Chapter 2: Microplastic Contamination in Horse Husbandry

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Abstract:

The presence of MPs in water source, two types of diets, and in equine feces in Guayas province, Ecuador was assessed. The aims were to measure MPs in water and feed, and their presence in feces. A cross-sectional, descriptive, observational field-based research was carried out. A total of 200 samples (100 samples of feces, 50 from water, and 50 feed) were obtained from the Guayaquil Country Club and the Miguel Salem Dibo Racecourse. Isolation of BmβCope1 was performed by flotation using a modification of the Willis-Molloy method, and observation was carried out with an optical microscope. The SPSS software was applied for statistical analysis. Results MPs were detectable in all samples, with a concentration of 58% of the river water and 42% of the cistern water, and 66% of the balanced feed and 34% of the alfalfa. In terms of presence of MPs in feces samples, the 65% corresponded to GC and 35% represented HT. In the category of MP types, fibers made up 86% of all the MPs detected in this study. The major color was transparent which constitute 58.23% of MPs. The high prevalence of these particles in all samples indicates a ubiquitous environmental contamination of the equine settings evaluated. Additional studies are requested to assess MPs adverse effects on equines and to devise ways of reducing the environmental burden.

Keywords: Equines, microplastics, pollution, presence, quantification

1 Introduction

Microplastics (MPs) have been neglected for ages in the assessment of their occurrence and effects on living organisms. Plastic waste degrades very slowly and it decays by the action of living organisms, water, radiation, and wind to form MPs (Del Rosario et al., 2020).

In addition to developing knowledge about the impact and implications of plastic pollution for ecosystems, there is a need to examine the presence of plastics in human sources including water, food amongst others and the potential health impacts. According to Li et al. (2018), such plastic pieces have the capacity to transport toxic

https://doi.org/10.70593/978-93-7185-104-6_2

compounds from industrial practices, with serious health implications for both human and animals. Fish studies have revealed that MPs and their related toxins are accumulated into their body, damaging their intestines, and altering their metabolisms.

Microplastics are very little in size, smaller than 5 mm (Mora, 2022). They are the smaller plastics ripped from bigger ones, like bottles and bags. These small particles may end up in waterways via multiple routes, such as urban or farming runoff. In water, they may be consumed by many species of animals, leading to concerns about potential negative health impacts.

Horses receive much of their drinking and feed water from natural and artificial water sources. Furthermore, they may be also fed with different forages and concentrated feeds that might be contaminated with MPs. The evaluation of equine exposure to MPs is not only essential to evaluate possible health and welfare effects, but also to consider the potential implications for food safety and human health (Semmouri et al., 2022).

The identification and quantification of MPs in water samples and other matrices, such as diets and equine feces, is challenging as MPs are small and require sensitive and selective methods to analyze. This is crucial to comprehend the extent and the consequences of MPs contamination in these animals (Pérez et al., 2021).

General Objective:

- Assess the occurrence of microplastics in water sources and the diets given to horses in Guayas province, Ecuador.

Specific Objectives:

- Determine the levels of microplastics in the different types of water supplied to equines, in order to evaluate their potential as a pathway for contaminants to enter the organism.
- Determine the amount of microplastics present in the different diets offered to equines, considering their possible implications in the digestive tract.
- To evaluate the amount of microplastics present in horse manure, excluding the contributions coming from water and diet, as an indirect indicator of absorption and excretion as a function of equine metabolism.

II. Theoretical Framework

Origin of plastic

According to Caballero et al. (The poet 2019) "Plastic (its other name is celluloid), was invented in the United States in 1860, but when it was dirst discovered, it was used as a substitute for ivory in many applications. Its application immediately transcended to all parts of the world resulting in a substantial raise of the production after polyethylene was introduced.

Plastic was introduced as a make and packaging material for human use. 1In the long run, it turned out that plastic cause a problem because it has a low recyclation potential. According to Yulu et al. (2024), polyethylene terephthalate (PET) and polyethylene are among the most widespread types of plastic and show particularly long decomposition times (over 10 years).

Types of plastics

Plastic consists of larger particles (polymers) made of smaller particles (monomers). The most popular types are polyethylene terephthalate (PET), applied for the manufacture of food containers; polyethylene (PE), used in the automotive and food industries; polypropylene (PP), found in medicine bottles and packaging; polystyrene (PS), used in disposable cutlery and cups; polyvinyl chloride (PVC), present in water bottles and shampoo containers; high-density polyethylene (HDPE), used for toys and detergent bottles; and low-density polyethylene (LDPE), occurring in plastic food bags (Liu et al., 2024).

Origin of microplastics

In the last fifty years, production and usage of plastic materials has steadily increased worldwide, becoming a major environmental issue because of the difficulties in recycling. This implies that small plastic debris (microplastics (MPs), for instance) and nano plastics, are present in one way or another, across the ecosystems of the globe (Urli et al., 2023).

Murray et al. (2021) argue that, in contrast to organic waste breaking down by bacterial activity, plastic degradation reduces the size of fragments and produces further fragmentation; plastic does not biodegrade. Bottles, bags and containers are slowly whittled into microscopic pieces by solar radiation, marine salinity, precipitation and temperature.

The MPs consist predominantly of five types of polymeric materials; polypropylene, polyethylene, polystyrene, polyvinyl chloride, and polyethylene terephthalate. Such synthetic origin microparticles, which originated by human activities, could potentially have a severe and long-term effect on ecosystems (Karthick and Siddhuraju, 2024).

Classification of microplastics

Castañeta et al. (2020) assert that MPs are found in the environment in different shapes and forms such as spheres, pellets, foam, fragments, fibers and foil. These forms are dependent on the original shape of the primary plastic and the degradation process it has experienced including the conditions of erosion.

Over time, plastic litter that is introduced into soil decomposes into small fragments, fibers, and particles (Quan et al., 203).

Microplastics in water

Freshwater systems serve as one of the main repositories where MPs tend to accumulate in the environment. Powerful motivation to prompt us to find an exact value of their small synthetic particles today (Ermolin, 2024). Presently, the primary contributors of these particles are certain neglected plastic objects, industrial intermediates, personal care products and clothes made from synthetic fibers (Upadhyay et al., 2024).

Use of biodegradable or oxo-degradable products has not been reduced, and, thus, the direct chemical or physical impact to marine or terrestrial ecosystems has not been reduced. Plastics Although their physical appearance belies a sense of kinetic stability, plastics can be progressively subjected to physical and chemical degradation processes, eventually leading to the formation of smaller entities such as microplastics (MPs) and nano plastics (Bollaín et al., 2019).

Huerta Lwanga et al. (2017) note that globally, the principal sources of plastic pollution include the oceans, waste dumps, landfills, and home gardens. Plastic packaging alone makes up 37% of all plastic waste. The most commonly used polymer in plastics worldwide is polyethylene. Even in freshwater systems, the degree of disequilibrium between the numbers of MPs polluted can be as high, or higher, than that of marine environments.

Determination of amount and toxicological effect of MPs in freshwater ecosystem is largely neglected, unnoticed, and less reported than marine water bodies. The occurrence, bio-concentration, and toxic effect of MPs in freshwater biota is equally relevant and considerable as that in marine environment (Khan et al., 2024).

Another pressing and significant issue in the future is that microplastic particles are becoming efficient hosts for some microbial pathogens, and their genes could be carried on plasmids resistant to antibiotics (ARGs) in marine, freshwater, wastewater, and urban river ecosystems. These drug-resistant bacteria associate with microplastic to create synthetic community referred as "plastispheres," which create an overall conducive environment for biofilm formation (Kawaljeet et al., 2022).

According to Sáenz et al. (2023) the emission of wastewater into natural water bodies adds microplastics and additives to environmental compartments. These are degradative to the environment and harmful to the diversity of the biota.

Microplastics in terrestrial ecosystems

The abundance of plastic fibers and particles generally fluctuates between ecosystems. The greatest levels were found in grasslands and were significantly higher than those in tropical rainforest, pine plantations and savannah (where these presented similar and lower levels). One long-term rising trend in microplastics (fibers and fragments) is seen in parallel with an increase in clay and sand content of the soil (Álvarez et al., 2021).

Kasa et al. (2022) have noted that recent studies on MPs have revealed their ubiquity across all habitats in terrestrial ecosystems. They have been released from a number of sources including, but not limited to, organic fertilizers (e.g., compost and sewage sludge), plastic films utilised in agriculture, atmospheric deposition, plastic waste in landfills. The MP found in the agriculture field may be associated with the use of treated sewage sludge with synthetic fibers from household products.

In contrast, Spieker and Kleunen (2023) also speculate that plants can arguably be not just victims of plastic pollution in the environment but are likely able to respond actively to it as a potential environmental stressor. What is clear is that microplastics in the soil induce changes in the physical and chemical properties of the soil, leading to a decline in the environment in which soil microorganisms live, and to a certain extent cause a serious negative effect on plant growth (Chen et al.

Microplastics in Food Products

The use of plastics as protective coverings in farming operations represents a key source of plastic pollution in rural areas. These plastic sheets are not always cleared from fields, even after harvesting. Subsequently, those remaining fragments fractural into smaller particles that are commonly carried away by the action of wind and surface runoff water (Beriot et al., 2021).

According to Canga et al. table salt is one of the positive major sources of microplastics (MPs), and a ratio of approximately x 100 was observed between the studies with the highest and lowest reports of MP content.

Similar average levels of about 0.14 microplastic particles per gram tissue of cattle and sheep in different edible organs (muscle/viscera/intestines) were detected. The cow muscle tissue contained the highest levels, approximately 0.19 fragment/g. The most abundant found fibers in these samples were of nylon polymers and other synthetic fibers (Bahrani et al., 2024).

Microplastics in Equine Feed

Diet is everything when it comes to horse breeding and welfare. Although they are herbivorous they are non-ruminants therefore knowledge of the nutritional composition of the grass that they consume is important in the evaluation of their diet (Pellegrini et al., 2018).

Microplastics in Feces

One way to create and spread the contamination is by using fresh or processed animal manure as natural fertilizer on croplands. This human practice is an avenue for small-sized plastic particles to permeate cultivated soils (Sheriff et al., 2023).

Liu et al. (2023) discovered MPs in the two south-sourced soil samples and also in the feces of Equus kiang which is the Tibetan wild ass. The average of median MP concentration was 102 and 4.01 particles per g of dry feces and soil, respectively. Fifty micron long slender fibers were the primary morphology of MPs in both samples. The total number of different types of MPs was generally higher in feces than in soil samples.

González-Puetate et al. (2024) carried out an observational cross-sectional study on 300 ruminant fecal samples. The flotation by saturated saline solution approach showed that more than three-fourths of the samples (75.67%) were found to be positive for microplastics.

Pinzón and Ayora (2024), in their research, realized 77 fecal samples obtained from 100 different horses showed the presence of MPs. Hence no MPs were detected in 23% of the samples analyzed. The modified Willis-Molloy technique was employed to perform the analyses.

Samples of feces from 100 pigs were analyzed following a modified Willis-Molloy flotation method (Peñafiel and Peñafiel, 2024). The process used an super- saturated saline solution. The results of the research were shocking, with microplastics detected in 47% of samples.

Interactions of Microplastics with Organisms Living organisms cannot readily distinguish microplastic from their own food sources and filters when the size of microplastics is made to fit, and their ingestion, accidental as it may be, is possible [11,12].

Several organisms can ingest or uptake MPs in different methods, and this may result in metabolic, physiological and health disorders. Furthermore, MPs owning large surface areas could uptake the diverse contaminants, heavy metals (HMs) and persistent organic pollutants (POPs), which have severe impacts on human health (Chang et al., 2022). This was confirmed by the finding of MPs in biological wastewater, blood fluids and placental tissues. The accumulation of these particles can lead to adverse effects in tissues and organs, presenting an additional health risk (Hao et al., 2023).

They may also affect the reproductive system (infertility, uterine and neuro toxicity) (Afreen, 2023). Moreover, liver injury, gallbladder and pancreas impairment, weight loss, low lipid reserves, inflammatory responses, oxidative stress which causes apoptosis, genetic damage, intestinal disorders, kidney failure and muscle wasting have been recorded (Huang et al., 2021).

The liver is likely to be at risk of potential micro/nano plastics toxicity. Yiling et al. (2023) exhibited inflammation, liver dysfunction, fibrosis, and metabolic disturbances.

Jiang et al. (2024) used a mouse model to investigate the impact of such ingestion of MPs. The effects of MPs in long-term hematopoietic system hematopoiesis They found that exposure to MPs in a long-term and cumulative manner caused significant damage to the hematopoietic system. Both OR MPs and in vivo intestinal microbiota transfer from pre-MP-treated mice modified the self-renewal and regenerative capacities of HSCs. Notably, MPs did not kill HSCs directly, but induced changes in intestinal structure and permeability.

Identification of Microplastics

The Willis-Molloy method allows concentration of parasitic forms and microplastics by means of microscopy of the supernatant. This upper layer has a more appropriate density and is formed by mixing the feces sample with a supersaturated solution, causing the elements measured (of lesser density than the supersaturated liquid) to automatically flow upwards and be concentrated at the surface for analysis (Carrasco et al., 2023).

Syamsu et al. (2024) state that the optical microscope is a method for characterization of microplastics, i.e., to find out its features (quantity, morphology, etc.). For the polymer type contribution of microplastics, Fourier-transform infrared in attenuated total reflectance (ATR-FTIR) mode is used.

Coloration of Microplastics

Gómez et al. (2024) observed the presence of microfibers of different colors; black was the most common color. Therefore, these results indicate that sea lions can act as bioindicators of the occurrence and transfer of microplastics across the food web of the study area.

A total of 54 microplastic particles were observed in his study, representing various colours (Percca, 2021). Blue particles constituted the majority: 20 units (37% of the total), lower were yellow: 17 units (31%). 6 microplastics (11%) were light blue; 11 (21%) were others (fuchsia, green, white and orange).

II. Methods

Methodology

Unit of Analysis

- The equines

Type of Research

This was a field based, non-experimental, observational study conducted as cross-sectional descriptive and analytical study.

The on swelling investigation seeks to explore and describe one phenomenon. The researcher has no role in doing anything to, or manipulating, variables and instead simply observes and records features of the phenomenon. They can be either descriptive or analytic in nature depending on the main purpose of the investigation. This model is recognized for its speed, simplicity and for allowing the prevalence of a condition to be estimated directly. In such research projects, exposure and effect are assessed concurrently at the same time (Cvetković et al., 2021).

Population and Sample

Convenience sampling was carried out and a total of 200 samples were collected: 100 fecal samples, 50 food samples and 50 water samples. Fifty percent of the samples were taken at the Guayaquil Country Club, and the other 50% at the Miguel Salem Dibo Racetrack.

Hernández (2021) reported that convenience sampling is largely influenced by the level of access and the availability of the participants who are considered most relevant to the researchers and enable the researchers to generate their own number of sample size during data collection.

Variables

Dependent variable: Presence of microplastics

Explanatory variables: Kind of drinking water, type of diet, feces

Measurement Instrument

Microscope

Statistical Analysis

Data were analyzed in percentages, frequency tables and cross-tabulations with SPSS (Statistical Package for the Social Sciences) version 26.

SPSS is a powerful program which can be used for a wide range of purposes. It is able to execute various mathematical operations such as arithmetic, algebraic, and trigonometric calculations. It is also a sophisticated data manipulation system that allows users to interactively query data in real-time. One of its strengths is the ability to display results in a personalized manner, which is conducive to the interpretation of analyzed data (Mayorga et al., 2021).

Sampling Procedure

The horses were selected randomly. Disposable wooden tongue depressors were used for sample collection. The samples were first placed into glass vessels, then maintained within a thermal transporting container. The samples remained stable and were transported without contamination and at the necessary temperature through this method.

Sample Processing Procedure

The methodology presented in this study is adjusted to perform in schools or in veterinary universities teaching animals and veterinary Human anatomy if not counted by appropriate facilities in each institution. The procedure was adapted from the proposed by previous studies using the flotation technique (Agrocalidad, 2018):

1. They removed each glass jar containing the fecal sample from the thermal container.
2. The samples were weighed after the weight of the watch glass was subtracted from the total weight and the unit of measure was fixed at 2.5 grams.
3. The samples were subsequently transferred to test tubes containing a 28 mL of supersaturated saline solution.
4. The test tubes were agitated with a glass stirrer until the contents were thoroughly mixed.
5. The test tubes were incubated for 4 hours at 37.5 °C.
6. The supernatant was removed with a glass pipette and a drop was pipetted out and placed on a microscope slide keeping it for about 1-2 minutes and then covered with a coverslip.
7. Samples were observed under a conventional optical microscope starting with 10x objective and 40x to confirm the existence of microplastics.

Procedure for the Detection of Microplastic in Alfalfa and Balanced Feed:

1. All food stocks were maintained in small glass jars at room temp.
2. Samples were weighed by means of a digital scale with watch glass, 1 g of food being considered as 1 unit.
3. Each sample was homogenized in 10% sodium hydroxide (20 mL in a test tube with a glass stirrer).
4. The test tubes were incubated at 37.5°C for 72 h.

5. 1 mL was then drawn from each sample and added to 10 mL of 96% ethanol in another test tube and allowed to sit for 24 hours.
6. A drop of the supernatant was aspirated using glass pipette, added to microscope slide and covered with a coverslip for examination, after the incubation.
7. The sample was observed by optical microscope by field 10x and 40x to verify the microplastics.

Protocol to Determine the Occurrence of Microplastics in Water:

1. The water samples were kept in glass bottles at a natural thermal condition.
2. Ten mL from each sample was removed and filtered layer of filter paper used as a funnel. Each filtration was carried out in a different test tube.
3. The filter papers were incubated and dried.
4. After drying to completion, the filters were moved to an optical microscope and preparing visualizing slides with 10x and 40x objectives to identify MP presence.

III. Results and Discussions

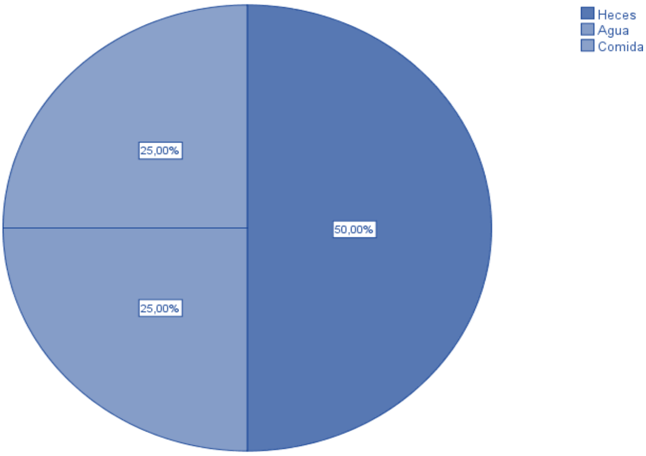
For this study, 100 fecal, 50 water from two different water sources, and 50 diets from two types were analyzed, with a total of 200 samples. Microplastics (MPs) were detected in each type of sample. Fecal and food samples were examined by the modified Willis-Molloy flotation technique, and the water by a technique of filtration and drying. The MPs visualization on the samples was performed by optical microscopy, which met the requirement of identification and quantification of the microplastics.

Table 1

Presence of Microplastics in Fecal Samples, Water Sources, and Types of Diet

Presence of Microplastics in Each Sample Type	Frequency		Percentage	
	Presence	Absence	Presence	Absence
Feces	100	0	50%	0%
Water	50	0	25%	0%
Food	50	0	25%	0%
Total	200	0	100%	0%

Figure 1
Graphical representation of the presence of microplastics in fecal samples, water sources, and diet types.



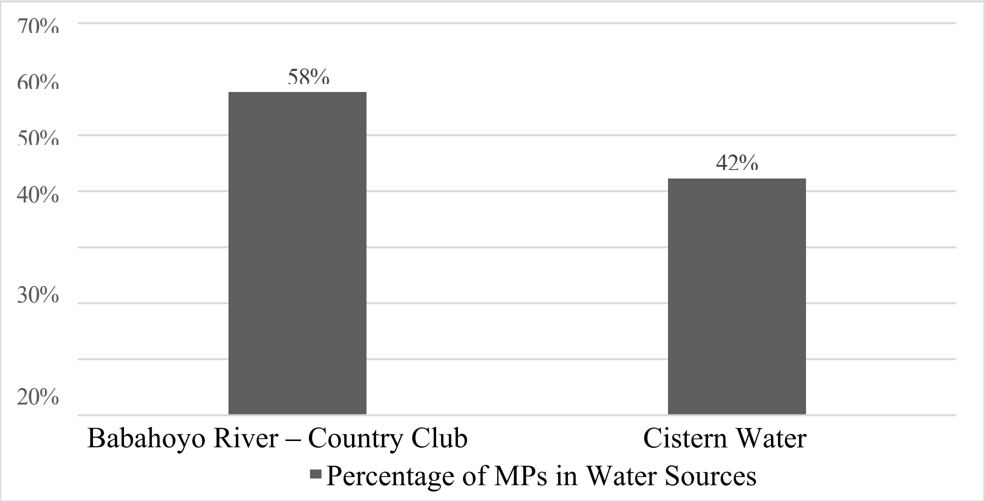
According to the data shown in Table 1 and Figure 1, the 100% incidence of microplastics is found in all the samples analyzed (sample types in—fecal, water, and feed), that is, complete contamination in the components evaluated. This is in a striking contrast with the report of Pinzón and Ayora (2024), some percentage microplastic free of all samples analyzed, even though the animals belong to the same species. In Peñafiel and Peñafiel (2023) 47% of pig samples were also contaminated with microplastics and the difference was in 53% in which these particles were not present.

For the studies it represents, we are seeing a dramatic difference in the microplastics that are here today. This increase could be the result of diverse reasons as for example different locations of the sampling, the types of water sources, the diet given to the animals, or even some sort of species-related variation. These results emphasize the necessity of further studies of the environmental and management factors that promote microplastics contamination in livestock.

Table 2
Quantification of Microplastics in Water Sources

Microplastics in Water Sources	Frequency	Percentage
Babahoyo River – Guayaquil Country Club	150	58%
Cisterns – Miguel Salem Racetrack	110	42%
Total	**260**	**100%**

Figure 2
Graphical representation of the quantification of microplastics in water sources



Based on the comparison between the 25 river water samples and that of the 25 cistern water samples as presented in Table 2 with reference to Figure 2, it was revealed that microplastics were more prevalent in rivers as discussed earlier with presence of 58 %. Nevertheless, both percentages are alarming. This tendency is corroborated by the study by Pinzón and Ayora (2024), as they observed that of 72 horses that drank river water, 55 presented microplastic contamination and of 28 that drank potable water, 22 presented it.

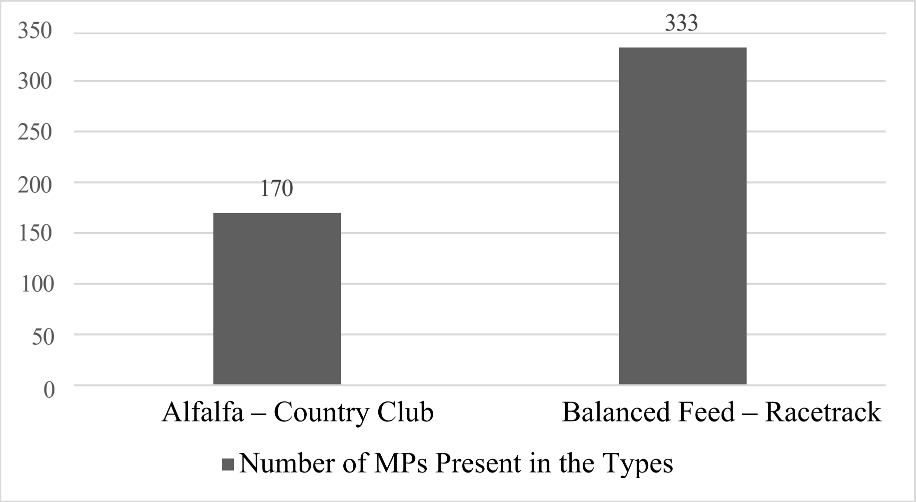
Studies like those of Bollaín et al. (2019) noted that the application of the 'biodegradable' or 'oxo-degradable' products has not decreased and thus has its impact on marine environment. These plastics become physically and chemically degraded to form small pieces such as microplastics (MPs) and nano plastics. According to Sáenz et al. (2023), wastewater releases due to this problem further confound this issue, and lead aquatic systems to continuous and pervasive pollutants including MPs and other particles.

All this indicates that ultimately, water sources, naturally or externally treated, become significant vectors for MPs.

Table 3
Quantification of Microplastics in Types of Diets

Microplastics in Types of Diets	Frequency	Percentage
Alfalfa – Guayaquil Country Club	170	34%
Balanced Feed – Miguel Salem Racetrack	333	66%
Total	**503**	**100%**

Figure 3
Graphical Representation of Microplastic Quantification in Types of Diets



Tables 3 and Figure 3, on the quantification of MPs in kinds of diets, show that the prevalence of MPs was among the 25 balanced feed samples, 66 %, from this finding are based on Pinzón and Ayora (2024) study. They found microplastics in 69 out of 88 samples of balanced feed and grass, or 78.4 percent.

Among the forage herbs, 34% of MPs measured in 25 samples of alfalfa. Another significant factor is the silage/forage preservation methods which are frequently used with substances that facilitate the forming of microplastic. In fact, Beriot et al. (2021) also noted that plastic covers in agricultural areas were the primary source of plastic pollution.

Table 4
Amount of microplastics present in feces not related to water sources and types of diet

Microplastics in Feces	Frequency	Percentage
Feces - Guayaquil Country Club	693	65%
Feces - Miguel Salem Racetrack	366	35%
Total	1059	100%

Figure 4
Graphical representation of the amount of microplastics in feces not related to water sources and types of diet

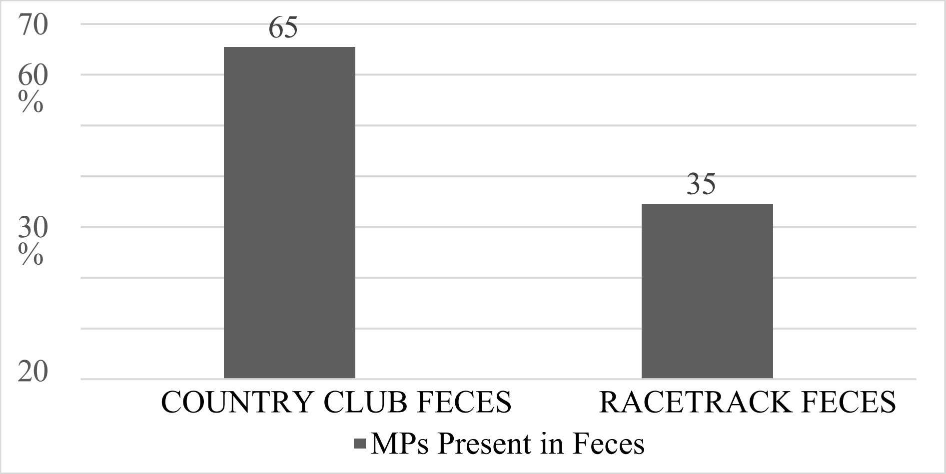


Table 4 and Figure 4 present the 50 fecal samples analyzed from the Guayaquil Country Club and 50 additional samples from the Miguel Salem Racetrack, which showed 65% and 35% prevalence of MPs, respectively. In both operations, a high level of microplastics is found. The occurrence of microplastics in the 100 fecal samples also exceeds the findings of the research conducted by Pinzón and Ayora (2024), where microplastics were reported in 100 fecal samples from 100 different horses with a prevalence of 77%, the remaining 23% being devoid of any of these contaminants.

In contrast with published work on other species, the analysis of González-Puetate et al. (2024) have detected MPs in 75.67% of 300 fecal samples from ruminants. This is consistent with the result of Liu et al. (2023) who identified MPs in the excrement of Equus kiang (Tibetan wild ass). Their study found a median 102 particles per gram of dry feces. So these contaminants are prevalent and not only in horses.

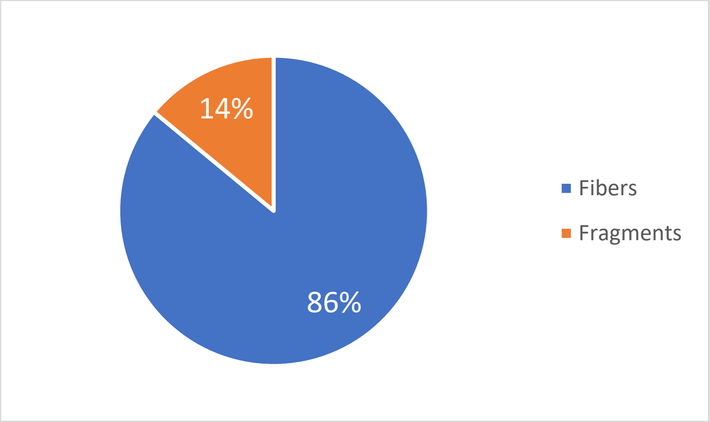
Table 5

Quantification of Microplastic Types

Types of Microplastics	**Frequency**	**Percentage**
Fibers	1560	86%
Fragments	262	14%
Total	1822	100%

Figure 5

Graphical representation of the quantification of microplastic types



The microplastic (MP) polymers occurrence by morphology in Table 5 and Figure 5 shows that the fibers are more abundant. Of the 50 samples analyzed, 86% was lineal shapes or fibers, and the other 14% was fragments. This is emphasized in the work of Castañeta et al. (2020) MPs exist in a plethora of forms predominantly in the spheres, pellets, foam, fragments, fibers, and flakes. Other morphs are however quite rare or even lacking in some analysis, apart from fibers and fragments.

A study conducted by Pinzón and Ayora (2024), fibers and fragments made up 97.4% of all samples analyzed, indicating that fibers are the most prevalent shape of microplastic.

Table 6
Frequency of Microplastic colours

Microplastic Color	Frequency	Percentage
Blue	183	10.04%
Red	15	0.82%
Yellow	16	0.88%
Green	547	30.02%
Transparent	1061	58.23%
Total	1822	100%

Figure 6
Graphical Representation of the Frequency of Microplastic Colors

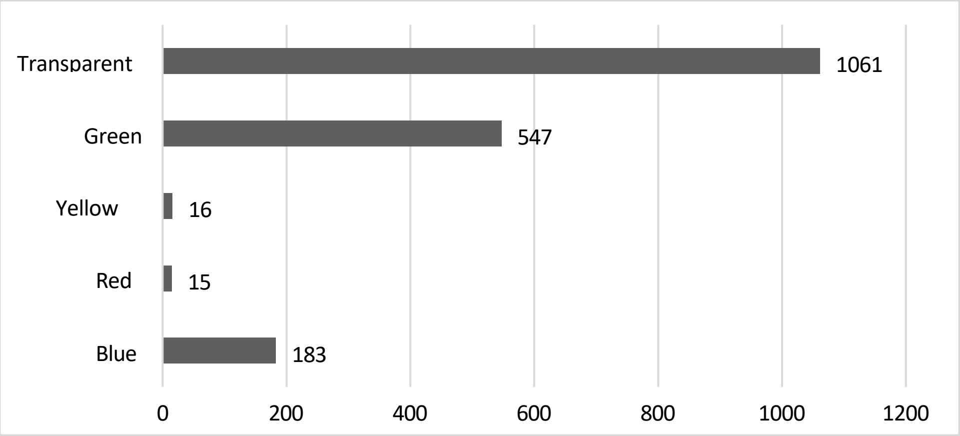


Table 6 and Figure 6 found that transparent microplastics are the most prevalent among the 200 samples, including feces, water sources and diet types, resulted from the frequency analysis of the predominant color of microplastics. This is a feature that is known to oscillate, as it was demonstrated by Gómez et al. (2024), who also screened the 18 fecal samples from sea lions and detected that black was the most common microplastic color.

In the study by Percca (2021), guano revealed the highest detection for microplastics, where blue color was the most recurring color type (37%; 20 MP in 54). The study even discovered fuchsia and light blue microplastics.

Information about the color of microplastics in fecal materials, natural waters and diet category available is limited. Therefore, the cause of the coloration remains unclear (plastic type, food, fragmentation, environmental exposure).

The presence of plastic particles in horses highlights their ability to enter and circulate in the animal's body. Younger specimens showed higher concentrations of particles, which suggests a possible vulnerability in early stages of development. These findings raise concerns about the long-term effects of plastic particles on equine health, especially in productive contexts and as indicators of human health (Culcay-Troncoso et al., 2025).

IV. Conclusions

This study has provided a remarkable insight to the microplastic (MP) in equine studies. Through the successful attainment of the specific aims proposed, there has been made progress in conceptually advancing and understanding this new area.

Microplastics were successfully quantified in the types of water sources offered to the Equidae. This is especially relevant because natural and treated water sources are potentially important vectors for MP contamination and, thereby, for the health and welfare of the animals. MP contamination was higher in river water used in the Guayaquil Country Club (GCC) (58%) in comparison to cistern water in the racetrack, Miguel Salem Dibo (42%).

The MPs were also measured in the various diets offered to the equids. This endpoint was useful since feed, especially compensated or concentrated diets, can be an important source of exposure to these contaminants. The methods and analysis employed showed a higher MPs content in Racetrack balanced feed samples (66%) than that of the Country club alfalfa feed samples (34%).

Furthermore, MPs were found in equine feces derived from non-water source or diet type, suggesting that horses could be exposed to MPs by other pathways as well. The screening revealed a high MP occurrence in the feces from the GCC (65%) and the Racetrack (35%), pointing out new questions about sources and pathways of MP exposure in horses.

In the future, this elevated environmental burden of MPs will likely threaten the health of all living entities and their environmental networks. As such, it is very important to invest into research, prevention and mitigation with regard to this area. One suggestion can be to test the long-term impacts of MP chronic exposure on the health of horses and other species (GI, respiratory and reproductive systems, among others). Additional study of other MP exposure pathways such as inhalation, dermal absorption, and even transplacental transfer is also recommended.

Acknowledgments: Jorge O., Richard L.

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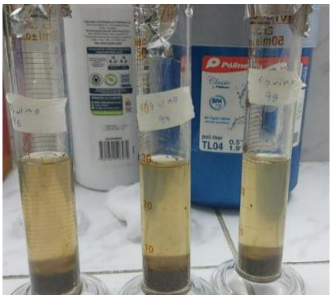
II. Annexes

Annex 1

Identification and selection of equines for fecal sample analysis

Annex 2

Processing of feces, water, and food samples



Annex 3

Types and shades of microplastics identified through optical microscopy