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Short Communication

Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers

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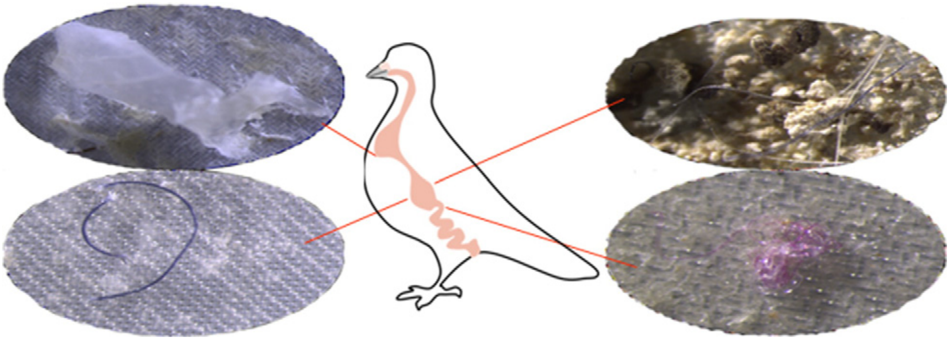
HIGHLIGHTS

- Microscopic litter in the digestive tracts of terrestrial birds is firstly studied.
- Natural fibers (136 items) accounted for 37.4% of the total microscopic litter.
- Two hundred fibers and 28 fragments were classified as microplastic particles.
- Microscopic litter was ubiquitous in the terrestrial ecosystem of the study area.

GRAPHICAL ABSTRACT



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ABSTRACT

The level of contamination by microscopic anthropogenic litter (0.5–5 mm) in terrestrial ecosystems is not well understood. After chemical digestion in 10% KOH, microscopic anthropogenic litter from the gastrointestinal tracts of 17 terrestrial birds was identified and categorized under a stereomicroscope based on its physical properties and melting tests. In total, 364 items from 16 birds were identified as microscopic anthropogenic litter, ranging in size from 0.5 to 8.5 mm. No relationship between plastic load and body condition was found. Natural fibers, plastic fibers and fragmented plastics represented, respectively, 37.4% (136 items), 54.9% (200 items) and 7.7% (28 items) of total litter items. Small sample sizes limited our ability to draw strong conclusions about the metabolism of natural fibers, but the decline in the proportion of natural fibers from the esophagus to stomach to intestine suggested that they may be digestible. Particles smaller than 5 mm represented more than 90% of the total number of pollutant items. Particles with colors in the mid-tones and fibrous shapes were overwhelmingly common particles. The results reflect pollution by microscopic anthropogenic litter in the terrestrial ecosystem of the study area. Microscopic natural fibers, which may disperse and adsorb chemical pollutants differently from microplastic and may pose an even greater risk, are in urgent need of further research.

1. Introduction

Anthropogenic debris (man-made material that enters the biosphere) and its associated toxic chemicals have raised growing concerns about the ingestion and transfer of anthropogenic debris by wildlife

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(Rochman, 2015). Plastic debris is responsible for the majority of anthropogenic debris in the marine environment (Bergmann et al., 2015). In the past decade, a new type of plastic contaminant, termed microplastic (<5 mm), has attracted attention (Barnes et al., 2009; Thompson et al., 2004). Due to its size and resemblance to prey species, microplastic can be ingested indiscriminately by a variety of lower trophic organisms, such as zooplankton (Cole et al., 2013), barnacles (Goldstein and Goodwin, 2013), sandhoppers (Ugolini et al., 2013) and fish (Foekema et al., 2013). Higher trophic levels can also passively ingest microplastic during routine foraging behavior or mistake particles for natural food items (van Franeker et al., 2011; Wright et al., 2013). However, microplastics are not the only material that poses such threats. Micro-sized artificial debris that is based on natural materials (e.g., natural or natural polymer fibers) is also ingested by organisms, though the reports are not very frequent. More than half of anthropogenic particles ingested by fishes in the English Channel were rayon, which is made of cellulose compounds (Lusher et al., 2013). Among detritivores, 27.6% of the digestive tracts of nine invertebrates colonizing the dead leaves of Neptune grass contained various sized and colored man-made cellulose fibers (Remy et al., 2015). By inspecting fish and shellfish on sale, Rochman et al. found that 28% and 25% of sampled fish from Indonesia and the USA and 33% of USA shellfish contained anthropogenic debris. All particles in fish from Indonesia were plastic, in contrast to those in the USA, which were primarily textile fibers (Rochman et al., 2015). The negative impacts of ingesting anthropogenic debris on wildlife range from physical harm such as entanglement, abrasion or blockage of the gut to chemical damage from leaching plastic additives, persistent organic pollutants (POPs) adsorbed from the environment (Teuten et al., 2009), and associated toxic dyes (Remy et al., 2015). Moreover, the global increase in wildlife cancer is thought to be a result of plastic pollution (Erren et al., 2009; Meyer-Rochow et al., 2015). Nevertheless, almost all research on anthropogenic debris ingestion is focused on aquatic organisms. This raises a question: “Are animals in terrestrial ecosystems safe from anthropogenic debris?” If not, what is the contamination level?

Birds occupying high trophic levels (Naert et al., 2007) not only ingest plastic debris directly but also indirectly via secondary ingestion (Verlis et al., 2013). Research on plastic ingestion by birds largely focuses on seabirds (Acampora et al., 2014; Auman et al., 2004; Connors and Smith, 1982; Lindborg et al., 2012; Ryan, 1987; Spear et al., 1995; van Franeker and Law, 2015; Wilcox et al., 2015). Adverse effects of plastic ingestion by seabirds include nutritional deprivation (Pierce et al., 2004), reduced body mass (Danner et al., 2009), decreased fat deposition (Connors and Smith, 1982), physical damage to the gut (Pierce et al., 2004) and chemical toxicity (Colabuono et al., 2010; Tanaka et al., 2013). An average of 29% of individual seabirds in the literature between 1962 and 2012 had plastic in their guts, and today, up to 90% of seabirds are estimated to ingest plastics. If these trends continue, this proportion could reach 99% by 2050 (Wilcox et al., 2015). Studies suggest that feeding behavior and the abundance of plastic in the environment are important determinants of plastic ingestion by birds (Shealer, 2002). Terrestrial birds have more diverse habitats and diets compared to aquatic birds, including seabirds (Sun et al., 2012). Anthropogenic debris pollution is believed to be pervasive in the terrestrial ecosystem as well as the marine environment (Ramos et al., 2015; Rillig, 2012; von Moos, 2010), and microplastic in terrestrial ecosystems has been documented in freshwater including lakes and rivers (Eerkes-Medrano et al., 2015; Wagner et al., 2014). Consequently, anthropogenic particle ingestion by terrestrial birds is plausible, but to the best of our knowledge, no data are available on anthropogenic particle ingestion by terrestrial fauna.

To bridge this knowledge gap, the present study was designed to address microscopic anthropogenic particle contamination in terrestrial birds from Shanghai, China. According to a common definition of microplastic (Thompson et al., 2004), particles less than 5 mm were defined as microscopic anthropogenic particles. This is the first specialized research on microscopic anthropogenic litter ingestion by terrestrial birds. We wanted to explore three questions: 1) can microscopic anthropogenic plastic be ingested by terrestrial birds? 2) What is the pollution level? 3) What are the characteristics of ingested particles (i.e., type, color and size)?

2. Materials and methods

2.1. Sampling

A total of 17 birds were obtained opportunistically from wildlife rehabilitators and birders in Shanghai. The 12 species examined were the common buzzard (*Buteo buteo*, N = 1, carnivorous); black kite (*Milvus migrans lineatus*, N = 1, carnivorous); common kestrel (*Falco tinnunculus*, N = 3, carnivorous); large hawk-cuckoo (*Cuculus sparverioides*, N = 1, carnivorous); cattle egret (*Bubulcus ibis*, N = 1, carnivorous); little grebe (*Tachybaptu sruficollis*, N = 1, carnivorous); dunlin (*Calidris alpina*, N = 1, carnivorous); common sandpiper (*Actitis hypoleucos*, N = 1, carnivorous); eyebrowed thrush (*Turdus obscurus*, N = 1, omnivorous); grey-backed thrush (*Turdus hortulorum*, N = 1, omnivorous); common magpie (*Pica pica*, N = 2, omnivorous); and spotted dove (*Streptopelia chinensis*, N = 3, herbivorous). In this study, all birds were received dead, died during attempted rehabilitation, or were euthanized due to serious injuries. No birds were killed for the purpose of this study. Once the specimens were available, body mass (g) and wing length (cm) were recorded (Supplementary material). The ratio of mass to wing length is referred to as the body mass index (BMI) (Acampora et al., 2014). The specimens were stored at −20 °C until dissection and analysis.

2.2. Contamination prevention

Milli-Q water and the reagents (ethanol, methanol, sodium iodide solution and 10% KOH) used in this study were filtered through Whatman GF/F filters prior to use. To avoid contamination, work surfaces were thoroughly cleaned with 100% filtered alcohol, and hands and forearms were scrubbed. Gloves (nitrile) and lab coats were worn throughout the study. Instruments were cleaned with filtered Milli-Q water after every specimen to avoid cross-contamination. The entire process was conducted in a chemical laminar hood. Nylon membrane filters (20 μm, NY2004700, Millipore) and glassware were checked under a stereo-microscope prior to use. Three blanks were run by sucking air through three nylon filters in the chemical hood.

2.3. Processing, separation, sorting and identifying

In the laboratory, specimens were dissected according to standard methods (Van Franeker, 2004). For each specimen, the complete digestive tract (esophagus, stomach and intestine) was removed. A new procedure modified from two previous studies was used in the present study (Foekema et al., 2013; van Franeker et al., 2011). Specifically, it was as follows: 1) Stomach (including proventriculus and gizzard) contents in each bird were poured into saturated sodium iodide solution (NaI, 1.6 g/cm^3). The mixture was shaken vigorously and allowed to settle overnight. The supernatants were poured through a 20-μm Nylon membrane, and retained items were transferred to a sealed petri dish for further analysis (van Franeker et al., 2011). 2) The esophagus and intestines of each bird were immersed in two jars filled with 10% KOH solution at room temperature for 2 to 3 weeks to dissolve the organic material completely. Then, the jar contents were sieved through nylon filters to collect the indigestible residue (Foekema et al., 2013). The filters were placed in an ultrasonic bath for 10 min in the NaI solution. The beakers carrying the filters for sonication were always covered with tin foil. After settling overnight, the supernatants were concentrated on the nylon filters, which were kept in the glass Petri dishes until analysis. All samples were extracted at one time.

Potential microscopic anthropogenic litter on the filters was evaluated under a stereomicroscope (Leica M165 FC at magnification 160×) by the following steps:

Step I (man-made particle identification): Based on the surface characteristics, internal morphology, and physical response, microscopic anthropogenic litter was identified (Desforges et al., 2015; Norén, 2007; Zhao et al., 2014). The detailed criteria were as follows: 1. no cellular or organic structures are visible; 2. fibers should be equally thick, not tapered at the end and should have a three-dimensional bend; 3. fibers are not segmented nor do they appear as twisted flat ribbons; 4. colored items are clear and homogeneously colored; 5. potential microscopic anthropogenic litter that is transparent or whitish is examined with extra care and under higher magnification; 6. particles should not be lustrous; and 7. fibers were bendable or soft. These selection criteria were applied to ensure that only MP particles (>0.5 mm) were considered in the present study (Hidalgo-Ruz et al., 2012; Rochman et al., 2015). Once classified as artificial material, particles were transferred into glass Petri dishes and ready for the following steps. The identification of artificial particles was performed conservatively, i.e., if the origin of a particle was hard to determine based on the protocol mentioned above, it was not included in the study.

Step II (characteristics of man-made particles): Individual items of microscopic anthropogenic litter were classified into two groups: fragments (e.g., hard and flexible pieces) and fibers (Zhao et al., 2015). Colors of microscopic anthropogenic litter were recorded and identified as one of three tone types: light (white, yellow, yellow-brown); mid (green, red, blue, etc.); dark (gray, black, dark red, etc.) (Carey, 2011). The longest dimension (mm) of each particle was measured.

Step III (plastic particle identification): Plastic particle identification was based on a melting test, which is reliable for particles larger than 50 μm (Enders et al., 2015; Gorokhova, 2015; Magnusson and Norén, 2014; Strand et al., 2015). Specifically, an insect pin was heated by flame and immediately placed in contact with the artificial particles. If the material melted like plastic and produced the odor of melting plastic, it was confirmed as a plastic particle. For each piece of microscopic anthropogenic litter, the melting test was repeated in triplicate.

Once confirmed plastic items were removed from the sample, the remaining items were categorized as natural particles.

2.4. Statistics

"Items" was used as the unit to describe the number of anthropogenic particles in this study. The Shapiro and Bartlett test were used because of the normality of the residues and homogeneity of their variances. The Kruskal-Wallis statistical test was used to analyze for multiple comparisons. If the test indicated significant differences, pairwise comparisons were performed with the Wilcox test. Differences were considered statistically significant when $p < 0.05$, and values are reported as the mean ± SD. Generalized linear models (GLM) were used to estimate the relationship between the quantity of ingested debris and the BMI of the birds. R 3.0.2 was used in all analyses.

3. Results and discussion

This study is the first account of microscopic anthropogenic litter in terrestrial birds. No particle contamination was detected by the procedural blanks in the chemical hood. Materials that were removed from the chemical hood were protected by tin foil. Although the lack of anthropogenic particles in the processed specimens supports the results of the blanks, even the clean blanks do not completely exclude the possibility of contamination being generated during the dissections and extractions. In future studies, blanks should be run throughout the processing, not only in the beginning.

In total, an estimated 364 items of microscopic anthropogenic litter, ranging in size from 0.5 to 8.5 mm, were found in 16 of 17 (94.1%) specimens, an average of 22.8 (±33.4) items per bird (Figs. 1a and 2). The particle identification was relatively reliable in that the synthesized criteria were able to unambiguously distinguish artificial particles larger than 0.5 mm from the others (Desforges et al., 2015; Rochman et al., 2015). However, the melting test to detect plastic-specific melting behavior and odor is more subjective. Other analytical approaches such as pyrolysis-gas chromatography–mass spectrometry (Pyr-GC–MS) (Fries et al., 2013) or Raman and Fourier transform infrared (FT-IR) spectroscopy (Lenz et al., 2015) should be employed to determine microplastics in the future. The mean abundance (22.8 ± 33.4 per bird) in the present study was an order of magnitude greater than the maximum density (5.9 ± 5.1 per fish) in the guts of seafood on sale in Indonesia as recorded by Rochman et al. (2015). Two outliers (*B. buteo*, 116 particles and *C. sparverioides*, 100 particles) in the experimental data might be responsible for the higher average abundance as well as for the high standard deviation (Fig. 1c). The fact that only dead birds were sampled in this study might lead to a selection bias regarding the abundance of microscopic anthropogenic litter in their digestive tracts. It is possible that some specimens died from complications or contamination related to ingesting the anthropogenic particles. Additionally, the common buzzard (*B. buteo*) is an opportunistic species, primarily preying on small mammals, birds, and a variety of other animals including insects, earthworms, snakes and carrion. This variable diet might increase the risk of anthropogenic litter ingestion via prey (secondary ingestion). Furthermore, the digestive crop of the common buzzard, used for food storage, may extend the retention time of the food as well as the anthropogenic debris. A lot of prey remains were observed in the digestive tracts of *B. buteo*. Large hawk-cuckoo (*C. sparverioides*) feed mainly on smaller insects (e.g., caterpillars), which may result in a higher ingestion rate of anthropogenic particles from the ground as they capture prey. Additionally, large amounts of food were retained in the digestive tract of the specimen. Finally, the small sample size also contributed to the high abundance of particles detected in this study. After rejecting the two outliers, the mean density by number in all specimens was 10.6 ± 6.4 particles per bird (Fig. 1b),

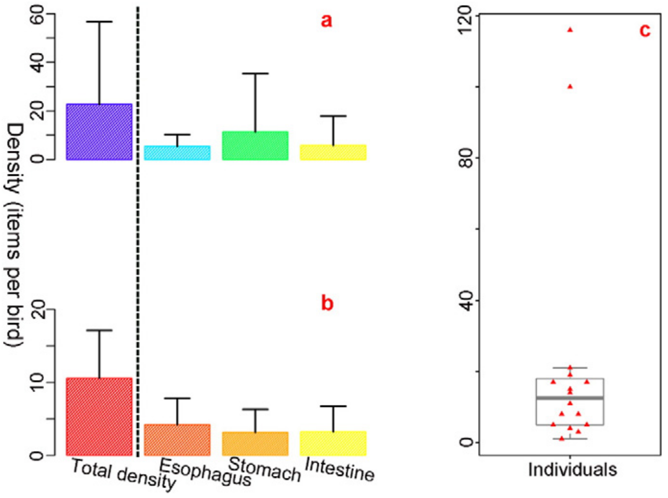

Fig. 1. MAL density (mean ± SE) in all the specimens and different digestive tracts (esophagus, stomach and intestine) with (a) and without (b) two outliers. Boxplot of number of MAL within bird individuals.

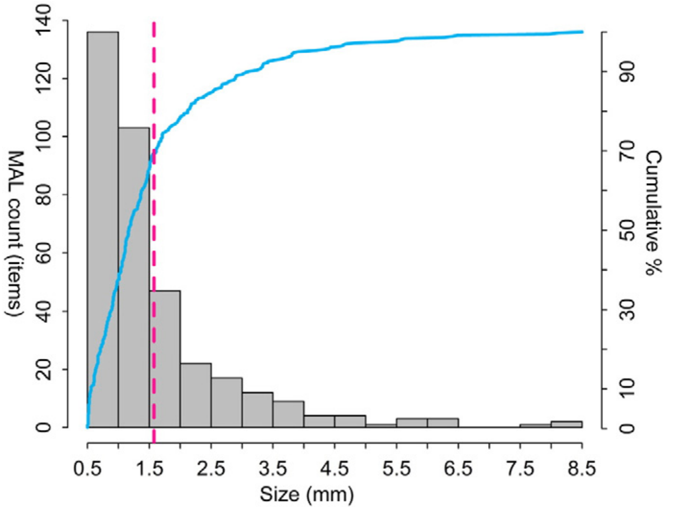

Fig. 2. MAL sample size histogram. The line represents cumulative percentage. Dashed line represents measured mean size.

which is similar to the average number of anthropogenic particles in seafood organisms from the USA and Indonesia (Rochman et al., 2015). Although the biased sampling method in the present study does not represent a random sample of the population, the results obtained do provide first-hand information on microscopic anthropogenic litter ingestion by terrestrial birds. These results are of conservation concern because three of the species are threatened and endemic to the Mediterranean basin. The common buzzard, black kite and common kestrel are included on the list of endangered and protected species of China.

No significant correlation between BMI and number of plastic particles per bird was found. Plastic sources, feeding habits and foraging areas may contribute to the variation in plastic traits among specimens. The 9 species of birds studied had variable feeding strategies and habitats. In addition, the detrimental effects of ingesting litter, such as absorbing toxins, may be hidden or delayed. Assessing the presence of other plastic-associated contaminants such as POPs and added plasticizers (Fries et al., 2013) is crucial to understanding the potentially hidden effects of anthropogenic litter ingestion and developing conservation plans for these species. In contrast to marine anthropogenic particles, microscopic anthropogenic litter in terrestrial ecosystems may be perceived as an environmental issue that is "closer to home," resulting in more scientific and public attention (Rillig, 2012).

The mean length of artificial particles was 1.6 ± 1.2 mm and ranged from 0.5 to 8.5 mm. Particles (<5 mm) accounted for more than 90% of the total items by number (Fig. 2). Due to the small size (0.5–8.5 mm) and primary fibrous particles in this study (Figs. 2 and 3), it is unlikely that birds actively ingested those particles by misidentifying them as food items. The microscopic anthropogenic litter in the birds suggested accidental ingestion while foraging. Additionally, particles in the guts of carnivorous birds might derive from the secondary ingestion of debris that was consumed by their prey. Another hypothesis is that the small plastic particles were the result of fragmentation of larger particles in the digestive tracts of birds. With regard to color, 81.6% (297 items) was mid-tone, followed by light 10.4% (38 items), and dark particles 8.0% (29 items) (Fig. 4). Colored particles were more abundant than light or dark ones (Kruskal-Wallis, $\chi^2 = 21.8796$, $df = 2$, $p = 1.774e-05 < 0.05$; Wilcox test, $V = 136$, $p = 0.0002375$, $0.0002349 < 0.05$). The dominance of mid-tone particles (red, blue, pink, etc.) may be the result of their prevalence in the environment, easy detection, or their resemblance to food, resulting in an actual color preference by the biota at lower trophic levels (Zhao et al., 2014). Fibrous and fragmented pieces accounted for 92.3% (336 items) and 7.7% (28 items) of the particles by number (Fig. 3). Fibers were more abundant than fragments (Wilcox test, $V = 136$ $p = 0.0002 < 0.05$). Two reasons might be responsible for the high fiber abundance. First, the smaller overall size of fibers that are folded, knotted or intertwined into an aggregate may increase their likelihood of ingestion. In fact, the fibers observed were often inextricably twisted (Fig. 3). Second, the ubiquity of fibers in clothing, furniture, female hygiene products and diapers may make them more bioavailable. The global fiber market reached a volume of 82.1 million tons in 2012. China dominated the fiber market both as the final consumer, with 30% of global demand, and as a textile processor, with 53% (Pci Group, 2013). In the Yangtze estuarine system, fibrous geometry is the most frequent form of floating microplastic (Zhao et al., 2014), which supports the finding of prevalent fibers in this study.

Based on the characteristics of artificial materials and the melting test (Desforges et al., 2015; Enders et al., 2015; Magnusson and Norén, 2014), 28 (7.7%) fragmented and 200 (54.9%) fibrous particles were identified as plastic debris in the items detected (Fig. 3). The demand for man-made fibers, including synthetic and man-made cellulose fibers, in 2014 was 55.2 million tons, accounting for 62.6% of the global fiber production. In 2014, 46.1 million tons of polyester dominated chemical fiber production. China accounted for 69% of all polyester fiber production (http://www.textileworld.com/Issues/2015/2014/F- iber_World). Fragmented microplastics ingested by birds here could cause both physical harm (such as inflammation, blockage, or cellular necrosis in the gastrointestinal tract) and chemical toxicity (from plastic additives or chemicals accumulated from the surrounding environments) to the biota (Rochman, 2015). In plastic marine debris, 231 different chemicals have been detected (Rani et al., 2015).

In total, 136 (37.4%) items from our specimens were categorized as natural fibers. Natural fibers in the present study theoretically contained both fibers of a natural origin and man-made cellulose fibers manufactured from natural raw material such as wood. Of the 89.4 million tons of global fibers produced in 2014, cotton, man-made cellulose and wool fibers accounted for 29.4%, 6.7% and 1.3%, respectively (http://www.lenzing.com). Natural fibers pose some similar environmental concerns as synthetic fibers because both have been associated with a 'cocktail of chemicals'. The chemicals associated with natural fibers include dyes, additives and substances adsorbed from the environment. Although natural fibers may seem to be environmentally friendly, a complex mixture of associated chemicals could be bioavailable and potentially affect the health of ingesting organisms. Two dyes (Direct Blue 22 and Direct Red 28) associated with cellulose fibers in the guts of vagile macrocrustaceans have been identified, and Direct Red 28 is thought to be carcinogenic for vertebrates (Remy et al., 2015).

The abundance of microscopic anthropogenic litter was higher in the stomach than in the esophagus and intestine. However, particle concentration did not vary significantly among the three parts of the digestive tract (Kruskal–Wallis, $\chi^2 = 1.0284$, $df = 2$, $p = 0.598 > 0.05$), which implies that any potentially toxic anthropogenic particles would not be immediately excreted from the digestive tracts. Longer retention of microscopic anthropogenic litter could aggravate its potential to create physical and chemical hazards for the ingesting wildlife. However, natural fibers play a different role than synthetic fibers in the dispersion of hazardous chemicals. Compared to the slow degradation of synthetic materials, natural fibers could be metabolized quickly once ingested, leading to a release of their associated chemicals and potentially to their greater bioavailability. The proportion of natural fibers declined from the esophagus (40.2%), to the stomach (41.5%) to the intestine (26.6%), although the three regions did not differ statistically (Fig. 4). This result suggests the digestion of natural fibers by the birds examined in our study. Due to the opportunistic specimen collection of only 17 individuals, our ability to draw conclusions about the bioavailability of natural fibers to birds is limited. Documenting the levels of natural fiber ingestion in more specimens would be necessary for a comprehensive understanding of the fate of natural fiber pollution. The distinct surface properties (e.g., electronegative surface and a less negative zeta potential) of natural fibers create different chemical sorption behaviors than those of plastic

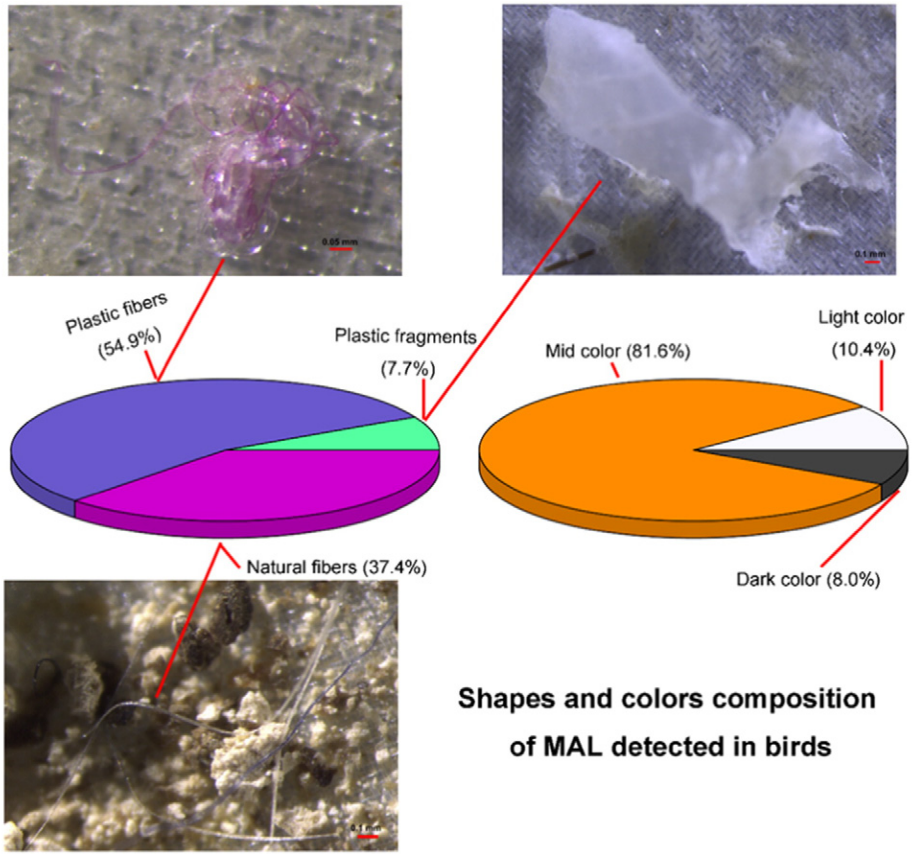

Fig. 3. Shapes (left) and colors (right) composition of MAL detected in birds (in % of the total count of anthropogenic particles detected in birds). Images are examples of each shape type of MAL.

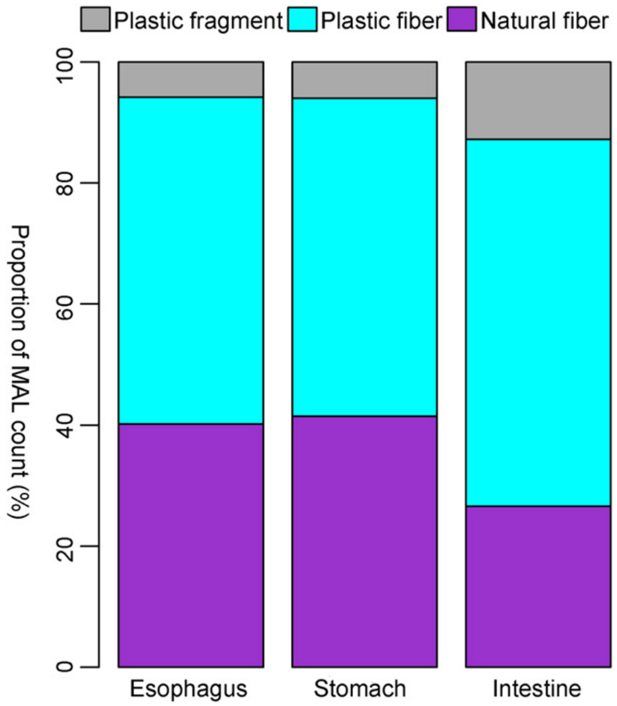

Fig. 4. Plastic fragment, plastic fiber and natural fiber composition in the esophagus, stomach and intestine of specimens (in % of the total count of MAL detected in each part).

particles (Ladewig et al., 2015). Out of 82.1 million tons of fibers produced in 2012, natural fiber accounted for approximately 37.4%, while synthetics accounted for 62.6% (Pci Group, 2013). Even so, natural fibers have been largely ignored in research on environmental pollution, especially in terrestrial ecosystems.

This paper provides the first report of the ingestion of microscopic anthropogenic particles by terrestrial birds. Among our specimens, 94.1% ingested a total of 364 microscopic anthropogenic items. After outliers were removed, we found an average of 10.6 ± 6.4 items per bird, which reflects the relative pollution level in the terrestrial ecosystem of the study area. Terrestrial microscopic anthropogenic particles are thus a type of pollution that is “close to home” and merits more public attention. Particles smaller than 5 mm represented more than 90% of the particles detected. Natural fibers, microplastic fibers and microplastic fragments accounted for 37.4%, 54.9% and 7.7% of the items, respectively. Mid-tone and fibrous particles dominated the color and shape. Microscopic anthropogenic litter and its associated complex of chemical contaminants have the potential to transfer to the wildlife. Natural fibers that can disperse toxic chemicals via different pathways than plastic polymers deserve further investigation. In addition, the chemical composition of the pollutants associated with microscopic anthropogenic litter should be identified to allow the understanding of its specific role in the biosphere.

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Appendix A. Supplementary data

Supplementary data accompanies this paper in the excel file. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2016.01.112.

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