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Research Article

Microplastic contamination in human breast milk: A disquieting disparity linked to seafood consumption in an economically disadvantaged fishermen community settled along the Karachi coast

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Abstract

Plastic pollution has emerged as a pervasive global concern, with plastic waste contributing to the generation of microplastic particles (MPs) that have now integrated seamlessly into the human food chain. Intriguingly, early life exposure to MPs in children may occur through breast milk. This preliminary involved the analysis of human breast milk samples collected from 15 healthy breastfeeding mothers of the fishing community (BMSF) with daily habits of sea-food consumption and 8 healthy breastfeeding mothers (BMSH) with no history of seafood consumption in the past month and analyzed using microscopy and micro-Fourier-transformed infrared spectroscopy. In the BMSF group, a total of 276 MPs while 49 MPs were found in the BMSH group, indicating a substantial difference in MPs presence. The most prevalent MPs in both groups were fibers and pellets which fell within the size range of 5 to 18 µm, with polyethylene, polyvinyl chloride, and polypropylene being the most frequently observed types. Statistical analysis revealed a significant difference in the abundance of MPs between the two communities (F=13.57, P=0.001; Mann-Whitney U test, p<0.05). These findings emphasize the urgent need for further research to explore the potential health implications of MPs exposure through breast milk, especially in economically disadvantaged and vulnerable communities' *i.e.* pregnant women, lactating mothers and children, with high seafood consumption.

Introduction

The widespread usage of plastic products, inappropriate disposal, and their persistent nature make plastic pollution a global issue and a worldwide threat to aquatic ecosystems (Qiu *et al*., 2015; Dris *et al*., 2016; Su *et al*., 2016; Zhang *et al*., 2017; Teng *et al.*, 2019; Arshad *et al*., 2023). It is estimated that an average of 5 to 13 million tons of plastic enter the world's oceans each year (Wang *et al*., 2019).

Plastic waste gradually breaks down and is degraded into smaller particles by environmental and natural processes, which leads to the formation of MPs (Sighicelli *et al.*, 2018). Microplastics (MPs) range from 5mm to 100nm and are grouped based on their origin as primary and secondary MPs. Primary MPs are micro-sized synthetic polymers designed for commercial use, such as cosmetics, health care products, dish scrubbing pads, etc. (Kannan and Vimalkumar, 2021; Sridharan *et al.*, 2021). Secondary MPs are the fragmented form of macro or mesoplastics with a diameter or length of less than 5mm and are mostly produced as a result of biodegradation, photo degradation, thermo-oxidative degradation, thermal degradation, and hydrolysis (Jiang, 2018).

The presence of MPs has raised great concern about ecosystems and human health safety, as they can be ingested and accumulated in marine organisms, such as bivalves, crustaceans and fishes, which are considered an important human food source. The intake of MPs through fish consumption is estimated between 518 and 3078 MPs/year/individual (Barboza *et al*., 2020). Considering the number of people who are highly dependent on the seafood diet, and assessing potential hazards of MPs associated with seafood consumption, it is preferred to have a limiting intake of seafood, especially for the most susceptible groups *i.e.* the pregnant or lactating women and their children, respectively for their health safety (Akhbarizadeh *et al*., 2019). Breastfeeding is widely acknowledged as a crucial and optimal source of nutrition for infants, providing essential nutrients, immunological protection, and fostering emotional bonds between mothers and their babies. However, the potential exposure of breastfeeding infants to MPs, has raised questions about the implications for both human health and the delicate balance of the mother-infant relationship.

Several studies have reported the occurrence of MPs in human placenta, human breast milk and infant stool (Schwabl *et al*., 2019; Ragusa *et al*., 2021; Ragusa *et al*., 2022). The present study aims; I) to the study occurrence of MPs in human breast milk samples (HBM) of lactating mothers of the cosmopolitan city of Karachi, II) to compare the abundance of MPs in HBM collected from two different ethnic populations grouped based on their dietary habits (those who consume sea food regularly and those who haven't consumed any kind of seafood in past one month). This research underscores the potential influence of seafood intake on the presence of MPs in human breast milk, which is concerning due to the risk of newborns being exposed to these harmful pollutants.

Materials and methods

Cohort selection

This is a pilot baseline study consisting of two cohorts of consenting lactating mothers and it was conducted in strict adherence to ethical guidelines, which encompassed compliance with the Code of Ethics established by the World Medical Association (Declaration of Helsinki) for studies involving human subjects. Cohort A consisted of 15 healthy lactating mothers of economically underprivileged fisherman communities (BMSF) who consume seafood daily as the main source of protein. Cohort B consisted of 8 consenting healthy mothers with no seafood consumption in the past month (BMSH). Exemption criteria were: a) any infectious disease e.g., hepatitis, HIV, TB, or serious illness demanding medical treatment, b) use of antibiotics within two weeks of sampling, c) diarrhea, constipation, or any gastrointestinal pathology d) use of drugs affecting intestinal resorption. The mother participants were visited at home by members of the study team for human breast milk (HBM) sample collection. Participants who decided to contribute samples for this research were asked to sign an informed consent form and to fill in a questionnaire to record their food consumption, with a special focus on seafood consumption, food storing containers, utensils and personal care habits/products containing microbeads and glitters. Only those mothers were selected to donate HBM samples whose daily habits were compatible i.e. use of plastic-free clothing, utensils, personal care products, and makeup containing glitters.

Sample collection

For sample collection, mothers were led on a manual expression procedure, to produce the maximum milk output and to prevent any pain or injury to the breast tissue, as recommended by the World Health Organization in the presence of a trained health care professional. To prevent contamination from the plastic components, no breast pumps were allowed. The manual expression technique, in brief, involves cupping the breast with one hand while using the other to form a C-shape with the thumb and forefinger 3-4 cm from the base of the nipple. Pressure was applied by pushing towards the ribcage, squeezing with the thumb and forefinger, and then releasing the pressure. Pressure, squeeze, and release were repeated until an ideal amount of milk sample was attained. Milk samples were placed into the impermeable-to-air glass flasks and stored in the ice box until the samples were reached to the laboratory of the Institute of Marine Science, University of Karachi, where these samples were weighed and stored at -20°C.

Analysis and MPs characterization

A 10% solution of potassium hydroxide (KOH) was prepared for digestion using 1.6 µm prefiltered deionized water with KOH tablets sourced from Sigma-Aldrich. The milk samples were treated by adding the KOH solution to each flask in a ratio of 1:10 (w/v) as described by Ragusa *et al*. (2022). These flasks were sealed and left to incubate at rest at room temperature for 48 h. Subsequently, the digested samples underwent filtration using GF/A (Whatman) glass microfiber filter paper with a pore size of 1.6 µm.

To identify and characterize MPs, the filter papers were examined using a compound microscope (Micros Austria-

MC30) and imaged using Micros Cam 500 at magnifications ranging from 20 to 80. The assessment of MPs involved determining their quantity, size, shape, and colors (Lusher *et al*., 2015, 2017; Ragusa *et al*., 2022). Morphologically, MPs were categorized into three distinct categories: fibers, pellets and fragments (including irregular particles) (Li *et al*., 2016; Lusher *et al*., 2017) and their identification was confirmed through physical attributes (Kolandhasamy *et al*., 2018). The color spectrum for MPs encompassed black, blue, green, red, white, and transparent. For detailed analysis, 45 selected MPs particles (25 from BMSF and 20 from BMSH) were subjected to identification using a micro-Fourier-transformed infrared spectroscope (μ-FTIR) unit (Shimadzu-8900, Japan). The μ-FTIR unit operated in reflection mode with a spectrum range from 4000 to 500 cm^{-1} and collected data over a 3-second interval with 16 co-scans for each measurement. To validate the polymer type, obtained spectra were cross-referenced with the FTIR spectrum database, with a threshold of >70% match considered acceptable based on criteria established by Thompson *et al*. (2004).

Quality assurance and control (QA/QC)

Contamination from air-borne particles and reagents is a matter of concern in MPs analytics (Prata *et al*., 2021). MPs can be present in the reagents used for the digestion of organic matter, laboratory apparatus, laboratory environment, and distilled and ultrapure water (Barrows *et al*., 2017; Prata *et al*., 2021) which makes quality assurance challenging. All sampling and experimental procedures were handled with care throughout the study to avoid cross-contamination. Only glass and stainless-steel apparatus were used for sampling, preservation, alkaline digestion and filter paper analysis. Deionized water was used to rinse the apparatus and glass-ware was filtered with 1.6 μm GF/A filter paper before use. 10% KOH solution was filtered with 1.6 μm (pore size) GF/A filter papers before use. The workstation was cleaned with prefiltered 70% alcohol. Filter papers were examined under a microscope for air-borne contamination before use. After filtration, filter papers were placed in glass Petri dishes with lids rinsed with prefiltered deionized water. All the experimental procedures and observations were made at the earliest convenience.

Blanks were made and tested for microplastic contamination from the laboratory environment and procedures. Each batch of milk samples included a procedural blank, which was made using prefiltered milli-Q water following the same process as the samples were collected and analyzed but without milk. Each procedural blank was opened as many times as milk samples containing glass flasks. For environmental blanks, a wetted filter membrane soaked with 1.6 μm filtered deionized water was placed in an uncovered Petri dish at a designated work station each day. A Stereomicroscope was used to evaluate filters derived from environmental and procedural blanks.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences *(SPSS) program for Windows, version 26.0 (IBM, America)*. One-way analysis of variance (ANOVA) and Mann-Whitney U test were conducted to determine the relationship between MPs abundance in HBM samples collected from two communities. $p<0.05$ was considered to indicate statistical significance.

Results

A total of 276 MPs particles were recorded from HBM samples collected from the healthy breastfeeding mothers of the fishermen community (BMSF), and 49 MPs were found in the 8 HBM samples collected from healthy breastfeeding mothers (BMSH) with no seafood consumption in the past one month. The results of the preliminary investigation suggest a notably higher occurrence of MPs in the breast milk of lactating mothers belonging to an economically disadvantaged fishing community compared to lactating mothers with no history of seafood consumption. For QA/QC protocols, we analyzed 17 environmental and 23 procedural blanks. The environmental blanks had just 20 fibers ranging in size from 650 mm to 4500 mm. The filters from the procedural blanks showed no MPs contamination. Given the diameters of the fibers and their absence in the milk samples tested, there was no need to adjust the findings for blank correction, as they were not conducive to transfer into breastmilk. The most dominant types of MPs observed in both samples were fibers and pellets (Tables 1 and 2), whereas the majority of the observed MPs had a size ranging from 5-18µm (Fig. 1C and D). The dominant colors of MPs observed in the BMSF and BMSH were black, orange, transparent and black, orange, and red, respectively (Fig. 1E and F). The identified MPs in HBM samples exhibited a variety of characteristics, including types, size, colors, and composition where the most abundant MPs ranged from 5 to 18 µm in size. The most observed MPs were polyethylene (28% BMSF, 25% BMSH), polyvinyl chloride (20% BMSF, 15 % BMSH), and polypropylene (16% BMSF, 15 % BMSH) (Fig. 1G and H). The results of the study show a significant statistical difference in the abundance of MPs between the two studied communities ($F=13.57$, $P=0.001$). Mann-Whitney U test revealed a significant statistical difference ($p < 0.05$) in the distribution of MPs between the two communities.

Table 1: Information about healthy women from the fisherman community who regularly consume seafood (age, quantity of milk sample) and morphological characteristics of identified MPs (MPs).

Sample	Age	Milk quantity (g)	MPs/g	Colour	MPs types			Total no. of MPs
					Fiber	Fragment	Pellet	
BMF1	18	8.65	1.96	Black	2	0	0	17
				Blue	2	0	0	
				Green	1	0	2	
				Orange	0	0	2	
				Red	3	0	0	
				Transparent	5	0	0	
BMF2	25	10.95	2.92	Black	3	0	1	32
				Blue	4	0	2	
				Green	0	2	3	

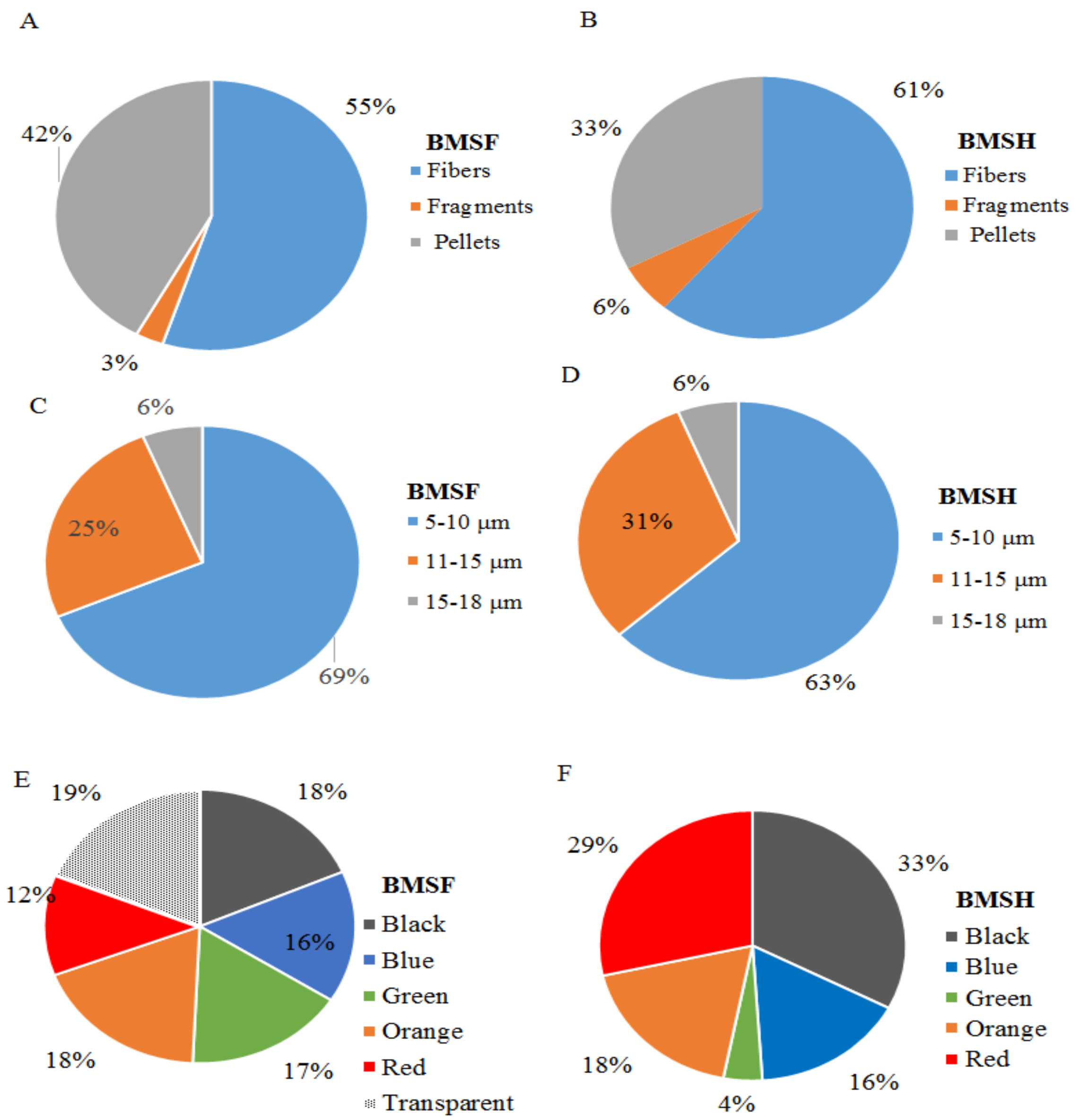
Sample	Age	Milk quantity (g)	MPs/g	Colour	MPs types			Total no. of MPs
					Fiber	Fragment	Pellet	
				Orange	3	1	3	
				Red	4	0	0	
				Transparent	2	0	4	
BMF3	27	8.25	1.93	Black	1	0	0	16
				Blue	2	0	0	
				Green	3	0	2	
				Orange	0	0	2	
				Red	3	0	0	
				Transparent	0	0	3	
BMF4	35	10.03	1.39	Black	1	0	0	14
				Blue	0	0	3	
				Green	1	0	3	
				Orange	2	0	0	
				Red	2	0	0	
				Transparent	2	0	0	
BMF5	20	8.41	0.83	Black	1	0	0	7
				Green	0	0	2	
				Orange	0	0	1	
				Red	2	0	0	
				Transparent	0	0	1	
BMF6	24	8.74	3.43	Black	8	1	0	30
				Blue	5	0	1	
				Green	1	0	0	
				Orange	2	0	2	
				Red	3	0	0	
				Transparent	7	0	0	
BMF7	32	8.98	0.66	Black	3	0	0	6
				Blue	1	0	0	
				Orange	0	0	2	
BMF8	26	11.39	1.05	Black	2	0	0	12
				Blue	2	0	0	
				Green	0	0	1	
				Orange	0	0	3	
				Red	4	0	0	
BMF9	34	10.6	2.83	Black	3	0	2	30
				Blue	9	0	0	
				Green	3	0	3	
				Orange	0	0	2	
				Red	2	0	0	
				Transparent	5	0	1	
BMF10	23	9.42	2.33	Black	2	0	0	22
				Blue	4	0	0	
				Green	0	2	2	
				Orange	0	0	5	
				Red	5	0	0	
				Transparent	0	0	2	
BMF11	24	10.85	1.93	Black	5	0	2	21
				Blue	0	0	0	
				Green	0	0	1	
				Orange	1	0	5	

Sample	Age	Milk quantity (g)	MPs/g	Colour	MPs types			Total no. of MPs
					Fiber	Fragment	Pellet	
				Red	2	0	0	
				Transparent	0	0	5	
BMF12	26	7.95	1.5	Black	2	0	1	12
				Blue	0	0	0	
				Green	1	0	2	
				Orange	0	0	3	
				Red	0	0	0	
				Transparent	0	0	3	
BMF13	37	9.45	1.79	Black	2	2	0	17
				Blue	0	0	0	
				Green	1	0	2	
				Orange	0	0	5	
				Red	0	0	0	
				Transparent	1	0	4	
BMF14	20	12.66	2.44	Black	5	0	2	31
				Blue	6	0	0	
				Green	3	0	3	
				Orange	2	0	3	
				Red	2	0	0	
				Transparent	2	0	4	
BMF15	22	9.94	0.8	Black	0	0	0	8
				Blue	2	0	0	
				Green	0	0	2	
				Orange	0	0	2	
				Red	1	0	0	
				Transparent	0	0	1	

Table 2: Information about healthy women with no seafood consumption in past month (age, quantity of milk sample) and morphological characteristics of identified MPs (MPs).

S. No.	Sample	Age	Milk quantity (g)	MPs/g	Colour	MPs			Total no. of MPs
						Fiber	Fragment	Pellet	
1	BMH1	30	10.12	0.39	Black	0	1	1	4
					Blue	1	0	0	
					Red	1	0	0	
2	BMH2	25	9.43	0.74	Black	0	0	1	7
					Blue	1	0	0	
					Orange	1	0	1	
					Red	2	0	1	
3	BMH3	29	10.32	0.29	Black	2	0	0	3
					Red	1	0	0	
4	BMH4	28	9.42	1.32	Black	0	1	0	10
					Blue	2	0	0	
					Green	1	0	0	
					Orange	0	0	3	
					Red	3	0	0	
5	BMH5	28	7.52	0.26	Black	0	0	1	2
					Blue	1	0	0	

S. No.	Sample	Age	Milk quantity (g)	MPs/g	Colour	MPs			Total no. of MPs
						Fiber	Fragment	Pellet	
6	BMH6	30	7.5	0.8	Black	1	0	0	6
					Blue	2	0	0	
					Green	1	0	0	
					Red	2	0	0	
7	BMH7	26	11.11	0.72	Black	1	1	2	8
					Orange	0	0	2	
					Red	2	0	0	
8	BMH8	27	12.5	0.72	Black	3	0	1	9
					Blue	0	0	1	
					Orange	1	0	1	
					Red	1	0	1	



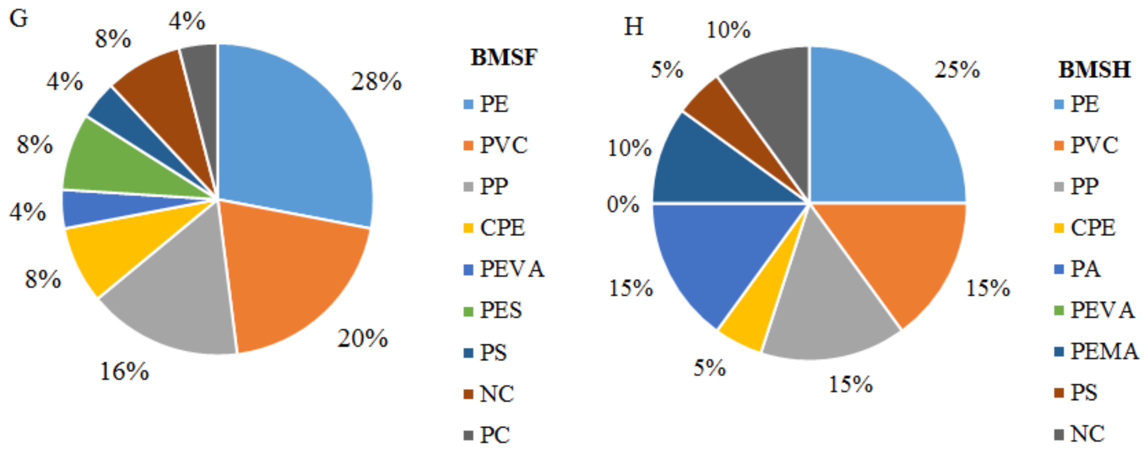


Figure 1: MPs types in BMSF (A) and BMSH (B), MPs size in BMSF (C) and BMSH (D), MPs color in BMSF (E) and BMSH (F) and MPs chemical composition in BMSF (G) and BMSH (H).

Discussion

This study highlights a concerning discrepancy in the presence of MPs in breast milk, with a significantly higher occurrence in lactating mothers from an economically disadvantaged fishing community, who consume seafood daily. The presence of pollutants in breast milk is a major cause for concern. As a vital source of nutrients and immune support, breast milk is vital to an infant's development. Infants are particularly vulnerable throughout their key early developmental phases, thus exposure to dangerous compounds like MPs and other pollutants can have a long-lasting impact on their physical and mental health (Ragusa *et al.*, 2022). This problem emphasizes how important it is to keep a close eye on environmental contaminants and how they affect human health, especially in susceptible groups like young children (Danopoulos *et al.*, 2021; Han *et al.*, 2022).

The investigation revealed 276 MPs in their breast milk compared to 49 MPs in a control group with no recent seafood consumption living in the cosmopolitan city of Karachi. The identified MPs in HBM samples exhibited a variety of characteristics, including types, size, colors, and composition where the most abundant MPs ranged from 5 to 18 µm in size whereas Ragusa *et al.* (2022) have already reported a size range of 2-12 µm from HBM samples. The most observed MPs were polyethylene, polyvinyl chloride and polypropylene. Similar findings have been reported by Ragusa *et al.* (2022). The elevated presence of MPs in breast milk collected from the underprivileged fishing community was found to be attributed to the consumption of contaminated seafood. The significant disparity in the abundance of MPs between the two groups of lactating mothers indicates that seafood consumption plays a pivotal role in the presence of MPs in breast milk. Due to the substantial reliance of many communities on a seafood diet, it's advised to limit seafood intake, especially among those who are more susceptible, due to the potential risks associated with consuming MPs through seafood. This group includes pregnant and lactating women and their children, as proposed by Akhbarizadeh *et al*. (2019). Additionally, it is advisable to avoid the consumption of marine fishes from coastal and estuarine environments, as fishes from coastal habitats tend to accumulate a higher concentration of MPs compared to those from open sea or deep-sea environments (Arshad *et al*., 2023).

Conclusions

Despite the existence of several limitations, the study involved a small number of participants, A larger sample size would provide more robust results and increase the generalizability of the findings. The study provides data about MPs presence in breast milk at a single point in time. Longitudinal data tracking MPs levels over time would provide a better understanding of temporal trends and potential changes in exposure levels. Other than sea-food consumption, factors like maternal occupation, lifestyle, and proximity to plastic waste sites could also influence MPs levels in breast milk. Addressing these limitations through larger-scale studies with diverse populations, longitudinal designs, uniform analytical methods, and consideration of potential confounding factors will contribute to a better understanding of the health implications of MPs exposure through breast milk and inform effective strategies for mitigating plastic pollution. These findings underscore the critical importance of reducing plastic usage and adopting sustainable fishing practices to mitigate the accumulation of plastic waste in the environment, ultimately minimizing its impact on the food chain. Adverse impacts of MPs during early-life exposure have not been reported in humans. Therefore, this study accentuates the necessity for additional research to assess the potential health consequences of MPs on individuals. The present study concludes and furnishes evidence of the existence of MPs in human breast milk and their association with seafood consumption. These results accentuate the urgency to take mitigation measures to curtail plastic pollution and promote sustainable practices to safeguard a healthy environment for future generations.

Conflicts of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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