Chapter 1: Microplastic Contamination in Pig Production

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Abstract:

This research aimed to study the presence of microplastics in different water sources and diet types using observational research and data collection in the city of Manta. Three variables were considered: feces, water, and feed which pigs ingested. A total of 200 samples were included with 100 (50%) being fecal samples, 50(25%) water samples and 50 (25%) feed samples. The prevalence of microplastics in all analyzed sources was 100%, greater than previous studies. Comparison of MPs concentrations was performed using Student's t-tests and resulted with the p-values of 0.25 for the water sources, 0.33 for the diet types and 0.83 for fecal samples not related to water or feed. Hence, the null hypotheses were retained, and the alternative hypotheses rejected, showing no significant level of differences among the three samples. These results indicate that the problem of global microplastic pollution is emerging, and has caused a significant deposition of microplastics in animal tissues. This accumulated damage as relayed to humans through the food chain, carries severe public health implications.

Keywords: Colours, Contamination, Fibers, Microplastics, Pigs

1 Introduction

Plastic has had a strong presence in ecosystems and, in spite of a variety of attempts undertaken over the years to control waste, it has continued to accumulate, leading to deteriorating quality of life to those living in contaminated environments. This continued increase in plastic waste illustrates the pressing need for novel, more sustainable strategies to reduce the environmental impact of plastics (Martínez, 2024).

Microplastics are harmful because these are able to give birth to diseases in organisms. It is known that in pigs raised for food consumption (Sus scrofa domesticus) such material can cause cancer cells and intestinal obstructions . As a consequence, the ingestion of such meat increases the possibility for contact with these pollutants, which can develop major health problems (Rivera et al., 2023).

Microplastics are estimated by the UN (2021) to have generated as much as a remarkable 1.7 gigatonnes of CO_2 in 2015 and projected to rise to 6.5 gigatonnes of CO_2 by 2050, which would be 15 % of the maximum annual limit memo set.

This shows that as much as it might be an unbelievable thought to many, much of what we consume has plastic traces in them. Because of environmental influence and their availability, plastics disintegrate into tiny particles that are invisible to the naked eye, referred to as microplastics. One of the most important sources of these particles is the disposal of fibers by clothing in the form of microplastics, being of crucial concern to deal with the presence of microplastics in the consumption cycle and their potential health and environmental effects (Martínez, 2024).

The objective of this work was to assess the presence, quantity, and composition of microplastics in water sources and types of diet in pigs destined for human consumption in different pig farms.

General Objective

Assess the occurrence of microplastics in water supplies and pig's diets.

Specific Objectives

- Quantify the microplastics in the various water sources received by the pigs used in the study.
- Quantify the microplastics available in the various diet types offered to the experimental pigs.
- Quantify the levels of microplastics in pig feces unrelated to water sources or types of diet.

II. Theoretical Framework

History of Plastic

Today, microplastics are a part and parcel of human's life and are found everywhere. With the advent of industrialization, mankind has grown more dependent on the use of synthetic materials and their products for various utilizations. With the passage of time, these materials have morphed and changed to satisfy a multitude of purposes, transitioning from extraordinary pieces to ordinary objects as they became a necessity to the advancement of technology and society (Luna, 2020).

Plastics: Through History The first indications of plastic materials date to some 150 years ago. That's when 'so-ho' means the crude material used to substitute for everyday items. This novel material was soon adopted widely due to its ease of production and affordability and over time, that replaced a diverse set of other materials (i.e., metal, clay, stone), revolutionizing many industries and many everyday habitats (Murray et al., 2021).

Importance of Plastic

Plastics are a carbon-based, petroleum-boiled, chemical compound that typically includes small carbon-hydrogen and polymer molecules. Plastic has been heavily mass-produced and distributed since the 1950s. This has led to plastic becoming one of the most prevalent materials in the world for everything from day to day living to industry. Due to its abundance, versatile nature and cost effectiveness, plastic turns out to have widespread use across industries, shaping the modern-day life and fastening the pace of technology (Lema, 2021).

Types of Plastics

In the classification of plastics, different polymeric derivatives are found, and have different applications in sectors such as manufacturing, construction, agriculture, telecommunications, and the like (Castañeta et al., 2020).

Most Commonly Used Plastics

Since it was developed people have wanted plastic in ever greater volumes because of the diversity of its formats. The following are some of the most common forms:

- Low-Density Polyethylene (LDPE),
- High-Density Polyethylene (HDPE),
- Polypropylene (PP),
- Polystyrene (PS), and
- Polyester (PES).

Each one has certain qualifications set by the makers. Due to its variations in chemical and physical properties, it is widely applicable in packaging, textile, electronics, construction materials and so on (Obaco & Pin, 2022).

Impact of Plastic on Ecosystems

The problem of plastic is not new and was even analyzed in several opportunities revealing it to be a high pollutant agent, despite being the main raw material of human lives. The worry is that this stuff, made in huge quantities every year, finds its way into the ecosystem, doing untold damage to flora and fauna. There are initiatives to recycle this material but the waste is too large to manage it and this poses a major environmental challenge (Giraldez et al., 2020).

Plastic Pollution

Plastic production is still expanding, as current measures to reduce or eliminate its use have not been sustainable. Plastics diversity is growing by leaps and bounds. It's estimated that there are already 7.8 billion tons of plastic—basically a ton for every person alive today. Most of this plastic is non-biodegradable or may take hundreds or even thousands of years to decompose (Buteler, 2019).

Plastic pollution is humans' indefatigable enemy. Despite ongoing initiatives by companies in the waste management, plastic pollution remains intractable but worrisomely, it continues to evolve, constituting a menace to marine life. Microplastics are now present in a variety of animal species, enter the food chain and also harm other species with the cycle of contamination also causing problems to ecosystems and human health (Orbegozo, 2023).

Plastic Waste Management

In the country, the recycling of and second lives of plastics can be a major topic in the management of plastic waste. The issue is with plastics that do not degrade naturally — that is to say, those that cannot break down, or be recycled. These plastics have to be transported by a vehicle to the treatment factories and they contaminate, even in controlled conditions (Agreement Ministerial, 2014).

Plastic Degradation

However, in spite of its numerous advantages and applications, the large-scale availability of plastic products has caused a pollution problem. The groups working to clean up the environmental mess caused by plastics have a human labor problem. This is not surprising, with the large amount of plastic employed and thrown away every day (Loaiza, 2022).

Definition of Microplastics

The concept of microplastics was introduced by the United States Air Force in 1968 to describe small plastic particles from industrial processes and analysis of environmental exposure factors. It was registered that for microplastics size under 5 mm and over 1 μm (Crawford & Quinn, 2017).

Origin of Microplastics

The major types of microplastics can be classified as follows:

- Indirect primary microplastics: in small particle shape initially manufactured for certain purposes.
- Microplastics of secondary origin: these result from the fragmentation of other larger plastic and its decomposition over time into smaller particles (León Muez et al., 2020).

Identification of Microplastics

According to Mariano et al. (2021) there are several observations methods for microplastics. One is observation on the flat of a microscope. Transmission electron microscopy (TEM) also permits the observation of extremely small materials in fine detail. Another technique is spectroscopy, which uses infrared light to study the composition of a sample — each form of microplastic leaves a distinct infrared signature. Alternatively, also the Raman method can be employed which works with a laser beam whose light tells the chemical composition of the material.

For investigations of microplastics in feces, the Willy-Mollis technique (altered for parasite detection in feces; modified Willy-Mollis technique) is recommended. This procedure is based on dilution of feces in a saturated sodium chloride solution (table salt). When it’s been left to stand, differences in density cause the solid microplastics to float. This approach enables the determination and investigation of microplastics in faces (Alaín et al., 2021).

Classification of Microplastics

Microplastics can be identified with a number of methods, with microscopy being the most preferred and applied, as the pros outnumber the cons, and they are commonly used and convenient for the researchers. The microscopy method requires the slide to be read to determine the nature, size, and color of the microplastic (Segarra, 2023).

Most definitions place microplastics into either primary or secondary, based on size and intended use. Primary microplastics are typically incorporated into products such as facial scrubs and cleansers, while secondary microplastics, which measure between 5 and 1 mm in diameter, come from the degradation of bigger plastics. They are also categorized according with their shape, to further understanding (Purca & Henostroza, 2017).

Fibers or Filaments

Some research has demonstrated that fibers might be bad for animal health. Microplastics can cause cancer, since cells exposed to the microplastics could change their structure, scientists claim particles are the key to cancer – and are linked to strokes and heart disease. They also harbor toxins that can pass between different kinds of animals. This pollution does not only affect individuals, but also has potential consequences at the level of entire food chains, and even ecosystems (Cisneros et al., 2021).

Such materials can often be seen in lakes, oceans, rivers, and other water bodies intended for human use, which have become contaminated with waste. Their occurrence in the aquatic system is of great concern to environmental health and water quality because they can impose dietary risk to aquatic life and human health (Miller et al., 2024).

Fragments

Microplastic particles generally have sharp, rigid edges that can be hazardous to aquatic life with potential laceration of organs and tissues of marine species (Cruz et al., 2020). Also, broken plastic fragments serve as carriers of organic pollutants as DDT and PCBs type, which act as endocrine disruptors in biota (Torrez-Pérez et al., 2021).

Pellets

Pellets, like microbeads, are one of the primary microplastics to be intentionally produced at this form, not fragmented due to time or environmental conditions. Such particles are prevalent in the sea and are intentionally ingested by marine animals foraging for food such as shellfish. There have been cases of seagulls defecated with this type of microplastic (López-Monroy & Fermín, 2019).

Microbeads

Microbeads, as their name implies, are minuscule ball shaped particles, usually measuring 500µm or smaller, found in a variety of personal care and cosmetic products such as exfoliating scrubs, facial washes, creams, gels and toothpaste. They are also cautiously applied in the field of medicine (Rojo-Nieto & Montoto, 2017).

Films

Macro plastics refer to plastic items that can be seen with the naked eye; however, microplastic films are thin layers or sheets that come loose from larger plastic objects, like bottles, bags, and containers, as they degrade and absorb environmental components. These films are airborne and may be washed into oceanic or other water bodies and contribute to pollution (Löder et al. pool sand filter676 2015).

Toxicity of Microplastics to the Gut

Trophic chains are impacted by plastic waste and litter that has been transported to deep-sea. It also leads to further health issues on animals and organisms that ingest these substances (Barros & Léon, 2024).

Upon ingestion and accumulation in the gastrointestinal tract, microplastics may trigger the development of gut toxicosis, that is to say as the execute of the toxic chemical additives, oxidative stress and disturbance of the gut microbes (Barboza et al., 2018).

Consequences of Microplastic Ingestion

Apart from being hazardous to the environment, plastics have direct biological effects when they are ingested. At least, according to the IARC (International Agency for Research on Cancer), some plastics and their derivatives are considered as carcinogens (Bollain & Vicente, 2020).

The small intestine has an important function of absorption of nutrients. This organ is considered to be general and if an animal is fed with a diet containing heavy metal, then this part of body will probably be affected. Contamination can attenuate intestinal integrity, affect absorptive capacity, and elicit inflammatory reactions contributing to a poor state health (Julià, 2020).

Microplastics with different types and sizes have toxicity in different species, they can be showed through the physiological response (Yang et al., 2021).

General Information About Pigs

The domestic pig (Sus scrofa domestica), a member of the Suidae family, is a mammalian artiodactyl. It is distributed throughout the world, and respectively has typically short legs with three-toed hooves and a close barely-there tail, as well as a small rounded body and long tubular snout. They have multicolored bodies with coarse hairs. Pigs are high consumers and reach puberty around 110 kg (Moreira, 2021).

The breeding of pigs now is very popular and well received. Besides beef and chicken, pork is one of the most widely-consumed meats globally, due in part to the fact that it facilitates the production of "good" cholesterol, which is made in the liver and more readily expelled (García, 2023).

Transmission of Microplastics

The specific way the microplastics get into people from eating the tainted pork is less clear. We can conclude, however, based on Alcocer et al. (2019) have reported that aquatic organisms consume these materials and they are integrated inside their body and finally in the food chain. Inferring from this, contaminated pork can also be a pathway for microplastics transmission to humans.

III. Methods

Procedure

Sampling and laboratory analyses

Fecal Samples:

- Feces were collected in glass jars and stored in a cooler.
- The samples were sent to the Laboratory of the FMVZ, University of Guayaquil to be analyzed under biosafety measures in place.

- Processing The technique used for processing was the modified Willis-Molloy technique.
- All samples were weighed using a digital balance and a watchglass with a standardized weight of 2 g per sample.
- 25 ml of supersaturated saline solution was added with a pipette to each of 50 ml test tubes, which were stirred with a spatula for homogenous mixture. Test tubes were sealed with aluminum foil.
- Tubes were incubated at 37.5°C for 4 h.
- After incubation, 50-μl supernatant was mounted on a microscope slide using a micropipette and a coverslip, and the sample was read under a microscope at 10x and 40× magnification.

Feed Sample Preparation:

- The feed samples were obtained directly from the pigs' feeders with gloves and a glass jar.
- Labeled the samples to avoid confusion.
- In the FMVZ laboratory the samples were ground and 10 g were placed in a flask with 10 ml of 10% potassium hydroxide. 3× sample volume of KOH was added to the sample.
- The blended product was covered with aluminum foil to avoid potential environmental contamination and allowed to stand for 72 h.
- The organic matter was broken down with the help of potassium hydroxide, but, importantly, the microplastics were preserved.
- 1 ml of sample was collected with a 5 ml pipette at 72 hours.
- 10 ml 25 ml Polythene opened Extract and 10 ml 96% ethyl alcohol were measured out and transferred to a 25 ml graduated cylinder.
- The sediment was left to stand for 24 h.
- One drop of the supernatant was then transferred onto a glass slide and covered by a coverslip.
- Microplastics were observed under a light microscope at x10 and x40 magnification (Lino, 2019). Top of Form

Water Sample Preparation:

- Sample was taken directly from the drink source of the animals with gloves and glass container.
- The sample tag contained the specific animal information for identification and to prevent confusion.

- The samples were transferred to the FMVZ laboratory and a drop of the water sample was placed over a filter paper using a pipette.
- The sample was then air dried and the filter paper was transferred to a slide for microscopic examination.
- The sample was observed with an optical microscope with 10x and 40x objectives in search of microplastics.

IV. Results and Discussions

A total of 200 samples were collected in this study, representing 100% of the sample population. Of these, 100 were feces samples, 50 were water samples, and 50 were feed samples provided to the pigs.

Variable	N (Valid)	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
MPs/g Feces	100	41	2	43	10.76	1.096	7.753
MPs/ml Water	50	24	1	25	11.30	0.701	4.958
MPs/g Feed	50	26	1	27	9.20	0.777	5.493
Valid N (by list)	50	—	—	—	—	—	—

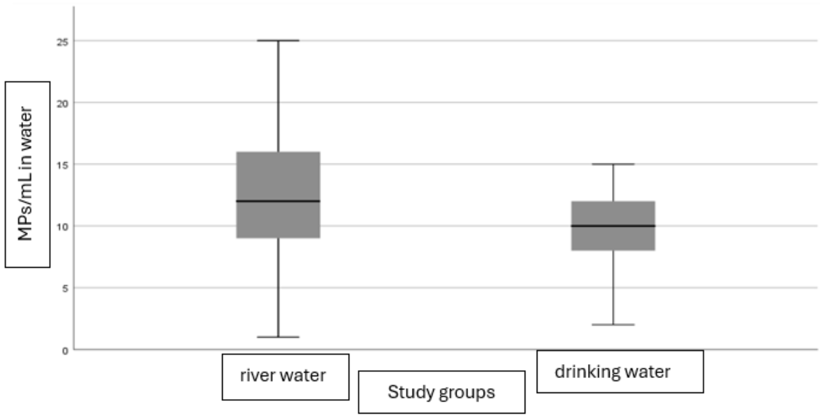
Sampling based on 200 processed-samples 100 from feces, 50 from water sources, 50 from diet type showed 100% occurrence of microplastic in processed samples.

This is significantly higher than those found in the previous studies of Peñafiel & Peñafiel (2023) (47% calculated) and Chusan & Cruz (2023) who found 54%._ Once compared to the findings of Peñafiel & Peñafiel (2023) (47%/_ calculated) and Chusan & Cruz (2023) (54%) there is a considerable increasing presence of microplastics in the present time. This increase may be a result of increased environmental pollution, or current management systems.

Classification	Variable	Group 1	Group 2	Mean (1)	Mean (2)	Mean Difference (1–2)	95% CI Lower	95% CI Upper	p-value
Water Sources	MPs	Potable	River	10.48	12.12	-1.64	-4.45	-1.17	0.2462

Figure 1

Box Plot Representation of MPs/mL in Water Sources (River Water and Potable Water)



In the study the number of MPs in the potable water that is used in Porcinos S.A. industrial farm has an average of 10.48 MPs/mL and the average of the water from the Manta River was 12.12 MPs/mL. On average, this equates to 11.30 MPs/mL 5 of water from the sources studied (Tab. 1).

An independent samples t-test was used to compare MP levels between the two water bodies, and a p value of 0.24 was obtained. This finding has led to rejection of the alternative and hence acceptance of the null hypothesis that there are no statistically significant differences between the sample groups (cf Table 2 and Fig. 1).

MPs were detected in 70.83% of Manta River water used to feed pigs and in 29.17% of potable water from Porcinos S.A. Our results indicate that less-purified water has more MP contamination. Not even bottled water -- purified via reverse osmosis, and then stored in glass bottles -- was free from such contamination, which perhaps indicated how widespread microplastics were in drinking water, and how they were bringing contaminations down the food chain. Flores & Orozco (2022) in their study observed 60%–89% MPs prevalence in purified water.

Furthermore, a study of the quantification of MP in water by Ayora & Pinzón (2024) has reported for the detection of MPs of 55 of 72 horses drinking river water. And 22 of 28 horses that drank treated water also tested positive. These results are in line with the present study by illustrating that MPs were more abundant in short-treated waters and assuming that such water was also a vector for MPs in pigs.

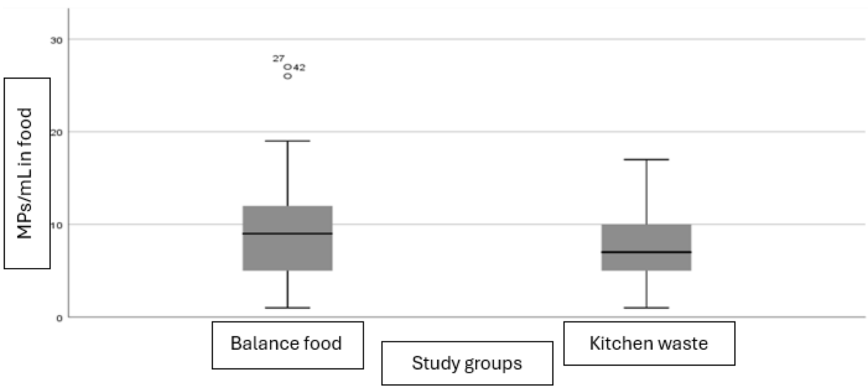
Table 3

Independent Samples t-Test: Statistical Summary of MP Quantification in the Types of Diets Provided (Balanced Feed and Kitchen Waste)

Classification	Variable	Group 1	Group 2	Mean (1)	Mean (2)	Mean Difference (1–2)	95% CI Lower	95% CI Upper	p-value
Diet Types	MPs	Balanced	Kitchen waste	9.92	8.49	1.52	-1.61	4.65	0.3334

Figure 2

Box Plot Representation of MPs/g in Balanced Feed and Kitchen Waste (Lavaza)



The average of the Amounts of MPs/g was 9.20 (Table 1). When calculating to the average value of MPs/g in g of feed, 9.92 yield and 8.49 MPs/g were observed in balanced and kitchen waste (lavaza), respectively. The independent samples t-test showed no significant differences between the two feeds ($p = 0.33$) with regard to MP concentrations. Such finding accepted the null hypothesis and rejected the alternative hypothesis, which means that there are not significant differences between the type of test used during sample analysis, as seen in Table 3.

The quantity of microplastic consumed by pigs is alarming as the accumulation of these lets reward of the in their gastrointestinal track led to various forms of damages. An inconsistency in the concentration of the microplastics was noticed in the two different feed samples examined in this study. Lavaza, which consists of peels, legumes, and other food scraps, displayed a relatively high content of microplastics (46.37%).

Counterintuitively, commercial pelletized balanced feed had an even higher concentration (53.63%) of microplastics. This problem may be related to the manufacturing process and the utilization of synthetic fiber plastic sacks for the packaging of these feeds. It emphasizes the significance of not to underestimate the contribution of microplastics that are present in commercial feeds that might contribute as a major cake play in contaminating to the pigs and which play onward, to the human consumers of the meat obtained from the pigs (Mazzoleni et al., 2023).

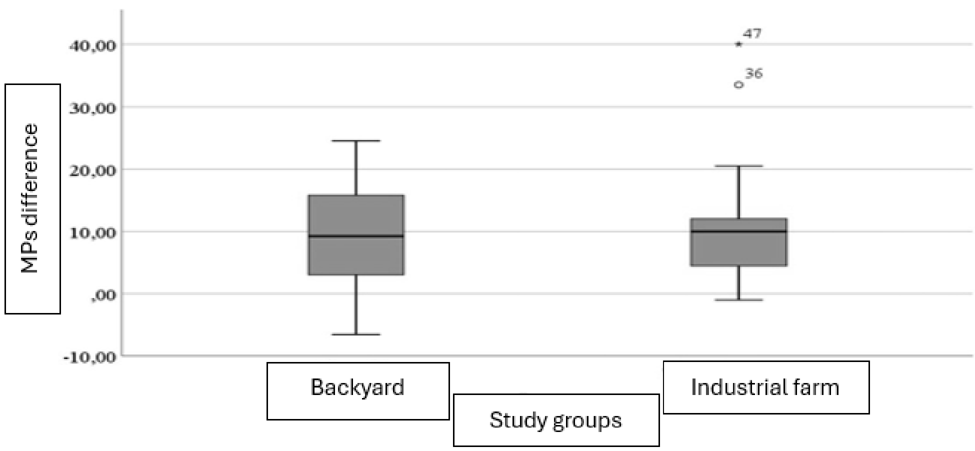
Table 4

Wilcoxon Test for Independent Samples: Determination of MP Concentration in Pig Fecal Samples Unrelated to Water Sources and Diet Types

Classification	**Variable**	**Group 1**	**Group 2**	**Mean (1)**	**Mean (2)**	**SD (1)**	**SD (2)**	**W**	**p (2-tailed)**
Location	MP difference	Industrial Farm	Backyard	10.74	9.35	9.53	9.15	648.5	0.8309

Figure 3

Graphical Representation of MP Quantities in Pig Fecal Samples Unrelated to Water Sources and Diet Types



When analyzing the volcano difference in MPs, a non-parametric test was performed to compare two groups: industrial farm and backyard. The Wilcoxon test for independent samples was applied and returned the pvalue of 0.83, which greater than the pvalue threshold of 0.05. This led to rejection of the alternative hypothesis and acceptance of the null hypothesis that there is no significant difference between the two groups.

Difference values were calculated by adding up the quantified MPs in water and feed from both sampling sites and subtracting the MPs that were present in the feces of a group. A normality test was also conducted which revealed that normality was violated for this data, therefore Wilcoxon test was considered.

Castañeta et al. (2020) explain that inhalation, ingestion, and direct skin contact are the primary methods of human exposure to MPs. These results indicated the potential of another ways of entry not considered in the pigs of this research, that is, inhalation and skin contact. According to the authors, MP smaller than 130 µm is capable of infiltrating tissues and inducing responses in the face of pollutants as well.

The findings of initial fecal samples indicate that in feces of backyard pigs the proportion is 52.35% and 47.65% is from Porcinos S.A. These are all extremely large numbers, as it seems most of these microplastics are still left in the soil, leading to the cycle of pigs in the backyard still being exposed to them. On the contrary, despite pigs on factory farms experience cleaning treatments, the MPs can be detected, although in lower amounts, because microplastics cannot be entirely removed from the environment.

In the research of González-Puetate et al. (2024), different frequencies in species were observed: 80% in sheep, 93% in goats, and 54% in cattle. These findings are in direct contrast to the findings from the present study. It seems that the diet and management of the animals as well as the diet and source of the MPs may influence the MP contents in the tissue. For instance, goats, as lower-maintenance and free-foraging animals, are also more likely to ingest microplastics—like pigs.

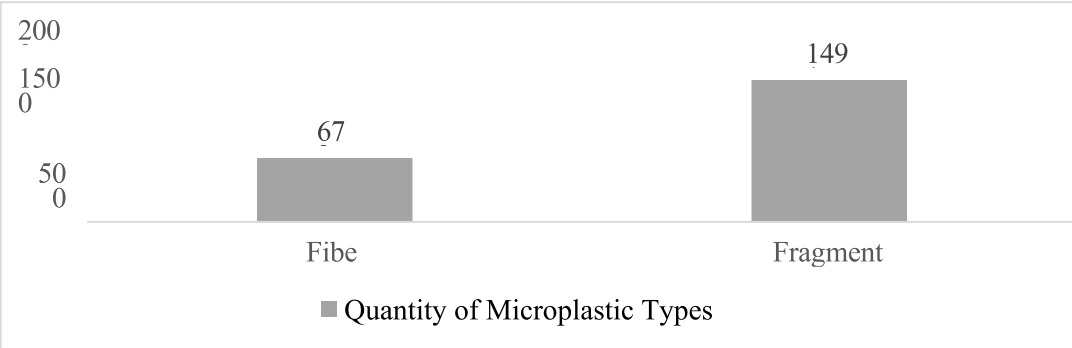
Table 5

Quantification of Microplastic Types

Microplastic Types	**Frequency**	**Percentage**
Fibers	673	31.10%
Fragments	1491	68.90%
Total	**2164**	**100.00%**

Figure 4

Graphical Representation of the Quantification of Microplastic Types



In the microplastics study, several types were recorded, such as fibers (31.10%) and fragments (68.90%), of 5 mm to 500 mm. In the specific study area analysis, for the type of plastic utilized and the different routes through which they entered the environment, microfibers and fragments were the most important. Microfibers were less frequent in the study, however, most common source of them was also covers and clothing indicating the contribution of textile products to microplastic pollution (Arboleda et al., 2024).

Conversely, Sánchez & Guanoluisa (2024) found a 93% presence of fibers and a 31% presence of fragments in goats from a canton in Manabí. This variation is indicative of differences seen across different animal species. Fiber and fragment content vary depending on the environmental conditions the animals are conditioned in and the grade of contamination they are exposed to.

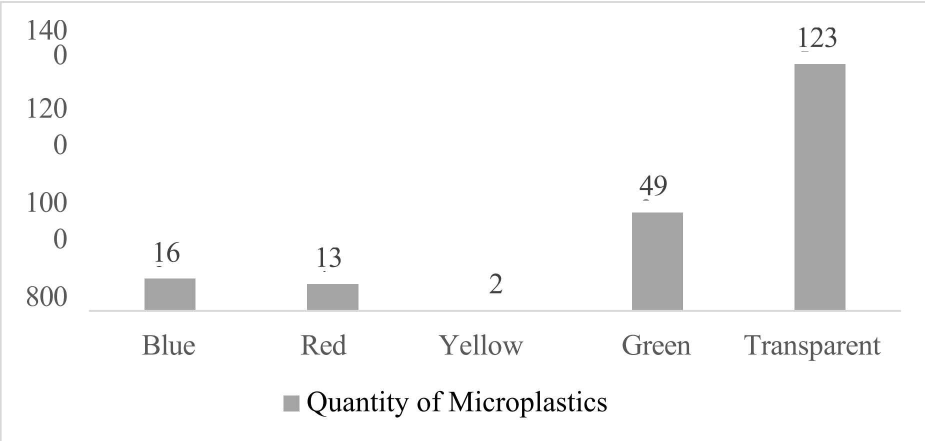
Table 6

Color Frequency of Microplastics

Microplastic Color	Frequency	Percentage
Blue	160	7.91%
Red	134	6.62%
Yellow	2	0.10%
Green	493	24.36%
Transparent	1235	61.02%
Total	**2024**	**100.00%**

Figure 5

Graphical Representation of the Frequency of Microplastic Colors



In the present study, microplastics were mainly transparent (61.02%), as well as blue (7.91%), red (6.62%), green (24.36%) and yellow (0.10%). In a study by Añaños (2024), it was found that the black catfish (Rhamdia quelen) had the highest frequency of white microplastics (30.70%), while the Peruvian sardine (Creagrutus yanatili) had the highest levels of blue (27.77%) and white (27.77%) microplastics. Nevertheless, no final judgment is made with respect to the influence of color on the results, the author writes. However, even the colour of microplastics betrays something about their likely source (polystyrene is usually clear). In addition, here you should bear in mind that in some cases the color of plastic is modified with the help of colorants in order to match the product needs.

However, Sánchez and Guanoluisa (2024) found a different result - black accounted for 40.9% of the microplastic pieces. This is unlike the present study in which the prevalence of transparent microplastics (61.02%) was greater.

V. Conclusions

A higher prevalence of microplastics, indicating more severe contamination via multiple pathways (e.g., animal feed and drinking water), confirmed in the previous studies and this research.

The quantities of microplastic measured in rivers and other water bodies are high enough to regard such poorly purified water sources as a source of contamination. But the other worrying thing is that drinking water made for human consumption is also a direct exposure pathway for these contaminants. Moreso, the presence of microplastics in different diets fed to pigs it's sinful because not even forages or commercial feed is free from microplastics.

The global occurrence of microplastics in fecal samples, water and diet is a clear indicator for the need to propose future strategies to reduce production of plastic and reduce waste. Environmentally friendly practices might have the potential to drive down the annual carbon footprint produced due to manufacturing of these substances.

Two forms of microplastics were recognized in this study, fragments and fibers, with the fragments being more dominant (1,491) than the fibers (673). This indicates that plastics are being degraded and are releasing different forms into the environment.

Variation was observed in color of the microplastics; this is consistent with the fact that these materials have diverse origins, with a predominance of visible microplastics, presumably resulting from the breakdown of consumer products (which are potential sources of contamination in the environments, and in this case, contaminated pigs).

Moreover, we found other potential pathways for microplastic exposure including inhalation and skin contact which were not tested for pigs in the current study. It is suggested that additional studies to investigate these exposure pathways be performed.

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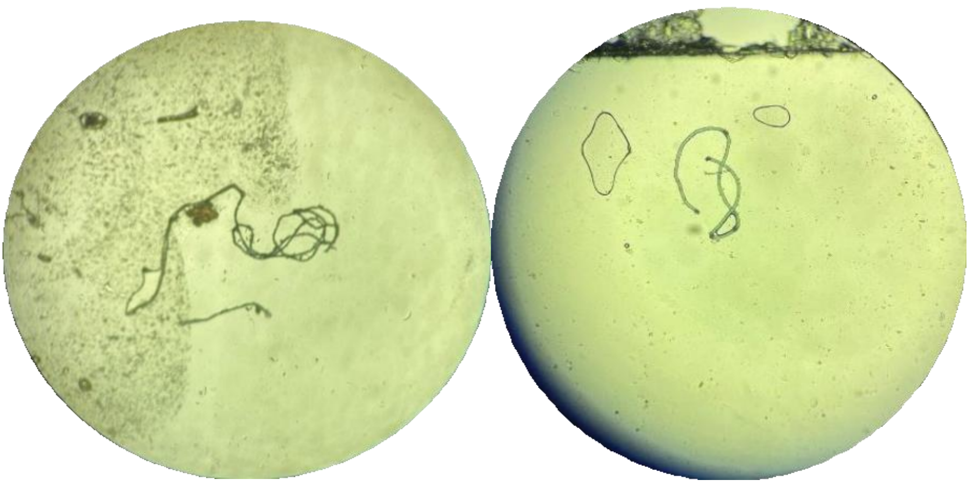
Annexes

Annex 1

Red-colored fiber-type microplastics observed under the microscope

Annex 2

Transparent-colored fiber-type microplastics



Annex 3
Fragment-type microplastic in transparent and red colors

Annex 4
Green-colored fiber-type microplastic