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First report on the presence of microplastics in bovine and porcine livers from Costa Rica

Primer reporte de la presencia de microplásticos en hígados de bovinos y porcinos de Costa Rica

Primeiro relatório sobre a presença de microplásticos em fígados de bovinos e suínos na Costa Rica

Natalia Soto-Barrientos[1], Karol Ulate-Naranjo[2], Catalina Víquez-Murillo[3], Andrea García-Rojas[2] *

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Abstract

[Objective] Given the ease with which microplastics (MPs) are transported, ecosystems around the world are being polluted with these particles. Reports on the presence of MPs in marine organisms are alarming, but research on the presence of such particles in the terrestrial environment is still very scarce in Costa Rica, and even scarcer for the Central American region. Given this situation, it was considered urgent to carry out a multidisciplinary investigation to obtain information about MP contamination of animal tissues destined for human consumption. **[Methodology]** Samples of 20 livers from bovines and 18 from porcine, were purchased from local markets in 4 of the main meat-producing regions of the country. All samples were chemically digested with potassium hydroxide for the extraction of microplastic particles. **[Results]** Ninety percent of the bovine livers were positive for the presence of MPs, as were 83.3% of the porcine livers. Fibers and films, ranging from 220 µm to 4.5 mm, most of which were blue-green in color, were found. **[Conclusions]** These findings provide information about the types of MPs that were present in the samples analyzed, but they do not aid in determining the exact origin of the particles and how they reached livers of the animals. Nonetheless, it is now clear that these pollutants are reaching human and animal populations, and since adverse effects have already been reported due to the presence of these particles in the human body, these results must be taken into account by healthcare professionals when considering any emerging diseases.

Key words: Bovine; contamination; liver; microplastics; porcine.

* Corresponding author
Natalia Soto-Barrientos, natalia.soto.barrientos@una.ac.cr , Orcid ID: https://orcid.org/0000-0002-1540-0563
Karol Ulate-Naranjo, karol.ulate.naranjo@una.ac.cr , Orcid ID: https://orcid.org/0000-0001-5687-555X
Catalina Víquez-Murillo, catalina.viquez.murillo@una.ac.cr , Orcid ID: https://orcid.org/0000-0002-3957-2992
Andrea García-Rojas, andrea.garcia.rojas@una.ac.cr , Orcid ID: https://orcid.org/0000-0003-3451-7094
1 Clínica de Especies Mayores, Escuela de Medicina Veterinaria. Universidad Nacional, Costa Rica
2 Laboratorio de Estudios Marino Costeros (LEMACO), Escuela de Ciencias Biológicas. Universidad Nacional, Costa Rica
3 Laboratorio de Histología, Escuela de Medicina Veterinaria. Universidad Nacional, Costa Rica.

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Resumen

[Objetivo] Dada la facilidad con la que se transportan los microplásticos (MP), los ecosistemas de todo el mundo se están contaminando con estas partículas. Los informes sobre la presencia de MP en organismos marinos son alarmantes, pero la investigación sobre su presencia en el medio terrestre es aún muy escasa en Costa Rica, y aún más insuficiente en la región centroamericana. Ante esta situación, se consideró urgente realizar una investigación multidisciplinaria para obtener información sobre la contaminación por MP en tejidos animales destinados al consumo humano. **[Metodología]** Se adquirieron hígados de 20 de bovinos y 18 de cerdos, en mercados locales de 4 de las principales regiones productorasde carne del país. Todas las muestras fueron digeridas químicamente con hidróxido de potasio para la extracción de partículas de microplásticos. **[Resultados]** El 90 % de los hígados de bovinos, al igual que el 83,3 % de los hígados de cerdos, dieron positivo a la presencia de MP. Se encontraron fibras y películas de entre 220 μm y 4,5 mm, la mayoría de color azul verdoso. **[Conclusiones]** Estos hallazgos permiten conocer los tipos de micropartículas presentes en las muestras analizadas, pero no nos ayudan a determinar el origen exacto de ellas, ni cómo llegaron al hígado de los animales. No obstante, ahora es evidente que esos contaminantes están afectando a la población humana y animal, y dado que ya se han reportado efectos adversos, debido a la presencia de estas partículas en el cuerpo humano, los profesionales sanitarios deben tener en cuenta estos resultados al considerar cualquier enfermedad emergente.

Palabras clave: bovino; contaminación; hígado; microplásticos; porcino.

Resumo

[Objetivo] Devido à facilidade com que os microplásticos (MPs) são transportados, os ecossistemas de todo o mundo estão sendo contaminados por essas partículas. Os relatórios sobre a presença de MP em organismos marinhos são alarmantes, mas as pesquisas sobre a presença dessas partículas no ambiente terrestre ainda são muito escassas na Costa Rica e ainda mais escassas na região da América Central. Em vista dessa situação, foi considerada urgente a realização de uma pesquisa multidisciplinar para obter informações sobre a contaminação por MP em tecidos animais destinados ao consumo humano. **[Metodologia]** Fígados, 20 de bovinos e 18 de suínos, foram adquiridos em mercados locais de quatro das principais regiões produtoras de carne do país. Todas as amostras foram digeridas quimicamente com hidróxido de potássio para a extração de partículas de microplástico. **[Resultados]** 90% dos fígados bovinos, bem como 83,3% dos fígados suínos, apresentaram resultados positivos para a presença de MP. Foram encontrados fibras e filmes entre 220 μm e 4,5 mm, a maioria de cor azul-esverdeada. **[Conclusões]** Essas descobertas fornecem informações sobre os tipos de micropartículas presentes nas amostras analisadas, mas não nos ajudam a determinar a origem exata das partículas ou como elas chegaram ao fígado dos animais. No entanto, agora está claro que esses poluentes estão afetando a população humana e animal e, como já foram relatados efeitos adversos devido à presença dessas partículas no corpo humano, os profissionais de saúde devem levar em conta essas descobertas ao considerar quaisquer doenças emergentes.

Palavras-chave: microplásticos; bovinos; suínos; fígado; contaminação.

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Introduction

It is well established that microplastic (MP) contamination is affecting ecosystems at a global level (Cox *et al.*, 2019). MPs have been detected in diverse environments, from the deep sea and polar regions to coastal areas across all continents (Blumenröder *et al.*, 2017), as well as in freshwater and terrestrial systems (Horton *et al.*, 2017; Blettler *et al.*, 2018; Dris *et al.*, 2018; Ramachandraiah *et al.*, 2022). The United States Environmental Protection Agency (U.S. Environmental Protection Agency, 2024) defines MPs as plastic particles ranging in size from 5 millimeters (mm) to 1 nanometer (nm). Their widespread distribution is further documented by findings from 2019, which revealed that MPs travel through atmospheric water condensation and are deposited via precipitation, even in remote and uninhabited regions (Allen *et al.*, 2019).

The growing number of publications documenting the presence of MPs in marine organisms is alarming, but similar research on the presence in terrestrial environments is still scarce, although Horton *et al.*, 2017 showed that the amount of plastics released into the terrestrial environment is 4 to 23 times greater than that released into the marine environment. In addition, despite the abundance of terrestrial MPs and their potential to cause deleterious biological effects, there are few studies related to the impact of MPs in terrestrial organisms, although there are already reports of MPs found in milk, meat, feed, blood (Van der Veen *et al.*, 2022), earthworms and chickens for human consumption (Huerta-Lwanga, *et al.*, 2017).

The Central American region is far behind in this regard. Only a few reports on MPs in marine environments have been published (Davidson, 2012; Delvalle de Borrero *et al.*, 2020; Kutralam-Muniasamy *et al.*, 2020; Mazariegos-Ortíz *et al.*, 2020; Fallon & Freeman, 2021; Oldenburg *et al.*, 2021; Aldana Aranda *et al.*, 2022; Orona-Návar *et al.*, 2022), and the lack of relevant literature in the region has been mentioned by authors including Ivar Do Sul & Costa, 2014. The first report on MPs in Costa Rica appeared in 2018, and since then five other reports have been published (Johnson *et al.*, 2018; Bermúdez-Guzmán *et al.*, 2022; Astorga *et al.*, 2022a; Astorga *et al.*, 2022b, Rojas-Jimenez *et al.*, 2022). The information generated so far has primarily focused on marine organisms, highlighting a significant gap in our understanding of Costa Rican terrestrial ecosystems, making it a critical area of study. Studies of MPs in terrestrial animals, especially those consumed by humans, are essential to broaden our knowledge and address this public health concern in the country.

The liver, through which blood from the stomach and intestine passes, has already been determined to be one of the routes through which microplastics are spread throughout the human body (Osman *et al.*, 2023; Kalra *et al.*, 2024). In Costa Rica, bovine and pork liver is widely consumed, with bovine liver typically prepared as a steak and pork liver often made into pâté (Chacón Villalobos, 2021; Madrigal-Meneses & Caravaca-Rodríguez, 2020). This underscores the importance of understanding the potential health implications of contaminants in this widely consumed organ. The objective of the study was therefore to determine the presence of microplastic particles in meat products from Costa Rica that form part of the human diet.

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Materials and methods

- *Samples*

For this study, bovine and porcine livers were obtained from butcher shops located in the centers of cities. This implies that they were healthy organs that had successfully passed veterinary inspections at the slaughterhouse. The livers were randomly collected, bought as a whole and not sliced, just as they are customarily sold to the public, and were transported in coolers. A total of 20 bovine livers and 18 porcine livers were processed, obtained from markets in the regions of greatest meat production, to provide a representative sample of both types of liver (Figure 1).

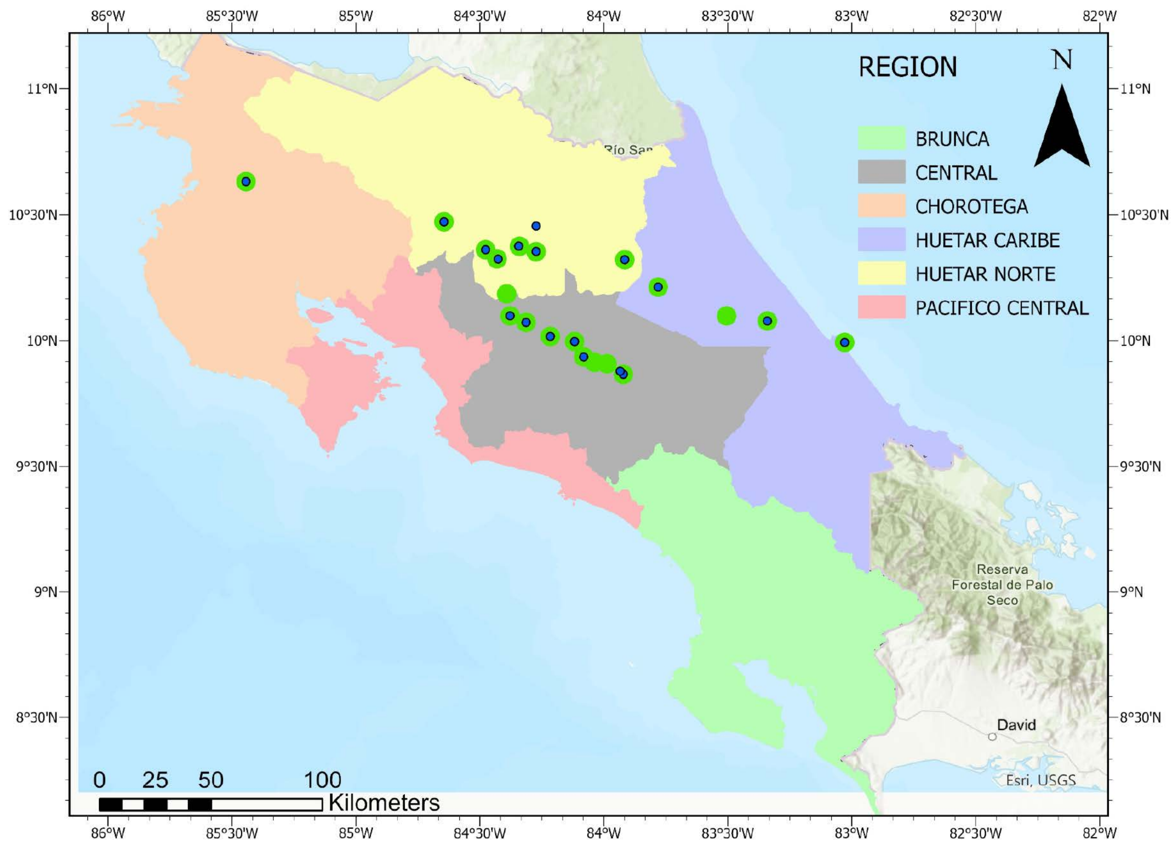

Figure 1. *Distribution of sample points in the regions of greatest meat production in Costa Rica. Green points are bovine samples, and blue points are porcine samples. Source: data obtained during this investigation.*

- *Tissue processing*

The first step in preparing the sample analysis was a thorough cleaning of the working areas of the laboratory. To prevent contamination by microplastic particles in the air, work was carried out in a laminar flow chamber, mainly for the preparation of solutions, filtration and sieving. In addition, all glassware used in the process was washed three times, then dried in a laboratory oven and covered

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with aluminum foil to avoid cross-contamination, following the methodology applied by Astorga *et al*., 2022b.

For chemical digestion, a sample of 100 grams was taken from the inside of each liver, taking into consideration the central axis of the organ to ensure that the sample allowed part of the hepatic veins and arteries to be seen. To extract the MPs from liver samples, the tissue was chemically digested following the method described by Cole *et al* (2014), Kühn *et al*. (2017), and Bessa *et al*., (2019). A 10% KOH solution was added to the liver tissue sample, ensuring that the liquid covered the sample and did not exceed 50% of the total volume of the Erlenmeyer flask (250 or 500 ml). Next, all Erlenmeyer flasks were covered with aluminum foil and placed in an incubator with orbital shaking at 60 °C and 300 rpm for 24 hours. Once the chemical process was completed, the digested contents were sieved through a 63 µm stainless steel sieve and transferred to a clean glass Petri dish. To remove excess water, the glass Petri dishes were covered with aluminum foil with small holes to allow water evaporation and prevent possible contamination by airborne plastic and placed in an oven at 45 °C for 30 hours (Enders *et al*., 2020).

As a quality control measure for the experiment, controls were performed with distilled water and KOH without any sample during chemical treatment of the livers, as well as during observation, identification and validation of microplastics (Jabeen *et al* 2017). Fibers were the most abundant microplastic particles identified in the control samples, and all these plastic microparticles in these samples were excluded from the data analysis.

- *Identification and validation of microplastic particles*

An OPTIKA SZ-ST2 stereoscope with camera (AMSCOPE MU1000) was used for the identification, measurement and photographing of each microplastic particle observed in the processed samples. For data analysis, only plastic particles smaller than 5000 µm were considered (Andrady, 2011), which were classified by type as fibers, fragments, pellets or films, and categorized by color (Hidalgo-Ruz *et al*., 2012; Qiu *et al*., 2016; Martin *et al*., 2017; Enders *et al*., 2020).

Results

Out of the 20 bovine livers processed, 90% (n= 18) were positive for the presence of MPs. All the particles found were classified as fibers with an average size of 1530 µm (range 230 µm to 3680 µm) and were most frequently (38.10%) blue-green in color. Other colors of fibers detected were black (21.43%), blue (16.67%), purple (7.14%), red (7.14%), white translucent (4.76%), brown (2.38%) and sky blue (2.38%) (Figures 2 and 3). The amount of MPs per gram of liver tissue processed varied from 0.009 to 0.074 with an average of 0.0203 MPs g^{-1} (Figure 4).

On the other hand, of the 18 processed porcine livers, 83.3% (n = 15) were positive for the presence of MPs. Ninety-five (98.96%) of the particles found were classified as fibers with an average size of 1240 µm (range 220 µm - 4510 µm) and most of them (32.29%) blue-green in color. Other colors of the fibers detected were red (22.92%), blue (15.63%), translucent white (11.46%), black (9.38%), green (5.21%), and purple (3.13%) (Figure 2, 3). The other particle found was classified as a film, red in

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color, with a size of 2150 µm. The amount of MPs per gram of liver tissue processed varied from 0.013 to 0.2948 with an average of 0.063 MP g^{-1} (Figure 4).

A summary of these findings, including the percentage of positive samples and MP size ranges for both species, is presented in **Table 1**.

Table 1. *Comparison of microplastic (MP) presence in bovine and porcine livers from Costa Rica.*

Parameter	Bovine Livers	Porcine Livers
Total samples processed	20	18
Positive samples (%)	90% (n=18)	83.3% (n=15)
MP classification	100% fibers	98.96% fibers, 1.04% film
Average MP size (µm)	1530 µm	1240 µm
MP size range (µm)	230 – 3680 µm	220 – 4510 µm
Most common MP color (%)	Blue-green (38.10%)	Blue-green (32.29%)
Other MP colors (%)	Black (21.43%), blue (16.67%), purple (7.14%), red (7.14%), translucent white (4.76%), brown (2.38%), sky blue (2.38%)	Red (22.92%), blue (15.63%), translucent white (11.46%), black (9.38%), green (5.21%), purple (3.13%)
MP per gram of liver (range)	0.009 – 0.074 MP g^{-1}	0.013 – 0.2948 MP g^{-1}
MP per gram of liver (average)	0.0203 MP g^{-1}	0.063 MP g^{-1}

Source: data obtained during this investigation.

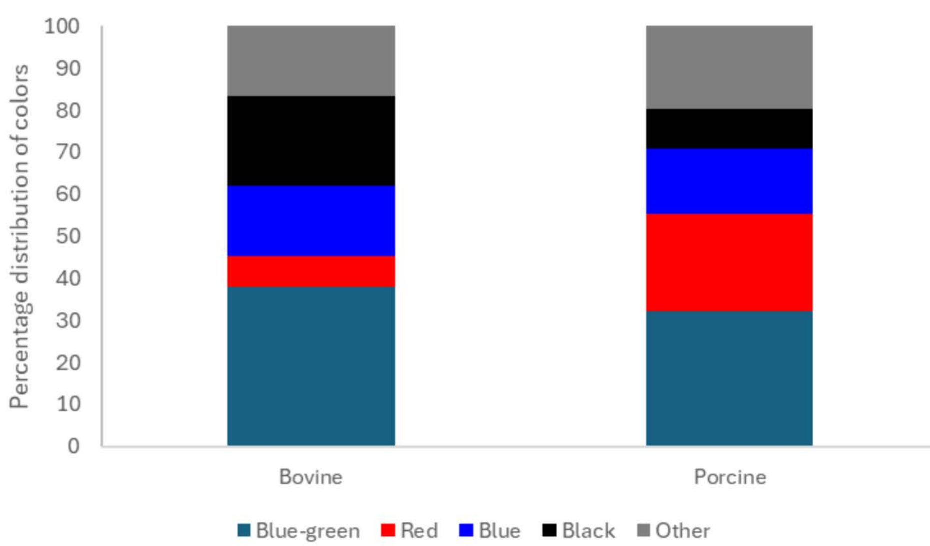

Figure 2. *Percentage distribution of colors found in bovine and porcine livers. Source: data obtained during this investigation.*

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Figure 3. *Microplastic particles found in bovine (A) and porcine (B) livers from the regions of greatest meat production of Costa Rica. Source: data obtained during this investigation.*

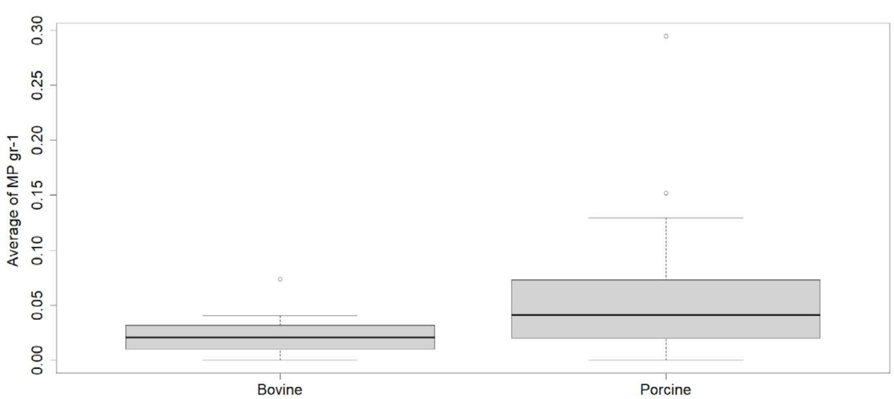

Figure 4. *Average of microplastics (MPs per gram of tissue) found in bovine and porcine livers. Source: data obtained during this investigation.*

Discussion

Due to the ubiquity of MPs, living organisms are inevitably taking them into their bodies through ingestion or inhalation (Galloway et al., 2017). Since the objective of the study was to investigate if the Costa Rican human population was consuming MPs in products of animal origin, without attempting to compare the quantity of microplastics between regions or trace the origin of the microplastics, there is no information on the specific food supplied to each of the animals whose livers were sampled. However, in Costa Rica, both dairy and beef cattle are generally fed similar feed ingredients—primarily forage, supplemented with corn, soybean meal, and sugarcane molasses in varying proportions. In specialized dairy systems, the proportion of concentrate in the diet is typically higher compared to beef cattle, whose feed is predominantly pasture-based with minimal supplementation. It is also important to note that feedlot systems, such as those commonly used in the United States for beef cattle fattening, are not used in the country. On the other hand, most pigs are kept housed and have a diet of fattening concentrated feed based mostly on corn and soybean meal (Instituto Nacional de Estadística y Censos, 2022).

The presence of MPs has been documented in salt (Yang *et al*., 2015; Kim *et al*., 2018), soybean meal (Walkinshaw *et al*., 2022), sugarcane (Liebezeit & Liebezeit, 2013) and corn (Haluska, 2020; Garrido

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& Costanzo, 2022; Shi *et al*., 2023), all of which are ingredients often used in the animal feed industry. Also, MPs of marine origin could be incorporated into foods of terrestrial animals due to the use of derivatives of fish in the manufacture of feed concentrates (Bouwmeester *et al*., 2015; Walkinshaw *et al*., 2022). Another possible route of contamination could be drinking water; in a systematic study carried out by Danopoulus *et al* (2020), it was found that microplastics were nearly ubiquitous and were present in drinking water.

The results of our study show the presence of microplastics (MPs) in liver samples intended for human consumption. Although cross-contamination during processing, from the slaughter of the animals to their subsequent market commercialization in the country, cannot be completely ruled out (Garrido & Costanzo, 2022), this is considered unlikely. Extensive precautions were taken to process the samples carefully, including taking digestion samples from inside the organ and implementing all possible measures to avoid contamination in the laboratory. Regardless of the contamination source, our study sought to determine the presence of microplastics in the product that ultimately reaches the consumer.

In this study, the amount of MPs per gram found in animal livers (bovine: average 0.024 MPs g^{-1}; porcine average 0.074 MPs g^{-1}) is lower than that found in human livers, which in healthy humans were found to have a number of microplastic particles ranging from 0 to 2.2 per gram of tissue (with an average of 1 MP g^{-1}), while in patients with liver disease (cirrhosis) this number increased by more than 8 times (an average of 8.3 MP g^{-1}) (Hovartis *et al*., 2022).

In our studies, the size of the MPs found was bigger in both bovine (average 1530 µm) and porcine (average 1240 µm) livers than the size of the particles found in cirrhotic human liver tissue, which ranged from 3.0 to 29.5 µm (average 9.8 µm); however, it has not been possible to determine whether the accumulation of MPs is the cause or the consequence of liver disease in human subjects (Hovartis *et al*., 2022), although they have been reported to damage liver cells, and to alter fat metabolism and enzyme activities in crabs, fish and mice (Lu *et al*., 2016; Deng *et al*., 2017; Lu *et al*., 2018; Chae *et al*., 2018; Yu *et al*., 2018).

While the scientific literature has reported translocation of MPs ranging from 1 to 500 µm in size into different tissues (liver, cell membranes, the blood-brain barrier and even the placenta) of several species (Hussain *et al*., 2001; Vethaak & Leslie, 2016; Triebskorn *et al*., 2019; Elizalde-Velazquez *et al*., 2020; Yee, *et al*., 2021) causing oxidative stress, cell damage and inflammation, some authors have recently challenged whether microplastics are capable of being translocated (Schür *et al*., 2019). Currently, the disagreement is related to the size of particles that can be taken up by cells and translocated among tissues (Triebskorn *et al*., 2019).

Haave *et al*., (2021) carried out a study between 2017 and 2019 on wild animals (mammals, birds and fish), and found MPs in the liver of anchovies. They mentioned that there was a translocation of MPs in the tissues of the animals studied, and that MPs that cross the epithelial tissue of the digestive tract could then be transported through the portal system to the liver (Garrido & Costanzo, 2022).

Based on the criteria for visual identification of microplastics described by Zhang *et al*., 2020, information regarding the shape, size, and color of the MPs that were encountered in this study provides important information about the types of plastic found,

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but not about their origin and how the animals may be being contaminated. In the case of shapes, fibers in urban areas have been linked to industrial activities, while flakes are attributed to mechanical and chemical degradation. Colors are due to pigments that are used in the fabrication of plastics – for instance, black plastics have been shown to contain PU, transparent plastics indicate polypropylene, opaque plastics point to LDP, and white plastics indicate polyethylene (Ramachandraiah *et al*., 2022).

Plastics are generally considered to be inert materials. However, the following have been proposed as possible damages that MPs might cause in the body: vascular embolization, inflammatory responses (associated with cell phagocytosis of particles and increased autoimmune response) (Wright & Kelly, 2017; Yee *et al*., 2021; Hu *et al*., 2023), gastrointestinal alterations such as dysbiosis and altered mucus secretion (Garrido & Costanzo, 2022), cytotoxicity due to oxidative stress mechanisms (Schirinzi *et al*., 2017; Solomando *et al*., 2020; Urli *et al*., 2023), liver damage (Garrido & Costanzo, 2022), alterations in energy and lipid metabolism (Deng *et al*., 2017), and neurotoxicity (Garrido & Costanzo, 2022).

In some plastics, additives (such as bromates as flame retardants, plasticizers such as phthalates, and heat stabilizing metal compounds) may constitute a major fraction of their composition, and the toxicity of a large part of the additives is well known. Some well-documented examples in humans include reproductive toxicity (e.g., bis(2-ethylhexyl) phthalate [DEHP] and bisphenol A [BPA]), carcinogenicity (vinyl chloride and butadiene), and mutagenicity (benzene and phenol) (Campanale *et al*., 2020). In addition to additives, MPs can release residual monomers and degradation products that have formed on the surface through chemical or photochemical reactions during their stay in the environment (Lucas *et al*., 2008; Ramachandraiah *et al*., 2022). Studies of the chemical composition of plastic in field samples are scarce due to the high cost of the tests, but they are necessary to understand the toxicity of these particles.

Finally, consumption of the species studied by humans could increase their consumption of MPs, as other studies have shown. Yee *et al*., 2021 showed that the human food chain is a major source of MP consumption, based on a review of MP pollution in food and other ingested substances (seafood, sugar, honey, salt, alcohol, bottled water, tap water and air), and we now have evidence of MPs in terrestrial animals which are consumed by humans.

Conclusions

Although the origin of the MPs in the livers studied in this investigation cannot be determined, their presence in these products suggests a high likelihood that the human population consuming them is being exposed to microplastic contamination. This research at the local and regional level has produced several significant findings. Firstly, the identification of microplastics in livers intended for human consumption is crucially important, shedding light on environmental contamination and contributing to public awareness about food safety. These findings do not indicate that consumption of these products should be avoided (this study is not intend to discourage consumption of these products); however, they should be taken into account by healthcare professionals, since the presence of these particles in tissues has been reported to potentially trigger adverse reactions in the digestive and respiratory systems, skin reactions, reproductive issues, and more. Secondly, the findings highlight environmental impacts, emphasizing the interaction between plastic pollution and the food chain, which could encourage initiatives to mitigate plastic contamination and protect local natural resources. Thirdly, the findings may influence policy-making and regulations, leading to stricter measures in waste management and food safety practices, ultimately benefiting public health and the environment at both the local and regional levels. Lastly, this pioneering study opens avenues for further research into microplastic presence and impacts on other organs and food sources, thereby advancing global understanding of this issue.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contribution statement

All the authors declare that the final version of this paper was read and approved. Authors and CRediT Roles: N. S. B., K. U. N., C. V. M. & A. G. R.: Conceptualization, Methodology & Supervision; K. U. N. & A. G. R.: Data Curation, Formal Analysis; N. S. B., K. U. N., C. V. M. & A. G. R.: Validation, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization.

The total contribution percentage for this paper was as follows: N.S.B. 25%, K.U.N. 25%, C.V.M. 25% & A.G.R. 25%.

Data availability statement

The data supporting the results of this study will be made available by the corresponding author, [A.G.R.], upon reasonable request.

Preprint

A preprint version of this document has been deposited at: Zenodo.org https://zenodo.org/records/13380154

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