Isolation, Characterization, and Quantification of Microplastics and Heavy Metals from Meconium, Breast Milk & Formula Milk

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A thesis submitted to the Department of Microbiology in partial fulfillment of the requirements for the degree of

B.Sc. in Microbiology

Department of Microbiology

BRAC University

May 2026

Declaration

It is hereby declared that

1. The thesis submitted is our original work while completing the degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. We have acknowledged all main sources of help.

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Ethics Statement

Our thesis paper contains less than 15% plagiarism.The Experimental procedures of this research were carried out following the ethical guidelines of Helsinki Ethical Guideline and approved by the Institutional Review Board (IRB) under the supervision of Dr. Nadia Sultana Deen, Department of Microbiology, BRAC University. The IRB Protocol was maintained and overseen by the supervisor and research assistant. No funds were received.

Abstract

Heavy metals (HMs) and microplastics (MPs) have become ubiquitous environmental pollutants that have detrimental effects on the health of mothers and newborns. In order to further comprehend early-life exposure pathways, this study aimed to identify, characterize, and quantify MPs and HMs in human breast milk and neonatal feces. Neonatal samples were collected and processed utilizing filtration, density separation, and standardized digesting methods designed for biological matrices. Raman spectroscopy was implemented to identify and characterize microplastics according to their shape, size distribution, and polymer type. Through the use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS), heavy metals that include lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) were identified. The findings demonstrated that a variety of microplastic particles, notably fibers and pieces, were present. These particles ranged from 0.01 mm to 0.22 mm in breast milk and from 0.1 mm to 1.48 mm in neonatal feces, reflecting maternal transfer and early postnatal exposure. Polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) were discovered to be the most common polymers. Although heavy metal concentrations fluctuated between samples, with higher quantities frequently encountered in neonatal feces, suggesting potential accumulation or additional environmental inputs. These outcomes showcase the early-life existence of toxic metals and microplastics in maternal and neonatal biological systems and emphasize the need for increased monitoring, exposure-reduction measures, and additional research into possible health effects during crucial developmental stages.

Keywords : Heavy metal, Neonates, Microplastic, Meconium, Breast milk, Baby products.

Acknowledgement

First and foremost, we would like to express all the praise to the Almighty for the consent throughout the journey. We also would like to express our deepest respect and appreciation to our supervisor Dr. Nadia Sultana Deen, Associate Professor of the Microbiology program, and M.Mahboob Hossain, Professor of the Microbiology program for their unwavering support, insightful advice, inspiration and encouragement along with sharing their immense knowledge and experience to complete our undergraduate thesis. Their constructive and thoughtful comments made us believe in ourselves along the journey. Besides, we would like to extend our sincere gratitude and appreciation to our co-supervisor, Ifthikhar Zaman, Lecturer of the Biotechnology program, for his invaluable guidance, motivation, and constant support throughout this journey. We also express our heartfelt gratitude to him for his immense patience in embarking on the journey. We would not have the project if he hadn't motivated us in such a way.

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List of Acronyms

AAS	Absorption Spectroscopy
ABS	Acrylonitrile Butadiene Styrene
GC/MS	Gas Chromatography/Mass Spectrometry
HMs	Heavy Metals
HDPE	High Density Polyethylene
H_2O_2	Hydrogen Peroxide
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LDPE	Low-Density Polyethylene
MPs	Microplastics
PAHs	Polycyclic Aromatic Hydrocarbons
PPM	Parts Per Million
PMS	Polymethylstyrene
PET	Polyester
PS	Polystyrene (PS)
PVA	Polyethylene Vinyl Acetate
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscope
VOCs	Volatile Organic Compounds
XRF	X-ray Fluorescence

Chapter 1

1. Introduction

1.1. Micropollutant Exposure in Early Life is a Global Problem

To newborn health, microplastics and heavy metals are nowadays seen as a serious problem, because in early life they can enter and affect growth and immunity (Ragusa et al., 2022). For the first time, scientists found microplastics in human breast milk in 2022, showing that babies are directly exposed through feeding (Ragusa et al., 2022). Microplastic particles have also been shown by studies of neonatal feces with meconium, which means from bottles, packaging, and maternal transfer, newborns are already ingesting them (Rodrigues et al., 2022). Lead, cadmium, arsenic, and mercury, which are heavy metals, can pass through breast milk and may harm brain development and the immune system (Gatti et al., 2024). Projections of the future suggest that exposure will especially rise in South Asia and Africa, where there is high industrial pollution and waste (State of Global Air Report 2024 | State of Global Air, n.d.). The most common plastics that are found are polyethylene and polypropylene, which often come from bottles and packaging (Ragusa et al., 2022). Furthermore heavy metals are detected in ug/L for meconium and ug/L for breast milk (Gatti et al., 2024). To estimate long-term exposure, risk models combine daily intake and biological half-life (State of Global Air Report 2024 | State of Global Air, n.d.). Also, for public health implications, newborns are more vulnerable because they do not have fully developed detox systems (Ragusa et al., 2022). Gut bacteria may be disturbed by microplastics. It may cause increasing risk of antimicrobial resistance and weak immunity (Rodrigues et al., 2022). Mother's body burdens are shown by contamination of breast milk and also by linking environmental pollution directly to infant health (Gatti et al., 2024). The urgent need for stricter rules on plastic use, food packaging, and industrial pollution is highlighted by these findings.

1.2. Heavy Metals and Microplastics as Emerging Toxicants

Coming from packaging, textiles, consumer products, and the breakdown of larger plastics in the environment, microplastics are a common contaminant. These nanoscale plastics that range from millimeter -sized can enter cells and move through the body's system. Polymers such as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS) in meconium and breast milk have been found in some studies, showing direct exposure in infants (Ragusa et al., 2022).

Microplastics have a small structure and can cross the epithelial barrier. Also disturbs gut balance. And toxic chemicals are carried by them. No safe level of exposure for infants to heavy metals like lead, cadmium, mercury, and arsenic toxins (Gatti et al., 2024). By building maternal tissues, these metals can be passed into breast milk and enter through contaminated water into infant formula (Serreau et al., 2024).

Furthermore, metallic ions which are positively charged attach with microplastics which are negatively charged, increase the bioavailability of heavy metals, which makes mixed toxicity more dangerous and this technique is called Trojan Horse effect. Heavy metals and microplastics both can damage cells by causing oxidative stress, breaking cell membranes, interfering with protein- DNA binding, and mitochondrial dysfunction. Long term effects in infants can be caused by these effects, such as impaired brain development, weakened immune system, hormonal imbalance, metabolic changes, etc (Gatti et al., 2024).

1.3. Pathways in Mothers and Infants and Exposure Source

Mainly through eating,breathing and skin contact, mothers are exposed to microplastics. Also, the major entry points are cosmetics, drinking water, household dust, drinking water, processed foods, food packaging, etc. (Rodrigues et al., 2022). Lead, cadmium, mercury, and arsenic, which are major heavy metals, enter the body through contaminated food, dirty water, polluted water, and occupational or environmental hazards. Both heavy metals and microplastics travel through the bloodstream once they are absorbed and can be released into breastmilk (Ragusa et al., 2022).

Directly and indirectly, infants are exposed to heavy metals and microplastics, and one of the major sources is breastfeeding. Moreover, from different populations, microplastics and heavy metals are found (Ragusa et al., 2022 ; State of Global Air Report 2024 | State of Global Air, n.d.). Babies who are formula fed are exposed to plastic bottles, rubber nipples, and sippy cups with shed microparticles during sterilization, shaking, and heating (Rodrigues et al., 2022). High concentrations of microplastics are often contained in neonatal feces or meconium that describes that infants have limited ability to process and release these contaminants. The importance of monitoring both meconium and breast milk is highlighted by a combined mother-infant exposure system to identify and understand the health risks and exposure pathways (State of Global Air Report 2024 | State of Global Air, n.d.).

1.4. The Reason for Biomonitoring Breast Milk and Neonatal Feces

Maternal exposure reflected by breast milk: The mother's internal accumulation of contaminations are shown by it, and what infants consume during lactation is provided by direct evidence (Janakiraman et al., 2025). Microplastics in breast milk: Also, how plastics transfer from mother to child is measured by detecting microplastics (Zainuddin et al., 2026); (*State of Global Air Report 2024 | State of Global Air*, n.d.).

Heavy metals in breast milk: For making it a critical matrix for risk assessment, metals such as lead, cadmium, and mercury can pass into milk (Gatti et al., 2024). Meconium sample: Both prenatal and postnatal exposure represented by this sample and show what infants ingest and release (Zhang et al., 2025).

Firstly,Biomonitoring meconium reveals such as microplastics excrete types and concentrations.Secondly, metals and metal-plastic complex presence. Also, physiological interactions with contaminants of infants (Microplastics Exposure during Perinatal Period: Impacts on Neonatal Immune and Metabolic Programming - a Scoping Review | Journal of Perinatology, n.d.). Combined analysis: Full pictures of exposure given by both meconium and breastmilk studies, which help to identify risk factors and preventive strategies (State of Global Air Report 2024 | State of Global Air, n.d.).

1.5. Analysis and Characterization Methods :

1.5.1. For The Microplastic Analysis Technique :

Raman spectroscopy (Zainuddin et al., 2026). The difference between incoming and scattered light helps to understand how the molecules in the sample are vibrating .

1.5.2. For The Heavy Metals Analysis Technique :

ICP-MS (Inductively Coupled Plasma Mass Spectrometry) : With this method, from a dried meconium sample, highly sensitive trace metals are detected.

1.5.3. For Avoiding Contaminants:

In sterile, non-plastic containers, samples must be collected.And also to prevent false positives, controlled laboratory environments and standardized protocols are essential (Zainuddin et al., 2026).

1.5.4. Combined Analysis :

Exploring both Microplastics and heavy metals together helps to evaluate most possible synergistic effects, since both metallic ions and increased toxicity carried by plastics (Zhang et al., 2025).

1.6. Toxic Effects of Heavy Metals and Microplastics in Early Development :

Through four main toxic pathways microplastic and heavy metals harm infants. Firstly in generation of oxidative stress and ROS, too many oxygen species (ROS) are produced by microplastics and heavy metals. Also, the infant's antioxidant defenses are overwhelmed by this, causing cell damage (Ragusa et al., 2022).

Secondly, in cell membrane integrity disruption, epithelial membranes can be physically damaged by microplastics. This also changes the permeability and weakens the cell stability (Rodrigues et al., 2022). Thirdly, in DNA and protein interference, heavy metals mainly attach to DNA and proteins, which leads to strand breakage, enzyme activity reduction, and altered gene expression. In addition, microplastics carry metal ions, other toxic substances which are called Trojan Horse Effect and make this situation worse (Zhang et al., 2025).

Finally in microbiome modification and gut barrier dysfunction, microbial diversity is reduced by heavy metals that disrupt metabolism. While gut microbiota are disrupted by microplastics that damage mucosal layers. These changes together increase inflammation and alter immune programming (Microplastics Exposure during Perinatal Period: Impacts on Neonatal Immune and Metabolic Programming - a Scoping Review | Journal of Perinatology, n.d.).

1.7. Motivation of This Study :

Heavy metals and microplastics in breastmilk and meconium sample effects are still poorly understood in many populations, though these are growing worldwide (State of Global Air Report 2024 | State of Global Air, n.d.). Therefore, identifying exposure sources, measuring early -life body burden, and evaluating maternal to infant transfer are critical. Also, the study may guide environmental regulations, breastfeeding safety recommendations, public health policies, and provide important information by filling this gap (Zhang et al., 2025); Microplastics Exposure during Perinatal Period: Impacts on Neonatal Immune and Metabolic Programming - a Scoping Review | Journal of Perinatology, n.d.).

1.8. Hypothesis

It has been hypothesized that microplastics and heavy metals could be detected in meconium and milk samples and an association between the abundance of microplastics and heavy metals could be found on the same neonate-mother dyad.

1.9. Objectives :

1. Abundance of microplastics and heavy metals in meconium and milk samples.
2. Association in the abundance of microplastics in meconium and milk samples on the same neonate-mother dyad.
3. To check "Trojan Horse Effect" in meconium samples.
4. To check the effect of microplastics and heavy metals on gut dysbiosis.

Chapter 2

2. Methods

The overall work methods followed in this study is given in the following flow chart.

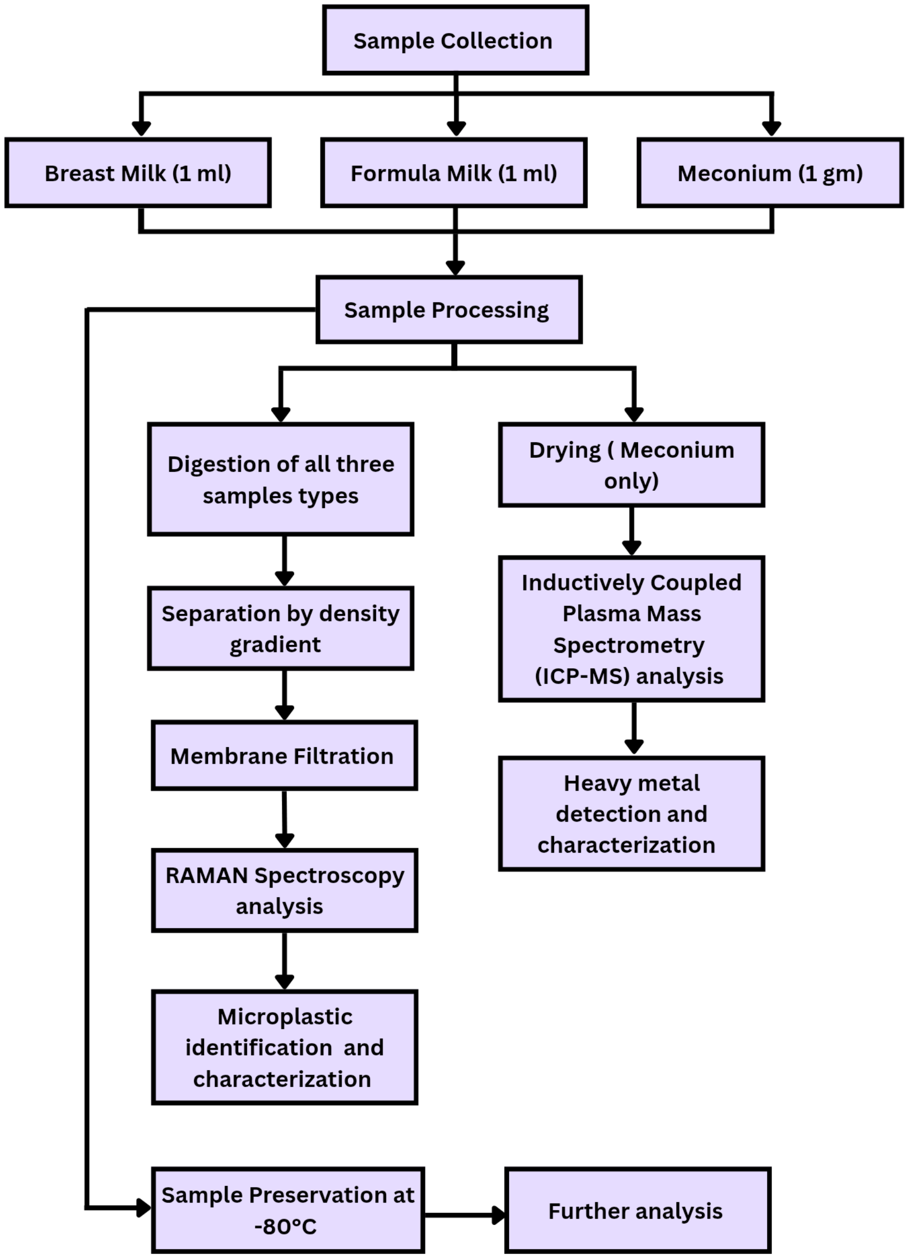

Figure 2 : Flowchart of the overall study workflow .

2.1. Materials

The laboratory materials used in this study have been carefully chosen to obtain accurate results while avoiding any form of plastic contamination throughout the entire experimental procedures. For sample handling and reaction processes, 250 mL conical flasks were chosen. A magnetic stirrer with a hot plate, or a shaking incubator, was also chosen to obtain a constant heating reaction throughout the digestion process.

A vacuum filtration unit was also chosen to separate particles from liquid media, which can contain microplastics. This unit uses 0.1µm Whatman GF/F glass microfiber filters, which are known to be effective in retaining microplastics. Microplastics are generally defined as plastic particles measuring less than 5 mm in size, with a commonly reported size range spanning from approximately 1 µm to 5 mm, while particles smaller than 1 µm are often categorized as nanoplastics. The selection of a 0.1 µm pore size allowed the effective capture of microplastics across the lower size spectrum, including very small particles (e.g.,1–100 µm) that are frequently present in environmental and biological samples. Filters with larger pore sizes (such as 0.45 µm or 1 µm) may permit the passage of these smaller particles, leading to potential underestimation of microplastic abundance. Therefore, the use of a 0.1 µm membrane filter enhanced particle recovery by enabling the retention of a broader size range of microplastics, including those approaching the nanoplastic boundary, thereby improving the sensitivity and reliability of subsequent analytical characterization.

To avoid microplastic contaminants, only glass and metallic equipment were chosen to carry out this study, avoiding plastic materials wherever necessary. Aluminum foil was used instead of plastic caps to cover containers, further reducing the risk of contamination. Additionally, desiccators were used to preserve and store filter papers in a moisture-free environment prior to analysis.

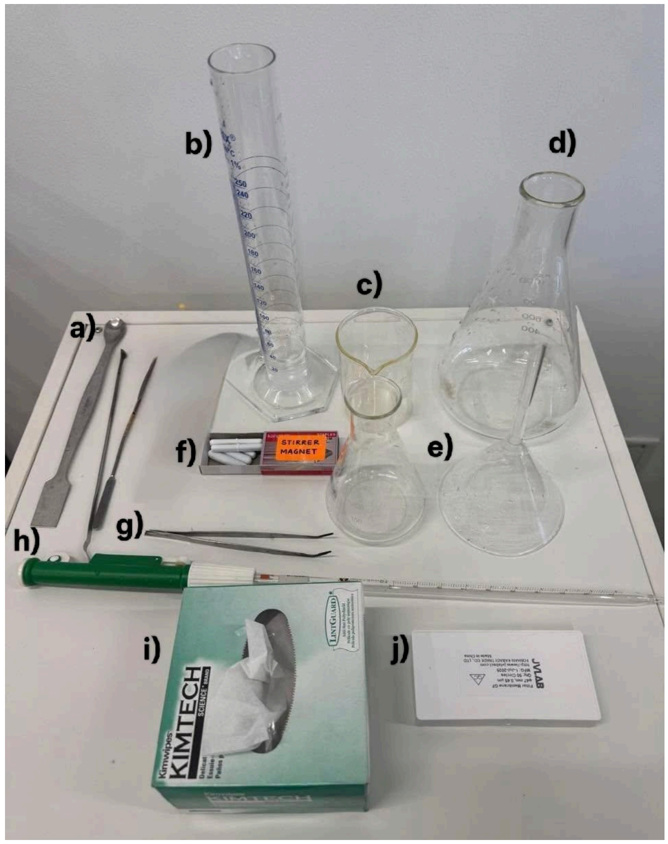

Figure 2.1 : Laboratory materials used in the experiment. (a) Spatula, (b) Measuring Cylinder, (c) Beaker, (d) Conical Flasks, (e) Glass Funnel, (f) Magnets, (g) Forceps, (h) Glass Pipette, (i) Kimwipe Tissue, (j) 0.22 µm Whatman GF/F glass microfiber filters

Figure 2.2 : A Magnetic Stirrer with Digested Samples .

2.2. Reagents

All the reagents used in the study were of analytical grade, and the selection was made with the aim of achieving reliable results in the study. Hydrogen peroxide with a concentration of 30%, was used as a strong oxidizing agent in the Fenton digestion process, with about 40 mL used in the process per sample. Iron (II) sulfate heptahydrate was used as a catalyst for Fenton's reaction. The iron (II) sulfate concentration was maintained at a constant of 0.05 M throughout the entire experiment. Distilled water was used throughout the experiment; importantly, all distilled water was pre-filtered to eliminate any potential microplastic contamination.For the experimental processes and solution preparations, this was used. This water was also used for rinsing and preventing impurities that might interfere with the analysis. For the density separation technique, saturated sodium chloride solution with a concentration of approximately 5.8 M or 350 g/L and a volume of around 50 mL was utilized. This solution was effective in the separation of the particles based on density differences.

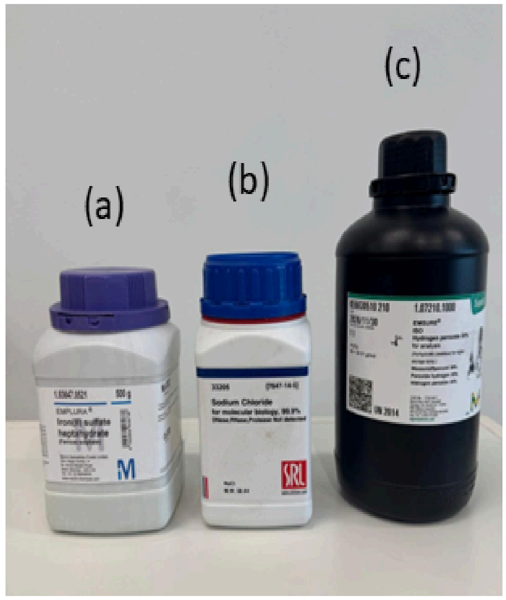

Figure 2.3 : Reagents used in the experiment. a) Iron sulphate heptahydrate, b) Sodium Chloride c) Hydrogen Peroxide

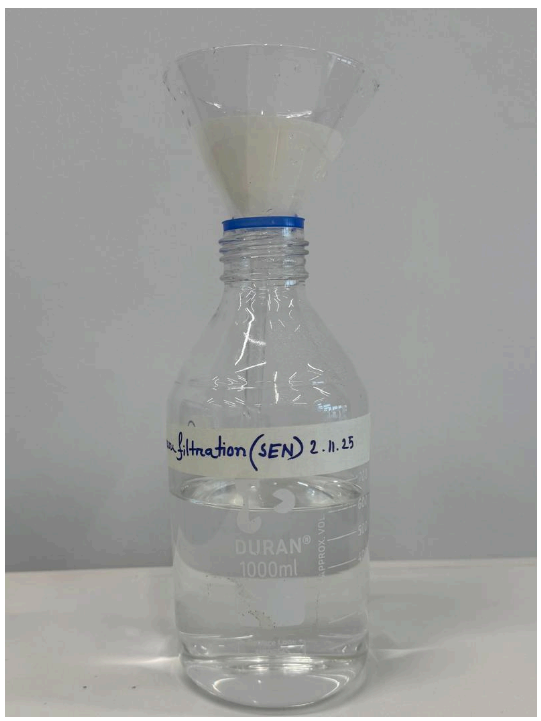

Figure 2.4 : Filtered distilled water to eliminate contamination.

2.3. Sample Collection

The sample collection was conducted aseptically at Ad-din Medical College Hospital, Dhaka. For the collection of the meconium and breast milk samples, separate glass vials were used, which was important for the prevention of cross-contamination. In addition, an extra vial was used for the collection of the meconium samples for future use. During the collection of the meconium and breast milk samples, special attention was given to the prevention of exposure to external contaminants.The meconium samples were collected by using a clean metal spatula from the cloth material (katha) and the diaper materials (Neocare). After the collection of the meconium samples, they were immediately placed in the glass vials. For the prevention of the meconium samples from the possible contaminants such as microplastics present in the air, the glass vials were completely sealed with aluminum foil instead of plastic caps. After conducting the sample collection process, samples were placed in an ice box and transported to the university laboratory. It is important to maintain a low temperature during sample transportation in order to avoid any kind of alteration in the physical and chemical properties of samples.

Figure 2.5 : Collection of the Meconium and Milk Samples in Separate Glass Vials.

2.4. Sample Processing

2.4.1. Sample Processing for Micro-Plastics Analysis

a. **Fenton Digestion -** The collected samples were subjected to Fenton digestion, a chemical oxidation process commonly used for the removal of organic matter from environmental and biological samples. The process is very useful in the analysis of microplastic particles or inorganic particles, as the organic matter is destroyed selectively while the synthetic polymers are left intact. The Fenton process is based on the catalytic decomposition of hydrogen peroxide in the presence of ferrous ions, which produces highly reactive hydroxyl radicals that are strong oxidizing agents, effective in the degradation of complex organic matter (Krupińska, 2024).

b. **Mechanism of Fenton Digestion -** Fenton digestion is an advanced oxidation process in which the oxidation-reduction reaction between hydrogen peroxide and iron ions produce highly reactive oxidizing agents, which are effective in the degradation of organic matter in the sample. The Fenton process is based on the cyclic oxidation-reduction reactions between ferrous ions and ferric ions, resulting in the continuous production of hydroxyl radicals, which are the main oxidizing agents in the process.

i. Initiation: Formation of Hydroxyl Radicals - The Fenton process starts with the oxidation-reduction reaction between ferrous ions and hydrogen peroxide, as indicated in the following equation (Eq. 1)

: $Fe^{2+} + H_2\ O_2 \rightarrow\ Fe^{3+} + OH^- \ + \bullet OH$ .... (1)

In this step, Fe^{2+} acts as a catalyst and promotes the decomposition of hydrogen peroxide, resulting in the formation of ferric iron , hydroxide ions , and hydroxyl radicals. The hydroxyl radical is a strong, non-selective oxidizing agent with a very high oxidation potential, allowing the oxidizing agent to quickly react with a wide range of organic compounds in the sample matrix.

ii. Propagation: Regeneration of Ferrous Iron -

To continue the reaction, ferric iron is reduced back to ferrous iron by reacting with a second hydrogen peroxide molecule: (Eq. 2)

$Fe^{3+} + H_2 O_2 \rightarrow Fe^{2+} + HO_2 \bullet + H^+$ ………………………………………………(2)

This reaction regenerates Fe^{2+}, enabling the catalytic cycle to proceed. At the same time, hydroperoxyl radicals and protons (H^+) are formed. The regeneration of Fe^{2+} is essential, as this ensures the continuous production of hydroxyl radicals during digestion.

iii. Secondary Radical Reactions and Formation of Reactive Oxygen Species - The formed radicals can participate in further reactions, leading to the production of other reactive oxygen species: (Eq. 3) & (Eq. 4)

$\bullet OH + H_2 O_2 \rightarrow HO_2 \bullet + H_2 O$……………………………………………..(3)

$HO_2 \bullet \Leftrightarrow O_2 \bullet^- + H^+$…………………………………………………………(4)

This reaction contributes to the production of hydroperoxyl radicals and superoxide radicals, which participate in organic matter oxidation, though not as effectively as hydroxyl radicals.

iv. Oxidation and Degradation of Organic Matter - Hydroxyl radicals formed during the reaction react with organic matter, represented by RH, through hydrogen abstraction or electron transfer : (Eq. 5)

$$RH + \bullet OH \rightarrow R\bullet + H_2O \quad \text{...(5)}$$

Organic matter radicals ($R\bullet$) then react further with oxygen, forming intermediate compounds such as peroxyl radicals, which eventually degrade to simpler, stable compounds : (Eq. 6)

$$R\bullet + O_2 \rightarrow ROO\bullet \rightarrow CO_2 + H_2O \quad \text{..(6)}$$

(and other low-molecular-weight compounds)

v. Overall Mechanism - The overall mechanism of the Fenton reaction may thus be explained as a catalytic cycle for the continued oxidation of hydrogen peroxide into hydroxyl radicals: (Eq. 7)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH \quad \text{..(7)}$$

The above mechanism has been shown to result in the effective oxidation and mineralization of complex organic materials such as proteins, fats, carbohydrates, and other biological materials. From the perspective of sample preparation, the Fenton digestion is significant in the elimination of organic materials that may otherwise interfere with the analysis. This allows for the isolation and accurate identification of hard particles such as microplastics or inorganic materials.

For the digestion process, 40 mL of 30% hydrogen peroxide was added to the conical flask containing the sample. Hydrogen peroxide acts as the oxidizing agent in the Fenton reaction, which produces hydroxyl radicals to decompose organic matter. Iron(II) sulfate heptahydrate was employed as the catalyst to initiate the reaction and decompose the hydrogen peroxide to hydroxyl radicals. Iron(II) sulfate heptahydrate solution was prepared by dissolving 0.56 g of Iron(II) sulfate heptahydrate in 2.5 mL of distilled water and then added dropwise to the hydrogen peroxide solution to achieve a 0.05 M concentration. During the reaction, bubbling was observed due to the release of oxygen gas, demonstrating the active decomposition of hydrogen peroxide. Finally, the mixture was placed on the magnetic stirrer and heated to about 60°C with continuous stirring for 7-8 hours. This heating and stirring process enhances the rate of the reaction to facilitate the digestion of the organic matter. The main aim of this digestion process was to remove the biological and organic matter from the samples, allowing any other particles, including microplastics and other inorganic particles, to be isolated and separated easily.

Figure 2.6 : Bubbling observed in conical flask, demonstrating the active digestion process.

c. **Density Separation :** After the completion of the digestion procedure, the sample was left to cool to room temperature. The digested solution was then placed in a separatory funnel, where density separation was carried out to separate potential microplastic particles from heavier particles. Density separation is a separation technique where particles are separated on the basis of differences in densities of particles relative to the surrounding medium. The primary importance of this technique lies in its ability to selectively isolate low-density microplastic particles from heavier particles without causing any structural or chemical alteration to the particles.

In this separation technique, a high-density solution is used to separate particles of different densities, where particles with lower densities than the solution will float on the surface, while those with higher densities will settle at the bottom of the container holding the solution. Most common plastic polymers, such as polyethylene (PE) and polypropylene (PP), have densities lower than that of saturated sodium chloride solution, causing them to float, while heavier inorganic particles settle at the bottom. About 50 mL of a saturated solution of sodium chloride ($NaCl$) (5.8 M) was added to the digested solution. Sodium chloride solution is a moderately dense solution used to separate various types of plastic polymers commonly found in microplastics.The separatory funnel containing the solution and digested particles was left undisturbed overnight. During this period, heavier particles settled at the bottom of the funnel, while lighter particles were suspended in the solution and floated on the surface of the solution in the funnel.density separation enhances the accuracy and reliability of microplastic identification and quantification by concentrating the target particles into a smaller, more manageable fraction. Overall, density separation plays a vital role in improving sample purity, reducing analytical errors, and ensuring precise detection of microplastics in environmental and biological samples.

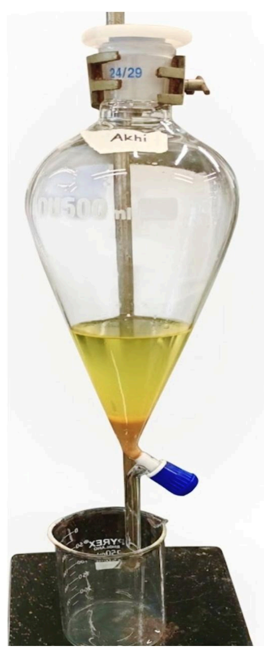

Figure 2.7 : Separation by density gradient using a separating funnel.

d. **Filtration and Drying -** The collected supernatant liquid was then subjected to membrane filtration by a vacuum filtration unit with a 0.10 µm Whatman GF/F glass microfiber filter. Membrane filtration is an important step for the collection of very small particles suspended in the liquid phase. Glass microfiber filters with fine pores are used for the collection of microplastic particles while the liquid passes through the filter. After filtration, the filter paper with the collected particles was handled with care to be transferred to a clean petri dish for drying. Filter papers were left to dry completely in a desiccator overnight. Drying the filter papers completely removes the moisture from the samples. This drying process occurs in a controlled environment to avoid contamination from the atmosphere. This step ensures that the filters remain clean, dry, and suitable for further analytical procedures.

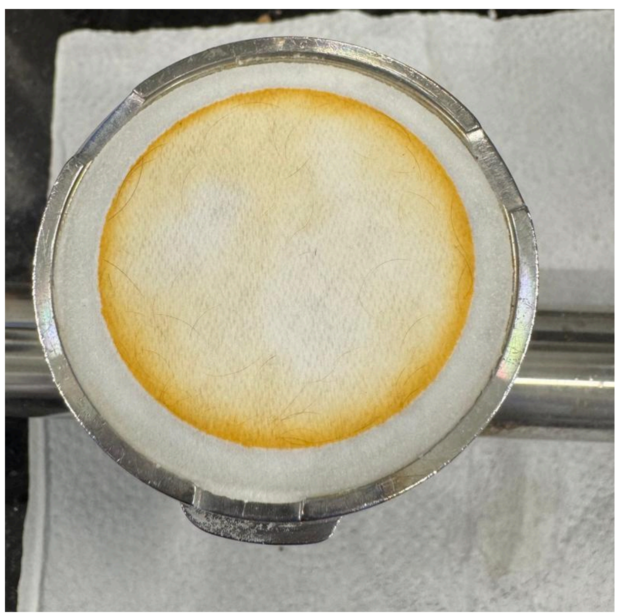

Figure 2.8 : Membrane filtration by a vacuum filtration unit with a 0.10 µm Whatman GF/F Glass Microfiber Filter.

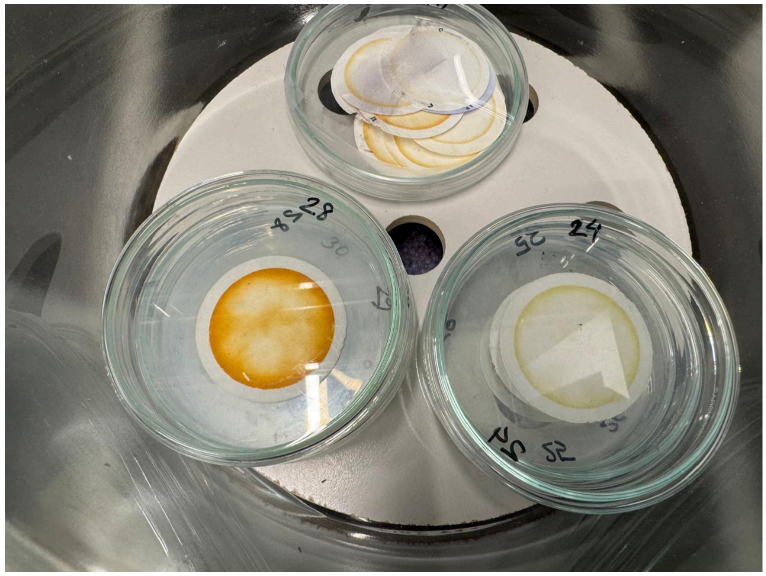

Figure 2.9 : Filter papers were left to dry in a Desiccator .

2.4.2. Sample Processing for Heavy Metal Analysis

a. **Drying**

After collection, the samples were carefully prepared for heavy metal analysis through a controlled drying process. The samples were placed in a laboratory drier and maintained at a constant temperature of 40°C to remove all moisture while preserving the chemical integrity of the sample. Using a relatively low drying temperature is critical to prevent the degradation of heat-sensitive components and to minimize the potential volatilization or loss of trace heavy metals, ensuring that their natural concentrations remain intact.The drying process was continued until the samples achieved a complete removal of residual moisture. Eliminating water from the samples is essential for accurate analytical measurements, as excess moisture can dilute the sample, interfere with precise weight-based calculations, and affect the efficiency of subsequent digestion and instrumental analysis. Additionally, drying inhibits microbial activity and chemical alterations that could otherwise modify the sample composition over time.

Once dried, the samples were carefully removed from the oven and allowed to cool to room temperature in a clean, contamination-free environment. The dried samples were then stored in a controlled environment to maintain their stability until further processing. This careful drying and handling process ensures that the samples remain stable, homogeneous, and suitable for precise quantitative analysis of heavy metal content, thereby enhancing the reliability and reproducibility of the results.

Figure 2.10 : Samples were placed in a drier at a constant temperature of 40°C to remove all moisture .

2.5. Characterization of Microplastics and Heavy Metals

2.5.1. Raman Spectroscopy

Raman spectroscopy is widely used for the identification and characterization of microplastics in environmental and biological samples. This technique enables the accurate identification of different polymer types based on their unique molecular vibration spectra. It is capable of detecting and distinguishing common plastic polymers such as polypropylene, polystyrene, and polyethylene (Bumbrah & Sharma, 2015).

It also allows the characterization of microplastic particles by determining their chemical composition and polymer structure. In addition, it can analyze very small particles, making it suitable for studying microplastics at the microscopic level. The technique provides non-destructive analysis, meaning the sample remains intact during measurement (Bumbrah & Sharma, 2015). So, Raman spectroscopy analysis was carried out to identify and characterize the microplastics present in the samples.

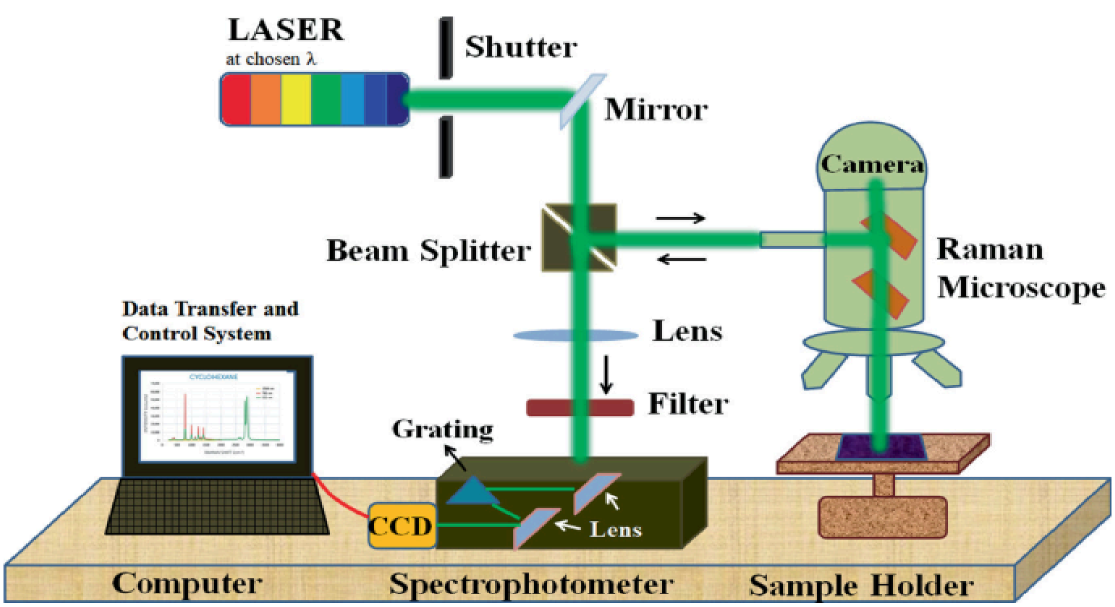

Figure 2.11: A schematic diagram of raman spectroscopy (Pandey et al., 2021).

The collected stool samples were first digested and, finely filtered by Whatman GF/F glass microfiber filter, and preserved in a desiccator. The filter paper were analyzed using a Raman spectrometer within the spectral range of 4000–400 cm^{-1}. The obtained spectra were compared with reference spectral libraries to identify characteristic vibrational bands corresponding to different polymer types. The distinct Raman peaks were further analyzed to confirm the presence and composition of microplastics in the stool samples.

2.5.2. ICP-MS Analysis

Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) is widely used for the detection and characterization of heavy metals in biological and environmental samples. It enables highly sensitive identification of trace metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As). The technique can detect metals at very low concentrations (parts per billion or parts per trillion), making it suitable for studying toxic metal exposure (Wilschefski & Baxter, 2019).

It also allows simultaneous analysis of multiple heavy metals in a single sample, providing comprehensive elemental profiling. In addition, it enables accurate quantification of metaconcentrations (Wilschefski & Baxter, 2019). So, the dried samples obtained from the dryer were then subsequently analyzed using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). This analysis enabled the identification and quantification of heavy metals present in the samples at trace levels.

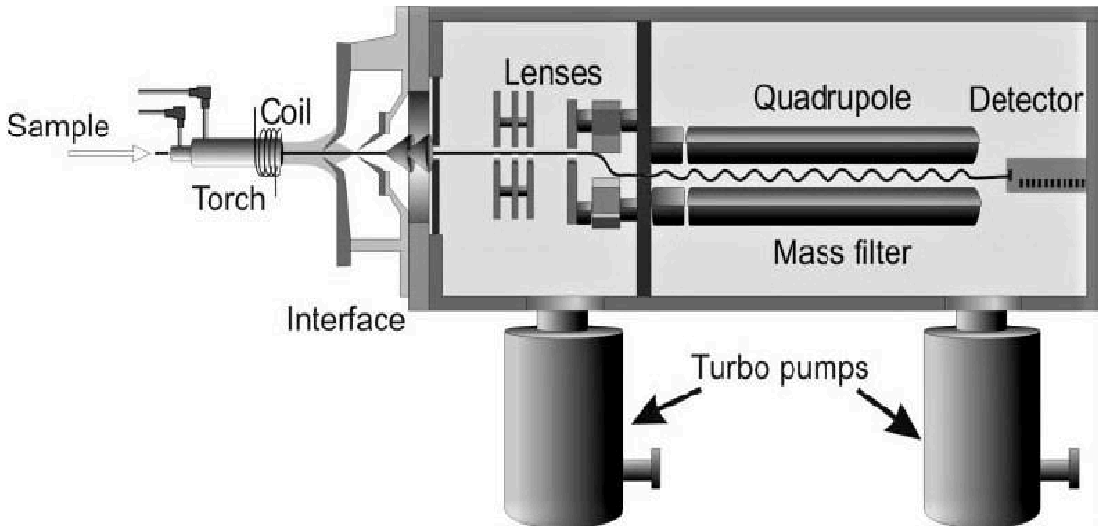

Figure 2.12: Cross section schematic of an ICP-MS (Wilschefski & Baxter, 2019).

The dried stool samples were then introduced into the ICP-MS system for elemental analysis. The instrument was calibrated using standard solutions, and measurements were carried out to detect trace levels of metals. The resulting data were analyzed to identify and quantify the concentration of various heavy metals present in the stool samples.

2.6 Metagenomic Analysis of Meconium Samples

For future experiments, meconium samples exhibiting the highest and lowest abundance of microplastics were selected for comparative metagenomic analysis to investigate the relationship between microplastic exposure and neonatal gut symbiosis. Total microbial DNA was extracted from the selected meconium samples using a sterile, contamination-controlled protocol and a commercially available microbial DNA extraction kit optimized for low-biomass samples. The quality and concentration of the extracted DNA were assessed using spectrophotometric and fluorometric methods, followed by agarose gel electrophoresis to evaluate DNA integrity.

Subsequently, metagenomic sequencing libraries were prepared according to the manufacturer's instructions and subjected to high-throughput shotgun metagenomic sequencing using an Illumina sequencing platform. Taxonomic profiling was then performed to identify microbial communities present in the neonatal gut, including bacterial, archaeal, viral, and fungal taxa. Functional annotation analyses were also conducted using established genomic databases to identify genes and metabolic pathways associated with gut symbiosis, immune modulation, and potential plastic-degrading activities.

Microbial diversity analyses, including alpha and beta diversity measurements, were used to compare microbiome composition between samples with high and low microplastic abundance. Differential abundance analysis further identified microbial taxa and functional genes significantly associated with microplastic exposure. Correlation analyses between microplastic concentration and microbial functional profiles were performed to evaluate possible relationships between environmental contaminant exposure and neonatal gut dysbiosis.(Liu et al., 2023)

Chapter 3

3. Result

3.1 Source of Miconium

Figure 3.1a demonstrates the gender distribution of neonatal meconium samples. Meconium samples were collected from 30 neonates, among whom 19 were male (63.3%) and 11 were female (36.7%), suggesting that the samples predominantly represented male.

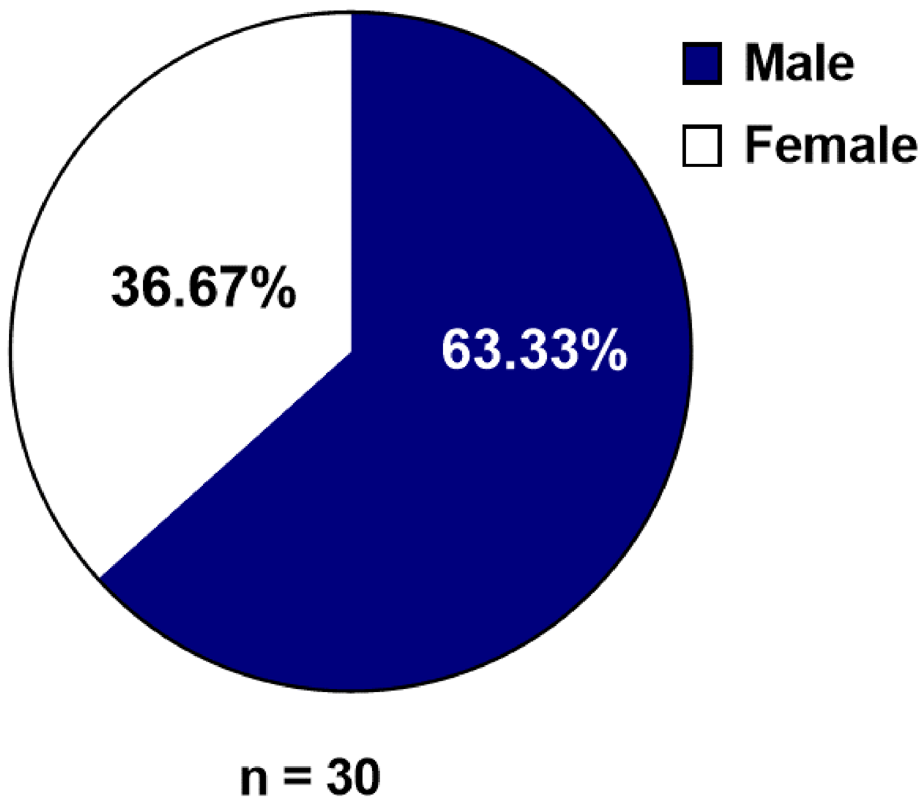

Figure 3.1a. Distribution across male and female newborns.

Figure 3.1b shows the bedding materials from which meconeum was collected. uAmong the 30 neonates included in the study, 56.67% used katha, while 43.33% used disposable diapers. The findings suggest that traditional cloth-based materials were more commonly used than commercially available diaper products within the study population.

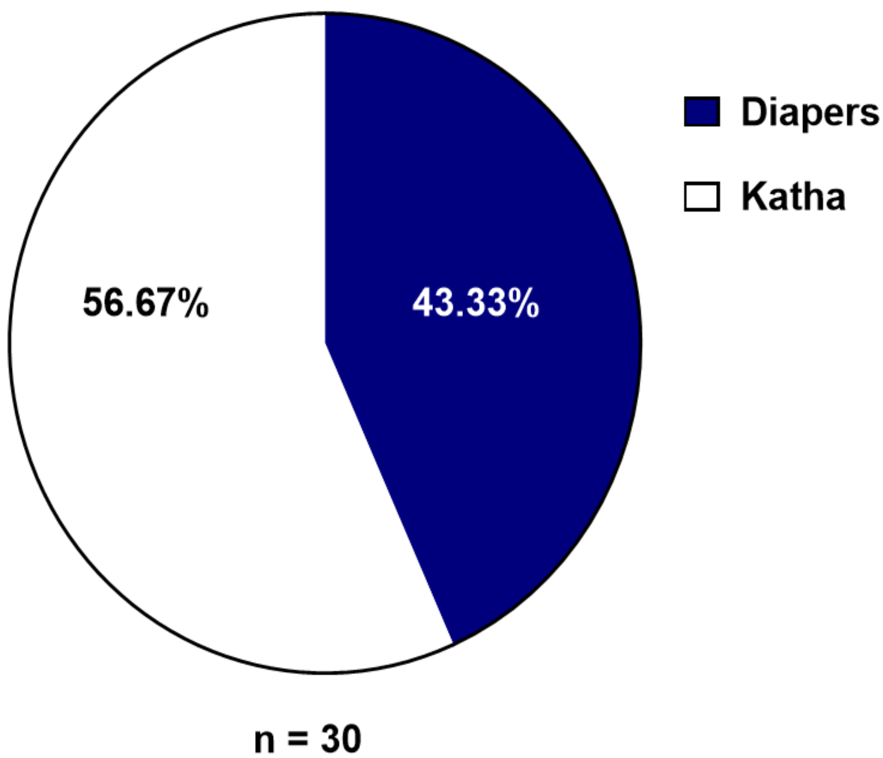

Figure 3.1b: Materials from which meconium was collected.

Figure 3.1c describes the number of preterm and term neonates from whom meconium was collected. Among the 30 neonates in the study , 36.67% were preterm .And on the other hand 63.33% were term .The finding suggests that at term the majority of neonates were born rather than preterm.

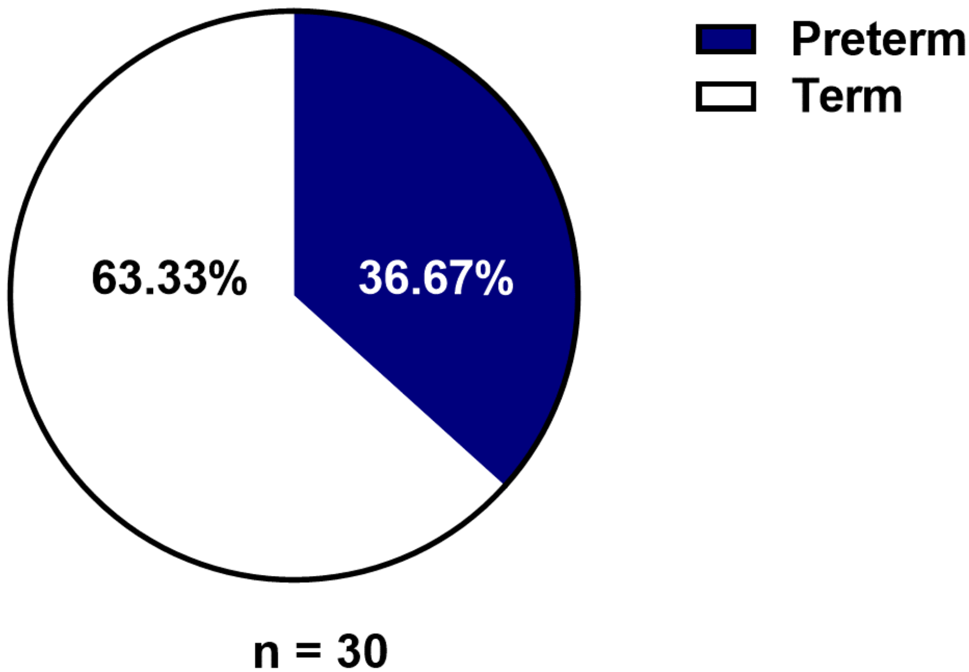

Figure 3.1c : Meconium collected from term and preterm neonates.

3.2 Source of Milk Samples

Figure 3.2 indicates that 15 out of 30 neonates were exclusively breastfed, making breast milk the most common source of milk samples. Mixed feeding, the second most common feeding method was used by 13 neonates and only 2 neonates exclusively used formula, making this the least common feeding method.

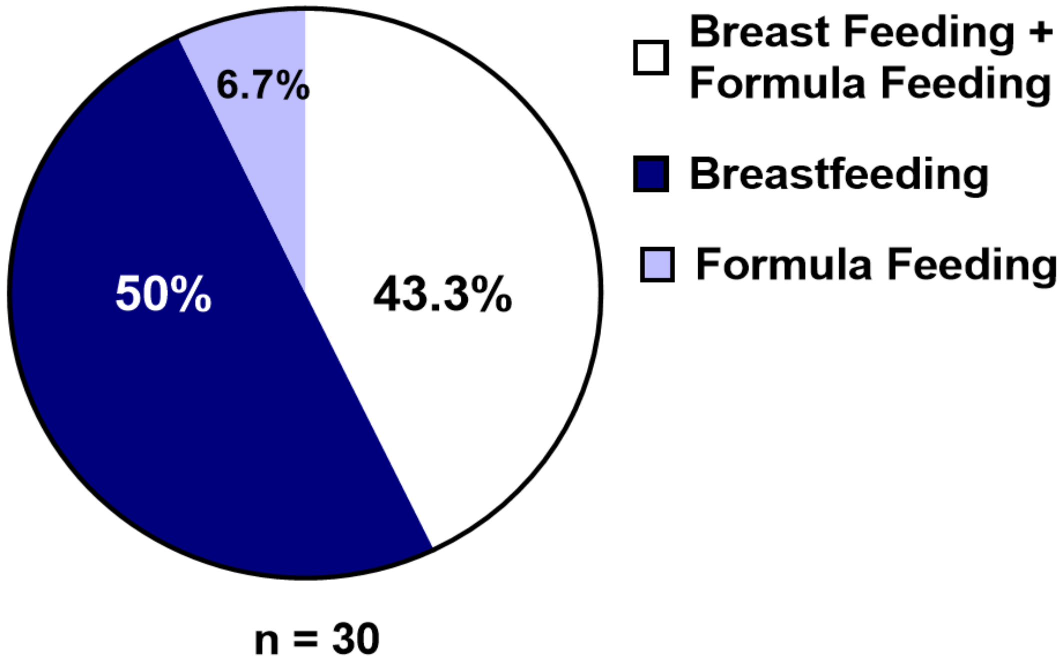

Figure 3.2: Source of milk samples.

3.3. Distribution of Microplastics in Meconium Samples

3.3.1. Distribution of Microplastic Fragments in Meconium Samples

A comprehensive analysis of the distribution of microplastics (MP) profile in 28 meconium samples is demonstrated in figure 3.3.1, which reveals notable polymer composition variation. The complete prevalence of polyester (PET), which was observed in 100% of the cohort and identified as the main pollutant, is a crucial finding. Polyethylene Vinyl Acetate (PEVA), which is a variation of the low-frequency category in the context of the current research, and Polyvinyl Alcohol (PVA) showed the lowest occurrence, with PVA being completely absent. Polystyrene (PS) and High-Density Polyethylene (HDPE) were found in about 45% of samples, with a medium-range frequency. Interestingly, samples S19, and S21 have the most internal diversity, with more than five different types of polymers, suggesting exposure to many sources throughout the gestational period.

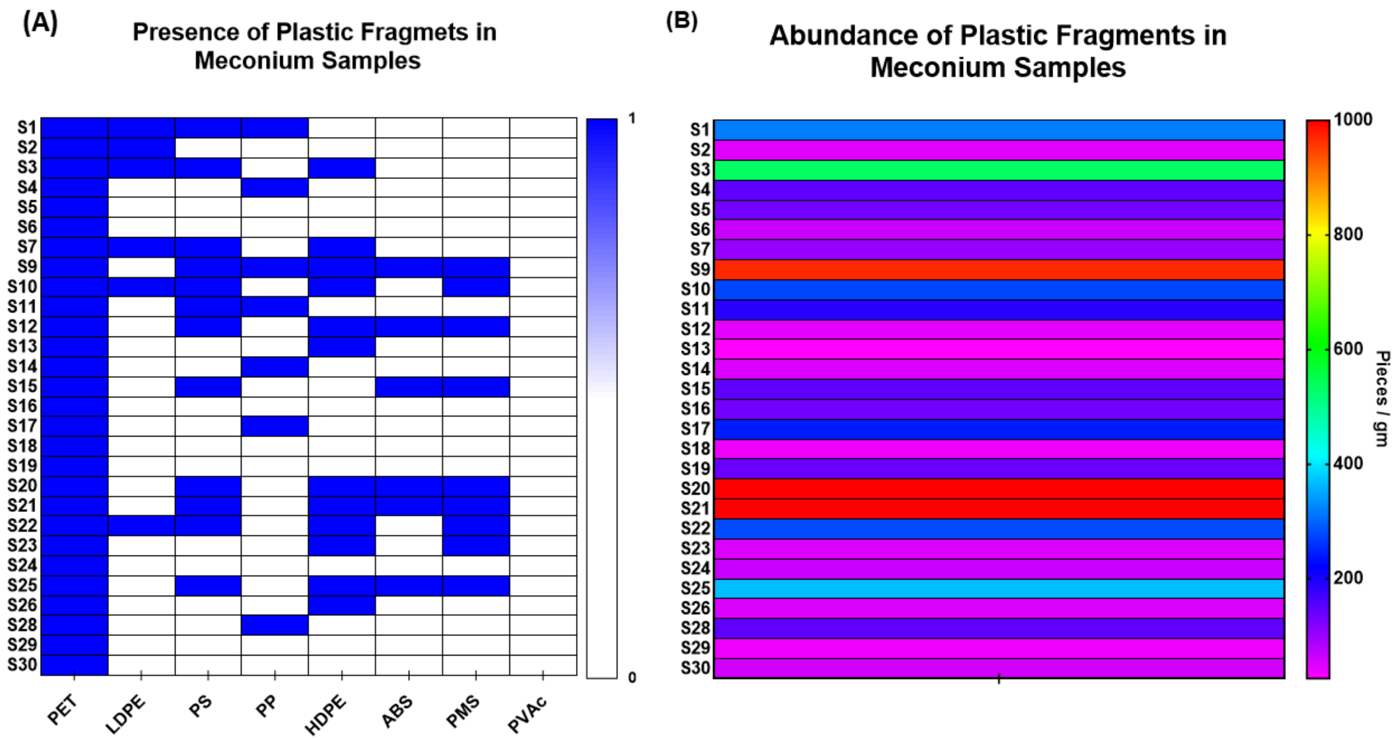

Figure 3.3.1: Distribution and polymer profiles of microplastic fragments in meconium samples.

3.3.2. Distribution of Microplastic Fiber in Meconium Samples

The pie chart in figure 3.3.2a illustrates the prevalence of plastic fiber (nylon) in meconium samples among the study population (n = 28). A substantial majority of samples (89.28%) tested positive for the presence of nylon fibers, while only a small proportion (10.72%) showed no detectable presence. This indicates a markedly high occurrence of microplastic contamination in neonatal samples. Overall, the findings suggest that exposure to nylon-based microplastics may be widespread even at the earliest stages of human life.

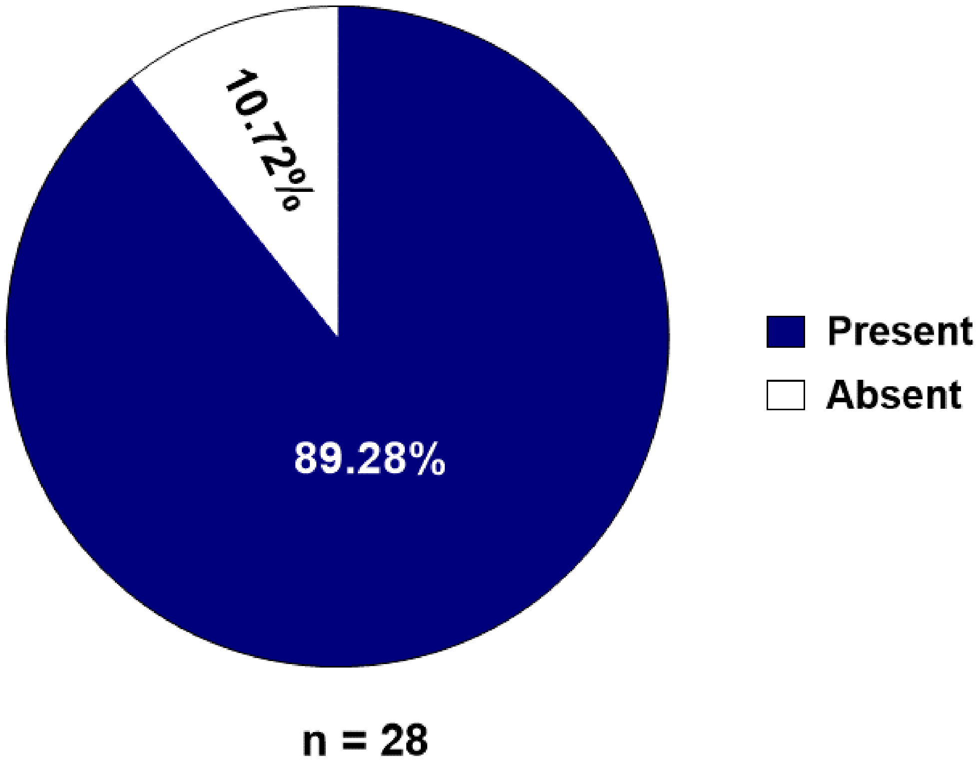

Figure 3.3.2a : Distribution of plastic fiber (nylon) in meconium samples.

The abundance of nylon fibers in meconium samples is depicted in the figure 3.3.2b, which shows usually low and fairly consistent quantities throughout the majority of samples. Sample S17 is a noteworthy exception, showing a significantly higher abundance than the others, suggesting a possible outlier. The remaining samples show slight differences, but there is no discernible pattern or clustering. Overall, the results point to little variation in the presence of nylon fiber, with one case showing noticeably higher levels.

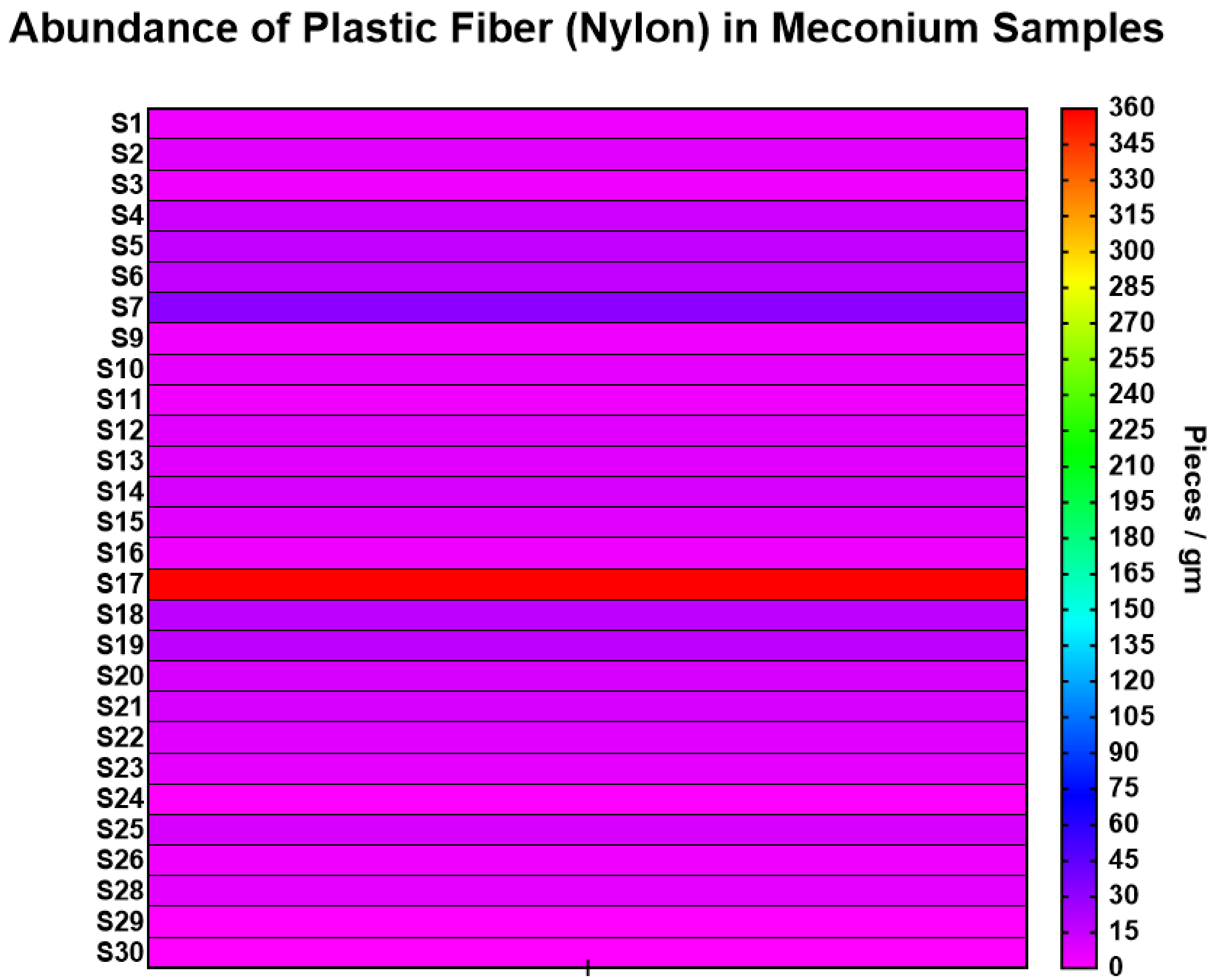

Figure 3.3.2b: Microplastic fiber distribution in meconium samples.

3.4. Distribution of Microplastics in Milk Samples

3.4.1. Distribution of Microplastic Fragments in Milk Samples

The heatmap in figure 3.4.1 reveals a diverse microplastic profile within the milk samples, characterized by the absolute predominance of Polyester (PET), Polypropylene (PP), and High-Density Polyethylene (HDPE). While PET was detected across the entire cohort, industrial polymers such as ABS and PEVA occurred at negligible frequencies, often approaching zero. Significant heterogeneity was observed, with samples BM5, BM16, BM18, BM19, BM20, FM1, and FM2 exhibiting a far broader polymer diversity and high co-occurrence of PET, PS, PP, and HDPE compared to the isolated polymer types found in the rest of the population. Notably, formula milk samples (FM1 and FM2) displayed a nearly saturated profile across the primary polymer categories, indicating a substantially higher cumulative burden than the irregular distribution seen in samples like BM6 or BM14. In contrast to the relatively isolated polymer detections found in samples like BM6 or BM14, this indicates that certain samples are subject to cumulative exposure pathways, most likely due to the mechanical deterioration of feeding infrastructure in formula samples and high-frequency maternal environmental contact in the enriched breast milk samples.

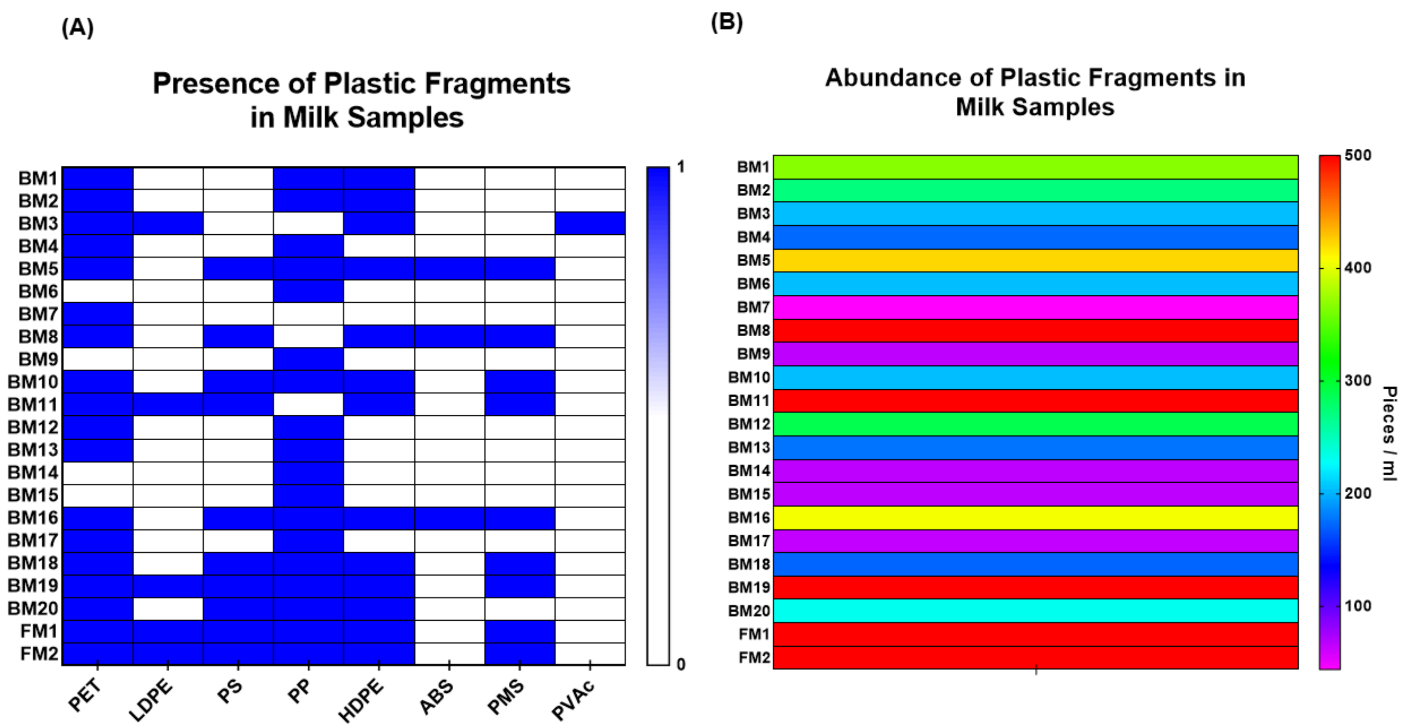

Figure 3.4.1: Presence and abundance of plastic fragments in milk samples.

3.4.2. Distribution of Microplastic Fiber in Milk Samples

The pie chart in figure 3.4.2a demonstrates the prevalence of plastic fiber (nylon) in milk samples within the study population (n = 28). A majority of the samples (77.27%) showed the presence of nylon fibers, whereas 22.73% were found to be free from detectable contamination. This indicates a notably high occurrence of microplastic contamination in milk samples, although lower than that observed in meconium. Overall, the findings suggest that exposure to nylon microplastics may extend through early dietary sources.

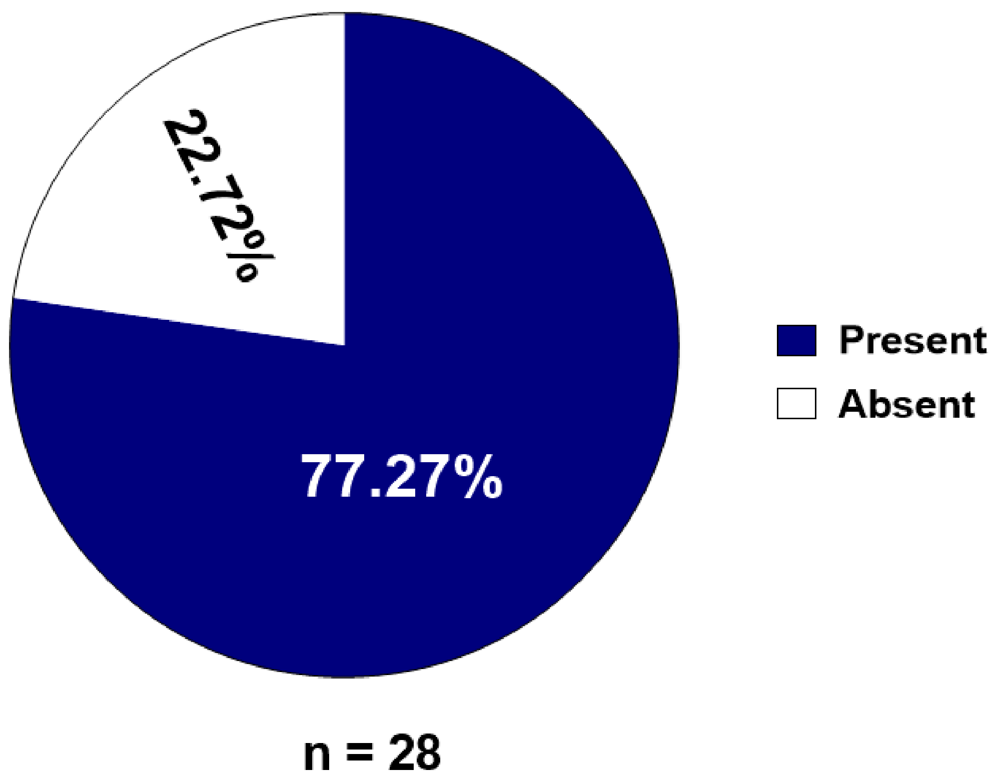

Figure 3.4.2a: Distribution of plastic fiber (nylon) in milk samples.

The heatmap in figure 3.4.2b of nylon fiber abundance in milk samples indicates low to moderate variability across samples, with most values ranging between 2 and 4 pieces/ml. A few samples, such as BM4 and BM5, show relatively higher concentrations, while several samples (e.g., BM2, BM6, BM14, BM17, and FM2) exhibit negligible or zero counts. Overall, the distribution suggests sporadic presence of nylon fibers without a consistent pattern, highlighting moderate heterogeneity among milk samples.

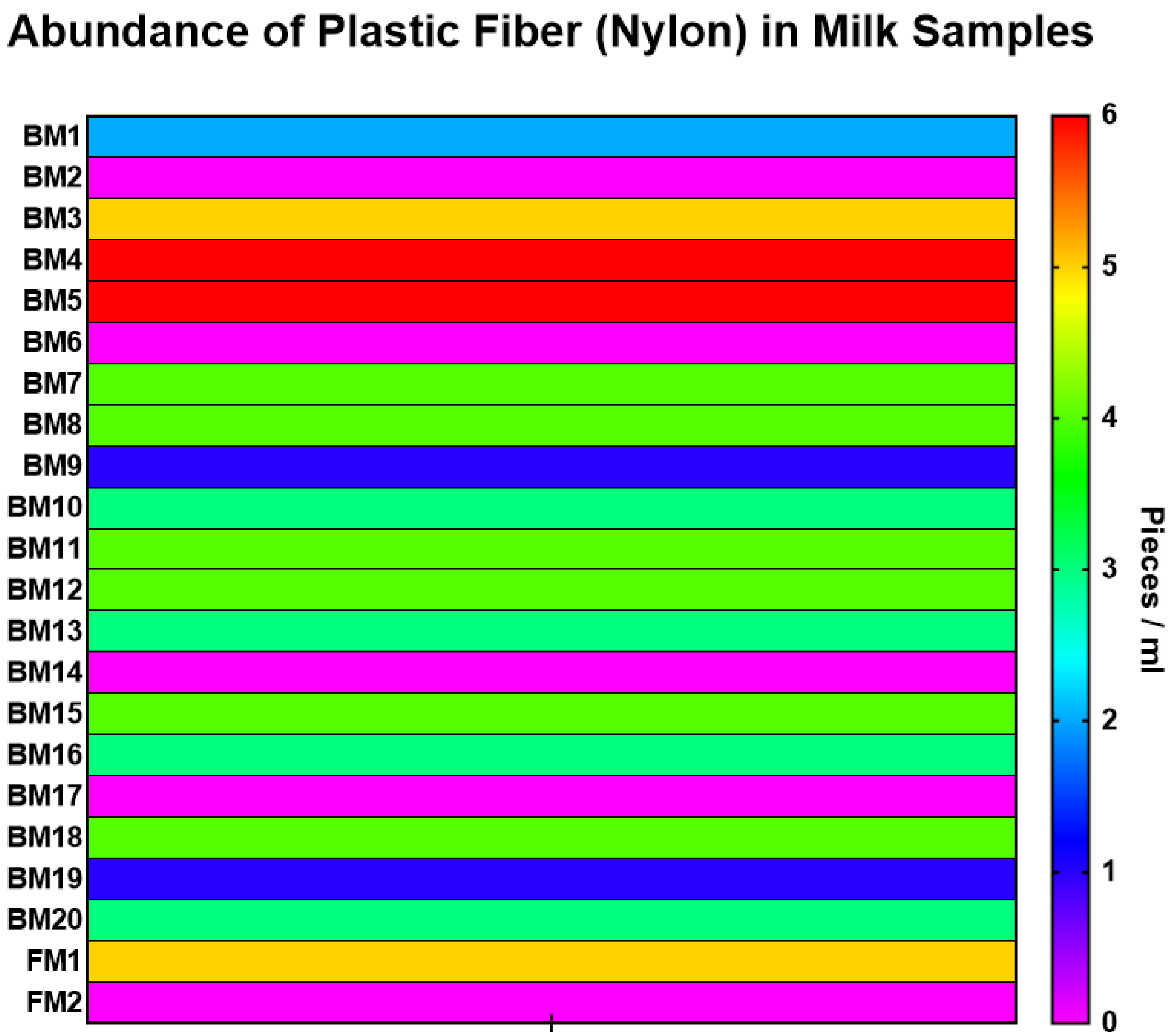

Figure 3.4.2b: Microplastic fiber distribution in milk samples.

3.5. Distribution of Heavy Metals in Meconium Samples

Heavy metals concentration in meconium samples were analyzed through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in figure 3.5. The color gradient in the heatmap indicates a distinct variation in concentration intensity, indicating a heterogeneous distribution of heavy metals throughout the tested meconium samples in Parts Per Million (PPM) unit. We tasted a total 15 heavy metals exposure among all stool samples. While certain metals such as Cu 63, Mn 55 (Figure 3.9) show significant fluctuation with clear high-intensity clusters in particular samples, others like Al 27, Be 9 and Co 59 show consistently low expression throughout the majority of samples, indicating little exposure or limited bioaccumulation. Remarkably, a particular group of samples including Zn 66 and Fe 57 (Figure 3.9) demonstrates simultaneous elevation of several metals, suggesting similar contamination sources or potential cumulative exposure. In contrast, certain samples such as sample number 11, 26, 30 (Figure 3.9) consistently display low metal loads, which indicates relatively less exposure to the environment or to biological agents. As a whole, the heatmap depicts that different feces samples accumulate varied amounts of heavy metals, suggesting that the neonates have diverse exposure profiles.

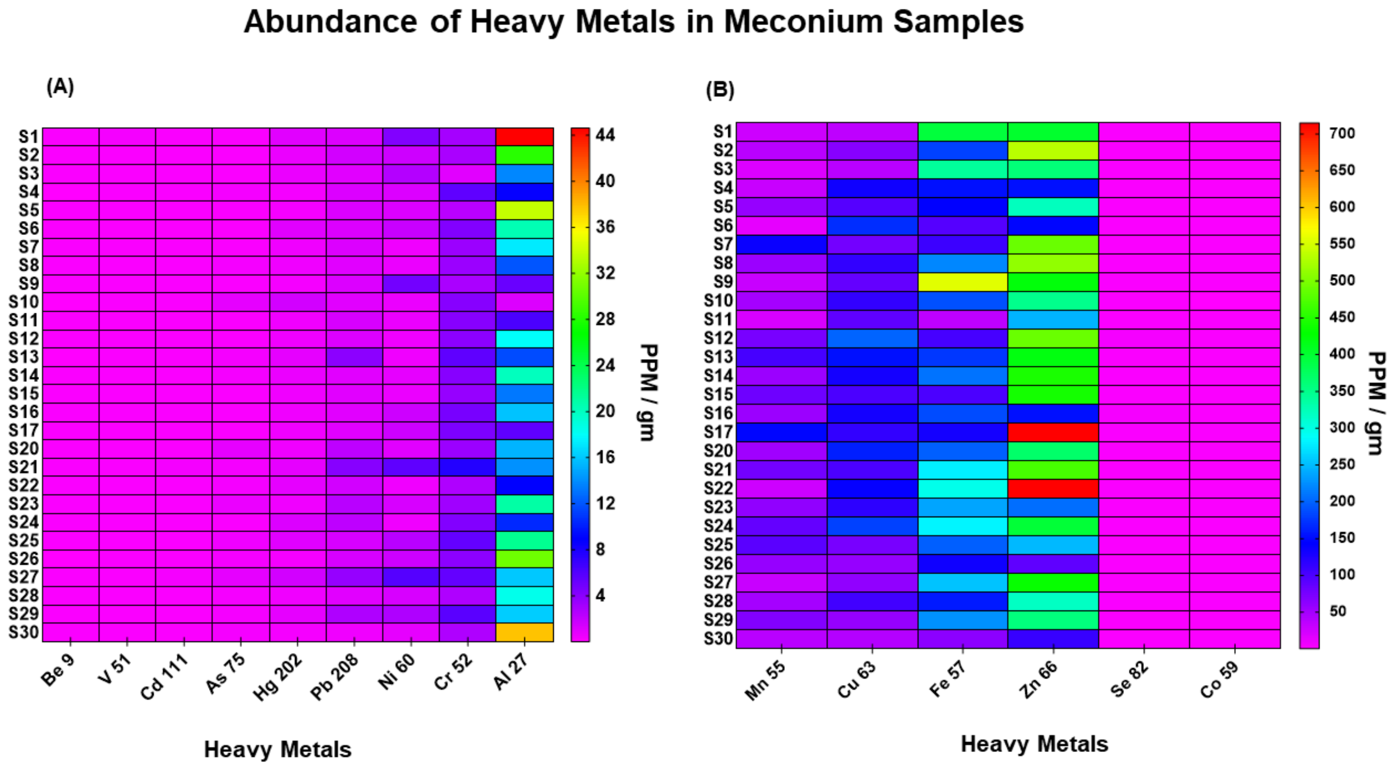

Figure 3.5 : Assessment of 15 different heavy metals exposure in meconium samples.

3.6. Comparative Analysis of Microplastics

3.6.1. Analysis of Microplastics Present between Male and Female

The comparative analysis of microplastic abundance by gender figure 3.6.1 indicates no statistically significant differences between male and female neonates across all measured categories. Specifically, the number of microplastic fragments (Figure 3.6.1a) was higher in males compared to females; however, this difference was not significant (P = 0.2190), suggesting substantial intra-group variability. Similarly, the abundance of microplastic fibers, particularly nylon figure 3.6.1b, showed comparable distributions between genders, with females exhibiting a slightly higher median value, though the difference was not statistically significant (P = 0.6737). In terms of total microplastic load figure 3.6.1c, males again demonstrated a higher overall count than females, but the difference approached, yet did not reach, statistical significance (P = 0.0758). Overall, while a consistent trend of higher microplastic presence in males is evident in both fragment and total counts, the lack of statistical significance (ns) across all comparisons suggests that gender does not play a decisive role in microplastic accumulation in meconium samples in this study cohort.

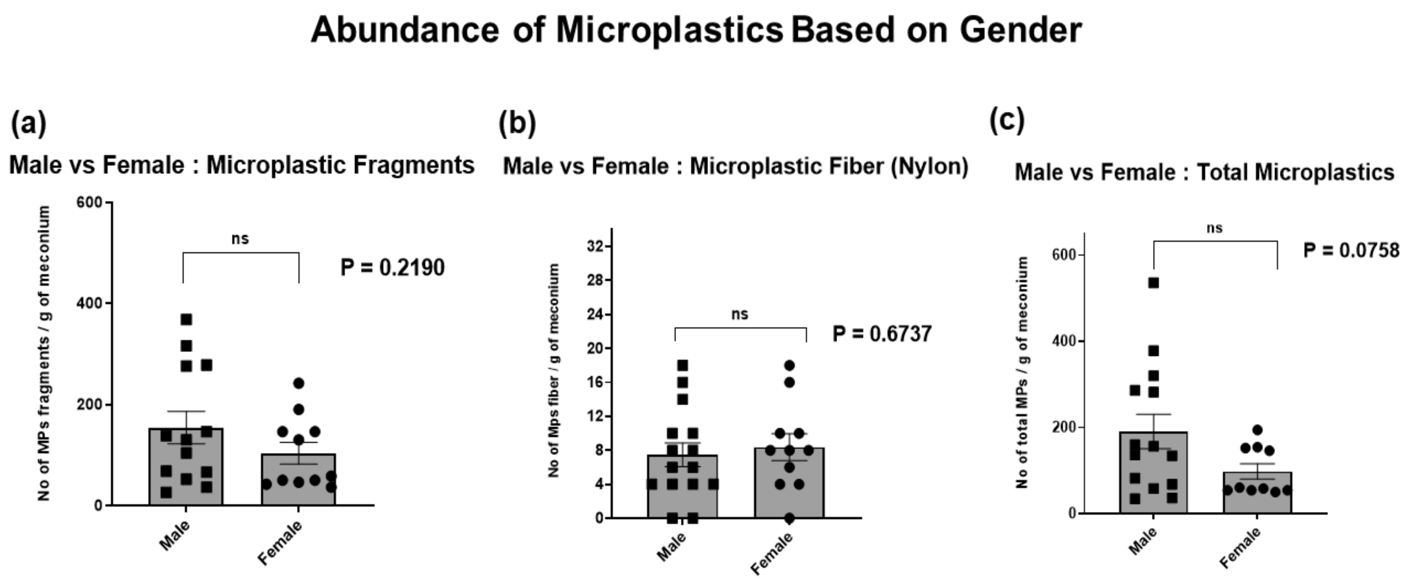

Figure 3.6.1: Microplastic count based on gender.

3.6.2. Analysis of Microplastic Fragments Present in Pre-term and Term Neonates

Figure 3.6.2 shows the comparative analysis of microplastic abundance based on gestational age, which shows no consistent statistically significant differences between preterm (34–36 weeks) and term (37–40 weeks) neonates. The number of microplastic fragments (Figure A) was higher in term samples and reached statistical significance ($P < 0.05$), indicating a possible increase with advancing gestational age. However, total microplastic counts 3.6.2b and 3.6.2c were also higher in term neonates but remained statistically non-significant (P=0.2051, P = 0.2951), reflecting notable intra-group variability. Overall, despite a trend of higher microplastic presence in term neonates, the lack of consistent significance suggests gestational age is not a strong determinant of microplastic accumulation in this cohort.

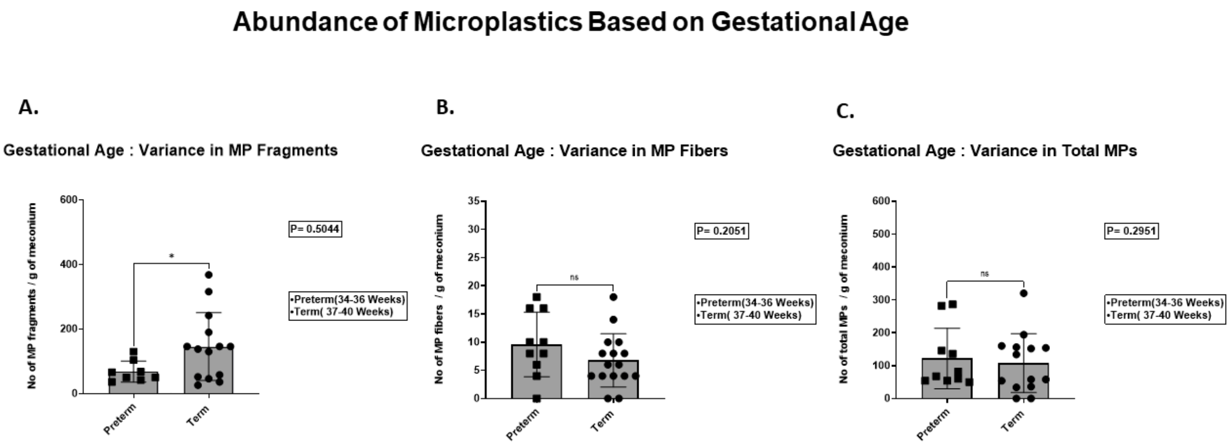

Figure 3.6.2: Variance in MPs based on gestational age.

3.6.3. Analysis of Microplastics Present between Exclusive Feeding and Mixed Feeding

Graph 3.6.3a describes the difference in fragment count (fragments per gram of meconium) between Exclusive Breast Feeding and Mixed Feeding. Overall Exclusive breast feeding shows a higher mean number of microplastic fragments than Mixed feeding mean number of fragments. It is observed that both groups represent wide variability with several higher value outliers in the Exclusive feeding type. Also, in statistical tests, the result is not significant according to the apparent difference in averages. This indicates that while Exclusive breastfeeding may visually appear to increase microplastic exposure in meconium, the difference is not statistically significant (P = 0.20; ns). Though a meaningful association with microplastic abundance in neonates is not described by feeding type.The graph 3.6.3b illustrates a comparative description of microplastic fiber counts (fragments per gram meconium) among Exclusive Breast Feeding and Mixed Feeding neonates. In the Exclusive breastfeeding group, the average number of microplastic fibers appears slightly higher compared to the mixed feeding group. Both types describe moderate variability in individual measurement. Also, in statistical analysis, the difference is not significant (P = 0.28) and that indicates between feeding type and the abundance of microplastic fiber in neonatal meconium there is no meaningful association.The graph 3.6.3c of total microplastic count (per gram of meconium) compares Exclusive Feeding and Mixed Feeding types. The averages are slightly different, though it approaches the central tendency. Both groups show the spread of data points, and it indicates that individual infants have different levels of microplastics. Moreover, statistically, there is no significant difference between the two types (P = 0.37). This means no meaningful effect on total microplastic abundance in neonatal meconium of feeding type. Even though averages look slightly different, the difference is so small that it is considered statistically meaningless.

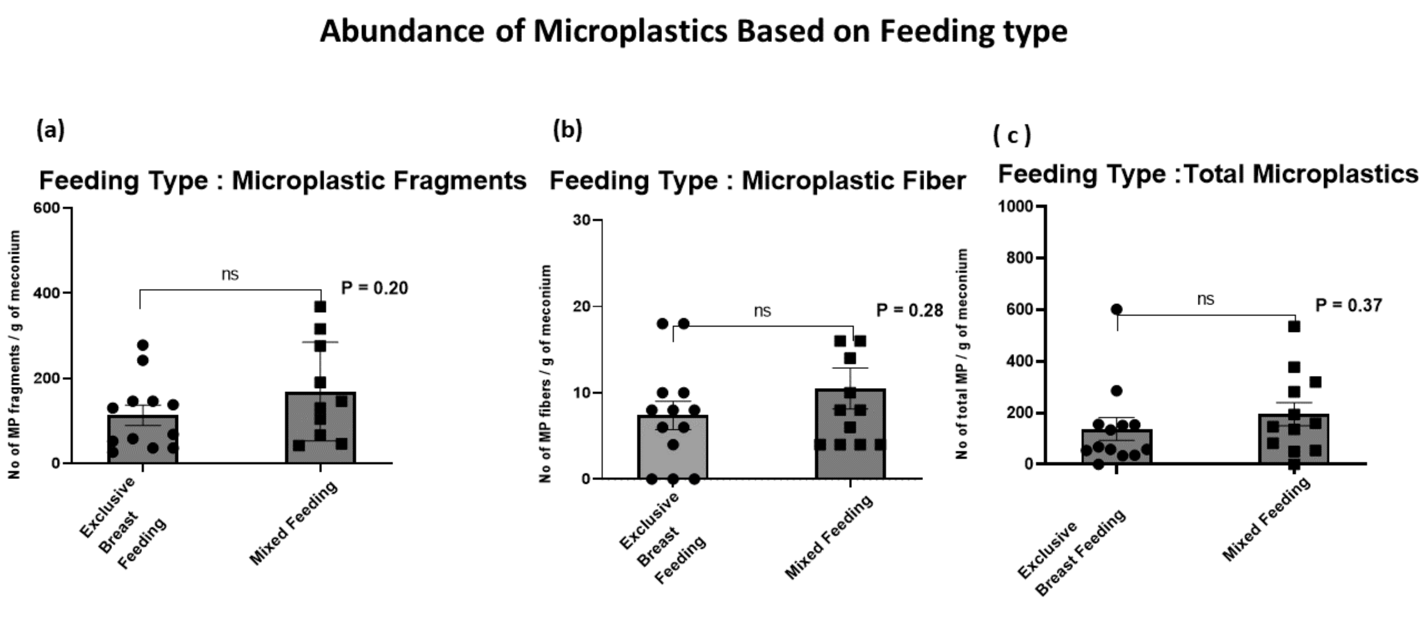

Figure 3.6.3: Microplastic count based on feeding type.

3.6.4. Analysis of Microplastic Fragments Present in Materials from Which Meconium was Collected

Analysis of microplastics in meconium samples collected using diapers and traditional katha (cloth) revealed comparable results across all measured parameters. As shown in figure 3.6.4a, the number of microplastic fragments per gram was slightly higher and more variable in the katha group; however, this difference was not statistically significant ($P = 0.85$), indicating no meaningful influence of collection material. Similarly, Figure 3.6.4b demonstrates that microplastic fibers, particularly nylon fibers, were detected in both groups, with a relatively higher median in the katha group, though again without statistical significance ($P = 0.10$). In terms of total microplastic particles, Figure 3.6.4c shows that both diaper- and cloth-collected samples exhibited considerable variability, with the katha group presenting a marginally higher median count. Despite these observed differences, statistical analysis confirmed no significant variation between the two groups ($P = 0.99$). Overall, these findings suggest that the type of material used for meconium collection does not significantly affect the detection or quantification of microplastic fragments, fibers, or total particles.

Abundance of Microplastics Based on Materials from Which Meconium was Collected

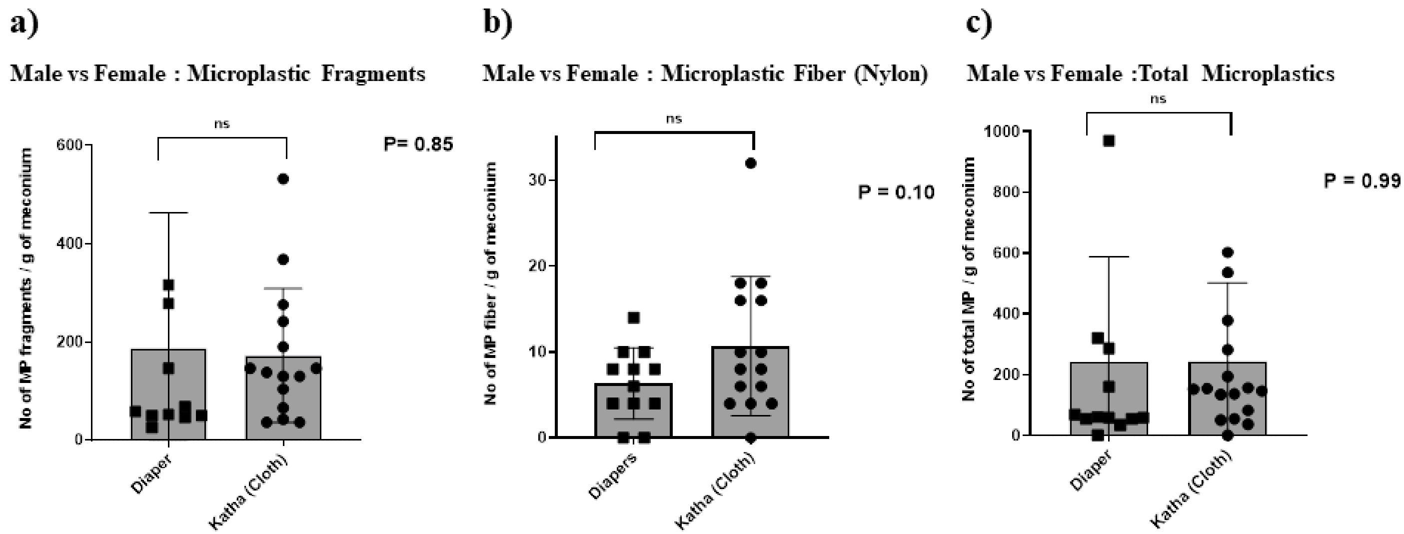

Figure 3.6.4: Microplastics count based on meconium collection material.

3.7. Comparative Analysis of Abundance of Toxic Heavy Metals in Meconium

3.7.1. Analysis of Abundance of Toxic Heavy Metals (Arsenic) in Meconium

The figure 3.7.1 illustrates the variation in arsenic (As) concentration in meconium across gender, feeding type, and gestational age. A statistically significant difference was observed between male and female neonates ($P = 0.0402$), where males exhibited comparatively higher arsenic levels and greater variability, suggesting potential gender-based differences in utero exposure or metabolism. Similarly, feeding type showed a significant association ($P = 0.0228$), with exclusively breastfed infants demonstrating higher arsenic concentrations than those receiving mixed feeding, indicating a possible postnatal exposure pathway or maternal transfer influence. In contrast, no significant difference was identified between preterm and term infants ($P = 0.3373$), implying that gestational age may not be a determining factor in arsenic accumulation in meconium. Overall, the results highlight that biological sex and feeding practices are more influential determinants of arsenic abundance compared to gestational maturity in this cohort.

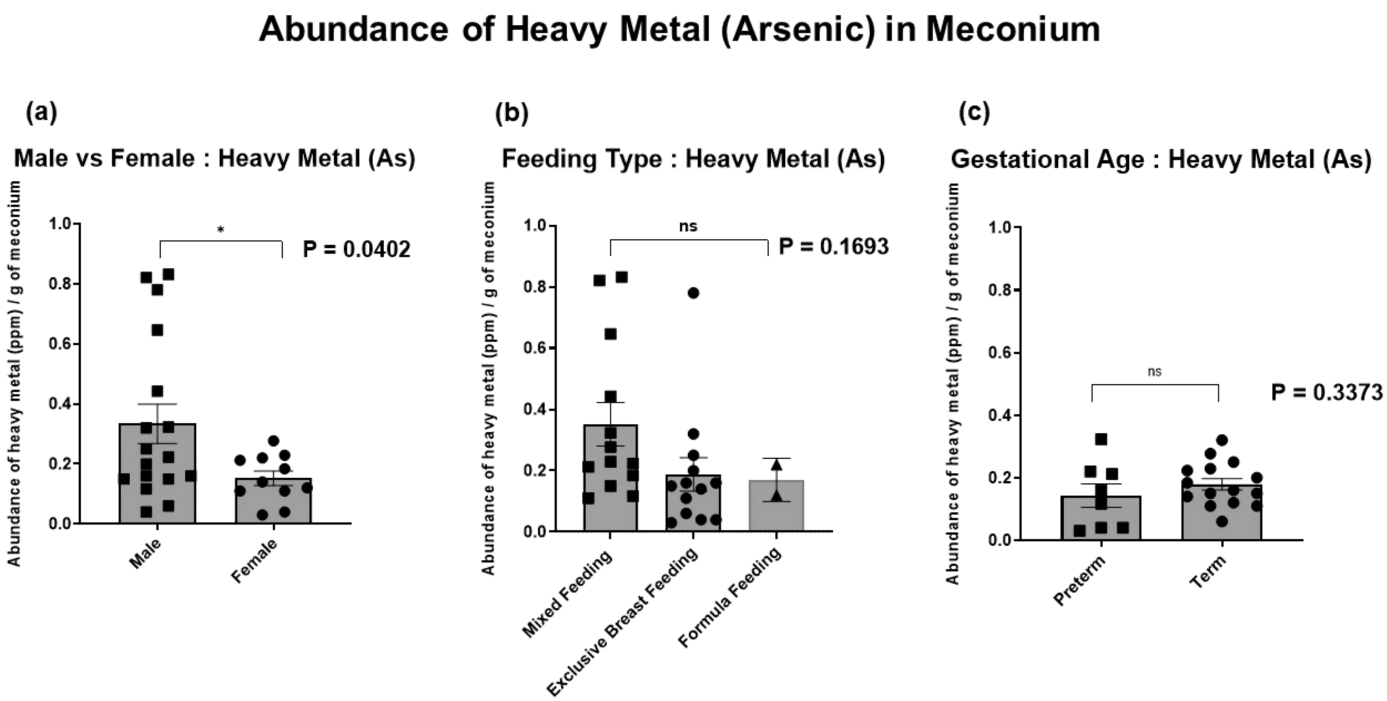

Figure 3.7.1: Distribution of arsenic in meconium samples.

3.7.2. Analysis of Abundance of Toxic Heavy Metals (Lead) in Meconium

Figure 3.7.2 presents the distribution of lead (Pb) concentrations in meconium across gender, feeding type, and gestational age. No statistically significant differences were observed among any of the compared groups. Although male neonates exhibited slightly higher mean Pb levels than females, this difference was not significant ($P = 0.0696$), indicating only a marginal trend toward gender-based variation. Similarly, feeding type showed no significant association with Pb concentration ($P = 0.3852$), despite minor variability between mixed feeding and exclusive breastfeeding groups. In terms of gestational age, preterm infants demonstrated somewhat higher Pb levels compared to term infants; however, this difference was also not statistically significant (P = 0.1706). Overall, compared to arsenic, lead exposure appears more uniformly distributed across demographic and clinical variables, with no strong determinants identified within this cohort.

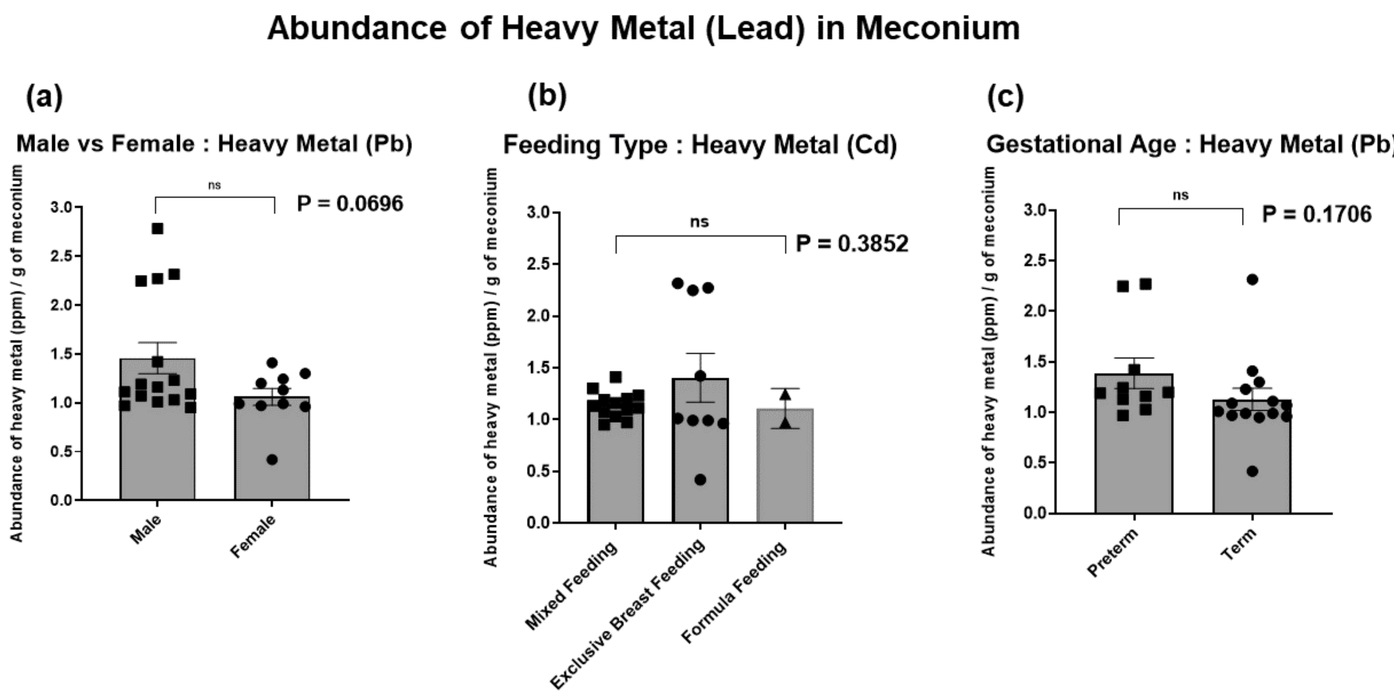

Figure 3.7.2: Distribution of lead in meconium samples.

3.7.3. Analysis of Abundance of Toxic Heavy Metals (Cadmium) in Meconium

The figure 3.7.3 depicts cadmium (Cd) concentrations in meconium across gender, feeding type, and gestational age, revealing no statistically significant differences among the examined groups. Cd levels were comparable between male and female neonates (P = 0.7331), indicating no evidence of gender-based variation in exposure. Similarly, feeding type did not show a significant association with Cd concentration (P = 0.1332), although exclusively breastfed infants exhibited slightly higher variability compared to mixed and formula-fed groups. In terms of gestational age, term infants demonstrated marginally higher Cd levels than preterm infants; however, this difference was not statistically significant (P = 0.2348). Overall, in contrast to arsenic, cadmium appears to be more uniformly distributed across demographic and clinical categories, similar to the pattern observed for lead, suggesting limited influence of these factors on Cd accumulation in this cohort.

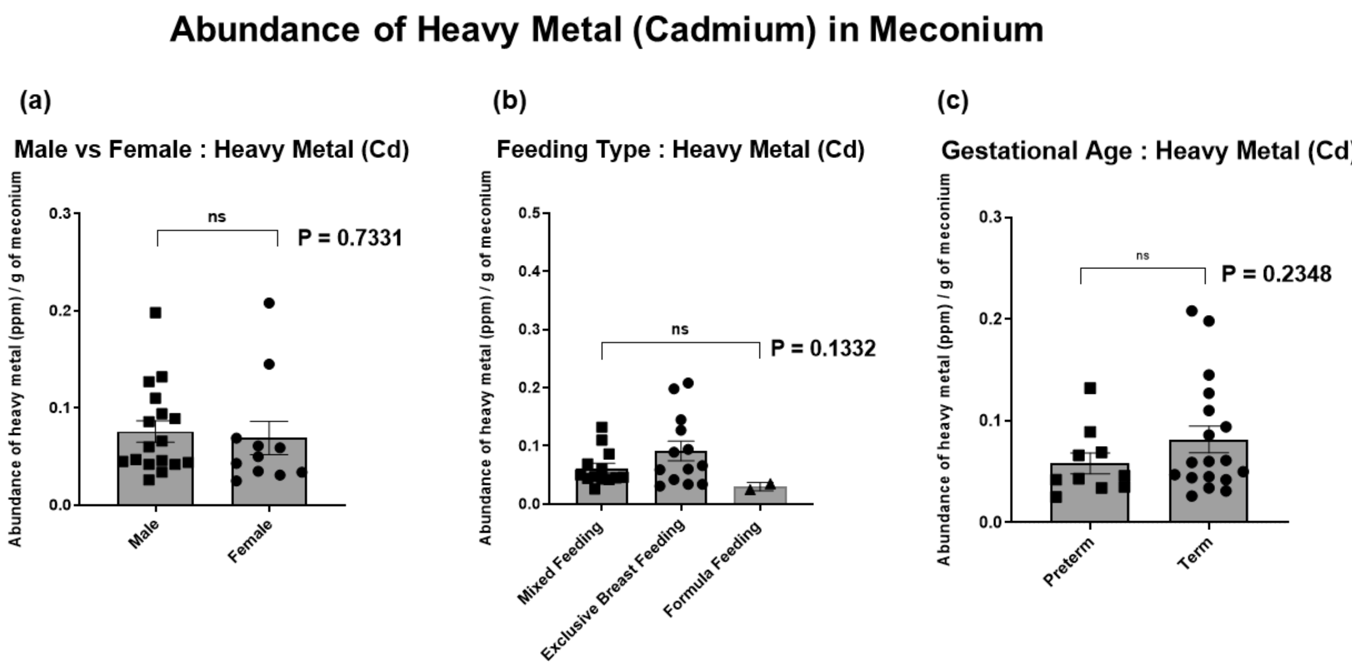

Figure 3.7.3: Distribution of cadmium in meconium samples.

3.7.4. Analysis of Abundance of Toxic Heavy Metals (Chromium) in Meconium

Figure 3.7.4 illustrates chromium (Cr) concentrations in meconium across gender, feeding type, and gestational age, demonstrating no statistically significant differences among the compared groups. Although male neonates exhibited slightly higher mean Cr levels than females, this difference was not significant (P = 0.2381), indicating minimal gender-based variation. Similarly, feeding type showed no significant association with chromium concentration (P = 0.5819), despite minor fluctuations among mixed, exclusive breastfeeding, and formula feeding groups. In terms of gestational age, term infants displayed marginally higher Cr levels compared to preterm infants; however, this difference was also not statistically significant (P = 0.6112). Overall, chromium distribution appears relatively consistent across all demographic and clinical variables, aligning with the patterns observed for lead and cadmium, and contrasting with the significant variations identified for arsenic in this cohort.

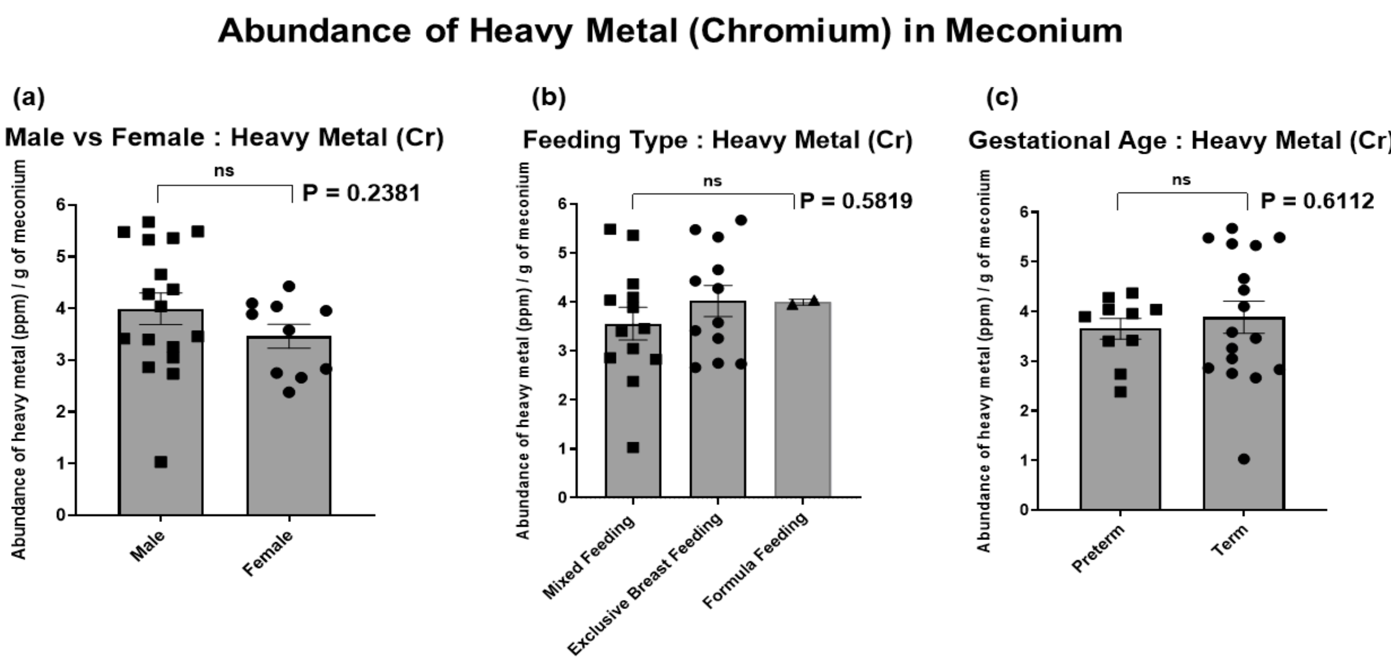

Figure 3.7.4: Distribution of chromium in meconium samples.

3.7.5. Analysis of Abundance of Toxic Heavy Metal (Vanadium) in Meconium

Figure 3.7.5 shows the change of Vanadium (V) levels in meconium for gender, feeding type, and gestational age. Female babies have lower Vanadium levels than male babies. And the difference is not statistically significant (P = 0.5), meaning gender does not affect exposure or metabolism. On the other hand, Exclusively Breastfed babies have higher Vanadium levels than mixed-fed babies. This difference is also not significant (P = 0.8), and that suggests Vanadium may not pass from mother to baby through breast milk. Also, there is no significant difference between preterm and term babies (P = 0.3). This means Vanadium levels are not strongly affected by gestational age.

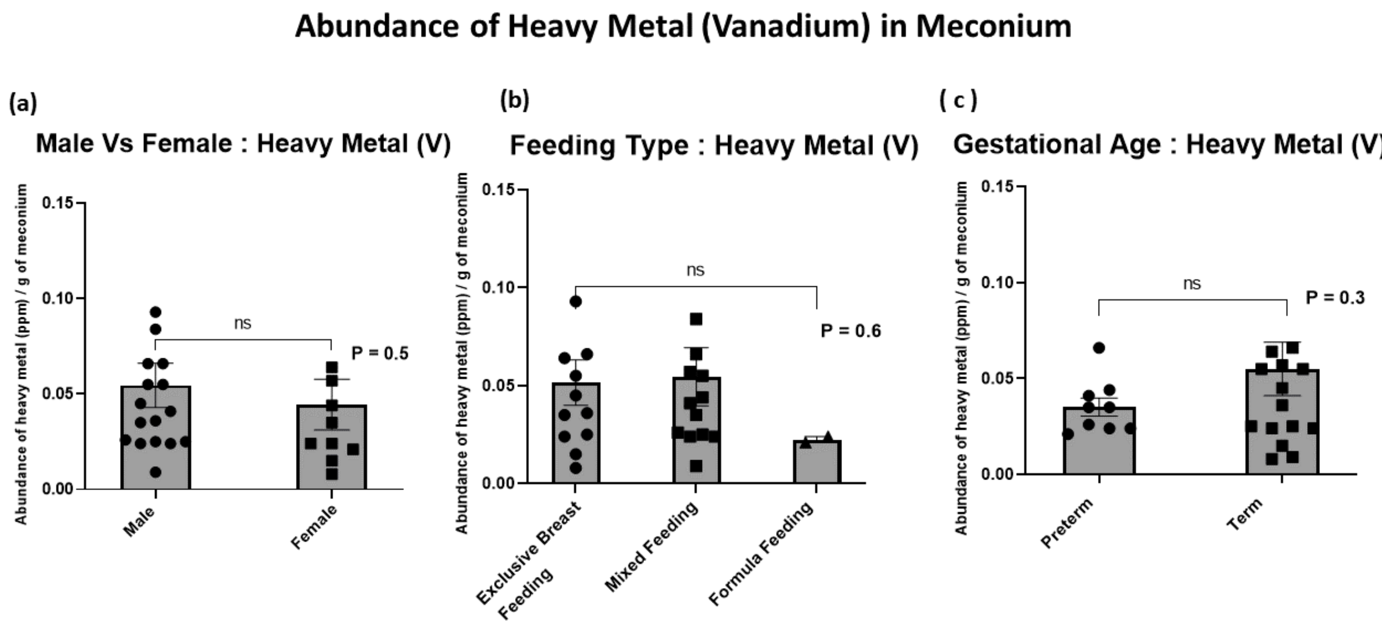

Figure 3.7.5: Distribution of vanadium in meconium samples.

3.7.6. Analysis of the Abundance of Toxic Heavy Metal (Nickel) in Meconium

Figure 3.7.6 shows a comparative analysis of nickel (Ni) abundance in meconium, which shows mostly non-significant differences across groups. Specifically, Ni levels were slightly higher in males compared to females figure 3.7.6a, but this difference was not statistically significant (P = 0.1714), indicating considerable intra-group variability. Similarly, no significant variation was observed among different feeding types in figure 3.7.6b, with comparable Ni levels across exclusive breastfeeding, mixed feeding, and formula feeding groups (P = 0.8578). In contrast, gestational age in figure 3.7.6c showed a statistically significant difference, where term neonates exhibited higher Ni levels compared to preterm (P = 0.0187). Overall, while gender and feeding type do not appear to influence Ni accumulation, gestational age may play a role, with increased levels observed in term neonates.

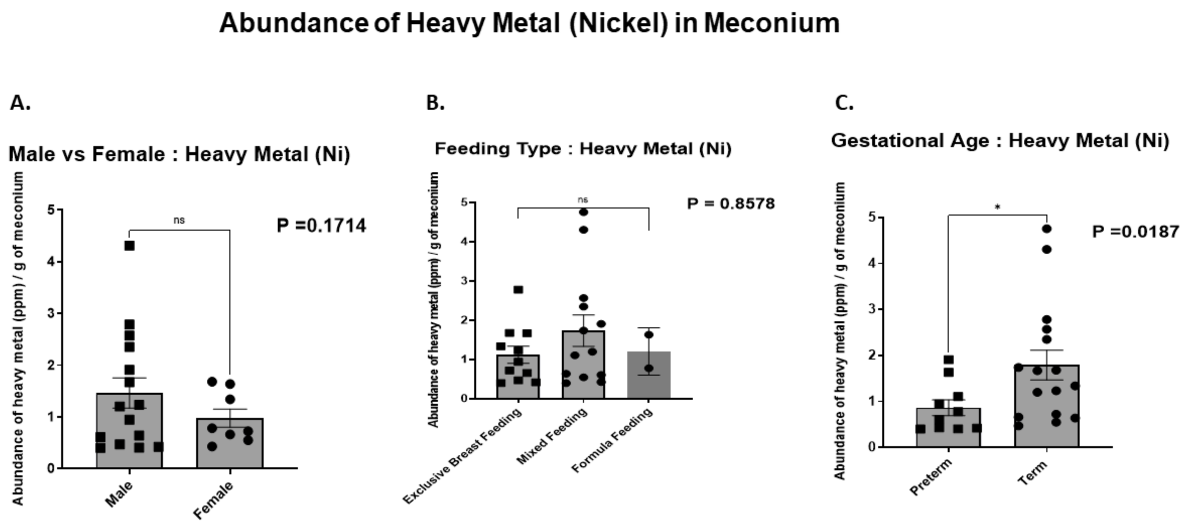

Figure 3.7.6: Distribution of nickel in meconium samples.

3.7.7. Analysis of the Abundance of Toxic Heavy Metal (Mercury) in Meconium

Figure 3.7.7a shows a comparative analysis of mercury (Hg) abundance in meconium, which reveals selective statistical significance across groups. Hg levels were higher in males compared to females in figure 3.7.7a, and this difference was statistically significant (P = 0.0490), indicating a potential gender-related variation. In contrast, no significant difference was observed between preterm and term neonates in figure 3.7.7b, with similar Hg distributions (P = 0.5070). Likewise, feeding type in figure 3.7.7c showed no significant variation in Hg levels across exclusive breastfeeding, mixed feeding, and formula feeding groups (P = 0.6378). Overall, while gender appears to influence Hg accumulation, gestational age and feeding type do not show a significant effect in this cohort.

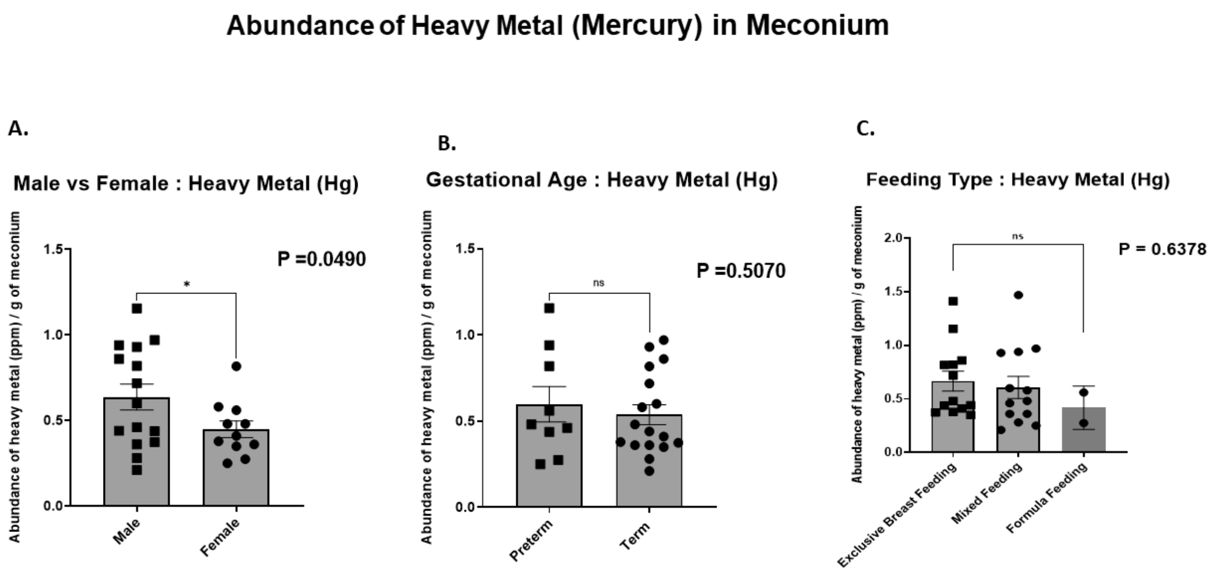

Figure 3.7.7: Distribution of mercury in meconium samples.

3.7.8. Analysis of the Abundance of Toxic Heavy Metal (Aluminium) in Meconium

Figure 3.7.8 shows a comparative analysis of Aluminium abundance in meconium, which reveals selective statistical significance across groups. Aluminium levels were higher in females compared to males (figure 3.7.8a), and this difference was statistically non-significant (P = 0.1331), indicating a non-potential gender-related variation. In contrast, no significant difference was observed between preterm and term neonates (figure 3.7.8b), with similar Al distributions (P =0.3490). Likewise, feeding type (figure 3.7.8c) showed no significant variation in Al levels across exclusive breastfeeding, mixed feeding, and formula feeding groups (P = 0.1503). Overall, gender, gestational age, and feeding type do not show a significant effect in this cohort.

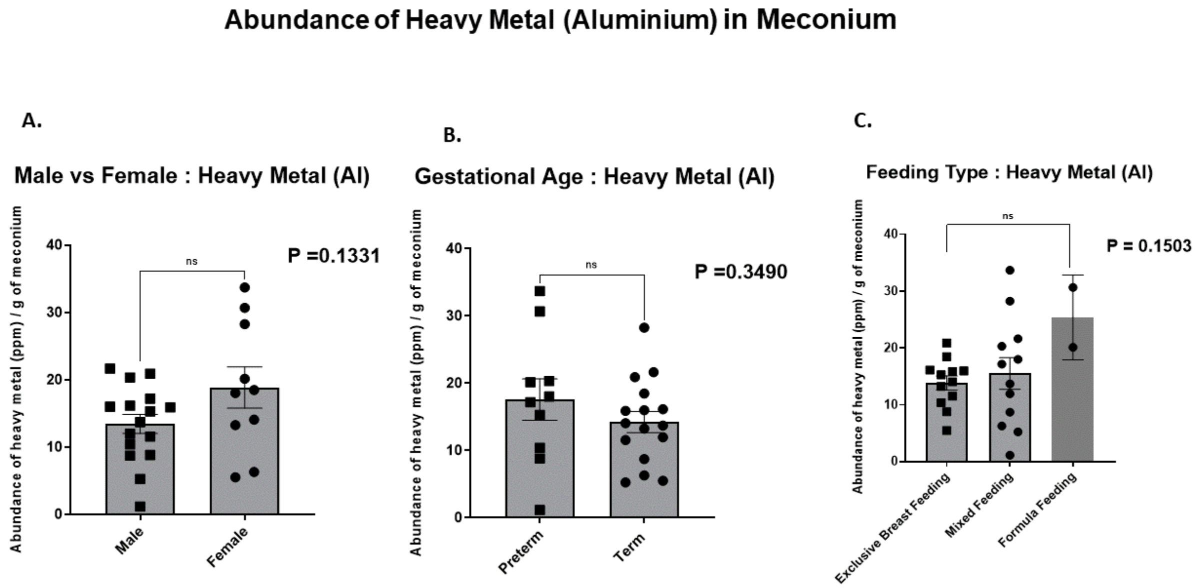

Figure 3.7.8: Distribution of aluminium in meconium samples.

3.7.9 . Analysis of the Abundance of Toxic Heavy Metal (Be) in Meconium

Figure 3.7.9 shows a comparative analysis of Beryllium (Be) abundance in meconium, showing mostly non-significant differences across groups. Specifically, Ni levels were slightly higher in males compared to females (figure 3.7.9a), but this difference was not statistically significant (P =0.7888), indicating considerable intra-group variability. Similarly, no significant variation was observed among different gestational ages (figure 3.7.9b), with comparable Be levels across preterm and term groups (P = 0.0934). In contrast, feeding type (figure 3.7.9c) showed a statistically significant difference, where exclusively breastfeeding neonates exhibited higher Be levels compared to mixed feeding and formula feeding (P = 0.451). Overall, while gender and gestational age do not appear to influence Be accumulation, feeding type may play a role, with increased levels observed in exclusive breastfeeding neonates.

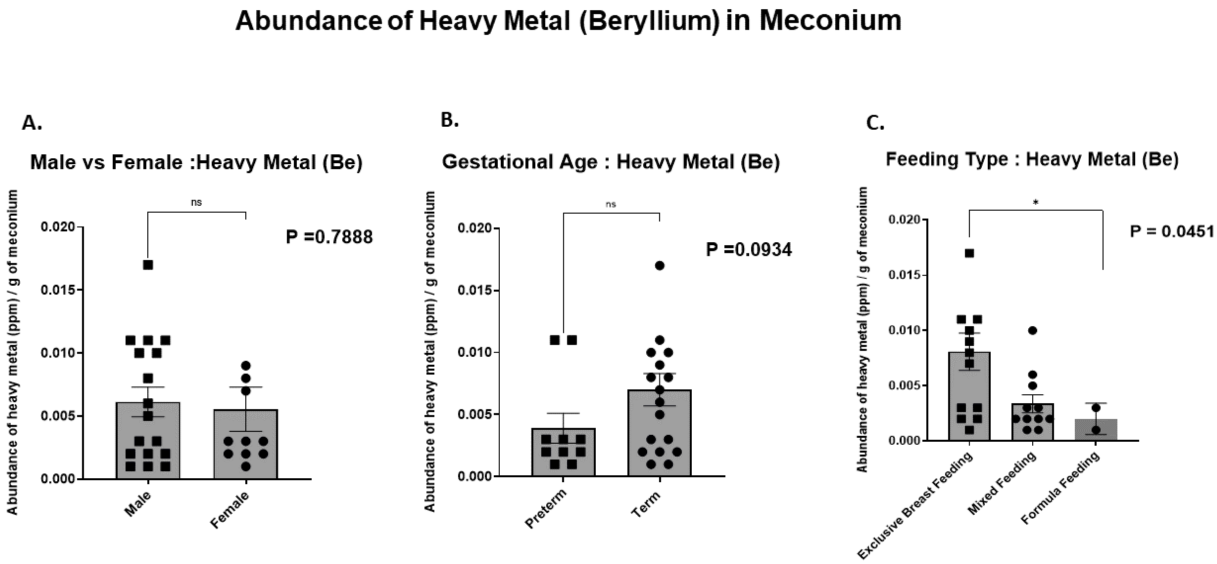

Figure 3.7.9: Distribution of beryllium in meconium samples.

3.8. Comparative Analysis of Abundance of Essential Heavy Metals in Meconium

3.8.1. Analysis of Abundance of Essential Heavy Metal (Selenium) in Meconium

Figure 3.8.1 shows the change of Selenium (Se) levels across meconium for gender, feeding type, and gestational age. Male babies have higher Selenium levels than female babies. And the difference is not statistically significant (P = 0.3), which means gender does not affect exposure or metabolism . Additionally, Exclusively Breastfed babies have higher Selenium levels than mixed-fed babies. This difference is also not significant (P = 0.4), and that suggests Selenium may not pass from mother to baby through breast milk. Also, differences among preterm and term babies are significant (P = 0.00). This means that selenium levels are strongly affected by gestational age.

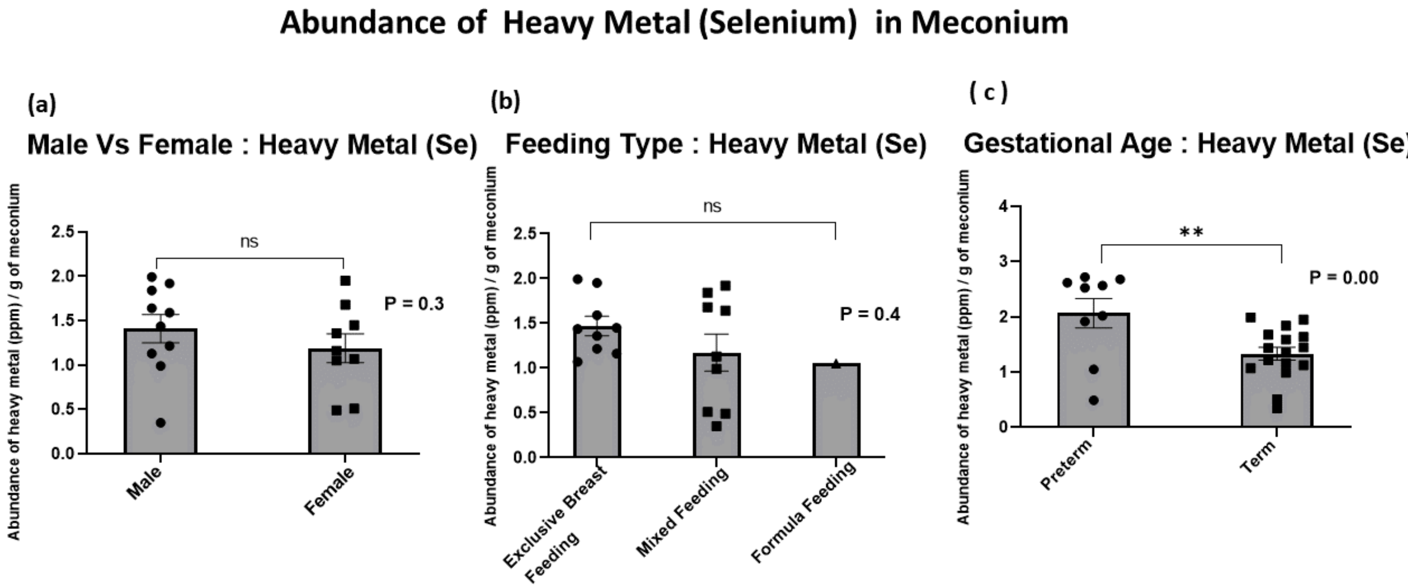

Figure 3.8.1: Distribution of selenium in meconium samples.

3.8.2. Analysis of the Abundance of Essential Heavy Metal (Copper) in Meconium

The figure 3.8.2 illustrates the difference in copper (Cu) levels across meconium for gender, feeding type, and gestational age. Male babies have higher Copper levels than female babies. And the difference is not statistically significant (P = 0.5), which indicates that gender does not affect exposure or metabolism. Moreover, Exclusively Breastfed babies have higher Copper levels than mixed-fed babies. This difference is also not significant (P = 0.7), and that suggests Copper may not pass from mother to baby through breast milk. Additionally, differences among preterm and term babies are not significant (P = 0.2). That means copper levels are strongly affected by gestational ag**e**

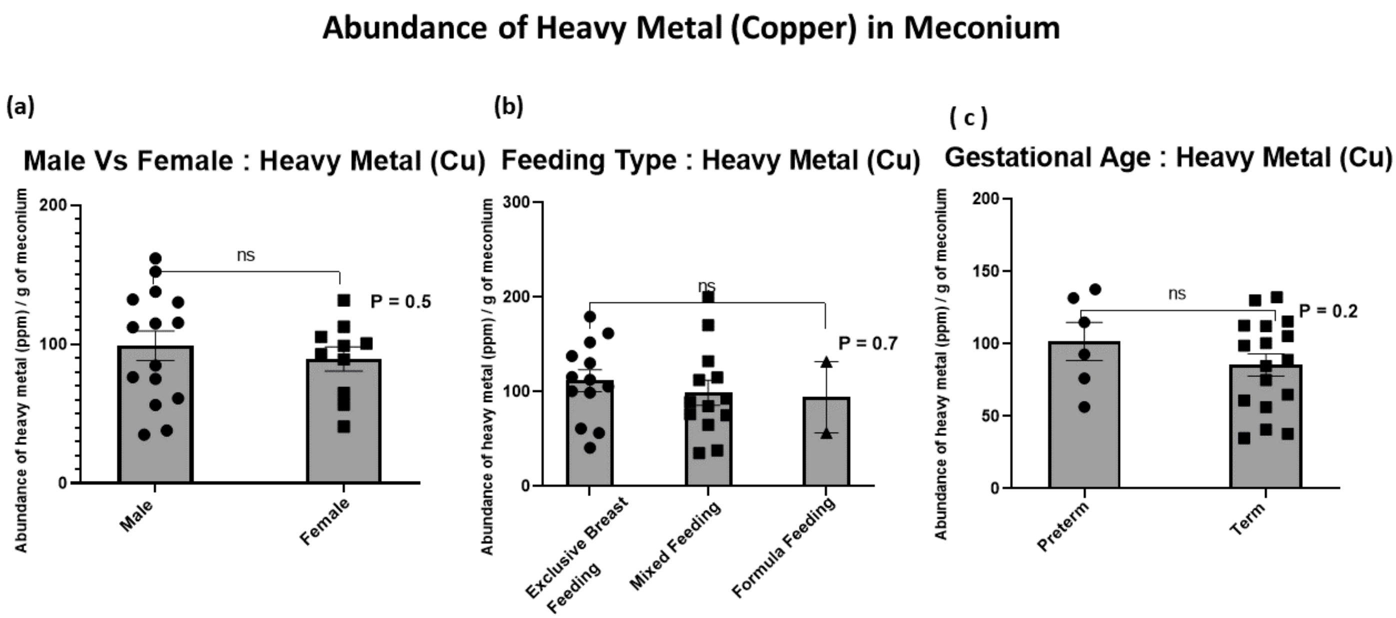

Figure 3.8.2: Distribution of copper in meconium samples.

3.8.3. Analysis of the Abundance of Essential Heavy Metal (Zinc) in Meconium

The figure 3.8.3 compares zinc (Zn) abundance in meconium (ppm per gram) across three categorical variables: infant sex (male vs. female), gestational age (preterm vs. term), and feeding type (mixed feeding, exclusive breastfeeding, formula feeding). Each dot represents an individual newborn, and p-values are shown for each comparison. No statistically significant differences were observed in meconium zinc levels across any of the subgroups. The comparison between male and female infants yielded a p-value of 0.766, indicating no sex-based difference. Similarly, zinc levels did not differ significantly between term and preterm neonates ($p = 0.515$). Among feeding types, the p-value was 0.64, showing no association with meconium zinc concentration. Overall, these findings suggest that sex, gestational age at birth, and early feeding type do not significantly influence zinc levels in meconium.

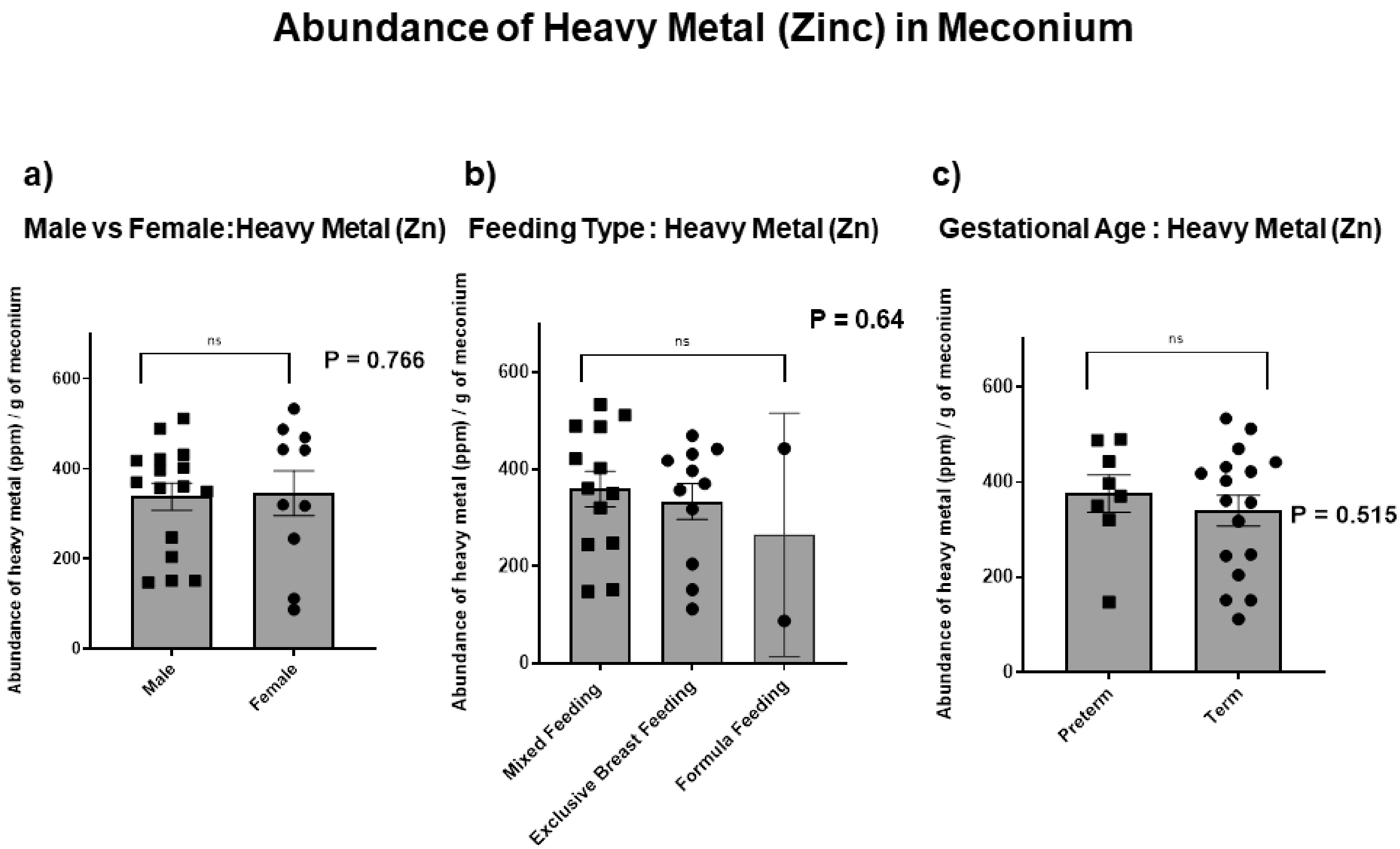

Figure 3.8.3: Distribution of zinc in meconium samples.

3.8.4 . Analysis of the Abundance of Essential Heavy Metal (Manganese) in Meconium

Figure 3.8.4 illustrates manganese (Mn) abundance in meconium (ppb per gram) across three subgroups: male versus female infants, feeding type (mixed feeding, exclusive breast feeding, formula feeding), and gestational age (preterm versus term). Statistical analysis revealed no significant differences: sex comparison yielded (P = 0.348), feeding type (P = 0.397), and gestational age (P = 0.433). All p-values exceeded the conventional threshold of 0.05, indicating no statistically meaningful associations between meconium manganese levels and any of the three variables. Nevertheless, these findings must be interpreted with caution, as the extremely small sample size renders the study underpowered and the p-values inherently unstable. Overall, no statistically significant differences were observed in meconium manganese levels across any of the compared subgroups. Therefore, based on these findings, there is no association between meconium manganese abundance and infant sex, feeding type, or gestational age.

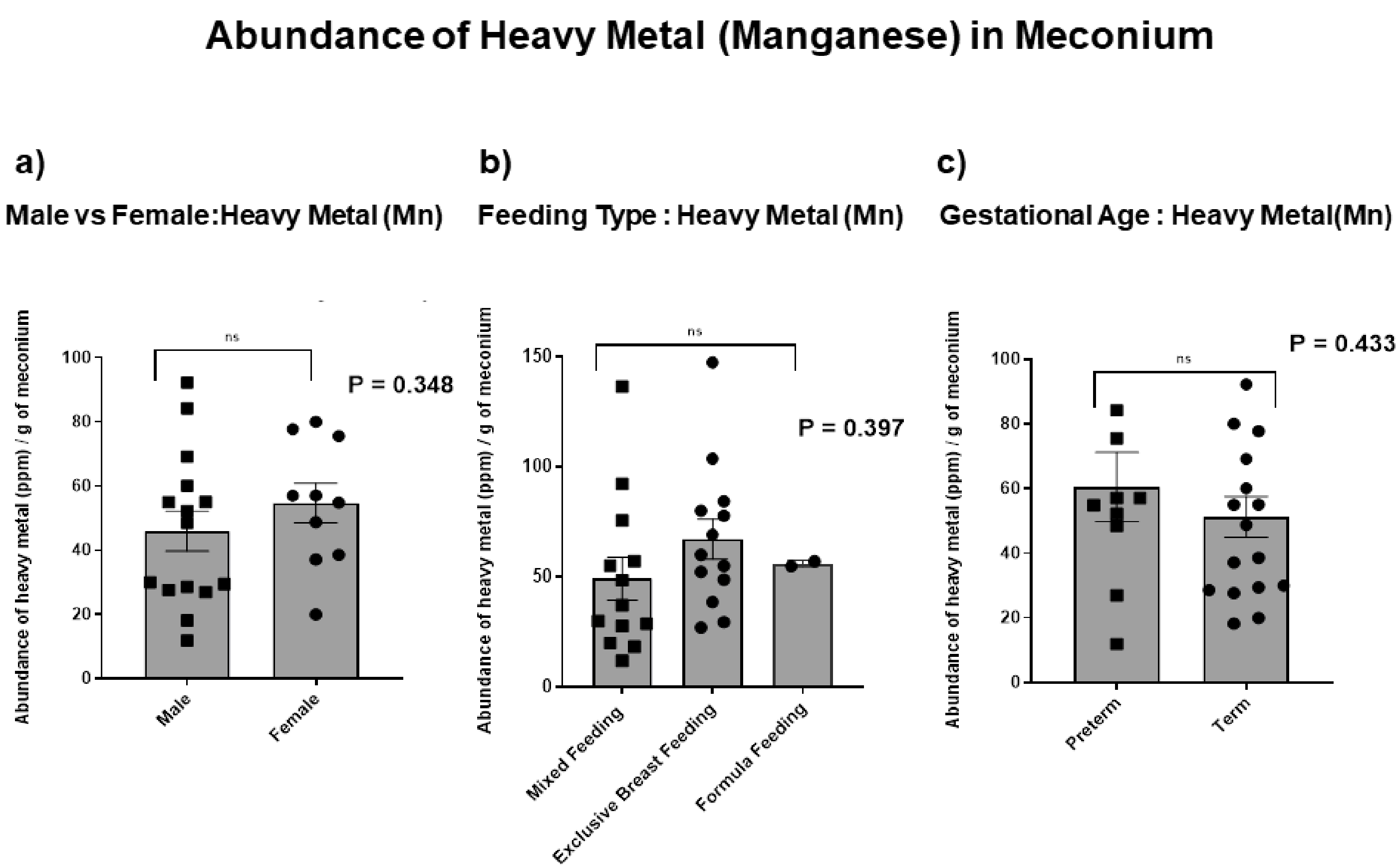

Figure 3.8.4: Distribution of manganese in meconium samples

3.8.5. Analysis of the Abundance of Essential Heavy Metal (Cobalt) in Meconium

Figure 3.8.5 presents cobalt (Co) abundance in meconium (ppm per gram) across three comparisons: male versus female infants, feeding type (mixed feeding, exclusive breastfeeding, formula feeding), and gestational age (preterm versus term). Statistical analysis revealed no significant difference in meconium cobalt levels between males and females (P=0.86), not significant). Similarly, gestational age showed no association with cobalt abundance (P=0.19), not significant. In contrast, feeding type yielded a statistically significant difference (P=0.007), indicating that cobalt levels in meconium vary significantly depending on whether the infant received mixed feeding, exclusive breastfeeding, or formula feeding. Among the three variables examined, only feeding type demonstrated a statistically significant association with meconium cobalt levels, while sex and gestational age did not. This finding suggests that early postnatal or prenatal dietary exposure may influence cobalt accumulation in meconium. The lack of significance for sex and gestational age indicates that these factors are unlikely to be major determinants of cobalt levels.

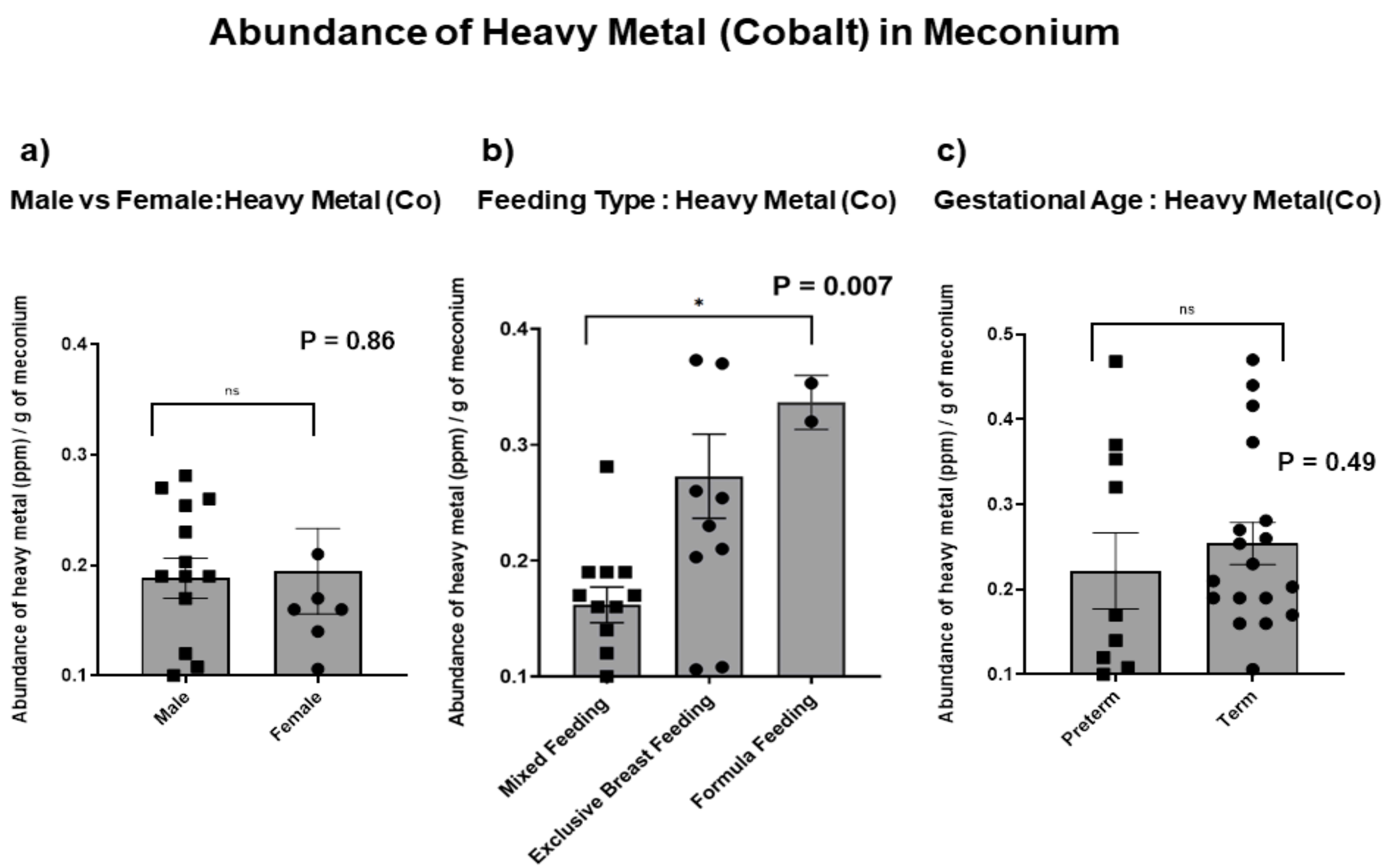

Figure 3.8.5: Distribution of cobalt in meconium samples.

3.8.6. Analysis of the Abundance of Essential Heavy Metal (Iron) in Meconium

Figure 3.8.6 illustrates iron (Fe) abundance in meconium (ppm per gram) across three comparisons: male versus female infants, feeding type (mixed feeding, exclusive breastfeeding, formula feeding), and gestational age (preterm versus term). Statistical analysis revealed a statistically significant difference between males and females (P = 0.0092), with male infants showing higher mean meconium iron levels than females. In contrast, no significant association was observed for feeding type (P = 0.898) or gestational age (P = 0.35), indicating that iron levels did not differ meaningfully across feeding categories or between preterm and term neonates. Of the three variables examined, infant sex emerged as the only factor significantly associated with meconium iron abundance, with males exhibiting higher levels than females. Conversely, feeding type and gestational age showed no statistically significant relationships with meconium iron levels, implying that these factors are unlikely to be major determinants of iron burden in this sample. Overall, these results highlight infant sex as a key variable in meconium heavy metal research, particularly for iron.

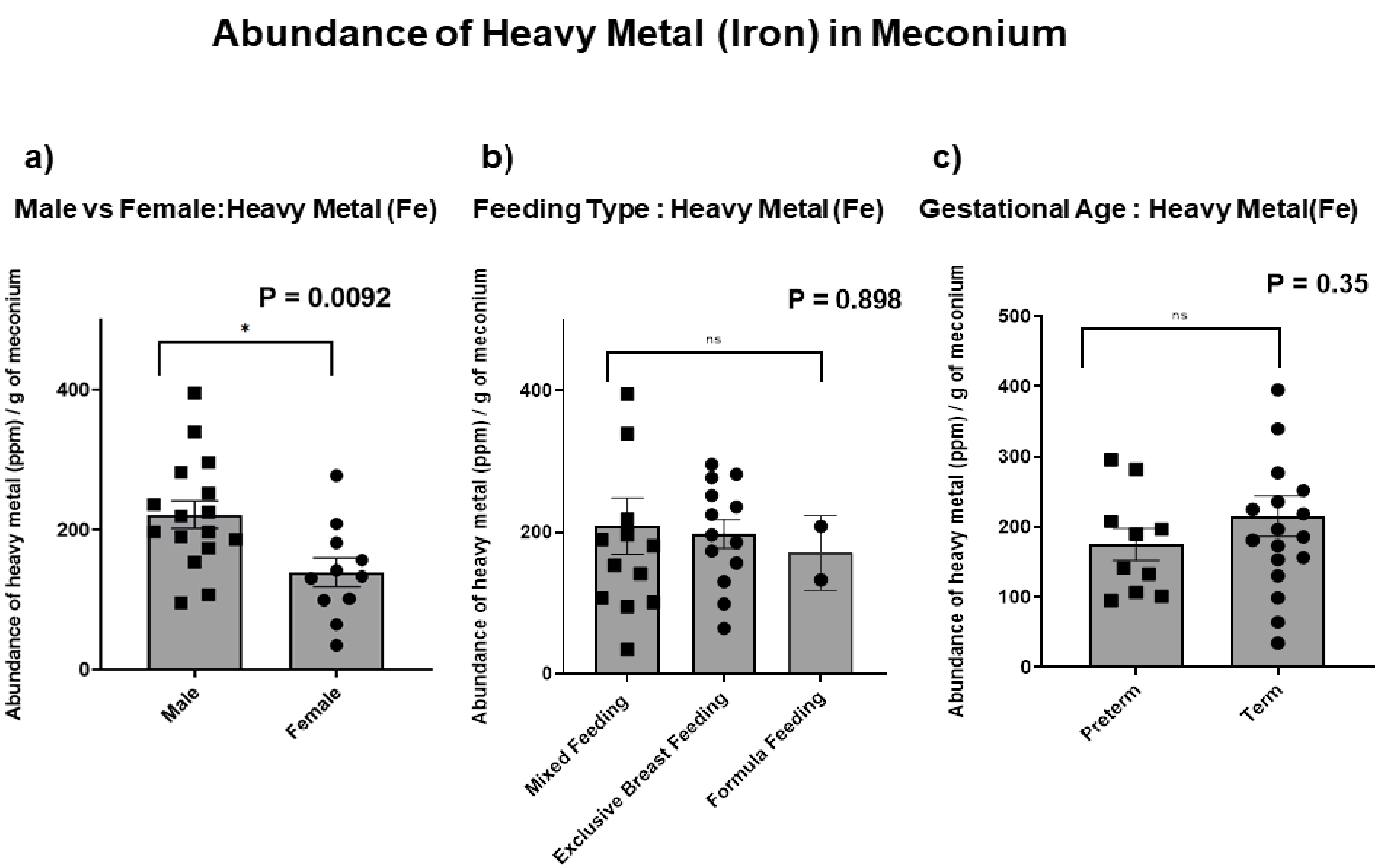

Figure 3.8.6: Distribution of iron in meconium samples.

3.9. Trojan Horse Effect in Samples

We aimed to explore a potential link between the "Trojan Horse Effect" and the samples analyzed. However, our study did not find any conclusive evidence of such an association. While we successfully detected, quantified, and characterized both microplastics and heavy metals in the samples, these results alone do not provide definitive proof of the "Trojan Horse Effect" occurring in the tested samples.

3.10. Effect of Microplastics on Gut Dysbiosis

To observe the potential impact of microplastics on gut dysbiosis, six meconium samples were selected, including three samples with relatively high microplastic counts and three samples with relatively low microplastic counts. The objective was to explore whether variations in microplastic abundance could influence the neonatal gut microbiome. Therefore, metagenomic analysis was performed on the selected samples to evaluate alpha diversity and beta diversity within the microbial communities. The resulting data haven't yet been fully analyzed.

Chapter 4

4. Discussion

4.1. Purpose of the Study

This study aims to characterize and quantify the microplastic fragments and fibers found in meconium samples and also their mothers' milk samples. We wanted to correlate whether there is any association between mothers' and infants' plastic type exposure, and also analyzed heavy metal profiling from neonatal meconium samples to understand the association between their industrial pollutant exposure burden and microplastic accumulation in the early stage of life. Additionally, we wanted to analyze if there any association between the abundance of heavy metal in meconium samples with the metal-specific associations with neonatal biological characteristics. Again, we wanted to determine if there is any influence of neonatal biological characteristics in abundance of microplastics in meconium samples.

In this study, the abundance of microplastic types & fragments in meconium samples and breast milk from corresponding neonate mothers was analyzed via Macro-Raman spectroscopy. Additionally, numerous types of heavy metals were also determined from meconium samples using the ICP-MS method. The present study provides important insights into neonatal hygiene practices, infant feeding patterns, gestational age distribution, birth weight outcomes, and environmental exposures such as heavy metals and microplastics among the study population. These findings contribute to a broader understanding of environmental and behavioral factors influencing neonatal health in developing regions, where socioeconomic conditions and traditional practices continue to shape maternal and infant care.

4.2. Usage of Bedding Materials for Neonates

The analysis of neonatal hygiene practices revealed that 56.67% of neonates were cared for using traditional cloth (*katha*), while 43.33% used disposable diapers. This finding indicates that traditional infant care practices remain common within the study population. The continued use of reusable cloth materials reflects the persistence of cultural caregiving practices despite the increasing availability of commercial disposable products. In contrast, studies conducted in high-income countries report significantly higher use of disposable diapers. For instance, a study in Brazil reported that 91.4% of infants used disposable diapers, while only 8.6% relied on cloth diapers (Uber et al., 2025). Similar trends have been observed in Europe and North America, where disposable diapers dominate infant care due to their convenience, superior absorbency, and perceived hygienic benefits (Cordella et al., 2015). These differences highlight disparities in infant hygiene practices between developed countries and many developing regions. However, disposable diapers may introduce additional environmental exposure risks. These products are commonly manufactured from polyethylene and polypropylene layers, super-absorbent polymers, adhesives, and chemical additives. Previous studies have identified chemical compounds such as phthalates, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and trace heavy metals in disposable diapers, raising concerns about potential chemical exposure during infancy (Nyamukamba et al., 2023). Such materials may contribute to environmental contamination and increase exposure to potentially harmful substances during early development.

4.3. Source of Milk Samples

Neotanes feeding practices represent another critical determinant of neonatal health, growth, and immune system development. The findings of the present study showed that 50% of respondents practiced exclusive breastfeeding, 43.3% practiced mixed feeding, and 6.7% relied exclusively on formula feeding. Exclusive breastfeeding was therefore the most common feeding practice among the respondents. However, the relatively small difference between exclusive breastfeeding and mixed feeding suggests that maintaining exclusive breastfeeding for the recommended six-month period may be challenging for some mothers. Globally, the prevalence of exclusive breastfeeding among infants under six months is approximately 44%, according to the World Health Organization (Global Breastfeeding Scorecard 2023, n.d.). The rate observed in this study is therefore slightly higher than the global average. Healthcare providers also play an important role in promoting breastfeeding by educating mothers about its nutritional and immunological benefits (Global Breastfeeding Scorecard 2023, n.d.).

Economic considerations may further influence feeding practices, as formula feeding is relatively expensive for families in low-income settings. However, the high proportion of mixed feeding observed in this study may reflect challenges faced by breastfeeding mothers, including perceived insufficient milk supply, maternal illness, employment demands, or the belief that infants require supplementary foods before six months of age. Exclusive breastfeeding remains strongly recommended because of its substantial health benefits. Breast milk contains essential antibodies, nutrients, and bioactive compounds that support immune development, digestion, and healthy growth (Global Breastfeeding Scorecard 2023, n.d.). Research consistently demonstrates that exclusive breastfeeding reduces the risk of gastrointestinal infections, respiratory illnesses, allergies, and malnutrition. Research has reported microplastic contamination in infant formula products and breast milk, suggesting that environmental plastic exposure may be transferred from mothers to infants (Kadac-Czapska et al., 2024).

Additionally, polypropylene baby bottles have been shown to release large numbers of microplastic particles when exposed to high temperatures during formula preparation (D. Li et al., 2020). These findings indicate that infants may be exposed to microplastics through both breastfeeding and formula feeding pathways. Moreover, recent studies suggest that microplastics present in breast milk may influence the microbial composition of human milk, potentially affecting infant gut health and immune development (Ragusa et al., 2022). Although breastfeeding remains the most beneficial feeding method, environmental contamination represents an emerging concern requiring further investigation.

4.4. Distribution of Gestational Age

Gestational age distribution is another important indicator of neonatal health outcomes. According to the World Health Organization, preterm birth refers to birth occurring before 37 weeks of gestation, and globally approximately 10–20% of infants are born preterm (Preterm Birth, n.d.). Late preterm births (34–36 weeks) represent the subgroup of preterm births worldwide. In the present dataset(n = 30), the proportion of late preterm births appears higher than international benchmarks. For example, data from the United States indicate that late preterm births account for approximately 7–8% of births (Martin & Osterman, 2023). Similarly, European studies report preterm birth rates between 5% and 9% (*EPHR2010_w_disclaimer.Pdf*, n.d.; EPHR2010_w_disclaimer.Pdf, n.d.-b). The relatively higher proportion observed in this study may reflect increased rates of induced labor or cesarean deliveries before full-term gestation. Both WHO and CDC guidelines emphasize that 39–40 weeks of gestation represents the optimal period for birth, as infants born during this period experience the lowest health risks (MNCAHA & QoC Indicator Metadata Toolkit, n.d. Martin & Osterman, 2023).

4.5. Microplastic Fragments and Fibers Analysis

In our present study, we did not find an association between the abundance of microplastics in the mother-neonate dyed. There could be many reasons behind the abundance of microplastics in meconium samples including placental transfer, breastfeeding, environmental exposure, mothers dietary habits etc. According to recent research, there was no discernible or statistically significant relationship between neonatal gender, gestational age, or feeding type and the microplastic count in meconium. This suggests that maternal prenatal environmental exposure, dietary practices, plastic use, indoor dust, and transplacental transfer may have a greater impact on microplastic accumulation in meconium than infant-specific biological factors. This interpretation aligns with research conducted in Asia, where Liu et al. found microplastics in placenta, meconium, breast milk, infant feces, and formula, indicating both prenatal and postnatal exposure pathways. However, the study focused more on exposure sources like water intake, personal care products, breastfeeding, bottles, and plastic toys than neonatal sex or gestational age as significant predictors (Liu et al., 2023).

In a similar vein, a pilot study conducted in South China found microplastics in meconium, placenta, and cord blood, indicating fetal exposure prior to delivery (Zhang et al., 2025). Microplastics were discovered in 75% of breast milk samples in Europe, according to an Italian study. However, there was no discernible link between the use of personal care products or the consumption of plastic-packaged food by mothers, indicating widespread ambient exposure. Zhang et al. found PET and PC microplastics in infant feces and newborn meconium in the

United States, suggesting early-life exposure; however, the sample size was modest and no significant correlations with demographic characteristics were found (Zhang et al., 2025). Therefore, gender, gestational age, and feeding type may not be able to independently account for differences in meconium microplastic abundance, but the current investigation supports the notion that microplastic exposure in newborns is probably broad and complex when compared with worldwide evidence.

Microplastic analysis revealed that polyester (PET) was the dominant polymer detected in the meconium samples. Studies conducted in China have similarly identified PET as a dominant polymer in biological samples (Li et al., 2025), while research in Italy confirmed the presence of PET in human placentas, indicating that small fibers may cross the placental barrier (Ragusa et al., 2021). Other polymers detected included polypropylene (PP) and high-density polyethylene (HDPE), commonly used in plastic feeding bottles and packaging materials, which have also been identified in human breast milk (Ragusa et al., 2022). In contrast, polyethylene vinyl acetate (PEVA) and acrylonitrile butadiene styrene (ABS) were detected at very low levels, consistent with findings from studies conducted in the United States (Braun et al., 2021).

Table 4.1 : Global Distribution of Dominant Microplastic Polymers Found in Meconium Samples.

Region	Dominant Microplastics Identified	Reference
Bangladesh (Dhaka)	Polyester (PET) (100%), HDPE, POLS	Present study (2026)
Asia (China)	● Main dominant polymers: ○ Polyamide (PA) ○ Polyurethane (PU)	(Liu et al., 2022)
Europe (Italy)	Polypropylene (PP), PET	(Ragusa et al., 2021)
USA	PE, PU, PP	(Braun et al., 2021).

4.6. Heavy Metals Analysis in Meconium

The current investigation found metal-specific correlations between neonatal features and the concentration of heavy metals in meconium. While gestational age was not substantially correlated, arsenic (As) showed considerable variation by gender and feeding type, with higher amounts seen in males and exclusively breastfed newborns. Lead (Pb), cadmium (Cd), and chromium (Cr), on the other hand, did not exhibit statistically significant differences by gender, feeding type, or gestational age, indicating that maternal environmental exposure, diet, occupation, residence, and placental transfer may have a greater influence on these metals than neonatal factors. Meconium-based research demonstrating that meconium represents cumulative fetal exposure throughout pregnancy, especially starting in the second trimester, lends credence to this interpretation. Heavy metals were found in neonatal meconium in Asian studies from China and Pakistan, which mostly connected exposure to maternal environmental circumstances and industrial pollution rather than infant features.

Pb and Cd were discovered in the meconium of babies from an industrial city in Europe by Türker et al., who also documented connections with residential proximity to pollution sources and parental occupation (Turker et al., 2006). Meconium is a biomarker of intrauterine metal exposure; McDermott et al. found metals in newborn meconium from South Carolina and Montana in the United States (McDermott et al., 2020). Moreover, gender, gestational age, and feeding method may have limited and metal-specific effects, but overall, the current findings are consistent with worldwide data that heavy metal accumulation in meconium is essentially a sign of prenatal environmental exposure.

The analysis of heavy metal concentrations in meconium samples revealed that zinc (Zn) and iron (Fe) were the most abundant metals detected. This finding aligns with previous studies, as both Zn and Fe are essential trace elements required for fetal growth, immune function, and enzyme activity (Turker et al., 2006). Environmental monitoring studies in Bangladesh frequently report zinc and iron as among the most prevalent trace elements in soil and food sources. Similar findings have been reported in neonatal studies conducted in Pakistan, where Zn and Fe concentrations were higher than those of toxic metals such as lead or cadmium (Aziz et al., 2017). In contrast, studies conducted in North America and Europe typically report lower concentrations of toxic metals due to stronger environmental regulations and improved pollution control measures (McDermott et al., 2020).

Table 4.2. : Global Distribution of Heavy Metal Exposure in Meconium Samples Measured According to Their Average Concentration in PPM. Source: Adapted from (Türker et al., 2006), (Gheissari et al., 2022), (Michelsen-Correa et al., 2021), (Huang et al., 2021) and global meconium metal exposure review studies.

Types of Heavy Metals Metal (Molecular weight)	Present Study (ppm)	Asian Countries (China) (ppm)	European Countries (Italy) (ppm)	United State of America (ppm)
Arsenic 75	0.28	0.35	0.18	0.20
Selenium 82	1.77	1.80	1.20	1.30
Lead 208	1.53	1.70	0.90	1.00
Cadmium 111	0.07	0.08	0.04	0.05
Chromium 52	3.95	3.80	2.20	2.50
Nickel 60	1.73	1.90	1.10	1.20
Cupper 63	106.66	110	80	90
Zinc 66	379.24	370	250	300
Mercury 202	0.61	0.70	0.40	0.45
Manganese 55	60.29	65	40	45

Cobalt 59	0.26	0.28	0.15	0.18
Aluminum 27	16.95	18	12	14
Beryllium 9	0.00	0.02	0.01	0.01
Vanadium 51	0.05	0.06	0.03	0.04
Iron 57	202.49	210	150	170

N.B. : PPP (Parts Per Million)

Overall, the findings of this study highlight the complex interaction between traditional caregiving practices, socioeconomic conditions, environmental exposures, and neonatal health outcomes. Although breastfeeding practices remain encouraging within the study population, the relatively high proportion of mixed feeding and late preterm births suggests areas where maternal and neonatal healthcare interventions could be strengthened. Furthermore, the detection of heavy metals and microplastics in neonatal samples underscores the growing influence of environmental pollution on early human development. Future research should focus on identifying major exposure pathways and implementing strategies to reduce environmental contaminants affecting maternal and neonatal health.

4.7. Trojan Horse Effect in Samples

The "Trojan Horse Effect" describes the ability of microplastics to adsorb toxic heavy metals onto their surfaces and transport these contaminants into the human body. Due to their high surface area and hydrophobic nature, microplastics can bind metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As), forming microplastic–metal complexes that may enter tissues after ingestion or inhalation. Once inside the body, these complexes can release toxic metals, thereby enhancing bioaccumulation, oxidative stress, inflammation, and cellular toxicity (Gatti et al., 2024). Yet, we weren't able to find any association of the "Trojan Horse Effect" in our study. But we will analyze more samples in the future in order to detect the "Trojan Horse Effect".

4.8. Effect of Microplastics on Gut Dysbiosis

The detection and characterization of microplastics in meconium and breast milk have raised serious concerns regarding early-life exposure and its potential impact on infant gut health, particularly gut dysbiosis. Meconium, the first stool passed by newborns, is considered an important biomarker for prenatal exposure, while breast milk represents a major postnatal exposure route. Recent studies have identified various types of microplastics, including polyethylene (PE), polypropylene (PP), and polystyrene (PS), in both meconium and human breast milk, indicating that microplastics can cross biological barriers and enter the maternal–fetal system. Once ingested by infants, these particles may interact with the developing gut microbiota, disrupting the balance of beneficial and harmful microorganisms during a critical stage of immune and metabolic development. Microplastics can induce oxidative stress, intestinal inflammation, and increased gut permeability, which may contribute to gut dysbiosis and long-term gastrointestinal or metabolic disorders. Furthermore, microplastics often adsorb toxic chemicals, heavy metals, and environmental pollutants, enhancing their adverse biological effects. Overall, the presence of microplastics in meconium and breast milk highlights a growing environmental health issue and emphasizes the need for further research into their effects on neonatal gut microbiota and infant health outcomes (Fournier et al., 2022).

Chapter 5

5. Conclusion & Future Prospects

5.1. Strengths

The study demonstrates several notable strengths, particularly its comprehensive and integrative methodological approach. It combines advanced analytical techniques such as Micro-Raman spectroscopy and ICP-MS to ensure precise identification and quantification of microplastics and heavy metals. Additionally, the use of both breast milk and neonatal meconium provides a holistic assessment of maternal-infant exposure pathways. Rigorous contamination control measures, including the use of non-plastic equipment and controlled laboratory conditions, further enhance data reliability. Moreover, the study addresses an underexplored area in early-life environmental exposure, contributing valuable insights to public health research.

Considering that the literature currently in publication demonstrates that research on microplastics and heavy metals in neonatal biological matrices is still limited and dispersed, especially in Bangladesh and larger South Asian contexts, this study exhibits a notable strength in its distinctiveness and regional contribution. Comprehensive research incorporating microplastics and heavy metals in meconium and breast milk are rare, despite the fact that a small number of studies in nations like Thailand and Italy have found microplastics in breast milk and only a few worldwide have looked at such exposure pathways (Adjama et al., 2024). As a result, this work closes a significant methodological and geographical gap in environmental health research and offers new baseline data.

5.2. Limitations

Although determining the presence of microplastic and heavy metals in meconium samples via Micro-Raman Spectroscopy and ICP-MS, the current study has certain drawbacks. The present study has several limitations that should be considered when interpreting the findings. First, publicly accessible clinical databases lack widely accepted reference ranges for heavy metal contents in neonatal meconium. Meconium is often used as a biomarker for determining prenatal exposure to environmental pollutants, although there are still few established reference values, and reported amounts tend to vary according to the mother's environmental exposure and geographical location (Pavilonis et al., 2022).

Second, the results might not be regarded as widely applicable due to the very small sample size. There were a lesser number that could be analyzed since some samples were inadvertently lost during the digesting procedure that reduced biological components. Such losses during sample preparation are recognized challenges in trace metal analysis of biological matrices (Papadopoulos et al., 2022).

Lastly, the hospital's logistical limitations during sample collection prevented the collection of neonatal birth weight data. Birth weight is a crucial measure of neonatal health and is frequently used in environmental health research to investigate the association between developmental outcomes and prenatal exposure to pollutants (*Neonatal Heavy Metals Levels Are Associated with the Severity of Neonatal Respiratory Distress Syndrome: A Case–Control Study | BMC Pediatrics | Springer Nature Link*, n.d.). Therefore, in order to boost the reliability of results pertaining to prenatal environmental exposure, future studies ought to incorporate more sample numbers and more comprehensive clinical data.

Another limitation of our study is the sample collection procedure. We collected meconium samples from baby cloth (katha) and also some samples from diapers. Diapers are probable contaminates of plastic in our sample. Disposable diaper products are commonly manufactured from polyethylene and polypropylene layers, super-absorbent polymers, adhesives, and chemical additives. Previous studies have identified chemical compounds such as phthalates, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and trace heavy metals in disposable diapers, raising concerns about potential chemical exposure during infancy (Nyamukamba et al., 2023).

5.3. Conclusion & Future Prospects

Multiple heavy metals, even at sub-toxic individual levels, may exert additive or synergistic effects, potentially influencing neonatal growth and immune or microbiome development. Interpretation is limited by the absence of neonatal fecal reference ranges; therefore, corroborative biomonitoring and environmental source assessment are recommended. The presence of heavy metals and microplastics in neonatal meconium reflects exposure to environmental pollutants during pregnancy and may be harmful to neonates' health. Lead, cadmium, and mercury are examples of heavy metals that might affect prenatal growth, brain development, and immune function, raising the possibility of developmental abnormalities (Health Risks of Heavy Metals from Long-Range Transboundary Air Pollution, n.d.). Similarly, microplastics found in meconium indicate that these tiny fragments may be able to pass through biological barriers during pregnancy and build up in fetal organs, where they may induce inflammation and oxidative stress (Braun et al., 2021; Ragusa et al., 2021). Additionally, the presence of microplastics in human breast milk may expose neonates to plastic particles while they are being breastfed and may change the microbial makeup of human milk, which may have an impact on the immune system and gut health of neonates (Ragusa et al., 2022; Saraluck et al., 2024). Moreover, these findings of heavy metals and microplastics in biological samples from newborns raise serious concerns about environmental pollution and its possible effects on the health of neonates.

To correlate the data with gut symbiosis in the neonatal body, the meconium samples with the highest and lowest abundance of microplastics need to undergo metagenomic analysis in future experiments. The identification of microbial taxa, functional genes, and metabolic pathways linked to early-life gut symbiosis is made possible by metagenomic analysis, which offers a high-resolution, culture-independent method for characterizing the neonatal gut microbiome. Because metagenomics can identify changes in microbial diversity, dysbiosis, and the enrichment of plastic-degrading or stress-response genes that may indicate host–microbe interactions under environmental stress, it is especially beneficial in the context of microplastic exposure identified in meconium samples. By connecting metagenomic profiling with microplastic detection, researchers may evaluate how such contaminants might impact the initial colonization and symbiotic equilibrium of the neonatal gut, as meconium reflects in utero microbial and chemical exposures. Recent research has shown that environmental contaminants, such as microplastics, may alter the composition and function of the gut microbiota, which may have an impact on early life immunological development and metabolic consequences (Ragusa et al., 2021; X. Liu et al., 2022).

Chapter 6

6. References

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