Mekelle University

Concentration of Heavy Metals and Microbial Quality in Cows' Fresh Milk in Eastern and South Eastern Zones of Tigray Ethiopia

By

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A thesis

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DECLARATION

I, Ms. **Asmeret Kidane Tekle**, hereby present for consideration by the **Animal Rangeland and Wildlife Science Department** within the College of Dryland Agriculture and Natural Resources at Mekelle University, my dissertation in partial fulfillment of the requirement for the degree of Masters in **Livestock Production and Pastoral Development** with thesis research title' **Concentration of Heavy Metals and Microbial Quality in Cows' Fresh Milk in Eastern and Southeastern Zones of Tigray**. I sincerely declare that this thesis is the product of my own efforts. No other person has published a similar study which i might have copied, and at no stage will this be published without my consent and that of the **Animal Rangeland, and Wildlife Sciences Department.**

Name of the student ______________________ Signature & date ____________________

Approval

Name of the major adviser__________________ Signature & date ___________________

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Name of Department head ___________________Signature & date__________________

BIOGRAPHICAL SKECH

The author, Asmeret was born in April 14, 1998 at Kebele Lese Muguna-Andi, Tahtay Adyabo district. Northwestern Tigray, Ethiopia. She attended her elementary education at Erdi Weyane Elementary School from 2005 to 2012. She pursued her secondary education at Barud Secondary School in Adi-Hage ray from 2013 to 2014, and later at Sheraro preparatory School from 2015 to 2016. In the 2016/2017 academic year, she joined Mekelle University and graduated with a Bachelor's degree in Animal Production and Technology in July 2019. Following her graduation, she was employed by Mekelle University, College of Dryland Agriculture and Natural Resources, in the Department of Animal, Rangeland, and Wildlife Sciences as Assistant lecturer on September 2019. She began her postgraduate studies in October 2019, specializing in Livestock Production and Pastoral Development.

ABSTRACT

Cow milk is important foods of daily nutrient especially, for infants, vulnerable groups, and elderly people. But milk may contain various toxic pollutants and microbial contaminants which are dangerous for health. Therefore, this study aims to analyze the concentration of heavy metals and microbial quality of cows' fresh milk in the eastern and southeastern zones of Tigray. Two districts were selected from each zone: Kilite Awulaelo and Tsirae Wenberta (eastern), and Degu'a Tembien and Enderta (southeastern), with one tabia sampled per district; Aynalem, Mahbere Weyni, Limat, and Mahbere Genet, respectively. A total of 120 local breed lactating cows (30 per tabia), reared for over five years, were sampled. Fresh milk (50 ml per cow) was collected in sterile plastic bottles and transported at on dry ice. Heavy metal concentrations (Fe, Cd, Cr, Cu, Ni, Pb) were analyzed using Atomic Absorption Spectrometry following acid digestion, HNO_3 *and* H_2O_2*. Microbial quality, including Total Bacterial Count (TBC), Total Coliform Count (TCC), and Total Yeast & Mold Count (TYMC), was assessed using standard culturing methods. Data was analyzed using SPSS software and Microsoft excel. The result showed that, the highest concentration recorded were Fe (26.660±15.076 mg/l) and Cd (0.0442±0.025 mg/l) in Tabia Aynalem, Cr (0.0725±0.048 mg/l) in Mahbere Genet, and Cu (1.519±0.314 mg/l), Ni (0.0506±0.030 mg/l), and Pb (0.0807±0.053 mg/l) in Tabia Lim'at. All heavy metals except Fe showed statistically significant variation among sites* ($P \leq 0.05$)*. Fe was higher from all the selected elements in all study areas, Cu, Cd, Cr, Ni and Pb exceeded from FAO/WHO permissible limits in most areas. Microbial results showed the highest means of TBC (6.23±0.88 Log10 CFU/ml) and TYMC (5.36±0.58 Log10 CFU/ml) in Mahbere Weyni, and TCC (5.22±0.89 Log10 CFU/ml) in Aynalem. However, there were no statistically significant differences* ($P > 0.05$) *in microbial counts across study areas. Generally this study indicates most of the selected heavy metals were higher from standards and microbial quality: TBC mostly higher from acceptable standards but TCC was classified as 'very good' in all study areas. Further study on heavy metals and microbial quality in milk on these study areas is important.*

Key words: Cows' fresh milk, heavy metals, microbial quality

DEDICATION

This thesis is dedicated to my Father **Kidane Tekle** and my mother **Mtslal W/siea**, whose unwavering support and encouragement have been my guiding light throughout this journey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
ANOVA	Analysis of Variance
Ca	Calcium
Cd	Cadmium
Cr	Chromium
Cu	Copper
Fe	Iron
K	Potassium
Mg/L	Milligram per Liter
Ni	Nickel
P	Phosphorus
Pb	Lead
PDA	Potato Dextrose Agar
PPB	Parts Per Billion
PPM	Parts Per Million
Se	Selenium
SPC	Standard Plate Count
SPSS	Statistical Package for Social Science
TBC	Total Bacterial Count
TCC	Total Coliform Count

VRBA	Violet Red Bile Agar
WHO	World Health Organization
YMC	Yeast and Mold Count
Zn	Zinc

CHAPTER 1: INTRODUCTION

1.1 Background and justification

Milk is composed of various essential nutrients, including water, fat, lactose, minerals, and trace amounts of biological proteins and enzymes (Odongo et al., 2022). It provides a significant portion of daily nutritional requirements for many countries, importantly for infants, vulnerable groups, and elderly people (Forcada et al., 2023). Ethiopia possesses the largest livestock population in Africa, with an estimated 70 million cattle, 42.9 million sheep, 52.5 million goats, 2.15 million horses, 10.8 million donkeys, 0.38 million mules, and approximately 8.1 million camels (Getaneh et al., 2023). Among this**,** female cattle account for approximately 56%**,** while male cattle were 44**%**. In the Tigray region**,** the total cattle population is 4,908,964**,** with the number of milking cows recorded at 778,498 in Tigray, the total milk production 209,169,047 liters is for Tigray region (CSA, 2020/21).

Ensuring food safety is vital for public health, monitoring contamination of foodstuffs ensure that safe food is availed to the population and safeguards products of high nutritional value don't contain contaminants beyond recommended maximum levels in milk (Biryomumaisho et al., 2022). Then milk must be of acceptable quality in order to protect the health of the community. It should be obtained from healthy animals. The collection and storage procedure should be under hygienic conditions that avoid contamination (Hymete et al., 2021).

Among the contaminants mineral concentrations and microbial load are important determinants of milk quality (Birhanu and Asamnew, 2019). Because of the buildup of harmful substances and the existence of harmful bacteria, contaminated milk can pose major health hazards. Milk can become contaminated by heavy metals like cadmium (Cd), chromium (Cr), lead (Pb), Copper(Cu), Iron(Fe), Nickel (Ni) and arsenic (As) from a variety of environmental sources, including soil, water, air, and animal feed (Ali et al., 2019). These pollutants come from both man-made (industrial emissions, agricultural runoff, mining, wastewater discharge, and vehicle exhaust) and natural (volcanic eruptions, sea-salt sprays, forest fires, and rock weathering) sources (Abdel-rahman et al., 2022). Cows' can absorb heavy metals through the consumption of contaminated feed, water, or dust particles and certain pharmaceutical products contribute to the bioaccumulation of heavy metals in milk. Long-term exposure to these metals can lead to serious health issues, including metabolic disorders, immune system suppression, lung tumors, heart disease, kidney failure, infertility, neurological impairments, and brain damage (Hymete et al., 2021).

In addition to heavy metal contamination, milk is also highly susceptible to microbial contamination, which can occur during production, handling, and storage (Fadaei et al., 2014). Consuming milk with high microbial contamination can lead to different foodborne diseases, spoilage, and reduced consumer safety. Microbial contamination originates from primary sources infected or sick lactating cows, and secondary from poor hygienic practices during milking, unclean milk handling equipment, contaminated water, and improper storage conditions (Umer and Bongase, 2023).

According to the World Health Organization (WHO), foodborne pathogens were responsible for approximately 600,652,361 cases and 418,608 deaths worldwide in 2010 (Asfaw et al., 2023).

Assessing the concentration of heavy metals and microbial quality in cows' fresh milk is important for ensuring food safety and consumer health. By analyzing these factors, it is possible to detect potential contamination and minimize health risks associated with consuming contaminated milk.

1.2 Statement Problem

Despite the significant health risks, there is limited research on heavy metal contamination in cows' fresh milk in the Tigray region, creating a knowledge gap that needs to be addressed. Besides, there is a limited study of the milk microbial quality in the study areas. Therefore, this study is intended to fill the gap of information on the analysis of the concentration or level of heavy metals (Cu, Fe, Cd, Pb, Ni, Cr) and microbial quality of cow's fresh milk in East and Southeastern zones of Tigray, Ethiopia.

1.3 Objectives

1.3.1 General objectives

To analyze the concentration of heavy metals and microbial quality in cows' fresh milk in eastern and southeastern zones of Tigray.

1.3.2 Specific objectives

The specific objectives of the study were:

- To investigate the concentration level of heavy metals in cows' fresh milk in the study areas.
- To investigate the microbial quality in cows' fresh milk in the study areas.

1.4 Hypothesis of the study

- H_0: The concentration level of the selected heavy metals in cows' fresh milk does not vary among the study areas.
- H_0: The microbial quality in cows' fresh milk does not vary among the study areas

1.5 Research questions

- What is concentration of the selected heavy metals is present in the cow's fresh milk?
- Is there any difference in the concentration of heavy metals in cows' fresh milk in different study areas?
- Which heavy metal exhibits the highest concentration among the study areas?
- Is there any difference in the microbial quality of cows' fresh milk in different study areas?

1.6 Significance of the Study

The preliminary data on the levels of heavy metals specifically iron (Fe), cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni), and lead (Pb) and microbial quality in fresh cow's milk in Eastern and Southeastern Tigray. The concentration levels identified help as a scientific basis for assessing the potential health risks related with long-term exposure to toxic metals through daily dietary intake. Understanding these concentration levels is significant for developing strategies to protect public health and to ensure the safety of milk quality. The results can inform policymakers and regulatory bodies as they work to establish or update food safety (milk quality) standards, and can support agricultural extension services in promoting safer feeding and grazing practices for dairy. Besides, this study establishes a valuable baseline for future study about heavy metals and microbial quality in fresh cow's milk in the present study areas and it adds to the scientific literature on food safety (milk quality).

CHAPTER 2: LITERATURE REVIEW

2.1 Milk Production in the World

The mammary glands of mammals create milk, a white liquid, to nourish their young. The glands naturally release milk after the newborn is born. The FAO/WHO Codex Alimentarius Commission states that milk is a substrate that is meant for human consumption, regardless of whether it is processed, semi-processed, or raw (Sanjulián et al., 2025). Humans have a long tradition of consuming milk produced by animals, and cows' milk is the most popular milk consumed in both developed and developing countries(Tenaw et al., 2024).

Milk and dairy products are necessary sources of important nutrients for human beings (Berhe et al., 2020). Any food made from animal milk is referred to as a dairy product, The main source of dairy products for human consumption, aside from breastfed newborns, is milk cows; other mammals that humans consume include sheep, goats, camels, horses, and others (Haftom et al., 2023). Milk is a complete food for young animals and is consumed by humans due to its high nutritional value; all of its nutrients are good for human health. Milk, excluding water, contains complete nutrients that are a source of protein, lipids, carbohydrates, vitamins and minerals (Tenaw et al., 2024).

The average annual rate of milk production per cow has increased by 10.6 percent from 2010 and the average number of milk cows on farms in the United States during 2019 was 9.34 million head, down 0.7 percent from 2018, the average number of milk cows was revised to 4,000 head for 2019. The average annual number of milk cows has increased by 2.3 percent from 2010 studied by (Japaro et al., 2021).

2.1.1 Milk Production in Africa

East Africa is the leading milk-producing region in Africa, representing 68% of the continent's milk output (Gobezie and Aysheshim, 2020). Milk production remained stable in Africa with 49 million tone's (Hailemariam et al., 2022). However, Ethiopia, South Africa, and Kenya, among others, registered declines. The majority of the world's low-income tropical livestock producers are in South Asia and Sub-Saharan Africa where dairy is produced in mixed crop-livestock systems, ranging from low input, and extensive grazing to more specialized intensive enterprises. intensive systems are common in countries such as Egypt, Sudan, Kenya, Algeria, and South Africa which are the top milk-producing countries on the continent in terms of milk volume, producing about 52% of total African milk (Hailemariam et al., 2022).

Apart from southern Africa, where high outputs have been reported, the rest of Africa is challenged to meet the milk demand of their respective countries milk. Much of the milk gained from extensive systems is for home consumption or sale at local markets, with only 5% of milk produced sold through commercial markets. Nevertheless, milk production on the continent has doubled since 1996 with major growth taking place in Kenya, North Africa, and South Africa with the other countries in Sub-Saharan Africa experiencing high relative growth but from an extremely low level (Japaro et al., 2021).

2.1.2 Milk Production in Ethiopia

The milk production in Ethiopia mainly depended on the milk of cows and the milking months of local breed exist in countries are almost seven months of lactation period and cows actually milking potential average milk yield per cow per day is about 1.48 liters day and average. Total cow milk production potential of the country is about 3.89 billion liters (Tenaw et al., 2024). Ethiopia's rural dairy production system is mainly determined by agro pastoralists, pastoralists, and mixed crop-livestock farmers. This traditional, subsistence-based system contributes approximately 98% of the nation's total milk production (Tenaw et al., 2024).

From the African continent, Ethiopia has the highest record of livestock. But its productivity and maximum financial gain are unsatisfactory. In Ethiopia, cows are the major milk production source which produces 83.4% of the total yearly milk product of the country (Abegaz et al., 2022) . It is 9th in the world with the number of livestock, being first among the African countries of Sub-Saharan Africa's developing countries (Endale et al., 2022). However, milk production is unsatisfactory and the milk production in Ethiopia mostly depends on the milk of cows and the time of milking of local breeds present in the countries are almost seven months of lactation. Currently, the total milk production from 12.57 million milking cows in the country is estimated at 3.89 billion liters (Japaro et al., 2021). The production per cow is 1.48 liters of milk per cow per day and the average lactation length is about seven months (Eshete et al., 2023).

In Ethiopia, there is an increase in milk production from year to year, and milk consumption and its price increase from time to time because of different factors. From these factors population growth and nutritional focus towards dietary are the major ones (Gebretsadik et al., 2020). In some countries with low socio-economic status, income growth and urbanization have led to almost doubled consumption of milk and dairy products (Berhe et al., 2020).

The traditional smallholder system is a component of the farming system, which has agro-pastoral pastoralists, and mixed crop-livestock producers, Peri-urban milk production has been practiced in areas where the population becomes high and the agricultural land is scarred due to urbanization around major cities and urban milk production system depend on in major cities and regional towns, which have a high demand for milk, and they are the most important source of milk producers (Mes and Sha, 2022).

2.1.3 Milk production in Tigray

Ethiopia has the huge livestock population in Africa the estimated livestock population in Ethiopia is 70 million head of cattle, 42.9 million sheep, 52.5 million goats, 2.15 million horses, 10.80 million donkeys, 0.38 million mules, and about 8.1 million camels (Getaneh et al, 2023). Out of this total cattle population, female cattle constitute about 56 percent, and the remaining 44 percent are male cattle. Out of Ethiopia's total cattle population, the Tigray region accounts for approximately 4,908,964 cattle. In this region, the number of milking cows was reported to be 778,498 in Tigray, with 76,280 in Eastern Tigray and 71,504 in Southern Tigray. Similarly, total milk production was estimated at 209,169,047 liters for Tigray, 17,955,411 liters for Eastern Tigray, and 2,082,804 liters for Southern Tigray, based on lactation periods of 6-7 months, respectively (CSA, 2020/21)

Cattle Population in Tigray is the fourth-highest region in Ethiopia for livestock production, with a cattle population of approximately 4.79 million, contributing about 8.05% to the national cattle population (Adem et al., 2020). Milking Cows: The region is home to around 862,441 milking cows,milk Yield: The average lactation yields are 415 liters for local cows and 1,712 liters for crossbred cows (Misganaw et al., 2020).Milk production in the Eastern Zone of Tigray, Ethiopia, is a vital component of the region's agricultural economy, predominantly managed by smallholder farmers within mixed crop-livestock systems. Despite the region's substantial cattle population, dairy productivity remains relatively low due to various challenges.

The Tigray region is home to an estimated 3.4 million cattle, accounting for approximately 7.16% of Ethiopia's total cattle population. In detailed localities such as Wukro-Kilte Awlaelo, the livestock population is estimated at around 60,000. Despite the significant cattle population, the average milk yield per cow remains relatively low. For example, in the Northwestern and Western Zones of Tigray, cows produce an average of 1.27 to 1.36 liters of milk per day during a typical six-month lactation period (Bahita and Hailay, 2025).

2.2 Milk and milk products

Milk is a complex, bioactive substance that promotes growth and development of mammalian infants (Kochare et al., 2015). There is a lot of potential for developing new products using cows' milk, Cows' milk is well-liked by the general public due to its advantageous nutritional value. In nature, the quality of food and food ingredients cannot be isolated from a variety of influences, such as environmental conditions, which serve as a standard for food to obtain eligibility for consumption. Fresh cows' milk is a liquid made from the udders of healthy and clean cows after proper milking; its natural content is still pure without being reduced or added to anything, in the event of pollutants, the quality of milk will decline (Sanjulián et al., 2025).

2.2.1 Factors Affect Milk Quality

Numerous factors pertaining to animal health, milking methods, genetics, environmental factors, and milk handling and processing all have an impact on milk quality. Careful management of these elements, such as healthy diet, illness prevention, sanitary procedures, and effective milking methods, is necessary to guarantee high milk quality (Sanjulián et al., 2025).

Cow milk can be contaminated with heavy metals from the environment, such as Pb, Cu, Fe, Ni, Cd and Cr, milk contamination can be caused by several things, such as microbes, pesticide residues, and heavy metal buildup (Kochare et al., 2015)

For a balanced diet, milk and milk products are generally a complete food. Toxic metals in milk or milk products, however, can be harmful and hazardous to human health. Therefore, as metal levels rise, milk or milk products become less safe. Because milk is frequently ingested by infants and children, the presence of hazardous metals in milk and milk products presents a special risk (Abdel-rahman et al., 2022) The levels of heavy metals in milk and dairy products depend on the genetic factors of the animal, stage of lactation, metal contamination from the equipment during production, nutritional type of the animal, environmental factors, and manufacturing practices(Abdel-rahman et al., 2022).

Dairy animals that graze on the contaminated plants accumulate toxic metals in their tissues, as well as milk if lactating (Hasan and Kabir, 2022). Human activities that lead contamination of heavy Metals are such as metal mining, foundry smelting, automobile, landfill, and road construction. from agricultural activities such as the use of pesticides, insecticides, and fertilizers, and natural causes such as metal corrosion, volcanic activity, soil erosion, and geological weathering (Pirhadi et al., 2021). The other causes of environmental pollution all over the world are rapid urbanization and industrial development (Igucu et al., 2016).

Heavy metals have toxic effects on human health, and the removal of these metals has been increased by industrial and anthropogenic activities and modern industrialization (Hasan and Kabir, 2022). Water and air pollution by toxic metals is an environmental issue and hundreds of millions of human beings are being affected around the world, for human and animal health contamination of food with heavy metals is another concern. The concentration of heavy metals in air, water resources, and food is assessed in this regard (Balali-mood et al., 2021). The heavy metal concentration level of water in Akaki is higher than the natural elemental levels in freshwater (Prabu et al., 2021). This shows that the heavy metal concentration in Akaki water is due to the waste from municipal, industrial, and domestic activities in the neighborhoods. Among the metals

Cr could be added into the river from effluents of tannery industries, Pb may be from gas emissions from the traffic in Addis Ababa, and Fe from effluents of metal industries (Prabu et al., 2021).

Contamination of milk globally with unwanted substances through animal feeds, heavy metals, mycotoxins, biotoxins, and similar pollutants has gained great concern to public health due to their toxic effects on humans and animals (Odongo et al., 2022). The environmental conditions are also conducive for milk spoilage, microbes which can either harm the milk consumer or use in milk processing and natural fermentation get into milk from a various bases and, Milk is the most perishable of all farm output products, milk is continuously harvested and consumed either fresh or after fermented which is perhaps produced from unhygienic farm, milk quality is paramount important for the consumer and also for milk processing plants. (Aleli et al., 2024).

2.2.2 Heavy Metals

2.2.2.1 Definition of Heavy Metals

Different definitions have been proposed about heavy metals, with some based on densities, others on atomic quantity or atomic mass, and still others on biochemical properties or poisonousness (Abreham and Girma, 2024) and (Abdel-rahman et al., 2022). Heavy metals are regularly classified into two classes and are not biodegradable in the natural environment. The first group consists of hazardous metals (such as Pb, Cd, and As) that are unwanted, harmful at all concentrations, and have no biological benefits for human health. The second group consists of essential metals (Cu, Zn, Mn, Fe, Ni, and Cr, for example), which are desirable and beneficial to human health at low concentration but toxic at high concentration (Abdel-rahman et al., 2022). And produced from natural and anthropogenic (human activities, industries, agricultural activities etc.) sources (Olowoyo et al., 2024) and (Peter et al., 2022)

2.2.2.2 Sources of Heavy Metal Contaminant

Heavy metals are introduced into the atmosphere in various ways globally, they can rise into the atmosphere from wind-blown dust, aerosols formed at the aquatic surface, forestry fires, volcanoes, and biological processes (Sopaj et al., 2021).

Heavy metals such as iron (Fe), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb) can contaminate cow's milk through a variety of environmental and agricultural pathways. Major sources include environmental pollution, where heavy metals from industrial emissions, vehicle consume, and atmospheric deposition settle on pastures and feed crops, which are then consumed by cattle. Soil adulteration is another route, often resulting from irrigation with polluted water or the application of contaminated fertilizers, allowing metals to be absorbed by plants that serve as animal feed (Peter et al., 2022). Applying manure from animals exposed to heavy metals can lead to soil contamination, affecting the feed crops grown on that soil, using equipment contaminated with heavy metals during milk processing can introduce these metals into the final product (Odongo et al., 2022).

Heavy metals are persistent contaminants in the environment that can cause serious environmental and health hazards. They are among the most dangerous contaminants in the environment and are released into the environmental from natural as well as an anthropogenic activity (Peter et al., 2022)

Natural and man-made sources are the two categories of sources that release heavy metals into various environmental media, including soil, air, and water and rock weathering, forest fires, volcano eruptions, sea salt sprays, biogenic sources, and wind-borne soil particles are examples of natural sources (Abdel-rahman et al., 2022), Unlike most organic pollutants, trace metals occur naturally in rock forming and are minerals, pollution gives rise to high concentrations of the metals relative to the normal background levels; therefore, the presence of the metal is insufficient evidence of pollution. (Malhat and Hagag, 2012). Anthropogenic causes include mining, metallurgical, oil and gas, motor and chimney emissions, wastewater discharge, and industrial and agricultural processes, pesticides, paint manufacturing, textile printing, earth's crust, hair dyes, and Polyvinyl chloride pipes are the primary sources of lead (Pb); cadmium (Cd) is found in rechargeable batteries, tobacco, phosphate fertilizers, pigments, industrial wastes, and agrochemical wastes; chromium (Cr) is found in stainless steel, alloy production, wood saving, textile dyes, leather tanning, and electroplating; nickel (Ni) is found in kitchenware, machine parts, batteries, copper, and nickel (Ni); volcanic activities, pyrolysis of biomass, mining, ship paint, wastewater discharges (Peter et al., 2022)

Most of the contamination of milk from moderate agricultural practices, industrial pollutants in the environment, animal feeds and use of sewage sludge in agriculture is increasing and therefore requires urgent attention because of the risk this contamination poses especially to the health of the consumers, Contamination of milk globally with unwanted substance through animal feeds, heavy metals, mycotoxins, biotoxin's and similar pollutants has gained great concern to public health due to their toxic effects on humans and animals (Odongo et al., 2022).

Heavy metals are usually nonbiodegradable and tend to accumulate in the food chain including edible animal products like meat and milk of exposed livestock populations (Odongo et al (2022,). Significant number of heavy metals can be transferred from contaminated soil, water and air to plants and grass, causing accumulation of these metals in grazing animals and subsequently their transfer to food chain via milk and meat. Thus, accumulation of toxic metals in animals is not only associated with toxic effects in food producing animals, but also poses health risks to humans consuming milk and meat contaminated by the toxic metals. (Gelaye and Musie, 2023).

There are several reports from different parts of the world indicating higher concentration of heavy metals in milk of cows reared in the contaminated environment, In Iran, 1.9% of the bovine and 8.1% of the sheep milk samples were found to contain higher levels of lead than the newly established Codex Standard indicating its public health hazards, Presence of lead in edible animal products like milk (Gelaye and Musie, 2023).

Contamination of milk is a major threat to consumer health and needs to be addressed without delay. Mycotoxins, heavy metals, dioxins, animal feeds, and other pollutants can be hazardous to both humans and animals, making them a significant public health concern in terms of milk contamination (Olowoyo et al., 2024).

2.4 Impact of Heavy Metal on the Environment, Animal and Human Health

2.4.1 Impact of Heavy Metal on Environment

Heavy metals obviously occur in the environment and are vital for existence, but they may become hazardous when they accumulate in organisms and a few of the most frequent heavy metals that contaminate the environment include cadmium, mercury, arsenic, chromium, lead, copper, and nickel (Mitra and Chakraborty, 2022). Potentially toxic elements, mainly heavy metals and metalloids, face a growing hazard in the environment. (Olowoyo et al., 2024). Those elements known as heavy metals are indicated by their high atomic mass and density, Their persistence in the soil, reflecting their resistance to degradation, makes them the most toxic group of inorganic contaminants (Forcada et al., 2023). The periodic table consists of heavy metals to a special portion with high density and atomic weight. Among them, the majority are found in the biosphere, such as in soil, water, and rocks, and are also removed from the surroundings from anthropogenic resources, mostly commercial and industrial (Mitra, Jyoti, et al., 2022). Environmental pollution is linked with one in every six global deaths, and this amounts to about nine million deaths annually.

Since the growth of global industrialization and fossil fuel exploration, toxic heavy metals such as Lead (Pb), Arsenic (As), Cadmium (Cd), Mercury (Hg), and have progressively been detected in the environment (Philip-slaboh and Id, 2023).

Urbanization and Rapid industrialization have caused pollution of the environment by heavy metals, and their rates of transport and mobilization in the environment have greatly increased. Natural sources in the environment include volcanic eruption and weathering of metal-containing rocks, while principal anthropogenic sources include mining, industrial emissions, smelting, and agricultural activities like the application of pesticides and phosphate fertilizers. The combustion of fossil fuels also contributes to the release of heavy metals such as cadmium (Cd) into the environment (Ali et al., 2019). Most of the toxic heavy metals including lead, thallium, cadmium, and antimony, are common in industrial operations and are substantial polluters of the environment (Mitra, Jyoti, et al., 2022). Heavy metals also harmful effects on human health, and exposure to these metals has been increased by industrial and anthropogenic activities and modern industrialization. Contamination of water and air by toxic metals is an environmental concern and hundreds of millions of people are being affected around the world. Food contamination with heavy metals is another concern for human and animal health. The concentration of heavy metals in water resources, air, and food is reviewed in this regard (Balali-mood et al., 2021).

2.4.2 Impact of Heavy Metal on Animal Health

Heavy metals such as copper (Cu), zinc (Zn), arsenic (As), chromium (Cr), lead (Pb), and cadmium (Cd) are potential bio-accumulative toxins of the dairy production system (Ali et al., 2019).The long-term accumulation of heavy metals in agricultural soils has the potential to reduce soil productivity by inhibiting soil microbial and fauna populations and may pose a risk to animal, human, and ecosystem health in a closed system, the cycling of many heavy metals through the dairy food chain is likely to be limited by the soil-plant barrier (Kochare et al., 2015).

Heavy metals can enter animal's tissues through the food chain if animal grazes on pastures irrigated with contaminated industrial waste water or drink water from a contaminated source, this is because heavy metals in polluted soils can be absorbed by plants and grasses, eventually making their way into the animal's body (Olowoyo et al., 2024). Lactating cows may be exposed to high quantities of toxic metals in the environment by air, water and through grazing on grass/feeds from polluted fields or near roads with heavy traffic. Dairy animals may ingest metals while grazing on the pasture. Though, in the cow transfer of minerals to milk is highly variable (Odongo et al., 2022).

2.4.3 Impact of Heavy Metal on Human Health

Toxic metal contamination of plants, animals, irrigation water, and agricultural soils results in their entry into the food chain and poses a serious risk to human health (Singh et al., 2019). The widespread, non-biodegradable, and non-thermos degradable nature of heavy metals causes their harmful effects on human health to multiply. These effects include anemia, gastrointestinal issues, cancer of the lungs and blood, skeletal damage, osteoporosis, cell damage, hormonal imbalances, renal failure, and gastrointestinal issues (Ismail et al., 2017). For example, Lead (Pb) has a toxic effect on human health leading to central and peripheral nervous system, kidney failure, and damage to the reproductive system (Prabu et al., 2021). and Pb can also affect human health by damaging the liver, anemia, heart and bled vessels, the immune system, the genital system, the development of cancer and various cancers, the digestive tract, and cause encephalitis hepatitis (Parisa and Faezeh, 2018). Even though they are present in trace amounts, heavy metals pose a major risk to human health because they can lead to anemia, dementia, neurological abnormalities, psychological disorders, inhibition of enzyme activity, problems with iron absorption, kidney damage, muscular atrophy, and cancers of the mouth, lungs, and genital glands (Odongo et al., 2022). The possible health dangers to humans from ingesting heavy metals through milk, including both non-carcinogenic and carcinogenic risks. About two million deaths worldwide were caused by water pollution in 2015, whereas about six million deaths were caused by contaminated air (Philip-slaboh and Id, 2023).

2.5 Concentration Level of Same Heavy Metal in Milk and Standard for Milk and Milk Products

Lead concentration values of 4.40 ± 1.60 mg/l were found to be reported by (Malhat and Hagag, 2012). The WHO's recommended limit for human consumption was obviously exceeded by the high levels of lead (3.80 ± 0.42 mg/l) found in raw cow milk samples collected in Slovakia (Olowoyo et al., 2024)

Heavy metals have been found in milk and other milk products in several studies, reported high Pb concentrations in all their milk samples that exceeded the maximum permissible limit (0.02 mg/kg) established by Codex standard (Olowoyo et al., 2024) For some heavy metals in cow's milk, the International Dairy Federation (IDF) has established Maximum Permissible Limits of 0.026 mg/kg for cadmium (Cd), 0.01 mg/kg for copper (Cu), 0.02 mg/kg for lead (Pb), and 0.0328 mg/kg for zinc (Zn) (Fenta, 2014). Furthermore, according to the FAO and WHO recommendations, Pb levels in milk can be as high as 0.02-0.1 mg/kg. To ensure food safety and safeguard the public's health, it is crucial to monitor the levels of heavy metals in cow's milk because their presence may have negative health effects (Lebsir et al., 2023).

The toxicity of heavy metals and their capacity for accumulation in the human organism make it necessary to conduct monitoring of their concentration in food. Toxic elements like cadmium, are absorbed with food and drinking water. Therefore, they can undergo bioaccumulation in products of animal origin and inclusion in the human food chain. Because milk and milk products are primarily consumed by infants and children, residues of lead, cadmium, arsenic, and mercury are of great concern (Babu et al., 2018) . Their levels in food and food products must be monitored and controlled. Heavy metal level measurement is useful not only for determining risks to human health and assessing environmental quality but also for maintaining the high quality of final food and food products (Chandrakar et al., 2018).

Microelements such as copper (Cu), Iron (Fe), selenium (Se), and Zinc (Zn) are known to be essential for human growth. However, heavy metals such as Arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb) have health impacts on human well-being if it is more than the maximum permissible level (Babu et al., 2018). High permissible limit for example Cd in milk recommended by FAO/WHO is 0.01mg/l (FAO and WHO, 2011). According to Indian regulations, the maximum permissible limits for Pb in milk are 0.02 and 0.1mg/l (Sujka et al., 2019).

Table 2. 1: Concentration level of heavy metals in fresh cows' milk from different countries and maximum permissible limits by regulations

Country	**Concentration (mg/L(kg))**						**Reference**
	Cd	Cu	Pb	Fe	Ni	Cr	
Saudi Arebia	0.003	0.42	0.004	1.13	------	------	(Muhib et al., 2016)
Egypt	0.051	0.0953	0.214	8.994	------	------	(Meshref et al., 2014)
Ethiopia	-------	0.206	-------		------	0.064	(Muhib et al., 2016)
-Butajira and Meskan	0.03	1.1	12.1	1.9	-------	0.1	(Hymete et al., 2021)
-Borina zone		0.109				0.868	(Belete et al., 2014)
FAO/WHO	0.01	1.50	0.02-0.1	-----	0.02	0.05	(FAO and WHO, 2011), (Rewini et al., 2020)
EU (2006)	-------	-------	0.1	-----	-----	-----	(Rewini et al., 2020)
Uganda (AberSub County)	0.13	-----	0.17	-------	------	6.84	(Odongo et al., 2022)
Nigeria	0.008	0.343	0.028	0.611	0.078	------	(Peter et al., 2022)
Pakistan	-----	0.388	-----	0.241	0.223	0.172	(Nazir et al., 2015)

2.6 Bacteriological Content of Milk

Once milk is secreted out of the udder of the cow, the retention of milk requires cleanliness, sanitation and cooling. Additionally, milk drawn from a healthy cow normally contains a low microbial load of less than 10^3cfu/ml But, the bacterial load may increase up to 100-fold or more if stored for sometimes at ambient (30 to 35°C) temperature. Milk produced under hygienic conditions from healthy animals (Wanjala and Mathooko, 2017) and (Mohamed, 2018).

Milk is made and sold to consumers in Ethiopia without being pasteurized or held to a quality standard. Approximately 98% of the nation's milk is produced annually by subsistence farmers in rural areas, where smallholder dairy processing is essentially the only option and product sanitary standards are typically low (Bereda et al., 2019).

The microbial content of milk is a major factor in determining its quality because of its complex biochemical composition and high-water activity, which make it an excellent culture medium for the growth and multiplication of many different kinds of microorganisms. Because of the specific production, it is impossible to avoid contamination of milk with microorganisms, so the number of organisms that cannot grow at refrigeration temperatures remains low, suggesting that temperature plays a significant role in the prevalence and proliferation of particular organisms in the milk (Reta and Addis, 2017).

Traditional milk products like Irgo (traditional Ethiopian yogurt) and kibbe (traditional Ethiopian butter) are made from raw milk without any heat treatment, and the microbiological composition of milk is a key factor in determining its quality. As a result, there is an excessive chance of contamination with pathogenic organisms. When milk and milk products are handled improperly, harmful microorganisms may be introduced into the goods (Babege et al., 2020)

Personal cleanliness and appropriate clothing are essential for maintaining high milk quality, as long as they are free of diseases that spread from person to person. Poor postharvest technologies and unhealthy milk harvesting practices have an overall negative impact on milk quality, and youngsters in pastoral areas directly consume (suckle) from dirty teats just like their infants do. (Aleli et al., 2024).

2.6.1 Milk Contamination by Microbial

Milk is contaminated easily, and adverse environmental condition significantly affect the quality of milk and milk products, high temperature of the area where the climate is hot and humid, the raw milk gets easily fermented and spoiled during storage. However, less awareness of milk handling in rural areas, such storage facilities are not readily available in rural areas and cooling systems are not feasible due to lack of the required dairy infrastructure (Gemechu et al., 2016). Furthermore, milk protection refers to the lack of pathogenic organisms and other contaminants that could endanger the health of the consumer, while milk quality refers to a combination of chemical, physical, bacteriological, and cosmetic characteristics that enhance the acceptability of the milk product (Bereda et al., 2019. The consistency and protection of milk are determined by its specific gravity, chemical composition, and microbiological state, The chemical composition and microbiological presence of milk products are significant variables that impact their quality and protection (Abera and Lammifyad, 2022). Besides, milk contains important nutritional components such as carbohydrates, fat, protein, minerals, and vitamins, all of which are required for the body’s growth and maintenance, milk, on the other hand, is an ideal medium for the growth of pathogenic microorganisms, the transmission of chemicals, and the transmission of other impurities (Desye et al., 2023). Along with keeping the milk quality and safety, a great deal of milk safety and quality measures should be put in place at any segment of milk production, handling, processing and storage to ensure the milk offered to the consumer is of high quality, safe and wholesome. Even though bacteria cause serious health problems (Merwan et al., 2018).

Table 2. 2: Microbial quality in cows' milk in different countries/study areas and standards for milk by different regulations

Study areas	**Microbial quality tests in log10cfu/ml**			**Reference**
	TBC	TCC	Y&MC	
AdissAbebeba and its surrounding	6.15			(Tekilegiorgis and Ababa, 2024)
Hawassa	7.23+0.19	7.43±0.23 7.38±0.23	7.46±0.211 7.38±0.21	(Nurfeta and Gobena, 2023)
Tigray	4.9419			(Gebremichael et al., 2024)
Bahir Dar	7.11+0.13	5.57±0.26	4.12±0.14	(Birhanu et al., 2019)
BenchMaji-Zone -		5.203±0.230 5.187±0.211 4.911±0.324	4.001±0.588 3.944±0.346 3.762±0.68	(Gemechu, 2016)
Nairobi		4.56		(Wanjala et al., 2017)
Ethiopia			9.82	(Aleli et al., 2024)
European Commotion R egulation No.1662/2006	5.00			(Kačániová et al., 2017)
(ESA) (Ref No ES 3460: 2009		<5.3		(Tekilegiorgis and Ababa, 2024)
EU		**2.00**		**(Gebremichael et al., 2024)**

2.6.2 Impact of Contaminated Milk in Human Health

Milk can be contaminated with pathogenic bacteria or bacterial toxins, which can cause health problems like diarrhea, food poisoning, tuberculosis and salmonellosis. Several microorganisms, including *Salmonella species, Escherichia coli and Staphylococcus* species, are responsible for foodborne illnesses and milk-borne pathogenic bacteria pose a serious threat to human health, and constitute about 90% of all dairy- related diseases Staphylococcus aureus, *Salmonella spp*., *Listeria monocytogenes*, Escherichia coli O157:H7 and Campylobacter are the main microbiological hazards associated with raw milk consumption (Berhe et al., 2020).

Food-associated diseases are responsible for 33 to 90% of the deaths of children in Africa, and represent serious problems of the continent. In developing countries, particularly Ethiopia, milk is an important source of foodborne diseases and other infectious diseases. This happens when the production of milk and various dairy products occurs under unsanitary conditions and bad production practices (Aliyo and Teklemariam, 2022).

CHAPTER 3: MATERIAL AND METHODS

3.1 Description of the study area

The study was conducted in eastern and southeastern zones of tigray region. From eastern zone kilite awulaelo and tsirae wenberta , from south eastern zone degu’a tembien and Enderta were used for this research.

3.1.1 kilteawulaelo

Kilte awulaelo is one of the district in easten zone of Tigray region.

Table 3. 1: Information about kilteawulaelo district

Site description		Detail information
Location	Latitude	13°30' to 14°00' N
	Longuited	39°30'to 39°45' E
Physiograpy	Elivation	From1500metrs to lowlands to over 2700 m.a.s.l
	Soils	Shallow, rocky, soils ,Leptosols andVertisols**.**
	Climate	-semi arid tosub humid -rain fall is seasonal -the avarage annual rainfall is 400-600mm
Population	Human	-Men 49,239 -Women 50,429
	Livestock	-cattle ,119590 -sheep and goat,100,000 -donkey , 10,000 -poltry, 50,000 -Mixed crop-livestock system

Source : Tesfsy, W. (office of Agriculture and natural resources, kilteawulaelo). personal comminication, April 20,2024.

The farming system in the study areas is mixed farming in which crop production is the dominant livelihood source supplemented by the livestock sector. (Etsay et al., 2022).

3.1.2 Tsirae Wen Berta

Locality within the Kilte awulaelo district in the Eastern Zone of the Tigray Region. But specific information about Tsirae wenberta is limited because is a newly established district.

3.1.3 Enderta

One of the southeastern zones of Tigray region. The detailed information is as following table.

Table 3. 2: Information about Enderta district

Site description		Detail information
Location	Latitude	13°10′ to 13°30′ N
	Longuted	39°20′ to 39°40′ E
Physiograpy	Elivation	From200metrs to over 2600 m.a.s.l
	Soils	shallow, moderately fertile soil.
	Climate	-Semi-arid to sub-humid highland, highly seasonal**,** with over 80% falling between June and September, the avarage annual rainfall is 400-700mm.
Population	Human	-Men 57,738 -Women 59,405
	Livestock	-Cattle 90,000 -Sheep and goat,109,000 -Donkey , 12,000 -Poltry, 58,450 -Livestock are often mixed with crop farming

Source: Birhan, G and Yemane, k. (development agent of Enderta) personal information, October 19,2024)

3.1.4 Degu’a Tembien

Table 3. 3: Information about Degu’a tembien district

Site description		Detail information
Location	Latitude	13°35′ to 13°50′ N
	Longitude	38°40′ to 39°00′ E
Physiography	Elevation	1,800 meters in valley areas to over 2,800 meters at the highest.
	Soils	Shallow, stony soils on steep slopes and rock outcrops Vertosols.
	Climate	-Sub-humid highland climate -rain fall is seasonal -Annual rainfall: 500–800 mm
Population	Human	-Men 50,000 -Women 51,420
	Livestock	-Cattle,70,000 -Sheep 40,000 Goat37,00 -Donkey, 9,000 -Poultry, 49,779 -Mixed crop-livestock system

Source: Mies ,T. (Developmental agent, Lim’at) personal comminication, November 01,2024)

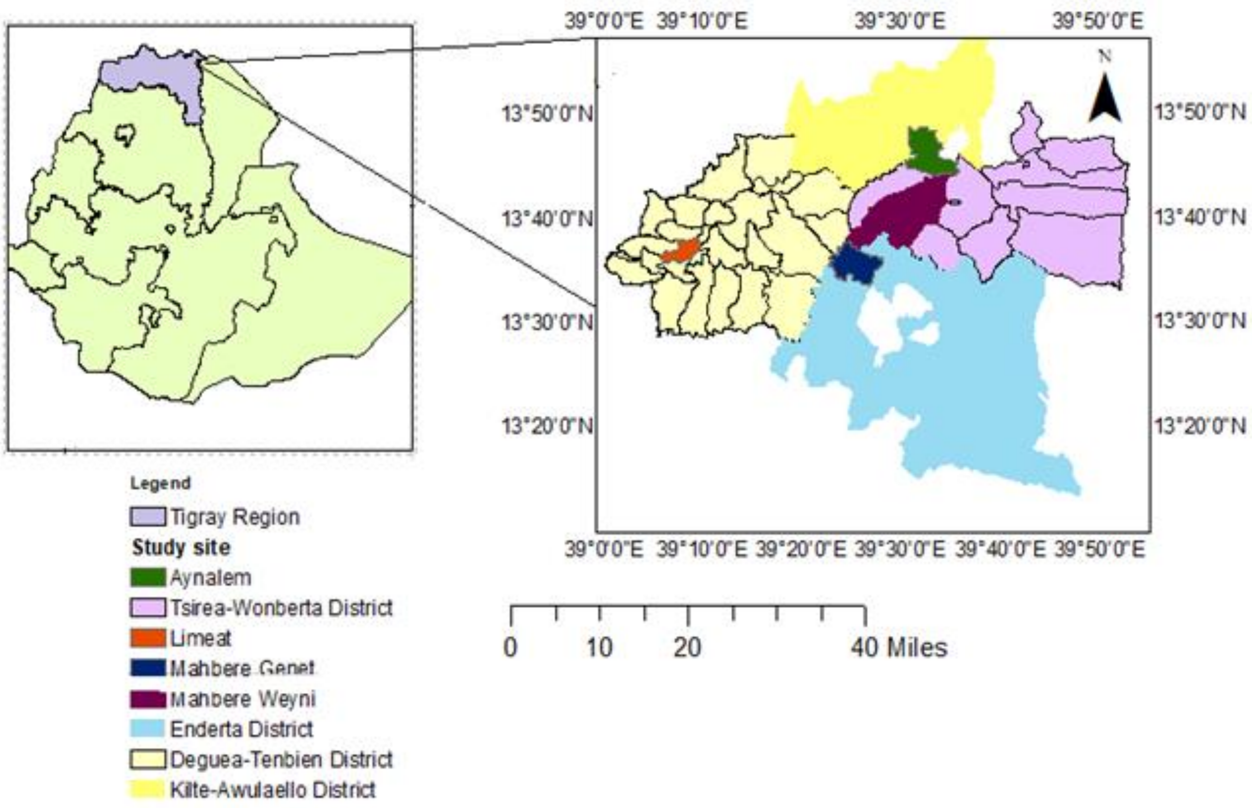

Figure 3. 1: Map the study areas

3.2 Site Selection

For this research, two zones were selected in Tigray, namely Eastern zone and Southeastern Zone. From each zone two districts were selected for this research. Purposively selecting of Tabias based local cattle breed type, a lactating cow who lived for more than 5 years was performed by communicating with the developmental agents. Accordingly, from eastern zone; two district was select, kilite awulaelo (tabia Aynalem) and Tsirae wenberta (tabia Mahbere weyni), whereas from southeastern zone, degu’a tembien (tabia Limat maygua) and Enderta (tabia Mahbere genet) were selected.

3.3 Research Design

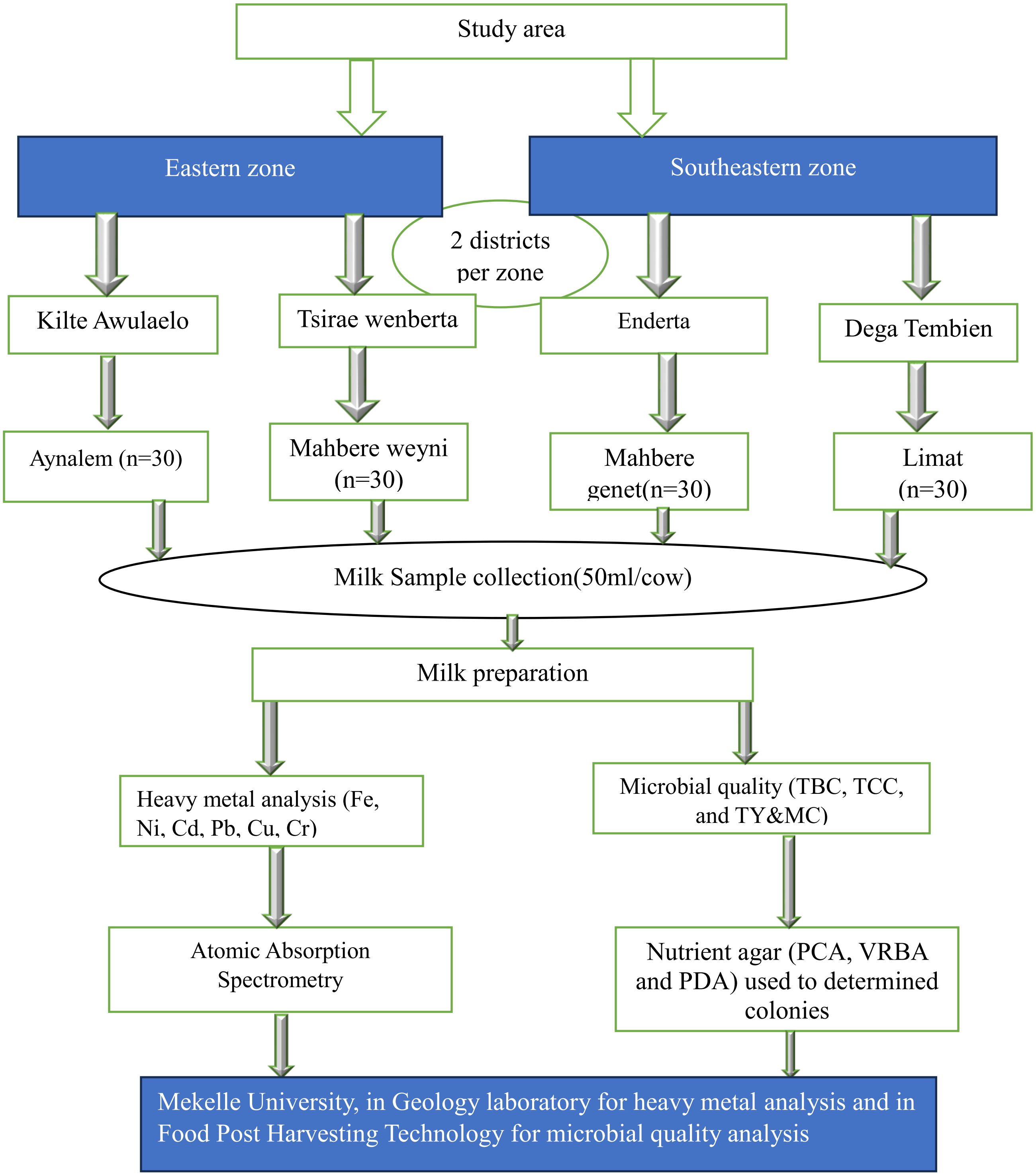

Figure 3. 2: Research design

3.4 Milk Sample Collection and Sampling Technique

Table 3. 4: Total number local dairy cow per tabia and number of selecting lactating cows for this research

Zones	District	Tabia	Number of available cows	Number of selected lactating cows
Eastern	Kilte awulaelo	Aynalem	3808	30
	Tsirae wenberta	M/weyni	2496	30
Southeastern	Enderta	M/genet	2077	30
	Deguatemben	Lim'at	3049	30

Source: Tabia Development Agents unpublished report, personal communication.

For this research, was used a total of 120 local breed lactating cows, which lived for five years and above, were selected for milk sample collection. Specifically, 30 lactating cows were chosen from each of the following Tabias: Aynalem (n = 30), M/ Weyni (n = 30), M/Genet (n = 30), and Limat (n = 30). For the research. Milking was performed using a hand milking by the farmers to their local containers (plastic vessels and calabashes) the milking time for all the sites was at early morning 12:30 to 2:00 local time. Afterwards, representative 50 ml milk samples were taken immediately from each cow, 50 ml of fresh milk was collected into a 50 ml plastic tube. Prior to milk collection, plastic tube was soaked in 10%HNO_3 and rinsed with deionized water to avoid any contamination. Afterwards, the sample milk was transported to the laboratory in a dry ice the temperature of 4°C and keep at refrigerator until they started preparation and analysis.

3.5 Milk Sample Preparation

Milk sample was prepared individually and pooled once. From each tabia, milk samples from randomly selected 5 individual cows (50ml) was analyzed individually and the others were pooled in a group of 5cows per pool.

For the pooling, the sample collected from the 25 cows was pooled in to 5 groups and each group consists of 5 cows. After that samples were coded as follow; the individual sample as (IS1, IS2, IS3, IS4 and IS5). The pooled samples (PS1, PS2, PS3, PS4 and PS5). A mixed milk from (cow 6-10) as PS1, the pooled sample combine milk from (cows11-15) PS2, combine milk from (cow 16-20) as PS3, pooled sample combine milk from (cow 21-25) as PS4 and pooled sample combine milk from (cows 26-30) PS5. From the pooled samples 50ml per pool was used for the experiment. The purpose of pooled (mixed) samples was to minimize cost for determination of heavy metals and microbial quantity analysis there is analytical work (Teklu et al., 2023). Then after preparation labeling the individual and pooled samples for heavy metal and microbial quality analysis.

3.6 Milk Sample Digestion and Analysis of Heavy Metal by Atomic Absorption Spectrometry

The milk sample were brought into clear solution for analysis by the Atomic Absorption Spectrometer. For this reason, the milk samples were digested with chemicals (nitric acid and hydrogen per oxide) where the organic matrix of milk was destroyed and left the element into a clear solution (Wet Digestion method) (Ishak et al., 2015).

Prior to heavy metal analysis, milk samples were digested using wet digestion method. For this, milk sample (3ml) was digested using concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) on a hot plate. In detail, a mixture of 6 mL of 69% HNO_3 was added to a beaker containing 3 mL milk samples. Next to that, 1 mL of 30% H_2O_2 was poured to the mixture and mixed thoroughly and placed on a hot plate. Afterwards, digested was performed using slow heating with a temperature of 90°C until the solution becomes clear (around 50 minutes up to 1 hour). The digestion vessels were then cooled to room temperature to avoid foaming and splashing on opening, and the digested samples were filtered through Whatman 42 filter paper to remove any suspended residues.

The filtered sample solution was transferred into a 50 ml volumetric flask and diluted with distilled water to bring the volume of the solution to 50 ml following the procedure as stated in (Hymete et al., 2021). Measurement of levels of iron (Fe), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni) and lead (Pb) analysis using an Atomic Absorption Spectrometer (VARIN-50B). AAS methods were observed to exhibit good linearity, confirming its reliability for determining trace levels of the heavy metals in cows' milk. Concentrations of each metal was determined from the absorption verses, concentration plotted calibration curves. For each sample, triplicate determinations were performed, and average results were recorded.

3.6.1 Calibration Curves and Measurement of Metal Concentrations

The calibration curves were drawn for Fe, Cu, Cd, Ni, Cr and Pb using linear regression analysis of the concentrations of the standard solutions versus absorbance values. Six series of working standard solutions of metals were prepared by diluting the intermediate standard solution with de-ionized water (a stock solution containing 1000 mg/l). Each standard solution was measured three times and the mean was plotted.

3.7 Microbial Quality Analysis Method

Three types of nutrient agar were used to count the number of bacterial loads in milk samples. Plate Count Agar (PCA) for Total Bacterial count (TBC), Violet Red Bile Agar (VRBA). for Total Coliform Count (TCC) and Potato Dextrose Agar (PDA) for Yeast and Mold Count are methods used to growth or counting the number of colonies forming unit (CFU/ml). which were determined in Food Post Harvesting Technology laboratory. After counting and recording bacterial colonies in each petri-dish/plates the number of bacteria in milliliter was calculated by the following formula given by APHA (Birhanu and Asamnew , 2019).

$$N\ (cfu/ml) = \frac{\sum colonies}{[(1 \times n_1) + (0.1 \times n_2)d]}$$

Where,

N is number of colonies per milliliter of milk

$\sum c$ is sum of colonies on plates counted

n_1 is number of plates on lower dilution counted

n_2 is number of plates in next higher dilution counted and

d is dilution from which the first counts are obtained (dilution factor)

3.7.1 Total Bacterial Count

The Total Bacterial Count was determine using the Plate Count Agar (PCA). Preparation the PCA was according to manufacturer instructions typically, dissolve the specified amount of PCA powder in distilled water was used 23.5 g /l by assuming that calculated the amount of PCA for the surface of 15-20ml Petri dish was 4.7g/Petri dish and then sterilized by using Autoclave the prepared medium at 121°C for 15 minutes. Afterwards allow the agar to cool to about 45-50°C before spread into sterile Petri dishes and the agar was solidified at room temperature. prepare the sample of serial dilution of the milk sample use serial dilution tubes and add 9 ml of sterile distilled water, transfer 1ml of milk sample in to 1st tube 10^{-1} (1:10) mix thoroughly and perform further dilution up to 10^{-6} (1:100,0000) by transfer 1ml from the previous diluted in to the next one after dilution was completed transferred 0.1ml on the surface of PCA used micropipettes after transferred distribute the sample across the agar surface used sterile spreader. Plates were invert and incubated at 37^0C for 48 hours. After that examine the plates for colony growth, and count all the distinct colonies on plates with between 30 and 300 colonies forming units/ml. this method is similar procedure to the (Desye et al., 2023).

3.7.2 Total Coliform Count

Total Coliform Count was measured using a sterile Violet Red Bile Agar (VRBA). Preparation the VRBA was according to manufacturer instructions typically, dissolve the specified amount of VRBA powder in distilled water was used 51.53 g/l by assuming that calculated the amount of VRBA for the surface of 15-20ml Petri dish was 10.306g/Petri dish or plate and then sterilized by using Autoclave the prepared medium at 121°C for 15 minutes. Afterwards allow the agar to cool to about 45-50°C before spread into sterile Petri dishes and the agar was solidified at room temperature. prepare the sample of serial dilution of the milk sample use serial dilution tubes and add 9 ml of sterile distilled water, transfer 1ml of milk sample in to 1st tube 10^{-1} (1:10) mix thoroughly and perform further dilution up to 10^{-6} (1:100,0000) by transfer 1ml from the previous diluted in to the next one after dilution was completed transferred 0.1ml on the surface of VRBA used micropipettes after transferred distribute the sample across the agar surface used sterile spreader. Plates were invert and incubated at 37^0C for 48 hours. After that examine the plates for colony growth, and count plates with colonies ranging from 15 to 150cfu/ml were chosen. This is similar procedure to the (Desye et al., 2023).

3.7.3 Total Yeast and Mold Count (TY&MC)

Potato Dextrose Agar (PDA) was used to determine Total Yeast and M mold Count. Preparation the PDA was according to manufacturer instructions typically, dissolve the specified amount of PDA powder in distilled water was used 39 g/l by assuming that calculated the amount of PDA for the surface of 15-20ml Petri dish was 7.8g/l Petri dish or plate and then sterilized by using Autoclave the prepared medium at 121°C for 15 minutes. Afterwards allow the agar to cool to about 45-50°C before spread into sterile Petri dishes and the agar was solidified at room temperature. prepare the sample of serial dilution of the milk sample use serial dilution tubes and add 9 ml of sterile distilled water, transfer 1ml of milk sample in to 1st tube 10^{-1} (1:10) mix thoroughly and perform further dilution up to 10^{-6} (1:100,0000) by transfer 1ml from the previous diluted in to the next one after dilution was completed transferred 0.1ml on the surface of PDA used micropipettes after transferred distribute the sample across the agar surface used sterile spreader. Plates were invert and incubated at 30^0C for 5 days. After that examine the plates for colony growth, and count plates with colonies ranging from 10 to 150 cfu/ml were chosen. This is similar procedure to the (Desye et al., 2023).

3.8 Statistical data analysis

Microsoft excel spreadsheet was used to create the calibration curves and used for transformation to logarithmic base 10 values of the microbial quality before the data recorded to SPSS software.

Basic variation statistical values (mean, standard deviation, maximum and minimum values) were calculated using SPSS (version 26), The data was analyzed using one-way analysis of variance (ANOVA) to examine statistical significance at 95% ($P<0.05$) confidence level Tukey HSD, Post Hock multiple comparison test was used to evaluate the significant difference among the study areas.

3.9 Materials used with Function

3.9.1 Laboratory and Field Equipment

Table 3. 5: List of materials used in field and laboratory works with function

Equipment’s		Function
Field equipment	Dry Ice (ice box)	Used to keep the samples until collection complete and transported.
	Plastic tube	used to collect milk samples
Lab equipment	Bottle flasks	different size Bottle flasks of used in the heavy metal analysis and sterilize of the nutrient agars.
	Hand glove	To control contaminants
	Hot plate	Used for digesting milk samples (produce heat)
	YP-series Precision Balance (YP-20002)	
	Atomic Absorption Spectrometry (VARIN 50B)	used to analysis the concentration of the elements.
	Oven drier (DHG 9023A)	used to dry the bottle flask after cleaning the contaminated materials.
	Refrigeration (HT903WA)	Used to keep the samples until starting the samples analyses
	Autoclave (WAC-60)	Used to sterilized by autoclaving at 121°C for 15 min of the nutrient agars.
	Incubator (DHP-9082)	
	Colony counter	used for if the colony is not seen.
	Test tubes/Dilution tubes	using for sample dilution
	Pipettes/micropipettes	was used to take the amount of sample
	Spreader	used to spread the nutrient agar and milk sample in the petri-dish

3.9.2 Chemical and Reagents

Analytical grade (AnalaR) chemicals, reagents and distilled(deionized) water was used throughout the study. Nitric acid, HNO_3, (69%, Sigma Aldrich, Steinin, Germany) and hydrogen peroxide, H_2O_2 (30%, BDH Chemicals Ltd, Poole, England) was used for the digestion of milk samples. Deionized water was used for sample preparation, dilution, and rinsing before analysis according to (Hymete et al., 2021). A stock standard solution (Buck Scientific Puro-Graphic, USA) ICP multi-element standard solution with concentrations of 1000 mg/ L^{-1} Cu, Fe, Cr, Ni, Cd, and Pb were used for the preparation of calibration standards before starting the analysis (reading) according the AAS machine.

3.9.3 Nutrient Agar

Plate count Agar (Indian) Violet Red Bile Agar (Indian) Potato Dextrose Agar (Indian): was used for identification of Total Bacterial Count, Total Coliform Count, and Yeast and Mold Count respectively.

CHAPTER 4: RESULTS

4.1 Calibration Curve of the Six Heavy Metals in Atomic Absorption Spectrometer

The correlation coefficients of calibration curves were given in the following figures. The correlation coefficient of more than 0.99 showed that there is strong linear relationship between concentrations and absorbance.

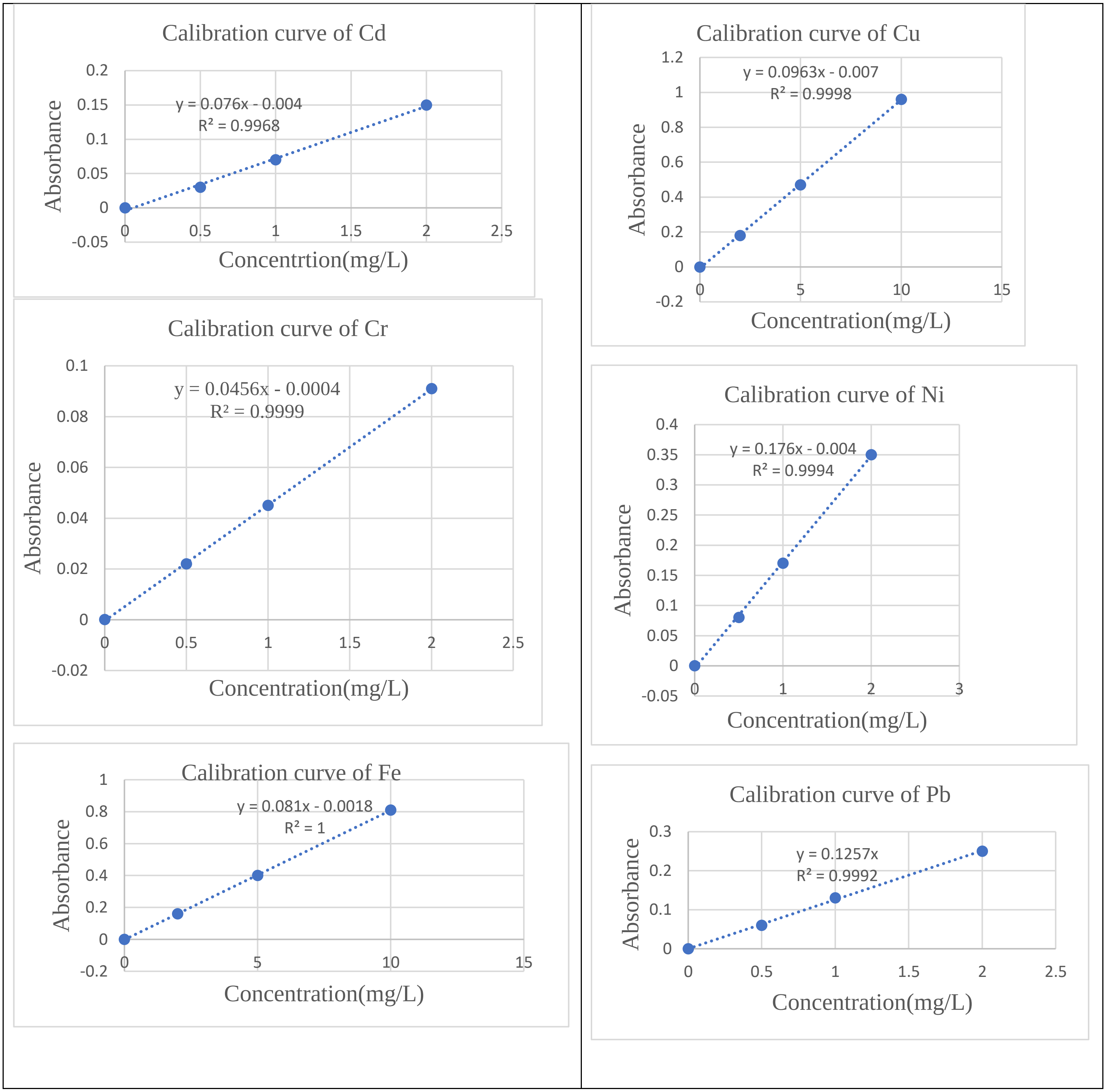

Figure 4. 1: Calibration curves of the six heavy metals in sample analysis tabia Aynalem

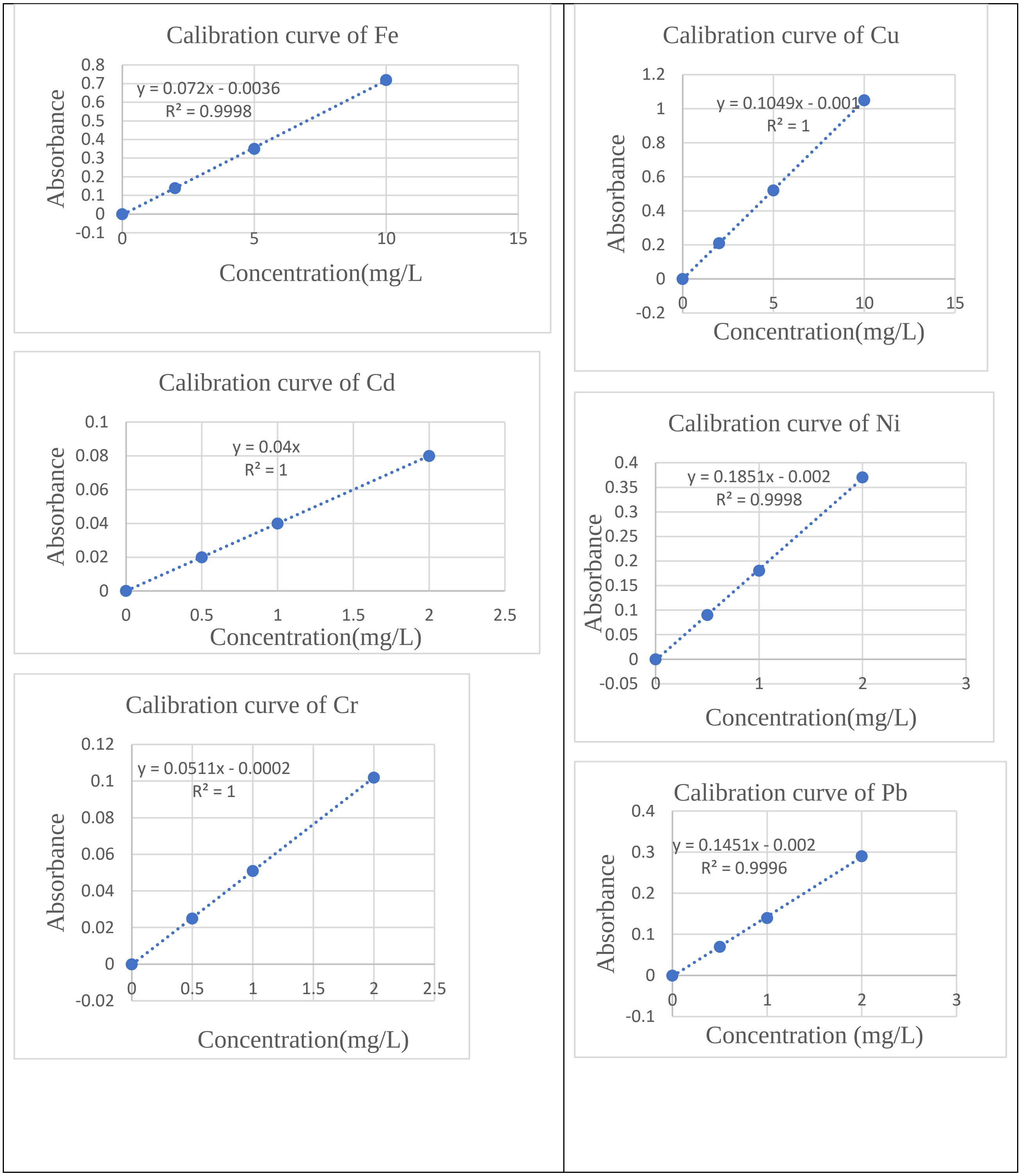

Figure 4. 2: Calibration curves of the six heavy metals in sample analysis tabia Mahbereweyni

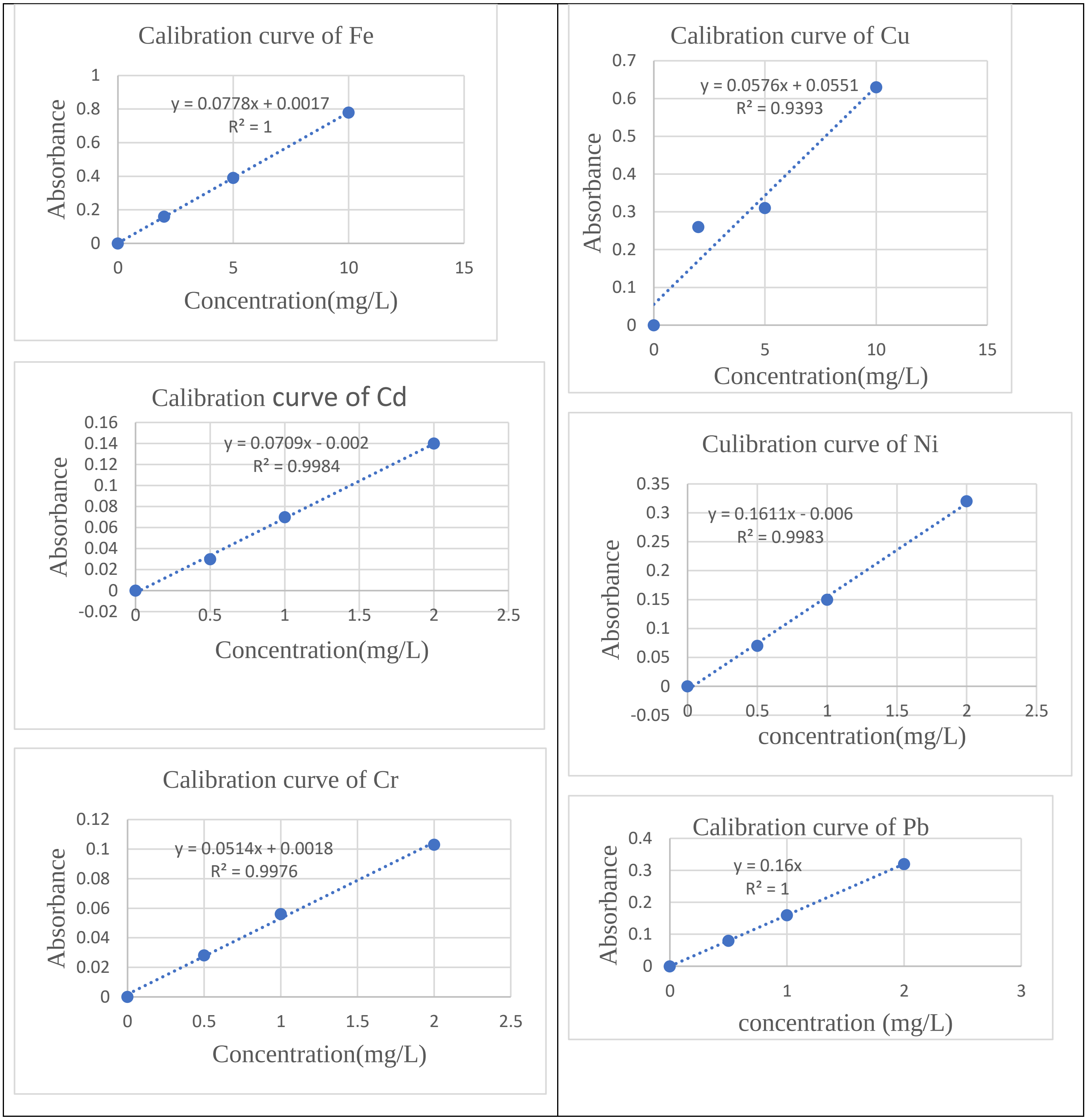

Figure 4. 3: Calibration curves of the six heavy metals in sample analysis Mahbere genet

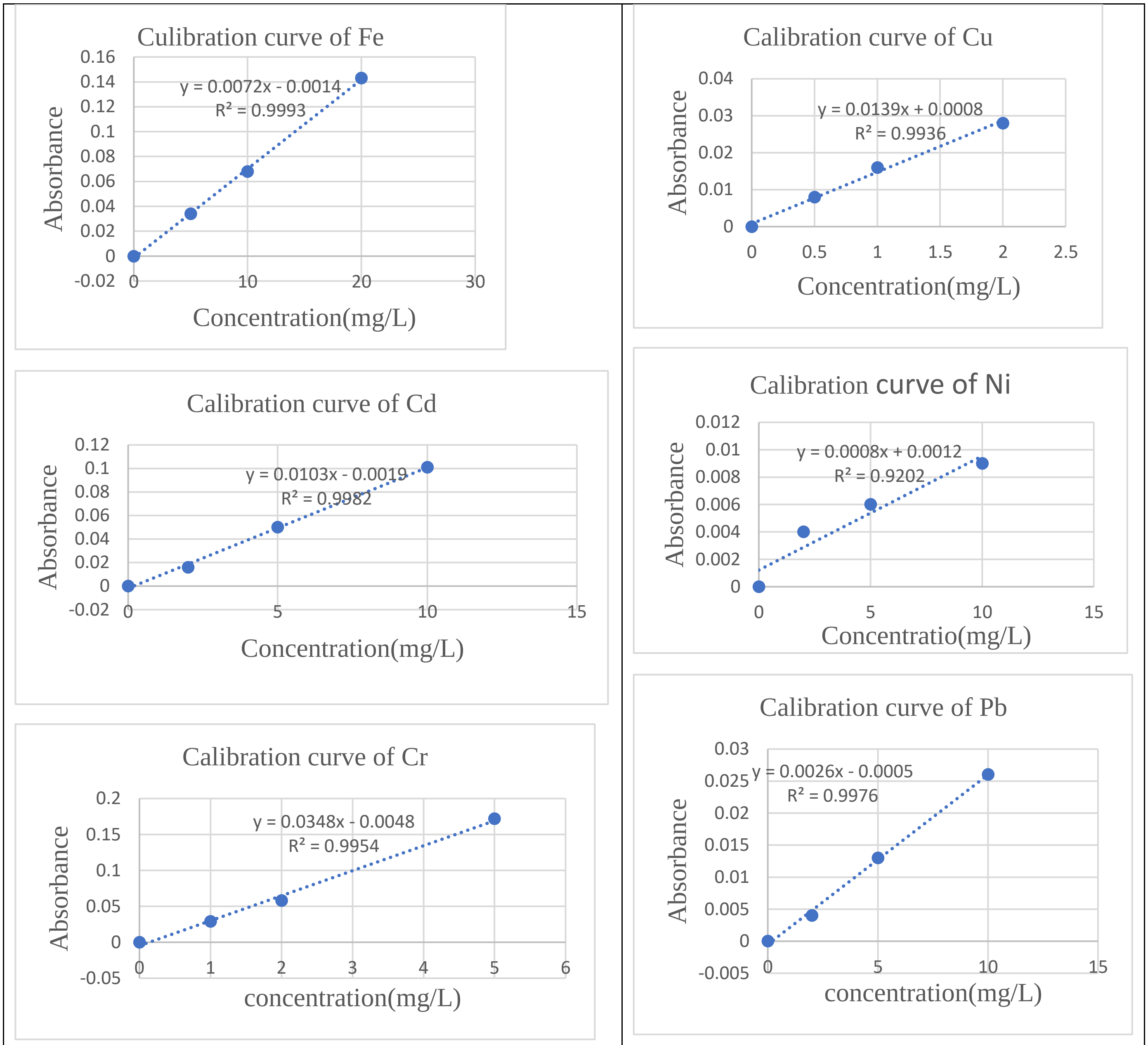

Figure 4. 4: Calibration curves of the six heavy metals in sample analysis Limat

4.2 Concentration Level of Heavy Metals in Cows' Fresh Milk

4.2.1 Concentration Level of the Six Selected Heavy Metals Based on Sample Type

Table 4. 1: Mean concentration of the six heavy metals for individual samples

Study areas(tabias)	Concentration in mg/l (ppm)					
	Fe	Cd	Cr	Cu	Ni	Pb
Aymalem (n=5)	23.48	0.044	0.162	0.504	0.212	0.036
M/weyni (n=5)	15.22	0.024	0.010	0.465	0.023	0.084
M/genet (n=5)	21.56	0.015	0.009	0.551	0.019	0.014
Lmiat (n=5)	24.71	0.005	0.006	1.266	0.052	0.067
Average (n=20)	21.26	0.0225	0.0094	0.6969	0.0279	0.0404

mg/l: milligram prelitter (ppm): parts per million, n: sample size

Table 4. 2: Mean concentration of the six heavy metals for pooled samples

Study areas (tabias)	Concentration in mg/l (ppm)					
	Fe	Cd	Cr	Cu	Ni	Pb
Aymalem (n=5)	29.840	0.043	0.021	0.461	0.028	0.0304
M/weyni (n=5)	21.061	0.022	0.014	0.327	0.025	0.020
M/genet (n=5)	20.22	0.017	0.005	0.533	0.027	0.033
Limat (n=5)	12.661	0.002	0.002	1.771	0.073	0.068
Average (n=20)	20.74	0.0215	0.0110	0.7863	0.0357	0.0411

mg/l: milligram prelitter (ppm): parts per million, n: sample size

Table 4. 3: Concentration of heavy metal in cow fresh milk in Mean ± Std. Deviation of based on study area.

Parameters/elements in mg/l(ppm)	Study areas (Tabias)			
	Aynalem (n=10)	M/weyni (n=10)	M/genet (n=10)	Lim ‘at(n=10)
Fe	26.660±15.0756	18.140±6.1381	20.880±1.9742	18.720±15.7210
Cd	0.0442±0.0248	0.0234±0.0137	0.0165±0.009	0.0041±0.0038
Cr	0.0189±0.0043	0.0127±0.0034	0.0725±0.048	0.0021±0.0005
Cu	0.4823±0.3201	0.4232±0.2801	0.5421±0.2819	1.5189±0.3136
Ni	0.0245±0.0209	0.0246±0.062	0.0276±0.0194	0.0506±0.0296
Pb	0.0335±0.0179	0.0293±0.0236	0.0196±0.0094	0.0807±0.0532

mg/l: milligram prelitter (ppm): parts per million, n: sample size

4.3 Comparison of the Concentration Level of Heavy Metals in Cows’ Fresh Milk at Different Study Areas

The overall mean of the concentration of these selected metals in all studying areas were found to be the higher concentration of Fe and the lowest concentration of Cr in fresh milk.

4.3.1Measurement of the Concentration of Iron (Fe) at Different Study Areas

The measurement of Iron (Fe), lead (Pb), chromium (Cr) and cadmium (Cd), nickel (Ni), and copper (Cu) were determined as indicated in (Table 4.3). The study's findings indicate that, while all six elements (heavy metals) were found in all study sites. However, the concentration levels of these elements in fresh cows’ milk varied among the study areas. The element Fe was found to be higher in all sites. In detailed the concentration level of Fe in cows’ fresh milk in tabia Aynalem is higher than the other three tabias in mean concentration, like concentration level of Fe ranges from 13 to 58.8mg/l with the mean concentration of 26.660±15.0756 mg/l in tabia Aynalem was higher than the ranges from 17.4 to 23.8mg/l with the mean concentration of 20.880±1.9742 mg/l in tabi

M/genet, the ranges from 9.2 to 61.6mg/l with the mean concentration of 18.720±15.7210 mg/l in tabia Limat, and ranges from 12.8 to 32.8 with the mean concentration of 18.140±6.1381 mg/l in tabia M/genet (Table 4.3). but there is no significant difference among the four study areas (p-value >0.05) (Appendix-I)

4.3.2 Measurement of the Concentration of Cadmium (Cd) at Different Study Areas

Cadmium (Cd) is also presented in all the study areas, the concentration level of Cadmium (Cd) varied among the four study areas as follows: The concentration of Cd ranges from 0.01 to 0.087mg/l with the mean concentration of 0.0442±0.0248 mg/l for tabia Aynalem, 0.011 to 0.051mg/l with the mean concentration of 0.0234±0.0137 mg/l for tabia M/weyni, 0.008 to 0.034mg/l with the mean concentration of 0.0165±0.009 mg/l for tabia M/gent, and 0 to 0.0118mg/l with the mean concentration of 0.0041±0.0038 mg/l for tabia Limat, (Table 4.3)

There was a significant difference in Cd concentration among the four sites. Tabia Aynalem showed a significant difference compared to tabia M/weyni, tabia M/genet, and tabia Limat, as the p-value was less than 0.05, tabia M/weyni was significantly different from tabia Aynalem and tabia Limat but showed no significant difference from tabia m/genet, as the p-value was greater than 0.05 (Appendix -II).

4.3.3 Measurement of the Concentration of Chromium (Cr) at Different Study Areas

The concentration of Chromium (Cr) in the four study areas was as follows: the concentration of Fe ranges from 0.001 to 0.012mg/l with the mean concentration of 0.0725±0.048 mg/l at tabia M/gent, 0.013 to 0.025mg/l with the mean concentration of 0.0189±0.0043 mg/l at tabia Aynalem, 0.008 to 0.019mg/l with the mean concentration of 0.0127±0.0034 mg/l at tabia M/weyni, and

0.0013 to 0.0024mg/l with the mean concentration of 0.0021±0.0005 mg/l at tabia Limat (Table4.3) Overall, Cr concentration showed significant differences among all study Areas (Appendix -III).

4.3.4 Measurement of the Concentration of Copper (Cu) at Different study Areas

The mean concentration of Copper (Cu) in the four study areas was as follows: the concentration of Cu ranges from 1.087 to 1.979mg/l with the mean concentration of 1.5189±0.3136 mg/l in tabia Limat, 0.006 to 0.815mg/l with the mean concentration of 0.5421±0.2819 mg/l in tabia M/gent,0.006 to 0.897mg/l with the mean concentration of 0.4823±0.3201 mg/l in tabia Analem, and 0.004 to 0.746mg/l with the mean concentration of 0.4232±0.2801 mg/l in tabia M/weyni (Table 4.3).There was no significant difference in Cu concentration between tabia Aynalem and M/weyni or between tabia Aynalem and tabia M/genet; however, tabia Aynalem showed a significant difference compared to tabia Limat, Tabia limat was showed significantly different from all the study areas P – values less than 0.05 (Appendix-IV).

4.3.5 Measurement of the Concentration of Nickel (Ni) at Different study Areas

The concentration of Nickel (Ni) in the four study areas was as follows: the concentration of Ni ranges from 0.017 to 0.141mg/l with the mean concentration of 0.0506±0.0296 mg/l in tabia Limat, 0.013 to 0.059mg/l with the mean concentration of 0.0276±0.0194mg/l in tabia M/genet, 0.011 to 0.065mg/l with the mean concentration of 0.0246±0.062 mg/l in tabia M/weyni, and 0.012 to 0.081 with the mean concentration of 0.0245±0.0209 mg/l in tabia Aynalem. However, the differences in Ni concentration among the study areas were not significant difference, as the p-value was greater than 0.05 among all the study areas (Appendix-V).

4.3.6 Measurement of the Concentration of Lead (Pb) at Different study Areas

The concentration of Lead (Pb) in the four study areas was as follows: the concentration of Pb ranges from 0.014 to 0.156mg/l with the mean concentration of 0.0807±0.0532 mg/l in tabia Limat, 0.013 to 0.61mg/l with the mean concentration of 0.0335±0.0179mg/l in tabia Aynalem, 0.007 to 0.08mg/l with the mean concentration of 0.0293±0.0236 mg/l in tabia M/weyni, and 0.012 to 0.052mg/l with the mean concentration of 0.0196±0.0094 mg/l in M/genet (Table 4.3). Tabia Aynalem showed no significant difference (P-values greater than 0.05) from tabia M/weyni and M/genet. But was significantly different from tabia Limat. P-values less than 0.05 (Appendix-VI).

4.4 Analysis of Microbial Quality in Cows Fresh Milk at Different Study Areas

4.4.1 Microbial Quality Analysis Based on Individual and Pooled Samples

Table 4. 4: Mean of the individual samples of microbial quality in each study areas

Study areas(tabias)	Parameters (Log10 CFU/ml)		
	TBC	TCC	TY&MC
Aynalem(n=5)	5.554	4.910	5.022
M/weyni(n=5)	6.664	4.864	5.572
M/genet(n=5)	5.481	3.852	5.400
Lim’at(n=5)	4.302	2.784	4.244
Average(n=20)	5.500	4.102	5.059

Table 4. 5: Mean of the pooled samples of microbial quality in each study areas

Study areas(tabias)	Parameters (Log10 CFU/ml)		
	TBC	TCC	TY&MC
Aynalem(n=5)	5.554	5.044	5.320
M/weyni(n=5)	5.802	4.882	5.388
M/genet(n=5)	6.192	5.002	5.336
Lim'at (n=5)	6.050	5.640	5.782
Average(n=20)	5.899	5.142	5.456

Table 4. 6: Mean ± Std. Deviation of microbial quality of each study areas

Study areas	Parameters (Log10 CFU/ml)		
	TBC	TCC	TY&MC
Aynalem (N=10)	5.55±0.96	5.22±0.89	5.28±0.96
M/weyni (N=10)	6.23±0.88	4.87±0.89	5.48±1.01
M/genet (N=10)	5.83±0.99	4.42±1.73	5.36±0.58
Lim'at (N=10)	5.17±2.03	4.21±2.42	5.01±2.00
Over all mean (n=40)	5.70±1.31	4.68±1.60	5.28±1.22

Log10: logarithm values base 10, CFU: colony forming unit per milliliter, TBC: Total Bacterial Count, TCC: Total Coliform Count, TY&MC: Total Yeast and Mold Count, N: sample size

4.4.2 Total Bacterial Counts (TBC)

The results are indicated no significant difference ($P>0.05$) in TBC among the four study areas (Appendix-VII). The mean of 5.55±0.96 Log10cfu/ml, 6.23±0.88 Log10cfu/ml, 5.83±0.99 Log10cfu/ml, 5.17±2.03 Log10cfu/ml were respectively for tabia Aynalem, tabia M/weyni, tabia M/ genet and Lim'at. and the total bacterial count of four study areas are follows tabia M/weyni > tabia M/genet > tabia Aynalem > tabia Lim'at (Table 4.6).

4.4.3. Total Coliform Count (TCC)

Coliforms were found 5.22±0.89 Log10cfu/ml in Aynalem 4.87±0.89 Log10cfu/ml tabia M/weyni, 4.42±1.73 Log10cfu/ml in M/genet and 4.21±2.42 Log10cfu/ml in tabia Limat and these were tabai Aynalem>tabia M/weyni>tabia M/genet>tabia Limat in total coliform count of the four study areas (Table 4.6)., there is no significant differences among the four study areas (p-value > 0.05) (Appendix-VIII).

4.4.4 Total Yeast and Mold Count (TY&MC)

Total Yeast and mold also presented in all samples of in the four study areas like 5.28±0.96 Log 10cfu/ml in Aynalem 5.48±1.01 Log10cfu/ml M/weyni 5.36±0.58 Log10cfu/ml in M/genet and 5.01±2.42 in Limat and there were M/weyni> M/genet>Aynalem>Limat in Total Yeast and Mold Count of the four study areas (Table 4.6) but there is no significant difference (Appendix-IX)

CHAPTER 5: DISCUSSION

5.1 Concentration Level of the Heavy metals in Cows' Fresh Milk

One of the biggest issues with metals is that they can bioaccumulate. Since milk is primarily consumed by infants and children, metal residues in milk are especially dangerous because heavy metals are extremely toxic and cannot be broken down, even at low concentrations (Kochare et al., 2015). When living things are exposed to these substances from the food chain, like milk, they accumulate and store them more quickly than they can be metabolized and eliminated.(Teklu et al., 2023). From this research the average concentration of heavy metals in fresh cows' milk from the four studying areas the Fe, Cd, Cr, Cu, Ni and Pb are presented.

5.1.1 Mean Concentration Level of Heavy Metals in Individual and pooled samples

The highest concentration of the selected heavy metals obtained from individual samples 24.714mg/l for Fe, and the lowest values was 0.005mg/l for Cd and 0.006mg/l for Cr in tabia Limat (Table 4.1) and the highest concentration of heavy metals obtained from pooled samples 29.840 mg/l for Fe was in tabia Aynalem and the lowest values was 0.002mg/l for Cd and Cr in tabia Lim'at (Table 4.2) However the average of Fe in the individual sample was 21.26mg/l (Table 4.1) and 20.94mg/l for pooled samples (Table 4.2) were higher concentration if compared to the other studies, concentrations of Fe in cows milk with an average of 8.994mg/l a study by (Meshref et al., 2014).

The an average of concentration of Cd was 0.0225mg/l from individual sample(Table 4.1) and 0.0215mg/l from pooled (Table 4.2) were less than concentration of Cd in cows milk with an average of 0.051mg/l study by (Meshref et al., 2014) and the concentration of Cd in cows milk from the study areas of Meskan and Butajira was obtained 0.03mg/l also greater than the present study in individual sample and pooled samples (Hymete et al., 2021). The average concentration of Cr from individual sample was obtained 0.0094mg/l (Table 4.1) and 0.0110mg/l (Table 4.2) for pooled samples. These were lowest if compared to the higher level of Cr obtained from Borena zone in cows' fresh 0.868mg/l the study by (Belete et al., 2014). Basically the average concentration of Cr in both individual and pooled samples were not exceed the maximum permissible limits set by FAO/WHO was 0.05mg/l (FAO and WHO, 2011).

An average concentration of Cu also obtained 0.6969mg/l (Table 4.1) from individual samples and 0.7863mg/l (Table 4.2) from pooled samples. These also higher if compared to 0.0953 mg/l study by (Meshref et al., 2014) but lowest from the average concentration of Cu in cows milk obtained from Buta Jira and Meskan 1.1mg/l (Hymete et al., 2021). Additionally the an average of Cu in both individual and pooled sample were within the maximum permissible limits set by FAO/WHO was 1.50mg/l (FAO and WHO, 2011). An average concentration of Ni also 0.0279 mg/l (Table 4.1) from individual samples and 0.0357 mg/l (Table 4.2) from pooled samples both samples were lower compared to the Ni concentration in cows milk was 0.223 mg/l reported by (Nazir et al., 2015). But higher than the concentration level Ni for milk is 0.02 mg/l was the maximum permissible limit set by WHO as sited (Rewini et al., 2020). Pb with an average of 0.0404 mg/l (table 4.1) from individual samples and 0.0411(Table 4.2) from pooled samples. These were also lower from 12.1mg/l in cows fresh milk from Buta Jira and Meskan study by (Hymete et al., 2021). The average sample from individual and pooled were within the maximum permissible limits 0.1mg/l by EU (2006) (Rewini et al., 2020) and the 0.02-0.1mg/l set by FAO/WHO (FAO and WHO, 2011)

5.1.2 Measurement of the Concentration of Iron (Fe) at Different Study Areas

Iron is an essential mineral needed in human biological and physiological systems, iron deficiency causes anemia and there is a decrease in physical work, the excess of this substance is also the cause of organ failure (Rewini et al., 2020).

Irone (Fe) is presented in high concentration level from the selected six heavy metals, additionally the mean concentration of Fe in tabia Aynalem is higher than from all study areas. Concentration level of Fe ranges from 13 to58.8mg/l with the mean concentration of 26.660±15.0756 mg/l in tabia Aynalem. Here is the range from 17.4 to 23.8mg/l with the mean concentration of 20.880±1.9742 mg/l in tabia M/genet, the range from 9.2 to 61.6 mg/l with the mean concentration of 18.720±15.7210 mg/l in tabia Limat, and the range from 12.8 to 32.8 with the mean concentration of 18.140±6.1381 mg/l in tabia M/weyni (Table 4.3).

Mean concentration of Fe in this research is greater than the average concentration of Fe in cows fresh milk 0.611 mg/l reported by (Peter et al., 2022) and Fe levels in milk from cows fed only grass ranged from 1.43 to 1.80 mg/l was reported (Auni et al., 2021). The average concentration of Fe compared with this study with concentration ranges from (0.0 to 1.9) mg/l was lower in milk collected from Butajirra and Meskan Districts reported by (Hymete et al., 2021).

Cows' fresh milk from Khyber Pakhtunkhwa, Pakistan, from four study sites was reported like this 0.424±0.004 mg/kg from Bannu, 0.236±0.004 mg/kg from Lachi, 0.095±0.003 mg/kg from Karak and 0.126±0.004 mg/kg from Lucky Marwat (Nazir et al., 2015). High iron (Fe) concentrations with an average of 8.994 (2.961 - 45.619 mg/l) heavy metals and trace elements levels in cows milk and milk products a study by (Meshref et al., 2014).However these all reports concentration of Fe in cows' milk lower than this study.

5.1.3 Measurement of the Concentration of Cadmium (Cd) at Different Study

Cadmium is the most dangerous heavy metals with pronounced carcinogenic and mutagenic properties (Sarsembayeva et al., 2020). The concentration of Cadmium (Cd) varied among the four study areas as follows: the concentration of Cd ranges from 0.01 to 0.087mg/l with the mean concentration of 0.0442±0.0248 mg/l for tabia Aynalem, ranges from 0.011 to 0.051 mg/l with the mean concentration of 0.0234±0.0137 mg/l for tabia M/weyni, 0.008 to 0.034mg/l with the mean concentration of 0.0165±0.009 mg/l for tabia M/genet, and 0 to 0.0118 mg/l with the mean concentration of 0.0041±0.0038 mg/l for tabia Limat (Table 4.3). The result indicates the concentration of Cd was significantly exceeds themaximum permissible limit for milk set by FAO/WHO (FAO and WHO, 2011) is 0.01mg/l (ppm) in all the study areas except in tabia Limat (0.0041±0.0038 mg/l) within 0.01mg/l (ppm).

The same to that the concentration of Cd in cows milk from selected Areas of Zanzibar Island , Tanzania was present in the mean values 0.001 mg/l reported by (Ali et al., 2023) was less than from all the present study areas. But the mean concentration of Cd 0.13±0.05, 0.12±0.04 0.15±0.11, 0.19±0.13mg/l respectively for Aber , Acaba, Loro and Kamdini in investigation of levels of some selected heavy metals in raw bovine milk from Oyam District, Uganda by (Odongo et al., 2022) was greater than the present study in Cd concentration at the four study areas. Cd concentrations ranges from 0.008–0.104 with an average 0.0953 ± 0.0413mg/l was reported by (Meshref et al., 2014).This also greater than from all the present study.

5.1.4 Measurement of the Concentration of Chromium (Cr) at Different Study Areas

The concentration of Chromium (Cr) in the four study areas was as follows: the concentration of Cr ranges from 0.001 to 0.012mg/l with the mean concentration of 0.0725±0.048 mg/l at tabia M/genet, ranges from 0.013 to 0.025 mg/l with the mean concentration of 0.0189±0.0043 mg/l at tabia Aynalem, ranges from 0.008 to 0.019 mg/l with the mean concentration of 0.0127±0.0034 mg/l at tabia M/weyni, and ranges from 0.0013 to 0.0024 mg/l with the mean concentration of 0.0021±0.0005 mg/l at Tabia Limat (Table 4.3).

The mean concentration of Cr in the four study areas in present study is less than the study in cows fresh milk in Borena zone 0.868 ± 0.026 mg/l (Belete et al., 2014). And the mean concentration of Cr 6.84±2.03, 6.55±1.71 ,6.83±1.74 and 8.34±2.92 mg/l respectively for Aber , Acaba, Loro and Kamdini in investigation of levels of some selected heavy metals in raw cows' milk from Oyam District, Uganda by (Odongo et al., 2022). However, the maximum permissible limit of Cr is 0.05 mg/l in cows' milk set by FAO/WHO as sited by (Rewini et al., 2020). Generally, Cr is lower at all the study areas but significantly exceeds at tabia M/genet was (0.0725±0.048 mg/l).

Chromium (Cr) is an essential nutrient for plant and animal metabolism, but increasing in accumulation of Cr in the environment from industrial outputs has caused great concern because long term exposure to Cr can cause kidney and liver damage as well circulatory and nerve tissue problems (Auni et al., 2021).

5.1.5 Measurement of the Concentration of Copper (Cu) at Different Study

The mean concentration of Copper (Cu) at four study areas was as follows: the concentration of Cu ranges from 1.087 to 1.979 mg/l with the mean concentration of 1.5189±0.3136 mg/l in tabia

Limat, ranges from 0.006 to 0.815 mg/l with the mean concentration of 0.5421±0.2819 mg/l in tabia M/genet ,ranges from 0.006 to 0.897 mg/l with the mean concentration of 0.4823±0.3201 mg/l in tabia Aynalem, and ranges from 0.004 to 0.746 mg/l with the mean concentration of 0.4232±0.2801 mg/l in tabia M/weyni (Table 4.3).

The average concentration of Cu in this study is greater than the Cu concentrations ranging from 0.287 mg/l to 0.384 mg/l with a mean concentration of 0.343 mg/l the study by (Peter et al., 2022). The mean concentration of Cu in cows' fresh milk in Borena zone 0.109 ± 0.006 mg/kg (Belete et al., 2014). The average concentration of Cu in cows milk is 0.231 ± 0.020 ppm among five villages of in Dodoma Urban District , Tanzania it indicates that the cows' milk of the zone was poor source of Cu (Rao and Murthy, 2017) was also less than the present study at all the study areas. Besides, all the study areas were lower than the 1.50 mg/l maximum permissible limits set by WHO as sited by (Rewini et al., 2020) except 1.5189±0.3136 mg/l in tabia Lim'at was significantly higher than the maximum permissible limits.

5.1.6 Measurement of the Concentration of Nickel (Ni) at Different Study Areas

Nickel (Ni) is a widespread component of Earth's crust and is ubiquitous in the biosphere (Durguti et al., 2018)**.** The concentration of Nickel (Ni) in the four study areas was as follows: the concentration of Ni ranges from 0.017 to 0.141mg/l with the mean concentration of 0.0506±0.0296 mg/l in tabia Limat, ranges from 0.013 to 0.059 mg/l with the mean concentration of 0.0276±0.0194 mg/l in tabia M/genet, ranges from 0.011 to 0.065 mg/l with the mean concentration of 0.0246±0.062 mg/l in tabia M/weyni, and 0.012 to 0.081mg/l with the mean concentration of 0.0245±0.0209 mg/l in tabia Aynalem (Table 4.1).

Mean concentration of Ni in cow's milk 0.223±0.009 mg/kg from Bannu, 0.127±0.008 mg/l from Lachi, 0.169±0.007 mg/l from Karak, from 0.203±0.007 mg/kg and from Lucky Marwat study areas from Khyber Pakhtunkhwa , Pakistan reported by (Nazir et al., 2015), However the concentration of Ni in this report is greater than the present study at all study areas.

The mean concentration of Ni in cows fresh milk was 3.013 mg/l reported by (Auni et al., 2021) which is much higher compared to the present study. However, at all the present study area were significantly exceeds the concentration of Ni in cows' milk is Permissible limit by Codex for nickel (Ni) in milk is 0.02 mg/l (Durguti et al., 2018).

5.1.7 Measurement of the Concentration of Lead (Pb) at Different Study Araes

The concentration of Lead (Pb) in the four study areas was as follows: the concentration of Pb ranges from 0.014 to 0.156 mg/l with the mean concentration of 0.0807±0.0532 mg/l in tabia Lmat, ranges from 0.013 to 0.61mg/l with the mean concentration of 0.0335±0.0179 mg/l in tabia Aynalem, ranges from 0.007 to 0.08 mg/l with the mean concentration of 0.0293±0.0236 mg/l in tabia M/weyni, and ranges from 0.012 to 0.052 mg/l with the mean concentration of 0.0196±0.0094 mg/l in tabia M/genet (Table 4.3).

The concentration of lead detected ranged from 0.022 mg/l to 0.036 mg/l was greater than the mean concentration of lead in tabia Aynalem and tabia M/weyni but less than the concentration tabia m/genet and tabia Limat from present study (Peter et al., 2022). The concentration of Pb ranged from 0.05 to 0.51 mg/l , the mean concentration of 0.263 ± 0.031 mg/l reported by (Ali et al., 2023) is higher than from all the present study areas in Pb concentration. But the study mean concentration of Pb in cows milk in Almaty region, Kazakhstan in milk and milk products , in raw milk samples ranged from 0.0010 to 0.008 mg/l, with an average of 0.0045 mg/l

(Sarsembayeva et al., 2020). Also, lower than from all study areas of the present study. Pb concentration of the present study also lower than 0.1 mg/l set by FAO/WHO the maximum permissible limits for food and milk as sites by (Rewini et al., 2020).

Lead (Pb) is classified as a trace metal that is not beneficial for human health and primarily affects human nervous and vascular systems, poisoning can cause anemia, lethargy, kidney and brain damage, as well as death, Due to lead's ability to pass the placental barrier, pregnant women who are exposed to it expose their unborn children to it as well (Olowoyo et al., 2024).

5.2 Microbial Quality in Cows Fresh Milk at Different Study Areas

Health of the animal, feed and water qualities, the equipment, sanitation of the barn, personal hygiene are most significant factors that speed up microbials of fresh milk (Aleli et al., 2024). Therefore, continuous check- up related to microbial quality including TBC, TCC and TY&MC is vital to identify the milk quality. In this study was identification of the TBC, TCC, and TY&MC in the four study areas was performed.

5.2.1 Microbial Quality Analysis Based on Individual and Pooled Samples

From the above (Table 4.4) the highest colony forming units (CFU) obtained from individual samples TBC (6.664 log10cfu/m) (Table 4.4) in tabia M/weyni and (6.192log10cfu/ml) (Table 4.5) from pooled samples, the lowest values indicated 4.302log10cfu/ml from individual samples in tabia Limat. But the average value of TBC 5.500log10cfu/ml (Table 4.4) from individual samples was greater than the standard of TBC in milk was 5.00log10cfu/ml set by European commotion regulation No.1662/2006 (Kačániová et al., 2017).However the study in Bahri Dar was 7.11log10cfu/ml the study by (Birhanu and Asamnew , 2019) greater than this study.

The highest TCC(4.190log10cfu/ml) (Table 4.4) from individual samples in tabia Aynalm and (5.640log10cfu/ml) (Table 4.5) from pooled sample in tabia Limat. The lowest 2.784log10cfu/ml and 4.882log10cfu/ml from individual, pooled samples in tabia limat and tabia M/weyni respectively. But the average of TCC (4.102log10cfu/ml) (Table 4.4) from individual and (5.142log10cfu/ml) Table 4.5) from pooled samples. Both the average of individual and pooled samples were higher than the maximum recommended value of the microbiological standards of the European Union (EU) is 2.00 log10cfu/ml for Total coliform count (TCC) (Gebremichael et al., 2024)

TY&MC indicates high in tabia M/weyni (5.572log10cfu/ml) and lower in tabia Limat (4.244log10cfu/ml) (Table 4.4) from individual samples and higher in tabia Limat (5.782log10cfu/ml) and the lowest in tabia Aynalem(5.320log10cfu/ml) from pooled samples. However, the average of TY&MC (5.059log10cfu/ml) from individual and (5.456log10cfu/ml) from pooled samples were indicated higher number of colonies but if compared to the studies lower from 7.46log10cfu/ml in cows' milk in Hawassa study by (Nurfeta and Gobena, 2023), but greater than 4.12 log10cfu/ml was study by (Birhanu and Asamnew , 2019)

5.2.2 Total Bacterial Count (TBC)

The results were indicated no significant variation (P>0.05) in TBC among the four study areas (Appendix-VII) with an average of 5.55±0.96 Log10cfu/ml, 6.23±0.88 Log10cfu/ml, 5.83±0.99 Log10cfu/ml, 5.17±2.03 Log10cfu/ml are respectively for tabia Aynalem, tabia M/weyni, tabia M/genet and tabia Limat (Table 4.6).

The total bacterial count of four study areas are follows tabia M/weyni>tabia M/genet>tabia Ynalem>tabia Limat (Table 4.6). However all are above recommended maximum value of TBC 100 000cfu/ml (5.00 Log10cfu/ml) according the European Commission Regulation No.

1662/2006 (Kačániová et al., 2017). But less than the study in Beteseb dairy farm studied by korma the mean of TBC was 7.32 ± 0.19log10cfu/ml (Nurfeta and Gobena, 2023) greater than from all the present study areas. The Mean TBC in raw milk Samples Collected from Addis Ababa and its Surrounding in Ethiopia was 6.15log10cfu/ml conducted by (Tekilegiorgis and Ababa, 2024). this is higher than in this study from tabia Aynalem, tabia M/genet and tabia Limat but lower than from tabia M/weyni. Additionally the study in the central and northwestern zones of the Tigray region, focusing on three specific towns: Axum, Shire, and Sheraro, the mean TBC of milk samples collected directly from the udder, bucket, and cafeteria and yogurt samples collected from the udder was 4.94 log10cfu/mL reported by (Gebremichael et al., 2024) was lower than this study. However, 7.11±0.13 log 10cfu/ml of raw milk study in and around Bahir Dar was higher than the present study from all study areas (Birhanu and Asamnew, 2019). According to the Ethiopian standards Authority (2009) good quality milk should not contain a total bacterial count of more than 0-200,000 cfu/ml (5.301log10cfu/ml) (Alganesh et al., 2016). However, except tabia Lim'at (5.17±2.03 Log10cfu/ml) all are higher than the standard.

5.2.3 Total Coliform Count (TCC)

Coliforms were found 5.22±0.89 Log10cfu/ml in tabia Aynalem, 4.87±0.89 Log10cfu/ml in tabia M/weyni 4.42±1.73 Log10cfu/ml tabia M/genet and 4.21±2.42 Log10cfu in tabia Limat (Table 4.6) these are tabia Aynalem> tabia M/weyni>tabia M/genet>tabia Limat in total coliform count of the four study areas (Table 4.6) There is no significant differences among the four study areas (p-value > 0.05) (Appendix-VIII).

However, mean coliform counts in this study is tabia M/genet and tabia Limat are lower than the study in raw milk collected from rural areas of Nairobi was 4.56log10cfu/ml (Wanjala and

Mathooko, 2017). According to the Ethiopian Standards Agency (ESA) (Ref No ES 3460: 2009), the bacteriological quality of whole/raw cow milk coliform counts is set to be less than 200,000(5.3 log10cfu/ml), was for very good (Tekilegiorgis and Ababa, 2024), then in this study all the study areas were less than 200,000(5.3 log10cfu/ml.)

However TCC of this study was lower than the mean of coliform count observed in raw cow's milk samples collected from Mizan Aman, Debub and Shei Bench Woredas were 5.203±0.230, 5.187±0.211 and 4.911±0.324 log10cfu/ml, respectively the study by (Gemechu et al., 2016), 7.43 ± 0.23 $\log_{10}$cfu/ml and 7.38 ± 0.23 log10cfu/ml, from rural areas of Hawassa districts, Ethiopia reported by (Nurfeta and Gobena, 2023) and raw milk in and around Bahir Dar was 5.57±0.26 log10cfu/ml coliform bacteria was found in rural production system reported by (Birhanu and Asamnew, 2019) were higher than the present study from all study Areas.

5.2.4 Total Yeast and Mold Count (TY&MC)

Total Yeast and mold also presented in all samples of in the four study areas like 5.28±0.96 Log10cfu/ml in tabia Aynalem 5.48±1.01 Log10cfu/ml in tabia M/weyni 5.36±0.58 Log10cfu/ml in tabia M/genet and 5.01±2.42 tabia Limat and there is tabia M/weyni>tabia M/genet>tabia Aynalem>tabia Limat (Table 4.6) but there is no significant difference (Appendix-IX). The result of TY&MC in all the study areas were less than as compared with 9.82 Log10cfu/ml in cows raw milk reported by (Aleli et al., 2024), but greater than of 4.001±0.588, 3.944±0.346 and 3.762±0.468 log10cfu/ml for milk samples collected from the Mizan Aman, Debub and Shei Bench Woredas, respectively reported by (Gemechu et al., 2016). And 7.46 ± 0.21log10cfu/ml, 7.38 ± 0.21 log10cfu/ml, from rural areas of Hawassa districts, Ethiopia reported by (Nurfeta and Gobena, 2023) were higher than this study. However, 4.12±0.14 log10cfu/ml microbiological quality of raw cows’ milk in and around Bahir Dar City reported by (Birhanu and Asamnew, 2019) was lower than this study.

CHAPTER 6: CONCLUSION and RECOMMENDATION

6.1 Conclusion

The present study gives important information on the level's heavy metals and microbial quality of four study areas. All the heavy metals were investigated in all of the milk samples. Fe was high concentration levels from all the selected elements but not specific standard for Fe by FAO/WHO, Cd also, above the permissible limits set by FAO/WHO in all study areas except in Tabia Lim'at, Cr in tabia M/genet was higher than the maximum permissible limits, Cu was within the permissible limit by FAO/WHO except in Tabia Lim'at but higher than the maximum permissible limits for milk by European Union, Ni was also presented in all the study areas besides exceeds the maximum permissible limits in all study areas, Pb also higher than maximum permissible limits set by FAO/WHO in all study areas. Generally, there is a variation (statistically significant difference ($P<0.05$)) by the concentration level of the selected elements among the four study areas except Fe was no significance difference among the all-study areas ($P>0.05$)

In other hand TBC was a slight difference on the relative abundance among the study areas. However, did not observe a significant difference among the study areas. Tabia Limat was within the level of 'very good' for TBC set by Ethiopian standards Authority (2009) but the three study areas were in 'good quality'. this indicates that, most of the microbial contamination could be come from handling and processing. TCC also presented in high number at all study areas within the Ethiopian Agency standards ('very good') and not significant difference among the study areas ($P>0.05$). TYMC also counted, but not significant difference among the study areas ($P>0.05$). Usually higher observed colony forming unit in the present study could probably be due to use of unclean milking utensils and plastic containers for collecting, lack of knowledge about clean milk production, and keeping milk, initial contamination of the milk samples either from the udder of the cow or the milkers' hand and the poor hygienic quality of milking area.

6.2 Recommendation

Considering as a baseline for the findings of this study, the following recommendations are important. Special attention should be given to these heavy metals as once they are present in concentrations greater than the acceptable limit; it may become difficult to reduce them to an acceptable level during processing. Investigating heavy meals in milk are important for milk quality and human health. Therefore, there is a need for additional study on the source of high heavy metals in the milk including animal feed, water and other potential sources. Further study about heavy metals based on lactating period, age of lactating cows, based on different location, based on different season, based on different feeds for lactating cows, and other related areas will be important to get the improvement on the reduction of heavy metal consumption by the animal. Additionally, this suggests the need for enriched hygienic practices and educating the public on safety issues and personal hygiene in milk handling. It would be a great interest if further investigations are to be carried out to identify and isolate different species of microorganisms that might cause public health importance.

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APPENDEXICES

N.B site1 is for tabia Aynalem, site 2 for tabia M/weyni, site 3 for tabia M/genet, site 4 for tabia Limat

Appendix -I Mean difference of concentration of Fe (mg/l) each study areas (sites)

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	8.52000	5.07936	.350	-5.1599	22.1999
	site3	5.78000	5.07936	.669	-7.8999	19.4599
	site4	7.94000	5.07936	.412	-5.7399	21.6199
site2	site1	-8.52000	5.07936	.350	-22.1999	5.1599
	site3	-2.74000	5.07936	.949	-16.4199	10.9399
	site4	-.58000	5.07936	.999	-14.2599	13.0999
site3	site1	-5.78000	5.07936	.669	-19.4599	7.8999
	site2	2.74000	5.07936	.949	-10.9399	16.4199
	site4	2.16000	5.07936	.974	-11.5199	15.8399
site4	site1	-7.94000	5.07936	.412	-21.6199	5.7399
	site2	.58000	5.07936	.999	-13.0999	14.2599
	site3	-2.16000	5.07936	.974	-15.8399	11.5199

*. The mean difference is significant at the 0.05 level

Fe: iron, Std. Error: standard error, Sig.: significant

Appendix-II Mean difference of concentration of Cd (mg/l) in each study areas (sites)

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	.020800*	.006716	.019	.00271	.03889
	site3	.027700*	.006716	.001	.00961	.04579
	site4	.040068*	.006716	.000	.02198	.05815
site2	site1	-.020800*	.006716	.019	-.03889	-.00271
	site3	.006900	.006716	.735	-.01119	.02499
	site4	.019268*	.006716	.033	.00118	.03735
site3	site1	-.027700*	.006716	.001	-.04579	-.00961
	site2	-.006900	.006716	.735	-.02499	.01119
	site4	.012368	.006716	.271	-.00572	.03045
site4	site1	-.040068*	.006716	.000	-.05815	-.02198
	site2	-.019268*	.006716	.033	-.03735	-.00118
	site3	-.012368	.006716	.271	-.03045	.00572

*. The mean difference is significant at the 0.05 level

Cd: Cadmium, Std. Error: standard error, Sig.: significant

Appendix-III mean difference of concentration of Cr (mg/l) in each study areas (sites)

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	.006200*	.001669	.004	.00170	.01070
	site3	.011650*	.001669	.000	.00715	.01615
	site4	.016780*	.001669	.000	.01228	.02128
site2	site1	-.006200*	.001669	.004	-.01070	-.00170
	site3	.005450*	.001669	.012	.00095	.00995
	site4	.010580*	.001669	.000	.00608	.01508
site3	site1	-.011650*	.001669	.000	-.01615	-.00715
	site2	-.005450*	.001669	.012	-.00995	-.00095
	site4	.005130*	.001669	.020	.00063	.00963
site4	site1	-.016780*	.001669	.000	-.02128	-.01228
	site2	-.010580*	.001669	.000	-.01508	-.00608
	site3	-.005130*	.001669	.020	-.00963	-.00063

*. The mean difference is significant at the 0.05 level.

Cr: Chromium Std. Error: standard error, Sig.: significant

Appendix-IV Mean difference of concentration of Cu (mg/l) in each study areas (sites)

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	.059100	.133954	.971	-.30167	.41987
	site3	-.059800	.133954	.970	-.42057	.30097
	site4	-1.036600*	.133954	.000	-1.39737	-.67583
site2	site1	-.059100	.133954	.971	-.41987	.30167
	site3	-.118900	.133954	.811	-.47967	.24187
	site4	-1.095700*	.133954	.000	-1.45647	-.73493
site3	site1	.059800	.133954	.970	-.30097	.42057
	site2	.118900	.133954	.811	-.24187	.47967
	site4	-.976800*	.133954	.000	-1.33757	-.61603
site4	site1	1.036600*	.133954	.000	.67583	1.39737
	site2	1.095700*	.133954	.000	.73493	1.45647
	site3	.976800*	.133954	.000	.61603	1.33757

*. The mean difference is significant at the 0.05 level.

Cu: Copper Std. Error: standard error, Sig.: significant

Appendix -V Mean difference of concentration of Ni (mg/l) in each study areas (sites)

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	-.000100	.009905	1.000	-.02678	.02658
	site3	-.003100	.009905	.989	-.02978	.02358
	site4	-.026100	.009905	.057	-.05278	.00058
site2	site1	.000100	.009905	1.000	-.02658	.02678
	site3	-.003000	.009905	.990	-.02968	.02368
	site4	-.026000	.009905	.058	-.05268	.00068
site3	site1	.003100	.009905	.989	-.02358	.02978
	site2	.003000	.009905	.990	-.02368	.02968
	site4	-.023000	.009905	.112	-.04968	.00368
site4	site1	.026100	.009905	.057	-.00058	.05278
	site2	.026000	.009905	.058	-.00068	.05268
	site3	.023000	.009905	.112	-.00368	.04968

*. The mean difference is significant at the 0.05 level.

Appendix-VI Mean difference of concentration of Pb (mg/l) in each study areas (sites)

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	.004200	.013802	.990	-.03297	.04137
	site3	.013900	.013802	.746	-.02327	.05107
	site4	-.047200*	.013802	.008	-.08437	-.01003
site2	site1	-.004200	.013802	.990	-.04137	.03297
	site3	.009700	.013802	.895	-.02747	.04687
	site4	-.051400*	.013802	.004	-.08857	-.01423
site3	site1	-.013900	.013802	.746	-.05107	.02327
	site2	-.009700	.013802	.895	-.04687	.02747
	site4	-.061100*	.013802	.000	-.09827	-.02393
site4	site1	.047200*	.013802	.008	.01003	.08437
	site2	.051400*	.013802	.004	.01423	.08857
	site3	.061100*	.013802	.000	.02393	.09827

*. The mean difference is significant at the 0.05 level.

Pb: Lead, Std. Error: standard error, Sig.: significant

Appendix VII: Dependent Variable: TBC in log 10 cfu/ml

Tukey HSD

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	-.67900	.58519	.655	-2.2550	.8970
	site3	-.28200	.58519	.963	-1.8580	1.2940
	site4	.37500	.58519	.918	-1.2010	1.9510
site2	site1	.67900	.58519	.655	-.8970	2.2550
	site3	.39700	.58519	.905	-1.1790	1.9730
	site4	1.05400	.58519	.289	-.5220	2.6300
site3	site1	.28200	.58519	.963	-1.2940	1.8580
	site2	-.39700	.58519	.905	-1.9730	1.1790
	site4	.65700	.58519	.678	-.9190	2.2330
site4	site1	-.37500	.58519	.918	-1.9510	1.2010
	site2	-1.05400	.58519	.289	-2.6300	.5220
	site3	-.65700	.58519	.678	-2.2330	.9190

Appendix VIII: Dependent Variable: TCC in log 10 cfu/ml

Tukey HSD

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	.35400	.72507	.961	-1.5988	2.3068
	site3	.80100	.72507	.689	-1.1518	2.7538
	site4	1.01400	.72507	.508	-.9388	2.9668
site2	site1	-.35400	.72507	.961	-2.3068	1.5988
	site3	.44700	.72507	.926	-1.5058	2.3998
	site4	.66000	.72507	.799	-1.2928	2.6128
site3	site1	-.80100	.72507	.689	-2.7538	1.1518
	site2	-.44700	.72507	.926	-2.3998	1.5058
	site4	.21300	.72507	.991	-1.7398	2.1658
site4	site1	-1.01400	.72507	.508	-2.9668	.9388
	site2	-.66000	.72507	.799	-2.6128	1.2928
	site3	-.21300	.72507	.991	-2.1658	1.7398

Appendix IX: Dependent Variable: YMC in log 10 cfu/ml

Tukey HSD

(I) site identifier	(J) site identifier	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
site1	site2	-.19700	.56232	.985	-1.7115	1.3175
	site3	-.08500	.56232	.999	-1.5995	1.4295
	site4	.27000	.56232	.963	-1.2445	1.7845
site2	site1	.19700	.56232	.985	-1.3175	1.7115
	site3	.11200	.56232	.997	-1.4025	1.6265
	site4	.46700	.56232	.840	-1.0475	1.9815
site3	site1	.08500	.56232	.999	-1.4295	1.5995
	site2	-.11200	.56232	.997	-1.6265	1.4025
	site4	.35500	.56232	.921	-1.1595	1.8695
site4	site1	-.27000	.56232	.963	-1.7845	1.2445
	site2	-.46700	.56232	.840	-1.9815	1.0475
	site3	-.35500	.56232	.921	-1.8695	1.1595

Appendix -X: Photos (images) in sample collection and laboratory work

Appendix figure 1: photos in the milk sample collection time

Appendix figure 2: Photos in the laboratory work of microbial quality in cows' fresh milk (process from dilution to counting the growth of colonies/ bacteria's) in Food Post Harvesting Technology Laboratory

Appendix figure 3: photos of the laboratory work in sample digestion and analysis of the heavy metals in cows' fresh milk in Geology laboratory