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**ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**

**DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH**

# MICROPLASTIC POLLUTION AND ITS ASSOCIATED DETERMINANTS IN DONKEYS, HUMANS, AND THE ENVIRONMENT IN CENTRAL ETHIOPIA

**MSC THESIS**

**BY**

**MASRESHA GEBEYEHU**

**JUNE, 2026**

**BISHOFTU, ETHIOPIA**

# MICROPLASTIC POLLUTION AND ITS ASSOCIATED DETERMINANTS IN DONKEYS, HUMANS, AND THE ENVIRONMENT IN CENTRAL ETHIOPIA

Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in One Health

**By**

**Masresha Gebeyehu**

**JUNE, 2026**

**BISHOFTU, ETHIOPIA**

# APPROVAL SHEET

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## Department of Microbiology, Parasitology, and Poultry Health

As MSc in One Health research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Masresha Gebeyehu, entitled "Microplastics Pollution and its associated Determinants in Donkeys, Humans, and the Environment in Central Ethiopia." We recommend that it be submitted as fulfilling the MSc thesis requirement for the Degree of Master of Science in One Health.

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As members of the Board of Examiners of the MSc open defense examination, we certify that we have read and evaluated the thesis prepared by Masresha, entitled: *Microplastic Pollution and its Associated Determinants on donkeys, humans, and the environment in central Ethiopia*. We recommend that it be accepted to fulfill the thesis requirement for the Master of Science in One Health degree.

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## STATEMENT OF AUTHORS

Masresha Gebeyehu solemnly declares that the thesis entitled "Microplastics Pollution and its Associated Determinants in Donkeys, Humans, and the Environment in Central Ethiopia" is my original work and has not been previously submitted in whole or in part for the award of any degree, diploma, scholarship, or other academic title at any university or institution. All sources of information, including data, literature, and intellectual contributions used or cited in this work, have been duly acknowledged with proper citations and references. Any assistance received in the preparation of this thesis has been appropriately credited. This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in the One Health program at the College of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia.

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## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| BCS | Body Condition Score |
| FTIR | Fourier Transform Infrared Spectroscopy |
| KAP | Knowledge, Attitude, and Practice |
| LMICs | Low and Middle-Income Countries |
| MNPs | Micro and Nano-plastics |
| MPs | Micro-plastics |
| NPs | Nano-plastics |
| OR | Odds Ratio |
| POPs | Persistent Organic Pollutant |
| WHO | World Health Organization |
| WWTP | Waste Water Treatment Plant |

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# ABSTRACT

Microplastic (MPs) pollution has become a major global public health and environmental sustainability issue. In Ethiopia, the health implications of MP contamination remain understudied compared to global studies. This study aimed to detect MPs in donkey feces, human urine, and water samples collected from near and around some rivers in central Ethiopia; assess the determinants of plastic ingestion in working donkeys; to assess the awareness of community members and donkey owners about the effects of plastic pollution; and analyze the retrospective clinical records on colic cases in donkeys associated with plastic ingestion, within a One Health framework. This study employed a cross-sectional approach, sampling river water (27), human urine (36), and donkey feces (41). Additionally, 223 cases of retrospective veterinary records (2020-2026) of donkey colic cases were reviewed to examine the prevalence of plastic-related risks. Community member (110) and donkey owner (110) awareness surveys were conducted to assess the perceptions of plastic pollution. Descriptive statistics were applied to summarize contamination levels, and a one-way ANOVA was used to assess spatial differences. A multivariate logistic regression was applied to identify factors associated with donkey health outcomes. MPs were detected in all sample types, with water having the highest concentration (2.01 ± 0.91 particles/L). Donkey feces and human urine had concentrations of 1.36 ± 0.50 particles/g and 1.12 ± 0.89 particles/mL, respectively. ANOVA confirmed statistically significant variation among sample types (*p*<0.05). The dominant shape of MP particles was fiber across all sample types, with a size range of <500 µm. In the retrospective analysis, 95.7% of donkey colic cases were linked to plastic ingestion, with a mortality rate of 23.5% in Merkato and 56.1% in Bishoftu. A logistic regression indicated that no significant predictors of outcome were identified, including age, sex, or plastic risk. Community awareness of the health risks of plastic was moderate (40.9%), with higher levels in urban centers. MP contamination is widespread across environmental and biological samples in Ethiopia. This study underscores the urgent need for an interconnected waste management strategy, awareness campaign, strong policy, and One Health interventions to reduce plastic pollution.

**Keywords**: *One Health, plastic pollution, microplastics, donkey health, Ethiopia.*

## 1. INTRODUCTION

Plastic production and utilization increased in the early twentieth century. Plastics are widely used in packaging, healthcare, construction, and agriculture due to their affordability, durability, and wide applicability. However, their resistance to degradation has resulted in long-term buildup in the natural ecosystem. (Adekanmbi *et al.*, 2024). Currently, plastic waste contributes to a significant proportion of municipal solid waste, and its mismanagement has resulted in widespread contamination of the environment (Mariano *et al.*, 2021).

Microplastics (MPs) are plastic particles < 5 mm, while particles ranging between 1 nm and 1 mm are categorized as nano-plastics. These particles have received particular concern because of their ability to infiltrate ecosystems and enter food chains, causing risks to humans and environmental health, as well as animal health (Lee *et al.*, 2023). In recent years, there has been growing research on the presence and impacts of microscopic particles in the natural environment and public health worldwide (Bora *et al.,* 2024; Zheng *et al.,* 2024). In animals, consumption of plastic debris has been associated with digestive blockage, reduced nutrient intake, infertility, and, in some cases, mortality (Ali *et al*., 2025). Environmental effects include reduced soil productivity, deteriorated freshwater ecosystems, threats to biological productivity, and contributions to climate change, as plastics are derived from fossil fuels and release greenhouse gases during production and disposal (Beaumont *et al.,* 2019; Ho and Tang, 2023).

Despite high population density, rapid urbanization, and improper disposal of plastic waste, African countries are major contributors to ecosystem degradation and public health (Dube and Okuthe, 2024). Plastic packaging is the most common type of plastic (Bidashimwa *et al*., 2023). Among East and Central African countries, Ethiopia is one of the most common importers and consumers of plastic materials. The consumption of plastics rose from 0.044 million metric tons in 2007 to 0.38 million metric tons in 2020 (Seyoum, 2023). River water is a primary pathway for plastic pollution in terrestrial ecosystems. Plastics entering rivers degrade into microplastics through physical and chemical processes. These smaller particles enter the water used for drinking, irrigation, and livestock. The contaminants originate from multiple sources, including wastewater effluents, sewage sludge, industrial discharge, atmospheric deposition, and surface runoff (Saad *et* *al*., 2024). In Ethiopia, bottled water is widely used by the community, particularly in urban areas, due to the quality of tap water. (Aragaw, 2024).

Donkeys in Ethiopia, often fed in open environments, including dumping sites and riverbanks, especially free-ranging, are highly vulnerable to plastic pollution, and owners are less concerned about providing donkeys with decent feed of their preference, unlike other domestic animals. Donkeys are often left to roam around markets, riversides, and villages to find feed. These sites where donkeys are feeding have been reported to be polluted by plastic products used for carrying food items, water, and other chemicals (Biffa and Woldemeskel, 2006). This can result in gastrointestinal obstruction, reduced productivity, and death, leading to economic losses for owners (Priyanka and Dey, 2018). The detection of plastics in donkey feces serves as an important indicator of environmental contamination and an exposure pathway for humans and animals (Haddy *et al*., 2025).

Human exposure to MPs primarily occurs through consumption of contaminated food and water, and through inhalation of airborne particles (Winiarska *et al*., 2024). Biomonitoring approaches to detect MPs in urine samples provide evidence of internal exposure to plastic-associated chemicals (Rotchell *et al*., 2024). However, the health effects of MPs across the three components (human, animal, and the ecosystems) are still under investigation; emerging evidence suggests links to inflammation, endocrine disruption, and chronic diseases (Center for International Environmental Law, 2023).

Micro and nanoplastics (MNPs) can enter human and animal bodies through ingestion, inhalation, and skin contact, and may cause various health problems. However, gaps remain (Landrigan *et al*., 2023; Zheng *et al.,* 2024). Currently, no established guidelines from international organizations such as the World Health Organization (WHO) or the Food and Agriculture Organization (FAO) defining safe exposure limits or threshold concentrations of MNPs for humans or animals. Additionally, the health impacts of microplastics have been unclear due to insufficient data on their chemical properties, exposure levels, and pathways (Gebremedhin *et al.*, 2026). The quantitative dose-response relationship is still lacking. In Africa and most LMICs, the problems are largely due to weak regulatory policies and the absence of regular waste handling methods. Most research on plastic contamination focuses on environmental contamination rather than health outcomes (Dube and Okuthe, 2024).

In Ethiopia, previous studies examined the identification of MPs in sediments and water (lakes, rivers, tap, etc.) (Mhiret Gela and Aragaw, 2022; Gebremedhin *et al.*, 2026). Livestock effects include ruminal impaction, reduced productivity, and environmental degradation (Priyanka and Dey, 2018). However, there are no studies providing evidence of the direct health impacts of plastic exposure in human populations. This lack of integrated evidence highlights the need for a comprehensive One Health-based study to determine the impacts and determinants of plastic pollution effects. The objective of this study was:

- To detect the presence of MPs in human urine, donkey feces, and river water samples in the study areas
- To assess the determinants of plastic ingestion on the welfare and health status of working donkeys
- To assess the level of awareness among community members and donkey owners regarding plastic pollution on the health of humans, animals, and the environment
- To compile and analyze retrospective data on colic cases in donkeys in Merkato and Bishoftu clinics in relation to plastic ingestion by donkeys

# 2. LITERATURE REVIEW

## 2.1.Plastic Pollution as a Global Health Threat

Plastic contamination is currently a major concern for environmental and public health worldwide. The vast majority of plastic material was produced in the late 1950s. However, the environmental, human, and animal health risks were not immediately recognized. Initial scientific concern mainly emphasized visible plastic accumulation and its effects within aquatic ecosystems (Napper and Thompson, 2020). In the early 2000s, there was a major turning point following the introduction of MPs by Thompson, which highlighted the gradual breakdown of plastics into smaller pieces called MPs that are persistent and widely distributed  (Thompson *et al*., 2004). A recent study suggests that MP pollution is a critical global environmental and public health issue, with significant implications within the One Health approach (Cambridge Prisms, 2025).

## 2.2.Plastic Pollution in Ethiopia

In Ethiopia, almost half of plastic production is used in packaging, and 40 % of the waste is not properly managed (Seyoum, 2023). In the case of weak management of waste and poor enforcement of the law, it is difficult to manage wastes (Hirpe and Yeom, 2021). Plastic bags and other foreign materials can negatively affect livestock health and lead to economic losses for the owner. Because livestock often graze freely in open areas, they are more likely to consume these foreign objects. Animals affected by plastic ingestion commonly show abdominal swelling. Other signs include rolling behavior, weight loss, and reduced milk production (Ramaswamy and Sharma, 2016).

Rivers that pass through urban centers are heavily polluted with plastic waste, undermining water quality and causing ecosystem degradation (Awoke *et al*., 2016). MP pollution is generally higher in urban lakes than in distant areas, due to multiple sources of pollution, such as industries and population density. Plastics of different sizes can enter aquatic environments through several pathways, including wastewater discharge, stormwater runoff, and airborne MPs from activities such as road wear, tire degradation, and construction (Girimay *et al*., 2025). In addition, urban solid waste and wastewater effluents are important contributors to plastic contamination in lake ecosystems (Merga *et al*., 2020).

## 2.3.Impacts of Plastic Pollution on Donkey Welfare

Ethiopia has one of the largest donkey populations worldwide, with an estimated 9.9 million donkeys and 0.4 million mules. Of those, approximately 80% of equids live in rural areas and 20% in urban areas. Donkeys are the most common working animals in Ethiopia. They work in transportation, agriculture, and household economies (Duguma *et al.,* 2025). The most common stressors that Ethiopian donkeys face include inadequate nutrition, overloading, poor housing, and inadequate access to veterinary services (Tefera and Takele, 2024). The previous study conducted by Otsyina *et al*. (2018) on livestock showed that ingestion of macroplastics (e.g., plastic bags and packaging materials) is widespread in contaminated areas, such as open dumping, roadside, and riverbank

The ingestion of plastic materials by donkeys during foraging can result in accumulation in the gastrointestinal tract, leading to obstruction, impaction, ruminal or intestinal stasis, and recurrent colic. Results reduced feed intake, weight loss, decreased working capacity, and, in severe cases, mortality (Burn *et al*., 2010). This causes economic losses by increasing livestock mortality and decreasing productivity (Farooq *et al.*, 2023).

## 2.4.Mechanism of Microplastic Pollution

The most common mechanisms of MPs are driven by complex processes involving the generation, fragmentation, transport, and environmental fate of plastic materials. The major sinks of MPs are the sea and oceans, whereas freshwater and terrestrial environments are the main sources (Khalid *et al*., 2021). MPs can be classified into two main types: primary and secondary sources. Primary MPs are intentionally produced at a microscopic size for use in industrial and commercial applications (e.g., synthetic fibers in textiles, microbeads in personal care products), and secondary MPs are formed through the fragmentation of larger plastic items into smaller particles, as shown in Figure 1 below (Makmuang and Aït-Kaddour, 2025).

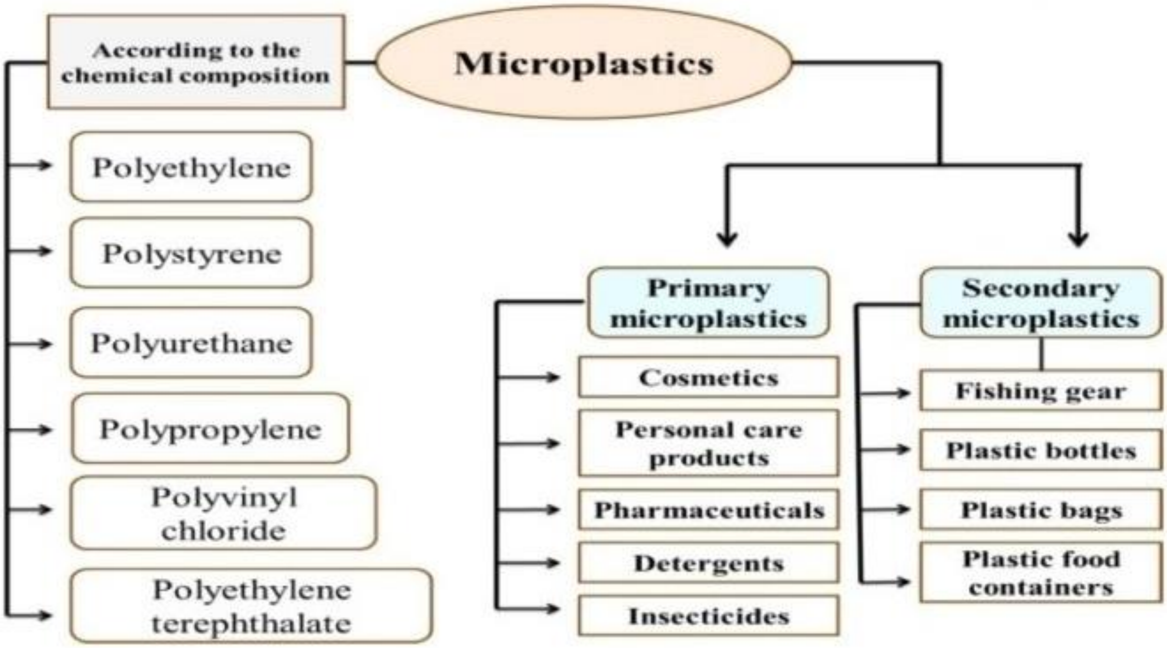

**Figure 1:** Microplastic polymer types and common sources (Pondala and Botsa, 2025)

### *2.4.1. Transport pathway*

Microplastics are transported and aggregate in terrestrial environments through multiple pathways, including hydrological, atmospheric, and terrestrial routes (Chen *et al*., 2023)**.** For hydrological pathways, rivers are major transporters of plastic materials from land ecosystems to lakes and oceans, with annual estimates of 1.15 to 2.14 million tons of MPs particles to the ocean. Additionally, winds can contribute to the long-distance atmospheric transport of lighter MP particles, particularly fibers. Sewage sludge and plastic mulching contribute to plastic contamination in the soil (Lebreton *et al.,* 2017).

### *2.4.2. Fragmentation process*

These processes are primarily focused on three mechanisms: (1) photodegradation through UV radiation exposure, (2) mechanical abrasion through physical stress and friction, and (3) biological activity through enzymatic degradation. These processes break down macro-plastics into smaller particles that aggregate in the ecosystem due to their resistance to complete biological decomposition (Bhardwaj *et al.,* 2024).

Microorganisms play an important role in the degradation of plastics by producing enzymes, which can also be utilized as carbon sources for growth and metabolism (Jain *et al*., 2023). The most prominent microorganisms for degrading MPs are bacteria and fungi due to their capability to break down larger plastic polymer structures into smaller particles (Demarquoy *et al.*, 2024). For example, several bacterial species have been reported to degrade polymers such as polyethylene and polyethylene terephthalate through enzymatic activities(e.g., hydrolase and oxidase), although the process is slow and influenced by environmental conditions, including temperature, PH, and oxygen availability (Parsaeimehr *et al.*, 2023).

### *2.4.3. Environmental fate and behavior*

The behavior and movements of MPs in the ecosystem are primarily determined by their size, shape, and density. MPs can also act as transporters of toxic substances because of their ability to attach pollutants such as trace metals and persistent organic pollutants(POPs). This process can increase the availability of these harmful substances to living organisms and increase their toxic effects. Overall, the persistence of MPs and the complexity of their pollution contribute to negative impacts on environmental quality, as well as the health of humans, donkeys, and water sources (Pondala and Botsa, 2025)**.**

## 2.5.Environmental Hotspots of Microplastic Pollution

High human activities and hydrological accumulation, including urban coastal areas, industrial river estuaries, and marine subaqueous areas, are the major environmental hotspots that increase MP accumulation (Ashokkumar *et al*., 2025). The marine and aquatic environments are the key environmental hotspots of microplastics, including river/fresh water environment, agricultural land, urban environments (roadside and ditch), and stormwater, and the key drivers are marine transportation and fishing, sewage and wastewater treatment effluent, coastal industry and tourism, and atmospheric deposits (Salunkhe-Patil *et al.,* 2024).

### *2.5.1. Waste treatment plant (WWTP)*

Wastewater treatment (WWT) facilities are important for the accumulation and redistribution of microplastic contaminants. They receive contaminants from domestic, industrial, and urban activities, such as synthetic fibers from textiles, microbeads from personal care products, and degraded plastic debris (Ou and Zeng, 2018). Conventional treatment methods can remove a large proportion of contaminants. However, they are not fully effective in achieving complete removal (Bhat *et al*., 2024). Earlier studies by Sun *et al*. (2019) indicated that sewage sludge is a major source of MPs. Sewage sludge used in agriculture can introduce contaminants into the land ecosystem. Furthermore, WWTPs are major accumulation zones (sludge storage), transformation sites (fragmentation and aging of plastics), and redistribution hubs (effluent discharge to rivers and soil).

Entry of microplastics: Influent can range from 1 to over 10,000 particles/L, while effluent concentrations remain detectable during treatment. Due to the high volume of wastewater processed, WWTPs can release millions to billions of MP particles daily into receiving environments (Carr *et al*., 2016). Removals depend on their characteristics, including shape, size, and color, which can affect the biological and physical treatment process (Murphy *et al*., 2016). The treatment process removes a large portion of MPs. Primary treatment removes larger particles, and secondary treatment, a biological process, enhances aggregation and settling, while advanced technologies, such as membrane bioreactors, achieve a higher removal efficiency of about 99.9% (Miino *et al.,* 2024; Zhang and Chen, 2019).

### *2.5.2. Agricultural soil*

Agricultural lands act as long-term storage environments for microplastic particles introduced through multiple agricultural activities. MPs enter soil environments through several pathways, including sewage sludge(biosolid), plastic mulching, irrigation with contaminated water, compost use, and fertilizer (Chalannavar *et al.,* 2025). MPs concentrate in soils and remain for long periods due to their slow degradation. As a result, they change soil quality and affect microbial communities and nutrient cycling processes (Rillig and Lehmann, 2020).

The previous study conducted by Arshad *et al*. (2026) revealed that MPs can be taken up by plant roots and transported into consumable tissues. This raises concerns for food safety and human exposure. Agricultural soils, therefore, function as long-term sinks of MPs, sources of secondary pollution through wind erosion and runoff, and critical entry points into the food chain. The previous study by Jadhav and Medyńska-Juraszek (2024) showed that most MPs are transported from the root to the shoot in plants, which causes stress. In developing countries, including Ethiopia, and in your study areas, where agricultural productivity is essential for food security, MPs' contamination of soils represents a significant threat. The use of untreated wastewater and sludge-based fertilizers from WWTPs for irrigation in agricultural areas is widespread, creating a direct method for the entry of MPs into the farming system (Mersha *et al*., 2025).

### *2.5.3. Atmospheric deposition and roadside environment*

Atmospheric deposition and roadside environments are considered major hotspots for the accumulation and transport of MP pollution. These methods are used for transporting MP pollution from urban, industrial, and agricultural sources into terrestrial and marine ecosystems (Hoon *et al*., 2024). These environments participate in both sinks for locally generated MPs and are used as a source for long-range transport, with an estimated 600 quadrillion MPs entering the atmosphere annually (Zhang *et al.,* 2020).

Atmospheric deposition processes play an important role in the distribution of MP particles across vast geographic areas and remote regions previously assumed to be pristine (Kole *et al*., 2017). Also, roads and streets are critical hotspots due to heavy traffic and human activity. Roadside dust is the main source of ambient levels of soil and atmospheric MPs pollution, with concentrations particularly elevated in urban centers and along major transportation corridors (Su *et al*., 2020). For donkey populations in Ethiopia, roadside environments are particular risk zones. Working donkeys traveling urban and peri-urban roads inhale airborne MPs from tire wear particles, synthetic textile fibers, and fragmented plastic debris.

### *2.5.4. Urban runoff and surface water system*

Stormwater runoff in urban areas is a pathway for the movement of plastic particles from land to water bodies. During rainfall, MPs that accumulate on surfaces such as roads, rooftops, and other urban areas are washed into drainage systems. These particles are then carried into rivers, lakes, and groundwater (Guti *et al.,* 2025). MPs in urban runoff mainly originate from roadside dust, tire wear particles, the breakdown of litter, and construction materials. The high concentrations of MPs occurred during storm events (first flush). It acts as a direct transport mechanism, a major contributor to freshwater pollution, and a link between urban activities and the aquatic ecosystem (Dris *et al*., 2015). These environmental hotspots and transport mechanisms create critical exposure pathways for MP contamination. Identifying where MPs accumulate and understanding their movement through the water, air, and soil systems is the most prominent step in assessing risks to human and animal health.

## 2.6.Impacts of Microplastics on Health

### 2.6.1. *Impacts of microplastics on human health*

The presence and accumulation of microplastics in natural environments are increasingly recognized as a significant concern for environmental and public health (Abbas *et al*., 2025). The major routes of exposure are inhalation, oral consumption, and skin contact (Kusyk *et al.,* 2025). Most previous studies confirm the presence of MPs in the human body by analyzing tissue, including blood, urine, stool, and other tissues (Callaghan *et al*., 2025; Mittal *et al*., 2023). It can cause both physical and chemical toxicity. Smaller plastic particles, specifically nanoplastics, can physically cross biological barriers and accumulate in tissue, causing inflammation and oxidative stress (Jayavel *et al.,* 2024). Chemically, MPs can emit hazardous substances, including endocrine-disrupting chemicals, heavy metals, and POPs; this may affect the hormonal system and metabolic processes. These combined effects cause chronic diseases such as cancer, heart disease, and fertility impairment (Ghosh *et al.,* 2023).

Airborne microplastics released from sources such as tire wear, synthetic textiles, and road dust can be inhaled and enter the respiratory system, where they may cause inflammation and breathing difficulties (Kaushik *et al.,* 2025). In addition, recent findings suggest that MPs can affect the human gut microbiome, leading to changes in microbial diversity and metabolic functions (Thin *et al*., 2025).

### 2.6.2. *Impacts of microplastics on animal health*

Animals can be exposed to MPs through contaminated food, water, and soil. In urban, peri-urban, and roadside areas, exposure may also occur through the inhalation of airborne particles. Terrestrial animals such as working donkeys are vulnerable to plastic pollution due to their natural feeding behavior, which involves close contact with soil and low-lying vegetation that may accumulate plastic debris and particles (Lwanga *et al*., 2017).

Several previous abattoir-based studies have shown that plastic materials are prevalent in the gastrointestinal tract of livestock. For instance, studies conducted in cattle, sheep, and goats revealed that a substantial proportion of slaughtered animals contained indigestible foreign bodies, with plastics accounting for the majority of materials, demonstrating the widespread nature of this exposure pathway (Kassahu and Tesfaye, 2017). MPs that have once been ingested cause both physical and physiological effects in animal health. Physically, MPs may irritate the gastrointestinal lining, cause blockages in severe cases, and induce false satiety, thereby reducing feed intake and nutrient absorption (Wright *et al.,* 2013).

Microplastics can act as carriers of hazardous chemicals and microbial pathogens, which increases their potential toxic effects. They can adsorb environmental pollutants such as heavy metals, pesticides, and POPs. These contaminants may be transferred into animal tissues through ingestion, leading to additional toxic effects (Teuten *et al*., 2009). Furthermore, MPs provide surfaces for microbial attachment, leading to the formation of complex biofilms known as the "plastisphere." The plastisphere is used as a community of pathogenic microorganisms, as indicated in Figure 2 (Zhai *et al.,* 2023).

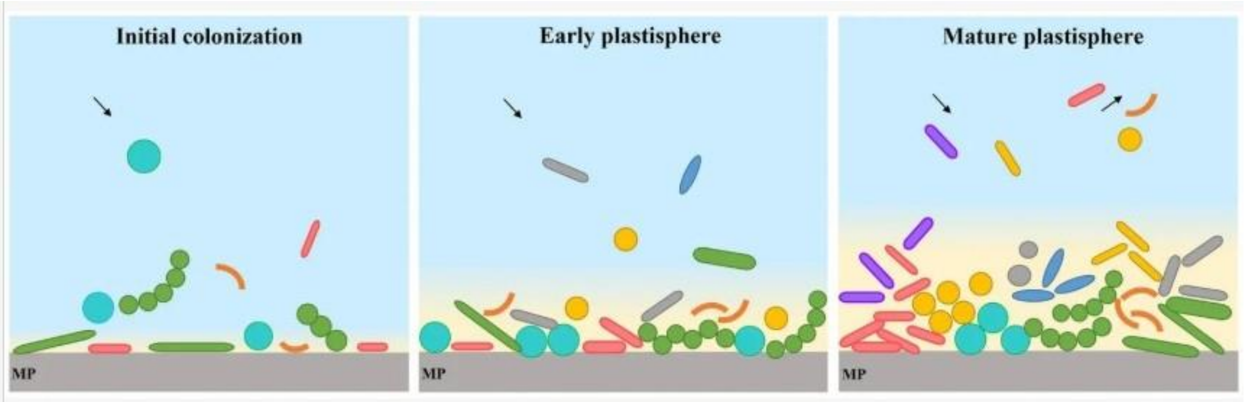

**Figure 2:** The formation of succession in the plastisphere.(Zhai *et al*., 2023).

Microplastics in animals cause endocrine disruption, reproductive health problems, and affect embryonic development in various species (Jeong *et al.,* 2024). Inhalation of airborne MPs constitutes an additional, often overlooked exposure pathway for donkeys, especially those working in urban and roadside environments. Airborne MPs from tyre wear, road dust, and urban activities cause respiratory problems and inflammation (Ullah *et al.,* 2025).

### *2.6.3. Impacts of microplastics on the environment*

The growing production of plastic and mismanagement of waste, especially in developing countries, have increased environmental exposure and related ecological risks (Geyer *et al*., 2017). MPs can harm ecosystems by lowering soil fertility and reducing plant growth (Machado *et al.,* 2019). They also interact with soil organisms such as earthworms and microorganisms, which can disrupt nutrient cycling and overall soil function. In addition, farming practices, including the use of sewage sludge from the WWTP, can lead to the buildup of MPs in soils (Zettler *et al*., 2013).

The most commonly affected environment by MP pollution is the aquatic environment (Barbuzano, 2019). MPs can be quantified and analyzed in freshwater systems, sediments, and drinking water, leading to risks to aquatic organisms and the environment (Koelmans *et al.,* 2019). In aquatic environments, MPs can be taken up by organisms such as plankton, fish, and invertebrates, resulting in physical injury, reduced appetite, and impaired reproduction. Moreover, MPs can alter habitat structure and provide a habitat for invasive species, thereby disrupting the ecological balance (Scaria *et al.,* 2023).

Microplastic pollution contributes to climate change and leads to global warming through multiple interconnected mechanisms. First, MPs contribute to greenhouse gas emissions by releasing carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$) from soils and sediments, affecting microbial activity and biogeochemical processes (Chen *et al*., 2023). Second, MPs disrupt the global carbon cycle, particularly in marine ecosystems, by interfering with phytoplankton, the primary organisms responsible for ($CO_2$) absorption through photosynthesis, causing weakened ocean carbon sequestration and reducing the Earth's natural capacity to regulate climate (Sunil *et al.,* 2024). From a broader ecological perspective, plastic pollution causes diversity loss and ecosystem degradation (Unuofin and Aboi, 2023).

### *2.6.4. Impacts of microplastics on the economy*

The widespread accumulation of MPs in the environment compromises the efficiency and sustainability of natural resources, resulting in measurable economic losses (UNEP, 2021). At the global scale, the cost of MPs has been linked to damage to marine ecosystems, reduced productivity, and increased management expenses, with estimated annual costs in the billions of dollars (Sutanto *et al*., 2024). MPs' contamination directly affects the economic output of fisheries and aquaculture by reducing the stock quality and increasing uncertainty in the seafood market (Kibria, 2023). In addition, contamination concerns can influence trade restrictions and consumer preferences, potentially decreasing export revenues and the market value of seafood products (Widyastuti *et al*., 2023).

The accumulation of MPs in agricultural soil affects its quality and productivity (Masciarelli *et al.,* 2024). These changes can raise production costs while lowering yields, thereby affecting farm income and national agricultural economies, particularly in LMICs dependent on subsistence farming (Cai *et al.,* 2024). In the natural environment, it can reduce aesthetic value and recreational quality, leading to decreased tourist inflow and revenue loss. Coastal and urban municipalities must also allocate substantial financial resources for cleanup operations and waste management, diverting funds from other development priorities (Asamoah *et al.,* 2022).

## 3. METHODS AND MATERIALS

### 3.1. Descriptions of the Study Areas

This study was conducted in central Ethiopia, specifically in Addis Ababa and Bishoftu, to assess microplastic pollution in humans, donkeys, and the environment, as well as community and donkey owner awareness. The selection of these study areas was based on their high levels of human–animal interaction, high population density, inadequate waste management practices, extensive irrigation activities, and the visible accumulation of plastic waste. These conditions make the sites suitable for investigating microplastic contamination within a One Health framework, as they represent urban, peri-urban, and industrial environments with high potential for plastic pollution.

Addis Ababa is located between 8°55′ and 9°05′ N latitude and 38°40′ and 38°50′ E longitude, covering an area of 540 km². The city is administratively divided into 10 sub-cities (kifle ketema) and about 99 kebeles, with an estimated population of approximately 3.5 million, an annual growth rate of 8%, and a population density of 5936.2 persons per km² (Mohammed and Elias, 2025). These characteristics contribute to increased plastic use and waste generation, which may lead to higher exposure to microplastics in humans and animals.

Bishoftu town is located between 8°43′–8°45′ N latitude and 38°56′–39°01′ E longitude, covering an area of 20,574 hectares, and lies at an altitude of 1850 m above sea level. It is situated approximately 47 km southeast of Addis Ababa within the Oromia National Regional State. The town's economy is supported by agriculture, manufacturing, tourism, and trade. Agricultural activities, small-scale industries, and tourism-related practices may contribute to plastic contamination of the environment. These factors, combined with close interactions between humans, donkeys, and the environment, make Bishoftu an appropriate site for evaluating microplastic pollution and assessing the awareness of the local community and donkey owners (Kebede *et al.,* 2025).

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The specific areas of this study were in and around the Little Akaki and Mojo Rivers. Both rivers are used for irrigation, livestock watering, domestic activities, and sand extraction during the dry season. The Little Akaki River is situated within a latitude of 8°46′–9°14′N and 38°34′–39°04′E longitude, covering an estimated area of about 1500 km$^2$ (Mamo *et al.*, 2021). The river flows across the area relatively gently, with the lower catchment characterized by grazing areas and irrigated vegetable farming (Mekuria *et al.*, 2020). However, the water quality of this river has been affected by the rapid urbanization of Addis Ababa, high population density, inadequate waste management, and poor hygiene (Kassegne *et al.*, 2018).

The Mojo River is located within the Awash basin, 38054′22″ to 39017′18″E and 08024′15″ to 09007′49″N, and has an elevation ranging from 1607 to 3091 meters above sea level (Alemu *et al.*, 2023). In the Bishoftu areas, where the current study was carried out, there is a notable seasonal fluctuation, with significant discharge during the primary rainy season and decreased or sporadic flow throughout the dry season.

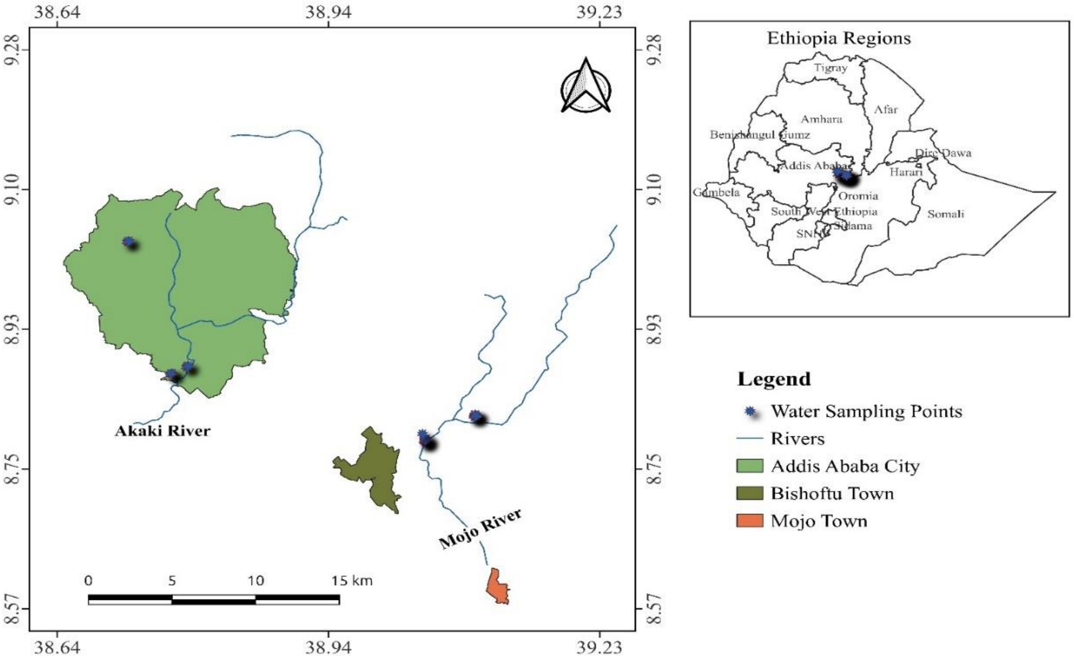

***Figure 3*****:** Map of the study area (prepared by the authors)

## 3.2. Study Population

The study was conducted across the three interconnected components: human, animal, and environment.

The human study population included healthy volunteers who lived near the Little Akaki and the Mojo (both seasonal and perennial) rivers for around five (5) years; for the awareness survey, participants lived in Addis Ababa and Bishoftu. Of those, the inclusion criteria for this study were participants who lived near both the Little Akaki and the Mojo River, non-alcoholic vegetarians and mixed food consumers, and individuals aged >=18. These are the participant characteristics to be included in this study. The exclusion criteria for this study included participants who lived less than five (5) years in the study area, alcohol and drug addicts, those aged <18, and those who did not use vegetarian diets.

The animal population in this study included donkeys that had lived near the Little Akaki and the Mojo River (Seasonal and Perennial), and were aged greater than five (5) months for microplastic analysis; for observation, donkeys were observed at the roadside, Merkato green market, and rural sites. For retrospective components, records of donkeys from Addis Ababa and Bishoftu were included. However, the exclusion criteria were that donkeys were seen during sampling but did not live in the sampling site.

The environmental samples in this study include river water collected from the Little Akaki River in Addis Ababa and from the Mojo River in Bishoftu (both seasonal and perennial).

## 3.3. Study Design and Sampling Technique

This research employed a cross-sectional design to evaluate the abundance and risk factors of MP pollution in humans, donkeys, and the environment in Addis Ababa and Bishoftu, central Ethiopia. This study also integrates a knowledge, attitude, and practice survey of community members and donkey owners, observations of donkey foraging behavior, and a retrospective review of veterinary records from the Merkato Clinic in Addis Ababa and the Bishoftu CVMA donkey sanctuary clinic from 2021 to 2026.

## 3.4. Sample Size Determination

This study employed a two-group comparison. Two different formulas were used: the first is for comparing two independent proportions (used for a binary outcome), and the two-mean formula for continuous outcomes.

For binary proportion/outcome;

$$N = \frac{\left(\frac{Za}{2}+Z\beta\right)^2 \times \left(P1(1-P1)+P2(1-P2)\right)}{(P1-P2)^2}$$

Where: N = required sample size per sub-group population, Za/2 = desired significance level at 5% (1.96), Zβ = power of the test set at 80% (0.84), P1 = previous prevalence report of exposed/nearby residents, P2 = Previous prevalence report of reference/distant residents

For a continuous outcome/ two mean formula;

$$N = \frac{\left(\frac{Za}{2}+Z\beta\right)^2 \times 2\sigma^2}{(\mu1-\mu2)^2}$$

Where: N = required sample size per subgroup population, $\frac{Za}{2}$ = desired significance level at 5 %(1.96), $Z\beta$ = power of test at 80 % (0.84 ), $\sigma^2$ = pooled standard deviation, and $\mu1 - \mu2$ = expected difference in means between groups.

The primary objective for human urine samples was to compare microplastic detection rates between residents near the Little Akaki River (industrial/urban pollution) and the Mojo River in Bishoftu (reference site). As no prior Ethiopian data exist, assumptions were based on Pironti *et al*. (2023), who reported microplastic detection in 29% of urban residents. We assumed a detection rate of 40% in the exposed group (Little Akaki) and 15% in the reference group (Bishoftu), representing a 25% point difference. using the two proportion formula: $N = \frac{(1.96+0.84)^2 \times [0.40(0.60)+0.15(0.85)]}{(0.40-0.15)^2}$ = 46.1≈46, Based on this formula, the minimum calculated sample size was 46 per group (92 total). However, human urine microplastic analysis remains an emerging field, and no previous studies have been conducted in Ethiopia to provide reliable prevalence estimates or methodological benchmarks. In addition, the analysis requires strict contamination-control procedures and specialized laboratory processing. Therefore, this study was designed as a baseline investigation, and a total of 36 urine samples were collected and analyzed, including 18 samples from the Little Akaki River area and 18 samples from the Mojo River area (9 from seasonal sites and 9 from perennial sites).

For donkey feces, the comparison was between free-grazing donkeys and controlled ones. Based on Kassahu and Tesfaye (2017), who reported 60% plastic ingestion prevalence in free-grazing Ethiopian livestock, and assuming 20% prevalence in controlled-feeding donkeys:

$N = \frac{(1.96+0.84)^2 \times [0.60(0.40)+0.20(0.80)]}{(0.6-0.2)^2} = 19.6 \approx 20$, The minimum calculated sample size was 20 donkeys per group (40 total). Accordingly, 41 fecal samples were collected.

In the river water samples, the comparison was between the locations that were divided into three groups, including upstream, downstream, and the midstream points (Little Akaki, Perennial Mojo, and Seasonal Mojo River), exposed to urban/industrial runoff, and the upstream points as the reference points. Based on the previous study conducted in Addis Ababa, a highly urbanized lake, Girimay *et al.* (2025) reported mean MP concentrations of 65 ± 20 particles/L downstream and 35 ± 15 particles/L upstream (pooled SD ≈ 18):

$N = \frac{(1.96+0.84)^2 \times 2 \times (18)^2}{(65-35)^2} = 5.64 \approx 6$, Then, the minimum sample size was 6 per group (a total of 18 ). Therefore, a total of 27 samples were collected, exceeding the minimum requirement.

In the KAP surveys, the comparison was between Addis Ababa residents (with higher exposure to plastic waste and awareness campaigns) and Bishoftu residents (as the reference group). However, there was no prior data in the study area, and assuming 60% awareness of plastic pollution impacts in Addis Ababa and 40 % in Bishoftu as references.

$N = \frac{(1.96+0.84)^2 \times [0.60(0.40)+0.40(0.60)]}{(0.6-0.4)^2} = 94.1 \approx 94,$ The minimum sample size was 94 per subgroup (188 total). Therefore, a total of 220 respondents, both community members and donkey owners.

In a behavioral observational study, the sample size was determined by feasibility rather than a statistical technique. A total of 110 individual donkeys (61 male, 49 female) were observed for 30 minutes each (3,300 observation minutes). Therefore, this study exceeded the previous study conducted on cattle foraging behavior (Papachristou *et al*., 2005; n = 40-60).

### 3.5. Sampling Methods

This study employed a purposive sampling technique to select participants and sites relevant to assessing the impact of plastic pollution (Tajik *et al*., 2024).

### 3.6. Sample Collection

Fecal samples from donkeys, water samples from the environment, and urine samples from humans were collected. In Addis Ababa, river water samples were collected manually using a stainless steel bucket. Nine (9) samples from 9 sampling points were collected from the Little Akaki River catchment. Of those, three samples from the three sampling points were collected from Sefereselam, around Autobus Tera, a tributary of the Akaki River, and labeled downstream (D1, D2, and D3). Another three samples were collected from the Gelan condominium area near Gelan Secondary School, close to Akaki-Kality, and labeled as upstream (U1, U2, and U3) (Ferraz *et al.*, 2020). The remaining three samples were collected from Akaki Meshalekia and labeled as midstream (M1, M2, and M3) *(*Hassen *et al.*, 2025).

In Bishoftu, samples were collected from two sections of the Mojo River: one near the Edi Administration office and the other near Lake Kilole. The Edi section is a seasonal (intermittent) river, while the Lake Kilole section represents a perennial (non-seasonal) system with continuous flow. From each section, 18 water samples were collected from 18 sampling points. At each section, nine (9) sampling points were established at approximately 200-300 m along the river transect. Points were categorized into upstream (U1, U2, U3), midstream (M1,M2,M3), and downstream (D1,D2,D3) to assess the contamination of microplastic particles. The sampling sites were chosen based on possible sources of MP pollution. These include areas with visible plastic waste, locations with human and animal activities, sites affected by industrial discharges, and areas used for irrigation and grazing (Sotnikova *et al.,* 2026).

For each sampling point, 10 liters of water were collected using a 2.5 L clean glass container with a triplicate method (Karing *et al.*, 2023). The samples were collected using on-site methods in the river at a depth of 1.2 m. We used a 0.25 mm (250 µm) and 63 µm stainless-steel mesh on-site to filter the sample (Schymanski *et al.*, 2021).

For donkey samples, a total of 41 donkey fecal samples were collected in Addis Ababa and Bishoftu, including 25 samples from donkeys living near the Mojo River (both the seasonal and perennial) and 16 samples from donkeys living near the Little Akaki River. Approximately 10 g of fresh fecal matter was observed during the observation period, before contact with the ground, to minimize environmental contamination (Haddy *et al.,* 2025). Metal utensils were used to collect samples. All materials were pre-washed with distilled water before use. Each fecal sample was first wrapped in aluminum foil, then placed in a clean glass container, and transported to the Environmental Science Department Laboratory in an ice box at approximately 4°C for microplastics analysis (Siña *et al*., 2025).

During the study period, a total of 36 human urine samples were collected from 18 healthy volunteer participants (7 males and 11 females) residing in Edi Kebele, Kilole Woreda, and from 18 (10 males and 8 females) volunteer participants in Addis Ababa residing near the Little Akaki River. Before collecting the samples, information on participants' demographic characteristics, dietary patterns, and overall health condition was documented. Participants regularly consumed vegetables and mixed food products cultivated along the nearby riverbanks. Approximately 10 mL of urine was collected from each participant using pre-cleaned Boro-Silicate glass collection tubes to minimize plastic contamination (Pironti *et al.*, 2023).

Foraging behavior was observed in 110 individual donkeys (61 males and 49 females) over a total observation time of 3,300 minutes. Pictures, ID notes, location, and other necessary information were taken. Focal animal sampling was conducted regularly to investigate donkey foraging behavior (Papachristou *et al*., 2005). Each focal donkey was observed for 30 minutes; during the observation, all occurrences and the time spent on feeding and social behaviors were recorded, including time spent in the plastic area, foraging time, injury/disease signs, stress indicators, and whether the materials were bitten, plastic, or other fabric materials. Observations were conducted in urban areas (donkeys in visible plastic areas and at Merkato green markets) and rural areas.

A structured questionnaire was prepared for donkey owners to assess the effects of plastic pollution on donkey health and welfare, human health, and the environment. The questionnaire collected demographic information of the respondents, including age, sex, location, educational level, as well as their knowledge, perceptions, and practices related to plastic pollution and its associated risk factors. Based on the responses, awareness was calculated.

Additionally, a structured questionnaire was prepared for community members to assess their knowledge, practices, and perceptions regarding effects on donkey health, human health, and the environment. The survey collected demographic information of the respondents, including age, sex, location, and education level. It also recorded behavior related to plastic use, such as the use of common plastic items (plastic bags, bottles, and food packaging) and the use of alternative materials after the ban (non-woven cloth bags and paper bags).

Retrospective veterinary records from Merkato Donkey Sanctuary Clinic and Bishoftu were collected from 2020 to 2026 and prepared for analysis.

### 3.7. Sample Preparation

For water samples, organic material in the filtered water was removed by stepwise chemical digestion. First, each sample was treated with 30% $H_2O_2$ at a 1:1 ratio and kept at 44 °C for 24 hours to break down organic matter. This was followed by treatment with 10% KOH at 44 °C for 5 days to further remove the remaining organic material. After digestion of the water samples using the combination of 30 % $H_2O_2$ for 24 h and 10% KOH for 5 days. After digestion, a NaCl solution (1.2 g/cm$^3$) was added to separate microplastic particles by density. The supernatant was then filtered using a custom-built filtration system with a 0.45 µm 47 mm cellulose nitrate filter. The filtered materials were placed in a clean glass petri dish and dried in a closed desiccator (Yang *et al*., 2023).

For fecal samples, the collected samples were first weighed wet, then wrapped in clean aluminum foil and dried in a fume hood. After drying, the entire sample was transferred to a 500 mL glass flask for digestion. The samples were treated with Fenton reagents, followed by digestion with 10% KOH at 44 °C in an incubator. After digestion, the supernatant was separated using a separatory funnel and filtered using a manually constructed filtration system. The filtered material was then placed in a clean glass petri dish and kept in a closed desiccator (Vazquez-cruz *et al.*, 2025).

For urine samples, they were first digested to remove organic debris with 10% KOH and incubated at 44°C for 5 days. After digestion, the samples were filtered using a custom-built filtration system. The filtered material was placed in a clean Petri dish and dried in a closed desiccator (Ji *et al*., 2025).

After the samples were dried inside a closed desiccator, they were examined using a stereomicroscope (OMAX, China) equipped with a digital camera. Toupview software was used to determine the shape, color, and size of microplastic particles with 4× and 10× objective lenses. Microplastic identification was based on visual characteristics, physical properties (rigid or flexible), and needle test response (Siña *et al*., 2025).

### 3.8. Quality Control/Quality Assurance

To reduce contamination, all materials and equipment were cleaned with distilled water and 97% ethanol before use. During sampling, sample preparation, and analysis, only non-plastic materials, such as glass flasks, glass beakers, an amber glass bottle, a glass petri dish, and glass tweezers/forceps, were used. Sample preparation was carried out in a cleaned laminar flow hood, and a lab coat was worn to limit airborne contamination. The analysis was conducted in triplicate, and blank procedures were conducted and analyzed as normal samples. Any particles detected in procedural blanks were subtracted from the corresponding sample counts.

### 3.9. Statistical Analysis

The statistical analyses for this study were performed using R software (version 4.2) to examine variations in microplastic (MP) concentrations across different sampling locations and sample types (water, feces, and urine). A two-way analysis of variance (ANOVA) was employed to determine the effects of these independent variables, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test to identify pairwise differences among groups. Statistical significance was established at $p < 0.05$. In addition, multiple linear regression and binary logistic regression analyses were conducted to assess the influence of plastic pollution and to identify key determinants affecting donkey health and welfare.

### 3.10. Ethical Approval and Considerations

Ethical clearance for animal aspects was obtained from the Institutional Animal Research Ethical Committee of the College of Veterinary Medicine and Agriculture. (Certificate Ref No.: VM/ERC/09/111/18/2026). The human samples were approved by the Aklilu Lemma Institute of Pathobiology Institutional Research Ethics Review Committee (Certificate Ref No.: ALIHR IRERC-010/2018/2026). The study adhered to the principles outlined in the Declaration of Helsinki (Shrestha and Dunn, 2020). All procedures were conducted in accordance with human and animal research ethics. The ethical certificates are attached for details in Annex 7 and Annex 8, respectively.

# 4. RESULTS

## 4.1. Microplastic Concentration

Microplastic particle concentrations were quantified across donkey feces, human urine, and river water samples (Table 1). In water samples, the highest mean concentration was observed in the perennial Mojo River, followed by the Little Akaki River and the seasonal Mojo River. In human urine samples, the highest mean concentration was observed among participants living near the perennial Mojo River, followed by those near the seasonal Mojo and Little Akaki rivers. A similar pattern was observed in donkey feces, with the highest concentrations recorded near the perennial Mojo River.

Prior to statistical analysis, ANOVA assumptions were evaluated. Residuals were normally distributed (Shapiro–Wilk test, $p = 0.316$), and homogeneity of variance was confirmed (Fligner–Killeen test, $p = 0.372$). Independence of observations was ensured through the study design. A one-way ANOVA (Table 2) revealed a statistically significant difference in microplastic concentrations among sample types ($F = 3.429$, $p = 0.045$). Furthermore, two-way ANOVA results (Table 3) indicated that both sample type ($p = 0.022$) and location ($p = 0.0044$) had significant effects on microplastic concentration. However, the interaction between sample type and location was not statistically significant ($p = 0.1629$), suggesting that their effects are independent. Post hoc analysis using Tukey's Honest Significant Difference (HSD) test identified significant pairwise differences between specific sample types and locations ($p = 0.021$).

**Table 1:** Mean (± SD) microplastic concentrations across sample types and locations.

| **Location** | **Sample type** | **MPs concentration (Mean ± SD)** |
|---|---|---|
| Little Akaki | River water | 1.78±0.59 ( particles/L) |
| | Donkey feces | 0.53±0.27 (particles/g) |
| | Human urine | 1.12±0.89 (particles/mL) |
| Perennial Mojo | River water | 2.01±0.91 (particles/L) |
| | Donkey feces | 1.36±0.50 (particles/g) |
| | Human urine | 1.31±0.71 (particles/mL) |
| Seasonal Mojo | River water | 0.92±0.81 (particles/L) |
| | Donkey feces | 0.85±0.44 (particles/g) |
| | Human urine | 1.20±0.78 (particles/mL) |

Note: MP = microplastics; SD = standard deviation.

**Table 2:** One-way ANOVA results comparing microplastic concentrations among sample types

| **Source of variation** | **Df** | **SS** | **MS** | **F-value** | ***p*-value** |
|---|---|---|---|---|---|
| Sample type | 2 | 3.73 | 1.86 | 3.43 | 0.045 |
| Residuals | 59 | 33.90 | 0.57 | | |

**Note :Df=degree of freedom, SS= sum of square, MS=mean square**

**Table 3:** Two-way ANOVA results showing the effects of sample type and location on microplastic concentration.

| **Source of variation** | **Df** | **SS** | **MS** | **F-value** | ***p*-value** |
|---|---|---|---|---|---|
| Sample type | 2 | 6.05 | 3.026 | 3.929 | 0.022 |
| Location | 2 | 8.88 | 4.442 | 5.768 | 0.004 |
| Sample*location | 4 | 5.25 | 1.312 | 1.704 | 0.163 |
| Residuals | 53 | 40.82 | 0.770 | | |

## 4.2. Physical Characteristics of Microplastic

Four MP shapes were identified in human urine, donkey feces, and environmental water samples: fragment, foam, pellet, and fiber. In donkey feces samples, fibers had 35% of the MPs, pellets (30%), fragments (20%), and foam (15 %), respectively. In urine samples, fiber was the dominant shape, with 50%, followed by pellets (25%), fragments (15%), and foam (10%). Similarly, the water samples' fiber was the dominant shape (50%), followed by pellets (25%), fragments (20%), and foam (15%) (Figure 4).

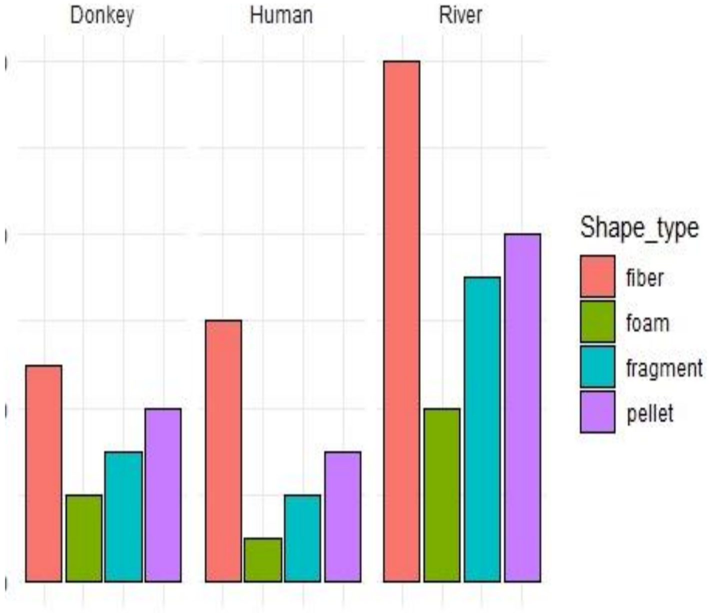

**Figure 4:** MPs shape distribution across the three samples

In this study, the color distributions were transparent, black, blue, white, yellow, orange, and green. Transparent, followed by black and light blue, were the most dominant colors in urine, feces, and water samples, accounting for 25-45 % (Figure 5).

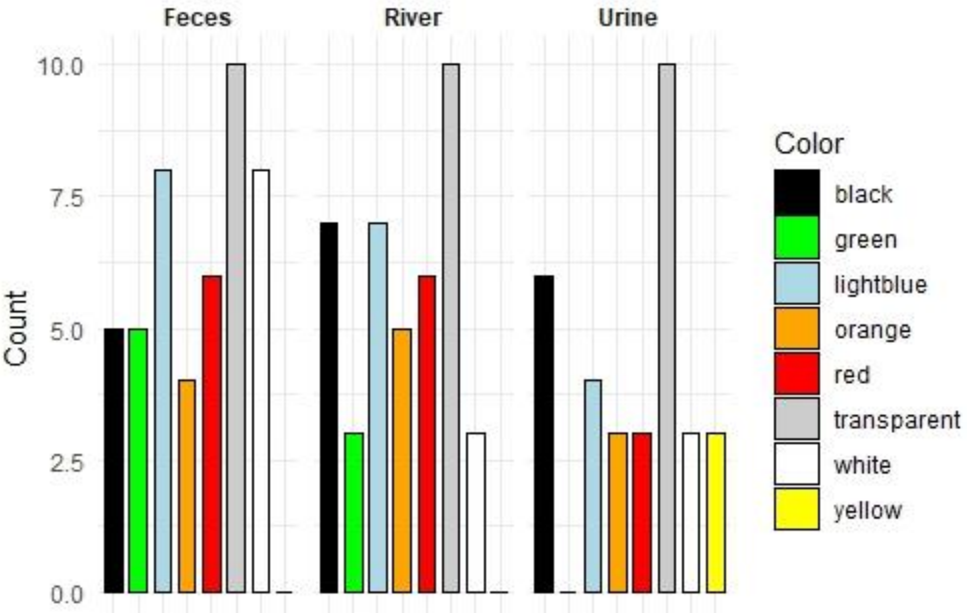

**Figure 5:** MPs' color distribution across urine, feces, and water samples

In this study, MP particles were grouped into three size categories: less than 500 µm, 500 µm-1000 µm, and greater than 1000 µm. The majority of particles (50-65%) were smaller than 500 µm, representing 55% in fecal samples, 50% in urine samples, and 65% in water samples. The medium-sized particles, ranging from 500-1000 µm, were 27% in water, 30% in feces, and 25% in urine samples. Larger MP particles accounted for 8% of urine, 20% in feces, and 25% of water samples.

Microplastic concentration in donkey feces was affected by their feeding type (crop residue vs grass), feeding practice (controlled vs free grazing), water type (river vs tap), and sex (male vs female). In this comparison, the controlled donkeys, crop residue feeders, female donkeys, and donkeys in river water had the lowest concentration compared to the grass feeders, free grazers, males, and donkeys that consumed tap water. The most significant predictors of MP in donkey feces were feeding practice (*p*<0.001), age (*p*=0.043), and location (*p*<0.001) (Table 4).

**Table 4**: Multiple linear regression analysis of factors associated with microplastic concentration in donkeys

| Variables | Comparison | MPs concentration | Percent difference | *p*-value |
|---|---|---|---|---|
| Feeding-practice | Controlled-feeding | 0.89 | 100% (baseline) | <0.001 |
| | Free-grazing | 1.54 | 173% | |
| Feeding-type | Grass | 1.12 | 100% (baseline) | 0.691 |
| | Crop residue | 1.19 | 106% | |
| Sex | Female | 1.08 | 100% (baseline) | 0.437 |
| | Male | 1.17 | 108% | |
| Location | Little Akaki | 0.53 | 100% (baseline) | <0.001 |
| | Perennial mojo | 1.48 | 279% | |
| | Seasonal mojo | 0.93 | 175% | 0.247 |
| Age | Per-1 years | +0.034 | +3.8% | 0.043 |

**Note**: Baseline categories were used as reference groups. Percent differences represent relative changes in microplastic concentration compared to the reference category. Age was included as a continuous variable.

Microplastic concentration in human urine was examined in relation to sex, water type, location, and age group. These results showed that none of these factors were statistically significant, including water type (p=0.68), sex (*p*=0.876), age (*p*=0.754), and location (*p*=0.682) (Table 5). Participants were categorized into three age groups (18–30, 31–50, and 51–65 years) based on commonly used epidemiological classifications to facilitate comparison across exposure groups. From those, the highest number of MPs was observed in participants aged 18-30. Regarding water type, individuals who used tap water had the highest MP abundance, whereas those who used both tap and bottled water had the lowest, compared with those who used only tap water.

**Table 5:** factors affecting MPs concentration in humans

| Variables | Categories | MPs concentration (mean ± ) | Compared to | Percent difference | *p*-value |
|---|---|---|---|---|---|
| Water type | Tap | 1.15 ± 0.87 | - | - | 0.68 |
| | Bottled | 0.90 ± 0.58 | Tap | -22% | |
| | Both | 0.98 ± 0.83 | Tap | -15% | |
| Sex | Female | 1.07 ± 0.77 | - | - | 0.87 |
| | Male | 1.13 ± 0.91 | Male | +6% | |
| Age group | 18-30 | 1.2 ± 0.91 | - | - | 0.75 |
| | 31-50 | 1.14 ± 0.88 | 18-30 | +1% | |
| | 51-65 | 0.30 ± 0.00 | 18-30 | -73% | |
| Location | Little Akaki | 1.12 ± 0.89 | - | - | 0.68 |
| | Perennial mojo | 1.20 ± 0.79 | Little Akaki | +7% | |
| | Seasonal mojo | 0.99 ± 0.82 | Little Akaki | -12% | |

The MPs concentrations in the three rivers (the Little Akaki, the Perennial Mojo, and the Seasonal Mojo), sampling point D3 in the Perennial Mojo, U2 in the Little Akaki, and D3 in the Seasonal Mojo rivers were the highest concentrations of MPs detected (Figure 6).

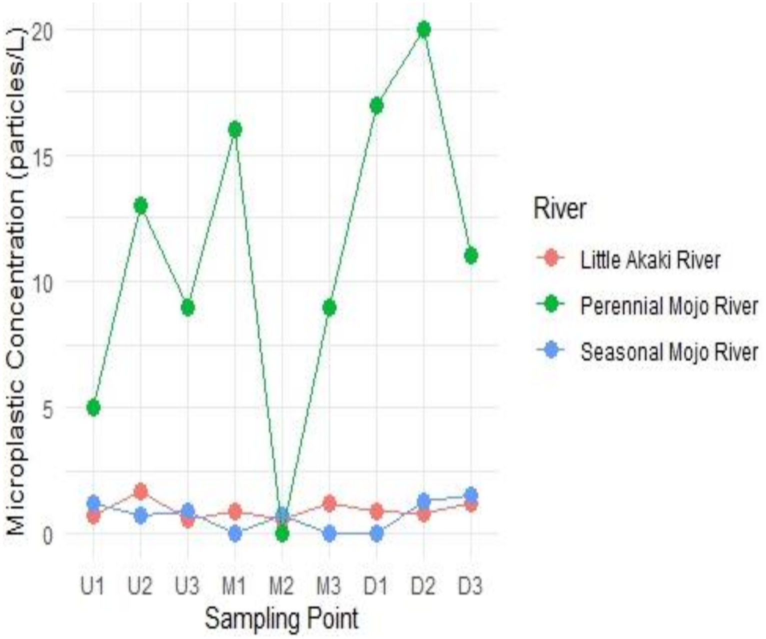

**Figure 6** : Microplastic particle concentration in water samples across the three rivers

The images of microscopic MPs encountered in different samples, including urine, feces, and water, captured during the study, are shown in the following figure (Figure 7).

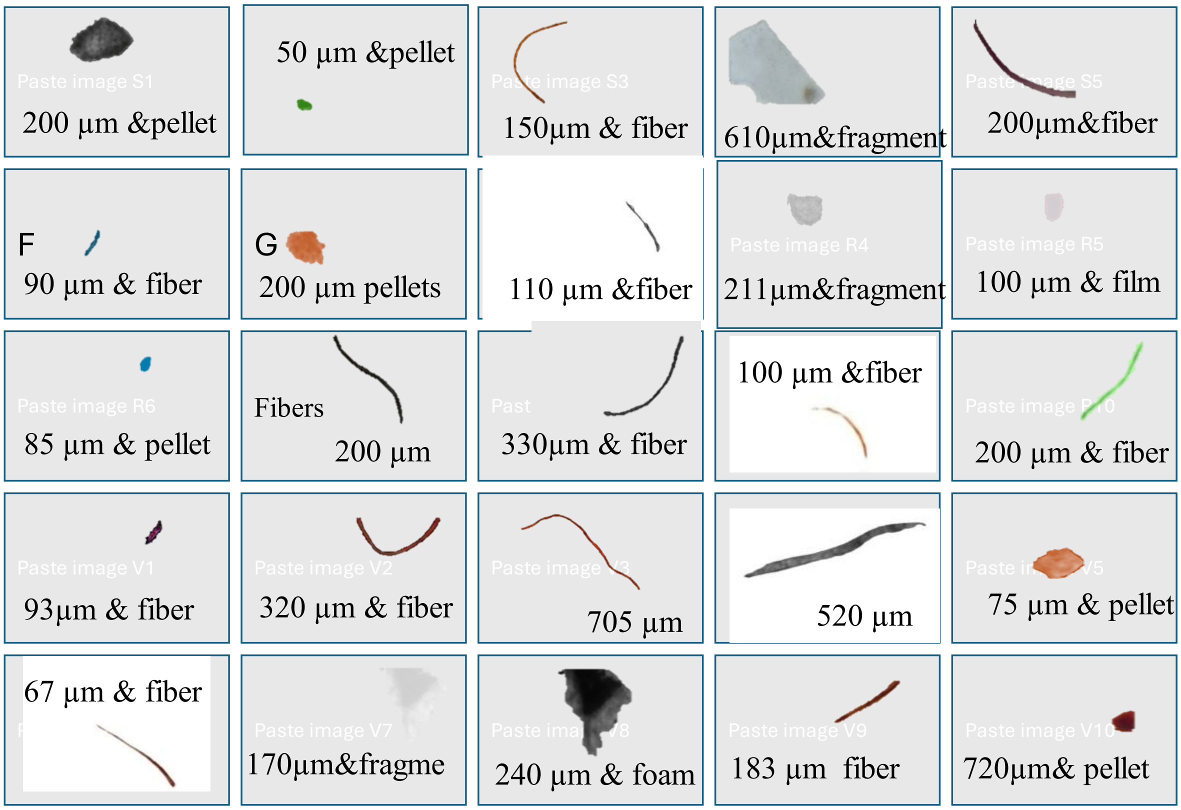

**Figure 7:** Microscopic images of MPs across urine, feces, and water samples

### 4.3. Determinants of Plastic Ingestion in Donkeys

A total of 110 donkeys were observed to assess the foraging behavior of donkeys and their determinants of plastic ingestion that affect the overall condition and well-being of donkeys within the study region by classifying the study area into three: rural sites, roadside, and Merkato Grain market. The prevalence of plastic ingestion across the three locations was similar, at 65.5% in Merkato, 67.3% in roadside environments, and 65.6% in rural sites, with no significant association among sites ($\chi^2 = 0.038$, $p = 0.981$). Donkeys that had an injury or disease had a 69.6% prevalence compared with donkeys without injury or disease, while there was no association between them ($\chi^2 = 1.863$, $p$=0.172) ( Table 6).

**Table 6:** Association between plastic ingestion and selected risk factors in donkeys

| Variable | Category | Yes n (%) | No n (%) | $\chi^2$ | *p*-value |
|---|---|---|---|---|---|
| Location | Merkato | 19 (65.5%) | 10 (34.5%) | 0.038 | 0.981 |
| | Roadside | 33 (67.3%) | 16 (32.7%) | | |
| | Rural | 21 (65.6%) | 11 (34.4%) | | |
| Injury/disease signs | Yes | 48 (69.6%) | 21 (30.4%) | 1.863 | 0.172 |
| | No | 25 (61.0%) | 16 (39.0%) | | |
| Stress indicator | Yes | 24 (66.7%) | 12 (33.3%) | 0.244 | 0.885 |
| | No | 49 (66.2%) | 25 (33.8%) | | |

**Note :$\chi^2$=Chi-square test statistic**

The multivariable logistic regression analysis indicated no significant predictors that affected plastic ingestion (*p*>0.05). Predictors, including exposure level, sex, location, body condition score, exposure %, and foraging time, did not have statistically significant effects on plastic ingestion of donkeys (as indicated in Table 7).

**Table 7:** Multivariable logistic regression analysis of predictors of plastic ingestion in working donkeys

| Variables | Categories | OR | 95% CI | *p*-value |
|---|---|---|---|---|
| Exposure level | High (Ref) | 1.00 | — | — |
| | Low | 0.32 | 0.01 – 7.82 | 0.482 |
| | Medium | 1.06 | 0.14 – 8.87 | 0.955 |
| Foraging time (in minutes) | Continuous | 1.02 | 0.95 – 1.10 | 0.609 |
| Exposure (%) | Continuous | 0.98 | 0.93 – 1.03 | 0.377 |
| Location | Merkato (Ref) | 1.00 | — | — |
| | Roadside | 1.13 | 0.38 – 3.32 | 0.830 |
| | Rural | 1.11 | 0.36 – 3.42 | 0.848 |
| Sex | Female (Ref) | 1.00 | — | — |
| | Male | 1.03 | 0.41 – 2.55 | 0.952 |
| Body condition score (BSC) | Continuous | 0.86 | 0.34 – 2.12 | 0.741 |

**Note : OR = Odds Ratio; CI = Confidence Interval; Ref = Reference category**

The linear regression model analysis showed that the only predictor that affects BCS is location. Donkeys observed along the roadside had higher BCS than those at Merkato ($\beta = 0.242, p = 0.028$). However, variables such as plastic ingestion, exposure level, foraging time, and rural location were not statistically significant predictors of donkey BCS (as shown in Table 8).

**Table 8:** Linear regression analysis of predictors of body condition scores (BCS) in working donkeys

| **Variable** | **Category** | **β (Estimate)** | **Std. Error** | ***p*-value** |
|---|---|---|---|---|
| Plastic ingestion | Yes | -0.028 | 0.094 | 0.768 |
| | No(Ref) | | | |
| Exposure level | Low | 0.154 | 0.113 | 0.175 |
| | High(Ref) | | | |
| | Medium vs High | 0.161 | 0.110 | 0.147 |
| Foraging time (min) | Continuous | -0.005 | 0.008 | 0.485 |
| Location | Roadside | 0.242 | 0.109 | 0.028 |
| | Merkato (Ref) | | | |
| | Rural | 0.075 | 0.121 | 0.536 |

**Note : β = Regression coefficient; Ref = Reference category**

### 4.4. Community Members' Awareness and Perception

A total of 110 participants in this study (55 from Addis Ababa and 55 from Bishoftu). Of those, 57.3% were females. No significant differences were found for gender (*p*=0.85) and education level (*p*=0.21). Regarding location (city), a significant difference was recorded (*p*=0.023). High awareness was recorded in Addis Ababa (56.4%), and low in Bishoftu (25.5%).

The respondents' awareness based on domain-specific domains was: 45.5% of respondents were aware of the risk factors of plastic contamination in environmental health, and significantly higher in Addis Ababa(*p*= 0.035); 56.4% were aware of animal health effects and significantly higher in Addis Ababa(*p*=0.0039); and 32.7% of respondents were aware of human health effects. However, the analysis indicated no significant variation between the two cities (*p*=0.839).

The most commonly used plastic type, according to the respondents, was plastic bags (57.3%), followed by bottles (35.5%), and food packaging (7.3%), while no significant variation was observed across the two cities (*p*=0.22). After the plastic ban, most respondents used non-plastic woven cloth (80%), while a small number used paper bags (20%). However, no significant variation was observed across the two cities (*p* = 0.09) (Table 9).

**Table 9:** Socio-demographic characteristics, awareness of plastic pollution impacts, and plastic use practices among respondents

| **Variable** | **Category** | **Addis Ababa (n=55)** | **Bishoftu (n=55)** | **Total (n=110)** | ***p*-value** |
|---|---|---|---|---|---|
| Gender | Female | 32 (58.2%) | 31 (56.4%) | 63 (57.3%) | 0.85 |
| | Male | 23 (41.8%) | 24 (43.6%) | 47 (42.7%) | |
| Education Level | None | 18 (32.7%) | 26 (47.3%) | 44 (40.0%) | 0.21 |
| | Primary | 10 (18.2%) | 10 (18.2%) | 20 (18.2%) | |
| | Secondary | 20 (36.4%) | 11 (20.0%) | 31 (28.2%) | |
| | Higher | 7 (12.7%) | 8 (14.5%) | 15 (13.6%) | |
| Overall Awareness Score | Low (0–1) | 24 (43.6%) | 41 (74.5%) | 65 (59.1%) | 0.023 |
| | High (2–3) | 31 (56.4%) | 14 (25.5%) | 45 (40.9%) | |
| Aware of Animal health | Not aware | 16 (29.1%) | 32 (58.2%) | 48 (43.6%) | 0.004 |
| | Aware | 39 (70.9%) | 23 (41.8%) | 62 (56.4%) | |
| Aware of Environmental Health | Not aware | 24 (43.6%) | 36 (65.5%) | 60 (54.5%) | 0.04 |
| | Aware | 31 (56.4%) | 19 (34.5%) | 50 (45.5%) | |
| Aware of Human Health | Not aware | 36 (65.5%) | 38 (69.1%) | 74 (67.3%) | 0.84 |
| | Aware | 19 (34.5%) | 17 (30.9%) | 36 (32.7%) | |
| Primary plastic use | Bottle | 21(38.2%) | 18(32.72%) | 39(35.45%) | 0.22 |
| | Food packaging | 6(10.9%) | 2(3.63%) | 8(7.27%) | |
| | Plastic bag | 28(50.9%) | 35(63.63%) | 63(57.27%) | |
| Post-ban plastic use | Non-woven cloth bag | 48(87.2%) | 40(72.7%) | 88(80%) | 0.09 |
| | Paper bag | 7(12.72%) | 15(27.27%) | 22(20%) | |

Logistic regression analysis, as shown in Table 10, age ($p$=0.021, OR=0.97) and city ($p$=0.0048, OR=0.313) were significant predictors of the level of awareness. However, gender and level of education were not significant predictors.

**Table 10:** Logistic regression analysis showing the awareness of the community

| Variables | Category | OR(95% CI) | *p*-value |
|---|---|---|---|
| Age | Per 1-year increase | 0.97 (0.95–0.99) | 0.021 |
| City | Addis Ababa (Ref) | 1.00 | — |
| | Bishoftu | 0.313 (0.14–0.72) | 0.0048 |
| Gender | Female (Ref) | 1.00 | — |
| | Male | 1.08 (0.52–2.24) | 0.84 |
| Education | None (Ref) | 1.00 | — |
| | Primary | 0.95 (0.35–2.60) | 0.92 |
| | Secondary | 1.42 (0.60–3.36) | 0.42 |
| | Higher | 1.67 (0.52–5.31) | 0.39 |

In the awareness score, there was a significant difference between the two cities (U = 1872.5, $p$ = 0.023), with a small effect size (r = 0.217).

## 4.5. Management and Owner Knowledge

A total of 110 donkey owners were interviewed about the health risks of plastic contamination in donkeys, humans, and the environment. Of those, 74.55% of respondents were aware of plastic pollution, while 25.5% were not. However, the level was almost similar between Addis Ababa and Bishoftu. However, awareness of the participants about the effects of plastic pollution in terms of education level ($\chi^2$=1.65, $p$=0.438), location ($\chi^2$=0.00, $p$=1.000), feeding system ($\chi^2$=1.77, $p$= 0.413), grazing location ($\chi^2$=3.63, $p$=0.304), or veterinary service ($\chi^2$=1.11, $p$=0.293) showed no significant variations(Table 11).

**Table 11:** Association between awareness and explanatory variables

| Variables | $X^2$ | Df | *p*-value |
|---|---|---|---|
| Education Level | 1.65 | 2 | 0.438 |
| Location | 0.00 | 1 | 1.000 |
| Feeding System | 1.77 | 2 | 0.413 |
| Grazing Location | 3.63 | 3 | 0.304 |
| Vet Visit | 1.11 | 1 | 0.293 |

The logistic regression analysis indicated that none of the variables were significantly associated with awareness. However, the Health-related variables, such as loss of appetite, were significant predictors of the health status of donkeys that ingest plastic materials (OR = 0.24, P = 0.055).

## 4.6. Retrospective Clinical Records in Donkeys

### *4.6.1. Retrospective records in Merkato Donkey Sanctuary Clinic*

A retrospective record of donkeys in the Merkato Donkey Sanctuary Clinic was collected from 2020 to 2026. There were 116 colic cases from January 2020 to March 2026. Of those, 23.5% died and 76.5% recovered. In this retrospective study of veterinary colic records, plastic-related colic was the most common case (95.7%), followed by parasitic colic (1.7%), diet-related colic (1.7%), and gassy colic (0.9%). In Fisher's exact test (Table 12), there was no significant association between diagnosis and outcome, address and diagnostic, or body condition and outcome.

**Table 12:** Association between categorical variables

| Comparison | Test type | p-value |
|---|---|---|
| Diagnosis group vs outcome (Feedback) | Fisher’s test | 0.3266 |
| Address group vs diagnosis group | Fisher’s test | 0.6992 |
| Body condition vs outcome | Fisher’s test | 0.2835 |

The colic case data were recorded annually from 2020 to 2026 and show variation in total cases and outcomes across years. In terms of location, donkeys in Kofe have the highest recovery rate, while donkeys in Gefersa have the lowest recovery rate compared to the other, as shown in Figure 8 below.

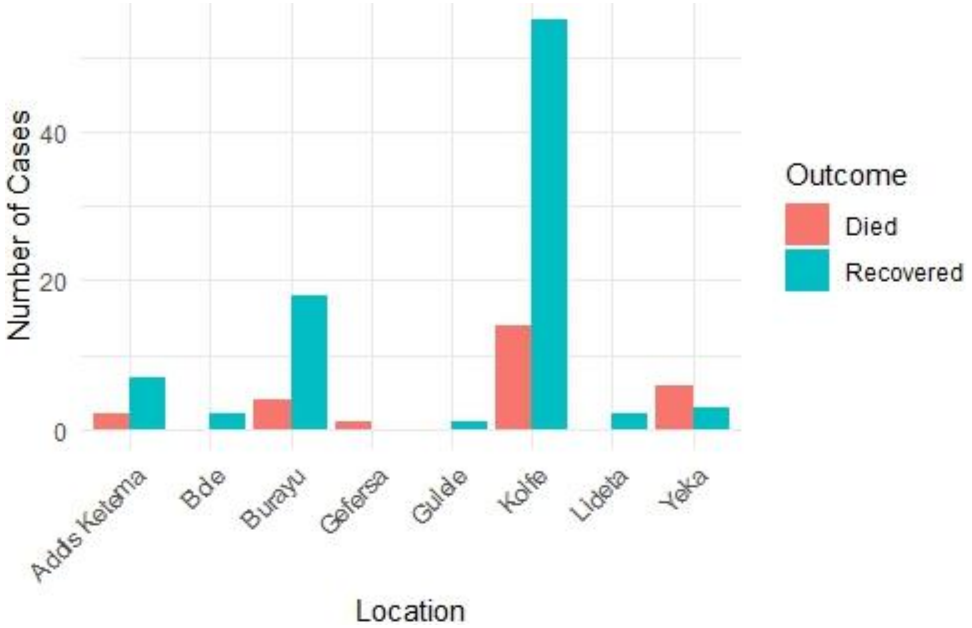

**Figure 8:** Outcome of colic cases in Merkato across locations

*4.6.2. Retrospective donkey colic case in Bishoftu*

A retrospective record of donkey colic cases from 2021 to 2024 was collected, yielding a total of 107 cases. Among those, 56.1% of donkeys died, and 43.9% recovered following treatment. In the chi-square and Fisher's exact test results (Table 13), statistically significant differences were not found for treatment outcome and sex (*p* = 1.000), BCS (*p* = 0.865), or plastic risk (*p* = 1.000). However, the association between addresses (location) and outcomes was a borderline significant association (*p*=0.069).

**Table 13:** Association between associated factors and outcome

| **Variables** | **Test Used** | **Statistic** | ***p*-value** |
|---|---|---|---|
| Sex | Chi-square | $\chi^2 = 0$ | 1.000 |
| Address | Fisher's Exact | – | 0.0699 |
| Age | t-test | t = 0.94 | 0.351 |
| BCS | Wilcoxon | W = 1435.5 | 0.865 |
| Plastic Risk (proxy) | Chi-square | $\chi^2 \approx 0$ | 1.000 |

The multivariable logistic regression analysis (Table 14) revealed that none of the predictors were statistically associated with treatment outcome. These included Age (OR = 0.94, 95% CI = 0.83-1.08, *P* = 0.368), BCS (OR = 0.90, 95% CI = 0.38-2.12, *P* = 0.813), sex (OR = 1.04, 95% CI = 0.48-2.26, *P* = 0.918), and plastic risk (OR = 1.03, 95% CI = 0.39-2.70, *P* = 0.947).

**Table 14:** Multivariable logistic regression analysis of factors in relation to treatment outcome

| **Variables** | **Category** | **OR** | **95% CI** | ***p*-value** |
|---|---|---|---|---|
| Age | Continuous | 0.94 | 0.83 – 1.08 | 0.368 |
| BCS | Continuous | 0.90 | 0.38 – 2.12 | 0.813 |
| Sex | Female (Ref) | 1.00 | – | - |
|  | Male | 1.04 | 0.48 – 2.26 | 0.918 |
| Plastic Risk | Low Risk (Ref) | 1.00 | – | – |
|  | High Risk | 1.03 | 0.39 – 2.70 | 0.947 |

In terms of location, Dambi, Kurkura, and Yerer had the highest recovery rates among Bishoftu locations. However, the specific locations, including Dire, Dukem, and Filtino, had the lowest recovery rate of colic cases in donkeys. Overall, the best recovery rate was recorded in Merkato (75%) compared with Bishoftu (43.9%), and the most common treatments for removing foreign bodies were Flunixin + Paraffin oil, used in both the Merkato and Bishoftu Donkey Sanctuary Clinics. However, there was no age or sex difference, and the most common BCS of donkeys in Merkato was 2.5 and 2 in Bishoftu.

# 5. DISCUSSION

This study confirmed that MPs were present in environmental (river water), animal (donkey feces), and human (urine) samples, indicating widespread contamination across interconnected systems. The detection of MPs in all river water samples confirmed that aquatic environments in the study areas were continuously exposed to plastic pollution. The higher concentration observed in the perennial Mojo River compared to the Little Akaki River and the seasonal Mojo River may be explained by continuous hydrological flow, which enhances the accumulation and transport of MPs from upstream anthropogenic sources and acts as a permanent sink for MPs. This finding is consistent with studies by Napper *et al.* (2021) and Windsor *et al.* (2019), who reported that rivers with constant flow tend to accumulate higher MP loads due to sustained urban runoff and wastewater discharge.

A study conducted in the Megeche River in Gonder by Esubalew *et al*.,(2026) showed that the highest concentration was recorded in urban tributaries compared to rural ones, with a concentration of 0.55 ± 0.31 items/L in surface water. However, in the present study, conducted in urban areas, the Little Akaki River was relatively low compared with the perennial Mojo. These differences may be related to sand mining activities and river sediments. Barrantes *et al.* (2025) and Radford *et al.*, (2024) reported that river sediments can act as a major sink for MPs, while sand mining can disturb these sediments, leading to the release, movement, and resuspension of MPs downstream.

In contrast, the relatively lower concentration observed in the seasonal river was attributed to intermittent flows only during the rainy season (approximately June to November), which exhibited the lowest MP concentration. This is consistent with the understanding that lower and intermittent river flow results in reduced transport capacity and lower MP loads (Zong *et al.*, 2025). However, variations in population density, waste disposal practices, and industrial activities may also contribute to these differences (Whitehead *et al.*, 2021).

The concentration of MPs in donkey feces showed a statistically significant variation among locations, indicating differences in environmental contamination across the study areas and supporting the role of donkeys as indicators of local plastic pollution. Similar observations have been reported by Sina *et al*. (2025), who found higher MP concentrations in non-selective feeders than in selective ones in Hong Kong. In the present study, free-grazing donkeys had higher MP concentrations than controlled donkeys, suggesting that the feeding system influences exposure, with controlled feeding potentially reducing MP ingestion. However, despite these differences in concentration, the prevalence of plastic ingestion remained uniformly high across locations, with no statistically significant variation. This lack of spatial difference, together with the absence of significant predictors, suggests that plastic pollution is widespread rather than confined to specific areas.

Microplastics were also detected in all human urine samples; however, no statistically significant associations were identified with age, sex, water source, or location. This suggests that exposure may be relatively homogeneous across the population. Similar findings have been reported in human biomonitoring studies, with MPs detected in biological samples such as placenta, blood, and stool, regardless of demographic variation (Ragusa *et al.*, 2021) and ( Leslie *et al.*, 2022). The absence of significant predictors in this study may indicate that exposure occurs through multiple pathways, including ingestion of contaminated food and water as well as inhalation of airborne particles (WHO, 2019). As a result, it becomes difficult to isolate specific risk factors.

The physical characteristics of the identified MPs, particularly the predominance of fibers and fragments, are consistent with global findings. Fibers are commonly associated with textile sources, while fragments result from the degradation of larger plastic materials such as packaging. Similar patterns have been reported in environmental and biological samples worldwide Tursi *et al.* (2022) and Zhang *et al. (*2022). The presence of smaller-sized particles in this study suggests advanced fragmentation processes, which increase the likelihood of ingestion and biological interaction. This is of concern, as smaller particles have a greater potential to penetrate biological tissues and may pose higher toxicological risks (WHO, 2019). Most of the transparent MP particles are likely derived from common plastic materials, especially single-use items such as plastic bags, cups, and bottles, which have short service lives. The sample digestion process may also have caused some particles to lose their original color and appear transparent (Esubalew *et al*., 2026). This may also result from prolonged exposure to UV light or abrasion against sediments (Lora *et* *al.*, 2024).

In this study, a comparable prevalence of plastic ingestion was observed in both Addis Ababa and Bishoftu, suggesting that plastic pollution is widespread and not confined to specific localized environments. The lack of association between plastic ingestion and injury/disease or stress indicator ($p>0.05$) is consistent with (Terefe *et al.,* 2019), which states that colic cases due to foreign bodies cause few clinical cases, suggesting that many donkeys ingest plastic sub-clinically without immediately detectable illness. Ashinde *et al*. (2017) reported that poor body condition was associated with wound prevalence, but not ingestion behavior.

Another study conducted in Ethiopian abattoirs by Dubie *et al*. (2026) reported a relationship between foreign body ingestion and poor body condition and found that cattle with poor BCS had the highest prevalence of foreign bodies (38.1%) at the Kombolcha ELFORA Abattoir, with a statistically significant association ($p = 0.022$). In a similar study, Tenaw (2025) found that cattle with medium body condition had a higher prevalence (50%) of foreign bodies compared to those with good body condition (12%) at the Debremarkos Municipal Abattoir, and this difference was statistically significant ($p < 0.001$).

Overall, 69% of respondents demonstrated good knowledge related to proper plastic waste management. Plastic bags were identified as the most commonly used type of plastic (57%). Similarly, in the present study, the most common plastic type was plastic bags (57.3%), followed by bottles (35.5%) and food packaging (7.3%). In the previous study conducted in Kenya by Haddy *et al.* (2025), livestock owners had better knowledge of animal health risks than human health risks, because the consequences of plastic ingestion in animals are more immediately visible than in humans; the health effects in humans may be chronic. This study is consistent with the present study, which showed that participants had better knowledge of plastic risks in animal health (56.4%), followed by environmental health (45.5%), and human health (32.7%).

Currently, the Ethiopian government's ban on thin plastic bags (Proclamation No. 513/1999; recently updated in Proclamation No. 1383/2025) has led respondents to use alternative bags, such as non-woven cloth (80%) and paper bags (20%); plastic bags remain the most commonly used plastic type (57.3% of respondents). The continued high prevalence of plastic bag use indicates incomplete policy implementation and enforcement.

These findings agree with Aragaw (2025), who reported that compliance with the plastic bag ban remains low due to several factors, including limited access to affordable alternatives, weak enforcement, low public awareness, and the continued use of plastic bags in everyday shopping. In addition, Aragaw  (2021) indicated that poor waste management practices and weak enforcement of regulations have contributed to the current levels of MP pollution in water systems across African countries.  A study conducted by Hussein *et al.* (2021) in Gode town stated that the main challenges of plastic pollution and environmental and health risks are poor municipal management, limited resources, and the absence of legislation. Similarly, Ogato *et al.* (2026) Conducted a study at Bole Sub-City, Addis Ababa, where the most common challenges to developing sustainable waste management are a lack of regular collection service, limited resources for waste collection, and a lack of awareness of plastic waste collection.

The retrospective analysis of 223 colic cases (116 from Merkato, 107 from Bishoftu) revealed that plastic-related colic was the predominant diagnosis, accounting for 95.7% of cases. This finding aligns with veterinary studies from Ethiopia and other LMICs, where free-roaming cattle have unrestricted access to plastic-contaminated environments. Similarly, Nugusu *et al*. (2013) reported that recurrent bloat (34.8%), suspended rumination (17.4%), and distended abdomen (21.7%) were common clinical symptoms in ruminants with foreign body ingestion in Gondar town.

The marked difference in mortality rates between Merkato (23.5%) and Bishoftu (56.1%) can be attributed to several interrelated factors. Although surgical intervention (rumenotomy) is available in both locations, differences may exist in the timeliness of presentation, case severity at admission, and overall perioperative management. Donkeys in Merkato may be presented earlier for treatment, for more favorable surgical outcomes, whereas delayed presentation in Bishoftu could increase the likelihood of complications and mortality.

Evidence from Nugusu *et al.* (2013) supports the variability in surgical outcomes, reporting that 66.7% of animals recovered successfully, 11.1% developed postoperative complications, and

22.2% died following treatment for foreign body ingestion in Gondar. Furthermore, the lower mortality rate observed in Merkato may reflect better owner awareness and improved husbandry practices. Donkey owners in Merkato are more likely to provide feed in clean conditions using troughs, reducing exposure to plastic and other foreign materials. In addition, donkeys are more often kept under controlled management systems rather than being allowed to roam freely, thereby minimizing the risk of ingesting indigestible waste. In contrast, less controlled management and lower awareness in Bishoftu may contribute to increased exposure and delayed intervention, ultimately leading to higher mortality.

Differences in treatment protocols: While both clinics used Flunixin + Paraffin oil as primary treatment, the availability of surgical intervention (rumenotomy) for severe impactions may differ between facilities. The logistic regression analysis indicates that there were no significant predictors of treatment outcome, including age, sex, BCS, or plastic risk classification. This null finding suggests that once a donkey presents with colic, survival may depend on factors not captured in the retrospective records, particularly timeliness of presentation, quality of veterinary care, and severity of impaction at the time of treatment, rather than on patient demographics.

This study provides useful information on MP pollution within a one-health framework that considers human, animal, and environmental health. However, certain limitations may exist. First, using a 63 µm mesh sieve to filter river water during sample collection excludes smaller MP particles. Second, the study relied on microscopic identification and physical verification methods rather than polymer-specific spectroscopic confirmation. While this approach is widely used for preliminary detection and characterization of microplastics, incorporating FTIR or Raman Spectroscopy in future studies would further improve the accuracy of polymer identification. Third, the cross-sectional design cannot establish causality between MP exposure and health outcomes; these gaps warrant further research.

# 6. CONCLUSION AND RECOMMENDATION

The findings of this study highlight the growing significance of plastic pollution as an integrated environmental, animal, and public health concern within the One Health framework in central Ethiopia. MPs were detected in river water, donkey feces, and human urine samples across all sampling sites, with significant spatial variation. The predominant shape of MP particles across the three sample types was fiber, and the size was particles < 500 µm. Community awareness was moderate but did not translate into reduced plastic use despite the national ban. This study highlights the need for interconnected interventions in environmental contamination, animal management, and human behavior through a One Health framework. Based on the above concluding remarks, the following recommendations are proposed;

- Strengthen plastic waste management and enforcement of plastic bans (Proclamation No. 513/1999; recently updated in Proclamation No. 1383/2025) to reduce environmental contamination.
- Improve community awareness to promote controlled feeding systems for working donkeys and human health risks.
- Improve the accessibility of alternative materials such as cloth bags and paper bags
- Enhance One Health collaboration and further research on microplastic exposure and impacts

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# 8. APPENDICES

**Annex 1:** Verbal informed consent form

Study Title: Assessing microplastic pollution and determinants in donkeys, humans, and environmental health in central Ethiopia.

Principal Investigator: Masresha Gebeyehu, MSc Student (One Health)

Introduction. Good morning/afternoon. My name is ____________________. I am a data collector working on behalf of the principal investigator from Addis Ababa University. You are invited to participate in this research study. The purpose of this study is to understand how plastic pollution affects humans, donkeys, and the environment. The findings will support public health, environmental protection, and animal welfare efforts in Ethiopia. You are invited because you meet the study criteria. Your participation is not related to the services you receive, and refusal will not affect your care. If you agree, you will be asked a few questions, and a small amount of urine will be collected using sterile procedures for the analysis of MP. The process will take about 15–20 minutes. All information will be kept confidential. Your name will not be recorded. Do you voluntarily agree to participate in this study? ☐ Yes, I agree ☐ No, I do not agree

Date: ____________

Time: ____________

**Annex 2:** Structured Questionnaire Survey

**PART 1: Questionnaire for Donkey Owners**

**A. Socio-Demographic Information**

1. Age: ______
2. Sex: ☐ Male ☐ Female
3. Education level ☐ illiterate ☐ primary ☐ secondary ☐ higher
4. Occupation __________________
5. Location (Kebele) _________________

**B: Donkey Management Practices**

6. Main purpose ☐ transport ☐ agriculture ☐ other
7. Feeding system ☐ free-grazing ☐ control feeding ☐ both

8. Where is your donkey fed?
   ☐open fields ☐near the dump site ☐near the dump site ☐ roadside

**C: Exposure to Plastic Waste**

10. Are there any plastic waste materials in donkey grazing areas? Donkey's grazing area?
    ☐ Yes ☐ No
11. What types of plastic materials are commonly used? ☐ plastic bags ☐ bottles
    ☐ food packaging ☐ other
12. Have you ever seen your donkeys eat plastic materials? ☐ Yes ☐ No ☐ Not sure

**D: Donkey Health Conditions**

14. Do your donkeys show any plastic-related symptoms? ☐loss of weight ☐ appetite loss ☐ abdominal pain ☐ sudden death
15. Do your donkeys visit a veterinary clinic? ☐ Yes ☐ No

**E: Awareness and Practices**

18. Is there any awareness that plastic waste harms animals? ☐ Yes ☐ No
19. How is the plastic waste disposed of? ☐ open dumping ☐ burning ☐ other ______
20. What solutions do you suggest? __________________

**Part Two: COMMUNITY OWNERS QUESTIONNAIRE**

**Title:** Assessment of Plastic Pollution and Its One Health Impacts (Human, Animal, Environment) in Central Ethiopia

**A: Demographic Information**

1. Age: ______
2. Sex: ☐ Male ☐ Female
3. Education level: ☐ illiterate ☐ Primary ☐ Secondary ☐ Higher
4. Occupation: __________________
5. Marital status: ☐ Single ☐ Married ☐ Other
6. Household size: ______
7. Residence (Kebele): __________________

**B: Plastic Use and Disposal Practices**

8. Are there any plastic materials used in daily life? ☐ Yes ☐ No

9. Which plastic types are commonly used? ☐ Plastic bags ☐ Bottles ☐ Food packaging
10. Which methods did you use to dispose of plastic waste? ☐ open dumping ☐ burning ☐ municipal collection ☐ other: ______

## C: Environmental Pollution Awareness

12. Are there any plastic materials in your environment that you observe? ☐ Yes ☐ No
13. Which parts are mostly polluted by plastic waste?
    ☐ water system (lakes and rivers) ☐ Roads ☐ agricultural areas ☐ Market areas
14. Does plastic pollution affect environmental health? ☐ yes ☐ no ☐ not sure
15. If yes, how does it affect the environment? (multiple answers) ☐ contaminate the water system ☐ soil pollution ☐ block the ditch

## D: Effects on Human Health

16. Does plastic affect human health? ☐ Yes ☐ No ☐ Not sure
17. If yes, what health problems can result? (multiple answers) ☐ contaminated drinking water ☐ food contamination ☐ respiratory problem ☐ chemical exposure
18. Which purpose is used by river waters? ☐ Drinking ☐ Washing ☐ Irrigation ☐ Livestock

## E: Effects on Animal Health

19. Do animals eat plastic waste you observe? ☐ Yes ☐ No
20. Which type of species are mostly affected by plastic waste? ☐ Donkeys ☐ Cattle ☐ Goats ☐ Others
21. What signs are observed in animals after eating plastic waste? ☐ loss of weight ☐ digestive problems ☐ weakness ☐ death ☐ other: ______

## F: One Health Awareness (Integration)

23. Are humans, animals, and environmental health related? ☐ Yes ☐ No
24. Is plastic pollution interconnected to humans, animals, and environmental health? ☐ Yes ☐ No ☐ Not sure

## G: Awareness of Plastic Ban

25. Is the government currently banning plastics? ☐ Yes ☐ No
26. Which types of plastic are currently banned? ☐ thin plastic bag ☐ all plastics ☐ not sure

27. By what means is the ban heard? ☐ TV/Radio ☐ social media ☐ government campaign ☐ neighborhood

**H: Compliance with Plastic Ban**

28. Are used plastics allowed after the ban? ☐ Yes ☐ No
29. Why is plastic still used after the ban? ☐ easily available ☐ cheap ☐ no alternative ☐ weak enforcement
30. Is the plastic bag still available in the shop? ☐ Yes ☐ No

**I: Enforcement and Challenges**

32. Is plastic effectively banned in your area? ☐ Yes ☐ No ☐ Not sure

**J: Alternatives and Solutions**

34. Is an alternative to plastic used? ☐ Yes ☐ No
35. If yes, what type of alternative did you use? ☐ non-woven cloth ☐ paper bag ☐ baskets ☐ other
36. Is the ban supported by you? ☐ Yes ☐ No ☐ Not sure
37. Which solutions are better to ban effectively? ☐ create awareness campaign ☐ strong law enforcement ☐ improved waste management ☐ increase availability of alternatives

**Annex 3:** Laboratory Procedures for Sample Processing and Microplastic Extraction

**A. Processing of Human Urine Samples**

1. **Sample Preparation**
    - Collected urine samples were transferred into clean glass beakers.
    - The volume of each sample was recorded.
2. **Digestion of Organic Matter**
    - 10% KOH was added to the sample in a ratio of approximately 1:1.
    - The mixture was heated at 44°C for 5 days to digest organic materials.
3. **Filtration**
    - The digested sample was filtered using vacuum filtration using a cellulose nitrate filter membrane (0.45 µm)
4. **Drying**

- Filters were carefully removed and placed in covered Petri dishes.
- Samples were air-dried at room temperature.

5. **Storage**
    - Dried filters were stored in sealed Petri dishes for further microscopic analysis.

**B. Processing of Donkey Feces Samples**

1. **Sample Preparation**
    - Fecal samples were air-dried and weighed.
    - Large debris, such as stones and plant material, was removed manually.
2. **Digestion**
    - Approximately 10 g of dried fecal material was placed in a glass beaker.
    - Fenton reagents and 10% KOH were added to digest organic matter.
    - The mixture was heated at 44°C until complete digestion was achieved
3. **Density Separation**
    - A saturated sodium chloride (NaCl) solution was added to the digested sample.
    - The mixture was stirred and allowed to settle for 12–24 hours.
    - Floating (supernatant) was carefully decanted.
4. **Filtration**
    - The supernatant was filtered using vacuum filtration through filter paper.
5. **Drying and Storage**
    - Filters were dried in covered Petri dishes at room temperature.
    - Samples were stored for subsequent identification and characterization.

**C. Processing of River Water Samples**

1. **Sample Preparation**
    - Water samples were thoroughly mixed before processing.
    - 10 L of water was collected for each sample.
2. **Digestion**
    - For samples containing visible organic matter, 30% $H_2O_2$ was added, followed by 10%KOH.
    - The mixture was heated at 44°C for 24 h with $H_2O_2$ and 5 days with KOH.
3. **Density separation**
    - A saturated sodium chloride (NaCl) solution was added to the digested sample.

   - The mixture was stirred and allowed to settle for 12–24 hours.
   - Floating (supernatant) was carefully decanted.
4. **Filtration**
   - Samples were filtered directly using a vacuum filtration system with a 0.45 µm cellulose nitrate filter membrane.
5. **Drying**
   - Filters were transferred to Petri dishes and dried inside a closed desiccator.
6. **Storage**
   - Dried filters were sealed and stored until analysis.

**D. Quality Control and Assurance**

- Procedural blanks were processed alongside samples to detect contamination.
- All experiments were conducted in triplicate where applicable.
- Laboratory exposure was minimized during filtration and drying steps by performing them inside the fume hood.

**E. Preparation for Microscopic Analysis**

- Dried filters were examined under a stereomicroscope.
- Suspected microplastics were identified based on shape, color, and texture.

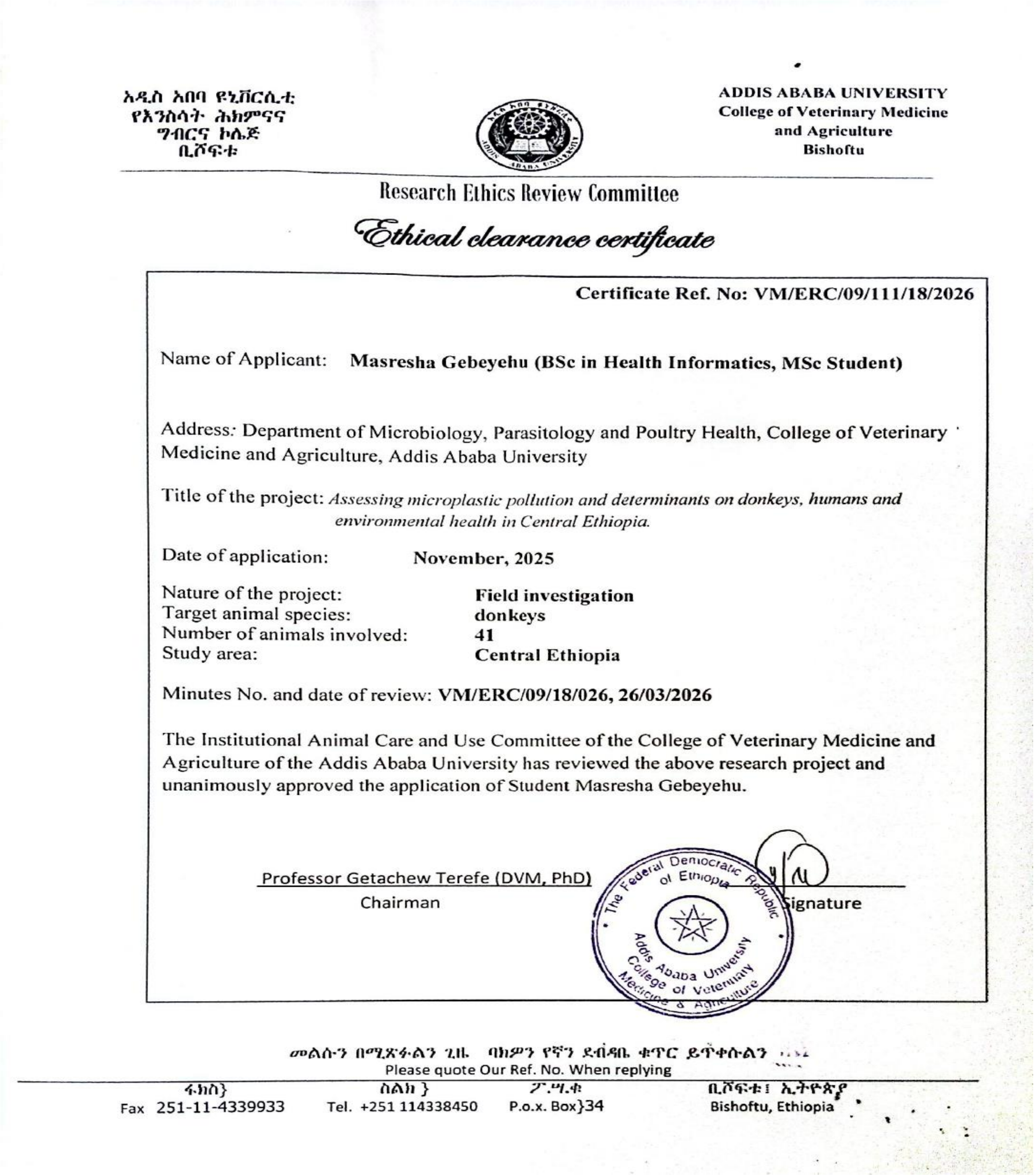
**Annex 4:** Laboratory materials (chemicals and instruments/apparatus)

| No. | Chemicals | Usage |
|---|---|---|
| 1 | Hydrogen peroxide (H2O2) | Digestion process |
| 2 | Iron (II) sulfate (Fe2SO4.7H2O) | Digestion process |
| 3 | Distilled water | Cleaning the apparatus |
| 4 | Ethanol | Pretreatment |
| 5 | Sodium chloride(NaCl) | Density separation |
| 6 | Potassium Hydroxide(KOH) | Digestion process |

**Annex 5**: Materials/apparatus and software to be used throughout the sample collection and analysis

| No. | Apparatus |
|---|---|
| 1. | Stainless steel bucket |
| 2. | Stainless steel sieve |
| 3. | Aluminum foil |
| 4. | Amber glass bottle |
| 5. | Glass flask |
| 6. | Glass petri dish |
| 7. | Cellulose nitrate filter membrane |
| 8. | Vacuum pump |
| 9. | Desiccator |
| 10. | Separatory funnel |
| 11. | Forceps |
| 12. | Stereomicroscope with digital camera |
| 13. | Toupview digital camera |
| 14. | ImageJ software |
| 15. | Glass beaker |
| 16. | Wash bottle |
| 17. | Icebox with an ice pack |

**Annex 6:** Ethical statement for animal-based research activities



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College of Veterinary Medicine
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Research Ethics Review Committee

*Ethical clearance certificate*

**Certificate Ref. No: VM/ERC/09/111/18/2026**

Name of Applicant: **Masresha Gebeyehu (BSc in Health Informatics, MSc Student)**

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Assessing microplastic pollution and determinants on donkeys, humans and environmental health in Central Ethiopia.*

Date of application: **November, 2025**

| | |
|---|---|
| Nature of the project: | **Field investigation** |
| Target animal species: | **donkeys** |
| Number of animals involved: | **41** |
| Study area: | **Central Ethiopia** |

Minutes No. and date of review: **VM/ERC/09/18/026, 26/03/2026**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Student Masresha Gebeyehu.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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Bishoftu, Ethiopia

**Annex 7:** Ethical statement for human-based research activities

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**Addis Ababa University**
**Aklilu Lemma Institute of Health Research**

**Ethical Clearance Certificate**

**Ref. No.:** ALIHR IRERC-010/2018/2026

**Date**: June 9, 2026

**Title:** [Assessing Microplastic Pollution and Determinants on Donkeys, Humans, and Environmental Health in Central Ethiopia]

**Principal Investigator**: Masresha Gebeyehu

**Recommendation by the ALIHR -IRERC**

Dear: Masresha,

The ALIHR-IRERC has reviewed your above mentioned research proposal and noted its merit. The IRERC would like to remind you, as PI, to submit progress reports of the work every 6 months and the final report upon completion of the study. Furthermore, you are expected to notify the ALIHR-IRERC ahead of time for any amendment or modification in the protocol or premature suspension or termination of the study.

**STATUS:** Approved

**National Ethics Review Board Clearance:** not required

IRERC Chairperson: Nigatu Kebede, Prof.
Signature: ____________

IRERC Secretary: Abebe Animut, PhD
Signature: ____________

**Approved by**
Name: Prof. Wakgari Deressa, Director, ALIHR
Signature: ____________
Date: ____________

CC:
- IRERC Office
- CPR Office
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## **Annex 8:** Images of site visit, clinic visit, sample processing, and analysis

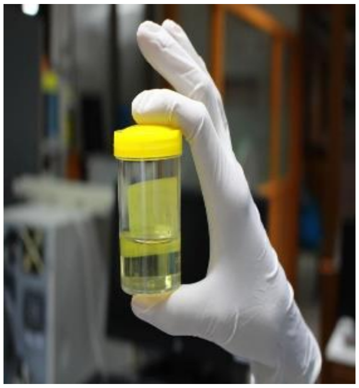
Water sampling sites

Focal observation of donkeys' foraging behavior in Merkato green market

Rectal removal of foreign body in a donkey following paraffin administration

Clinical signs of a donkey after eating foreign materials

Water Sample collection



urine samples

drying of feces sample inside a fume hood

Digestion of samples by hydrogen peroxide followed by KOH

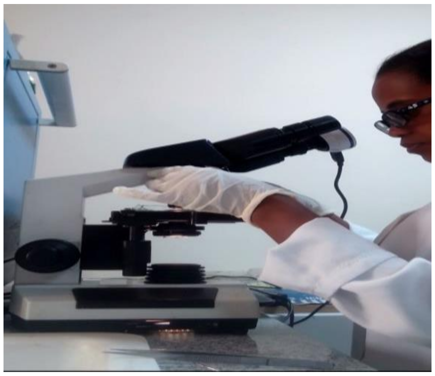
Separatory funnel

filtration system

samples inside a clean petri dish

Sample processing

Sample drying inside a desiccator



Microscopic examination