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# Evaluating Contaminants and Toxins in Mothers' Milk in Patna, Bihar

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***Abstract:*** *Human milk (HM) provides optimal nutrition to infants and is considered the gold standard for them, as it not only has a unique and dynamic composition of nutrients but also provides bioactive compounds, antibodies, enzymes, etc. HM composition changes based on maternal factors, such as gestational diabetes mellitus (GDM), hypothyroidism, body mass index (BMI), exposure to different contaminants, etc. In this study, we examined the nutritional content as well as contaminants and toxins (if any) in human milk from lactating mothers collected from Patna, Bihar. Total eleven breast milk samples were collected and evaluated for lead (Pb), arsenic (As), chromium(Cr), calcium and zinc using the Atomic Absorption Spectroscopy method.FTIR spectroscopy was used to assess the qualitative analysis of microplastics in the milk samples.The nutritional composition such as fat content of the milk was measured using the Rose-Gottlieb method, Protein presence was detected with UV absorbance at 280 nm, and carbohydrate content was recorded using the phenol–sulphuric acid technique.Detected ranges of HM components in our study were fat - 3.78 to 4.25%, calcium - 101.08 to 211.45 mg/L , and zinc - 0.112 to 0.290 mg/L. 70% of the samples contained lead, and the levels reached up to 76 µg/L, which is higher than the WHO guideline values for drinking water. Although drinking water guidelines are not directly applicable to breast milk, they are cited here for contextual comparison. Arsenic and chromium were less frequently found, and at lower levels.*

***Keywords:*** *human milk, nutritional composition, heavy metals, microplastics, Patna.*

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## Introduction:

Human breast milk is regarded as a complete source of nutrition for infants, as it provides all essential nutrients, immune components, and bioactive substances that support growth and development while protecting against various diseases (World Health Organisation, 2023). Therefore, exclusive breast feeding is particularly recommended during the first six months of life. The compositions of human milk are largely affected by several factors, such as mother's age, baseline anthropometric status of the mother, diet etc (Han et al., 2021). Besides being gold standard of infant feeding, breast milk may also serve as a means of transferring potentially dangerous substances such as pollutants, toxins and microplastics (MPs) from mother to baby

unknowingly (Samiee et al., 2019; Motas et al., 2021) highlighting the need for its continuous monitoring (EFSA, 2016). Numerous researchers are investigating the link between microplastics (MPs), nutrition and human breast milk, as MPs have been identified in human placenta, infant stool, and other biological samples (Ragusa et al., 2020). MPs and other contaminants may present in breast milk from various sources, including industrial emissions, farming practices, ingestion of contaminated water and food (Rebelo and Caldas, 2016; LaKind et al., 2023). Because these contaminants are lipophilic, they tend to accumulate in maternal tissues specially lipid rich components of breast milk and cause potential threat to infants. Prolonged exposure to heavy metals such as lead (Pb), arsenic (As), and chromium (Cr) has been linked to reduced IQ, behavioural abnormalities, and a higher risk of chronic diseases, primarily due to their disruptive effects on neurodevelopment, cognitive processes, and endocrine function (Cherkani-Hassani et al., 2016, Rebelo and Caldas 2016). Additionally, lead exposure during infancy, including through breast milk, has been associated with developmental delays and potential long-term cardiovascular outcomes (Ettinger et al. 2009).

In many developing countries, due to lack of strict governmental regulation excessive use of pesticides led to groundwater contamination and elevated human exposure to these chemicals compared to more stringently regulated industrialised countries (Tanabe and Kunisue, 2007). Several studies conducted in India have reported the presence of considerable levels of contaminants in breast milk. For instance, investigations in Punjab have identified residues of organochlorine pesticides (OCPs), including DDT and hexachlorocyclohexane (HCH), with concentrations among the highest recorded globally, reflecting past agricultural practices (Dev et al., 2018; Sharma et al., 2014). The report suggests that areas with concentrated electronic waste generate high level of persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), which can accumulate in breast milk.

In one study from 2024, 55% of the breast milk samples collected in arsenic-endemic regions of Bihar contained toxic levels of arsenic exceeding WHO's allowable limit and, as a result, posed a long-term toxicity risk to infants consuming them (Kumar et al., 2024). In a multi-institutional study, the presence of uranium in breast milk samples from six districts of Bihar indicated that nearly 70% of infants may exceed tolerable intake levels, posing potential non-carcinogenic health risks, with exposure largely attributed to geogenic sources and contaminated groundwater (Kumar et al., 2025).

While previous studies have reported a wide range of environmental contaminants in human tissues, the primary objective of the present study is to assess the presence of toxicants, including heavy metals (lead, arsenic, and chromium) and microplastics, in breast milk samples collected from lactating mothers in Patna, Bihar. Although microplastics and other contaminants have been increasingly detected in various human biological matrices, data on their occurrence in human breast milk—particularly within the Patna region—remain limited. Exploring the identification of MPs and heavy metals in human milk and determine the potential health risks of these contaminants for new-borns and infants, and finally promote awareness of mitigation strategies related to these environmental contaminants in human breast milks is the purpose of this study.

**Materials and methods:**

**Data Collection: Sampling and Questionnaire Administration**

Human milk samples were obtained from lactating mothers residing in Patna, Bihar, between October and November, 2025 from local hospitals, and community health centres. The sample included participants from varied socioeconomic background, age ranges and representing both primiparous and multiparous group. At the same time, participants were asked to complete survey forms about their background, lifestyle, diet habits, source of water, information regarding breastfeeding, chronic diseases as well as drug abuse (if any).

Total 11 samples were collected from different background and areas of Patna including health care centres. Approximately 10–20 mL of mature milk was manually expressed or pumped mid-feed (hind-milk) into pre-cleaned, sterile containers to minimise external contamination, following WHO guidelines for human milk sampling (WHO, 2007). Samples were stored at –20°C immediately after collection and transported to Central

Research Laboratory (CRL) Patna Women's College in icebox for analysis.

**Sample Processing and Analysis**

**Fat Analysis (Rose-Gottlieb Method):** Fat content was determined using the gravimetric Rose-Gottlieb method, suitable for accurate lipid extraction in dairy matrices. Briefly, 200µL of thawed, homogenised milk was mixed with 1 mL ammonia (to disrupt protein-fat emulsions), followed by sequential extractions with 10 mL ethanol, 10 mL diethyl ether, and 10 mL petroleum ether. The mixture was vigorously agitated, then centrifuged at 1500 rpm for 5 minutes, and the organic layer was decanted. Extractions were repeated twice, and the combined extracts were evaporated to dryness at 60°C under nitrogen. Fat residue was weighed gravimetrically and expressed as % fat. This method ensures complete fat recovery while minimising interference from non-lipid components.

**Protein Analysis (UV absorbance at 280 nm):** Protein analysis was done using UV absorbance at 280 nm (Scopes, 1974). This method is based on the intrinsic absorbance of aromatic amino acids, mainly tryptophan and tyrosine, and does not require further reagents.

**Carbohydrate Analysis (Dubois Method):** The total carbohydrate content of the breast milk samples was determined using the phenol–sulfuric acid method (Dubois et al. 1956), a widely used colorimetric assay for total sugars, particularly lactose, in human milk. Briefly, 1000 µL of appropriately diluted breast milk was mixed with 1000 µL of 5% phenol solution, followed by the rapid addition of 5 mL concentrated sulfuric acid. It was allowed to stand for 20 minutes at room temperature for colour development. At 490 nm absorbance was recorded using a UV–Vis spectrophotometer. Carbohydrate concentration was recorded using a glucose/lactose standard curve (0–100 µg/mL) and expressed as g/100 mL of milk. This method provides a reliable estimate of total carbohydrate content but does not differentiate individual sugars.

**Heavy Metals and Minerals (AAS):** Heavy metals (Pb, As, Cr) and minerals (Ca, Zn) were measured by Atomic Absorption Spectroscopy (AAS). 1 mL of each samples were wet-digested with HNO3/HCl in a fume hood at 180°C for 20 minutes, diluted to 100 mL, and analysed on a GBS Savant AA, Australia. Results were expressed in µg/L or mg/L.

**Microplastics (FTIR Spectroscopy)** Microplastics were identified using Fourier-Transform Infrared (FTIR) spectroscopy, following protocols from studies on food matrices (Cole et al., 2013). 5 ml of each samples were digested with 30% $H_2O_2$, 2.5 mL labolene and 25 µL Tween-80 reagent at 37°C for 24 hours, 7 mL concentrated NaCl was added and centrifuged at 8000 rpm at 4°C for 20 minutes to remove organic matter, filtered onto membranes, and scanned on a Bruker Alpha, Germany. Spectra were matched against polymer libraries (e.g., polystyrene, polyethene) using OMNIC software.

**Results and Discussion**

**Macro and micronutrients in human milk:** Macro and micronutrient compositions in human milk are shown in Table 1 (n=11). The fat content in the samples of breast milk varied between 3.78% and 4.25%, with an average of 3.99 ± 0.15% suggesting that there is moderate inter-individual variability. Similar results were recorded by Martin et al., (2016) and Ballard and Morrow (2013) in their studies. The observed variability reflects physiological rather than pathological differences, since the fat content of human milk is a dynamic process related to stage of lactation, time of sample collection, extent of breast emptying, and maternal metabolic state (Klotz et al., 2021; Innis, 2014).Multiple studies indicate that fat content in milk is the most variable among macronutrients and tends to increase progressively during the first month postpartum.

Protein values in the present study ranged from 76.9 to 99.8 µg/ml. The variation in protein or other macronutrient in human milk can be affected by maternal diet (Bravi et al., 2016).

A rise in lactose level along with a reduction in oligosaccharide content leads to an overall increase in the carbohydrate content of human milk after birth of the child. Lactose and human milk oligosaccharides are main components in human milk which not only support infant energy needs but also influence early gut maturation (Soyyılmaz et al., 2021). The carbohydrates concentration in the present study ranged between 22.3 and 56.5 mg/mL.

The distribution patterns of Calcium and Zinc differed markedly. Calcium concentrations ranged from 101.08 to 211.45 mg/L, whereas Zinc concentration varied between 0.112 and 0.290 mg/L, indicating differing degree of variability across individuals. Calcium levels in breast milk are known to remain relatively stable due to tight physiological regulation, even under conditions of variable maternal dietary intake. Thus ensuring adequate skeletal development in infants (Ballard and Morrow, 2013; Spahn et al., 2019). Several studies such as Repkova et al., (2023) reported similar ranges, suggest effective mobilization of Calcium from maternal reserves. In contrast, Zinc concentrations in later stages of lactation are more dependent on maternal body stores, which may account for the greater inter-individual variability observed (Pajewska-Szmyt et al., 2019).

Calcium and zinc are crucial trace nutrients in breast milk, contributing to the development of the skeleton, functioning of enzymes, immunity, and general growth of the infant (Spahn et al., 2019). Calcium and zinc levels in milk reflect mothers' diet. Though even when a mother's diet changes, the calcium in her milk stays fairly steady. The body adjusts to make sure babies get what they need for growing bones. This support happens naturally during breastfeeding. The values of calcium observed in this study are within the values previously reported herein, indicating sufficient mobilisation of calcium from maternal stores, despite probable dietary variation. Spahn et al., (2019) and Repková et al., (2023) observed similar zinc values as observed in the present study.

**Table 1. Macro and micro nutrients in human milk detected during the present study.**

| Sample | Age of Mother | Fat (%) | Protein (µg/mL) | Carbohydrate (mg/mL) | Ca (mg/L) | Zn (mg/L) |
|---|---|---|---|---|---|---|
| A | 22 | 3.8 | 88.75 | 26.01 | – | – |
| B | 24 | 4.15 | 89.18 | 22.33 | 101.08 | 0.175 |
| C | 25 | 4.03 | 99.69 | 36.87 | 210.89 | 0.131 |
| D | 21 | 3.78 | 92.75 | 47.99 | 156.01 | 0.220 |
| E | 22 | 4.25 | 76.94 | 56.53 | 112.09 | 0.112 |
| F | 24 | 3.97 | 92.92 | 22.94 | 189.78 | 0.131 |
| G | 27 | 3.93 | 78.31 | 39.73 | 112.78 | 0.246 |
| M | 23 | 4.07 | 85.31 | 27.10 | 186.07 | 0.290 |
| F21 | 31 | 3.99 | 98.12 | 35.64 | 189.23 | 0.273 |
| F22 | 34 | 4.15 | 93.49 | 45.26 | 205.09 | 0.220 |
| F23 | 32 | 3.86 | 99.77 | 50.72 | 211.45 | 0.242 |

**Heavy metals/metalloids in milk samples:** The concentration of heavy metals in milk samples, along with the corresponding WHO threshold values for each metal, are shown in Table 2. The pattern of heavy metal accumulation in breast milk samples followed the order: Pb (mean = 22 ± 28.3 µg/L) > Cr (mean = 2±1.84 µg/L) > As (mean = 0.03± 0.05 µg/L). It can be seen clearly that the concentration of Pb and Cr exceeded WHO's tolerable daily intake (3.6 µg/L for Pb and 0.9 µg/L for Cr) in most of the Human milk samples. Similar results were recorded by Kiomars et al., (2023) in their study. However, the concentration of Pb and Cr was recorded below detection limits in samples C, M and F 23 for Pb and Samples D, E and G for Cr. As concentration was recorded within permissible limits of WHO in all milk samples.

Among the heavy metals, lead (Pb) and arsenic (As) are of greater importance to human health. These elements are not readily metabolized in the body and can cause toxicity through their accumulation in the tissues over time (Rezaei et al., 2019). Concentrations of Pb, As, and Cr varied among human milk samples, pointing to a range of exposure to environmental contaminants. Breast feeding passes these metals to babies, since breast milk reflects a mother's diet, her environment, and substances stored in her tissues

(García-Esquinas et al., 2020). Lead was detected in numerous samples, consistent with earlier studies from India (Tripathi et al., 1999; Sharafi et al., 2023) indicating its continued occurrence in human milk and suggesting persistent environmental exposure and maternal accumulation. Chromium concentrations in the present study were found to be relatively low compared to previously reported values. For instance, studies such as Kiomars et al., (2023) reported chromium levels in human milk ranging from 11.02 to 128.72µg/L.

**Table 2. The heavy metals concentration in human milk (µg/L)) samples and TDI (Tolerable daily intake, WHO)**

| Sample | Pb | As | Cr |
|---|---|---|---|
| A | – | – | – |
| B | 5 | 0.07 | 2 |
| C | ND | 0.04 | 2 |
| D | 76 | ND | ND |
| E | 5 | ND | ND |
| F | 10 | 0.01 | 2 |
| G | 56 | 0.04 | ND |
| M | ND | ND | 5 |
| F21 | 34 | ND | 4 |
| F22 | 56 | ND | 3 |
| F23 | ND | 0.17 | 4 |
| Mean | 22 ± 28.3 | 0.03 ± 0.05 | 2 ±1.84 |
| Max | 76 | 0.17 | 5 |
| **TDI** | **3.6** | **0.3** | **0.9** |

Microplastics (MPs) detection in breast milk: Microplastics are plastic byproducts that arises from the degradation of plastic constituents. Currently, researchers globally are focused on the contamination of microplastics within the human body, which may badly affect human health by weaken the immune system. Microplastics have been detected in infant stool which may be due to contamination through placenta during embryonic stage, lactation, infant formula, feeding bottles or plastic objects (Ge et al., 2021). MPs were detected in 54.54% (6 out of 11) of the breast milk samples. The morphological and chemical characteristics of identified MPs are shown in Table 3. The most common MPs were PP and PS. The detected MPs were identified according to their shape and chemical composition. Apisith et al. (2024) detected MPs in 38.98% (23 of 59) of the milk samples which were mainly PP, PE and PVC. While previous literature has documented the presence of MPs in human milk, our study further supports the presence of MPs in human milk.

**Table 3. Morphological and chemical characteristics of the identified microplastics in 11 samples**

| Sample Code No. | Shape | Key FTIR spectral features observed | Interpretation |
|---|---|---|---|
| A | Irregular fragment | Distinct C–H stretching bands in the 2800–3000 cm $^{1}$ region | PE |
| B | Irregular fragment | C–H stretching and bending vibrations (~1450–1375 cm $^{1}$) | PP |
| C | ND | ND | ND |
| D | Thread like | Multiple overlapping polymer-associated absorption peaks | Mixed or degraded polymer signatures |
| E | Thread like | Broad alkyl C–H stretching and deformation bands | PP |
| F | ND | ND | ND |
| G | Irregular fragment | Polymer-characteristic absorption bands | PS |
| M | Irregular fragment | Weak but distinct polymer-related absorption bands | PA |
| F21 | ND | ND | ND |
| F22 | ND | ND | ND |
| F23 | ND | ND | ND |

*ND = Not Detected, PE = polyethylene, PP = polypropylene, PS = polystyrene, PA = polyamide.

**Conclusion:**

In summary, the present study reveals that contaminants such as heavy metals and microplastics (MPs) were detected in a substantial proportion of human milk samples. Among the heavy metals analysed, lead (Pb) and chromium (Cr) levels exceeded the World Health Organization's tolerable daily intake limits (3.6 µg/L for Pb and 0.9 µg/L for Cr) in most samples, raising significant concerns. In contrast, arsenic (As) concentrations remained within permissible WHO limits across all samples. MPs were detected in 54.54% of the samples. Overall, this study provides an integrated assessment of both nutritional components and environmental contaminants in breast milk from women in Patna, Bihar. Presence of lead, arsenic, chromium and MPs indicates ongoing maternal exposure to environmental pollutants, which highlights breast milk as a reflection of surrounding environmental conditions. Despite this, key nutrients – fat, protein, and calcium- were generally within expected physiological ranges. These findings suggest that while environmental exposures are evident, the fundamental nutritional quality of breast milk remains largely preserved. The study suggests urgent need for environmental monitoring targeted interventions to reduce maternal exposure to heavy metals and microplastics. Regular biomonitoring and improved nutritional guidance for lactating mothers are recommended to safeguard infant health while preserving the nutritional quality of breast milk.

Written consent was obtained from all participating lactating mothers included in the present study.

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