Journal of Hazardous Materials 492 (2025) 138052

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Dietary exposure and risk assessment of plastic particles in cow's milk stored in various packaging materials

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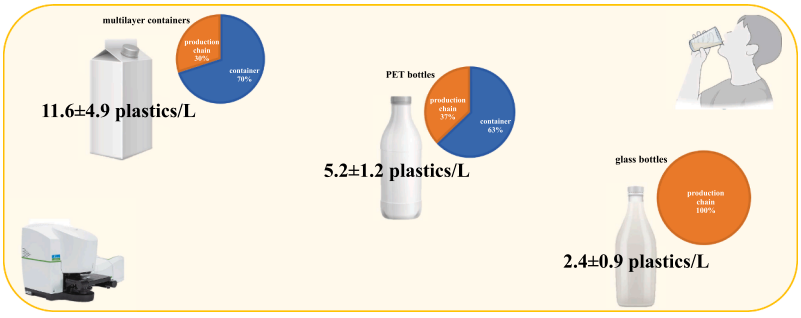
HIGHLIGHTS

- Plastic release in cow's milk depends mainly on the type of packaging.
- More plastics were detected in milk stored in multilayer packaging.
- Several polymers detected in milk come from the production chain.
- Daily plastic intake from milk is minimal compared to processed food.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Milk
Packaging
Risk assessment
Food
Polymers

ABSTRACT

Food packaging is a crucial step in the storage of many food products, but it raises several concerns related to the materials used in its production. Among these, various types of plastic particles are commonly used in food containers, posing a risk of migration into food. One of them frequently stored in different types of packaging is cow's milk. Despite its nutritional significance, very limited data are available on the occurrence of plastic contaminants in milk, and no study has investigated the influence of packaging type. To partially address this gap, the present study aimed to compare the quantity and types of plastic particles detected in 11 different cow's milk samples stored in multilayer containers, PET (polyethylene terephthalate) bottles, and glass bottles. In addition to a qualitative and quantitative comparison, we assessed dietary plastic intake and conducted a risk assessment based on quantitative and qualitative indices. The main findings revealed that milk stored in multilayer packaging contained a higher amount of plastic than milk stored in PET or glass bottles. Quantitative indices for risk assessment confirmed these differences, while the qualitative one highlighted that the presence of "unconventional" polymers increased the potential hazard for milk stored in PET and glass packaging.

1. Introduction

Plastic is now widely used beyond industry and has become part of everyday life. As a result, plastic production has been steadily increasing, reaching approximately 414 million tons (Mt) worldwide in 2023, representing an 8.2 % rise over the past five years [23]. Of the

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https://doi.org/10.1016/j.jhazmat.2025.138052
Received 3 February 2025; Received in revised form 5 March 2025; Accepted 21 March 2025
Available online 25 March 2025

total plastic produced, about 36 % is used for packaging, including single-use plastic products for food and beverage containers, as reported by the United Nations Environment Programme [28]. In Europe, a similar amount (34.7 %) is used for packaging, followed by construction materials (23.5 %) [24]. This shows that packaging is one of the main contributors to environmental plastic pollution but also a potential source for human exposure, particularly through food containers. A recent study [10] estimated that approximately 2977 microplastic particles (MPs) are ingested annually through take-out food, and those who eat packaged food 4–7 times a week may ingest between 12 and 203 microplastic particles weekly from the packaging. Plastic particles can enter food containers during manufacturing, via atmospheric deposition, handling, and transportation. More importantly, plastic packaging can release tiny particles into food due to wear and tear, temperature changes, friction, aging, and degradation over time [17].

The use of plastic materials in direct contact with food is regulated at the EU level by Commission Regulation No. 10/2011, which sets an overall migration limit of 10 mg of substances per 1 dm^2 of plastic surface area. For a cubic packaging unit containing 1 kg of food, this corresponds to a migration limit of 60 mg per kg of food [12]. Additionally, the European Commission has recently introduced requirements to improve product design, aiming to increase the reuse and refillability of specific packaging types, such as those for beverages and ready-to-eat takeaway meals, to mitigate production-related environmental impacts [13].

One of the most consumed packaged foods is cow's milk, a key source of nutrients, especially for children [22]. Some studies suggest that health benefits are linked to milk, such as reducing the risk of diabetes, stroke, colorectal cancer, obesity, metabolic syndrome, hypertension, osteoporosis, and cardiovascular disease. However, other studies have linked milk consumption to an increased risk of prostate cancer, Parkinson's disease, acne, and iron-deficiency anemia in infants [29].

India was the largest consumer of cow's milk (87 million metric tons, MMT), followed by the EU (23.65 MMT) and the United States (20.9 MMT) [25]. In Italy, milk consumption has dropped over the years, from 62.2 % in 1998 to 48.1 % in 2020 [3]. This decline contrasts with national dietary guidelines, which recommend three servings of milk per day (375 mL; [6]), while the average Italian consumes only about 115 mL daily [27].

Because milk is widely consumed, the right packaging must be chosen to prevent spoilage and contamination. The most common milk packaging materials include plastic, multilayer composite materials, metal cans (aluminum and tinplate) and, though less common, glass. Plastic used for milk packaging includes high-density polyethylene (HDPE), polyethylene terephthalate (PET), polycarbonate (PC), and low-density polyethylene (LDPE) [1]. Additionally, plastic is present in multilayer packaging, which is increasingly used for milk storage. These materials include polylaminate films composed predominantly of paper with plastic layers (EU code C/PAP 81; [9]/852) and multilayer composites consisting of paperboard, plastic, and aluminum (EU code C/PAP 84; [9]/852). The latter type is typically composed of printed paper coated with aluminum foil and polyethylene (PE) layers [1], with an average composition of 70 %, 5 %, and 25 %, respectively (www.tetrapak.com).

Despite the importance of milk and the potential risk of plastic contamination, few studies have been conducted on plastic particles in milk [2,5,7,8,18,19,30]. However, none of these studies have compared how different packaging materials affect plastic contamination in milk. To address this gap, this study aimed to quantitatively and qualitatively assess the plastic particles detected in 11 cow's milk samples, each analyzed in triplicate, stored in multilayer containers, PET bottles, and glass bottles, the most common packaging types in Italian supermarkets. Human exposure to plastic particles was also estimated based on milk consumption, and the risk was assessed using both quantitative and qualitative indices. To our knowledge, this is the first study in an EU-27 country to examine plastic contamination in cow's milk while considering the impact of different packaging materials.

2. Materials and methods

2.1. Selection of cow's milk samples

In this study, 33 one-liter containers of fresh cow's milk from 11 different brands were analyzed, considering various types of packaging: 4 samples (12 containers) were in multilayer packaging (MLn), 4 samples (12 containers) were in PET bottles (Pn), and 3 samples (9 containers) were in glass bottles (Gn). Detailed information about the 11 samples analyzed is provided in Table 1. The smaller number of glass samples was due to the difficulty in sourcing this type of packaging, which is almost unavailable in Italian supermarkets. Milk samples were purchased individually from October 2023 to March 2024 in various supermarkets in the provinces of Milan and Lecco (Northern Italy), as well as through direct sales from small and medium-sized companies in several provinces of Northern Italy. This approach was aimed at ensuring a more heterogeneous and representative sample pool. The samples were stored in the laboratory at 4 °C and processed as soon as possible, always before the expiration date, to prevent any release of plastic particles from the containers due to extended storage times.

2.2. Plastic particle separation

To minimize external contamination as much as possible, several precautions were implemented during the preparation steps. No plastic components (e.g. filter system components, Petri dishes) were used. Each component of the glassware was thoroughly washed with distilled water (which was previously checked to ensure the absence of plastic particles) before use. Personal protective equipment (latex gloves and cotton gown) was always worn. At the end of each filtration procedure, the glassware was first rinsed with distilled water and then subjected to an ultrasonic bath for 15 minutes. The entire preparation process was carried out under a laminar-flow hood to minimize potential atmospheric contamination, and blank filters (n = 33) were used throughout each stage of sample processing to detect possible environmental contamination. The containers were opened only once to minimize the release of plastic particles from the cap.

For each replicate, 1 L of milk was filtered using a vacuum pump and nitrocellulose filters with an 8 μm pore size (Sartorius™ 50 mm), lower than the instrumental detection limit (see below). Due to the casein micelles and fat globules, filtering 1 L of milk per sample required the use of multiple filters (ranging from 4 to 12) that were replaced when the milk flow through the vacuum pump became too slow or blocked. To reduce filter clogging, the procedure suggested by Basaran et al. [2] was followed, which involved heating the milk in glass beakers in a microwave oven for 30 seconds at a temperature not exceeding 40 °C.

To clean the filters of lipid residues and other impurities that could interfere with subsequent analysis, they were rinsed with a solution of Triton™ X-100 and Milli-Q water (1:5), applied in small amounts to the filter surface for 10 minutes, with the vacuum pump turned off. The vacuum pump was then restarted to drain the remaining liquid, ensuring the filter surface was completely dry. The rinsing solution was first filtered through glass fiber filters with a 1.2 μm porosity (Whatman GF/C 47 mm) and stored in a glass desiccator.

The filters were carefully removed from the filter holder using metal tweezers and placed on glass Petri dishes. To prevent the filters from folding during drying, they were secured along the outer edges at the base of the Petri dish with small pieces of paper tape. After drying completely, the Petri dishes containing the samples and controls were kept closed until the next step.

2.3. Instrumental analysis

A visual sorting was performed using a stereomicroscope to identify

Table 1
Characteristics of milk samples analyzed.

Sample acronym	Product	Notes	Packaging volume	Packaging colour	Packaging type	Inner layer	Cap
ML1	fresh semi-skimmed milk	-	1 L	white	C/PAP 81	PE	HDPE
ML2	fresh whole milk	-	1 L	white	C/PAP 81	PE	HDPE
ML3	fresh semi-skimmed milk	microfiltered	1 L	white	C/PAP 81	PE	HDPE
ML4	fresh whole milk	-	1 L	white	C/PAP 84	PE	HDPE
P1	fresh whole milk	-	1 L	yellow	PET	PET	HDPE
P2	fresh whole milk	-	1 L	green	PET	PET	HDPE
P3	fresh semi-skimmed milk	lactose-free	1 L	blue	PET	PET	HDPE
P4	fresh semi-skimmed milk	lactose-free and microfiltered	1 L	orange	PET	PET	HDPE
G1	fresh whole milk	-	1 L	-	glass	-	aluminum
G2	fresh semi-skimmed milk	lactose free	1 L	-	glass	-	aluminum
G3	fresh whole milk	-	1 L	-	glass	-	aluminum

C/PAP 81 = EU code for polylaminate film predominantly paper/cardboard with plastic.
C/PAP 84 = EU code for polylaminate film in paper and cardboard/plastic/aluminum.

and isolate all particles present on the filters, with any residual interfering materials that remained after the preventive rinsing process being removed to facilitate the subsequent instrumental analysis. Using metal tweezers, each visible particle was transferred to a clean nitrocellulose filter, within a defined area of approximately 1 cm^2, ensuring that particles did not overlap.

Each individual particle on the clean filter was then analyzed using the Spotlight 200i μFT-IR Microscopy System (PerkinElmer, Milan, Italy), with a detection limit of 10 μm. For each particle, the infrared (IR) spectrum was acquired in attenuated total reflectance (ATR) mode. The obtained spectra were then compared to those in the system's software database. For each match, the composition of the particle was identified and associated with a score, calculated based on the degree of spectral correspondence, considering only plastic particles with a match score $\geq$ 70 %.

For each particle analyzed, shape, color, and size were also evaluated. ImageJ software was used to assess the size. For size classification, the scale proposed by the International Organization for Standardization [16] was followed: macroplastics (>5 mm), large MPs (1 $\leq$ 5 mm), MPs (1 μm $\leq$ 1 mm), and nanoplastics (<1 μm). Particles were classified into five shape categories: fibers, fragments, lines, films, and pellets. Raw data are provided in Table S1, while the spectra for PE, PET, and PP are shown in Figure S1.

2.4. Plastic intake and risk assessment

2.4.1. Dietary exposure

The daily plastic intake (DPI) was calculated by this equation:

$$DPI = M_a \text{ x } M_c$$

where M_a is the average national daily amount of consumed milk (mL/day) in Italy, which is 115 mL per day [27], and M_c is the number of plastic particles found (plastic particles/mL) in milk samples.

2.4.2. Plastic pollution load index and plastic polymer risk index

The approach recently proposed by Basaran et al. [2] to assess plastic hazards was adopted, based on both quantitative evaluations using the plastic pollution load index (PPLi), and the polymer risk index (pRi), which also consider the toxicological risk of individual polymers detected in the milk samples. The acronyms used in the referenced study (plastics instead of MPs) were chosen to be modified because not all polymeric particles detected fall under the category of MPs, according to the classification applied [16].

To calculate the PPLi, the plastic contamination factor for each milk sample (PCFi) must first be determined using the following ratio [11]:

$$PCFi = \frac{PPi}{PPb}$$

where PP_i is the number of plastic particles revealed in each milk sample (i) and PP_b is the minimum plastic concentration (1.68 particles/kg) in processed food reported by Lin et al. [20]. Again, the acronym (P) for plastic particles has been used, as permitted by the fact that the authors of the previous study used the classical definition of MPs as particles smaller than 5 mm. This definition encompasses the size categories detected in all milk samples (MPs and large MPs), with the exception of one sample, in which two macroplastics (larger than 5 mm) were identified.

PPLi was then calculated following this equation [11]:

$$PPLi = (PCF_1 \text{ x } PCF_2 \text{ x } PCF_3 \text{ x } \ldots\ldots \text{x } PCF_n)^{1/n}$$

in which n is the total number of sample.

Finally, the pRi was calculated using the equation proposed by Basaran et al. [2]:

$$pRi = \sum\left(\frac{Pa}{Pt} \text{x } Rs\right)$$

where P_a is the number of each polymer found in the milk sample (a), P_t is the total number of plastic particles revealed in sample (a) and R_s is the chemical toxicity coefficient or risk score obtained by Lithner et al. [21] and adopted by Lin et al. [20] and Basaran et al. [2]. Risk scores used for each of the polymer found in our milk samples are shown in Table S2.

PCF and pRi were used to categorize the hazard related to plastic intake from milk consumption (Table 2). The different risk categories are the same as those used by Basaran et al. [2], derived from the criteria adopted by Ibeto et al. [15] for PCF and PPLi, and by Enyoh et al. [11] for pRi.

2.5. Statistical analysis

Significant differences in plastic particle content in the samples, evaluating in independent manner milk brand, packaging, and skimming, was investigated by one-way Analysis of Variance (one-way ANOVA) followed by Tukey HSD *post-hoc* test. ($p < 0.05$). A Multi-way

Table 2
Contamination levels and risk categories for PCF and PPL_i [15], and risk categories for pRi [11].

PCF	Contamination level	Risk Category	pRi	Risk Category
PCF< 1	low contamination	I	< 150	low
1 ≤PCF< 3	moderate contamination	II	150–300	medium
3 ≤ PCF< 6	significant contamination	III	300–600	considerable
PCF≥ 6	very high contamination	IV	600–1200	high
			> 1200	very high

Modified from Basaran et al. [2].

ANOVA could not be used because the three different types of packaging were not always available for each of the selected brands.

3. Results

It is important to emphasize that no plastic particles were detected in the 33 blank filters, in which only few cellulose particles were observed (Table S1). In the milk samples from 11 different brands, a total of 1755 particles were identified through microscopic observation, all of which were subsequently analyzed using μFT-IR spectroscopy. Among these, only 223 particles (12.7 %) were confirmed to be plastics, while 1248 particles (71.1 %) were classified as cellulose. Additionally, 284 particles (16.2 %) were determined to consist of various inorganic and organic materials, including silica, quartz, Arabic gum, monoolein, glass, calcium carbonate, magnesium silicate, limestone, and adipate. Furthermore, 257 particles remained unidentified due to factors such as surface contamination by fats and proteins, small particle size, and potential degradation over time. The 223 plastic particles, all ranging in size from 0.04 to 5.77 mm, were fully characterized and included in subsequent qualitative and quantitative analyses.

3.1. Quantitative characterization

Since the spectra of polyester (PEST) and PET cannot be distinguished by IR spectrometry, all particles identified as either of these polymers were classified exclusively as PET. This decision was based on two factors: first, a subset of the analyzed milk samples was stored in PET containers, and second, PET has been reported to have a significantly lower environmental risk score (Rs=4) compared to PEST (Rs=1177), as stated by Lin et al. [20]. This conservative feature might lead to an underestimation of the subsequent risk assessment, but it certainly does not result in an overestimation that could be too alarming for the consumption of that food.

From a quantitative point of view, highly significant effects of both milk brand ($F_{10,22}$=75.473; $p < 0.01$) and packaging type ($F_{2,30}$=13.286; $p < 0.01$) on plastic particle contamination in the samples were revealed by statistical analysis. Overall, the highest number of plastic particles was detected in the 3 L of milk from ML1, with a maximum of 53 plastic particles across the three replicates, whereas the lowest value was observed in G3, with only 4 plastic particles detected in total (Fig. 1A).

Milk stored in multilayer packaging was found to exhibit the highest amount of plastic particles, with a mean value of 35 ± 16 particles, followed by PET containers (16 ± 4 particles) and glass bottles (7 ± 3 particles). When plastic contamination was normalized to 1 L of milk and statistical comparisons were performed (Fig. 1B), considerable variability among samples stored in multilayer packaging was observed. Specifically, ML1 (17.7 ± 3.1 particles/L) was found to contain a significantly higher amount of plastic particles ($p < 0.05$) compared to ML4 (4.7 ± 2.1 particles/L), while ML2 (12.3 ± 8.5 particles/L) and ML3 (11.7 ± 4.0 particles/L) exhibited similar plastic contamination levels and did not differ statistically from either ML1 or ML4.

In contrast, no significant differences were detected among milk samples stored in PET bottles, but these samples were found to contain significantly fewer plastic particles ($p < 0.05$) than the most contaminated sample (ML1). Similarly, significantly lower plastic content was observed in milk samples stored in glass bottles compared to ML1 ($p < 0.05$). Among the glass-packaged samples, a statistically lower number of plastic particles ($p < 0.05$) was found in G3 (1.3 ± 2.3

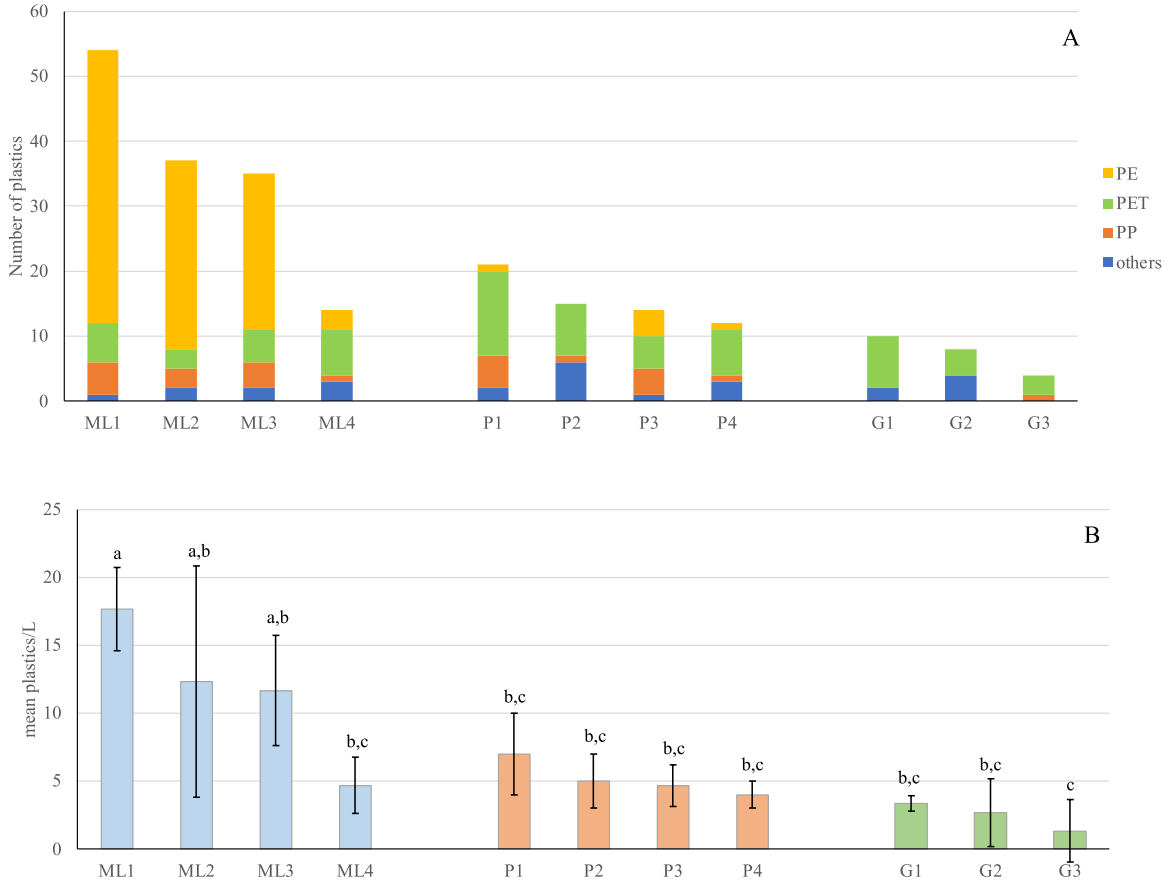

Fig. 1. Characterization of plastic particles detected in the 11 milk samples: A) number of plastic particles revealed in 3 L of each milk sample divided in the identified synthetic polymers; B) mean plastic particles/L ± standard deviation for each milk sample. Different letters show statistical difference ($p < 0.05$) between samples. ML = multilayer packaging; P = PET bottles; G = glass bottles.

particles/L) compared to G1 (3.3 ± 0.6 particles/L) and G2 (2.7 ± 2.5 particles/L) (Fig. 1B).

3.2. Qualitative characterization

In Fig. 1A, PE is shown as the most frequently detected polymer in the multilayer milk samples, accounting for 70 % of the total polymer particles identified across the four samples. Specifically, PE concentrations were found to range from 11.1 % in ML1 to 78.4 % in ML2, followed by PET, which constituted 15 % of the detected polymers, with values varying between 8.1 % in ML2 and 50.0 % in ML4. PP was represented in 9.3 % of the total, with the lowest percentage in ML1 (1.9 %) and the highest in ML3 (11.4 %).

Considerable differences in polymer distribution were observed in milk samples stored in PET bottles. In this group, PET was the most frequently detected polymer, accounting for 53.2 % of the total particles, with a range from 35.7 % in P3 to 61.9 % in P1. The second most abundant polymer was PP (17.7 %), with concentrations ranging from 6.7 % in P2 to 28.6 % in P3, followed by PE (9.7 %), which was entirely absent in P2 but reached 28.6 % in P3. Similarly, PET was predominantly detected (68.2 %) in milk samples stored in glass bottles, with values ranging from 25.0 % in G3 to 80.0 % in G1. However, PP was found only in G3 at a negligible percentage (4.5 %), while PE was not detected in any of the glass-packaged samples.

Notably, the highest proportion of "other" polymers (27.3 %) was identified in milk stored in glass bottles, compared to multilayer (5.7 %) and PET (19.4 %) samples. In addition to PET, PP, and PE, the glass-stored milk samples contained acrylonitrile-butadiene-styrene (ABS), polyurethane (PU), polyacrylate (PAK), styrene-butadiene-styrene (SBS), and polyamide (PA).

Fig. 2 illustrates the polymer composition across all milk samples, highlighting the detection of a single fragment of polyethylene glycol (PEG) in sample P2 and one pellet of polyvinylpyrrolidone (PVP) in ML1. These polymers are classified as water-soluble polymers (WSPs), also known as "liquid plastics".

As described in Section 2.3, plastic particle sizes were categorized according to ISO classification [16]. In all samples, MPs (1 μm ≤ 1 mm) were identified as the predominant size class and were the only size category detected in ML1 and G3 (Fig. 3). For the remaining samples, MPs accounted for percentages ranging from 47.6 % (P1) to 94.3 % (ML3). Large MPs (1 mm ≤ 5 mm) were identified at varying percentages, with the lowest occurrence in ML3 (5.7 %) and the highest in P1 (42.9 %). Notably, macroplastics (>5 mm) were detected only in P1.

Regarding shape identification (Fig. 4), fragments and fibers were found to be the two predominant plastic shapes, followed by lines and films, while pellets were detected exclusively in ML1 and ML2. In nearly all milk samples fragments were the most frequently identified shape, with proportions ranging from 14.3 % (ML4) to 75.5 % (ML1). Notably, ML4 and P1 were the only samples in which fibers constituted the dominant shape.

The relationship between the shapes of plastic particles and their polymer composition was particularly interesting to analyze (Fig. 5). The fibers detected across the three different packaging types were predominantly composed of PET, with proportions of 55.6 % in multilayer samples, 81.5 % in PET samples, and 100 % in glass samples. In multilayer packaging, PE fibers were also identified, accounting for 27.8 % of the total fibers, whereas this polymer was completely absent in the fibers from PET and glass containers. PE was also identified as the dominant polymer in both fragments (84.8 %) and films (68.4 %) detected in multilayer samples. However, its prevalence decreased to 25 % in lines and was completely absent in the two pellets found in these samples.

PET containers exhibited the most homogeneous shape distribution, as no films or pellets were detected in any of the 12 replicates analyzed. PET was found in all the lines identified and constituted 20 % of the fragments, although the majority of fragments were composed of PP (36 %) and PE (24 %).

In glass samples, PET was not only the sole polymer found in fibers but was also identified as the predominant polymer in fragments (77 %) and accounted for half of the lines detected. The remaining lines were composed of PA, which was also found to be the polymer forming the only film identified in these samples.

4. Discussion

The limited data available worldwide on the presence of plastic particles in cow's milk show considerable heterogeneity in both quantitative and qualitative characteristics (Table 3). The findings of the present study (mean of 6.8 ± 2.8 plastic particles/L), which represent the first data obtained in an EU-27 country, are remarkably similar to those reported for 14 milk samples from Turkey [2], where an average of 6.0 ± 5.0 plastic particles/L was detected, and for 23 milk samples from Mexico, in which a comparable concentration of 6.5 ± 2.3 plastic particles/L was found [19]. Conversely, significantly higher plastic contamination levels have been reported in other studies. For instance, 40 plastic particles/L were identified in 40 milk samples from Ecuador

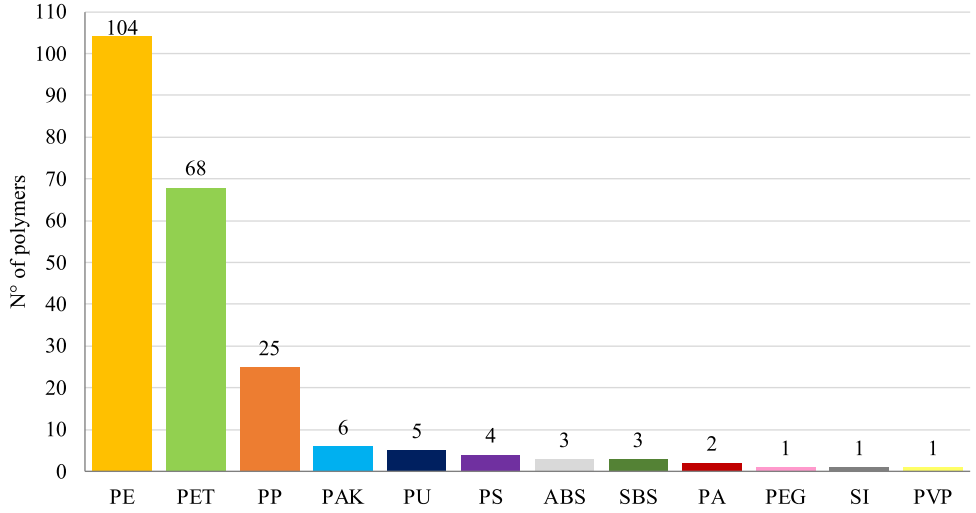

Fig. 2. Amount of all polymers detected in the 33 milk sample replicates. PE=polyethylene; PET=polyethylene terephthalate; PP=polypropylene; PAK=polyacrylate; PU=polyurethane; PS=polystyrene; ABS=acrylonitrile-butadiene-styrene; SBS=styrene-butadiene-styrene; PA=polyamide; PEG=polyethylene glycol; SI=silicone; PVP=polyvinylpyrrolidone.

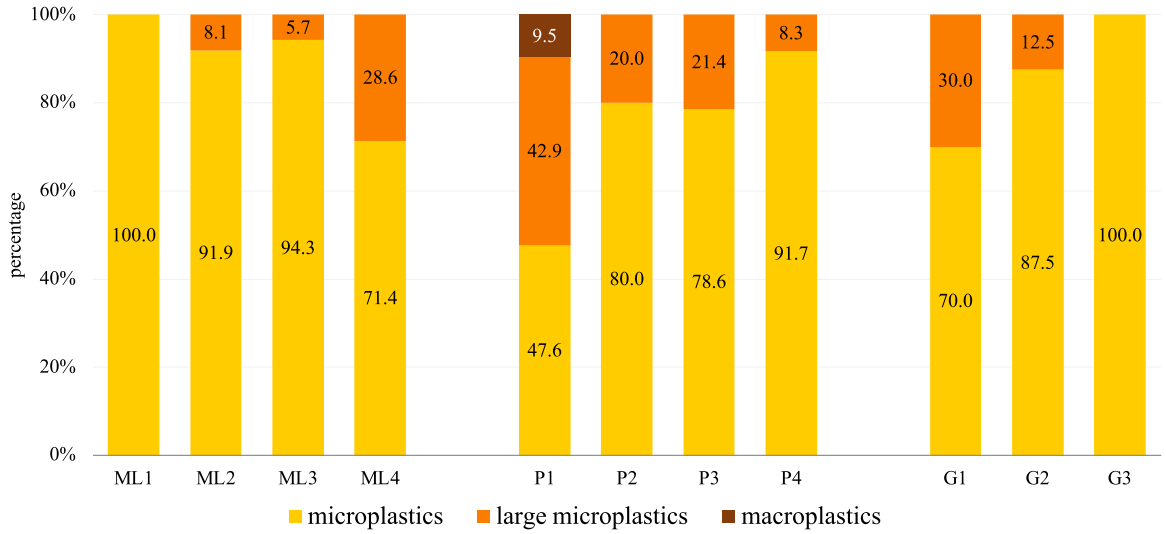

Fig. 3. Percentage of polymer sizes detected in each milk sample analyzed.

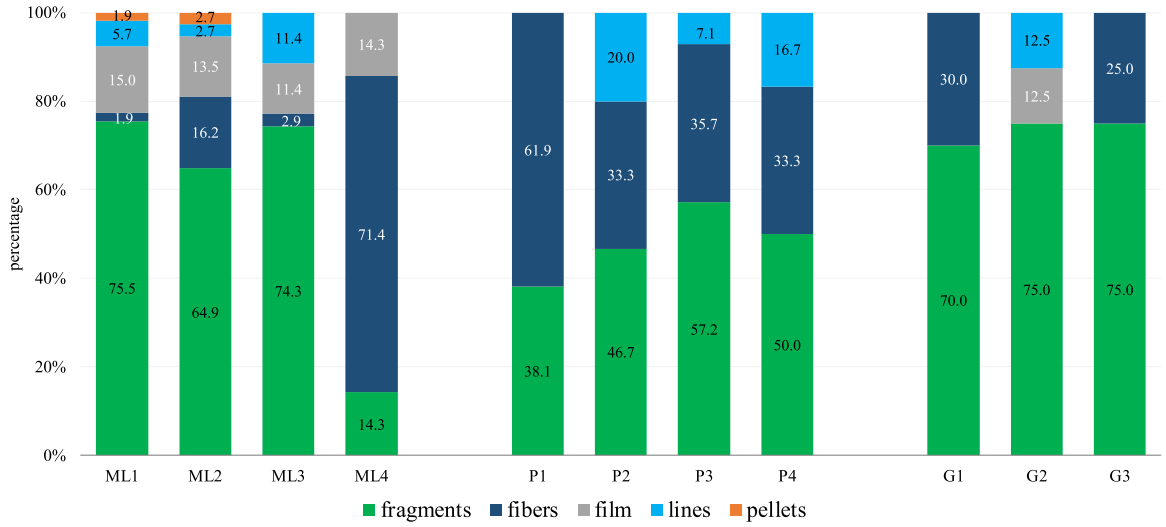

Fig. 4. Percentages of plastic shapes observed in each milk sample analyzed.

by Diaz-Basantes et al. [8], while 301 plastic particles/L were detected in 16 samples from India by Kiruba et al. [18] and 182 ± 55 plastic particles/L were revealed in 423 milk samples from Bangladesh by Chakraborty et al. [5]. The highest reported contamination level to date was recorded in 4 Swiss cow's milk samples by Da Costa Filho et al. [7], where as many as 2590 plastic particles/L were identified. The authors justify this high value by the use of the "point-to-point" Raman approach, with which it is possible to detect much smaller particles than visual sorting.

Differences are also evident in the polymeric composition of milk samples across studies. A broader range of polymers was identified in our analysis compared to previous research, though the majority of plastic particles consisted of PE, PET, and PP (Fig. 2). The polymeric diversity observed in our study is most comparable to that found in Swiss milk samples (Table 3).

4.1. Overall comparison among packaging types

One of the main objectives of this study was to compare plastic contamination in milk samples stored in different types of packaging. As an initial step, an investigation was conducted to determine whether one of the main processing steps, skimming, could contribute to an increase in plastic contamination. Although a slightly higher mean number of plastic particles was observed in semi-skimmed milk samples (8.1 ± 6.4 plastic particles/L) compared to whole milk samples (5.6 ± 3.8 plastic particles/L), suggesting a potential influence of this process, no statistically significant difference was found, in agreement with findings by Basaran et al. [2].

Overall, a significantly higher mean plastic contamination (11.6 ± 4.9 plastic particles/L) was exhibited by milk samples stored in multilayer containers ($F_{2,30}$=13.28647; $p < 0.01$) compared to those stored in PET bottles (5.2 ± 1.2 plastic particles/L) or glass bottles (2.4 ± 0.9 plastic particles/L), which, in contrast, did not show a statistically significant difference from each other (Fig. 6A).

Fig. 6B clearly illustrates that PE was the most frequently detected polymer (70 %) in milk stored in multilayer packaging. Given that the inner layer of these containers is composed of PE (Table 1), a direct release of plastic particles into the milk is suggested. Similarly, the predominance of PET (53.2 %) in milk stored in PET bottles (Fig. 6B) further supports the hypothesis that plastic particles are directly released from the container.

However, this was not the only source of plastic particles in the samples, as some of the detected polymer particles originated from outside the container. In fact, plastic contamination in milk can be

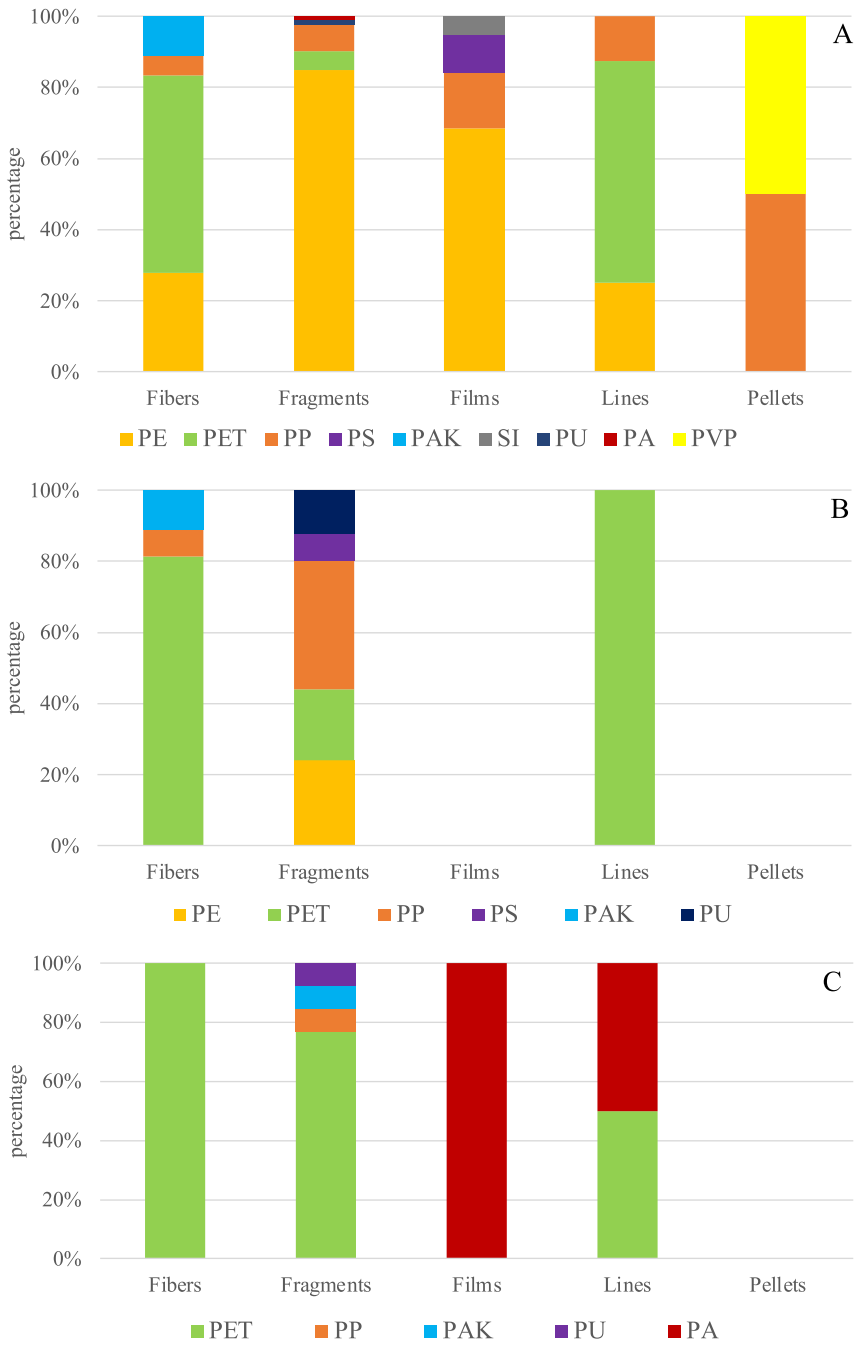

Fig. 5. Polymeric composition of each shape class identified for the 11 milk samples.

attributed to two main sources: (i) abrasion and fragmentation of the inner packaging layer after filling and sealing [17], and (ii) various processes throughout the production chain, including handling, storage, and transportation [20]. Evidence for this second source is provided by the detection of several plastic particles even in glass bottles (with aluminum caps) and by the identification of many other "unconventional" polymers mainly in PET and glass samples (Figs. 2 and 6B), confirming that their presence cannot be directly attributed to the packaging itself. Assuming that only PE particles originated from the multilayer packaging, we can roughly estimate that 70 % of the plastic particles detected in ML samples came from the container, while 30 % originated from the production chain. Similarly, considering that PET and PE (the material of the bottle caps) originate directly from PET bottles, it is possible to estimate that approximately 63 % of the polymers found in these samples derived from the container, while about 37 % came from the production chain. Obviously, all plastic particles detected in milk contained in glass bottles originated from outside the container. More in-depth hypotheses can also be formulated based on the relationships between shapes and polymers (Fig. 5). Assuming that the majority of PE originates from multilayer containers, as previously mentioned, it is reasonable to suppose that fragments, primarily composed of over 80 % PE, predominantly detach from the inner layer of these containers. Following the same reasoning, it is likely that PET bottles release mostly fibers and, to a lesser extent, lines of the same material. Given the very few plastic particles detected in glass bottles, speculation on their environmental origin based solely on these two characteristics is not considered appropriate.

Table 3
Data comparison with previous studies.

Country	Number of samples	Mean detected particles (plastics/L)	Ranges of detected particles (plastics/L)	Size	Polymers	Characterization method	References
Turkey	14	6 ± 5	3–48	25–5050 μm	PET, PP, PU, EVA, nylon−6	FTIR	Basaran et al. [2]
Mexico	23	6.5 ± 2.3	3–11	0.1–5 mm	PSU, PES	μ-Raman	Kutralam-Muniasamy et al. [19]
Ecuador	10	40	16–53	2.48–6742 μm	HDPE, LPDE, PAM, PP	FTIR	Diaz-Basantes et al. [8]
India	16	301	164–512	< 500 μm	PP, PE, PAM	FTIR	Kiruba et al. [18]
Switzerland	4	2590	1720–3480	≥ 5 μm	PA, PE, PS, PES, PVA, PTFE, PU, PP, PSU	μ-Raman	Da Costa Filho et al. [7]
Turkey	423	8.42	not available	17-5000 μm	PEA, EPC, HNBR, PAM	SEM-EDS; FTIR	Zipak et al. [30]
Bangladesh	6	182±55	95-250	>0.1 mm	PE, PP, PET, nylon-6, PA, PS	FTIR	Chakraborty et al. [5]
Italy	11 (in triplicate)	6.8 ± 2.8	4–53	0.04–5.77 mm	PE, PET, PP, PAK, PU, PS, ABS, SBS, PA, PEG, SI, PVP	μ-FTIR	Present study

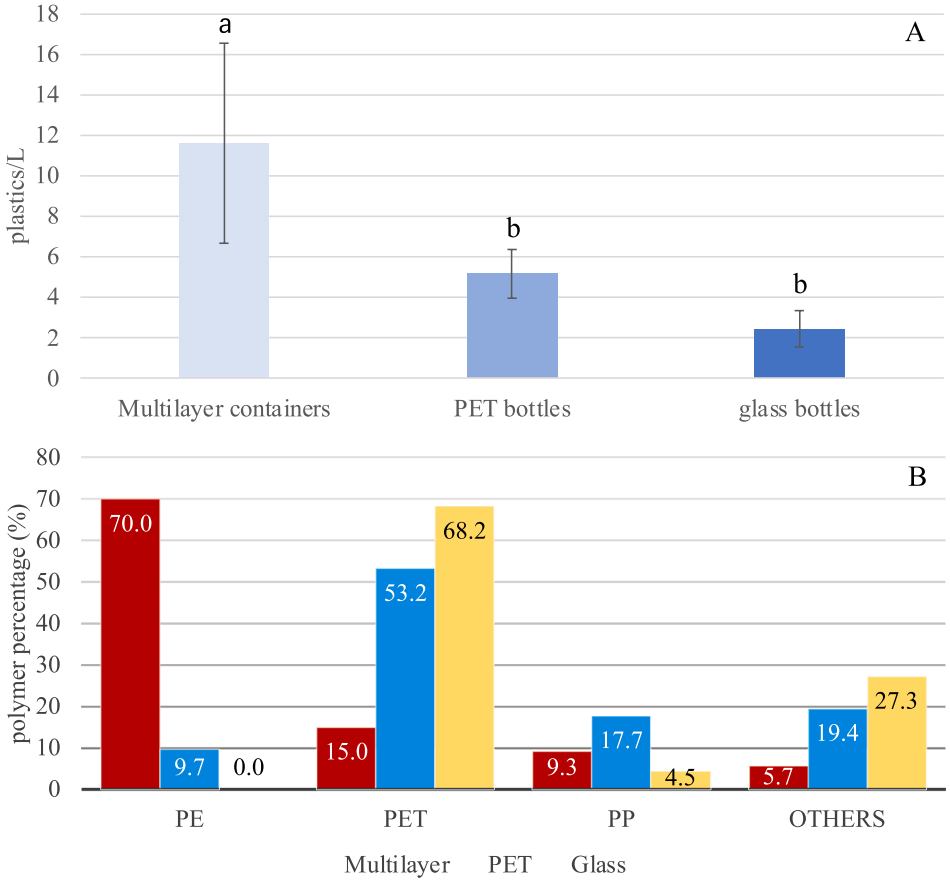

Fig. 6. A) Average polymeric particles (plastics/L ± standard deviations) found in milk samples in multilayers containers, PET bottles and glass bottles. Different letters show statistical difference ($p < 0.05$) between samples. B) Mean percentage of different polymers detected in the 3 types of packaging.

4.2. Plastic intake

Before addressing the issue of risk assessment for the plastic particles found in the milk samples, it is important to highlight that 71.1 % of the characterized particles were of non-synthetic origin, specifically cellulose. Assuming that at least the dyed cellulose fibers (n = 460; 36.8 % of cellulose particles) likely originated from garments, this contamination in milk should not be overlooked, even though it is not typically considered in hazard assessments. In fact, natural fibers used in clothing are often treated with dyes that may contain amines, metals, pentachlorophenol, chlorine bleach, halogen carriers, free formaldehyde, biocides, fire retardants, and softeners [4]. In this context, the potential risk posed by pollution from natural fibers treated with substances hazardous to human health should be considered.

Moving to the assessment of plastic intake, based on the data from the 33 milk sample replicates, the first value calculated was the daily dietary plastic intake (DPI), which was obtained using the formula provided in paragraph 2.4.1, as well as the corresponding yearly plastic intake (YPI). In Table 4 it is illustrated that, based on an average Italian consumption of cow's milk (115 mL/day, [27]), an average intake of 0.78 plastic particles per day was recorded from milk, consistent with the findings of Basaran et al. [2], though much lower than estimates

Table 4
Daily plastic intake (DPI) and annual plastic intake (YPI) calculated for average plastic concentrations found in milk samples and for the 3 types of packaging.

	M_a (mL/day)	average M_c (plastics/mL)	multilayers M_c (plastics/mL)	PET M_c (plastics/mL)	glass M_c (plastics/mL)
	115	0.0068	0.0116	0.0052	0.0024
DPI (plastics/day)		0.78	1.33	0.59	0.28
YPI (plastics/year)		284	486	217	103

M_a = daily amount of consumed milk in Italy; M_c = number of plastic particles found in milk samples; DPI = daily plastic intake; YPI = yearly plastic intake.

from other studies [18,20]. The calculated daily intake corresponds to an annual intake of 284 plastic particles (YPI) (Table 4).

Considering the average European consumption of cow's milk in 2023 (144.7 mL/day; [26]), a DPI of 0.98 particles per day (YPI=357 particles/year) was calculated, which is slightly higher than Italy's.

When comparing these values across the three types of packaging, the highest plastic intake was calculated in milk stored in multilayer packaging (DPI=1.33 plastic particles/day; YPI=486 plastic particles/year), followed by milk stored in PET bottles (DPI=0.59 plastic particles/day; YPI=217 plastic particles/year), and milk in glass bottles, where the lowest intake was recorded (DPI=0.28 plastic particles/day; YPI=103 plastic particles/year) (Table 4).

Recent estimates have suggested that a daily intake of MPs (particles<5 mm) from food ranges from 0.0002 to 1,531,524 MPs/day, with the intake from beverages (including soft drinks, coffee drip bags, milk, infant milk powder, fruit drinks, liquid tea, and beer) ranging from 0.002 to 18,140 MPs/day [14]. Taking the narrower range into account, the average plastic intake from the milk samples in our study was found to be 390 times higher than the lower extreme (0.002 MPs/day), but represented only 0.4 % of the estimated maximum daily intake (18,140 MPs/day) of plastic particles from beverages.

4.3. Risk assessment

The indices used for the risk assessment were calculated using the equations described in paragraph 2.4.2, and valuable insights are provided by them. A purely quantitative measure of the hazard posed by plastic particles in the milk samples is offered by the PCF and PPL indices, while the pR index also considers the toxicity of the individual polymers detected through their risk scores (Rs), allowing for a qualitative assessment as well.

According to the criteria proposed by Ibeto et al. [15], a PPLi of 3.2 was recorded for all our samples, placing them in risk category III (Table 2). This value is slightly higher than the PPLi of 2.8 (category II) which was calculated by Basaran et al. [2] for their samples from Turkey, but much lower than the PPLi of 22.8 (category III), which was derived from global data on milk contamination [20].

In Fig. 7A, the mean PCF index for each milk sample is shown, along

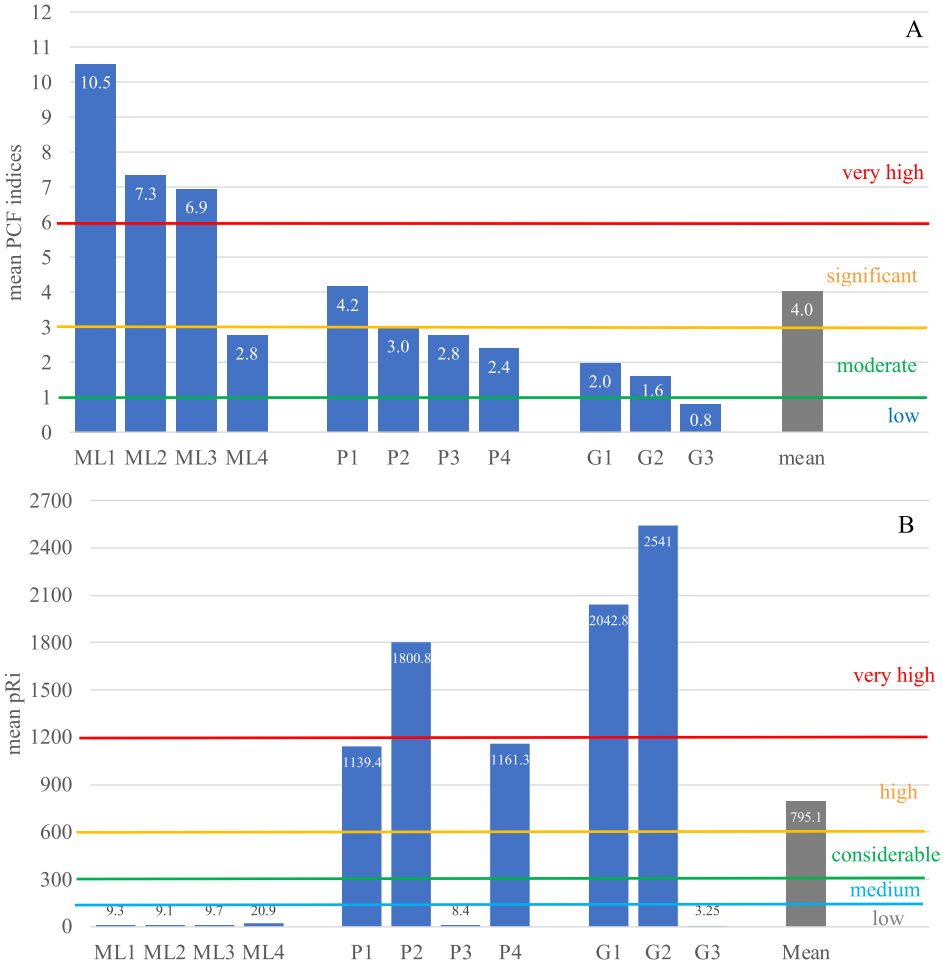

Fig. 7. A) Mean plastic contamination factor indices for the 11 milk samples. B) Mean polymer risk indices for the 11 milk samples. ML=multilayer; P = PET; G=glass.

with the average value for the 11 samples analyzed. Higher PCF values were observed for milk samples stored in multilayer packaging, ranging from 2.8 (ML4) to 10.5 (ML1). This corresponds with the level of plastic contamination, as very high contamination levels were exhibited by three samples, while only ML4 showed moderate contamination. This moderate contamination was observed in only half of the PET samples (P3 and P4), while the other two fell within the category of significant contamination. The three samples stored in glass bottles ranged from low contamination (G3) to moderate contamination (G1 and G2), with the lowest PCF values recorded among all samples tested.

An overall mean PCF of 4.0 was calculated, indicating a significant level of contamination across the entire pool of milk samples. This value is slightly higher than the average PCF of 2.8 (moderate contamination), which was calculated by Basaran et al. [2] for their 14 milk samples. In summary, based on this index, a very high contamination level was exhibited by 27.2 % of our samples, significant contamination was shown by 18.2 %, moderate contamination was classified in 45.5 %, and low contamination was found in only 9.1 %.

However, when not only the quantitative data but also the intrinsic hazard of the various polymers detected in the 33 replicates analyzed is considered, it is found that a low pRi was exhibited by the milk samples stored in multilayer containers, ranging from 9.1 (ML2) to 20.9 (ML4), placing them in the low-risk category (Fig. 7B). In contrast, higher risk categories were mostly assigned to samples stored in PET and glass bottles (Fig. 7B). Specifically, for PET bottles, only sample P3 was ranked in the low-risk category, while P1 and P4 were classified in the high-risk category, and P2 was categorized as very high risk. Although the lowest plastic content among the 11 samples analyzed was exhibited by milk samples in glass bottles, the pRi index reveals that G1 and G2 fall into the very high-risk category, while only G3 is placed in the low-risk category (Fig. 7B).

A mean pRi of 795.1 was calculated for our 11 samples, placing them in the high-risk category, which is higher than the pRi of 255 (medium risk) reported by Basaran et al. [2] and higher than the pRi of 337 (considerable risk) derived from worldwide data by Lin et al. [20].

The discrepancy between PCFi and pRi arises because PE (Rs = 4), PET (Rs = 4), and PP (Rs = 1) were predominantly found in the multilayer container samples, while a greater variety of "unconventional" polymers were contained in the PET and glass samples (Fig. 6), which have a higher Rs (Table S2). Specifically, PU (Rs = 13,844), SBS (Rs = 10,016), and ABS (Rs = 6552) were detected in these samples (Fig. 2; Table S1), significantly increasing the pRi, even though these polymers were present in very small quantities (1–3 particles per sample). This also explains the greater variability in pRi values for PET and glass bottle samples, where a low-risk level was shown by the P3 and G3 samples because these high-risk polymers were not contained in them. Despite the low percentage of these "unconventional" polymers, the risk of ingesting at least one of them from a 115 mL serving of milk appears low, being approximately 4 % for P4 and G1, 7 % for P1 and G2, and 11 % for P2. It is likely that these specific polymers originate from the production chain rather than the container itself, as they are not present in the packaging. However, it is particularly noteworthy that in the 12 milk samples stored in multilayer containers, these high-risk polymers were never detected (Table S1), suggesting that less external contamination occurs in the production of such packaging, or that it is more tightly controlled compared to the production or recycling processes for PET and glass bottles. It should be noted that some limitations exist in the application of pRi, as the hazard grade of polymers proposed by Lithner et al. [21] was determined by multiplying the monomer's weight fraction by its corresponding hazard score. However, a high hazard grade does not necessarily indicate that the polymer itself is hazardous. Instead, this classification suggests that the polymer contains hazardous monomers or additives, which may be released during its production or degradation, posing potential risks to human health. The hazard grade provides only a rough estimation of a polymer's potential risk and does not represent an absolute measure of hazard. Additionally, beyond chemical risks, the abundance of plastic particles in food is a key factor in risk assessment. Therefore, the pRi calculated in this section alone does not fully capture the overall risk of plastic particles in processed foods.

5. Conclusions

Although our data set cannot be considered fully representative of plastic contamination in cow's milk for human consumption, it is evident from our results that the level of plastic particle contamination in this valuable food product is strongly influenced by packaging.

Regarding the estimated plastic intake, the average values we found are comparable to the lowest global data available and contribute negligibly to the estimated total daily intake of plastic particles from food. Regarding possible impacts toward human health, it is important to emphasize that it will be necessary to propose a guideline that can achieve a proper risk assessment for food, as the application of different indices has yielded controversial results, bearing in mind also the intrinsic limitations in their application. Another limitation of this study is that only a small portion of the milk sold in Italy was considered. In the future, it would be interesting for such monitoring to be extended to a larger sample, possibly also considering the different age groups of consumers.

The findings of this study highlight the importance of controlling the milk production chain and carefully selecting the type of packaging for milk storage. Further investigation by milk producers and distributors is required to minimize plastic contamination as much as possible. Additionally, this information can help consumers make more informed choices when purchasing such a fundamental food.

Environmental implication

This study is the first to characterize at the EU level the plastic particles in human cow's milk samples and was the only one, so far, that compared plastics from different types of packaging (multilayer containers, PET and glass bottles) in order to assess particles from the environment, through the production chain, or directly from the packaging itself. From the point of view of the implications for human health, the study was not only limited to a qualitative-quantitative characterization of the plastics detected in milk samples, but a risk assessment was also performed, which showed conflicting data depending on the indices considered. Having also calculated the daily intake of plastics, it was also possible to assess the percentage of uptake in relation to total food ingestion.

CRediT authorship contribution statement

Magni Stefano: Writing – review & editing, Supervision, Conceptualization. **Cremonesi Cristina:** Writing – review & editing, Supervision, Formal analysis. **Tognetto Matteo:** Formal analysis. **Caorsi Giada:** Writing – review & editing, Validation. **Della Torre Camilla:** Writing – review & editing, Validation. **Binelli Andrea:** Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.138052.

Data availability

Data will be made available on request.

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