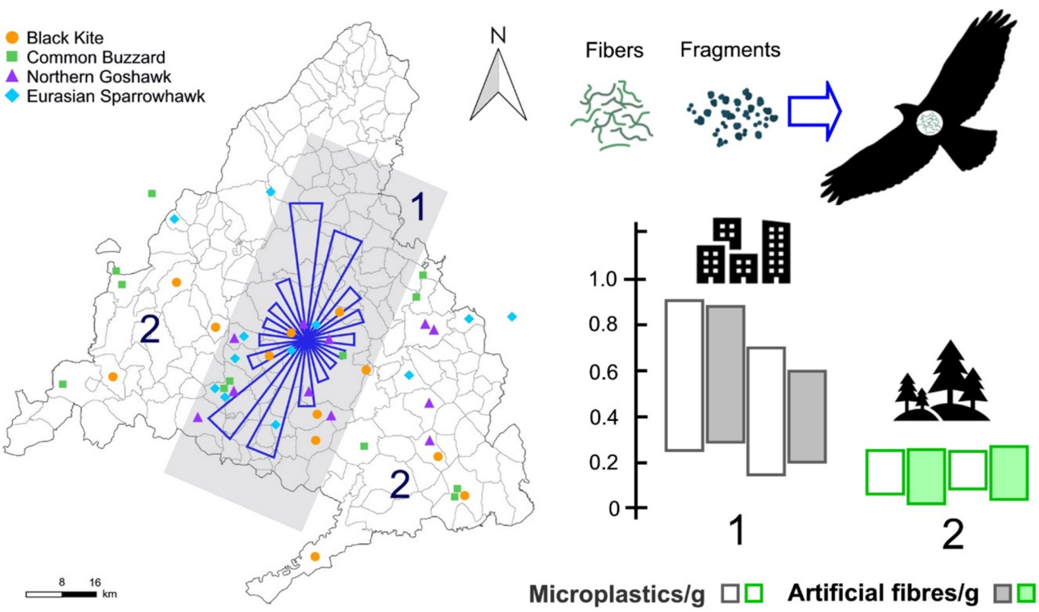
Accumulation of microplastics in predatory birds near a densely populated urban area

This manuscript version is made available in fulfillment of publisher's policy. Please, cite as follows:

Chloe Wayman, Miguel González-Pleiter, Francisca Fernández-Piñas, Elisa L. Sorribes, Rocío Fernández-Valeriano, Irene López-Márquez, Fernando González-González, Roberto Rosal. Accumulation of microplastics in predatory birds near a densely populated urban area. Science of The Total Environment 917, 170604, 2024.

https://doi.org/10.1016/j.scitotenv.2024.170604



Accumulation of microplastics in predatory birds near a densely populated urban area

Chloe Wayman[1], Miguel González-Pleiter[2], Francisca Fernández-Piñas[2,3], Elisa L. Sorribes[4], Rocío Fernández-Valeriano[4], Irene López-Márquez[4], Fernando González-González[4,5], Roberto Rosal[1,*]

[1]Department of Chemical Engineering, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain
[2]Department of Biology, Faculty of Science, Universidad Autónoma de Madrid, E-28049, Madrid, Spain
[3]Centro de Investigación en Biodiversidad y Cambio Global, Universidad Autónoma de Madrid. Darwin 2, 28049 Madrid, Spain
[4]Wildlife Hospital, Group of Rehabilitation of the Autochthonous Fauna and their Habitat (GREFA), Majadahonda, 28220 Madrid, Spain
[5]Departmental Section of Pharmacology and Toxicology, Faculty of Veterinary Science, Universidad Complutense de Madrid, 28020 Madrid, Spain

Abstract

The pollution due to plastic and other anthropogenic particles has steadily increased over the last few decades, presenting a significant threat to the environment and organisms, including avian species. This research aimed to investigate the occurrence of anthropogenic pollutants in the digestive and respiratory systems of four birds of prey: Common Buzzard (*Buteo buteo*), Black Kite (*Milvus migrans*), Eurasian Sparrowhawk (*Accipiter nisus*), and Northern Goshawk (*Accipiter gentilis*). The results revealed widespread contamination in all species with microplastics (MPs) and cellulosic anthropogenic fibers (AFs), with an average of 7.9 MPs and 9.2 AFs per specimen. Every digestive system contained at least one MP, while 65 % of specimens exhibited MPs in their respiratory systems. This is the work reporting a high incidence of MPs in the respiratory system of birds, clearly indicating inhalation as a pathway for exposure to plastic pollution. The content of MPs and AFs varied significantly when comparing specimens collected from central Madrid with those recovered from other parts of the region, including rural environments, suburban areas, or less populated cities. This result aligns with the assumption that anthropogenic particles disperse from urban centers to surrounding areas. Additionally, the dominant particle shape consisted of small-sized fibers (> 98 %), primarily composed of polyester, polyethylene, acrylic materials, and cellulose fibers exhibiting indicators of industrial treatment. These findings emphasize the necessity for further research on the impact of plastic and other anthropogenic material contamination in avian species, calling for effective strategies to mitigate plastic pollution.

1. Introduction

The exponential growth of plastics, which exceeded 400 million metric tons marketed in 2022, can be attributed to its cost-effective production and the versatile properties of plastic-based materials (PlasticsEurope, 2023). Simultaneously the global generation of plastic waste has emerged as a significant threat, as many nations are inadequately equipped to manage the escalating volume of debris (Borrelle et al., 2020; Jambeck et al., 2015). Geyer et al. estimated that out of the approximately 7,800 million metric tons of plastics produced since 1950, 60 % have ended up in landfills or become mismanaged debris (Geyer et al., 2017). Urban areas, with higher population density, produce more plastic waste, as evidenced by city residents generating twice as much waste as their rural counterparts, driven by the extensive use of packaged products and increased food waste (Hoornweg et al., 2013).

Improperly managed plastic litter has the potential to be transported by surface runoff, rivers, and wind, dispersing across all natural environments, even in remote areas (Emmerik and Schwarz, 2020; González-Pleiter et al., 2020; Woodall et al., 2014). However, the highest concentration is found in urban areas due to the proximity to human activities (Österlund et al., 2023). If the largest dimension of plastic particles is below 5 mm and greater than 1 µm, they are known as microplastics (MPs); classified as primary if manufactured in that size range or secondary, when they originate from the breakdown of larger materials (Wayman and Niemann, 2021). This breakdown occurs in the environment through processes such as photooxidation, mechanical forces, hydrolysis, and biodegradation (Gewert et al., 2015; Oberbeckmann and Labrenz, 2020).

The presence of MPs has been reported in different bird species (Kühn and van Franeker, 2020; Navarro et al., 2023). Sherlock et al. showed the presence of fiber MPs in the gastrointestinal tract of Tachicyneta

* Corresponding author: roberto.rosal@uah.es
Available online: February 2, 2024

bicolor, an insectivore nesting downstream from a wastewater treatment plant (Sherlock et al., 2022). Hoang and Mitten conducted a study on the abundance of MPs in the digestive systems of migratory birds. Their findings revealed limited differences in average concentrations at various sites, suggesting that the primary route of plastic exposure was likely through diet (Hoang and Mitten, 2022). Despite the crucial role of birds in ecosystems, terrestrial birds have received less attention compared to seabirds. Available studies indicate the widespread presence of plastic and other anthropogenic litter in terrestrial species. In particular, a study on Common Blackbirds (*Turdus merula*) and Song Thrushes (*Turdus philomelos*), two widely distributed terrestrial species, found MPs (> 100 µm) in the gastrointestinal tracts of all analyzed individuals. Most of the MPs were fibers, although no spectroscopic characterization was performed (Deoniziak et al., 2022). Another study in China found anthropogenic litter, including natural fibers representing 37.4 % of total litter items, in the gastrointestinal tracts of terrestrial birds (Zhao et al., 2016).

Predatory birds, occupying the top of food webs, play a crucial role in ecosystem dynamics, trophic interactions, and habitat regulation. Carlin et al. investigated plastic pollution in the gastrointestinal tracts of raptors in Florida, finding MPs in all individuals with an average of 11.9 ± 2.8, fibers per bird (Carlin et al., 2020). Winkler et al. examined MP presence in regurgitated pellets of the common kingfisher (*Alcedo atthis*), revealing MPs from three different polymers in 7.5 % of the pellets (Winkler et al., 2020). Plastic pollution was also assessed in regurgitated pellets from the great skua (*Stercorarius skua*) in the Faroe Islands, showing the highest prevalence of plastic in pellets containing remains of Northern fulmars (*Fulmarus glacialis*), consistent with the transfer of marine plastic debris from surface feeding seabirds to predatory species (Hammer et al., 2016). MPs were also quantified in pellets of the terrestrial predator barn owl (Tyto alba), attributed to diet transfer from rodents (Nessi et al., 2022). Overall, assessing plastic ingestion by apex predators is essential for understanding how plastic pollution permeates food webs.

MPs can enter trophic webs through inhalation or ingestion with widespread reports of their potential detrimental effects on wildlife and humans (Guzzetti et al., 2018; Sridharan et al., 2021). However, the effects of MPs on bird health remain poorly understood. MPs may induce mechanical effects, including polymer attachment to external surfaces and clogging of digestive tracts (Cole et al., 2011). Additionally, they can induce chemical harm and toxic effects at cellular and molecular levels. However, the effects of MPs on bird health remain poorly understood. Recent studies have shown that exposure to polystyrene (PS) MPs led to an imbalance in the antioxidant system, resulting in cell apoptosis and autophagy in lung tissues (Lu et al., 2023). In another study, exposure of chicken to PS MPs resulted in impaired myocardial development (Zhang et al., 2022). In the case of Japanese quails, the ingestion of aged MPs resulted in a reduction of body biomass and various biochemical alterations; however, these effects were not found to be dose-dependent (de Souza et al., 2022).

Beyond MPs, this study also investigated the presence of anthropogenic fibers (AFs), referring to fibers < 5 mm in length, made of materials other than synthetic polymers, used in textiles, household products, medical equipment, and building materials. AFs can have anthropogenic or natural origin and include artificial regenerated cellulose (viscose or rayon) and natural fibers that underwent some industrial processing before reaching their final form. AFs can be classified as anthropogenic pollutants because, despite being are derived from natural materials, they contain chemical additives, such as dyes and finishing agents that can be released upon degradation, and because once in the environment they may behave in a similar way to synthetic polymers (Suaria et al., 2020). In this study, cellulosic fibers were classified as AFs based on their non-natural colors or textures, excluding white or transparent fibers and cellulosic materials with natural textures, which were considered 'natural' (Finnegan et al., 2022; González-Pleiter et al., 2021; Stanton et al., 2019).

In this work, we studied the prevalence of MPs and AFs in birds of prey living in the highly urbanized region around central Madrid and its neighboring suburban and rural environments. We hypothesized that wildlife living near densely populated areas would exhibit a differential exposure to airborne particle pollutants, the quantification of which would contribute to the understanding of anthropogenic plastic pollution. The study investigates the presence of contaminants in the digestive and respiratory systems of the species Common Buzzard (*Buteo buteo*), Black Kite (*Milvus migrans*), Eurasian Sparrowhawk (*Accipiter nisus*) and Northern Goshawk (*Accipiter gentilis*). This information aims to contribute to advancing biomonitoring tool applications and lays the groundwork for using birds as biosamplers for microplastic pollution.

2. Materials and methods

2.1 Sample colection

A total of 47 birds, representing four species, were analyzed for this study. The species included were the Black Kite (*Milvus migrans*, N = 12), Common Buzzard (*Buteo buteo*, N = 12), Northern Goshawk (*Accipiter gentilis*, N = 11) and Eurasian Sparrowhawk (*Accipiter nisus*, N = 12). All specimens used were found in the Madrid region and its close vicinity, brought to the wildlife hospital center Grefa in Majadahonda, Spain, between 2020 and 2022. The birds used in our study were received at the Wildlife Hospital after experiencing accidents or being found dead in different situations. To minimize the risk of contamination with plastics or artificial fibers during their stay at the hospital, we exclusively selected birds that were either received dead or had died within 24 h of admission, provided they had not received any food during that period. MP concentrations within the animals were determined by extracting both the entire digestive and respiratory systems, including the tongue, trachea, and lungs. Subsequently, the samples were wrapped in aluminum foil and stored at -20 °C for further analysis. All samples were obtained in the necropsy room, following appropriate hygienic and sanitary protective measures. Additionally, all surgical materials were free of plastics to prevent sample contamination. The study area was divided in two zones: (1) central Madrid, with a population of over three million inhabitants, and the areas located to the north and south of it, following the direction of the prevailing winds; and (2) the rural and suburban areas, as well as smaller cities located to the east and west, which are relatively protected from the dispersion of pollutants originating from central Madrid.

2.2 Microplastics analysis and characterization

For MP analysis, each individual system, respiratory or digestive, was accurately weighed (details are shown in Table S1, Supplementary Materials, SM). Before digestion, the samples were rinsed with MilliQ water and then immersed in a beaker containing a 10 % KOH solution, serving as a contamination control. Immediately afterward, they were transferred to a new beaker and completely submerged in a 10 % KOH solution, undergoing chemical digestion for 48 h at 50 °C and 80 rpm. The KOH solution, prepared using MilliQ water in a 1 L graduated glass bottle, was kept closed to avoid contamination. Both the control and sample solutions were filtered through a stainless steel 25 µm mesh, using a filtering ramp system (stainless steel, ECOLAN model FL-S) connected to a vacuum pump. Digestive tracts were further treated with H_2O_2 (33 % w/v) for 24 h at 50 °C to dissolve any organic matter, such as insect exoskeletons and bones, that hadn't been previously digested. After this, they were sieved through 25 µm mesh again. Following filtration, samples were placed in Petri dishes and allowed to dry in an incubator at 37 °C until further analysis.

2.3 Microplastics analysis and characterization

Particle counting was performed using a stereomicroscope (Motic® SMZ-171 BLED Pole Type) equipped with an integrated camera (Moticam® X3). Particles were categorized based on their typology into fibers, fragments, and beads. All particles suspected of being anthropogenic pollutants were photographed, and their length and width determined using ImageJ software. In this work, we examined not only MPs, but also anthropogenic cellulosic fibers (AFs). For all positively identified MPs and AFs, we computed the diameter of the sphere with an equivalent volume as the particle (equivalent diameter, denoted as d_v) based on their two orthogonal projected dimensions: length and width for fragments and length and diameter for fibers. In the case of fragments, particle volume was estimated by approximating the particle as an ellipsoid, with the third unaccounted dimension being the average of the other two. For fibers, volume calculations assumed a cylindrical shape. The mass of individual particles was estimated using the tabulated average density for each polymer as shown in Table S2 SM). The use of d_v is due to the dynamic behavior of particles being a function of Stokes' diameter, defined as that of the sphere with unit (or reference) density settling with the same velocity as the particle, for which d_v can be used as an approximation. Additional details can be found elsewhere (Rosal, 2021).

For the chemical analyses, microparticles selected as putative MPs or AFs were placed onto KBr discs using a microneedle and subsequently analyzed using micro-Fourier transform infrared spectroscopy (micro-FTIR). The apparatus used was a Perkin-Elmer Spotlight 200i equipped with a mercury cadmium telluride detector, operating in transmission mode with a resolution of 8 cm^{-1} and a spectral range of 550-4000 cm^{-1}. The obtained spectra were compared to a database created by tour group, using the OMNIC 9 software (Thermo Fisher Scientific). A minimum of 70% similarity was required to confirm a positive match between the samples and the database or standards. This threshold represents a common compromise between accuracy and the necessity to

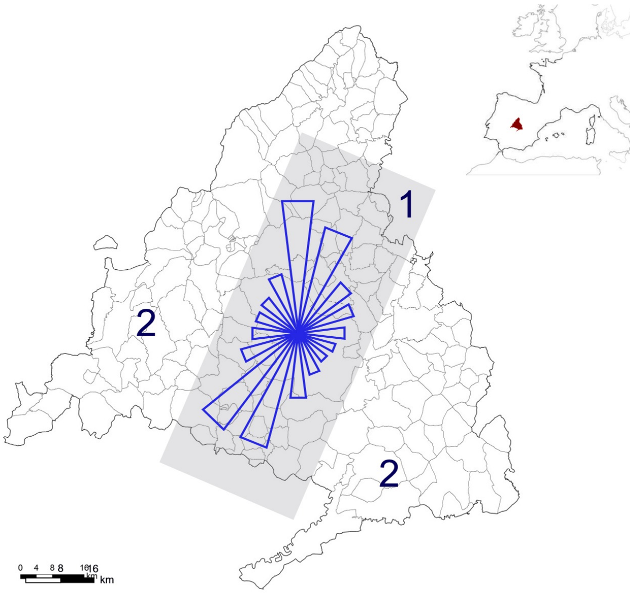


Figure 1: *Sampling zone. The compass rose shows the direction of the prevailing winds during the sampling period.*

account for the degradation observed in numerous polymers found in environmental samples (Carlin et al., 2020; Kanhai et al., 2020).

2.4. Prevention of contamination and QA/QC assessment

Throughout the entire sampling and analysis procedure, we adhered to strict quality assurance and quality control protocols to ensure the unbiased estimation of anthropogenic pollutants (Hung et al., 2021). Measures were implemented to prevent microplastic and artificial fiber contamination throughout the entire procedure. Plastic materials were avoided at all processing stages, with the exception of nitrile gloves used during experimental procedures and necropsies. In the laboratory, all personnel wore 100 % white cotton laboratory coats (white cellulose fibers were not considered artificial under any circumstances). Prior to use, all tweezers were rinsed with pure water, and glass beakers were covered with a double layer of aluminum and heated in an oven at 450 °C for 4 h to ensure the removal of all potential contaminants.

During sample handling, contamination controls were implemented. These controls consisted of open glass Petri dishes placed near the samples to monitor any potential pollution from the laboratory air. The controls were analyzed using the same methodology as the rest of the samples. If particles in the controls were found to have the same typology and composition as those in the samples, matching particles were removed from the affected samples. In the control samples, a total of 25 particles were identified as MPs, but only two polyester fibers matched some of the positively identified fibers found in the test samples. Further details regarding MPs and AFs can be found in Tables S3 and S4 (SM).

2.5. Statistical analysis

The software GraphPad Prism (GraphPad Software, Inc) was used for statistical analyses. Shapiro-Wilk and Levene's test were used to assess the normality and homoscedasticity of the data ($p > 0.05$). As not all results could be considered normal, se used the Kruskal-Wallis test to compare the abundance and concentration of contaminants among species. To assess differences between geographical zones, and between pairs the non-parametric Mann-Whitney-Wilcoxon test was used ($p > 0.05$).

3. Results

3.1. Occurrence of MPs and AFs

A total of 1040 particles with potential anthropogenic origins were recorded and analyzed, consisting of 723 from digestive systems and 317 from respiratory systems. These particles were collected from four species of birds of prey: Common Buzzard (200), Black Kite (431), Eurasian Sparrowhawk (170), and Northern Goshawk (239). Out of the 1040 particles spectroscopically analyzed with micro-FTIR, 301 particles were positively identified as MPs (228 from digestive systems and 73 from respiratory systems), and 393 particles were identified as AFs (257 from digestive systems and 136 from respiratory systems). Among the MP particles, 294 were fibers, and 7 were fragments. All AFs were identified as cellulose or regenerated cellulose materials, such as viscose. The

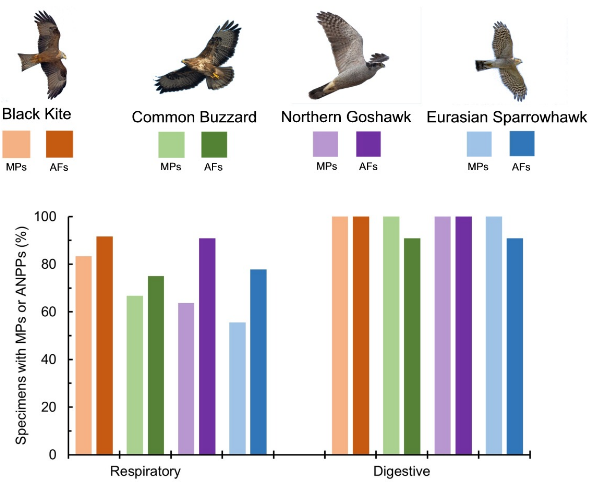


Figure 2: *Prevalence of anthropogenic pollution as percentage of specimens contaminated with MPs and AFs in their respiratory and digestive systems.*

identification of these anthropogenic pollutants was based on the presence of non-natural colors or textures. Consequently, cellulose with potentially natural colors was excluded from the AFs count. The remaining 346 particles were either non-artificial materials (such as vegetal debris) or couldn't be identified due to matching percentages falling below the established threshold.

The study confirmed the presence of MPs or AFs contamination in all the specimens analyzed (Fig. 2). Specifically, 100 % of the digestive tracts contained at least one MP, and 95 % contained AFs. In the respiratory systems, MPs were found in over 65 % of the samples. However, the percentages of MPs in the respiratory tract varied among species. Black Kites exhibited the highest percentage, with 10 out of 12 individuals containing MPs, while Common Buzzards had the lowest prevalence, with only 5 individuals contaminated by MPs. Meanwhile, the prevalence of AFs was slightly lower in the digestive tracts (found in 43 specimens) when compared to MPs (45 specimens), but higher in the respiratory systems (37 individuals compared to 30 for MPs).

The overall average number of MPs per specimen was 7.9, whereas for AFs the average was 9.2. When comparing contaminant abundance across species, Black Kites had the highest concentration of MPs per specimen (13.7 MPs/specimen), especially in their digestive system (12.0 MPs/specimen), followed by Northern Goshawk (6.6 MPs/specimen), Eurasian Sparrowhawk (6.2 MPs/specimen) and Common Buzzard (4.9 MPs/specimen). Black Kites also displayed the highest concentration of AFs (11.8 AFs/specimen). Overall, the data revealed a prevalence of anthropogenic particles (MPs and AFs) in

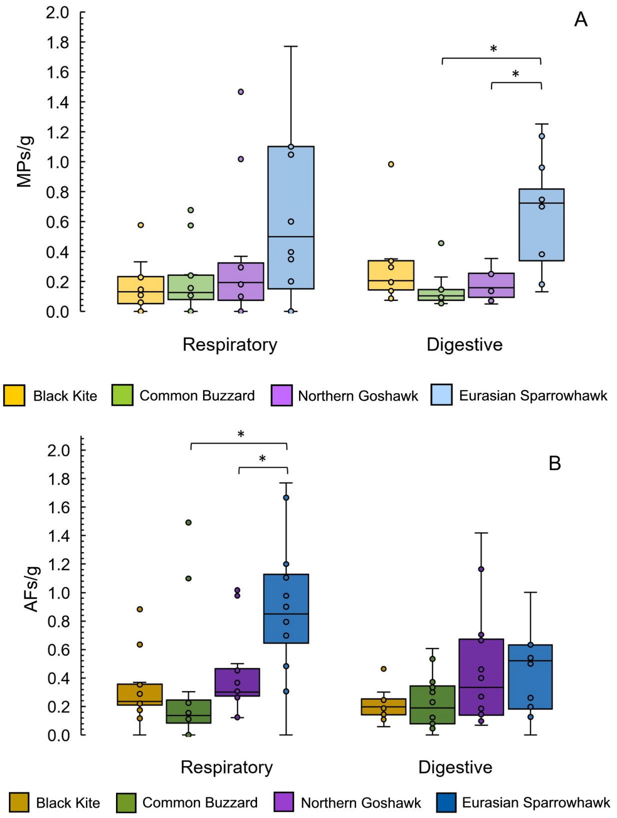


Figure 3: *MP and AFs abundance per weight of respiratory or digestive system in the four bird species. The asterisks indicate significant differences ($p < 0.05$).*

the range of 11.8 to 25.4 particles per specimen.

Considering the significant differences among birds (Table S5, SM), the variable that better describes the occurrence of anthropogenic pollutants is their concentration relative to the mass of the affected systems, be it digestive or respiratory (Carlin et al., 2020). The data are presented in Fig. 3 (A for MPs and B for AFs) and show median values in the ranges of 0.13-0.40 MPs/g and 0.14-0.79 AFs/g in respiratory systems and 0.10-0.75 MPs/g and 0.15-0.54 AFs/g in digestive systems. When comparing the abundance of both MPs/g and AFs/g between the two systems, a significantly higher abundance was found in the digestive than the respiratory system (paired Wilcoxon, $p \leq 0.001$). Despite morphological differences among species, the concentrations in both the respiratory and digestive systems were similar for the three birds of similar size. In fact, the only significant differences (paired Wilcoxon, $p < 0.05$) were observed when comparing the Eurasian Sparrowhawk with the Northern Goshawk and Common Buzzard (Figs. 3A for MPs and 3B for AFs).

Fig. 4 depicts the geographical distribution of the specimens studied in this work. Zone 1 corresponds to central Madrid and the areas located following the direction of the prevailing winds, while Zone 2 included suburban areas, and smaller cities relatively protected from the dispersion of pollutants from the most populated areas. The design was intended to test the hypothesis that anthropogenic airborne pollutants originate in areas with high human population density and disperse via prevailing winds.

The concentration of MPs and AFs in the respiratory and digestive systems of the birds recovered from Zones 1 and 2 are presented in Fig. 5. The comparison between zones showed that all concentration values were significantly lower in Zone 2 with respect to Zone 1 (Mann-Whitney U test, $p < 0.05$). No other differences were found, although the dispersion of results was somewhat higher for AFs as evidenced by the larger error bars for AFs in Fig. 5, which represent the interquartile range (IQR).

The results for the equivalent diameter, d_v, defined as the diameter of a sphere with the same volume as the particle, are displayed in Fig. 6. No significant differences were observed when comparing MPs or AFs from different bird species and geographical zones. The median equivalent diameter of MPs in respiratory systems was 52.2 µm (IQR, 43.6-63.5), while for MPs recovered from digestive systems, the median equivalent diameter was 57.9 µm (IQR, 47.7-69.4), only slightly higher. Noteworthy, 9.6 % of the MPs found in the digestive systems displayed an equivalent diameter > 100 µm. The largest MP in digestive systems was a PE fragment with projected dimensions 2550 x 1702 µm and the largest in respiratory systems was another PE fragment with length 255 µm and with 159 µm. The smallest particles in respiratory and digestive systems were a PES fiber with 239 µm length and 9.0 µm width, and a PE fiber with 164 µm length and 6.5 µm width. Regarding AFs, the equivalent diameters were also similar, with median equivalent diameters of 55.3 µm for respiratory systems and 58.6 µm for digestive systems without significant differences among species. The median length of AFs was 590 µm, with some ranging up to the millimeter range (11.4 % and 19.8 % in samples from respiratory and digestive systems, respectively). The longest cellulose fiber had a length of 2383 µm and a diameter of 19 µm.

3.2. Particle size and chemical composition

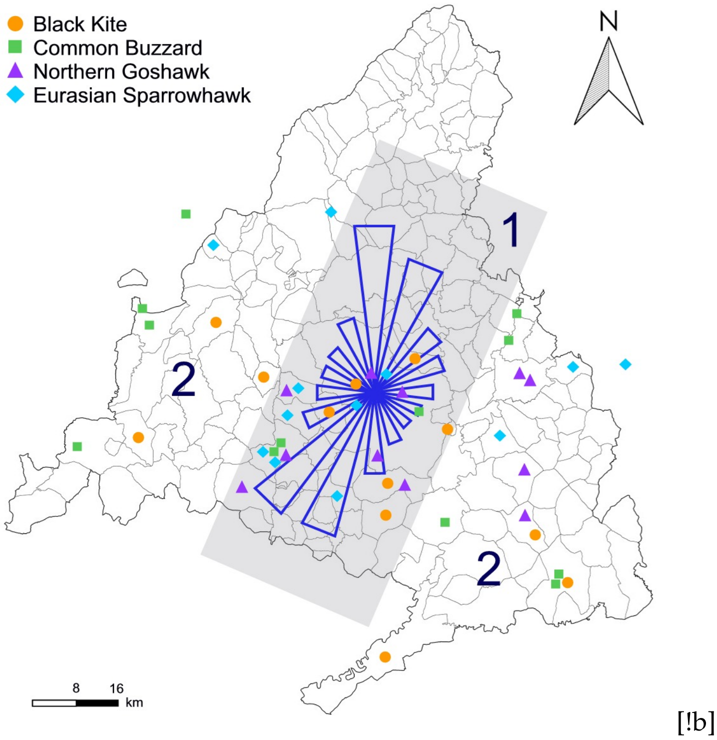
Out of the 301 microplastics, 228 (76 %) were found in digestive systems while 73 (24 %) were identified in respiratory systems. The majority of these MPs (294) were in the form of fibers, with only 6 fragments found in samples from digestive systems and 1 from respiratory systems. Seven different polymers were identified (as shown in Fig. 7 and Table S6, SM), with polyester (PES) being the predominant one accounting for 55.8 % of the polymers detected. Acrylic fibers (ACR) followed, representing 21.3 % of the polymers detected, and polyethylene (PE) accounted for 17.2 %. These three polymers collectively made up over 94 % of the plastic found. A similar pattern was observed in the results when broken down by species, as shown in Fig. S1 (SM).

4. Discussion

Plastic pollution has continuously increased over recent decades, leading to accumulation of plastic debris in ecosystems worldwide (Rios-Mendoza et al., 2021). This raises concerns about their presence and impacts on a wide variety of wild organisms (Carbery et al., 2018; McIlwraith et al., 2021; Pitacco et al., 2022). In this article, the presence of MPs and AFs was assessed for the first time in the digestive and respiratory system of four different species of predatory birds inhabiting a densely populated region. The purpose was to assess the level of contamination of the specimens with plastic and other anthropogenic particles, depending on their exposure to the densely populated areas of central Madrid.

All the digestive tracts analyzed contained at least one MP (Fig. 2). The presence of MP has been widely reported across avian species from different ecosystems (Kühn and van Franeker, 2020; Wang et al., 2021; Zhao et al., 2016). However, data on birds of prey ares very limited, despite their crucial role as predators in ecosystem functioning, their ability to accumulate plastic from their preys, and the fact that many species inhabit areas near human settlements. Carlin et al. reported the presence of MPs in the gastrointestinal tracts of all predatory birds examined in a study carried out in Florida (Carlin et al., 2020). The concentration reported in that study 0.3 (± 0.1) MPs/g of tract tissue, which included cellulose as well as synthetic polymers was comparable with our data, namely 0.14-0.63 (averages) or 0.10-0.75 (medians) MPs/g of digestive system (Fig. 3A). Conversely, Shutten et al., who studied the contents of plastic in the gastrointestinal tracts of predatory birds from 15 species mostly collected in the Vancouver area (Canada), found that only 5 out of 234 individuals contained anthropogenic litter. However, it is important to note that their study focused solely on particles larger than 2 mm, and no spectroscopic characterization was conducted (Schutten et al., 2023).

The majority of research of MPs in birds has been focused on the ingestion of plastic particles. However, it's important to note that inhalation can also serve as a potential entry pathway for organisms (Prata, 2018). To the best of our knowledge, only one previous study has explored this aspect in avian species (Tokunaga et al., 2023). Tokunaga et al. analyzed lung



[!b]

Figure 4: *Locations where the bird specimens were found.*

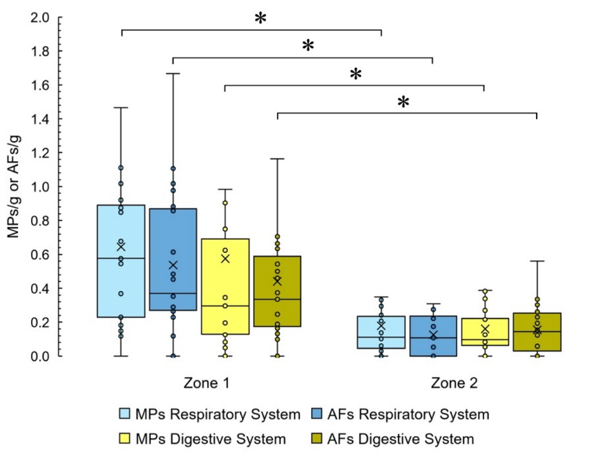


Figure 5: *Concentration of MPs and AFs per gram or respiratory or digestive systems in specimens recovered from Zones 1 and 2 as shown in Figs. 1 and 4. The crosses represent means and the asterisks statistically significant differences for comparisons between zones ($p < 0.05$).*

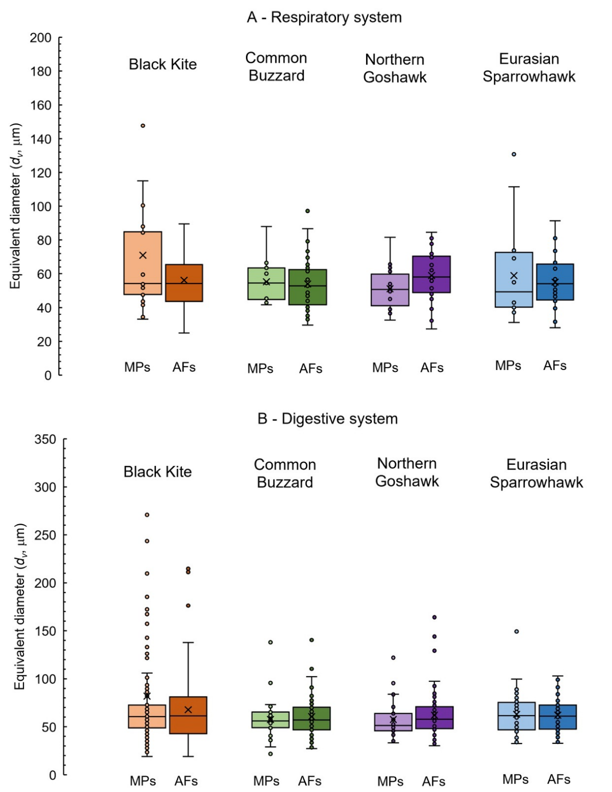


Figure 6: *Equivalent diameter, d_v, for MPs and AFs found in the respiratory (upper panel) and digestive (lower panel) systems for all species studied (expressed in µm).*

samples from several species of wild birds in Japan including Black Kites. They found only six plastics in a total of 3 out of 22 lungs, indicating a low incidence. In contrast, our study revealed 73 MPs and 136 AFs in the analyzed respiratory systems. Notably, almost all the MPs in our study were fibers (72 fibers and 1 fragment), in contrast to the six fragments reported by the Japanese team. Furthermore, no MPs were found in the Japanese Black Kites, while in this article 10 out of 12 individuals analyzed presented MPs in their respiratory systems. One possible explanation is the geographical situation of our sampling area, very far from the influence of sea winds and dominated by the highly populated area of central Madrid. In a previous study we obtained direct evidence of the presence of MPs in the atmosphere above central Madrid and surrounding areas. In that study, we demonstrated that MPs are present in the atmosphere, even at high altitude and showed a difference in the concentrations of MPs over central Madrid one order of magnitude higher than in rural areas (González-Pleiter et al., 2021). This concentration is higher than that reported for Tokyo in studies of ambient air in urban areas in Japan (Tokunaga et al., 2023). Considerable methodological differences may contribute to this variability, but assessing this aspect is challenging. Specifically, the size of MPs found in both studies was similar (43.6 µm on average compared to 58.4 µm in our study) and does not suggest a size bias. In summary, the understanding of the presence of MPs in the respiratory systems of avian species remains largely understudied, emphasizing the need for further research to better comprehend these distribution patterns.

In this work a comparison was conducted between urban and rural environments to elucidate how birds' habitats influence their exposure to plastic contaminants. It has been demonstrated that the atmosphere serves as a pathway for transporting plastics and related pollutants from their point of origin to less populated regions. Padha et al. collected data pointing to atmospheric transport (along with tourism when possible) as the major source of MPs in mountain environments (Padha et al., 2022). Atmospheric transport and deposition simulations using Lagrangian particle trajectory models showed that MPs could travel hundreds of km before being deposited (González-Pleiter et al., 2021). Measurements of atmospheric MPs in the marine boundary layer over the western Pacific Ocean revealed concentrations exponential decaying with the distance to coastal megacities, providing additional evidence that urban centers are the primary initial source for airborne MPs (Wang et al., 2022). Our results (Fig. 5) align with the assumption that urban centers disperse anthropogenic particles into less populated regions through the atmosphere. This phenomenon explains the abundance and distribution of plastic particles in remote areas and results in differential exposure for animals living in these different environments.

It is important to note that, despite variations in physical characteristics and life traits such as preferred preys and flying and hunting behaviors (Table S5, SM), the four species exhibit only slight differences in the presence of MPs and AFs in their respiratory and digestive systems. As shown in Fig. 3, the sole significant difference ($p < 0.05$) emerges in the content of MPs in the digestive system and AFs in the respiratory systems when comparing Eurasian Sparrowhawk with Northern Goshawk and Common Buzzard. Interestingly, Eurasian Sparrowhawk is the smallest bird studied, weighing 110-340 g, typically one-quarter the size of the other three species. This finding may suggest a higher propensity for smaller birds to accumulate anthropogenic particles relative to their weight, although the data are not robust enough to fully support this assumption. Another difference found was the relatively high proportion of larger plastics found in the digestive system of Black Kite specimens when compared to the other three species (Fig. 6). This discrepancy may be attributed to the fact that Black Kites are opportunistic scavengers, often reported to feed from landfills, while the other species primarily feed on other birds and small mammals (Blanco, 1994; Zhao et al., 2016). These results emphasize the importance of considering species-specific traits and physiological characteristics when evaluating the accumulation of microplastics in organisms.

Our research revealed the presence of six types of plastics in both the digestive and respiratory systems (Fig. 7). The predominant plastics were polyester (PES), which includes poly(ethylene terephthalate), polyethylene (PE), and acrylic fibers (ACR), collectively accounting for over 94 % of the plastics found. These plastics correspond to the materials commonly used in many everyday applications, primarily in textile production (Carlin et al., 2020). Interestingly, fibers represent the vast majority of the MPs found in this work (293 out of 301 MPs). High percentages of fibers have been found in terrestrial and seabirds (Carlin et al., 2020; Kühn and van Franeker, 2020; Zhao et al., 2016). There is considerable evidence that this shape of particles predominates in samples from air and soft tissues of animals (Dris et al., 2017; Salvador Cesa et al., 2017). Hoang et al. studied the occurrence of MPs in the gastrointestinal tract of several species of small birds and mostly found fibers of polyester, polyethylene and polyamide, which were mostly transferred to the birds via their diet, such as

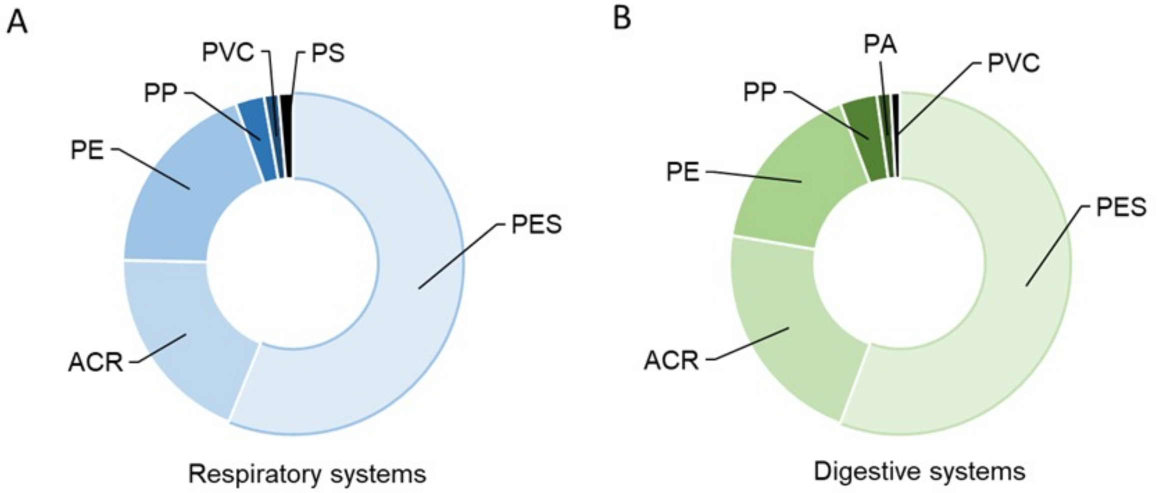


Figure 7: *Chemical composition of the MPs identified by micro-FTIR found (A) in the respiratory systems and (B) in the digestive systems. (PES: polyester; PE: polyethylene; PA: polyamide; ACR: acrylic; PP: polypropylene; PVC: polyvinyl chloride; PS: polystyrene).*

flying insects (Hoang and Mitten, 2022). This can be attributed to their high mobility, but it is also true that fibers are released into the environment in large amounts from the production, laundering of textiles, and the wear of synthetic clothes (Rebelein et al., 2021).

In our analysis, we also quantified the concentration of AFs, a type of pollutant often overlooked in this kind of research. Interestingly, we found a higher number of AFs (393) than microplastics (MPs) (301). Interestingly, the prevalence of AFs was higher than that of MPs in respiratory systems and slightly lower in digestive systems, as illustrated in Fig. 1. This can be explained by the fact that fibers make up the majority of anthropogenic particles in the atmospheric compartment, whether they are plastics or not, as shown elsewhere (Edo et al., 2023; Wright et al., 2020). All of these were fibers made from industrially processed cotton, including regenerated cellulose fibers. These types of fibers raise concerns similar to those associated with MPs, as both are manufactured using chemicals (including dyes and additives) and have the potential to adsorb pollutants, posing threats to the environment and the health of organisms (Cohen and Radian, 2022; Darbra et al., 2012). Wang et al. also identified a significant number of cotton and cellulose fibers, doubling the count of plastic particles, in samples collected over the western Pacific Ocean in transects near coastal megacities (Wang et al., 2022). The outcome of other studies suggests that cellulosic fibers may be even more prevalent than synthetic polymers in atmospheric samples (Carlin et al., 2020; Finnegan et al., 2022). However, quantifying fibers can be challenging due to the difficulty of identifying them using spectroscopic methods and determining their anthropogenic or natural origin. It is most likely that all studies underestimate the presence of this type of particulate pollution.

Another important issue when dealing with MPs pollution is estimating the mass of particles. This is essential in order to provide data in terms of mass concentration, rather than just number concentration. MPs and related particles are challenging to measure as they are small, often consisting of twisted fibers, and the only available information typically comes from two-dimensional images of the particles, usually in their most stable positions. Characterizing each particle individually within the entire set (or a representative set) is the only way to obtain reasonable estimates of the mass of anthropogenic particles in a given sample, thus avoiding the large errors usually associated with size averages (Pletz, 2022). In this study, we calculated the mass of MPs using the density corresponding to each polymer (Table S2, SM). In the respiratory systems, the measured mass of MPs ranged from 164 to 677 ng per specimen, with the highest value observed in Black Kites. Black Kites also exhibited the highest plastic concentration in their digestive systems, amounting to 393 µg per specimen, which was three orders of magnitude larger than the mass recorded for the other three species, falling in the 476-701 ng/specimen range. This difference was attributed to the presence of relatively large plastic fragments in the digestive systems of Black Kites, as indicated earlier and shown in Fig. 6B. The differences between Zones 1 and 2 become apparent when calculating the load of MPs in each zone. The average mass of MPs in the respiratory system amounted to 296 ng per specimen in Zone 1 and 164 ng per specimen in Zone 2. The difference was more pronounced in the digestive systems, with 1247 µg per specimen in Zone 1 and 575 ng per specimen in Zone 2. This difference was explained by the presence of some larger plastic particles in the digestive systems of the birds recovered from the area with a higher influence of central Madrid compared to those from more distant places.

This investigation was the first to quantify microparticle contamination in both the respiratory and digestive systems of different species of predatory birds. The results indicate a pervasive presence of microplastics (MPs) and artificial fibers (AFs) across species, with every examined bird containing at least one MP. The results demonstrated the widespread presence of MPs and AFs across species, as all studied birds had at least one MP. The main finding of this work underscores the exposure of wild birds to anthropogenic particle pollution from urban centers, offering new insights into the prevalence of MPs and other particle pollutants in avian species, particularly within their respiratory systems, which remains largely understudied. This information can also serve to increase the protection of wild species exposed to these pollutants underscoring the need for further research to understand how these contaminants spread through ecosystems and enter birds, including potential consequences. Such knowledge is essential to allow stakeholders to undertake actions against the pollution arising from MPs and AFs. This pollution is primarily linked to the inefficient circularity (limited recycling and reuse) in the life cycle of these materials. Specifically, the use of low-quality textiles, particularly in single-use applications, plays a significant role in spreading the specific type of pollution posed by fibers. Future studies should consider artificial fibers made from natural polymers, such as cellulose or regenerated cellulose fibers, as this is a relatively understudied component of the anthropogenic aerosol.

5. Conclusions

We monitored the presence of MPs and AFs in four species of prey birds living in the vicinity of a densely populated area. Seven different polymers were identified, with polyester, polyethylene, and acrylic fibers constituting the majority (94 %) of the detected plastics. All AFs were identified as cellulosic fibers, most likely originating from textiles. Every examined bird harbored at least one MP. The average mass of MPs per specimen was 936 ng in digestive systems and 225 ng in respiratory systems.

Clear differences were evident between specimens collected from areas influenced by central Madrid and those retrieved from rural and less populated locations. This finding supports the assumption that anthropogenic particles disperse from large urban centers through the atmosphere, resulting in varied exposure for wildlife residing in neighboring environments.

This study established that prey birds can effectively serve as biomonitors for plastic pollution, particularly for plastics entering the respiratory system. Such particles are likely more challenging to expel and can serve as indicators for the presence of atmospheric MPs across various species, including humans. We also showed that AFs are an important fraction of the anthropogenic airborne pollutants.

Acknowledgements

The authors acknowledge the funding provided by Plastics Europe and the support given by Research Network of Plastic Waste in the Environment, EnviroPlaNet (`www.enviroplanet.net`). The authors acknowledge the Ministry for Ecological Transition and the Demographic Challenge but does not express the opinion of the Ministry. The authors also acknowledge Ministry of Science of Spain, grants PID2020-113769RB-C21/C22, PLEC2021-007693 and TED2021-131609B-C32/33. Furthermore, the authors would like to thank the Interdepartmental Investigation Research Service of the Universidad Autónoma de Madrid (SIDI-UAM) for the use of their infrastructures and the student volunteers at GREFA for their help.

References

Blanco G. Seasonal abundance of black kites associated with the rubbish dump of Madrid, Spain. Journal of Raptor Research 1994; 28: 242-245.

Borrelle SB, Ringma J, Law KL, Monnahan CC, Lebreton L, McGivern A, Murphy E, Jambeck J, Leonard GH, Hilleary MA, Eriksen M, Possingham HP, De Frond H, Gerber LR, Polidoro B, Tahir A, Bernard M, Mallos N, Barnes M, Rochman CM. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science 2020; 369: 1515-1518.

Carbery M, O'Connor W, Palanisami T. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environment International 2018; 115: 400-409.

Carlin J, Craig C, Little S, Donnelly M, Fox D, Zhai L, Walters L. Microplastic accumulation in the gastrointestinal tracts in birds of prey in central Florida, USA. Environmental Pollution 2020; 264: 114633.

Cohen N, Radian A. Microplastic textile fibers accumulate in sand and are potential sources of micro(nano)plastic pollution. Environmental Science & Technology 2022; 56: 17635-17642.

Cole M, Lindeque P, Halsband C, Galloway TS. Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin 2011; 62: 2588-2597.

Darbra RM, Dan JRG, Casal J, Àgueda A, Capri E, Fait G, Schuhmacher M, Nadal M, Rovira J, Grundmann V, Barceló D, Ginebreda A, Guillén D. Additives in the textile industry. In: Bilitewski B, Darbra RM, Barceló D, editors. Global Risk-Based Management of Chemical Additives I: Production, Usage and Environmental Occurrence. Springer Berlin Heidelberg, Berlin, Heidelberg, 2012, pp. 83-107.

de Souza SS, Freitas ÍN, Gonçalves SO, Luz TM, Araújo AP, C., Rajagopal R, Balasubramani G, Rahman MM, Malafaia G. Toxicity induced via ingestion of naturally-aged polystyrene microplastics by a small-sized terrestrial bird and its potential role as vectors for the dispersion of these pollutants. Journal of Hazardous Materials 2022; 434: 128814.

Deoniziak K, Cichowska A, Niedźwiecki S, Pol W. Thrushes (Aves: Passeriformes) as indicators of microplastic pollution in terrestrial environments. Science of The Total Environment 2022; 853: 158621.

Dris R, Gasperi J, Mirande C, Mandin C, Guerrouache M, Langlois V, Tassin B. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environmental Pollution 2017; 221: 453-458.

Edo C, Fernández-Piñas F, Leganes F, Gómez M, Martínez I, Herrera A, Hernández-Sánchez C, González-Sálamo J, Borges JH, López-Castellanos J, Bayo J, Romera-Castillo C, Elustondo D, Santamaría C, Alonso R, García-Gómez H, Gonzalez-Cascon R, Martínez-Hernández V, Landaburu-Aguirre J, Incera M, Gago J, Noya B, Beiras R, Muniategui-Lorenzo S, Rosal R, González-Pleiter M. A nationwide monitoring of atmospheric microplastic deposition. Science of The Total Environment 2023; 905: 166923.

Emmerik T, Schwarz A. Plastic debris in rivers. WIREs Water 2020; 7.

Finnegan AMD, Süsserott R, Gabbott SE, Gouramanis C. Man-made natural and regenerated cellulosic fibres greatly outnumber microplastic fibres in the atmosphere. Environmental Pollution 2022; 310: 119808.

Gewert B, Plassmann MM, MacLeod M. Pathways for degradation of plastic polymers floating in the marine environment. Environmental Science: Processes & Impacts 2015; 17: 1513-1521.

Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Science Advances 2017; 3: e1700782.

González-Pleiter M, Edo C, Aguilera Á, Viúdez-Moreiras D, Pulido-Reyes G, González-Toril E, Osuna S, de Diego-Castilla G, Leganés F, Fernández-Piñas F, Rosal R. Occurrence and transport of microplastics sampled within and above the planetary boundary layer. Science of The Total Environment 2021; 761: 143213.

González-Pleiter M, Edo C, Velázquez D, Casero-Chamorro MC, Leganés F, Quesada A, Fernández-Piñas F, Rosal R. First detection of microplastics in the freshwater of an Antarctic Specially Protected Area. Marine Pollution Bulletin 2020; 161: 111811.

Guzzetti E, Sureda A, Tejada S, Faggio C. Microplastic in marine organism: Environmental and toxicological effects. Environmental Toxicology and Pharmacology 2018; 64: 164-171.

Hammer S, Nager RG, Johnson PCD, Furness RW, Provencher JF. Plastic debris in great skua (Stercorarius skua) pellets corresponds to seabird prey species. Marine Pollution Bulletin 2016; 103: 206-210.

Hoang TC, Mitten S. Microplastic accumulation in the gastrointestinal tracts of nestling and adult migratory birds. Science of The Total Environment 2022; 838: 155827.

Hoornweg D, Bhada-Tata P, Kennedy C. Environment: Waste production must peak this century. Nature 2013; 502: 615-617.

Hung C, Klasios N, Zhu X, Sedlak M, Sutton R, Rochman CM. Methods matter: methods for sampling microplastic and other anthropogenic particles and their implications for monitoring and ecological risk assessment. Integrated Environmental Assessment and Management 2021; 17: 282-291.

Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, Narayan R, Law KL. Plastic waste inputs from land into the ocean. Science 2015; 347: 768-771.

Kanhai LDK, Gardfeldt K, Krumpen T, Thompson RC, O'Connor I. Microplastics in sea ice and seawater beneath ice floes from the Arctic Ocean. Scientific Reports 2020; 10: 5004.

Kühn S, van Franeker JA. Quantitative overview of marine debris ingested by marine megafauna. Marine Pollution Bulletin 2020; 151: 110858.

Lu H, Yin K, Su H, Wang D, Zhang Y, Hou L, Li Jb, Wang Y, Xing M. Polystyrene microplastics induce autophagy and apoptosis in birds lungs via PTEN/PI3K/AKT/mTOR. Environmental Toxicology 2023; 38: 78-89.

McIlwraith HK, Kim J, Helm P, Bhavsar SP, Metzger JS, Rochman CM. Evidence of microplastic translocation in wild-caught fish and implications for microplastic accumulation dynamics in food webs. Environmental Science & Technology 2021; 55: 12372-12382.

Navarro A, Luzardo OP, Gómez M, Acosta-Dacal A, Martínez I, Felipe de la Rosa J, Macías-Montes A, Suárez-Pérez A, Herrera A. Microplastics ingestion and chemical pollutants in seabirds of Gran Canaria (Canary Islands, Spain). Marine Pollution Bulletin 2023; 186: 114434.

Nessi A, Winkler A, Tremolada P, Saliu F, Lasagni M, Ghezzi LLM, Balestrieri A. Microplastic contamination in terrestrial ecosystems: A study using barn owl (Tyto alba) pellets. Chemosphere 2022; 308: 136281.

Oberbeckmann S, Labrenz M. Marine Microbial Assemblages on Microplastics: Diversity, Adaptation, and Role in Degradation. Annual Review of Marine Science 2020; 12: 209-232.

Österlund H, Blecken G, Lange K, Marsalek J, Gopinath K, Viklander M. Microplastics in urban catchments: Review of sources, pathways, and entry into stormwater. Science of The Total Environment 2023; 858: 159781.

Padha S, Kumar R, Dhar A, Sharma P. Microplastic pollution in mountain terrains and foothills: A review on source, extraction, and distribution of microplastics in remote areas. Environmental Research 2022; 207: 112232.

Pitacco V, Orlando-Bonaca M, Avio CG. 4 - Plastic impact on marine benthic organisms and food webs. In: Bonanno G, Orlando-Bonaca M, editors. Plastic Pollution and Marine Conservation. Academic Press, 2022, pp. 95-151.

PlasticsEurope. Plastics - the fast Facts 2023. Plastics Europe, Brussels, 2023.

Pletz M. Ingested microplastics: Do humans eat one credit card per week? Journal of Hazardous Materials Letters 2022; 3: 100071.

Prata JC. Airborne microplastics: Consequences to human health? Environmental Pollution 2018; 234: 115-126.

Rebelein A, Int-Veen I, Kammann U, Scharsack JP. Microplastic fibers — Underestimated threat to aquatic organisms? Science of The Total Environment 2021; 777: 146045.

Rios-Mendoza LM, Leon-Vargas D, Balcer M. Microplastics occurrence and fate in the environment. Current Opinion in Green and Sustainable Chemistry 2021; 32: 100523.

Rosal R. Morphological description of microplastic particles for environmental fate studies. Marine Pollution Bulletin 2021; 171: 112716.

Salvador Cesa F, Turra A, Baruque-Ramos J. Synthetic fibers as microplastics in the marine environment: A review from textile perspective with a focus on domestic washings. Science of The Total Environment 2017; 598: 1116-1129.

Schutten K, Chandrashekar A, Bourdages M, Bowes V, Elliott J, Lee S, Redford T, Provencher J, Jardine C, Wilson L. Assessing plastic ingestion in birds of prey from British Columbia, Canada. Environmental Science and Pollution Research 2023; 30: 76631-76639.

Sherlock C, Fernie KJ, Munno K, Provencher J, Rochman C. The potential of aerial insectivores for monitoring microplastics in terrestrial environments. Science of The Total Environment 2022; 807: 150453.

Sridharan S, Kumar M, Singh L, Bolan NS, Saha M. Microplastics as an emerging source of particulate air pollution: A critical review. Journal of Hazardous Materials 2021; 418: 126245.

Stanton T, Johnson M, Nathanail P, MacNaughtan W, Gomes RL. Freshwater and airborne textile fibre populations are dominated by ‘natural’, not microplastic, fibres. Science of The Total Environment 2019; 666: 377-389.

Suaria G, Achtypi A, Perold V, Lee JR, Pierucci A, Bornman TG, Aliani S, Ryan PG. Microfibers in oceanic surface waters: A global characterization. 2020; 6: eaay8493.

Tokunaga Y, Okochi H, Tani Y, Niida Y, Tachibana T, Saigawa K, Katayama K, Moriguchi S, Kato T, Hayama S. Airborne microplastics detected in the lungs of wild birds in Japan. Chemosphere 2023; 321: 138032.

Wang L, Nabi G, Yin L, Wang Y, Li S, Hao Z, Li D. Birds and plastic pollution: recent advances. Avian Research 2021; 12: 59.

Wang X, Wei N, Liu K, Zhu L, Li C, Zong C, Li D. Exponential decrease of airborne microplastics: From megacity to open ocean. Science of The Total Environment 2022; 849: 157702.

Wayman C, Niemann H. The fate of plastic in the ocean environment – A minireview. Environmental Science: Processes & Impacts 2021; 23: 198-212.

Winkler A, Nessi A, Antonioli D, Laus M, Santo N, Parolini M, Tremolada P. Occurrence of microplastics in pellets from the common kingfisher (Alcedo atthis) along the Ticino River, North Italy. Environmental Science and Pollution Research 2020; 27: 41731-41739.

Woodall LC, Sanchez-Vidal A, Canals M, Paterson GLJ, Coppock R, Sleight V, Calafat A, Rogers AD, Narayanaswamy BE, Thompson RC. The deep sea is a major sink for microplastic debris. Royal

Society Open Science 2014; 1: 140317.
Wright SL, Ulke J, Font A, Chan KLA, Kelly FJ. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. Environment International 2020; 136: 105411.
Zhang Y, Wang D, Yin K, Zhao H, Lu H, Meng X, Hou L, Li J, Xing M. Endoplasmic reticulum stress-controlled autophagic pathway promotes polystyrene microplastics-induced myocardial dysplasia in birds. Environmental Pollution 2022; 311: 119963.
Zhao S, Zhu L, Li D. Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers. Science of The Total Environment 2016; 550: 1110-1115.

Supplementary Materials

Accumulation of microplastics in predatory birds near a densely populated urban area

Chloe Wayman[1], Miguel González-Pleiter[2], Francisca Fernández-Piñas[2,3], Elisa L. Sorribes[4], Rocío Fernández-Valeriano[4], Irene López-Márquez[4], Fernando González-González[4,5], Roberto Rosal[1,*]

[1]Department of Chemical Engineering, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain
[2]Department of Biology, Faculty of Science, Universidad Autónoma de Madrid, E-28049, Madrid, Spain
[3]Centro de Investigación en Biodiversidad y Cambio Global, Universidad Autónoma de Madrid. Darwin 2, 28049 Madrid, Spain
[4]Wildlife Hospital, Group of Rehabilitation of the Autochthonous Fauna and their Habitat (GREFA), Majadahonda, 28220 Madrid, Spain
[5]Departmental Section of Pharmacology and Toxicology, Faculty of Veterinary Science, Universidad Complutense de Madrid, 28020 Madrid, Spain

* Corresponding author: roberto.rosal@uah.es

Contents:

Table S1. Weight of respiratory and digestive systems.
Table S2. Polymers densities.
Table S3. Particles found in respiratory controls.
Table S4. Particles found in digestive controls.
Table S5. Main traits of the birds studied in this work.
Table S6. Plastic composition and their presence in the digestive and respiratory systems.
Figure S1. Chemical composition of the MPs identified by micro-FTIR found (A) in the respiratory system and (B) in the digestive system. PES: polyester; PE: polyethylene; PA: polyamide; ACR: acrylic; PP: polypropylene; PVC: polyvinyl chloride; PS: polystyrene.

Table S1. *Weight of respiratory and digestive systems.*

	Respiratory systems (weight, g)			Digestive systems (weight, g)		
	Median	Q1	Q3	Median	Q1	Q3
Black Kite	9.8	8.4	11.0	35.7	28.6	49.6
Common Buzzard	7.8	4.1	8.7	26.2	22.3	39.1
Northern Goshawk	10.1	8.2	12.3	20.7	14.8	26.1
Eurasian Sparrowhawk	2.9	2.3	3.1	7.7	6.5	9.4

Table S2. *Polymer densities.*

Polymer	Density (g/cm^3)
Polyester	1.39
Acrylic polymers	1.18
Polyamide	1.07
Polyethylene	0.95
Polypropylene	0.91
Polyvinylchloride	1.38
Polystyrene	1.04
Cellulose	1.50

Table S3. *Particles found in respiratory system controls.*

Species	Typology	Action taken in the affected samples
Common Buzzard	Blue, black, and green cellulose fibers	1 Blue and 1 black cellulose fibres removed
Common Buzzard	Black cellulose fiber	2 Black cellulose fibres removed
Eurasian Sparrowhawk	Transparent polyester fiber	1 Transparent polyester fiber removed
Black Kite	Blue cellulose fiber	1 Blue cellulose fiber removed
Black Kite	Black cellulose fiber	1 Black cellulose fibers removed

Table S4. *Particles found in digestive system controls.*

Species	Typology	Action taken in the affected samples
Common Buzzard	Blue cellulose fiber	1 Blue cellulose fiber removed
Common Buzzard	Black cellulose fiber	1 Black cellulose fiber removed
Common Buzzard	Green cellulose fiber	1 Green cellulose fiber removed
Eurasian Sparrowhawk	Green cellulose fiber	1 Green cellulose fiber removed
Eurasian Sparrowhawk	Black cellulose fiber	1 Black cellulose fiber removed
Eurasian Sparrowhawk	Black and blue cellulose fibers	1 Blue cellulose fiber removed
Northern Goshawk	2 Blue polyester fibers	1 Blue polyester fiber removed
Northern Goshawk	Black cellulose fiber	2 Black cellulose fiber removed
Black Kite	1 Blue and 1 green cellulose fiber	2 Green and 1 blue cellulose fibers removed

Table S5. Main traits of the birds studied in this work.

Species	Habitat and Altitude	Diet and Feeding Behavior	Average Weight	References
Black Kite (*Milvus migrans*)	Prefers forested areas and is typically found at altitudes below 1000 m above sea level. behavior.	Opportunistic feeding behavior. Varied diet including small preys. However, they are also known to scavenge alongside roads and landfill.	650-865 g	(Blanco, 1994; Palomino and Carrascal, 2006)
Common Buzzard (*Buteo buteo*)	Found from sea level to 2000 m altitude, prefers areas below 1000 m. Inhabits a variety of wooded terrains, often near wetlands. Also found in rocky areas and open lands in winter.	Opportunistic feeding behavior. Their primary diet consists of small mammals. They can also scavenge or even feed on food scraps in areas like landfills.	589-1250 g	(Tapia and Salvador-Milla, 2016)
Northern Goshawk (*Accipiter gentilis*)	Primarily a forest raptor. Prefers mature forests with structural complexity. Can nest in both native and novel forests.	Opportunistic predator feeding on any bird, mammal, or reptile ranging from a few grams to several kilograms.	805-1650 g	(Zuberogoitia and Martínez, 2015)
Eurasian Sparrowhawk (*Accipiter nisus*)	Prefers regions with a mix of open terrain and mixed forests. Also nests in large city parks and gardens.	Primarily feeds on passerine birds, though females can capture larger birds like corvids or woodpeckers.	147-263 g	(Newton, 2010)

Blanco, G. (1994). Seasonal abundance of black kites associated with the rubbish dump of Madrid, Spain. J Raptor Res. 28(4): 242-245.
Newton, I. (2010). The sparrowhawk. A&C Black.
Palomino, D., Carrascal, L.M. (2006). Urban influence on birds at a regional scale: a case study with the avifauna of northern Madrid province. Landscape and Urban Planning, 77(3), 276-290. https://doi.org/10.1016/j.landurbplan.2005.04.003
Tapia, L., Salvador-Milla, A. (2016). Busardo ratonero – Buteo buteo (Linnaeus, 1758). In: Enciclopedia Virtual de los Vertebrados Españoles. Salvador, A., Morales, M.B. (Eds.). Museo Nacional de Ciencias Naturales, Madrid. http://www.vertebradosibericos.org/
Zuberogoitia, I., Martínez, J.E. (2015). Azor común–Accipiter gentilis (Linnaeus, 1758). In: Enciclopedia Virtual de los Vertebrados Españoles. Salvador, A., Morales, M.B. (Eds.). Museo Nacional de Ciencias Naturales, Madrid. http://www.vertebradosibericos.org/

Table S6. *Plastic composition and their presence in the digestive and respiratory systems.*

		Respiratory		Digestive	
	Acronym	Total	%	Total	%
Polyester	PES	41	56.1	127	55.7%
Acrylic polymers	ACR	14	19.2	50	21.9%
Polyethylene	PE	14	19.2	38	16.7%
Polypropylene	PP	2	2.7	8	3.5%
Polyamide	PA	-	-	3	1.3%
Polyvinyl chloride	PVC	1	1.4	2	0.9%
Polystyrene	PS	1	1.4	-	-
		73		228	

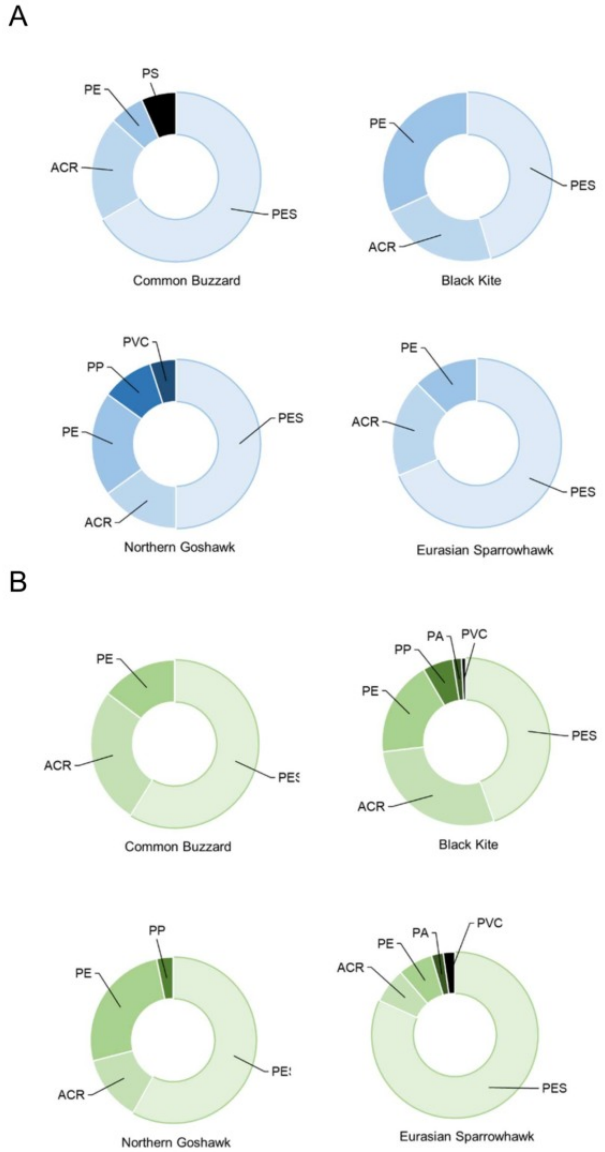


Figure S1: *Chemical composition of the MPs identified by micro-FTIR found (A) in the respiratory system and (B) in the digestive system. PES: polyester; PE: polyethylene; PA: polyamide; ACR: acrylic; PP: polypropylene; PVC: polyvinyl chloride; PS: polystyrene.*