Assessment of Occurrence and Fate of Microplastic Contaminants in Animal Manure

by

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A REPORT

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ABSTRACT

Animal manure commonly applied as organic soil amendments in crop cultivation is a beneficial by-product of animal husbandry. The application of animal manure could improve the properties and processes of agricultural soil while supplying essential plant nutrients. However, the application of low-quality manure that contains pollutants including microplastics (MPs) can be entered into agricultural soil. This results in the degradation of the quality of the agroecosystem and causes a risk to human health since MPs can move via food chains. However, there is no scientifically proven evidence for the level of MP contamination in agroecosystems from animal manure in Sri Lanka. Therefore, this study was to assess the level of MP contamination quantitatively and qualitatively in four types of animal manure including cattle, poultry, swine, and goat. Animal manure samples were collected from three segments in the manure value chain: Animal farms (source), manure sales centers (intermediary points), and agricultural farm fields (end users). Microplastic extraction was done by digesting the manure using a dual digestion protocol (Fenton's reagent and 69% HNO_3 digestion) and density separation with NaCl solution. Six measurements were obtained from the extracted MP samples including count, mass, colour, shape, total area, and total length. It was revealed that all types of manure available at sources are contaminated with MPs (142 – 12,468 particles/kg or 11 – 1,532 mg/kg). Moreover, among the studied manure, swine manure showed the highest contamination level up to 1,160 ± 125 mg/kg while goat manure remained at the lowest contamination (23.5 ± 7 mg/kg) at the source. It was also found that transparent (59.2%) and white (33.7%) particles were prominent colours. Fragments (54.4%) and fibers (24.6%) were the dominant shapes indicating that ingestion of plastics through animal feed and secondary contamination throughout the market value chain are the dominant contamination processes. The amount of MP contamination increased along the manure market value chain (311% increase in Poultry and 17% increase in cattle) confirming that not only the origin of the manure but also secondary contamination along the value chain accounted for the MP contamination.

Keywords: Agroecosystems, Contaminants, Manure, Microplastic, Value chain

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LIST OF ABBREVIATIONS

DOA: Department of Agriculture

LDPE: Low-Density Polyethylene

MNP/s: Micro and Nano Plastic/s

MP/s: Microplastic/s

OM: Organic Matter

PA6: Polyamide-6

PA66: Polyamide-66

PE: Polyethylene

PET: Polyethylene Terephthalate

PHA: Polyhydroxyalkanoates

PLA: Polylactic Acid

POPs: Persistent Organic Pollutants

PP: Polypropylene

PS: Polystyrene

PVC: Polyvinyl Chloride

SC: Supply Chain

SDGs: sustainable Development Goals

SLSI: Sri Lanka Standards Institute

1 INTRODUCTION

1.1 Background

Consumer awareness of environmental concerns significantly influences the demand for organic foods that align with eco-friendly farming practices. This shift highlights a global commitment to sustainability and environmental stewardship. The organic food market is projected to reach approximately $412,927.7 million by 2027, with a compound annual growth rate of 13.9% (Roy *et al*, 2024). Moreover, the government's development policy in Sri Lanka between 2010 and 2015 aimed to reduce synthetic fertilizer imports by 15% by promoting organic fertilizers (Dandeniya and Caucci, 2020).

Applying organic fertilizers derived from animal manure presents a sustainable alternative to chemical fertilizers, improving agricultural productivity and lowering farmers' costs. Beyond the economic benefits, this practice enhances soil quality, leading to better crop yields and supporting sustainable development goals (SDGs) through environmentally friendly farming (Patunah and Pradani, 2024). However, animal manure is also a source of soil contaminants, including antibiotics resistance genes, pathogens, and emerging pollutants such as microplastics (MPs), which originate from contaminated feed and plastic residues (Sheriff *et al*, 2023).

Applying MP-contaminated manure contributes to their accumulation in the soil, where they adsorb and transport organic and inorganic pollutants. This process degrades soil quality and poses significant risks to ecosystems and human health (Sheriff *et al*, 2023).

1.2 Problem Statement

What is the level of contamination, sources, pathways, and fate of MPs in animal manure at different stages of manure value chain?

1.3 Problem Justification

Microplastics are ubiquitous environmental contaminants, extensively studied in terrestrial and aquatic ecosystems worldwide due to their persistence and adverse impacts on soil health, biodiversity, and food security. Global studies have investigated various sources and pathways of MPs in ecological systems, including agricultural inputs. As an organic soil amendment, MP contaminants in animal manure were studied in different parts of the world (Sheriff *et al*, 2023; Zhang *et al*, 2022c; Zhang *et al*, 2022d). However, research on MPs in Sri Lanka has largely focused on other environmental compartments. For instance, studies have explored MPs in marine environments (Koongolla *et al*, 2021), riverine systems (Muhandiram *et al*, 2024), agricultural soils (Karthika *et al*, 2024), and compost (Ranasingha *et al*, 2024). Therefore, despite its widespread use as a soil amendment, no systematic investigations have been conducted on MPs in animal manure in Sri Lanka. This concerns animal manure, is often contaminated by plastics from feed, bedding materials, and packaging (Lackner *et al*, 2024). This could be a significant and overlooked pathway for MPs to enter Sri Lanka's agricultural soils.

Animal manure as an organic fertilizer is highly promoted in Sri Lanka, with the Department of Agriculture (DOA) recommending application rates of 10 tons per hectare per season for cow dung and 5 tons per hectare per season for poultry manure for vegetable farming (Makinde *et al*, 2009). Nevertheless, application rates of animal manure as organic soil amendment often exceed these recommendations, increasing the likelihood of MP accumulation in soils (Kahandage *et al*, 2023). Even though animal manure is used as an organic soil amendment in Sri Lanka, it cannot be guaranteed to be completely organic, since no scientific studies have investigated the extent of plastic contamination in manure, and its sources.

1.4 Objectives

1.4.1 General Objective

1. To quantitatively and qualitatively assess the microplastic contamination in animal manure available in Sri Lankan animal manure value chain

1.4.2 Specific Objectives

1. To identify various stages of poultry, cattle, swine, and goat manure value chain.
2. To develop an appropriate technique to quantify microplastic in animal manure samples
3. To quantify the level of microplastic contaminants in animal manure
4. To characterize the microplastic contaminants in manure
5. To assess the sources and fate of microplastic in manure

2 REVIEW OF LITERATURE

2.1 Plastics in the Environment

Plastic can be defined as a material that consists of synthetic or semi-synthetic organic compounds that are flexible and can be molded into solid objects. It has been estimated that the majority of the produced plastics (>80%) are thermoplastics. They are industrialized via polymerization to form high-molecular-weight polymers from low-molecular-weight monomers (de Souza Machado *et al*, 2018). Since plastics have special characteristics like versatility, durability, and low production costs, modern society highly relies on them. The cumulative production of plastics has exceeded 8 billion metric tons worldwide since the 1950s (Mazhandu *et al*, 2020). In 2023 alone, global plastic production reached approximately 413.8 million metric tons (Statista Research Department, 2024). This continuous increase in production reflects the growing demand for plastics and its environmental impacts (Adekanmbi *et al*, 2024).

Plastic waste possesses long-term environmental impacts on earth due to its low degradability (Pilapitiya & Ratnayake, 2024). Marine ecosystems are particularly vulnerable, as around 1 to 2 million tons of plastic waste (about 0.5% of total annual plastic waste), enter the oceans annually. This pollution disrupts marine life, contaminates food chains, and contributes to MP pollution (Ritchie *et al*, 2023). On the other hand, the accumulation of plastic waste in the terrestrial environment changes biodiversity on the land and soil composition (Paul *et al*, 2024). Furthermore, toxic chemicals emitted from inappropriate plastic waste disposal and open burning increase environmental and public health hazards (Velis & Cook, 2021).

2.2 Plastics in Agriculture and Their Impacts

The agricultural industry depends heavily on plastic materials to support plant and animal production. Isakov *et al* (2024) reported that agriculture accounts for a significant share of total annual global plastic usage, approximately 12.5 million metric tons. This trend is expected to expand more, with global demand for greenhouse films, mulching materials, and silage wraps projected to increase from 6.1 million tons in 2018 to 8 million tons by 2030 (Briassoulis, 2023). In particular, the agricultural plastic film usage in China in 2017 was 2,528,600 tons. However, despite their benefits, it highlights that extensive reliance on agricultural plastics could contribute to pollution and environmental concerns (Jia *et al*, 2023).

While plastics have enhanced agricultural efficiency, their environmental consequences aren't well understood. The widespread use of non-biodegradable plastics, such as Low-Density Polyethylene (LDPE), has led to significant soil and water contamination. Recycling these plastics is often expensive and labor-intensive, resulting in their accumulation in fields and landfills (Kyrikou & Briassoulis, 2007). Over time, these materials degrade into MPs, which are retained in soils and can adversely affect soil health and ecosystem functions (Qi *et al*, 2020).

2.3 Shift towards Biodegradable Plastics

Biodegradable plastics have been developed as alternatives to conventional plastics to address environmental concerns, particularly in agriculture. These materials are designed to decompose under natural conditions. It reduces the need for removal and minimizes long-term pollution risks (Brodhagen *et al*, 2015). Many modern biodegradable films are derived from renewable resources, such as polylactic acid (PLA) and polyhydroxyalkanoates (PHA). These also have demonstrated similar performance to conventional plastics in maintaining crop yield and soil moisture retention (Bandopadhyay *et al*, 2020).

However, the degradation of biodegradable plastics depends on specific environmental factors, including temperature, microbial activity, and moisture levels. In the absence of optimal conditions and proper disposal systems, these materials may persist in the environment, reducing their intended benefits (Niu *et al*, 2024). Biodegradable MPs exhibit lower aquatic eco-toxicity than conventional plastics. In contrast, their production and degradation result in higher greenhouse gas emissions, presenting a trade-off between reduced environmental pollution and increased carbon footprint (L *et al*, 2024).

Alternative biodegradable solutions such as cellulose-based mulches are being explored to address these limitations. These materials decompose more efficiently in natural environments while enhancing soil health, retaining nutrients, and suppressing weed growth (Riseh, 2024).

2.4 Microplastics

2.4.1 Plastic Degradation and Microplastic Creation

Plastic waste, including biodegradable plastics, is more susceptible to physical disintegration (fragmentation) than degradation (mineralization), which results in smaller-sized particles of plastics. Therefore, a large number of residual plastics in the open environment gradually break up and degrade, through a series of physical, chemical, and microbial processes. These processes continuously form a new type of pollutant called MPs (Gündogdu *et al*, 2023).

2.4.2 Definitions and Characteristics of Microplastics

Microplastics are defined as synthetic polymers ranging from 1 µm to 5 mm in size (Wijesooriya *et al*, 2023). These hydrophobic particles may have regular or irregular shapes. Based on their origin, MP particles can be categorized into primary and secondary. Primary microplastics are intentionally manufactured small-sized particles with microscopic dimensions (e.g. cosmetics, or toothpaste). Secondary microplastics are particles that result from the abrasion, degradation, and fragmentation processes of plastic materials (Čurlej *et al*, 2023).

MPs can be categorized based on their physical properties such as fibers, fragments, spheres, pellets, films, and foams (Issac & Kandasubramanian, 2021). Microplastic fibers are thin and flexible, have a uniform thickness along their length. Fragments are uneven in shape, range from round to angular. The spheres are round and have smooth surfaces. Pellets (nurdles) range in size from 3 to 5 mm and larger than spheres. Films are flat, thin, and flexible, often partially or entirely transparent. Foams are soft, compressible cloudlike structures typically white or opaque, but can also be colored (Rochman *et al*, 2019).

Microplastics are also categorized based on their polymer composition. Most common polymer types are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS). Therefore, according to their composition, physical and chemical properties and their distribution in the environment is changed. For instance, low-density MPs often float on water surfaces, while high-density particles can sink to sediments. Furthermore, unique physical characteristics of MPs, such as high surface area and chemical stability, allow them to persist in the environment for extended periods. Therefore, the degradation times of MPs ranging from decades to centuries. Additionally, their small size enables them to pass through filtration systems and bio-accumulate in living organisms (Li *et al*, 2020).

2.5 Impacts of Microplastics

2.5.1 Impacts on Soil Properties

Plastic residues, including MPs, are a threat to the soil ecosystem. Micro and Nano-plastics (MNPs) can potentially disrupt the finely balanced soil ecosystem (Boots *et al*, 2019). Due to its larger surface area, the soil has considerable potential to retain and accumulate plastic particles (Nizzetto *et al*, 2016). Therefore, soil acts as a temporary or permanent sink for MPs, eventually reaching levels that can affect soil quality (Rillig, 2012). There is a potential for MPs to alter fundamental soil properties which can directly affect microbial communities in the soil (de Souza Machado *et al*, 2018). Native microbial communities, which are necessary for the breakdown of organic matter and the cycling of nutrients, may be impacted by changes in soil's physical and chemical properties (Boots *et al*, 2019). These effects are not from just MP concentrations (% of soil), since specific particle properties (linear vs nonlinear, size distributions, polymer type, etc.) seem to be a matter. However, a significant relationship was observed between the concentration of individual MP types and microbial activities regardless of particle type (de Souza Machado *et al*, 2018).

MPs can affect soil water dynamics as well. Hydrophobic MPs repel water, like PE and PP. It causes isolated dry areas in the soil. This increases drought stress and decreases the amount of water available to plant roots, particularly in areas with little irrigation. On the other hand, certain MPs might combine with soil organic matter to form aggregates, which would temporarily improve water retention but eventually change the natural soil-water balance (Li *et al*, 2024).

MPs have become a threat to the survival, growth, and reproduction of the soil microbial community. Soils retain a diverse range of MPs. Microplastics alter or destroy microbial habitats by releasing chemicals as MPs decompose. The microorganisms also contribute to plastic degradation, and they are exposed to the harmful substances that consist of the MPs. It causes mutations within the microbial community. Therefore, it affects the changes in the growth and reproduction behavior of microorganisms (Yang *et al*, 2024).

2.5.2 Impact on Plants

The existence of MPs may negatively impact plant growth and development in multiple ways. Microplastics directly act as an obstacle that affects plant root growth. Moreover, MPs decrease soil productivity indirectly and inhibit plant growth (Jia *et al*, 2023). Therefore, MP stress reduced biomass production, shoots and leaves growth (Jia *et al*, 2023), and photosynthesis in plants (Yang and Gao, 2022).

In proportion to their size, MPs can physically block the seeds' pores in different plants, leading to a consistent reduction in the germination rate (Boots *et al,* 2019) and it decreases water uptake and the imbibition process (Bosker *et al,* 2019). Certain MPs, like PS MPs, are hydrophobic and readily absorbed on the root surface. It inhibits root growth (Jia *et al*, 2023). Additionally, because MP stress causes pore blockage in the cell wall and the hetero aggregation of opposite charges, it decreases nutrient uptake (Xu *et al*, 2022).

MPs affect the nutrient dynamics of soil in two ways. They can, on the one hand, absorb essential nutrients such as potassium, phosphorus, and nitrogen, making them less bioavailable for plant uptake. However, MPs can also expose the root zone to harmful additives or contaminants that have been adsorbed, like heavy metals and persistent organic pollutants (POPs). This release of contaminants may cause nutrient absorption processes to be further disrupted by competition with nutrients (Rillig & Lehmann, 2020; Nizzetto *et al*, 2016).

Recent research has demonstrated that plants can absorb MPs, especially through their root systems. Nanoplastics, or smaller MPs (less than 1 μm), have a higher tendency to enter root tissues and move to plant aerial parts. MPs are introduced into the food chain by this uptake. This raises questions regarding food safety and human health. MPs have the ability to disrupt cellular functions such as photosynthesis and nutrient transport once they are inside plant tissues (Li *et al*, 2020).

2.5.3 Impact on Human Health

Ingestion, inhalation, and dermal exposure are the common pathways for MP exposure in humans. There is evidence that MP exposure has a variety of detrimental effects on human health. Numerous studies indicate that MP may cause cellular impairment, inflammation, and toxicity in human cells (Ghosh, 2023). Furthermore, there may be a connection between exposure to MP and a higher risk of diabetes (Diamant, 2022).

2.5.4 Impacts on Animals

As a form of MPs, their size and interactions with soil fauna can also influence their impact on the soil ecosystem (Boots *et al*, 2019). MPs may significantly impact marine life (Porcino, 2022; Ghosh, 2023). When marine creatures like fish, sea turtles, and seabirds mistake MPs for food, it can seriously damage or even kill them (Roman, 2021). Additionally, MPs can disrupt marine animals' reproductive systems, which can decrease population size (Ghosh, 2023).

2.5.5 Positive Impacts

MP-induced stimulatory effects on seed germination have been reported. High concentrations of MPs in soil enhance wheat (*Triticum aestivum* L.) seed germination, whereas it was inhibited by low and medium concentrations. Additionally, due to exposure to Polyethylene terephthalate (PET) MPs, chickpea (*Cicer arietinum* L.) seed germination increased, as a result of MPs' priming effects on seed germination or their capacity to rupture seed coats and improve imbibition through improved water absorption through microscopic pores (Jia *et al*, 2023).

2.6 Sources of Microplastic Contaminants in Animal Manure

Plastic residues contaminated could be consumed by grazing animals, which could cause the plastic to break down in their digestive tract and eventually be expelled as manure (Beriot *et al*, 2021). Additionally, improper disposal of fertilizer and pesticide packaging is a major source of MP pollution. In 2018, fertilizer use in China generated 150,000 tons of plastic waste, primarily composed of PVC and PP, which can degrade into MPs and be ingested by farm animals (Li and Lu, 2021).

Additionally, animal feed contains MPs, which directly endanger poultry and cattle. The majority of the MPs consist of PP, PE, and PET that are contaminated in swine, chicken, and cattle feed (Wu *et al*, 2021; Xu *et al*, 2022b). The presence of polyamide-6 (PA6) and polyamide-66 (PA66) from nylon-lined feed bags has been connected to the contamination of poultry feed (Chen *et al*, 2023). Livestock may be exposed to even more plastic residues if they are fed expired packaged food (Cornelis *et al*, 2021). Additionally, the use of plastic farm equipment like water troughs, feed pipes, and plastic bowls increases exposure to MPs through wear and tears. Free-range livestock and poultry are at risk because they consume plastic particles from polluted food sources, especially from waste disposal places (Dong *et al*, 2023).

Livestock MPs contamination is made worse by open waste disposal. Animals that roam freely frequently consume food scraps and plastic waste, which can cause severe digestive problems or, in the worst situations, even death (Otsyina *et al*, 2018; Fasil, 2016; Anwar *et al*, 2013). A process known as "digestive fragmentation" may cause some ingested plastics to break up into smaller pieces inside of animals (Pérez-Guevara *et al*, 2021b). Furthermore, MPs present in fish muscles, gills, and intestines can be introduced into farm animals' diets through discarded fish parts (Selvam *et al*, 2021; Haque *et al*, 2023).

Once integrated into manure, MPs are not effectively decomposed by common management practices like composting or anaerobic digestion. Studies indicate that even after extended composting, a significant amount MPs remains intact. Consequently, when composted manure or slurry is applied as fertilizer, MPs are directly introduced into agricultural soils, leading to contamination (de Souza Machado *et al*, 2018). Therefore, MPs are pervasively contaminating the livestock and agricultural industries, endangering the health of both people and animals.

2.7 Environmental Fate of Microplastics

It is widely known that common routes for MP transport include soil, water, and air. Different densities of MPs show distinct transport behaviors. Lower-density MPs are more vulnerable to wind and surface runoff, which can cause them to enter surface water bodies and terrestrial systems. Higher-density MPs are more likely to stay in soil and be carried to deeper layers (Shamskhany and Karimpour, 2021). Since MPs are buoyant at the water's surface, they are frequently carried by water along rivers and into oceans by surface transport (advective transport) (Andrady, 2011).

Complex weathering processes such as photo-oxidation and biodegradation continuously alter the physicochemical characteristics of MPs, including size and surface charge. These factors make it challenging to predict their movement and final destination in freshwater environments due to their dynamic interactions with local conditions (Guo *et al*, 2024).

3 MATERIALS AND METHODOLOGY

3.1 Location of the Experiment

The experiment was conducted at the soil and water laboratory of the Department of Agricultural Engineering, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

3.2 Selection of Animal Species

Animal farming and the use of animal manure as inputs for agriculture have been practiced in Sri Lanka for centuries. The Department of Census and Statistics, Sri Lanka (2023) stated that the most commonly farmed animal species are poultry, cattle, swine, and goat. Therefore, manure from those four animal species was analyzed in this study.

3.3 Identification of Animal Manure Value Chains

The value chains were identified using field observation along the value chain using the snowball-sampling technique. Identification of each supply chain was started from the agricultural field (end user) where manure has been applied. Then according to feedback from the field study, agricultural inputs sales center (intermediary points) was identified from where manure has been sold to specific field. Finally, the animal farm (manure source) was identified from where manure has been sent those relevant sales centers. Accordingly, three supply chains were identified for each animal species and found that specific value chain is available only for cattle and Poultry manure. Goat and swine manure rarely flow through a market chain but through a direct link between producer and user. Therefore, samples of goat and swine manure were not collected from all possible segments of the value chain. However, samples were obtained from sources and end-user segments for all manure types.

3.4 Methodological Procedure

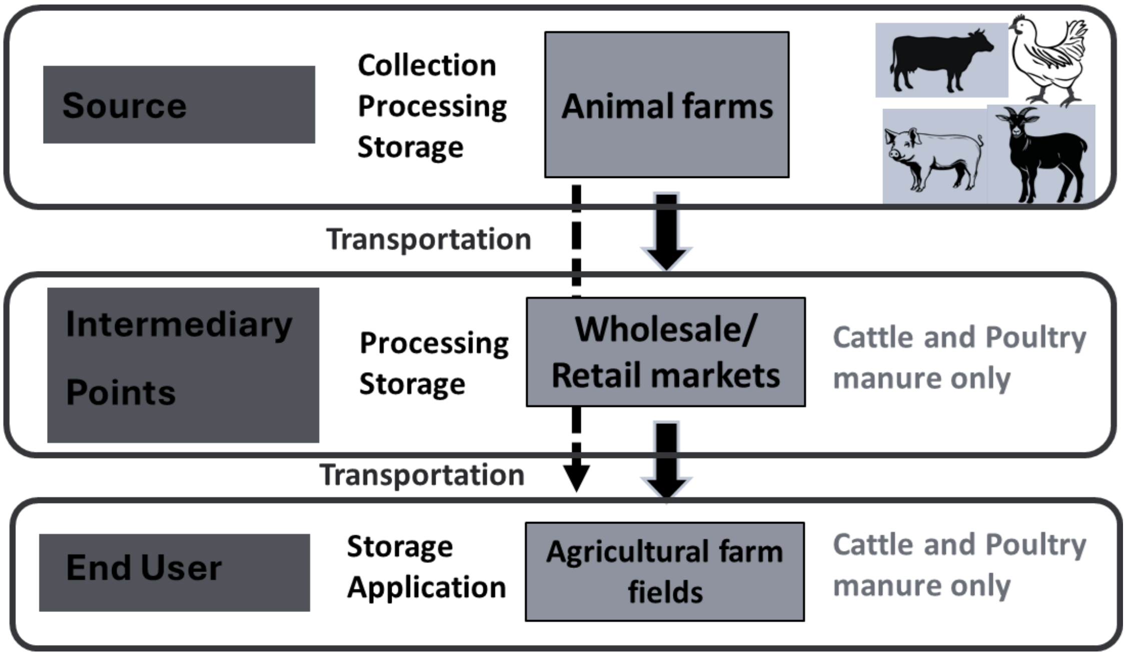

Figure 3.1 Animal manure sampling scheme

3.4.1 Identification of sampling Scheme and Animal Manure Sampling

In this study, manure samples were obtained from different stages of poultry, cattle, goat, and pig manure value chain. Animal manure samples were collected from three points in the manure value chain: animal farms (source), manure sales centers (intermediary points), and agricultural farm fields (end users). Cattle and poultry manure were sampled from three farms, three sales centers, and three agricultural fields (9 samples each). In contrast, swine and goat manure were collected only from three farms (3 samples each). Altogether twenty-four representative samples were taken from different locations, each containing ten kilograms according to the SLSI guidelines of compost sampling (SLS 1635:2019 and SLS 1634:2019).

From each of the above-mentioned manure samples, 100 g of sub-samples with three replicates were obtained to an aluminum foil and wrapped for further MPs identification. Accordingly, the total number of samples used in the study was seventy-two. Then they were packed into labeled paper bags separately. During sampling, all the required data (Initial Mass, Type of Packaging, Number of Animals in the Animal Farm, Management Practices used in Animal Farms/ Agricultural Field, Geographical

Coordinate of the location) were recorded at each sampling location. Then samples were brought to the laboratory for further processing and analysis.

Figure 3.2 Animal manure sampling according to the SLSI guidelines of compost sampling

3.4.1.1 Site Characteristics of Poultry Manure Sampling Locations

Poultry manure samples were collected from three poultry farms, three sales centers where poultry manure was stored for selling, and three agricultural fields where poultry manure was stored for application.

Table 3.1 Site characteristics of selected farms for poultry manure sampling

Farm	Location	Code	Description
Hettipola Farm	7.613194444, 80.181	PFH	Poly sack bags and plastic feeders have been used for feeding the poultry, while plastic waterers and plastic pipelines have been used for watering. The buildings have been covered with plastic shade nets on the side walls. Plastic egg trays have been used as part of the equipment for poultry management. Paddy husk bedding has been obtained and handled using poly sack bags. The manure age has been recorded as 14 weeks.

Farm	Location	Code	Description
Wariyapola Farm	7.608527778, 80.23272222	PFW	Feeding has been done using poly sack bags and plastic feeders, with plastic waterers and plastic pipelines having been used for watering. The buildings have been covered with plastic shade nets on the side walls. Equipment has included Plastic egg trays and rubber boots. Paddy husk bedding has been obtained and handled using poly sack bags. The manure age has been recorded as 26 weeks.
Mawelawatta Farm, Uda Peradeniya	7.252308, 80.609556	PFU	Poly sack bags and plastic feeders have been used for feeding, while plastic waterers and plastic pipelines have been used for watering. The buildings have been covered with plastic shade nets on the side walls. Equipment has included plastic egg trays and rubber boots. Paddy husk bedding has been obtained and handled using poly sack bags. The manure age has been recorded as 40 weeks.

Table 3.2 Site characteristics of selected sales centers for poultry manure sampling

Sales Center	Location	Code	Description
Seethaeliya Agro Product Center	6.933666667, 80.80636111	PMS	Polysack bags have been used for transportation and storage of manure. The storage period has been recorded as 8 weeks.
Nuwara Eliya (Mariyas Forage House)	6.976166667, 80.76527778	PMN	Polysack bags have been used for transportation and storage of manure.

Sales Center	Location	Code	Description
			The storage period has been recorded as 4 weeks.
Mawelawatta Farm Outlet	7.252361111, 80.60963889	PMU	Polysack bags have been used for transportation and storage of manure. The storage period has been recorded as 12 weeks.

Table 3.3 Site characteristics of selected agricultural fields for poultry manure sampling

Field	Location	Code	Description
Seethaeliya Vegetable Field	6.945083333, 80.79505556	PAS	The storage period has been recorded as 2 weeks. White-colored polythene has been used to cover the manure bags. Polysack bags have been used as a storage container. The present crop cultivated was leeks.
Nuwara Eliya Vegetable Field	6.966555556, 80.76613889	PAN	The storage period has been recorded as 2–3 weeks. Manure has been stored in a closed house. Polysack bags have been used as a storage container. The present crop cultivated was cabbage.
Mahakanda Home Garden	7.232222222, 80.60205556	PAM	The storage period has been recorded as 12 weeks. Manure has been stored in an open field. Polysack bags have been used as a storage container. The present crop cultivated was tomato.

3.4.1.2 Site Characteristics of Cattle Manure Sampling Locations

Cattle manure samples were collected from three cattle farms, three sales centers where cattle manure was stored for selling and three agricultural fields where cattle manure was stored for application.

Table 3.4 Site characteristics of selected farms for cattle manure sampling

Farm	Location	Code	Description
Hatton Farm	6.94327778, 80.7844444	CFH	Other than fresh forages, cattle feed has been given, which has been contained in poly sack bags. Watering has been done using Alkathine and PVC pipelines along with a plastic tank. The building has been constructed with concrete. Equipment has included a plastic milking machine and nylon ropes for tying cattle. Bedding has been made of concrete.
Nuwara Eliya Farm	6.9790412 , 80.7694891	CFN	Other than fresh forages, cattle feed has been given, which has been contained in poly sack bags. Water supply has been done using PVC pipelines and a plastic tank. The building has been made of a metal shed. Equipment has included nylon ropes for tying cattle. Bare land soil has been used as bedding.
Mawelawatta Farm, Uda Peradeniya	7.252596, 80.608596	CFU	Other than fresh forages, cattle feed has been given, which has been contained in poly sack bags. Water supply has been done using Alkathine and PVC pipelines along with a plastic tank. The building has been constructed with concrete. Equipment has included a plastic milking machine, rubber boots, ear tags, and a

Farm	Location	Code	Description
			plastic brush for floor cleaning. The bedding has been made of concrete.

Table 3.5 Site characteristics of selected sales centers for cattle manure sampling

Sales center	Location	Code	Description
Nuwara Eliya 1	6.93366667, 80.8063611	CMS	Transportation and storage have been done using a lorry without covering the manure. The storage period has been recorded as 2 weeks.
Nuwara Eliya 2	6.9768195, 80.7644483	CMN	Transportation and storage have been done using a lorry and the manure has been covered with a Tarpaulin sheet. The storage period has been recorded as 2 days.
Mawelawatta Farm Outlet	7.261858, 80.5960568	CMU	Storage has been done using transparent polythene bags in 2 kg packs. The storage period has been recorded as 4 weeks.

Table 3.6 Site characteristics of selected agricultural fields for cattle manure sampling

Field	Location	Code	Description
Seethaeliya Vegetable Field	6.94508333, 80.7950556	CAS	The cattle manure is stored by piling it up in an open field. The storage period has been recorded as two weeks. This manure has been used for leek cultivation.

Field	Location	Code	Description
Nuwara Eliya Vegetable Field	6.97837, 80.7661347	CAN	The manure has been piled up in an open field for a short period of two days. This manure has been used for potato cultivation.
Mahakanda Home Garden	7.229411, 80.603029	CAM	The cattle manure has been stored in polythene bags in an open field for one week. This manure has been used for flower cultivation.

3.4.1.3 Site Characteristics of Goat Manure Sampling Locations

Table 3.7 Site characteristics of selected farms for goat manure sampling

Farm	Location	Code	Description
Gampola Goat Farm	7.153972, 80.556833	GFG	Water has been supplied using plastic water buckets and a PVC pipeline. Fresh leaves have been provided on concrete for feeding. The shelter has been constructed with an asbestos sheet roof. The wooden bedding has been placed for the goats' comfort. Manure has been collected and cleaned weekly to maintain hygiene.
Goat Breeding Farm, Mathale	7.437083, 80.620333	GFM	Fresh leaves have been given as feed. Water has been made available through plastic water buckets and a PVC pipeline. The structure has been built with a metal sheet roof and wooden walls. Wooden bedding has been arranged for the goats. Manure has been collected and cleaned once every three months.

Farm	Location	Code	Description
Mawelawatta Farm, Uda Peradeniya	7.252361, 80.609639	GFU	Plastic buckets have been used to fill forages, and free ranging has been allowed for feeding. Water has been supplied through plastic water buckets and a PVC pipeline. The shelter has been made with wooden walls. Wooden bedding has been set up for the goats. Manure has been collected and cleaned weekly to ensure cleanliness.

3.4.1.4 Site Characteristics of Swine Manure Sampling Locations

Table 3.8 Site characteristics of selected farms for swine manure sampling

Farm	Location	Code	Description
Mahaberiyathenna NLBD Farm, Digana	7.286534, 80.752660	SFD	The trash from poultry and cattle units has been fed to the pigs. Water has been supplied using plastic water buckets and a PVC pipeline. The shelter has been constructed with a concrete floor to ensure durability. Manure has been collected and washed away daily to maintain cleanliness.
Kadana Pig Farm	7.0457406, 79.8985202	SFK	Swill from restaurants has been brought in plastic barrels and provided as feed. Water has been made available through plastic water buckets and a PVC pipeline. The building has been made with a concrete floor for easy maintenance. Manure has been collected and washed away daily to uphold hygiene.

Farm	Location	Code	Description
Mawelawatta Farm, Uda Peradeniya	7.2516302, 80.6127315	SFU	Swill from university premises has been used as swine feed. Water has been supplied using plastic water buckets and a PVC pipeline. A concrete floor has been constructed for feeding and as bedding. Manure has been cleaned and washed away twice daily to ensure sanitation

3.4.2 Animal Manure Sample Pre-preparation

First, each sample was air-dried until a constant Mass was achieved, and the initial moisture content was calculated after oven drying. Next, the manure samples were gently ground by hand with slight force to break down the clod/aggregates. Then, by using 5 mm stainless steel mesh, the manure sample was sieved to obtain manure samples for further analysis.

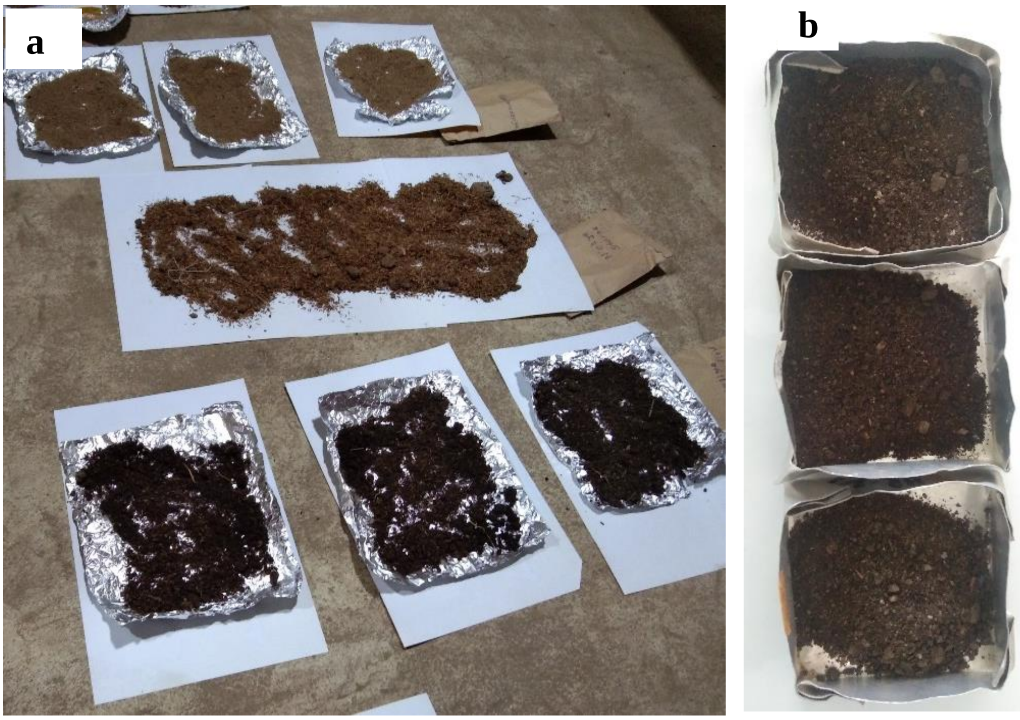

Figure 3.3 Animal manure sample pre-preparation; a) air drying, b) oven drying

3.4.3 Microplastics Extraction

3.4.3.1 Manual Separation

From each sieved sample, 10 g were obtained and passed through a series of stainless-steel sieves set with 5 mm, 2 mm, 1 mm and, 0.5 mm. After sieving through four sieves, manure retained on each sieve was spread onto Petri dishes separately into a thin layer, and visible MP particles were picked up manually using forceps while observing through the magnifying lens (3x). The separated MPs were counted (N1) and weighed (M1). Then the remaining manure of each sample was transferred into a pre-cleaned conical flask and closed using aluminum Foil.

Figure 3.4 Laboratory analysis; a) measuring manure, b) dry sieving of manure, c) and d) observing under magnifier

3.5 Selection of Suitable Methodology and Chemicals for Organic Matter Digestion

It is well known that organic compounds can be oxidized using oxidizing agents. However, samples which are rich in organic matter (OM) like animal manure, complete oxidation is challenging. Most difficult part of this study was also digestion of OM in animal manure to efficiently extract and clearly identify MPs. First, 30% H_2O_2 was used for 10 g of manure samples to oxidize OM as trail number one. In there, different volumes (15, 25, 50 ml) of H_2O_2 were used for each manure sample. However, it was not given sufficient results. Therefore, another trial was conducted using the Fenton's reagent. The Fenton's reagent was more successfully digested OM than H_2O_2 alone. However, it was also not fully oxidized the OM as expected. It consists of a mixture of hydrogen peroxide and iron salts. In this study, this reagent was prepared by mixing 0.05 M FeSO4 and 30% H_2O_2 solutions. It consists of a mixture of hydrogen peroxide and iron salts. There are chemical mechanisms that propose hydroxyl radicals as the oxidant species (Pignatello, 1992,) that are generated in the following chemical equation:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\cdot$ (1)

Hydroxyl radicals may be scavenged by reaction with another Fe2+:

$OH\cdot + Fe^{2+} \rightarrow Fe^{3+} + OH^-$ (2)

Fe3+ catalytically decomposes H2O2 following a radical mechanism that involves hydroxyl and hydroperoxyl radicals, including (1) and (2):

$Fe^{2+} + H_2O_2 \rightarrow Fe\text{-}OOH^{2+} + H^+$ (3)

$Fe\text{-}OOH^{2+} \rightarrow HO_2\cdot + Fe^{2+}$ (4)

$Fe^{2+} + HO_2\cdot \rightarrow Fe^{3+} + HO_2^-$ (5)

$Fe^{3+} + HO_2^- \rightarrow Fe^{2+} + H^+ + O_2$ (6)

$OH\cdot + H_2O_2 \rightarrow H_2O + HO_2\cdot$ (7)

The hydroxyl radicals are highly non-selective and can oxidize various bonds, including C-H, C-C, C-O, and others. The reaction between OM and the reagent can be written as follows:

Organic matter (CxHyOz) + $Fe^{2+} + H_2O_2 \rightarrow CO_2 + H_2O$ + other oxidation products

Even though the above-mentioned reagent reacted very rapidly with organic matter, it was not sufficient to clearly characterize MPs. Therefore, 69% of the HNO_3 solution was added to further oxidize residual OM.

CxHyOz + HNO3 $\rightarrow CO_2 + NOx + H_2O$ + other oxidation products

Additionally, the density separation was done using 25% (w/v) NaCl solution since MPs are buoyant on high density liquids.

3.6 Materials

- Manure samples collected at various stages of manure value chain from different places.
- Chemicals for manure digestion
  - 30% H_2O_2
  - NaCl
  - $FeSO_4.7H_2O$
  - 69% HNO_3
  - Distilled water to clean the laboratory equipment and to make required solutions
- Equipment for sample preparation and MPs analysis
  - Digital balance and analytical balance to weigh the required chemicals and samples
  - Laboratory oven to dry the filter papers.
  - Sampling scoop for sampling
  - Magnifier and tweezer to separate visible MPs
  - water bath to provide optimum digestion conditions at 70 ^{o}C
  - Freezer to store the sample until analysis at -4 ^{o}C
  - Stainless steel sieves (5 mm,2 mm, 1 mm, and 0.5 mm)
  - Magnetic stirrer to mix the digested samples at 400 rpm for 2 minutes.
  - Laboratory fume hood to prepare samples
  - Microscope to observe MPs under different magnification levels (40x and 100x)
- Additional materials for laboratory analysis
  - Paper bags (15 cm × 20 cm) for sampling and storing the samples
  - Labels and Markers for sample identification
  - Aluminium sheets for sampling manure.
  - Aluminum foil to store the samples and cover the conical flasks.
  - Petri dishes with lids to store each MP's sample within wrapped filter paper separately.
  - Filter papers (Whatman: 40) to filter the MPs from the digested solution
  - Conical flask (250 ml) for chemical analysis of samples
  - Image J Software for MPs identification

- Statistical Software for data analysis (SAS, Minitab and Microsoft Excel)
- Data Recording Tools: Notebooks, data sheets (Excel), and pens

3.6.1.1 Chemical Treatment (Dual Digestion Protocol)

The dual digestion protocol was followed in this study to extract MPs through a chemical treatment process. Therefore, the remaining manure sample was added to the 250 ml beaker after the manual separation of visible MPs. Then 10 ml of 0.05 M iron catalyst ($FeSO_4$) and 25 ml 30% hydrogen peroxide solution (H_2O_2) were added slowly (Ranasingha *et al*, 2024). After that, the beaker was heated up to 70 °C in a water bath while frequently stirring (40 rpm) until the sample got nearly dry and then kept until

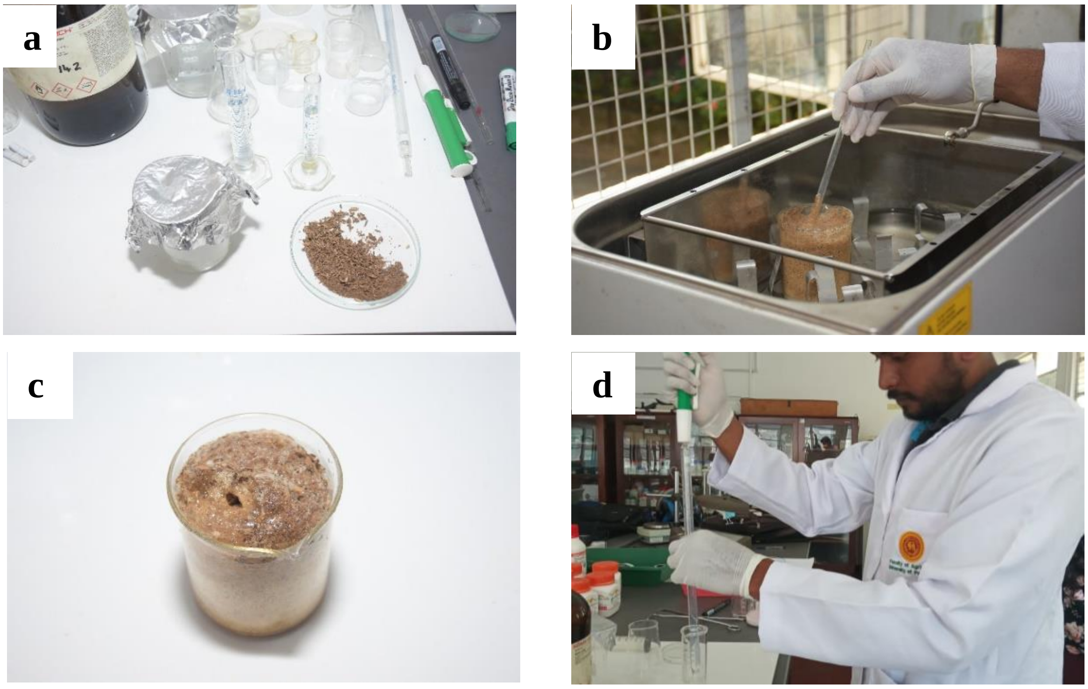

Figure 3.5 Laboratory analysis; a) manure sample and equipment for digestion with Fenton's reagent, b) heating sample in shaking water bath, c) digested sample from Fenton's reagent, d) adding hno_3 solution

cooling down to room temperature (~30 °C) for the downstream analyses. After that, 20 ml of 69% HNO3 solution was added to the beaker and kept overnight at room temperature (Masura *et al*, 2015). Finally, the beaker was placed in a water bath at ~50 °C for 2 hours for further digestion of organic matter.

3.6.1.2 Density Separation and Filtration

As the final step of the MPs extraction, 200 ml of 25% NaCl (w/v in water) was added to the beaker and stirred for 2 minutes by using a magnetic stirrer at 400 rpm. Then, the sample was transferred into a separating funnel covered with a lid and allowed to stand until the overlaying solution looked clear (About 12 hours). As the next step, Whatman No. 40 filter papers were dried at 70 °C in an oven until constant Mass (W2). Then sediment was removed through the tap to obtain the clear supernatant. Next, the supernatant was transferred through the pre-weighed filter paper using suction filtration. The separating funnel was thoroughly rinsed with distilled water and put onto the filter paper. The remaining NaCl solution on the filter paper was washed through itself using distilled water to ensure no remaining NaCl solution was on the filter paper before drying. After filtration, the filter paper was placed in the labelled crucible and covered using a lid.

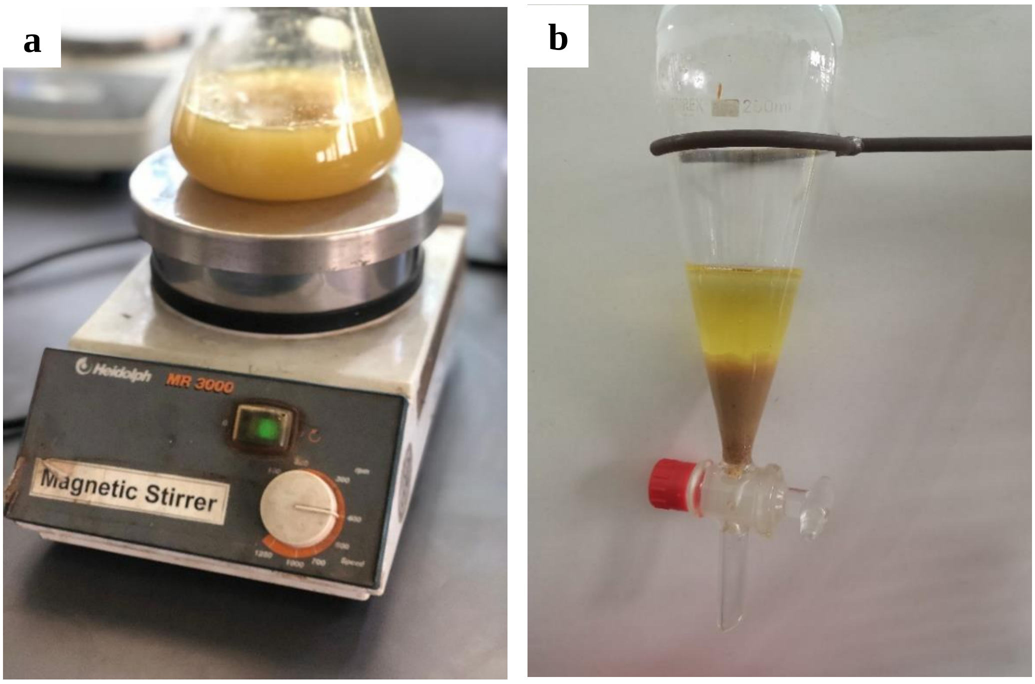

Figure 3.7 Laboratory analysis; a) stirring digested sample using magnetic stirrer, b) density separation using separating funnel

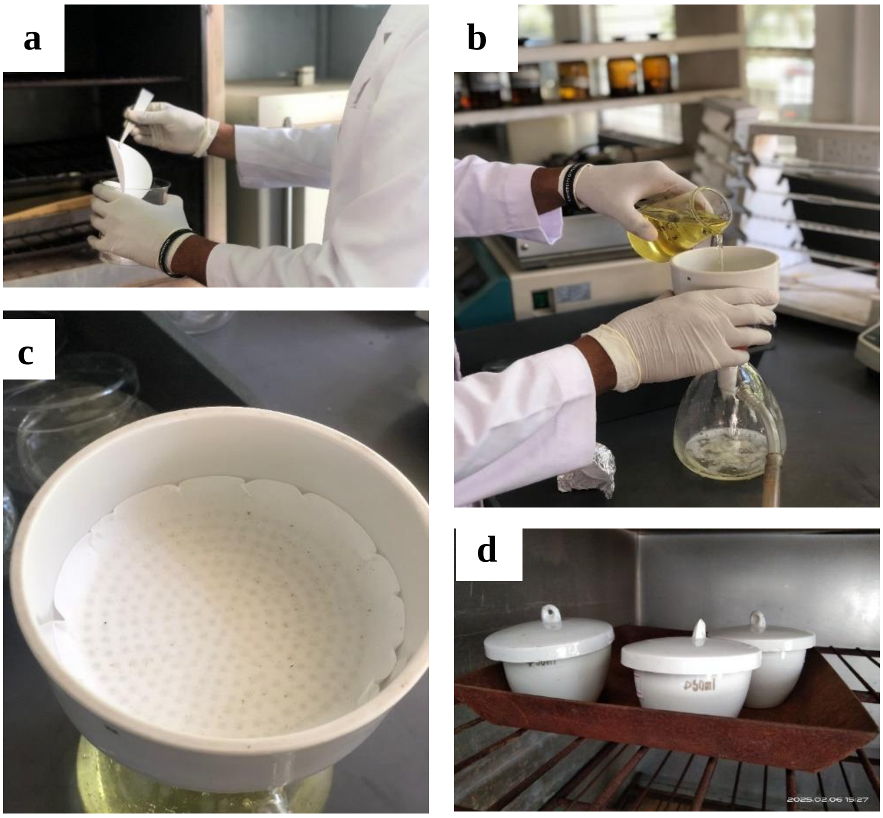

Figure 3.6 Laboratory analysis; a) oven drying filter paper, b) filtration, c) filter paper with MP residues, d) oven drying filter paper with MP residues

Then the crucible with filter paper was placed in an oven and dried at 70 °C until the filter paper got constant Mass. Finally, the Mass of the filter paper with the MPs was recorded (W3).

3.6.2 Microplastic Characterization

The sediment collected in the filter paper was transferred into a microscopic slide and observed under the magnification levels of 40x and 100x using a stereomicroscope. While observing through the microscope, MP particles were counted (N2) and images were taken covering the entire slide. The color and the type of MP particles were observed and recorded using those images. Then, the area and the length of MP particles were measured through "Optica Proview" software. Finally, all the MP Selected samples were separately prepared for FTIR analysis to identify different types of Polymers that were contaminated in the manure.

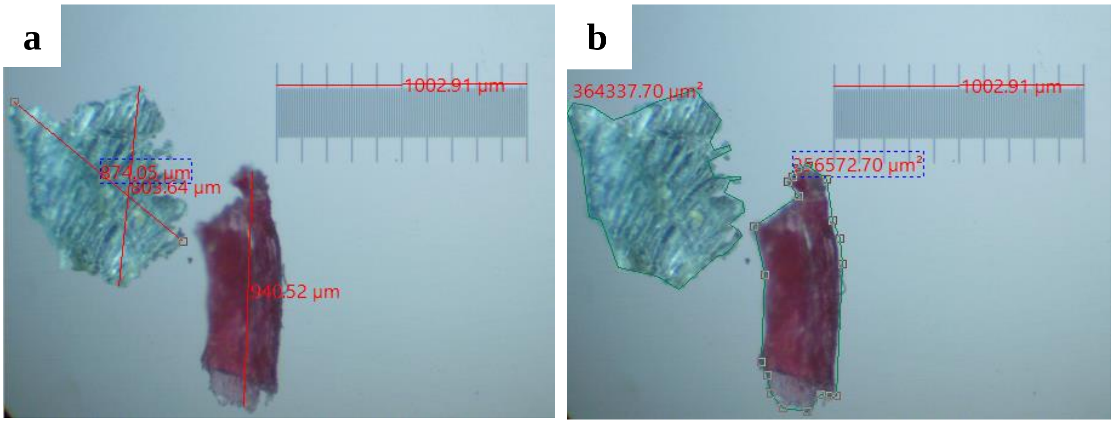

Figure 3.8 Microscopic images analysis using Optica View Software; a) measuring particle length, b) measuring particle area

3.6.3 Data Analysis

All the data obtained during the study were recorded in Microsoft Excel and further analysis was done accordingly.

The level of MP contamination was recorded as the number of MP items, the mass of MP items, the total area of MP items, and the total length of MP items in each replicate per kilogram of fresh manure.

$$\text{Mass of MPs in mg per Kg of fresh manure} = \frac{(W1 + W3 - W2)\ g \times (1 - MC\ \%)\ 100}{10\ g \times 1000}$$

Number of MP items per Kg of fresh manure $= \frac{(N1 + N2) \times \times(1 - MC\ \%)\ 100}{10\ g \times 1000}$

One-way and two-way ANOVA tests were used at 95% confidence level to analyze all the measurements with relevant categories and Tukey mean comparison.

4 RESULTS AND DISCUSSION

4.1 Summary of Microplastic Measurements in Manure of Different Animal Species and Locations

As the results of microplastic measurements, several microplastic related measurements were obtained and consequently estimations were made. Those measurements were number of MPs particles, mass, total area, total length, color and the type of MPs. These measurements have been shown as a summary in tables shown in Annex 1. These tables are relevant to the data of poultry manure in farms, poultry manure in sales centers, poultry manure agricultural field, cattle manure in farms, cattle manure in sales centers, cattle manure in agricultural field, goat manure in farms and swine manure in farms respectively.

4.2 Qualitative analysis of microplastics in manure

4.2.1 Morphology: Shape of Microplastic Particles

The microplastics detected in animal manure can be classified into three main morphological shapes: fragments, fibers, and films. The distribution of these shapes varies across animal manure types, possibly reflecting the different plastic materials used in their respective management systems. In goat manure, fragments account for 87%, suggesting that hard plastic items such as buckets or feeding containers may be the primary sources. Fibers (13%) could be derived from synthetic ropes or other woven materials occasionally used in shelters. Swine manure contains 84% fragments, likely originating from the use of plastic buckets, barrels, or containers. Films (12%) may have entered through plastic swill packaging or feed wrappers, while fibers (4%) could be from minor sources such as clothing or equipment.

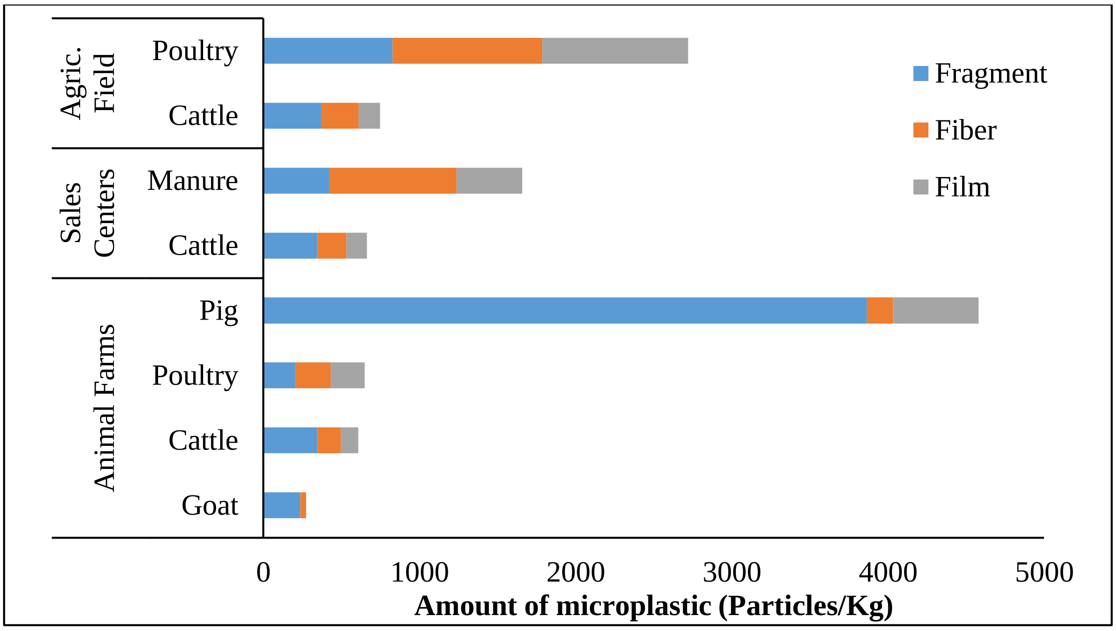

Figure 4.1 Morphological shape distribution of microplastic particles along the animal manure value chain

Cattle manure shows a more diverse distribution with 52.5% fragments, 28.6% fibers, and 20.9% films, which may result from a wider range of plastic usage including milking equipment, feed bags, plastic tanks, and synthetic ropes. In poultry manure, fragments make up 28.5%, while fiber (39.4%) and films (32.1%) are more dominant. These could be associated with materials like plastic shade nets, poly sack bags, and egg trays.

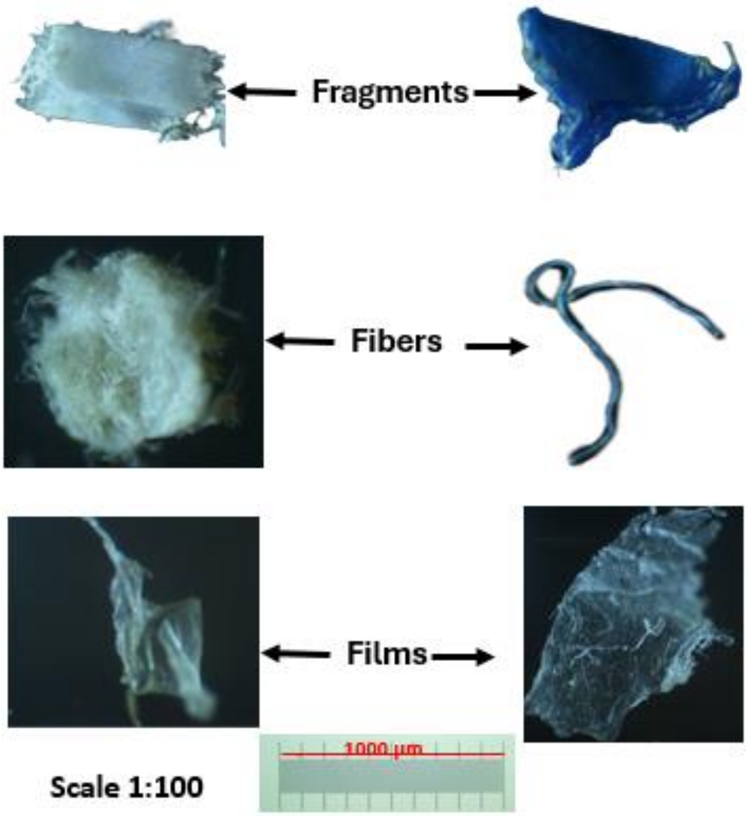

Figure 4.2 Shape variation of microplastic in animal manure

Overall, fragments (54%) are the most common microplastic type found in manure, followed by fibers (24%) and films (22%). These forms may arise from the gradual breakdown of various plastic components used throughout the animal husbandry value chain.

4.2.2 Colour Distribution of Microplastic Particles

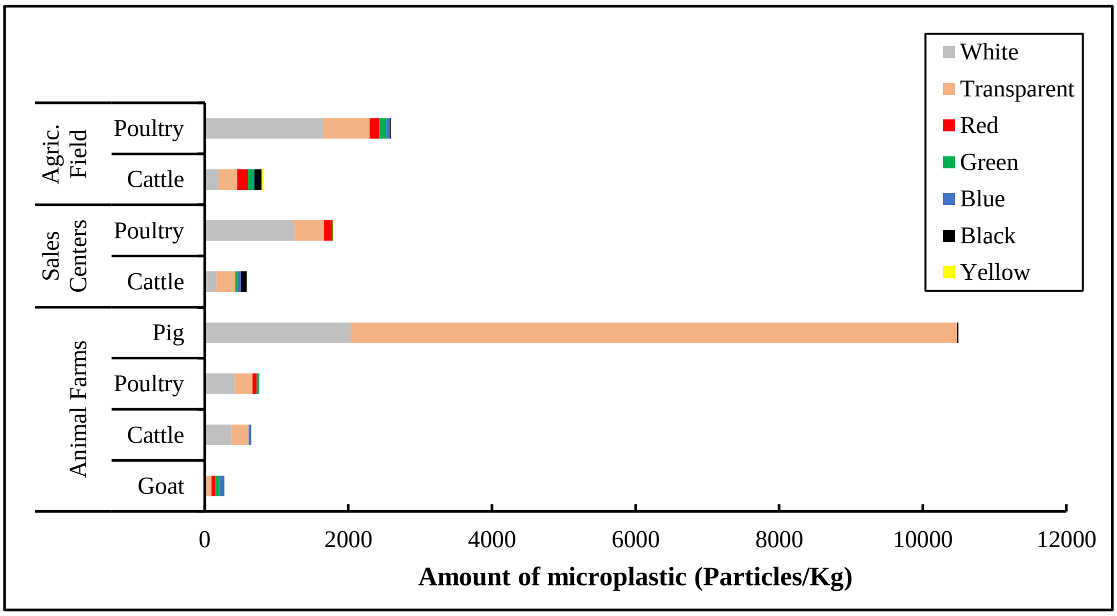

Figure 4.3 Colour distribution of microplastic particles along the manure value chain

Poultry manure was largely dominated by white (64%) and transparent (26%) particles, with only small traces of colored plastics, likely due to their confined housing and reliance on processed feed. Goat manure, though not from openly reared animals, showed notable colour diversity; blue (27%), green (21%), red (17%), and transparent (35%) suggesting exposure through feed, housing materials, or bedding. Swine manure exhibited a high proportion of transparent (80%) and white (19%) particles, with no colored plastic detected. This indicates limited but specific exposure, most likely through plastic-contaminated feed. These findings suggest that feed and management practices significantly influence the types and colours of microplastics ingested by livestock, with transparent and white particles being the most commonly encountered across all animal types.

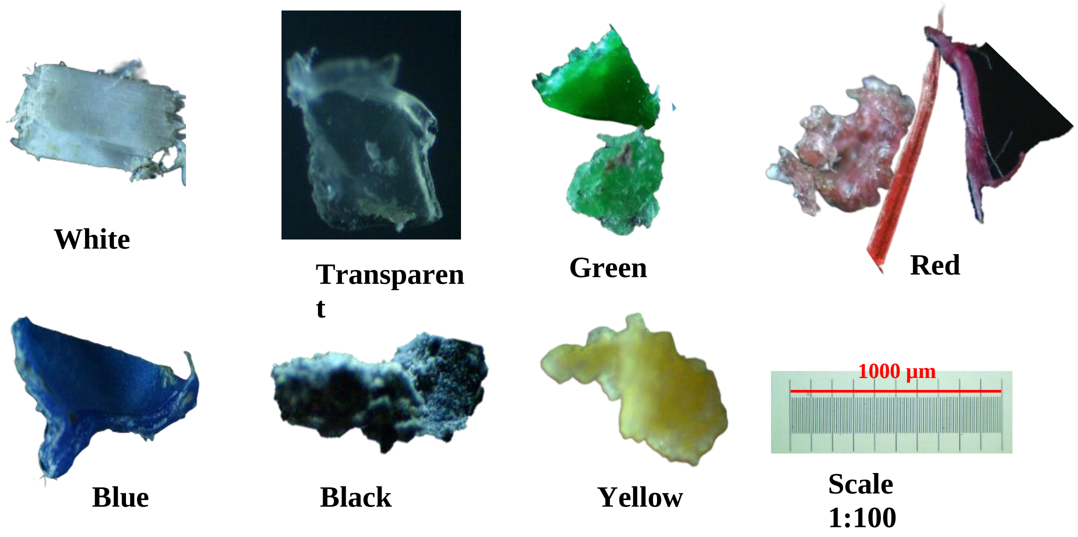

Figure 4.4 Colour variation of microplastic particles in animal manure samples

4.3 Quantitative Analysis of Microplastic in Manure Value Chain

4.3.1 Comparison of Amount Microplastic Particles in manures sourced from the source

As shown in table 4.1, there was not any significant difference between the locations of manure sources of each manure type. However, different contamination levels were observed at different source locations. It was most probably due to the different management practices each location has followed. According to figure 4.5 below, it was confirmed that all the source locations were contaminated with MPs. Swine farms showed the highest level of MP contamination followed by the poultry farms. Moreover, the Mawelawatta farm was the highly contaminated farm for all four types of manures.

Table 4.1 Probability value from the one-way ANOVA tests between amount of microplastic and animal farms

Manure Type	Goat	Cattle	Poultry	Swine
P value	0.656	0.341	0.121	0.101

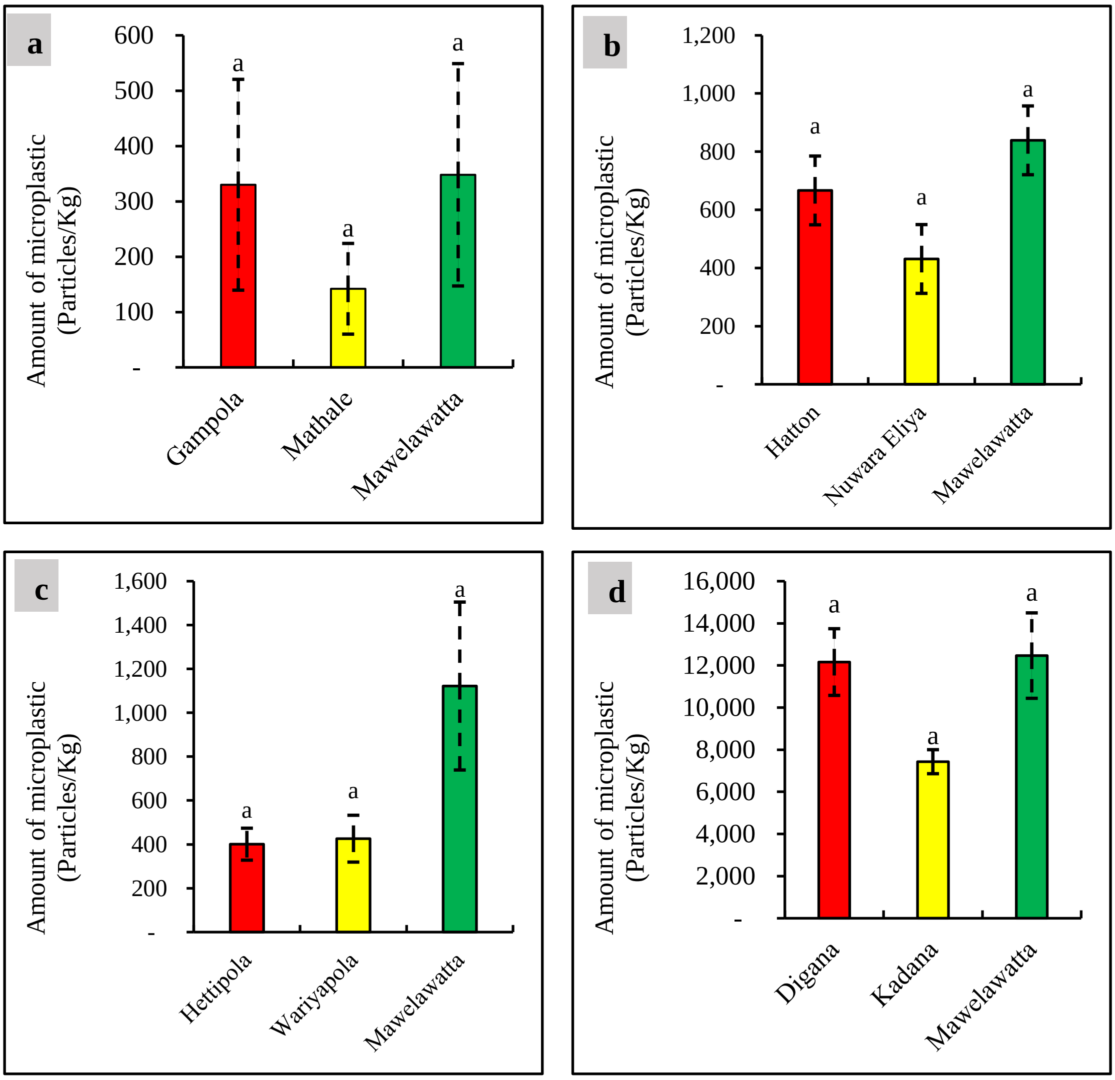

Figure 4.5 Quantity of microplastics expressed as number of particles in a) Goat, b) Cattle, c) Poultry, and) Piggery manure sources. Different letters on bar charts indicate the statistical difference ($p<0.05$) and the dash lines on each bar show the Standard Error (SE) for each source (*n*=*3*)

4.3.2 Comparison of mass of Microplastic in manures sourced from the source

The mass of MPs also followed the same pattern as their count. However, there was a significant difference between swine manure source locations. According to figure 4.6, the mass of MP particles in Mawelawatta and Digana piggeries were significantly higher than the Kadana farm.

Table 4.2 Probability value from the one-way ANOVA tests between mass of microplastic and animal farms

Manure Type	Goat	Cattle	Poultry	Swine
P value	0.619	0..34	0.880	0.003

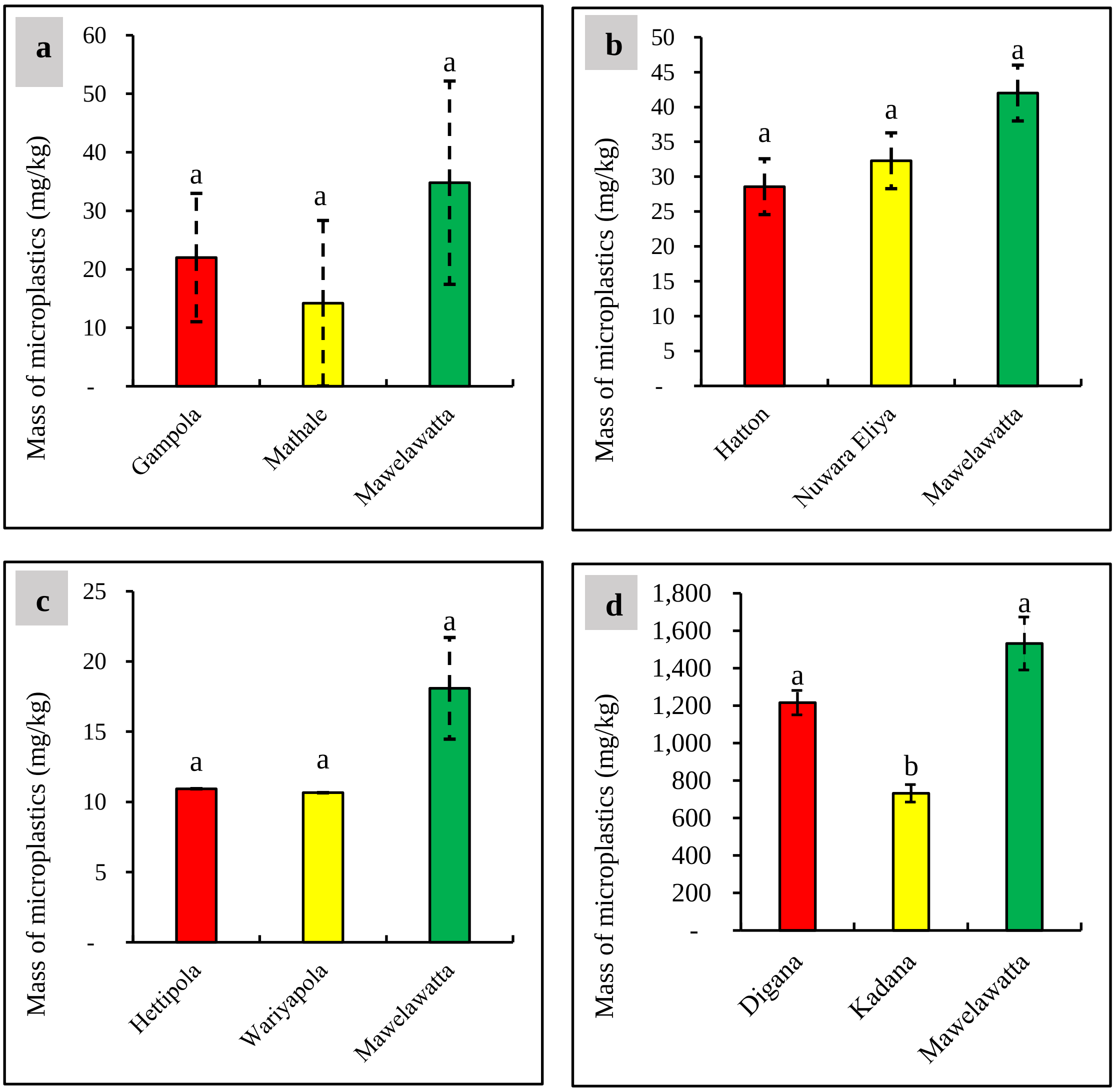

Figure 4.6 Quantity of microplastics expressed as mass on MP in a) Goat, b) Cattle, c) Poultry, and d) Piggery manure sources. Different letters on bar charts indicate the statistical difference ($p<0.05$) and the dash lines on each bar show the Standard Error for each source (*n=3*)

4.3.3 Comparison of Amount Microplastic Particles in manures sourced from the sales centers

Table 4.3 Probability value from the one-way ANOVA tests between the amount of microplastic and manure sales centers

Manure Type	Cattle	Poultry
P value	0.606	0.880

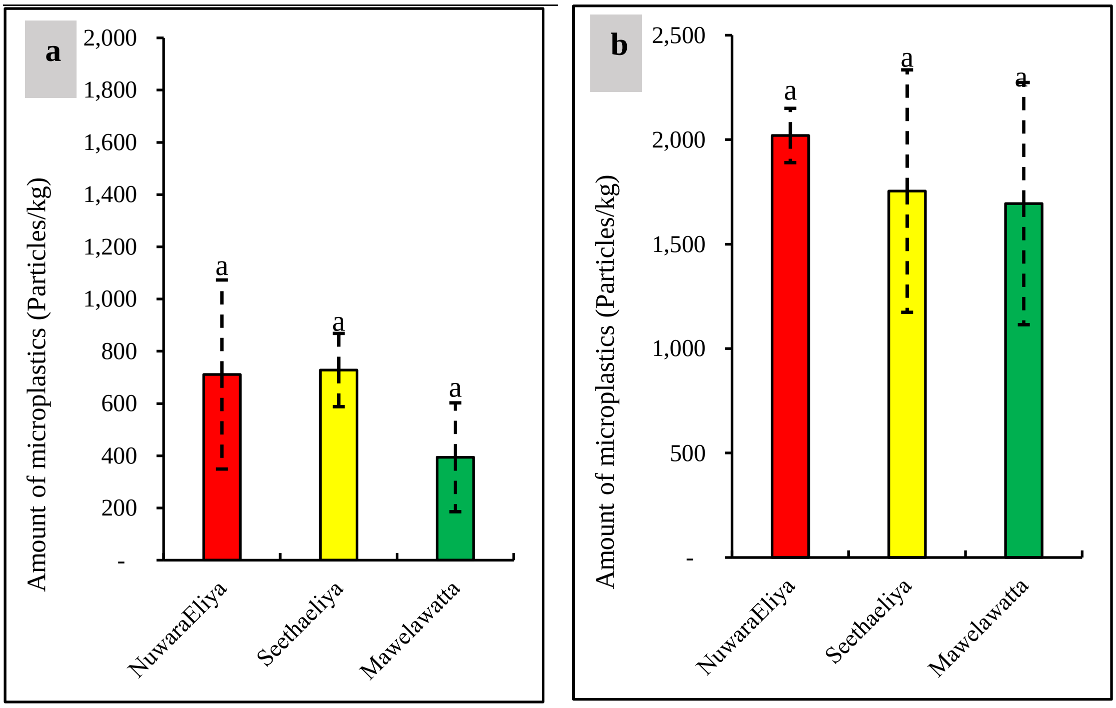

Figure 4.7 Quantity of microplastics expressed as number of MP particles in a) Cattle and b) Poultry manure sources at sales centers. Different letters on bar charts indicate the statistical difference (p<0.05) and the dash lines on each bar show the Standard Error for each source (n=3)

As shown in table 4.3, there was no significant difference in the level of MP contamination between the manure sales centers in each manure type. However, the amount of contamination is different location to location. It also observed that poultry manure was more highly contaminated than the cattle manure at the sales centers based on the results shown in figure 4.7 above.

4.3.4 Comparison of the mass of Microplastic in manures sourced from the sales centers

Table 4.4 Probability value from the one-way ANOVA tests between mass of microplastic and manure sales centers

Manure Type	**Cattle**	**Poultry**
P value	0.784	0.718

Figure 4.8 shows there is a slight difference in the mass of MPs between the sales centers where cattle manure is stored for selling. However, it was not significant according to the results obtained from the ANOVA test shown in table 4.4. The results obtained for the mass of MPs in poultry manure at sales centers show more or less similar values. The most important result obtained from the analysis of MPs contamination in sales centers is that even poultry manure contained higher number of MP particles, cattle manure shows the larger mass value per kilogram of dry manure. This could be due to the contamination of high-density MP particles in cattle manure.

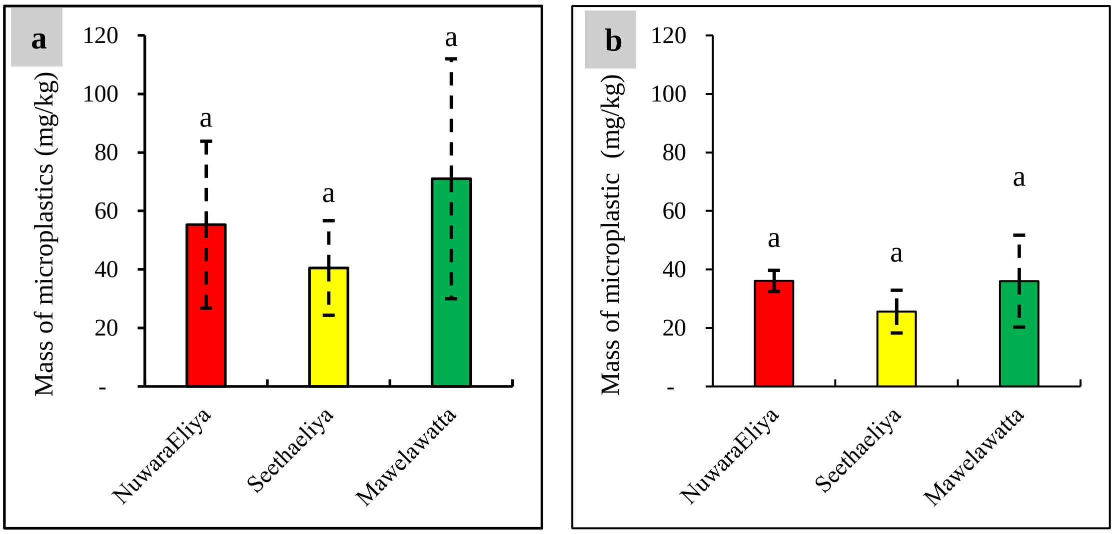

Figure 4.8 Quantity of microplastics expressed as mass of MP in a) Cattle and b) Poultry manure sources at sales centers. Different letters on bar charts indicate the statistical difference ($p<0.05$) and the dash lines on each bar show the Standard Error for each source (n=3)

4.3.5 Amount Microplastic Particles in manures sourced from the agricultural fields

Table 4.5 Probability value from the one-way ANOVA tests between amount of microplastic and agricultural fields

Manure Type	**Cattle**	**Poultry**
P value	0.044	0.961

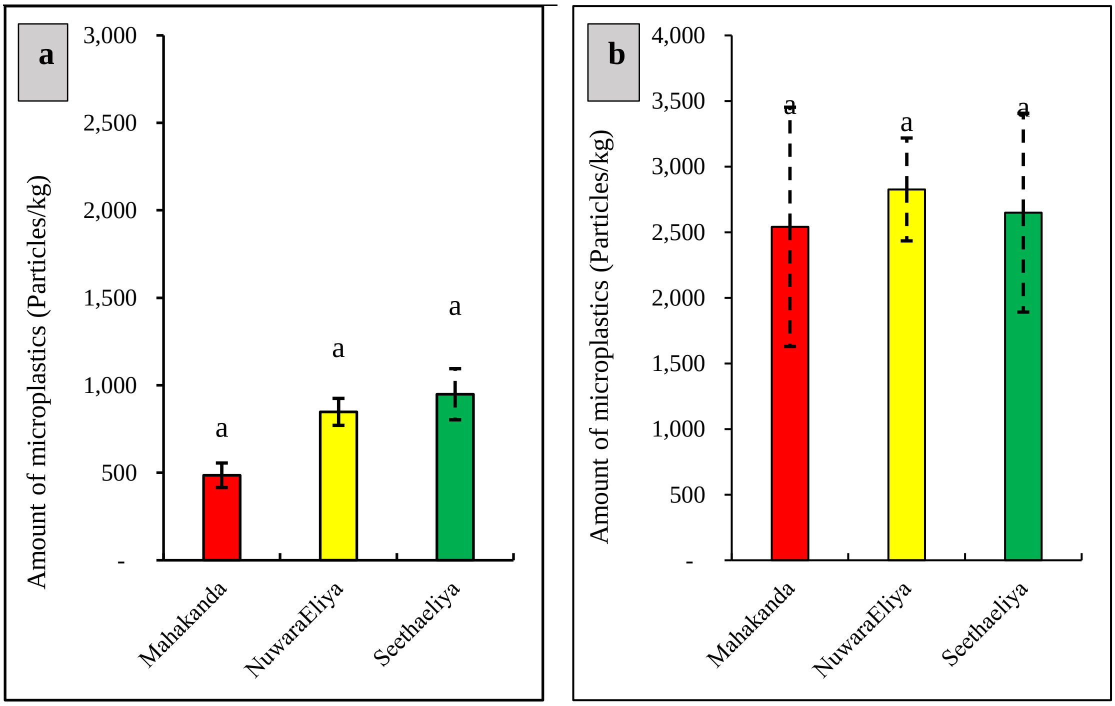

Figure 4.9 Quantity of microplastics expressed as number of particles in a) Cattle and b) Poultry manure sources at agricultural fields. Different letters on bar charts indicate the statistical difference ($p<0.05$) and the dash lines on each bar show the Standard Error for each source (n=3)

According to figure 4.9, poultry manure stored in the agricultural field for applying as soil amendments has shown higher level of MP contamination than the cattle manure. This increase may be the degradation of poly sack bags in which poultry manure stored.

However, there is not any significant variation among the locations of agricultural fields for each manure type. Comparison of Mass of Microplastic at Agricultural Fields

4.3.6 Comparison of masses of Microplastic in manures sourced from the agricultural fields

Table 4.6 Probability value from the one-way ANOVA tests between mass of microplastic and agricultural fields

Manure Type	Cattle	Poultry
P value	0.241	0.797

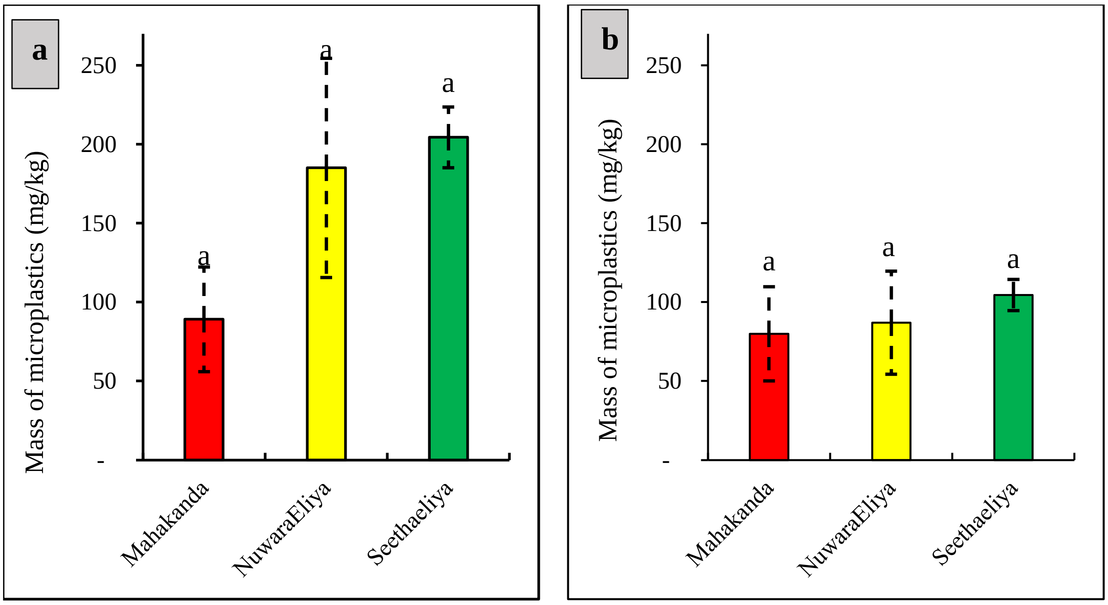

Figure 4.10 Quantity of microplastics expressed as masses of particles in a) Cattle and b) Poultry manure sources at agricultural fields. Different letters on bar charts indicate the statistical difference ($p<0.05$) and the dash lines on each bar show the Standard Error for each source (n=3)

The MP contaminants in cattle manure in the agricultural field shows the higher mass compared to the MP in poultry manure according to the figure 4.10 above. This reflects the cattle farming may have been used high density plastic materials causes releasing higher density MP particles. That figure also reflects that there is no significant variation of mass of MP contaminants in each manure type between the agricultural farm field locations.

4.3.7 Microplastic Contamination along the Cattle Manure Value Chain

Table 4.7 Probability value from the one-way ANOVA tests between amount and mass of microplastic along cattle manure value chain

Measurement	Count	Mass
P value	0.241	0.797

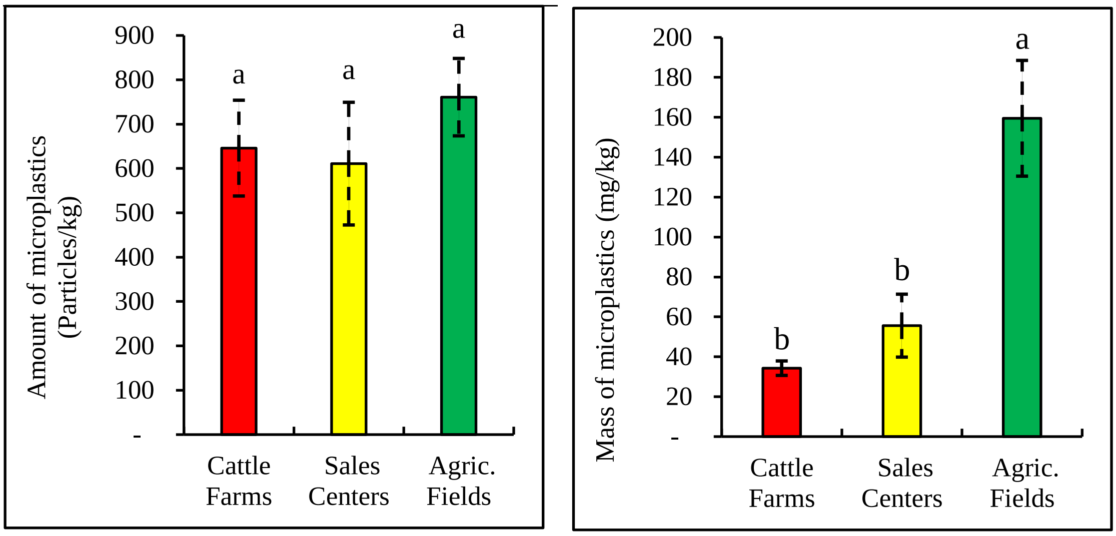

Figure 4.11 Quantity of microplastics expressed as number of particles along the cattle manure value chain. Different letters on bar charts indicate the statistical difference (p<0.05) and the dash lines on each bar show the Standard Error for each source (n=3)

The figure 4.11 reflects that although the number of particles is not significantly different, there is an increasing trend. However, the mass increase is significantly different according to table 4.7. Microplastic contamination increases along the value chain. The secondary contamination may be responsible for increase (Handling, storage, processing etc.)

4.3.8 Comparison of mass of Microplastic Particles along the Poultry Value Chain

Table 4.8 Probability value from the one-way ANOVA tests between amount and mass of microplastic along poultry manure value chain

Measurement	Count	Mass
P value	0.241	0.797

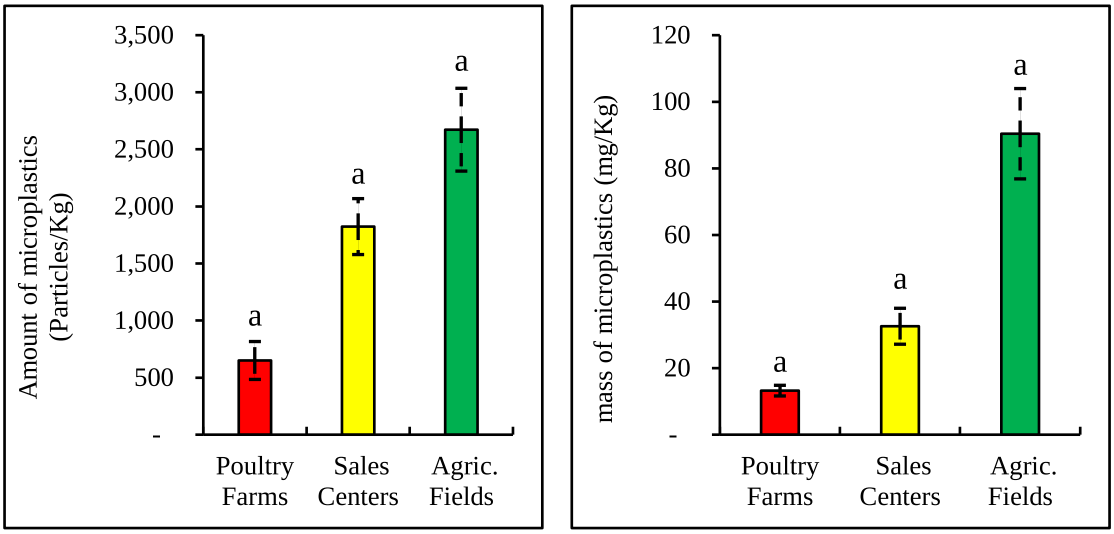

Figure 4.12 Quantity of microplastics expressed as mass of particles along the cattle manure value chain. Different letters on bar charts indicate the statistical difference ($p<0.05$) and the dash lines on each bar show the Standard Error for each source (n=3)

Table 4.8 shows that although the number and mass of particles are not significantly different, there is an increasing trend. Also figure 4.12 reflects that MPs contamination increases along the value chain. The secondary contamination may be responsible for these increases (Handling, storage, processing etc.).

4.3.9 Overall Comparison of Amount of Microplastic Particles along the Value Chain

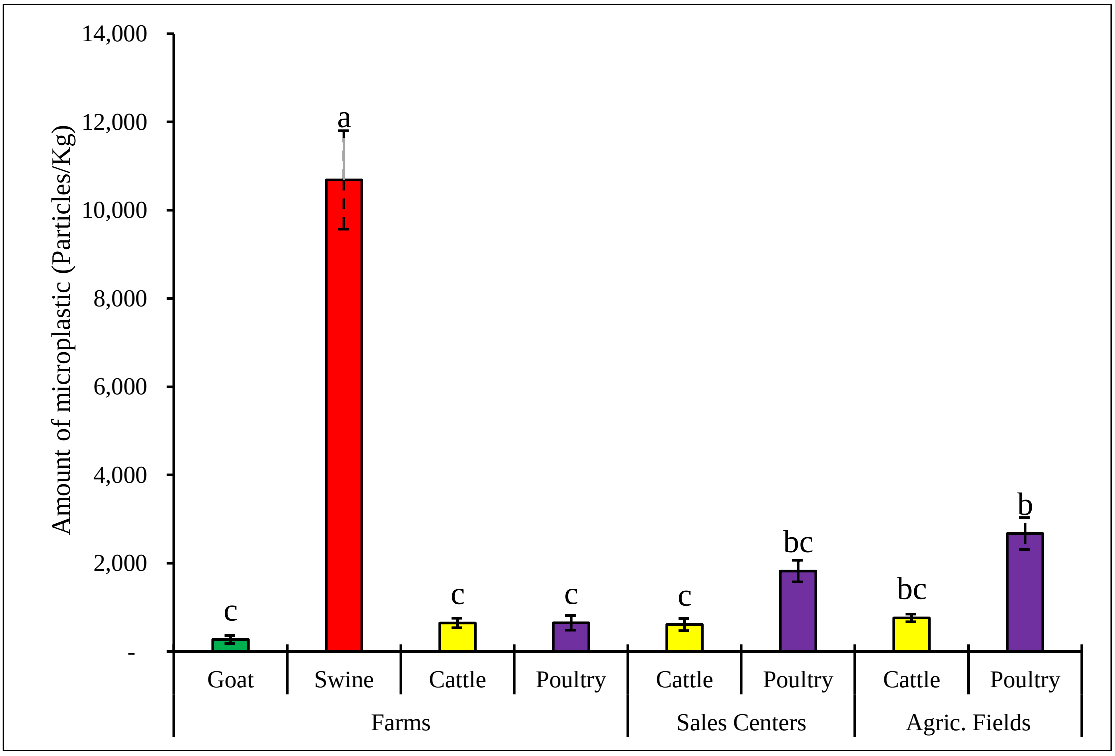

Figure 4.13 Microplastic Contamination expressed as number of MP particles along the Animal Manure Value Chain. Different letters on bar charts indicate the statistical difference ($p<0.05$) and the dash lines on each bar show the Standard Error for each source (n=3)

When considering the amount of MP contamination as the number of particles/Kg of dry manure, swine manure samples from the sources, poultry manure from the markets as well as from the sales centers, and cattle manure samples from the sales centers having a significantly higher level. This is confirmed by the lower probability value (<0.05) given by the two-way ANOVA tests conducted and the figure 4.13 shown above.

4.3.10 Overall Comparison of Mass of Microplastic Paricles along the Value Chain

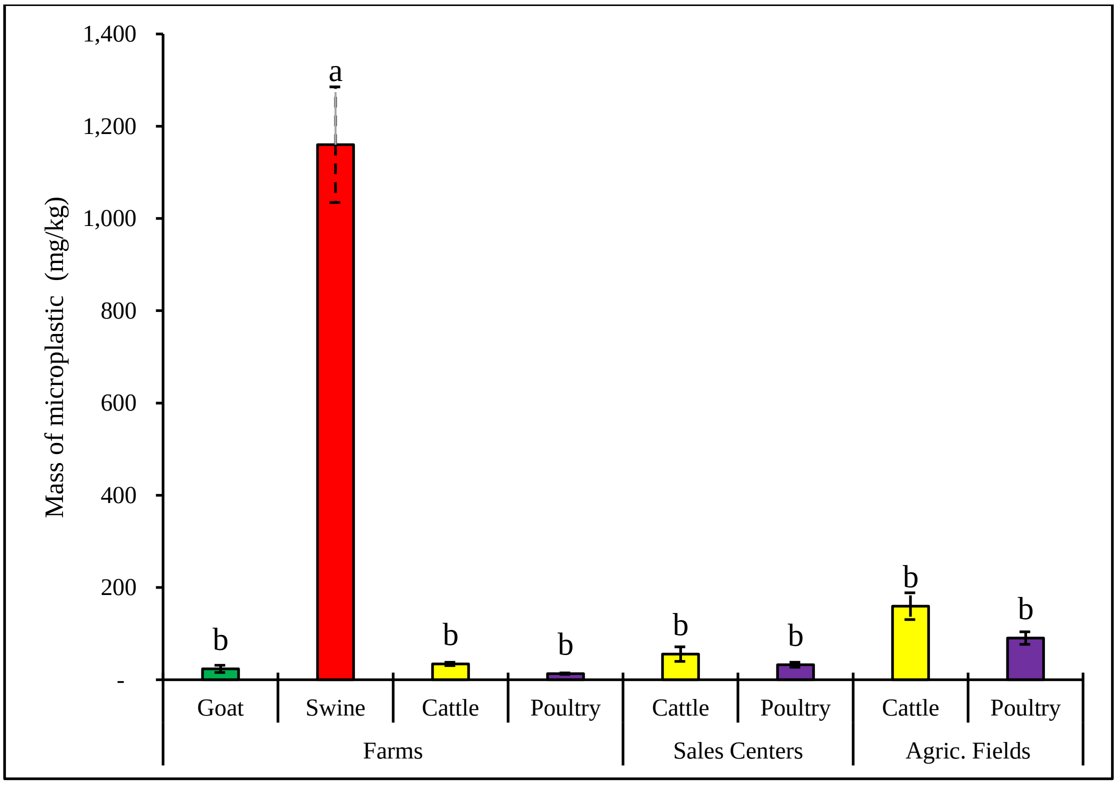

Figure 4.14 Microplastic Contaminants as expressed as maa of MP along the Animal Manure Value Chain. Different letters on bar charts indicate the statistical difference ($p<0.05$) and the dash lines on each bar show the Standard Error for each source (n=3)

However, other than swine manure, the mass of MP in the other three manure types in all the locations in the manure value chain shows significantly ($P<0.001$ from the two-way ANOVA test) lower levels according to the figure 4.14 above.

All manure types examined in this study were contaminated with MPs even at source locations, with concentrations ranging from 273 ± 91 to 10,687 ± 1,115 particles/kg, equivalent to 11–1532 mg/kg. Swine manure showed the highest contamination levels (10,687 ± 1,115 particles/kg), followed by poultry (650 ± 166 particles/kg), cattle (608 ± 108 particles/kg), and goat (273 ± 91 particles/kg). Also, compared to data reported by Sheriff et al. (2023), swine manure in Sri Lanka had significantly higher contamination than the reference value ($2,902 \times 10^3$ particles/kg), while poultry, cattle, and goat manure had slightly lower or comparable levels. The high proportion (95%) of white and transparent particles reflects that it could be contaminated from the feed sources because most of piggeries supply traces of poultry and cattle feeds and swills with transparent polythene bags as swine feeds.

Microplastic contamination was also found to increase along the manure market value chain. The poultry manure showing a 311% increase (from 667 to 2,738 particles/kg) and cattle showing a 17% increase (from 740 to 864 particles/kg), indicating the possibility of incorporation of MPs during storage, transport, or handling along the manure value chain.

Although all manure types in this study were contaminated with MPs, the contamination levels generally remained below the proposed international standards for organic soil amendments. For instance, the recorded levels were 0.02% by mass in poultry, 0.03% in cattle, and 0.03% in goat manure. These values are lower than the limits set by the EU and UK (<0.5%) and Switzerland (<0.1%). However, swine manure showed a contamination level of 0.12% by mass, which exceeds standards limit proposed by the Switzerland of 0.1%, though it remains within the broader thresholds set by the EU and UK. This indicates that while most manure types fall within safe limits, swine manure may pose a higher environmental risk.

4.4 Sources and Fate of Microplastic Contaminants in Animal Manure

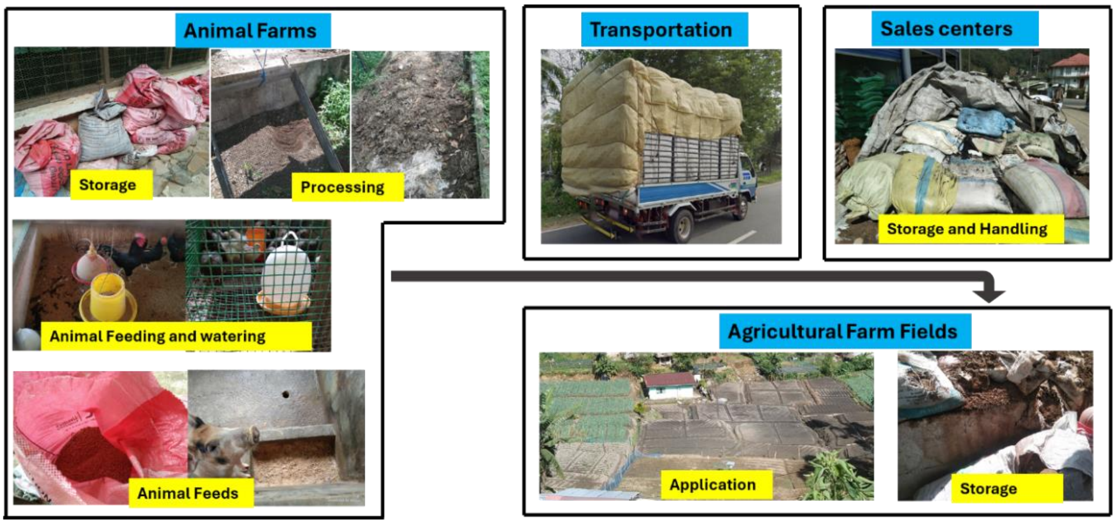

Figure 4.15 Pictures showing the potential sources and fate of microplastic contaminants in animal manure along the manure value chain in Sri Lanka

Microplastic contamination in animal husbandry arises from multiple sources along its value chain, particularly through the widespread use of plastic materials in feeding, watering, housing, equipment, storage, and manure handling. In poultry farms, poly sack bags, plastic feeders, waterers, pipelines, egg trays, and shade nets were commonly used, with manure often stored in poly sack bags for up to 40 weeks. Similar practices were observed in cattle farming, where PVC pipelines, plastic tanks, milking machines, poly sack feed bags, and nylon ropes are prevalent. Goat and pig farms also utilized plastic water buckets, PVC pipelines and plastic barrels for swill transport, all of which contribute to MP sources. Sales centers and agricultural fields that store manure in polythene or poly sack bags for extended periods further increase the risk of plastic degradation and MP release.

These materials can fragment into MPs due to weathering, physical stress, and microbial activity. Once released, MPs may persist in the soil for years, alter soil microbial activity, reduce fertility, and be taken up by crops, entering the food chain. Through manure application on agricultural fields, MPs from animal husbandry may ultimately reach ecosystems and human food systems, posing potential long-term environmental and health risks.

5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The swine and goat manure market shows only two segments: producers and consumers; however, poultry and cattle manure have three segments in their manure value chain: source, sales centers and consumers. All manure sampled from poultry, cattle, swine and goat farms were contaminated with microplastics with least in goat manure (23.5 ± 7 mg/kg) to highest in swine manure (1,160.2 ± 125 mg/kg).

Among the studied types of manure, swine manure was highly contaminated with microplastics particles from food sources, which was supported by the observed higher percentages of white and transparent particles (85%).

Amount of microplastic contamination increases along the manure value chain (400% increase in Poultry manure and 200% increase in cattle), indicating that secondary contamination along the market value chain make a significant contribution to the microplastics contamination of animal manure. The overall conclusion is that the animal manure in Sri Lanka has been contaminated with microplastic and the microplastic contamination in manure is not limited to the sources but also post handling and processing contribute to the contamination.

5.2 Recommendations for further studies

i. There is a need for an efficient and effective digestion protocol for MP extraction from highly organic samples like animal manure.
ii. Conducting ATR-FTIR and SEM is important to identify the polymer types and sources.

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7 ANNEX

Table 7.1 Summary of Microplastic Measurements in Poultry Manure of Selected Farms

Stage	**Poultry Farm**		
Location Code	PFH	PFW	PFU
Size Distribution			
Number of MPs items	401 ± 72.9	426 ± 106.6	1,122 ± 382.9
MPs Mass (mg)	10.9 ± 0	10.7 ± 0	18.1 ± 3.6
Total Area of MP particles (mm^2)	15.34 ± 0.3	24.02 ± 5.8	151.22 ± 54.9
Total Area of MP particles (cm)	16.97 ± 2.6	31.47 ± 2.9	82.98 ± 4.6
Colour Distribution (Number of MPs items)			
White	219	249	760
Transparent	109	293	175
Red	0	36	145
Green	0	0	36
Blue	0	0	36
Black	0	0	0
Yellow	0	0	0
Morphological Shape Distribution (Number of MPs items)			
Fiber	73	249	362
Fragment	219	71	326
Film	109	107	434

Table 7.2 Summary of Microplastic Measurements in Poultry Manure of Selected Sales Centers

Stage	Sales Center		
Location Code	PMS	PMN	PMU
Size Distribution			
Number of MPs items	1,754 ± 580.2	2,020 ± 130.1	1,694 ± 579.9
MPs Mass (mg)	25.6 ± 7.3	36.1 ± 3.6	36 ± 15.7
Total Area of MP particles (mm^2)	44.08 ± 16.5	24.14 ± 5.2	111.34 ± 3.5
Total Area of MP particles (cm)	81.46 ± 9.4	117.54 ± 22.2	123.58 ± 32.7
Colour Distribution (Number of MPs items)			
White	1,170	1,407	1,153
Transparent	439	462	386
Red	219	36	144
Green	110	0	0
Blue	0	0	0
Black	0	0	36
Yellow	0	0	0
Morphological Shape Distribution (Number of MPs items)			
Fiber	987	902	973
Fragment	292	613	288
Film	439	469	324
Foam	37	36	72

Table 7.3 Summary of Microplastic Measurements in Poultry Manure of Selected Agricultural Fields

Stage	**Agricultural Field**		
Location Code	PAS	PAN	PAM
Size Distribution			
Number of MPs items	2,649 ± 757.4	2,826 ± 391.9	2,541 ± 911.8
MPs Mass (mg)	104.5 ± 9.9	87 ± 32.6	79.9 ± 29.7
Total Area of MP particles (mm^2)	596.68 ± 77.1	61.02 ± 22.5	476.75 ± 181.6
Total Area of MP particles (cm)	254.13 ± 28	177.51 ± 52.7	214.07 ± 66.4
Colour Distribution (Number of MPs items)			
White	1,604	1,630	1,706
Transparent	336	929	581
Red	112	109	73
Green	336	0	109
Blue	0	36	73
Black	0	0	0
Yellow	0	0	0
Morphological Shape Distribution (Number of MPs items)			
Fiber	784	1,486	907
Fragment	1,045	507	944
Film	784	833	1,343

Table 7.4 Summary of Microplastic Measurements in Cattle Manure of Selected Farms

Stage	**Farm**		
Location Code	CFH	CFN	PFU
Size Distribution			
Number of MPs items	667 ± 95.2	431 ± 107.6	839 ± 277.6
MPs Mass (mg)	28.6 ± 0	32.3 ± 0	42 ± 10.5
Total Area of MP particles (mm^2)	12.96 ± 3.9	6.54 ± 4.7	11.02 ± 2.7
Total Area of MP particles (cm)	39.92 ± 13.4	19.06 ± 7.2	31.03 ± 5.3
Colour Distribution (Number of MPs items)			
White	381	215	525
Transparent	286	215	315
Red	0	0	0
Green	0	0	0
Blue	0	0	0
Black	0	0	0
Yellow	0	0	0
Morphological Shape Distribution (Number of MPs items)			
Fiber	190	108	105
Fragment	286	323	525
Film	190	0	210

Table 7.5 Summary of Microplastic Measurements in cattle Manure of Selected sales Centers

Stage	**Sales Center**		
Location Code	CMS	CMN	CMU
Size Distribution			
Number of MPs items	728 ± 140.1	711 ± 362.3	394 ± 208.7
MPs Mass (mg)	40.5 ± 16.2	55.3 ± 28.5	71 ± 41
Total Area of MP particles (mm^2)	9.26 ± 1.8	4.48 ± 2.2	15.32 ± 12.2
Total Area of MP particles (cm)	15.61 ± 3.3	8.65 ± 4.7	39.42 ± 28.5
Colour Distribution (Number of MPs items)			
White	243	79	158
Transparent	243	316	237
Red	0	0	0
Green	0	79	0
Blue	243	79	0
Black	0	79	0
Yellow	0	0	0
Morphological Shape Distribution (Number of MPs items)			
Fiber	243	158	158
Fragment	405	395	237
Film	162	158	79

Table 7.6 Summary of Microplastic Measurements in Cattle Manure of Selected Agricultural Fields

Stage	Agricultural Field		
Location Code	CAS	CAN	CAM
Size Distribution			
Number of MPs items	949 ± 146	848 ± 77.1	486 ± 70.1
MPs Mass (mg)	204.4 ± 19.3	185 ± 69.4	89.1 ± 33.1
Total Area of MP particles (mm^2)	6.35 ± 1.6	5.23 ± 1	3.6 ± 1.6
Total Area of MP particles (cm)	18.43 ± 4.1	39.01 ± 18.5	11.75 ± 1.3
Colour Distribution (Number of MPs items)			
White	219	231	121
Transparent	219	265	243
Red	219	231	0
Green	0	154	0
Blue	0	0	40
Black	438	0	81
Yellow	0	77	0
Morphological Shape Distribution (Number of MPs items)			
Fiber	292	308	121
Fragment	365	463	283
Film	292	77	40

Table 7.7 Summary of Microplastic Measurements in Goat Manure of Selected Farms

Stage	**Farm**		
Location Code	GFG	GFM	GFU
Size Distribution			
Number of MPs items	142 ± 141.7	330 ± 190.3	348 ± 173.9
MPs Mass (mg)	14 ± 14.2	22 ± 11	34.8 ± 17.4
Total Area of MP particles (mm^2)	15.67 ± 15.6	42.83 ± 22.2	51.71 ± 26
Total Area of MP particles (cm)	8 ± 7.9	30.08 ± 15.1	21.1 ± 10.6
Colour Distribution (Number of MPs items)			
White	0	0	0
Transparent	0	110	174
Red	0	0	0
Green	0	0	174
Blue	0	220	0
Black	0	0	0
Yellow	0	0	0
Morphological Shape Distribution (Number of MPs items)			
Fiber	0	110	0
Fragment	142	220	348

Table 7.8 Summary of Microplastic Measurements in Swine Manure of Selected Farms

Stage	Farm		
Location Code	SFD	SFK	SFU
Size Distribution			
Number of MPs items	7,432 ± 570.5	12,162 ± 1,583.2	12,468 ± 2,027.6
MPs Mass (mg)	732.2 ± 46.7	1,216.2 ± 65.2	1,532.5± 141.5
Total Area of MP particles (mm^2)	382.82 ± 147.5	367.05 ± 204.5	114.21 ± 76.4
Total Area of MP particles (cm)	233.88 ± 26.9	244.48 ± 43.5	185.87 ± 71.5
Colour Distribution (Number of MPs items)			
White	1,858	2,027	2,208
Transparent	3,770	10,135	10,065
Red	0	0	0
Green	0	0	0
Blue	0	0	0
Black	66	0	16
Yellow	0	0	0
Morphological Shape Distribution (Number of MPs items)			
fiber	191	209	104
fragment	2,027	4,101	5,468
film	443	615	584