AT (Atsbi), WU (Wukro), ET (Enderta), ME (Mekelle) and KT (Kola Tembien)

The findings on physicochemical properties analysis of honey collected from different agro-ecologies is presented below (Table 3). MC was reported highest in midland (19.93%) and lowest in highland (17.6%), followed by free acidity (8.5meq/kg in lowland to 16.5meq/kg in midland), HMF (9.75mg/kg in highland to 15.95mg/kg in lowland) and the least result was recorded ash content (0.045% in lowland to 0.267% in midland).

Table 3. Physicochemical properties of honey collected from different agro-ecologies

Parameters	Samples from Honey producers (n=6)			Significance (p-value)
	Highland (n=2) From (DT and AT)	Midland (n=3) From (WU, ET and ME)	Lowland (n=1) From (KT)	
MC (%)	17.6±0.283	19.93±0.188	18.6±0.000	0.000**
pH	4.523±0.159	4.627±0.094	5.505±0.205	0.00053**
HMF (mg/kg)	9.75±2.262	6.267±3.535	15.95±4.313	0.0226*
Ash content (%)	0.17±0.007	0.262±0.026	0.045±0.021	0.0000**
EC (mS/cm)	0.435±0.141	0.597±0.042	0.215±0.035	0.0000**
FA (meq/kg)	14.5±0.707	16.5±1.414	8.5±0.707	0.00023**

\*\* Significant at (p<0.001); \* significant at (p<0.05). ns No significant at (p>0.05). Number of Honey samples (n) = 6

The correlations (positively or negatively) with their significance between the physicochemical parameters were investigated using Pearson correlation (Table 4).

Table 4. Correlation coefficients between physicochemical parameters

Parameters	MC	pH	HMF	Ash content	EC	FA
MC	1					
pH	-14.195 0.000	1				
HMF	-9.891 0.000	4.303 0.064	1			
Ash content	-18.7383 0.000	-4.543 0.040	-8.846 0.000	1		
EC	-18.4542 0.000	-4.259 0.070	-8.562 0.000	0.284 1.000	1	
FA	-4.433 0.050	9.761 0.000	5.458 0.006	14.305 0.000	14.020 0.000	1

### 3.2. Levels of heavy metals in the honey samples

The results found for the total concentration of the mineral elements (Cu, Fe, Mn, Cr, Zn and Pb) in honey samples are presented in Table 6. From the analyzed metals (Cu, Zn, Mn and Fe) were detected in the honey samples. As shown in (Table 6), Fe was the most abundant metals analyzed in honey collected at all the study sites compared with other metals. The concentration of this element ranged from 6.58 to 25.36 mg/kg. However, no detection of metals Cr and Pb were reported in both study areas.

## Validation methods

In the case of validation method, more than 95% recovery test result was recorded (Table 5), which indicates that the test result is appeared to be credible enough, as all the recovery values are within the acceptable range of 80-120% for metal analysis (Bogale *et al.*, 2019).

Table 5. Detection and quantification limits for heavy metals in the honey samples

Metals	IDL (mg/kg)	MDL (mg/kg)	LOQ (mg/kg)	Recovery test (%)
Fe	1.95	2	0.02	97.5
Cu	2.03	2	0.01	101.5
Mn	2.1	2	0.02	105
Cr	2.05	2	0.02	102.5
Zn	1.91	2	0.05	95.5
Pb	2.05	2	0.02	102.5

IDL: Instrument Detection Limit, MDT: Method Detection Limit, LOQ: limit of quantification

The result in (Table 6) shows that heavy metals in honey from the samples were detected using FAAS (Flame Atomic Absorption spectrophotometry). Pb (lead) and Cr (chromium) were not detected in all honey samples, whereas Fe (iron) was recorded highest (25.36mg/kg) among all metals in Dogua Tembien but small amount of Cu (Copper), Mn (Manganese), Zn (Zinc) was recorded (0.08 to 0.19mg/kg, 0.20 to 2.74mg/kg, 0.01 to 1.90mg/kg) respectively.

Table 6. The level of heavy metals detected honey samples and compare with international guidelines

Metals	Location of Honey Sample from honey producers (n=6)						(p-value)	WHO,2022 in drinking water	CAC,1995 in Honey	FAO/WHO,2004 in foods (General nutrient guide line)
	DT	AT	WU	ET	ME	KT				
Cu(mg/kg)	0.18±0.036	0.04±0.020	0.09±0.030	0.08±0.264	0.19±0.036	0.11±0.264	0.0003*	2mg/l	-	2mg/kg
Fe(mg/kg)	25.36±2.495	9.25±0.661	7.49±0.944	11.70±1.374	6.58±1.368	15±2.000	0.0000**	-	-	30mg/kg
Mn(mg/kg)	0.38±0.121	2.74±1.264	0.06±0.017	0.26±0.046	1.10±0.200	0.20±0.050	0.0003*	0.4mg/l	-	-
Cr(mg/kg)	ND	ND	ND	ND	ND	ND	-	0.05mg/l	-	-
Zn(mg/kg)	0.41±0.036	0.44±0.053	1.90±0.264	0.01±0.000	0.26±0.020	0.21±0.026	0.0000**	0.01- 0.05mg/l	-	10mg/kg
Pb(mg/kg)	ND	ND	ND	ND	ND	ND	-	0.01mg/l	0.1-0.2mg/kg	1mg/kg

N.B. ND refers to no detect, Cu (Copper), Fe (Iron), Mn (Manganese), Cr (Chromium), Zn (Zinc), and Pb (lead). \*\* Significant at (p<0.001); \* significant at (p<0.05). Results are presented in Mean ± standard deviation, which is calculated from the triplicated sample value (each sample was repeated three times to avoid technical bias).

The correlations between the heavy metals were investigated using Pearson correlation (Table 7), and Fe had highly significance with Cu (positively related) and Mn, Cr, Zn and Pb (negatively relation).

Table 6. Correlation coefficients between heavy metals detected in the honey samples

<b>Metals</b>	<b>Cu</b>	<b>Fe</b>	<b>Mn</b>	<b>Cr</b>	<b>Zn</b>	<b>Pb</b>
Cu	1					
Fe	12.448 0.000	1				
Mn	0.675 1.000	-11.773 0.000	1			
Cr	-0.115 1.000	-12.563 0.000	-0.79 1.000	1		
Zn	0.423 1.000	-12.025 0.000	-0.251 1.000	0.538 1.000	1	
Pb	-0.115 1.000	-12.563 0.000	-0.79 1.000	0 1.000	0.538 1.000	1

Results in (Table 8) shows detection of heavy metals on different agro-ecology Fe (Iron) was reported highest in highland (17.305mg/kg) and lowest in midland (8.59mg/kg) followed by Mn (Manganese) 0.20mg/kg in lowland to 1.56mg/kg in highland, Zn (Zinc) 0.21 in lowland to 0.72mg/kg in highland) but Pb (Lead) and Cr (Chromium) were not detected in all agro-ecology of the study area.

Table 7. Heavy metals detected in honey samples collected from different agro-ecologies

Metals	Samples from Honey producers (n=6)			Significance (p-value)
	Highland (n=2) From DT and AT	Midland (n=3) From WU, ME and ET	Lowland (n=1) From KT	
Cu	0.11±0.028	0.12±0.11	0.11±0.264	0.0001**
Fe	17.305±1.578	8.59±1.229	15±2.000	0.0000**
Mn	1.56±0.693	0.473±0.088	0.20±0.050	0.0001**
Cr	ND	ND	ND	-
Zn	0.425±0.045	0.723±0.095	0.21±0.026	0.0000**
Pb	ND	ND	ND	-

\*\* Significant at (p<0.001); \* significant at (p<0.05). ns No significant at (p>0.05).

#### 4. CHAPTER IV: DISCUSSION

The findings of this study have unveiled the physicochemical composition of a pooled honey sample collected from six different sites, likely representing three distinct agroecologies. The moisture content, an indicator of maturity level, ranged from 16.8% to 20.5%, suggesting optimal harvesting conditions and a satisfactory degree of maturity. The moisture content of honey is the primary quality criterion that dictates its stability and ability to resist spoilage from yeast fermentation and mold growth. The moisture levels in the examined honey samples ranged from 16.8% to 20.5% (Table 1), which falls within the acceptable limit ( $\leq 21\%$ ) set by national and international quality standards (QSEA, 2005; CAC, 2001). Overall, the honey from Atsbi district exhibited the lowest moisture content ( $16.8 \pm 1.74\%$ ) among the samples analyzed, followed by Degua Tembien ( $18.4 \pm 0.283\%$ ), Kola Tembien ( $18.6 \pm 0.000\%$ ), Wukro ( $19.6 \pm 0.283\%$ ), and Mekelle ( $19.7 \pm 0.141\%$ ). In contrast, Enderta honey displayed the highest moisture content value at  $20.5 \pm 0.141\%$ .

The elevated moisture content in the Enderta district may result from harvesting unripe honey and/or improper handling during the honey harvesting process. The results of this study on moisture content were also in agreement with the findings of (Olubusayo *et al.*, 2024), who reported 13.9 to 19.04 %, (Anza *et al.*, 2022) who reported 16.7 to 20.7%, and (Mulugeta and Tadesse, 2017) who reported in the range of 16.61 to 18.64% in Ethiopian honey. Adudna *et al.* (2020) also reported similar moisture content values (14.23–19.2%) of honey samples from different origins. Higher moisture content was recorded in the previous studies by (Gebeyehu and Jalata, 2023; Amare and Arega, 2018; Tefera, 2021) who reported (17.8 to 23%, 15.3 to 21.9% and 16 to 22.9%. According to the Ethiopian Standard for moisture content, ES (2005), honey is classified into three grades: Grade A (17.5–19.0%), Grade B (19.1–20.0%), and Grade C (20.1–21.0%). The honey samples from three districts—Atsbi, Degua Tembien, and Kola Tembien in the Tigray region—are categorized as ‘Grade A’. Honey from two districts, Wukro and Mekelle, falls under “Grade B,” while honey from Enderta district is classified as “Grade C,” in accordance with the Ethiopian standard (ES, 2005). Higher moisture content increases the likelihood of honey fermentation during storage. Conversely, lower moisture levels ( $< 21\%$ ) extend honey's shelf life while in storage. However, honey's

moisture content is influenced by various factors, including the harvesting season, the maturity level achieved in the hive, and climatic conditions (Finola *et al.*, 2007). Overall, the low moisture content in this study indicates that all the samples have good storage ability and quality.

The ash content of the analyzed honey samples varies significantly. The values ranged from 0.045% to 0.29%, with the lowest recorded in Kola Tembien (0.045%) and Degua Tembien (0.065%), while the highest value was noted in Wukro (0.29%) (Table 1). The observed ash content (0.045%-0.29%) exceeds the findings of Mulugeta and Tadese (2017), who reported a range of 0.030% to 0.095%. Additionally, Gebremariam and Brhane (2014) noted lower values for honeys from Bizet (0.15%), Edagahamus (0.13%), and Fatsi (0.10%) compared to those from study areas Atsbi (0.275%), Mekelle (0.26%), Enderta (0.235), and Wukro (0.29%).

However, this is lower than the findings of Gebremariam and Brhane (2014), who reported ash content in honeys collected from Fireweyni (0.43%), Hawzien (0.34%), and Adigrat (0.54%). Olubusayo *et al.* (2024) observed values ranging from 0.099% to 0.600%, while Alemayehu *et al.* (2023) noted a range of 0.03% to 2.4%. Additionally, Yohannes *et al.* (2018) reported honey intended for commercial purposes with ash content recorded at Beza (0.46%) and Tutu (0.31%). The ash content was determined to be within acceptable quality standards, and variations in the ash content among different honeys may be attributed to soil characteristics and the mineral composition present in the nectars.

The study found that there was color diversity among the honey samples ranged from white to extra light amber, which is in accordance with the Pfund scale (Kayode and Oyeyemi, 2014). The diversity in honey color is common, and a previous study also reported the diversity ranged from colorless to amber and dark amber to black. Pfund color scale of honeys samples from Wukro, Atsbi, Degua Tembien, Kola Tembien, Enderta and Mekelle were 8, 8, 50, 50,50, and 50 (mm, Pfund) respectively. The color was determined in mm on the Pfund scale according to the U.S. Department of Agriculture classifications (water white: <9, extra white: 9–17, white: 18–34, extra light amber: 35–50, light amber: 51–85, amber: 86–114, dark amber: >114). According to the

Codex standard (Pfund color: 0–150 mm), Esriche *et al.* (2017) reported a higher value range (10.4 – 14.1 cm, Pfund) for honey samples from Mozambique. The difference between honey samples from (Wukro and Atsbi) and (Enderta, Mekelle, Degua Tembien, and Kola Tembien) might be floral sources, mineral contents, and locations could be classified as extra light amber and white (Table 1). These findings show that honey from these areas was of very high quality and confirms the results earlier reported by Babarinde *et al.* (2011). The commercially available honey varied greatly in quality due to its color, flavor, and density over the globe. The honey color is closely connected with botanical origin and is imperative to assess the honey quality. Light-colored honey has a mild flavor, while dark honey has more concentrated and richer flavor. (Kayode and Oyeyemi, 2014).

Free acidity is related to the source of nectar, bee species, and the action of enzymes or bacteria (Machado *et al.*, 2018). It indicates one of the quality parameters of honey and reveals whether the honey is fermented or not (Silvano *et al.*, 2014). International regulations specify a free acidity not higher than 50 meq/kg honey (CAC, 2001; EU, 2002)). There was a significant difference in the free acidity between the six types of honey samples ( $P < 0.05$ ). This result revealed the freshness of honey samples and the absence of unwanted fermentation (Reshma *et al.*, 2016), in which it ranged from  $8.5 \pm 0.707$  to  $23 \pm 2.828$  meq/kg (Table 1). These values are within the limit (below 50 meq/kg) indicating an absence of undesirable reactions (fermentations). The finding of this study was lower than the findings of (Anzal *et al.*, 2022; Amare and Arega, 2018; Raweh *et al.*, 2023) who reported 19.5 to 37, 28.80 to 40.40, 7 to 37 respectively but higher than the findings of (Gebeyehu and Jalata, 2023; Gebremariam and Tadese, 2014) who reported 5.8 to 11.8, 5.37 to 12.43 respectively.

According to (Marchini *et al.*, 2004) differences in acidity in honey might be due to floras or differences in a season of honey harvesting. Honey fermentation causes an increase in acidity value, i.e., as the honey acidity increases, the sourness of honey becomes higher. The acidity of Wukro district  $10.5 \pm 0.707$  meq/kg and Kola Tembien district  $8.5 \pm 0.707$  meq/kg of the honey was found similar to findings of (Mulugeta and Tadese, 2017) who reported 10.47 meq/kg and 8.502 meq/kg in the Oromia region of

Jeldu and Adaberga districts respectively. According to the findings of (Gebremariam and Brhane, 2014; Adugna *et al.*, 2020) who reported in different districts of Tigray region Adigrat in meq/kg (45.17, 33.3), Erop (31 meq/kg), Hawzien (30.6 meq/kg), was higher than the findings of this study whereas in Fatsi (4.58 meq/kg), Freweyni (4.59 meq/kg), Hawzien (3.99meq/kg), Mugulat (6.01 meq/kg), Zalambessa (4.15 meq/kg) was lower than the findings of this study. None of the samples exceeded the limit set, which may be taken as indicative of the freshness of all the honey samples in the study area. Variation in free acidity among different honeys can be attributed to the floral origin or variation in the harvest season (Reshma *et al.*, 2016).

Honey is acidic in nature and varies by the nectar and soil types (Nordin *et al.*, 2018). The pH values of the six honey samples were acidic 4.44 to 5.505 (Table 1) and are found within the range of the standard limit (3.40–6.10) (Codex Alimentations, 2001). According to (Gashaw *et al.*, 2024; Tefera *et al.*, 2021; Mulugeta and Tadese, 2017) the pH of the study area was higher than honeys of studied in the districts of Ethiopia Beyeda, D/markos, Gondar, Jimma, Adigrat, Janamora, Ambo, Dendi, Jeldu, Ejere, Adaberga and Tokekutaye (3.93, 3.91, 3.84,3.85,4.00, 3.83, 3.79, 3.820, 4.010, 4.222, 4.091 and 3.771) respectively but the similar result was recorded honeys of Dogua Tembien (4.44) with honeys of Debark(4.43) and honeys of Wukro (4.675) with honeys of Dabat (4.645). However, it is lower than reported by (Gebremariam and Brhane, 2014) in the study areas of Dogua Tembien (4.44) and Enderta (4.455) districts with honey from Edaghamus (4.65), Fatsi (4.58) and Fireweyni (4.59). The honey pH of the study was found within acceptable quality limits. Differences in pH might be due to factors like nectar source, the substances added by the honeybees, the method of processing, and soil pH (Haftu *et al.*, 2015). There was a significant difference recorded between the six studied types of honey concerning pH values ( $P < 0.05$ ). The low pH of honey has the advantage to prevent the presence and growth of microorganisms. In addition, the pH of honey mainly indicates the buffering action of the inorganic cation constituents of the acids present. pH values have great importance during the extraction and storage of honey as they influence the texture, stability and shelf life of honey (Reshma *et al.*, 2016).

The electrical conductivity (EC) of the investigated honey samples ranged from 0.215 to 0.645 mS/cm (Table 1). The Wukro, Atsbi, Mekelle and Enderta honey samples showed the highest EC ( $0.645 \pm 0.021$ ,  $0.62 \pm 0.141$ ,  $0.595 \pm 0.049$  and  $0.55 \pm 0.056$  mS/cm), respectively (Table 1). On the other hand, Kola Tembien and Dogua Tembien honey samples showed the lowest EC ( $0.215 \pm 0.035$  and  $0.25 \pm 0.0141$  mS/cm), respectively. All the values were below the maximum limit of electrical conductivity ( $\leq 0.800$  mS/cm) for honey recommended by the national and international quality regulations (QSEA, 2005; CAC, 2001; EU, 2002). There was a significant difference in the EC between examined samples ( $P < 0.05$ ). This result was lower than a previous study by (Olubusavo *et al.*, 2024; Amare and Arega, 2018; Raweh *et al.*, 2023) who reported (15 to 536mS/cm, 0.50 to 0.94mS/cm and 0.88mS/cm) respectively.

Whereas study by (Gashaw *et al.*, 2024) honey from selected areas of Ethiopia districts D/markos, Jimma, and Adigrat (0.14mS/cm, 0.09mS/cm and 0.18mS/cm) was lower than this study. Similarly lower results were recorded by (Tefera, 2021) honey from North Gondar in (Beyeda, Dabat, Debark, and Janamora) who reported (0.333mS/cm, 0.401Ms/cm, 0.392mS/cm, and 0.360mS/cm) respectively was lower than honey from these study areas (districts) in Wukro, Enderta, Mekelle and Atsbi (0.645mS/cm, 0.55mS/cm, 0.595mS/cm, and 0.62mS/cm) respectively. This variation might be depending on the botanical origin of the honey. The result of this study agreed with previous studies by (Mulugeta and Tadese, 2017) honey produced in the West Shewa zone, Oromia of different districts in Ambo ( $0.553 \pm 0.083$ ) and Dendi ( $0.545 \pm 0.065$ ) with honey from this study area in Enderta ( $0.55 \pm 0.056$ ) as well as previous study by (Gebremariam and Brhane, 2014) honey from Adigrat and its surrounding areas in Azeba, Dawhans and Mugulat who reported (0.22mS/cm, 0.26mS/cm and 0.20) was slightly similar with honey from this study areas of Dogua Tembien (0.25mS/cm) and Kola Tembien (0.215mS/cm).

Hydroxy methyl furfural content of the honey samples ranged from 0.8 to 15.95 mg/kg (Table 1) with Enderta and Kola Tembien having the lowest and highest values, respectively. According to (Abera and Alemu, 2023) who reported 22.7–65.1mg/kg, (Olubusayo *et al.*, 2024) who reported a maximum of 36.42mg/kg, (Gebeyehu and

Jalata, 2023) who reported (40.32 to 81.14mg/kg), (Gebremariam and Brhane, 2014) who reported in different Tigray districts, Adigrat (38.58mg/kg), Bizet (45.26mg/kg), Edagahamus (21.15mg/kg), Fatsi (30.52mg/kg), Fireweyni (23.26mg/kg), Hawzien (26.54mg/kg) and Zalambessa (26.33mg/kg) was recorded higher than this study. Lower results were recorded from a previous study by (Gebremariam and Brhane, 2014) from Tigray districts of honey from Mugulat, Azeba, and Dawhans (10.49mg/kg, 11.64mg/kg and 12.35mg/kg) respectively but results from this study districts were Kola Tembien and Mekelle (15.95mg/kg and 13.9mg/kg) respectively. This difference might be due to when honey is subjected to high temperatures, and inadequate storage conditions (Nascimento, 2015). The result of this study slightly agreed with the previous study which was reported by (Anzal et al., 2022) from 8.4 to 16.4mg/kg of honey produced in the ArbaMinch Zuria district of Gamo zone, Southern Ethiopia. There was no a significant difference recorded between the six studied types of honey concerning HMF values ( $P > 0.05$ ).

Several factors influence the levels of HMF, such as temperature and time of heating, storage conditions, pH, and type of honey, thus it provides an indication of overheating and storage in poor conditions (Ahmed, 2014). According to (Nascimento, 2015) when honey is subjected to high temperatures, inadequate storage conditions, or the addition of inverted sugar, the HMF content increases and it is one of the most common degrading products in honey, indicating its aging. The finding of this study was within the limit set by national and international standards (QSEA, 2005; CAC, 2001; EU, 2002) indicating that all the samples have good storage ability and quality.

Both parameters based on agro ecological had significantly influenced the honey quality, that is agro ecology had a highly significant ( $p < 0.001$ ) effect on the average MC, ash content, HMF, free acidity, pH and electrical conductivity of honey obtained from three agro ecologies (Table 3). MC (moisture content) is the primary criteria for honey quality. From the finding of this study MC, was compared based on agro-ecology with previous researches and was higher than (Yeserah, 2018) who reported  $16.82 \pm 0.81$  in midland and  $15.59 \pm 1.51$  in lowland but finding of this study was  $19.93 \pm 0.188$  and  $18.6 \pm 0.00$  in midland and lowland respectively. Similarly reports from (Thakur *et al.*, 2024) who reported (15.90%, 15.80% and 16.50%) in highland, midland and lowland respectively

were lower than MC of this study (17.61%, 19.93% and 18.6%) in highland, midland and lowland respectively. The difference in MC (moisture content) in agro-ecology may be due to different climatic conditions, hive temperature, extraction techniques, degree of storage and degree of maturity (Gariola *et al.*, 2013). The findings of the other parameters (pH, ash content, electrical conductivity, free acidity and HMF) of honey quality was found in the ranges of (agree) with the previous studies by (Thakur *et al.*, 2021; Anza *et al.*, 2022; Yeserah, 2018).

detection of heavy metals on different agro-ecology Fe (Iron) was reported highest in highland (17.305mg/kg) and lowest in midland (8.59mg/kg) followed by Mn (Manganese) 0.20mg/kg in lowland to 1.56mg/kg in highland, Zn (Zinc) 0.21 in lowland to 0.72mg/kg in highland) but Pb (Lead) and Cr (Chromium) were not detected in all agro-ecology of the study area.

The concentration of Cu, Fe, Mn, Cr, Zn, and Pb metals in the honey samples of six districts was determined by Flame Atomic Absorption Spectroscopy in honey harvested from Dogua Tembien, Atsbi, Wukro, Mekelle, Enderta, and Kola Tembien (Table 6). The concentration of essential minerals of Zn, Cu, and Mn became the highest value of the other metals, and the concentration of Pb in all samples was below the detection limits. The concentration of Iron is highest (6.58 mg/kg – 25.36 mg/kg) followed by Manganese (0.06 mg/kg – 2.74 mg/kg), Zinc (0.01 mg/kg – 1.90 mg/kg) and Copper (0.04 mg/kg – 0.19 mg/kg), whereas lead and chromium were not detected in all honey samples. The results show that the mean concentration values of the metals varied from one place to another.

There are no clear guidelines about the maximum permissible limits of metals in honey except Pb (lead) which is 0.1-0.2mg/kg. Codex Alimentarius Commission states that heavy metals in honey shall be at a level that does not pose a hazard to human health (CAC, 2001). But there is clear Guidelines for heavy metals in drinking-water quality (WHO, 2022) for (Chromium(Cr), Lead (Pb), Manganese (Mn), Copper (Cu) and Zinc (Zn) with guide line value of (0.05mg/Liter, 0.01mg/Liter, 0.4mg/Liter, 2mg/liter and 0.01-0.05mg/liter respectively, but no guideline value for iron (Fe) in drinking-water is proposed. Estimates of the minimum daily requirement for iron depend on age, sex,

physiological status and iron bioavailability and range from about 10 to 50mg/day. The current study shows Cr (ND), Pb (ND) and Cu (0.04- 0.18mg/kg) were below the permissible level heavy metals in drinking-water quality whereas Mn (0.06-2.74mg/kg), Zn (0.01-1.90mg/kg) and Fe (6.58-25.36mg/kg) were above the permit level of heavy metals in drinking-water quality which indicates that there is safety issue for consumers which great attention should be taken. The result of the current study were significant differences for (Cu, Fe, Zn, and Mn) in the study areas where ( $p < 0.05$ ).

As compared to the present study, higher mean concentrations of copper were reported as 0.176 to 0.243 mg/kg for different Anthropic Areas of Italy (Flamminii *et al.*, 2024), 4.524 to 288.298 mg/kg honey from three regions in Ghana (klutse *et al.*, 2024) and 1.72 to 2.91 mg/kg honey products from enugu metropolis ((Olubusayo *et al.*, 2024). while lower values were quoted honey from India (Bhalchandra and Baviscar, 2015) 0.004 to 0.015mg/kg, from different regions of Ethiopia in Bure 0.02 mg/kg and Bonga 0.026mg/kg (Adugna *et al.*, 2020), from commercially available honey in Ethiopia in Yeshi 0.09 mg/kg and Dima 0.091mg/kg (Yohannes *et al.*, 2018) which are lower than three districts of the present study in Dogua Tembien (0.18mg/kg), Mekelle (0.19mg/kg) and Kola Tembien (0.11mg/kg).

However, the range of the results obtained in this study is in agreement with those reported values of honey from different regions of Ethiopia (Adugna *et al.*, 2020) in Jimma (0.08mg/kg) and Erop (0.16mg/kg) agree with the result of this study in Enderta district (0.08mg/kg) and Dogua Tembien (0.18mg/kg) respectively. Similarly, honey from commercially available honey in Ethiopia (Yohannes *et al.*, 2018) from Yeshi (0.09mg/kg) agreed with the result of this study in the Wukro district (0.09mg/kg) hence, on average, the mean concentration of copper in the current study was within the ranges of previously reported values. The differences in the contents of copper in honeys of different regions/countries might be due to the floral type, the botanical origin, environment contamination, soil composition, production, and storage conditions (Pohl, 2009). In addition to this Crop mineralization, and the use of herbicides, pesticides, fungicides, and bactericides in the surrounding area contribute to the presence of copper in food products (Džugan *et al.*, 2017).

Among the heavy metals quantified in the six honey sample types, Fe content was the highest in Dogua Tembien honeys (25.36 mg/kg) and the lowest in Mekelle honeys. (6.58 mg/kg) (Table 6). The concentration of iron was higher compared to other honey from different regions in the world (3.267- 11.548mg/kg, 5.82- 15.31mg/kg, 0.495 – 3.346mg/kg, 0 – 6.76 mg/kg and 2.088 -8.794mg/kg) by (Kultse *et al.*, 2024; Alemu *et al.*, 2021; Czipa *et al.*, 2023; Mechoroa *et al.*, 2023; Aljedani, 2017) respectively. Besides, these values were lower than those found in honeys from Tlemcen Province, north-western and in United Arab Emirates honeys (11.7 – 59.6mg/kg and 1.15 – 110.79mg/kg) reported by (Bereksi *et al.*, 2020; Habib *et al.*, 2014) respectively, but similar Fe content was found honey from North Gondar, Ethiopia in four districts of Amhara region by (Tefera, 2021) in Beyeda, Dabat, Debark and Janamora ( 9.30mg/kg, 15.31mg/kg, 5.82mg/kg and 12.90mg/kg) respectively, agree with this study in Atsbi, Kola Tembien, Mekelle and Enderta (9.25mg/kg, 15mg/kg, 6.58mg/kg and 11.70mg/kg) respectively. Differences in concentration of Fe in honey might be, because plants in iron-rich regions transfer more iron into nectar, environmental pollution, farming practices, contamination, and botanical origin can all affect the amount of iron in honey.

Lead (Pb) and Chromium (Cr) were not detected in the analyzed honey samples, likely due to the low industrial activity and minimal environmental contamination in the sampling areas. These findings indicate the relative safety of honey produced in the selected districts. Whereas, there are reports where Pb and Cr are present in previous studies by (Flammini *et al.*, 2024; Klutse *et al.*, 2024; Adugna *et al.*, 2020; Czipa *et al.*, 2023) who reported (0.001 – 0.064mg/kg of Pb and 0.176 – 0.243mg/kg of Cr, 0.03- 0.828mg/kg of Pb and 0.042 – 0.078mg/kg of Cr, 0- 2.53mg/kg of Pb and 0 – 5.89mg/kg of Cr, and 0.0075 – 0.0147mg/kg of Pb and 0.015 – 0.0154mg/kg of Cr) respectively. This difference might be due to environmental contamination, the makeup of the soil and water, farming methods, and the botanical origin of nectar supplies, all of which differ by location. Furthermore, contamination can result from human activities including mining, industrial pollutants, and inappropriate management or storage, with closeness to pollution sources being a major factor. The investigation of this study also agrees with the report of (Tibebe *et al.*, 2022; Aljedani, 2017) who reported Pb and Cr was not detected in their research studies.

The manganese concentration of the analyzed honey types ranged from 0.06 mg/kg to 2.74 mg/kg with a maximum concentration of 2.74 mg/kg in Atsbi and a minimum in Wukro honey at 0.06 mg/kg. The investigated mean concentration of manganese was below all the reported results cited by (Adugna *et al.*, 2020; Bereksi *et al.*, 2020) who reported (0 – 7.29mg/kg and 1.36- 13.9) respectively. The result of this study is higher than the reports of many previous studies by (Tibebe *et al.*, 2022; Alemu *et al.*, 2021; Czipa *et al.*, 2023; Bibi *et al.*, 2008; Yohannes *et al.*, 2018) with a mean concentration of (0.83 – 1.01 mg/kg, 0.20 – 1.41 mg/kg, 0.25 – 1.185 mg/kg, 0.070 – 0.140 mg/kg and 0.16 – 0.88 mg/kg) respectively. But similar results were investigated with reports by (Gelaye, 2024; Aljedani, 2017) 0.952 – 2.545 mg/kg and 0.248 – 2.066 mg/kg respectively. The differences in manganese levels across various localities arise not only from the varying mineral content in the soil but also from the distinct types of plants that bees visit (Afzal *et al.*, 2014).

The Zinc (Zn) of the investigated honey samples ranged from 0.01 to 1.90 mg/kg. The Wukro honey samples showed the highest Zn ( $1.90 \pm 0.264$ ), (Table 6). On the other hand, Enderta honey samples showed the lowest Zn concentration ( $0.01 \pm 0.000$ ). There was a significant difference in the Zn between examined samples ( $P < 0.05$ ). The concentration of Zn in the present study was found higher when compared with reported concentration values from the pieces of literature (Amare and Arega, 2018; Bibi *et al.*, 2008; Aljedani, 2017; Mechoroa *et al.*, 2023) who investigated the concentration of (0.693 to 0.770 mg/kg, 0.1368 to 0.4717 mg/kg, 0.503 to 1.272 mg/kg and 0.46 to 1.16 mg/kg) respectively. This study was also lower than the previous research in different regions of the world cited by (Gashaw *et al.*, 2024; Adugna *et al.*, 2020; Yohannes *et al.*, 2018; Gebeyehu and Jalata, 2024; Flammini *et al.*, 2024; Alemu *et al.*, 2021; Bereksi *et al.*, 2020) who reported (4.2 to 10 mg/kg, 10.43 to 16.03 mg/kg, 1.10 to 4.22 mg/kg, 1.92 to 3.74 mg/kg, 0.157 to 2.956 mg/kg, 3.30 to 7.45 mg/kg and 0.2230 to 13.9 mg/kg) respectively. But fairly comparable with reports from (Bhalchandra and Bavisca, 2015; Czipa *et al.*, 2023; Tibebe *et al.*, 2022) who reported (0.01 to 1.75 mg/kg, 0.287 to 2.394 mg/kg and 1.97 to 2.04 mg/kg) respectively. The difference in concentration of zinc may be due to the floral type, the botanical origin, storage conditions anthropogenic factors such as season of the year, rainfall.

The Pearson correlation matrix was utilized to determine the relationship between the metal concentrations in honey and physicochemical characteristics. Free acidity, as shown in (Table 4) was strongly positively correlated with ash contents; pH, HMF, and electrical conductivity, indicating that increasing free acidity also increase these parameters (ash content, pH, HMF, and electrical conductivity). Electrical conductivity also showed a positive correlation with ash content, while moisture content was negatively associated with all these properties (free acidity, electrical conductivity, ash content, pH, and HMF), indicating that higher moisture reduces free acidity, electrical conductivity, ash content, pH, and HMF.

Regarding the metallic content, a negative correlation was observed Fe between all the metals (Mn, Cr, Pb, and Zn) in honey samples (Table 7) which correlation is significant ( $p < 0.05$ ), which suggests these metals may not arise from common sources as well as from chemical properties (Solomon *et al.*, 2018). But positive correlation was found between Fe and Cu, which suggests they may arise from similar sources. Significant correlations were not also found between the concentrations of Cu with (Pb, Mn, Cr, Fe, and Zn), Zn with (Mn and Cr), and Pb with (Mn, Cr, and Zn) all these correlations of metals were not significance ( $p > 0.05$ ).

## **5. CHAPTER V: CONCLUSION AND RECOMMENDATIONS**

The physicochemical analysis of honey samples from various locations revealed significant variations in quality parameters, which are crucial for determining the honey's marketability and safety. The moisture content of honey samples varied significantly, with the highest levels found in Enderta (20.5%) and the lowest in Atsbi (16.8%). High moisture content can lead to fermentation and crystallization, impacting the honey's preservation and storage. The pH values ranged from 4.44 to 5.505, with significant differences across locations. This range is within acceptable limits for honey but suggests that certain regions may produce more acidic honey, which could inhibit microbial growth. Hydroxymethylfurfural (HMF) levels were mostly below international standards, indicating good quality honey. However, free acidity showed variability that may affect taste and preservation. Electrical conductivity values also varied significantly, correlating with the mineral content of the honey, which is an important quality indicator. Among heavy metals analyzed, an essential element, iron was the most prevalent, while toxic metals like lead and chromium were not detected. The levels of copper and manganese varied significantly among regions, indicating potential environmental influences on honey quality. The physicochemical parameters of honey in these areas were generally within acceptable national and international standards, suggesting a good quality of honey production in the study area.

Based on the findings, several recommendations can be made to improve honey quality and ensure safety for consumers:

- Implement regular monitoring of physicochemical parameters and heavy metals (other potentially harmful heavy metals like cadmium or arsenic) to ensure compliance with national and international standards.
- Collaboration with Regulatory Authorities (collaborate with regulatory bodies to ensure adherence to food safety standards, Partnerships could help streamline training and monitoring efforts.
- Conduct further studies to assess environmental factors affecting heavy metal concentrations and physicochemical parameters in honey.

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## ANNEXES

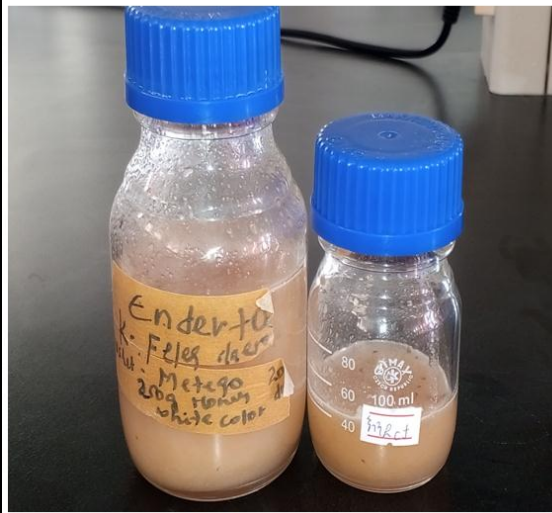
### Annex I. The honey sample collection and preparation process



Sample field collection



Pooled samples homogenized by vortex



Representative honey samples relabeled

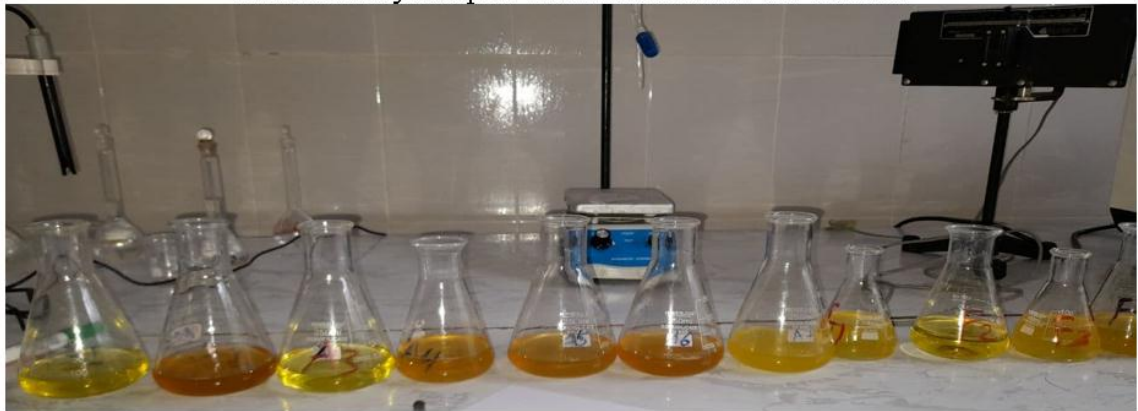


Measuring the representative honey samples

**Annex II.** Pooled honey samples preparation process for further analysis



Pooled honey samples collected from the six districts



Triplicates of the pooled honey samples




Sample preparation for physicochemical analysis




Sample preparation for heavy metals analysis

**Annex III. Physicochemical analysis: some analytical techniques, and the official report.**

Conducted at **Holeta Bee Research Center: Honet Quality Analysis Laboratory**




**Sample preparation**




**PH Meter**


Standards		
QSAE	EU	CAC
≤21	≤21	≤21
3.4-6.1	-	3.2-4.5
≤40	≤40	≤60
≤0.6	≤0.6	0.25-1
-	-	0.8
≤40	≤40	≤50



**Hot plate**



**Muffle Furnace**



**TEST REPORT**

Customer Name: Hagos Tsegay meles  
 Sample Submission Date: 30/02/2017  
 Sample Name: Honey

1. Analysis Objective: Thesis  
 2. Procedure and Results  
 To determine the quality of honey, the following quality parameters were measured.  
 a) HMF ,pH, free acidity ,moisture content and colour  
 b) Recommended method of test: International Honey commission (IHC,2009)  
 3. Results Recommended method of test: International Honey commission (IHC,2009)

Honey serial No.	Moisture content (%)		pH		Free acidity (meq /Kg )		HMF (mg/Kg)		Ash (%)		Electrical conductivity( mS.cm-1)		color
	ET	WU	ME	KT	AT	DT	ET	WU	ME	KT	AT	DT	
ET	20.4	20.6	4.47	4.44	21	25	0.7	0.9	0.26	0.21	0.59	0.51	Extra light amber
WU	19.8	19.4	4.5	4.85	11	10	2.7	5.5	0.30	0.28	0.66	0.63	White
ME	19.8	19.6	4.76	4.74	14	18	19.9	7.9	0.24	0.28	0.56	0.63	Extra light amber
KT	18.6	18.6	5.36	5.65	9	8	12.9	19	0.06	0.03	0.24	0.19	Extra light amber
AT	16.6	17	4.58	4.63	9	10	10.6	5.7	0.27	0.28	0.61	0.63	White
DT	18.2	18.6	4.42	4.46	19	20	10.6	12.1	0.07	0.06	0.24	0.26	Extra light amber
National and international Honey Quality standard according Codex A(2001),EU( 2002) and ESA (2005)	≤20		3.4-6.0		≤50meq/kg		≤ 40 mg/kg max		≤0.6 %		≤0.8 mS.cm-1		

4. Discussion

- Except, the water (moisture) content (ET) all parameter within the accepted range of honey quality standards
- The high water content might be due to harvesting unripe honey and /or inappropriate handling during harvesting

**Annex IV. Heavy metals analysis: method validation, sample analysis, official reports.**

Conducted at **Ezana Analytical Laboratory, Mekelle, Ethiopia**



**ENAO** Ezana Mining Development Plc Analytical Laboratory Mekelle, Ethiopia  
 Tel: +251 914 116128 Fax: +251 914 116128 P.O. Box 194, P.M.B. 1000, Mekelle, Ethiopia

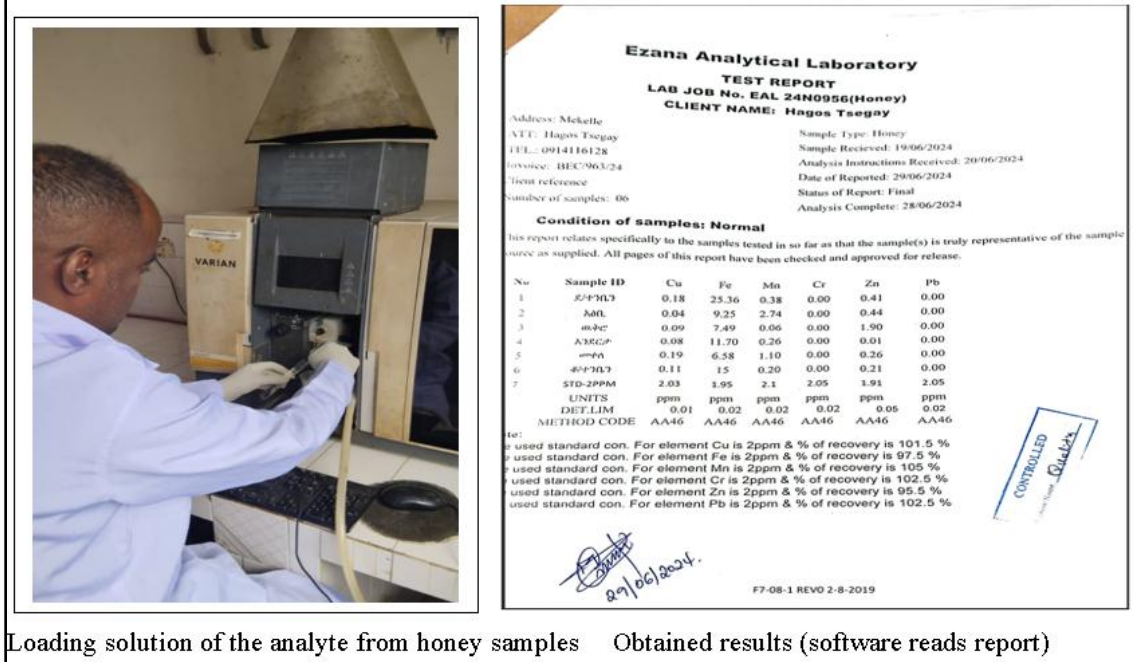
**TOTAL QUALITY REPORT**  
 Certificate of Analysis  
 Job Number - EAL 24A0956(Honey)  
 CLIENT NAME: Hagos Tsegay  
 Quality Parameter

Elements	Sample ID	Result-1	Result-2	Range	Mean range	Warning limit	Action limit
Cu	Lo	0.11	0.08	0.03	0.10	0.25	0.33
Fe		15.00	13.20	1.74	14.10	38.00	46.76
Mn		0.20	0.22	0.02	0.21	0.50	0.69
Zn		0.21	0.22	0.01	0.215	0.60	0.70
Cr		0.00	0.00	0.00	0.00	0.00	0.00
Pb		0.00	0.00	0.00	0.00	0.00	0.00

Prepared by: Yirga Abirha  
 Approved by: Quality Manager

**CONTROLLED**  
 Signature: *Yirga Abirha*

Validation process, and obtained results using stock solutions of the selected elements



**Ezana Analytical Laboratory**  
**TEST REPORT**  
 LAB JOB No. EAL 24N0956(Honey)  
 CLIENT NAME: Hagos Tsegay

Address: Mekelle  
 AT: Hagos Tsegay  
 TEL: 0914116128  
 Invoice: BEC/963/24  
 Client reference  
 Number of samples: 06

Sample Type: Honey  
 Sample Received: 19/06/2024  
 Analysis Instructions Received: 20/06/2024  
 Date of Reported: 29/06/2024  
 Status of Report: Final  
 Analysis Complete: 28/06/2024

**Condition of samples: Normal**

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

No	Sample ID	Cu	Fe	Mn	Cr	Zn	Pb
1	STP-1013	0.18	25.36	0.38	0.00	0.41	0.00
2	NAEL	0.04	9.25	2.74	0.00	0.44	0.00
3	AKPCE	0.09	7.49	0.06	0.00	1.50	0.00
4	AKRGP	0.08	11.70	0.26	0.00	0.01	0.00
5	AKPA	0.19	6.58	1.10	0.00	0.26	0.00
6	AKP103	0.11	15	0.20	0.00	0.21	0.00
7	STD-2PPM	2.03	1.95	2.1	2.05	1.91	2.05

UNITS: ppm ppm ppm ppm ppm ppm  
 DET LIM: 0.01 0.02 0.02 0.02 0.06 0.02  
 METHOD CODE: AA46 AA46 AA46 AA46 AA46 AA46

Note:  
 1. used standard con. For element Cu is 2ppm & % of recovery is 101.5 %  
 2. used standard con. For element Fe is 2ppm & % of recovery is 97.5 %  
 3. used standard con. For element Mn is 2ppm & % of recovery is 105 %  
 4. used standard con. For element Cr is 2ppm & % of recovery is 102.5 %  
 5. used standard con. For element Zn is 2ppm & % of recovery is 95.5 %  
 6. used standard con. For element Pb is 2ppm & % of recovery is 102.5 %

**CONTROLLED**  
 Signature: *Yirga Abirha*

F7-08-1 REV0 2-8-2019

Loading solution of the analyte from honey samples      Obtained results (software reads report)