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Research Article

Bioaccumulation of Microplastics in Thrushes: Analysis for Monitoring Environmental Quality by Comparing Different and Innovative Extraction Techniques

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Abstract

Over recent decades, the exponential increase in plastic use has led to significant environmental and biodiversity damage when improperly disposed of. Natural factors such as solar UV radiation, wind, and currents break down plastic into micro plastics (MPs) and Nano-plastics (NPs), which have become major environmental pollutants. Smaller plastic fragments are more likely to be ingested by wild animals. Birds, crucial in the global trophic network and indicators of biodiversity, pollution, and environmental change, are the focus of this study. This research aims to develop two innovative, cost-effective methods for detecting micro plastics in wild birds' stomachs while maintaining the micro plastics' integrity and avoiding contamination. The study investigates the bioaccumulation of MPs in Turdus philomelos, the migratory wintering thrush in Italy, using samples from 100 specimens hunted in the Bari countryside and donated by the "Arci caccia" association. The environmental quality of their feeding areas is assessed by analysing MPs in the stomachs and the birds' trophic regimes. The research seeks an alternative extraction method that avoids using chemical solvents such as potassium hydroxide (KOH), which can alter MP morphology, complicating physical characterisation. The results confirmed the presence of MPs, including filaments, fragments, and films of various colours, in all 100 thrush samples. These findings demonstrate that the two new flotation-based methods are effective tools for monitoring MP bioaccumulation and assessing environmental quality, given their simplicity and rapid analysis capabilities.

Keywords: Micro plastics; Birds; Pollution; Environment; Flotation; Flotac.

Introduction

The plastic industry has grown globally in the last five decades, with almost 370 million tons of plastic produced in 2019, of which 58 million tons were produced in Europe [1]. This increase in plastic consumption worldwide has led to a surge in plastic waste, which poses a growing threat to ecosystems and biodiversity. One particular concern is the breakdown of plastics into tiny particles known as micro plastics (MPs) and Nano-plastics (NPs), which are becoming increasingly recognized as pervasive pollutants. Plastic breaks down into small particles called micro plastics (MPs, <5 mm in diameter) or Nano plastics (NPs, <100 nm in diameter), which gradually accumulate in the environment under the influence of solar UV radiations, wind, currents, and other natural factors [2]. Micro plastics can be divided into two macro-categories: Primary micro plastics, which are fragments of plastics released directly into the environment at this size. The main source of this type of micro plastic is washing synthetic clothes (35% of primary micro plastics), followed by tyre abrasion while driving (28%) and micro plastics intentionally added in cosmetic products

(2%). Secondary micro plastics are fragments of plastics resulting from the gradual breakdown of larger wastes [3]. The term MP describes a heterogeneous mixture of particles that can differ in size (from a few microns to several millimetres), colour, and shape (from very different shapes of fragments to long filaments). It is estimated that about 14 million tons of plastic end up in the seas yearly [4,5]. Plastic debris and waste represent a major concern for marine ecosystems and shorelines in every continent, with a higher concentration in proximity to crowded tourist destinations and high-density populated areas. Once in the environment, MP generates various toxicological and physical effects on wildlife [6]. Recent research has shown that MP can impede animal movement [7]. When ingested, MP may cause damage and obstruction of the stomach, leading to reduced food intake, starvation, and direct mortality. Several chemicals used in the production of plastic materials are known to exert carcinogenic effects and interfere with the body's endocrine system, causing developmental, reproductive, neurological, and immune disorders in both humans and wildlife [8,9]. Plastic polymers are one of the most widely used materials owing to their versatility and durability. They are often released into the natural aquatic and terrestrial environments, thus continuously exposing the inhabitants to their hazardous constituents [10,11]. Thrushes (Turdus philomelos C.L. Brehm, 1831) arrive in Apulia (Southern Italy) in autumn, between the end of October and the beginning of November, where they stay in closed woods and Mediterranean scrubs, and leave again between the end of March and the first fortnight of April. Variations in the period of arrival and departure depend on the onset of the breeding season while the choice of the wintering areas is related to the environmental climatic conditions and the availability of trophic resources [12]. Due to their presence in different environments, these birds play a pivotal role in the global trophic network, serving as valuable bio indicators of environmental health, pollution levels, and broader ecological changes. Therefore, many authors investigated the presence of MP in Trush's stomach to study the plastic pollution condition of the environment [6], employing chemical solvents like potassium hydroxide (KOH) as described by Carlin et al., 2020. Our research aims to develop a new extraction technique to investigate the presence of micro plastics in the stomach of Thrushes, to contribute to a general understanding of environmental impacts on avian species and monitoring the degree of plastic waste pollution of the territories in which they feed more efficiently and effectively. We are proposing innovative extraction methods aimed to mitigate potential alterations in micro plastic morphology induced by such solvents and to provide a more accurate representation of micro plastic presence.

Materials and Methods

Sampling

For the trial, 100 thrushes hunted during the winter 2021-2022 in the provinces of Bari (Apulia region, Italy) were analysed. The birds were donated by members of the 'Arci Caccia' association for research purposes. Each bird was frozen within 12 hours of being caught and stored at -20°C up to the processing.

Sample preparation

After thawing, the carcasses were subjected to a necropsy, to extract the entire stomach. The thrush stomachs were put into a beker with 100 ml of fixative solution (70% ethanol) for five minutes to prevent infection risk; subsequently, the stomachs were chopped.

Extraction of Microplastics

According to the guidelines recommended by Prata et al. (2021), we used only glass and metal materials, including glass centrifuge tubes, and wore cotton lab coats; all equipment, containers and beakers were rinsed three times with filtered distilled water before and after the use and covered with aluminium foil to prevent contamination by airborne microplastics, providing a most quality and control procedures in all our experiments.

All the fluids used during the analysis (saline solution and distilled water) were filtered before the use with a cellulose nitrate filter membrane with a pore size of 1 µm and a diameter of 47 mm (Axiva Sichem Biotech, Delhi, India). Moreover, was prepared a blank extraction sample without tissue was per-formed to determine and correct any procedural contamination. For the detection of microplastics in bird stomachs were used two methodologies.

Simple Flotation (Method 1)

Flotation is a gravimetric separation technique based on the density difference between the object to be identified and the matrix in which it is located. For our trial, we used flotation to identify microplastics in the stomachs of Thrushes. As shown in Table 1, microplastics have a density that ranges from 0.89-1.4 g/cm3 [13]. Therefore, we used a solution with a density heavier than 1.2 g/cm3 to avoid the flotation of the biological matrix together with microplastics. Although NaCl solution (1.2 g/cm3) is commonly used due to its availability, cost-effectiveness, and eco-friendliness, it is limited to polymers of lower density. We opted for a NaCl solution with a higher density of 1.8 g/cm3, which was found to be the most effective way to separate all microplastics from the matrix. To carry out the flotation, we filtered the stomach solution through the Whatman 1 filter paper, which has a particle retention capacity of ~11 µm. Since microplastics have a size of ~100 µm, the solid part was left in the filter. A sample of 3g of solid part was added to 11 ml of NaCl solution in a glass tube and centrifuged it for 3 minutes at 1500 rpm. After centrifugation, we added a small amount of NaCl solution to each tube until a typical meniscus was obtained, which was capped with a coverslip. The coverslip was then observed first with a stereoscope and then with an optical microscope.

Plastic	Poly propylene (PP)	Low-density polyethylene (LDPE)	Polystyrene (PS)	Polyvinyl chloride (PVC)	Poly (adipic acid)/butylene terephthalate (PBAT)	Polybutylene succinate (PBS)	Polylactic acid (PLA)
Density (g/cm^3)	0.89–0.91	0.91–0.93	1.05	1.4	1.18–1.3	1.26	1.25–1.3

Table 1: The density of common plastic polymer types (LI, Chengtao, et al. 2021).

FLOTAC basic techniques (FBT) (Method 2)

The FLOTAC basic techniques (FBT) use a cylindrical device named FLOTAC that has 2 chambers of 5 mL each and a reading disk translation system. The technique consists of the centrifugal flotation of the sample suspension with the consequent upward migration of the parts interested in the analysis (in our case the microplastics) based on the different densities between the components in solution. This method has origins in veterinary parasitology, but then its use was extended to the field of human medicine and in the field of agricultural parasitology. It was decided to use Flotac in the extraction of microplastics as it exploits the flotation principle, as well as many micro plastic extraction techniques already mentioned in the literature [14] as an alternative to the previous method. Figure 2 shows, that after the sample collection, the addition of 70% ethanol, and the homogenisation step, the resulting solution was filtered through a 5mm wire mesh sieve. Next, 11 mL of the filtered solution was poured into a test tube and the sample was centrifuged for 3 minutes at 1500 rpm. After centrifugation, discard the supernatant, leaving only the sediment in the tube. Filled the tube with the 11mL of NaCl solution (1.8g/cm3) flotation solution (FS) everything was homogenized with a pipette. The new solution is then taken and inserted into the chambers of the Flotac device. The Flotac apparatus was then subjected to centrifugation (5 minutes at 1000 rpm) for observation under a stereomicroscope and optical microscope.

Quantification and characterization of MP Flotation

The microscope slides (Method 1) and the Flotac apparatus (Method 2), were observed under a stereomicroscope Leica M165c to analyse the presence of potential plastic particles and images were captured with a digital camera Tucsen GT CAM 5. The MPs were classified as films, fragments, and filaments according to guidelines by Rochman et al (2019) [2]. Colours were divided into black, brown, blue, silver, green, grey, red, white and transparent; the length of the detected particles was determined, and each particle was assigned to one of the four distinct size classes: 1-5 mm, 0.5-1 mm, 0.1–0.5 mm and 0.01-0.1 mm. The Capture 2.4 software for the imaging analysis was applied to the litter dimensional measurements. Each MP, after the description, was subjected to a hot needle test described by many authors [15-17], which was previously shown to be reliable for detecting MP larger than 50 µm.

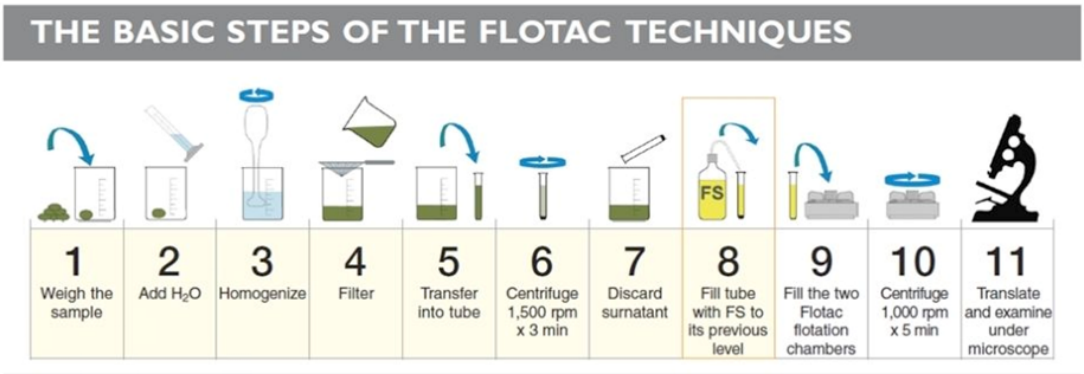

Figure 1: The Basic Steps of the Flotac Techniques (FLOTAC® Manual).

Figure 2: Steps Flotac Basic Techniques (FBT) for MP.

Results

At the end of the trial, only 17 thrushes did not test positive for the ingestion of MPs, which means microplastics were identified in 83% of samples. Within each positive sample, an average of 4.54 ± 2.88 particles were found per individual (Figure 3), therefore, on the total of 100 samples analysed, we detected 454 micro plastic particles (MP). With the use of the Simple flotation method (method 1) we detected 222 MP particles, while with the FLOTAC method (method 2) we identified 232 MP (Table 2). An analysis and subdivision of the samples according to MP type, colour, and size was carried out by the two methods used (Figures 4,5). Blue-coloured MP particles were the most abundant of the micro plastic particles identified, representing 50% of the results of the Simple flotation method (method 1) and 51.72% of the results of the FLOTAC method (method 2), for a total of 232 particles extracted through both methods. The brown-coloured extracted plastics presented a percentage of 28.13% in method 1 and a percentage of 24.57% in method 2, for a total of 120 MP particles. Similar percentages were found for silver-coloured (8.48% M1 - 9.91% M2), transparent (5.80% M1 - 5.17% M2), and white (7.59% M1 - 8.62% M2) particles (Table 2). The extracted particles were then categorized according to shape types (fragments, films, pellets, lines, and textile MFs) and size classes (5-1 mm; 1-0.5 mm; 0.5-0.1 mm; 0.1-0.01 mm). The criteria used for identifying shapes were those suggested by Viršek et al., [18]: fragments are rigid, thick objects with sharp edges and an irregular shape; films also have an irregular outline, but they are thin and flexible, and usually transparent; lines, also called filaments, may be long or short, of different thicknesses but with a regular diameter along the entire length of the particle and the clean, unframed ends [19-24]. Regarding the types of microplastics, the particles with the highest frequency percentage of 49.95% were filaments, followed by films (29.52%) and fragments (20.53%) (Table 3). No other types of MPs were found. Most of the MP particles detected in the samples were between 1-5 mm in size (41.32%), followed by particles in the range of 0.1 - 0.5 mm (25.62%), 19.79% between 0.5 - 1 mm, and finally, 7.27% between 0.01 - 0.1 mm (Table 4).

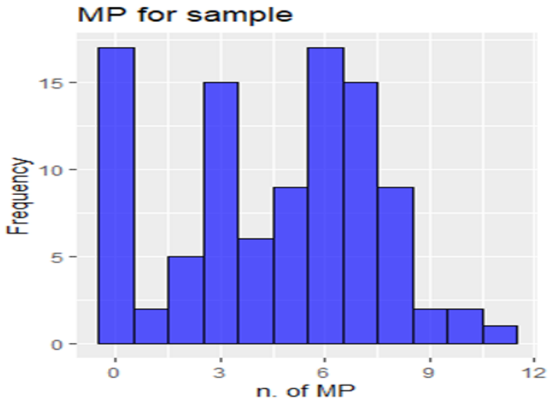

Figure 3: Frequency of MPs extracted per sample.

Colours MP	Simple Flotation Method (M1)		FLOTAC (FBT) for MP (M2)		M1 + M2	
	Total number	Frequency (%)	Total number	Frequency (%)	Total number	Frequency (%)
Blue	112	50%	120	51.72%	232	45.56%
Brown	63	28.13%	57	24.57%	120	23.53%
Silver:	19	8.48%	23	9.91%	42	8.24%
Transparent:	13	5.80%	12	5.17%	25	4.90%
White:	17	7.59%	20	8.62%	37	7.25%
Total Numbers of MP	222		232		454	

Table 2: Data analysis on the presence of MP in the samples divided by the colour of MP.

Sample	Film M1	Film M2	Fragment M1	Fragment M2	Filaments M1	Filaments M2	Total MP of 100 sample
Total Numbers for Type and Method	71	63	42	51	109	118	454
Total Numbers for Type	134		93		227		
Frequency (%)	29.52%		20.53%		49.95%		83%

Table 3: Data analysis on the presence of MP in the samples divided by typology of MP and extraction method M1 (Simple flotation method for microplastics) - M2 (FLOTAC basic techniques - method for microplastics).

Size of MPs	1 - 5 mm	0.5 -1 mm	0.1 -0.5 mm	0.01 -0.1 mm
N. of MPs	198	95	123	38
	41.32%	19.79%	25.62%	7.27%

Table 4: Data analysis on the presence of MP in the samples divided by typology of MP and extraction method M1 (Simple flotation method for microplastics) - M2 (FLOTAC basic techniques - method for microplastics).

Figure 4: Graphical representation of results by type (A), colour (B), and size (C).

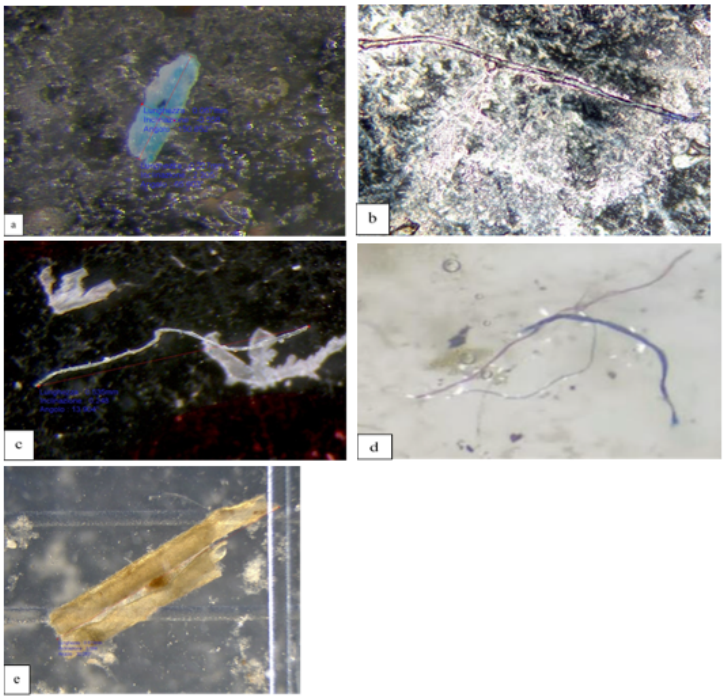

Figure 5: a MP Fragment – (Method 1); b MP Filaments - (Method 1); c MP Filaments (Method 1); d MP Filaments - (Method 2); e MP Film - (Method 2).

Discussion and Conclusions

This study aims to investigate the distribution of microplastics (MPs) in thrush species within the Apulia region, where limited information currently exists. The study found significant amounts of microplastics in the stomachs of migratory Thrushes, using two different analytical methods based on flotation. This research is innovative because it introduces two efficient methods for detecting microplastics in the stomachs of wild birds, which aim to reduce economic and environmental costs, maintain micro plastic integrity, and prevent contamination. The study also prioritized green chemistry by eliminating hazardous steps for human health and the environment. The motivation for finding alternative extraction methods was to prevent the potential alteration of micro plastic morphology caused by chemical solvents like potassium hydroxide (KOH), which is commonly used in existing techniques. The study highlights the innovative application of Flotac instrumentation for MP analysis. The research assessed the recently developed FLOTAC method, originally designed for parasitological studies, adapting it to detect the bioaccumulation of microplastics in Thrushes. The study introduced modifications to the procedure and aimed to compare it with widely used techniques for micro plastic analysis. The Flotac technique was successful in detecting the presence of microplastics for the first time. The study introduces two novel extraction methodologies that eliminate the need for chemical solvents, prioritizing simplicity, speed, and cost-effectiveness. The findings reveal a high rate of bioaccumulation in Thrushes. From the analysis of 100 samples, it emerged that 83% of the individuals had ingested microplastics, with an average of 4.54 ± 2.88 particles per individual. These results align with previous studies on micro plastic bioaccumulation in Thrushes, suggesting that the thrushes frequently come into contact with the microplastics present in the environment in which they live. This study also provides a detailed analysis of the particles found, revealing that blue particles were the most abundant, followed by brown particles. The distribution of silver, transparent, and white particles was relatively similar between the two methods. The classification of particles based on shape highlighted that filaments were the most frequent type, followed by films and fragments. Many particles fell within the range of 1 to 5 mm, with a significant presence also in the 0.1-0.5 mm category. The predominant presence of filaments could indicate a specific source of contamination in their diet or surrounding habitat.These results can contribute to the development of management and mitigation strategies to reduce micro plastic pollution, thereby protecting wildlife health and the ecosystem. The detection of microplastics in migratory thrushes raises important questions regarding environmental impacts and potential threats to wildlife. Further research could explore specific sources of contamination and assess the long-term impacts of the presence of microplastics in migratory Thrushes.

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