

Article

Unraveling Plastic Pollution in Protected Terrestrial Raptors Using Regurgitated Pellets

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Abstract: The threat of plastic pollution has escalated to unprecedented levels, with particular concern surrounding microplastics (MPs) and artificial fibers or particles (AFs) due to their wide distribution across ecosystems and their bioavailability to wildlife. Although research on the impact of plastic on wild birds is rapidly growing, knowledge of terrestrial species remains limited, especially regarding raptors, which have been significantly understudied. Here, we investigated the prevalence of MPs and AFs in regurgitated pellets from six protected terrestrial raptor species, namely the Cinereous Vulture (*Aegypius monachus*), the Bonelli's Eagle (*Aquila fasciata*), the Little Owl (*Athene noctua*), the Lesser Kestrel (*Falco naumanni*), the Red Kite (*Milvus milvus*), and the Barn Owl (*Tyto alba*), collected between 2022 and 2023. Our analysis revealed that 68% of the pellets contained MPs (47 out of 69), and 81% contained AFs (56 out of 69). Additionally, two macroplastics were found inside the pellets: a cable tie in a Red Kite and a bird identification ring in a Cinereous Vulture. The concentrations (mean \pm standard error of the mean) were 2.39 ± 0.39 MPs/pellet and 5.16 ± 0.72 AFs/pellet. The concentration of MPs and AFs varied significantly among some of the studied species; however, no significant differences were observed among urban, rural, and protected areas. This could indicate that contamination levels are mainly related to the type of species. Fibers emerged as the predominant contaminant shape, with six different polymers identified, among which PET, PE, and acrylics were the most prevalent. These findings highlight that plastic pollution has reached protected terrestrial raptors and that the impact of plastic on their life cycles needs to be assessed.

Keywords: artificial fibers; microplastics; One Health; raptors; regurgitated pellets



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1. Introduction

Currently, the ubiquity of plastic products is undeniable. Almost all aspects of daily life involve plastics, from clothing and packaging to construction materials [1,2]. Their widespread consumption, propelled by their versatile properties [3], is so extensive that sedimentary deposits are now recognized as a geological proxy for the beginning of the Anthropocene [4]. As scientists and policymakers strive to develop strategies to mitigate plastic production's ongoing expansion, the need for a One Health approach is becoming increasingly apparent [5]. These concerns stem from their long-lasting nature, which can

have negative implications, such as their distribution across ecosystems, both spatially and temporally [6–8]. Particularly concerning are microplastics from 5 mm to 1 µm in size (MPs) [9]. Their small size contributes to their easy dispersion across all ecosystems [10,11]. Moreover, their ease of ingestion by wildlife, whether accidental or intentional, exacerbates concerns about their ecological impact [12]. Often overlooked and understudied, additional polymer types, such as modified celluloses, can also be released and dispersed within ecosystems and be ingested by wildlife, complicating the issue [13,14].

The biodiversity of wild birds is declining due to a variety of factors, with plastic pollution emerging as a potential contributor [15,16]. Time and resource limitations, and the challenges associated with elusive species, make mapping all taxa to assess the extent of this impact unfeasible. This underscores the need for employing measurement shortcuts, such as the employment of bioindicator species [17,18]. Amongst potential indicator taxa, raptor species stand out because of their vital role in the ecosystem. Raptors, as long-lived apex predators that accumulate contaminants and are distributed across large geographical areas, can be used to track spatiotemporal trends of pollutants and identify adverse effects [19]. Several articles evaluate the impact of plastic pollution on these taxa, focusing on ingestion [20–22], accumulation [18], entanglement [23], and behavior, e.g., deposits in nests [24]. However, most of them are focused on marine raptors, while terrestrial raptors are less studied [22].

Studies of plastic pollution in raptors often include necropsies [18,22,25]. However, this approach proves unsuitable for systematic monitoring due to its reliance on the chance discovery of deceased raptors or animals treated within anthropogenic environments. Nevertheless, other methodologies exist since raptors can regurgitate indigestible material in the form of pelleted material, which consists of parts of their prey, such as bones, fur, feathers, scales, and other hard or fibrous materials [26]; these pellets can be considered as an alternative to necropsy samples. For decades, analyzing the composition of these regurgitated pellets has served as a non-invasive method to study diet composition, provide qualitative data on local populations [27,28], and conduct pollution monitoring [29–31]. Researchers are increasingly using raptor pellets to investigate macroplastic and MP ingestion [20,32–34]. Here, we investigated the prevalence of MPs and AFs in 69 regurgitated pellets from six protected terrestrial raptor species, which were collected during the spring and summer seasons of 2022 and 2023 (Figure 1; Table S1). Samples were gathered from various locations, including rural and urban area, as well as protected areas across Spain.

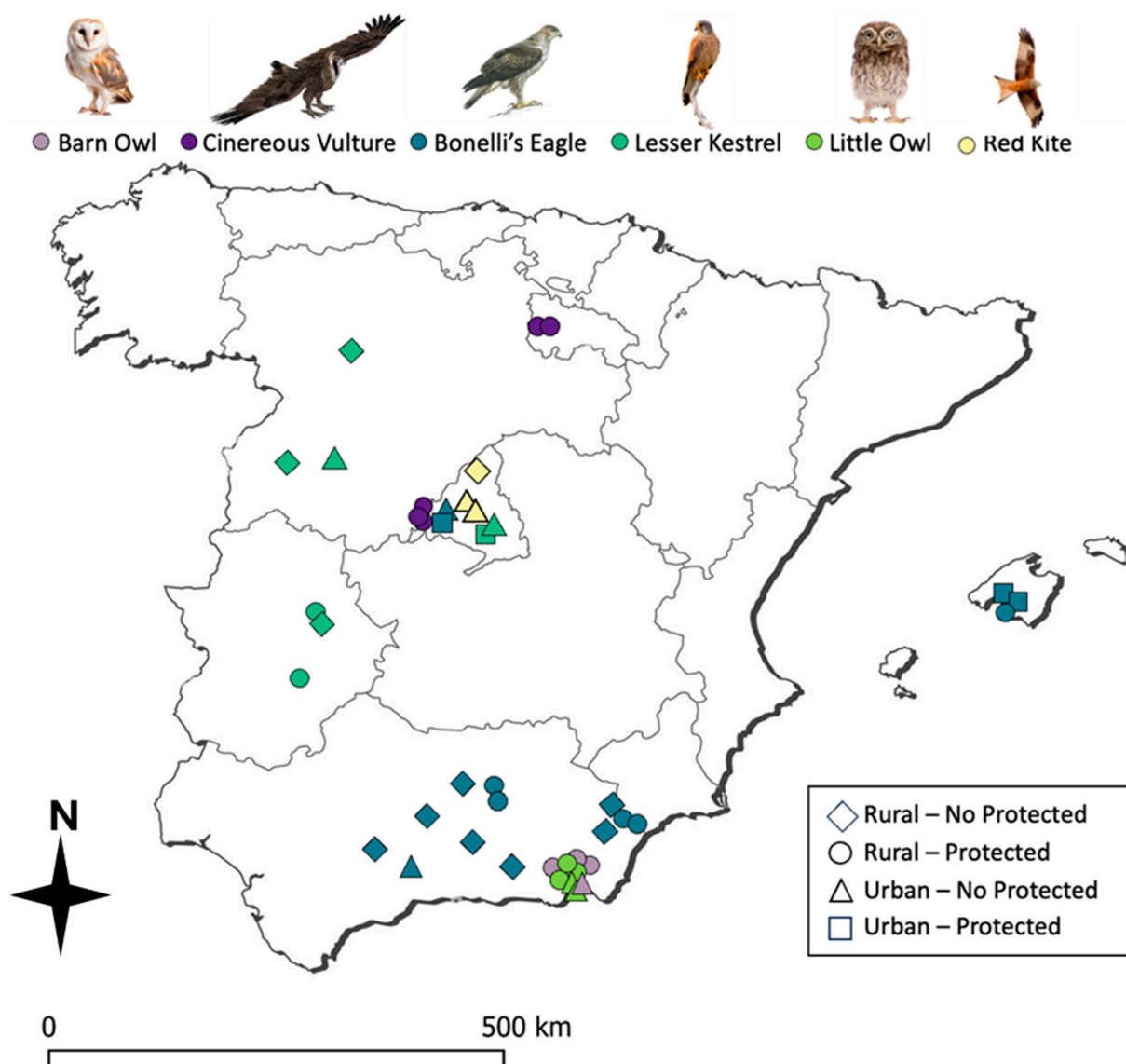


Figure 1. Geographical locations of sampling sites where regurgitated pellets of each protected terrestrial raptor species were found, collected between 2022 and 2023.

2. Materials and Methods

2.1. Sample Collection

The species included in the study (Table S2) were Cinereous Vulture (*Aegypius monachus*, N = 7), Red Kite (*Milvus milvus*, N = 7), Bonelli's Eagle (*Aquila fasciata*, N = 27), Lesser Kestrel (*Falco naumanni*, N = 13), Little Owl (*Athene Noctua*, N = 8), and Barn Owl (*Tyto alba*, N = 7). All of these species of terrestrial raptors are protected by Spanish and European legislations [35,36].

2.2. Sample Preparation

Each pellet was weighed and photographed and then digested in a 10% KOH solution for 48 h at a temperature of 50 °C and a stirring speed of 80 rpm. Following this initial digestion phase, the samples were sequentially filtered through meshes of varying sizes (300 µm, 150 µm, and 25 µm) to separate particles of different sizes. This was achieved using a filtering ramp system of stainless steel (ECOLAN model FL-S) connected to a vacuum pump. The filters underwent a second chemical digestion process using H₂O₂ (33% w/v) at a temperature of 50 °C for 24 h to eliminate any remaining organic material.

Following this step, the samples were filtered once more and then transferred to Petri dishes in preparation for further analysis.

2.3. Analysis and Classification of Microparticles

Potential anthropogenic particles were identified and photographed with a stereomicroscope (Motic[®] SMZ-171 BLED, Barcelona, Spain) with an integrated Moticam[®] X3 camera (Barcelona, Spain). To analyze the chemical composition of these particles, the micro-Fourier-transform infrared spectroscopy (micro-FTIR) method was employed using the Perkin-Elmer Spotlight 200 Spectrum Two system. Particles were placed on a potassium bromide (KBr) disk, and their spectra were compared against a database from the Analytical Chemistry, Physical Chemistry, and Chemical Engineering Department at the University of Alcalá using OMNIC 9 software. A match of 70% or higher was deemed accurate for categorizing the microparticles as MPs, considering the typical degradation observed in environmental samples [37,38]. Regenerated cellulose, such as cotton and linen fibers, which presented non-natural colors (white or transparent fibers were excluded in this study) and semi-synthetic fibers (rayon/viscose/cellophane), were classified as artificial fibers (AFs). They were assigned the same category since their spectra are closely identical, and, therefore, they are difficult to differentiate, especially in the case of the microparticles found in the environment due to weathering processes [39–41].

Following identification, the dimensions of all AFs and MPs were measured using ImageJ software 1.50d (Madison, WI, USA). The classification was based on shape, distinguishing between fibers or fragments. Additionally, the equivalent spherical diameter (d_v) was calculated based on these measurements. For fibers, volume and mass were estimated assuming a cylindrical shape, using average density values of the polymers [42]. For fragment particles, volume was estimated by approximating each particle to an ellipsoid shape, with the third dimension, which was not directly measured, assumed to be the average of the other two measured dimensions. The distribution of MP sizes was analyzed using a power law model, focusing on the cumulative frequency distribution function (CFD) and its relationship with particle size. The model parameters were estimated using maximum likelihood estimation, with bootstrapping ($n = 1000$) being used to determine standard deviations.

2.4. Contamination Prevention and Quality Control

Strict protocols were followed to prevent contamination throughout sample collection and processing. The use of any plastic material was avoided. Laboratory personnel wore 100% pink cotton lab coats. All metal, steel, and glass material were meticulously cleaned with Milli-Q water, wrapped with aluminum foil, and subjected to heating at 350 °C for 4 h to remove any potential organic microparticle residues that could interfere with the analysis. Environmental contamination was monitored by pairing each sample with a control that underwent the same process. When there was a positive match in typology and color between the control and the sample, all matching particles were excluded from the analysis. A total of 22 particles identified in the controls matched those in the samples and were therefore excluded (Table S3).

2.5. Statistical Analysis

The data were analyzed using RStudio (v 4.3.1; RStudio Team, 2023, Boston, MA, USA). The normality and homoscedasticity of the data were assessed using the Shapiro–Wilk and Levene’s tests, respectively. Due to the limited number of samples, we used the bootstrapping method employed to obtain 95% confidence intervals for the mean values [43], using the R package ‘boot’ [44] with a sample size of $n = 1000$. Permutation tests (Pesarin & Salmaso, 2010) from the R ‘perm’ package [45] were used to evaluate significant differences in contaminant presence across species and to determine whether contaminant levels varied based on habitat type, categorized as non-protected urban, protected urban, non-protected rural, and protected rural areas. Following the statistical results, a

False Discovery Rate (FDR) was applied. Lastly, due to the non-normal distribution of the data, Spearman correlations were employed to examine the relationship between the contaminant and the weight of species and pellets and their distribution.

3. Results

3.1. Characterization and Occurrence of Macroplastics in Regurgitated Pellets from Protected Terrestrial Raptor Species

Two macroplastics (plastic pieces larger than five millimeters long) were found in the regurgitated pellets of two different species (Figure 2). The prevalence per species was 14.3% (1 out of 7) for both the Red Kite and the Cinereous Vulture, resulting in a total pellet prevalence of 2.9% (2 out of 69).

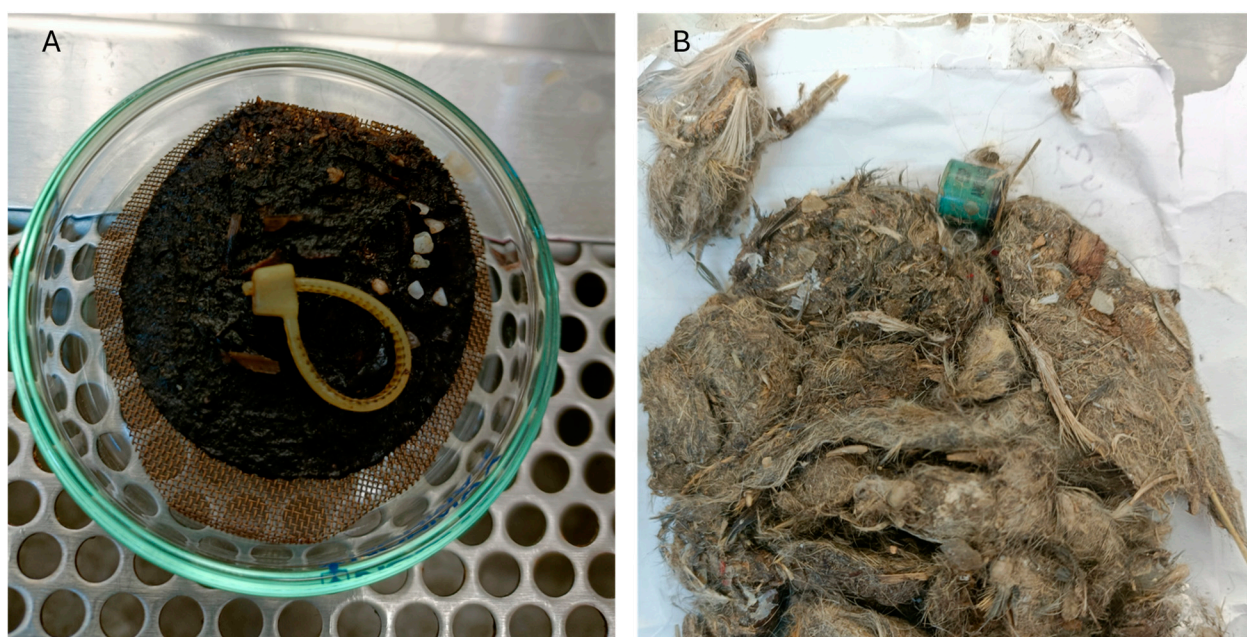


Figure 2. Macroplastics found in the regurgitated pellet of a Red Kite (A) and Cinereous Vulture (B). The photographs were taken by the authors.

Specifically, a white nylon zip tie was identified in a pellet retrieved from a Red Kite collected in a non-protected urban area located at the center of the Iberian Peninsula (Figure 2A). Additionally, a bird identification ring made of polymethylmethacrylate, likely originating from the breeding of ringed pigeons, was detected in a pellet obtained from a Cinereous Vulture in a protected rural area (Figure 2B).

3.2. Characterization and Occurrence of MPs and AFs in Regurgitated Pellets from Protected Terrestrial Raptor Species

3.2.1. Chemical Composition

A total of 590 microparticles were identified as anthropogenic. Chemical analyses categorized them as 130 MPs or 325 AFs. The other 135 items were either natural materials or were indeterminable due to identification confidence falling below the 70% threshold required [38,46]. Regarding MPs, a total of six polymers were identified (Figure 3). Polyester (PET) constituted 50% of all polymers found, polyethylene (PE) accounted for 25% of the plastics, acrylics (ACR) accounted for 15%, and polyamide accounted for 8%, while other plastics, including polyvinyl chloride (PVC) and polypropylene (PP), represented 1% each. Collectively, PET, PE, and ACR constituted 90% of the MPs, highlighting their prevalence among the environmental samples analyzed.

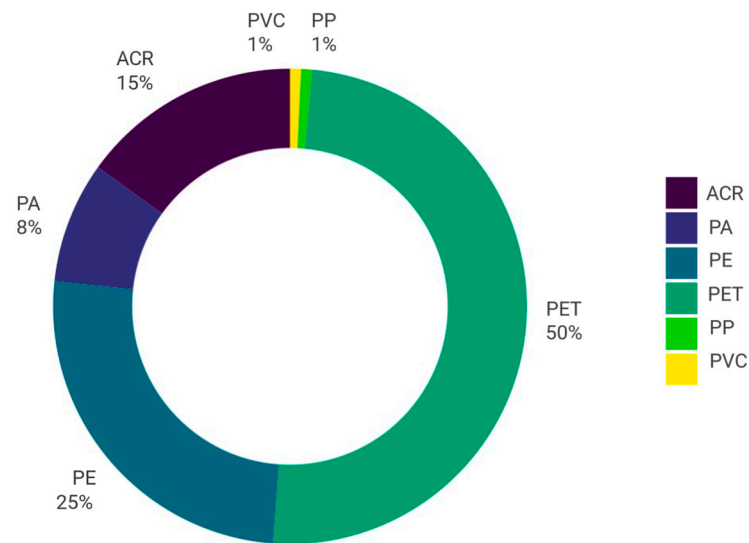


Figure 3. The chemical compositions of the MPs found in the pellets identified by micro-FTIR.

The prevalence of PET ranged from 29% to 59% across species (Table S4). PE ranged from 0% to 57%. ACR ranged from 0% to 36%. The prevalence of PA was notable, ranging from 0% (3 species) to 43% (Barn Owl). PVC was only found in the Little Owl, and PP was only found in Bonelli's Eagle.

3.2.2. Shapes and Sizes of MPs and AFs

Fibers represented 97.6% (444 of 455 microparticles identified) of contaminants. In the case of MPs, 97.7% (127 out of 130) were fibers, and the remaining microparticles were three irregularly shaped fragments found across a specimen of Cinereous Vulture, Bonelli's Eagle, and Lesser Kestrel. A similar trend was repeated for the AFs, where fibers accounted for 97.5% (317 out of 325), and eight fragments were found in total, of which four were found in Bonelli's Eagle, two in Cinereous Vulture, one in Red Kite, and lastly, one in Little Owl.

The equivalent diameter, d_v , was calculated based on the width and length, measured for all MPs and AFs (Table S5). d_v represents the diameter of a sphere with the same volume as the particle. The d_v s of MPs was 91.25 μm , and it was 74.64 μm for AFs. Significant differences were observed between species (Table S6). The AFs found in Red Kite were smaller than those present in Bonelli's Eagle (Kruskal–Wallis test, FDR correction, $p < 0.05$) and Lesser Kestrel (Kruskal–Wallis test, FDR correction, $p < 0.05$). In the MPs, there was also an observable difference between Lesser Kestrel and Red Kite (Kruskal–Wallis test, FDR correction, $p < 0.05$; Table S6), with those found in Red Kite being smaller.

Based on d_v and the density of each polymer, the mass of the polymers was calculated (Table S7). The mass of the MPs (721.85 ng) was higher than that for the AFs (512.92 ng). The difference in weight could be attributed to the bigger size of the MPs, but this difference was not significant (Mann–Whitney U test; $p > 0.05$). Furthermore, we calculated the probability distribution function [P(x)] of the MP, which was represented as a log-log plot, following a Normal Distribution model (Figure S1). The scaling exponent α was determined to be 4.71, which describes the slope of the distribution. The minimum particle size, $x(\text{min})$, observed in the distribution was 88.13, corresponding to the smallest particle size that can be accurately measured or has a physical significance in this context. The probability P(xmin) of observing the minimum particle size is 0.46, indicating a relatively high occurrence of particles around this size. The model shows a steep slope, highlighting the rarity of larger particles. Researchers have observed that smaller plastic fragments tend to break down into even smaller pieces due to environmental weathering or ingestion by organisms [47,48].

3.2.3. Abundance and Concentration of MPs and AFs per Regurgitated Pellet

The prevalence of both MPs and AFs was observed in all species, with varying percentages between them (Figure S2). For MPs, 68% of the pellets contained MPs (47 out of 69) varying from 62% in Lesser Kestrel to 86% in both Cinereous Vulture and Red Kite. In contrast, the presence of AFs was generally higher (81.2%; 56 out of 69), with the lowest recorded at 50% in Little Owl and the highest at 93% in Bonelli's Eagle. Notably, Cinereous Vulture displayed an equal presence percentage for both MPs and AFs at 86%.

The concentrations (mean \pm standard error of the mean) were 2.39 ± 0.39 MPs/pellet and 5.16 ± 0.72 AFs/pellet. Some statistically significant differences were found between species (Figure 4; Tables S8 and S9). Cinereous Vultures and Red Kites presented a significantly ($p < 0.05$) higher load of MPs per pellet (3.90 ± 1.56 and 4.21 ± 0.95 , respectively) than Lesser Kestrel (0.85 ± 0.46). Furthermore, Red Kite also showed a significantly ($p < 0.05$) higher load of MPs per pellet than Little Owl (1.59 ± 0.54). Regarding the AF concentration (Figure 4B), a significant difference ($p < 0.05$) between Little Owl (0.88 ± 0.48 AFs/pellet) and Cinereous Vulture (4.27 ± 1.23 AFs/pellet) and ($p < 0.001$) Bonelli's Eagle (7.60 ± 1.21 AFs/pellet) could be seen. Additionally, there were significant differences ($p < 0.01$) between Lesser Kestrel (2.60 ± 0.81 AFs/pellet) and Bonelli's Eagle (7.60 ± 1.21 AFs/pellet).

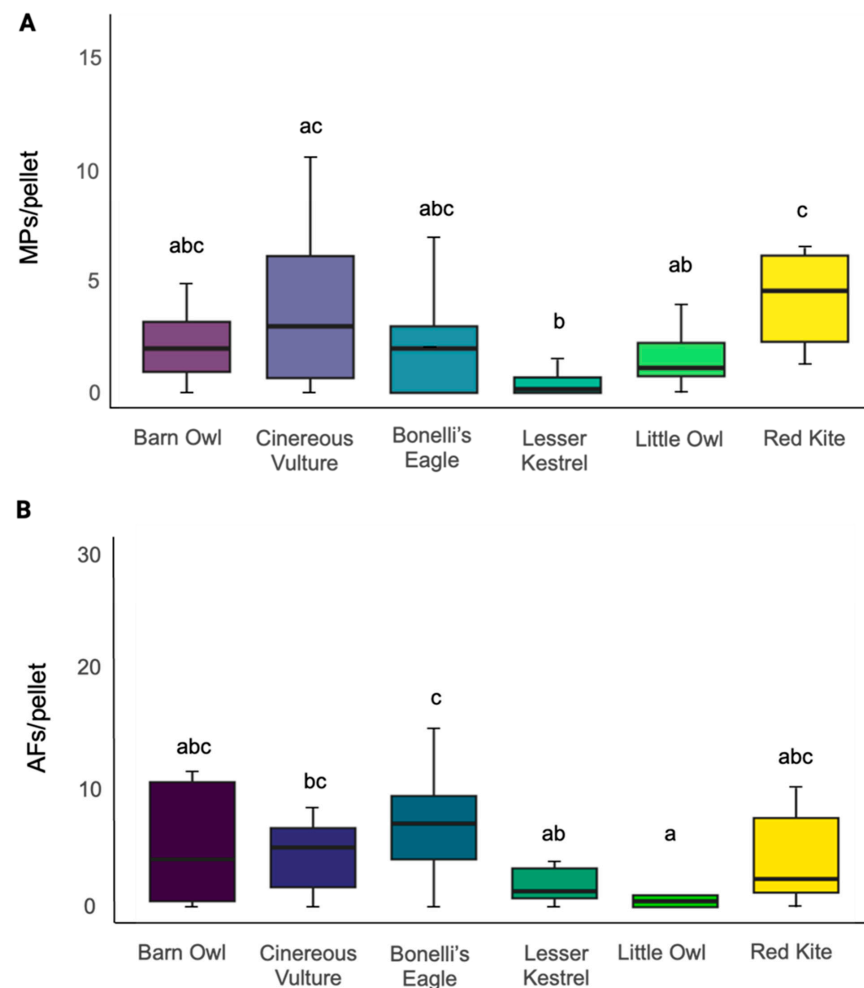


Figure 4. A boxplot of the total microplastics per regurgitated pellets of different bird species. In the upper panel (A), the total MPs/pellet is shown, and in the lower panel (B), the AFs/pellet is shown. Different letters indicate significant differences among MPs/pellet (A) or AF/pellet (B) in each species ($p < 0.05$).

To investigate contamination patterns, we divided the areas where the pellets were collected into four zones: urban and rural areas, and within those, protected and non-protected areas. The classification criteria were based on the distance (>10 km) to a city that has >15,000 inhabitants. Furthermore, the areas were subdivided into non-protected and protected areas based on Spanish regulation [49]. No significant differences were found in the MP/pellet and AF/pellet concentrations among the different areas (Table S10). Furthermore, we also tested the differences between the different areas within each specific species, but again, no significant differences were found (Table S11).

Given the absence of significant differences among the areas, it is important to identify which factors most influence contamination levels. To this end, we examined the correlation between the total contaminants per pellet (AF/pellet + MP/pellet) and the species weight (Spearman's $\rho = 0.4$, $p < 0.001$; Figure S3). The correlation between the total contaminants and the areas was also tested (Spearman's $\rho = 0.18$; $p > 0.05$), indicating no correlation between these two factors. These results demonstrate that, for this study, the species weight is among the predominant factors influencing the MPs/pellet and AFs/pellet concentrations, whereas the location cannot explain the variabilities observed.

4. Discussion

Raptors populations confront global and local threats, encompassing climate change, habitat loss, and pollution [50,51]. Among these threats, plastic contamination has emerged as a significant concern; however, the ingestion of MPs and AFs by raptor species is understudied. It is important to understand the ecological implications of plastic pollution on their health and biodiversity [18,25,51]. Identifying, characterizing, and assessing the impacts of plastic ingestion is essential for forecasting the ecotoxicological consequences for raptors populations [52]. Moreover, the repercussions of plastic pollution on terrestrial food webs are still largely understudied compared to its aquatic counterpart [53]. In this study, six protected terrestrial raptors were studied for MP and AF ingestion. All of these species share the common trait of regurgitating a portion of their indigestible diet, including bones, stones, and plastics, in the form of pellets. This regurgitation mechanism might reduce the retention of MPs in their digestive system. This could potentially lessen the adverse health effects of MPs compared to species that do not have such mechanism [54]. However, an unknown proportion remains in their digestive tracts. The studies of [18,25] demonstrated the accumulation of MPs in the gastrointestinal tracts of raptors. Thus, it is important to understand the proportion of contaminants expelled in the pellets and those that accumulate in the digestive tracts. Nevertheless, analyzing pellets can be valuable for assessing the spatiotemporal trends of contamination, particularly for monitoring plastic pollution in protected raptors [54,55]. In our case, the selection of these species for this work was deliberate due to their importance as emblematic species and their roles in conservation. Species are often chosen for conservation efforts based on factors such as their local presence, ecological importance, charisma, and urgent conservation status [56]. Despite debates regarding their definition and scientific rigor, these species hold immense relevance in biodiversity conservation [57]. Documenting their threats and highlighting their vulnerability can help enhance awareness. Moreover, our focus serves a larger purpose: indirectly protecting ecosystems through the development of targeted management policies to mitigate plastic pollution and its environmental and animal health impacts. This is essential in the context of the One Health paradigm [58,59].

As far as we know, among all of the species studied, MP concentrations in pellets have only been investigated in the Barn Owl [53,60]. Consequently, this study represents the first report on the ingestion of MPs in these species. Ref. [53] investigated the diets and MP concentrations in the pellets of Barn Owl, revealing the presence of MPs in 33% of the pellets. In a separate study, the presence of MP was reported in only 18% (22 of 122 pellets) [60]. In our study, 77% of the pellets presented MPs with an average concentration of 2.17 ± 0.70 MPs/pellet. These pellets were collected in the surrounding areas of the region in Spain with the highest concentration of greenhouses (Almeria).

In 2020, the total area of greenhouses in the region was 32,554 hectares. A significant proportion of the materials used in greenhouse construction includes plastics, which are subsequently released into the environment and degraded into MPs [61]. This accounts for the higher prevalence of MPs observed in this study. The pellets of the Little Owl were also collected in this region, with 75% of them containing MPs at an average concentration of 1.59 ± 0.54 MPs/pellet. These results emphasize the importance of considering species-specific and habitat characteristics when assessing the accumulation of MPs in organisms.

The substantial variability in contaminant concentrations can be attributed to morphological characteristics, specifically the weight of each species (Table S2). This is evident when comparing species such as the Lesser Kestrel, with an average weight of 150–200 g, with larger species like the Cinereous Vulture, weighing from 7 to 12.5 kg, or mid-sized species such as Bonelli's Eagle (1.9–2.5 Kg) and Red Kite (0.8–1.2 Kg) [62–64]. Larger animals tend to have higher exposure to MP and AF contamination due to their increased food and water intake. Consequently, this can explain why there were significantly higher concentrations of MPs in Cinereous Vultures and Red Kite than in the Lesser Kestrel and in Red Kite compared to Little Owl. Similarly, higher concentrations of AFs were found in Cinereous Vultures and Bonelli's Eagles compared to Little Owls and in Bonelli's Eagles compared to Lesser Kestrels. Another contributing factor could be their dietary preferences and feeding behaviors (Table S2). Raptors play a crucial role in maintaining ecosystem health. As nature's clean-up crew, they scavenge on carrion, facilitating the decomposition process. By feeding on deceased animals, they contribute to the cleanliness of their habitats and prevent the spread of diseases across different trophic levels within ecosystems [52]. In recent decades, other species such as Black Vultures (*Coragyps atratus*) have frequently been observed feeding in landfills [32,52,65]. Ref. [65] reported that MPs were present in 17.3% of the analyzed pellets from Black Vultures in the Argentinian Patagonia. Similarly, ref. [32] investigated the occurrence of contaminants, such as pesticides and metals associated with MPs, in Black Vultures' pellets collected from a landfill in Mexico, finding that 77% of the pellets contained MPs with an average concentration of 6.70 ± 5.8 MP total/pellet. In our study, we observed a higher occurrence of MPs at 86%, with a slightly lower mean concentration of 3.90 ± 1.56 MPs/pellet. These discrepancies can be attributed to the specific sampling locations and the human impact on those areas. For instance, in Mexico, the samples were collected in the surrounding areas of a landfill, whereas in Spain, they were collected in a rural area with a low population density.

Interestingly, no significant differences were observed for the concentrations of MPs and AFs among non-protected urban, protected urban, non-protected rural, and protected rural areas. This lack of variation was primarily due to species specificity rather than environmental location. When examining the distribution of pellets of the different species sampled, we found that species distribution was not uniform across the areas but rather species-specific. This evidence underscores the importance of considering these traits to conduct a comprehensive analysis of contaminant ingestion.

While most contaminants were microparticles, it is noteworthy that some macroplastics were detected in 14.3% of the pellets of Red Kite and Cinereous Vulture. The pattern of low macroplastic frequency aligns with findings from other studies. Refs. [22,66] found a low occurrence in Bald Eagles (*Haliaeetus leucocephalus*) and Barred Owls (*Strix varia*), where only 5 individuals out of 234 presented plastics larger than 2 mm. On the other hand, ref. [65] reported a higher presence of macroplastics in the pellets of Turkey Vultures (*Cathartes aura*; 24.5%). These results suggest that macroplastics are less ingested or retained in raptor species; however, the rate of ingestion also varies depending on the foraging strategy and dietary preferences. For example, Vultures are known as scavengers and are frequently found feeding in landfills [32,65], while Bald Eagles are generalist predators.

Regarding the chemical composition of contaminants, our findings suggest that the ingestion of different types of polymers is generally not selective. Rather, raptors randomly ingest the more abundant polymers within their respective ecosystems. The percentages of the different types of plastics are consistent with documented concentrations in the

Spanish region [18,37,67]. These studies investigated the concentrations of MPs and AFs in environmental compartments (such as air and water) and raptors. PET, PE, and ACR were the most represented polymers. These results extend beyond our study area and are consistent with global concentrations reported in other studies [25,68,69]. These plastics represent materials commonly used in everyday applications. PE, for example, is one of the most commonly used polymers globally, accounting for 22.1% of the total production in 2022 [70], making it highly prevalent in the natural environment and readily bioavailable for consumption by various organisms [71,72]. ACR is frequently employed in the textile industry. Recent studies show a substantial proportion of MPs in the environment and organisms originated from the textile industry [25,73]. Notably, many AFs released by the textile industry consist of natural materials like cotton or wool or celluloses that have been modified with chemical additives, such as dyes, to facilitate processing [40,74,75]. The concentrations of AFs are higher in the environment compared to MP fibers [13,76]. This trend was also observed in our study, where the concentrations of AFs in the pellets were higher for all species, except for the Little Owl. However, AFs are often overlooked in studies despite their potential to enter and harm organisms through inhalation and digestion [73,77].

To gain a comprehensive understanding of the impact of MP and AF pollution on the environment and organisms, it is essential to consider not only the chemical compositions and concentrations of these particles but also to obtain information about their characteristics, such as their size [42,78]. These characteristics play a crucial role in influencing their behavior, fate, and bioavailability for organisms [79]. The sizes of MPs can determine their transport mechanisms and eventual accumulation sites [80]. Particles smaller than 150 μm are expected to leave the gut and be translocated to other tissues, organs, or cells [81,82]. Additionally, particle shape can influence retention time, as fibers can become entangled in tissues and remain in the body for longer periods [80]. Furthermore, the type of polymer and the associated chemical additives can also have different influences on organism health [83]. Some plastics and additives are known to be carcinogenic, while others are more inert [1,84]. Therefore, investigating these properties is crucial for gaining new insights into mitigating and preventing environmental impacts [78].

5. Conclusions

This study investigated MPs and AFs in the regurgitated pellets of six emblematic raptor species across Spain. Among the eight polymers that were characterized as cellulosic fibers, PET, PE, and ACR were the most prevalent. The concentration of MPs and AFs varied significantly among some species, though no significant differences were detected between urban, rural, and protected areas. Fibers were the dominant contaminant by shape. These findings underscore that plastic pollution has reached protected terrestrial raptors. Future research could explore seasonal variations in contaminant presence and their impact on the life cycles of raptors.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microplastics3040041/s1>, Table S1. Information on the sampling sites where regurgitated pellets of each protected terrestrial raptor species were collected between 2022 and 2023. The criteria used to classify the areas were based on the fact that areas located at a distance > 10 km to a city that has >15,000 inhabitants were categorized as rural. Areas not meeting these criteria were designated as urban; Table S2. Species characteristics and traits; Table S3. Microparticles (MPs) and artificial fibers or particles (AFs) found in controls; Table S4. Prevalence in percentage of each type of polymer by species; Table S5. Size d_v (μm) of the MPs and AFs per species; Table S6. Size comparisons of the MPs and AFs per species. Kruskal-Wallis test, with a post-hoc FDR correction; Table S7. Polymers densities; Table S8. The concentrations of MPs and AFs in regurgitated pellets; Table S9. Results of Permutation test ($n = 1000$) of multiple comparisons between species for the Total Contaminant per pellet, with a post-hoc FDR correction; Table S10. Comparison between different areas (categorized as non-protected urban, protected urban, non-protected rural, and protected rural areas). Kruskal-Wallis test, with post-hoc FDR corrections;

Table S11. Comparison between different species per areas (categorized as non-protected urban, protected urban, non-protected rural, and protected rural areas). Kruskal-Wallis test, with post-hoc FDR corrections; Figure S1. Particle size distributions as CFD, $P(\text{size} > x)$; Figure S2. Prevalence of anthropogenic pollution defined as the number of pellets per species containing at least one particle (MPs or AFs) divided by the total number of pellets analyzed per species; Figure S3. Correlation plot for the species weight with the total amount of contaminants per pellet (MP/pellet + AF/pellet).

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