



Variation in the use of anthropogenic materials in tit nests: influence of human activities and pandemic restrictions

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Abstract

As urbanization expands, so does the presence of anthropogenic materials within bird's nests. However, our understanding of this phenomenon remains limited, particularly in terrestrial birds. This study describes and compares the use of anthropogenic materials in great tit (*Parus major*) and blue tit (*Cyanistes caeruleus*) nests in relation to the intensity of anthropogenic activities, distance to contamination sources and reproductive metrics, in nest boxes in a suburban wood in Coimbra, Portugal, during the breeding seasons of 2020 and 2021. Nests were collected after fledging and sorted to remove anthropogenic materials. From a total of 9.71% anthropogenic materials in great tit nests we observed an abundance of uncoloured tangles of mixed fibres, representing 78% of the total weight of the anthropogenic materials in great tit nests in 2021. The chemical composition of these mixtures was determined by spectroscopy analysis, using Fourier transform infrared spectroscopy (FTIR), and 56% of the nests (16/25) contained fibres from synthetic origin. The percentage of anthropogenic materials was higher in great tit than in blue tit nests and increased in 2021, comparatively to 2020. The proximity to potential contamination sources influenced positively the percentage of anthropogenic materials in nests, as those closer to areas with more intense anthropogenic activities (the bar/tennis court and the wastewater treatment plant) had a higher percentage of these materials. No effects on productivity were detected. Our results suggest that the use of these materials is probably related to the intensity of anthropogenic activities.

Keywords Anthropogenic waste · Nest boxes · Urbanization · Hole nesting birds · Human activities · Plastic pollution

Introduction

Increasing urbanization, projected to accommodate 68% of the global human population by 2050, poses a significant challenge to wildlife (Shochat et al. 2006; United Nations 2018). Its negative ecological effects include fragmentation, isolation and loss of natural habitats (Croci

et al. 2008), changes in the availability of resources for wildlife, including food and shelter, exposure to stress induced by urbanization and noise pollution (Lowry et al. 2011). However, wild species may differ in their ability to tolerate human pressure, explore resources, persist, or invade urban habitats, depending on specific characteristics related to their ecology, behaviour, and physiology (Bonier et al. 2007).

One of the main consequences of human pressure on ecosystems is the contamination by anthropogenic materials, with particular emphasis on plastics. In this context, anthropogenic materials are defined as any manufactured materials including paper, glass, textiles or plastics, as well as natural materials that have been processed through human activities, that occur in the environment. At present, plastic pollution is recognised as a significant concern due to its potential adverse effects on biodiversity (Thushari and Senevirathna 2020; Anunobi 2022). This is attributed to its durability and slow degradation, often

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resulting in fragmentation into smaller pieces (micro and nanoplastics) that can disperse rapidly in the environment (ter Halle et al. 2016; Andrady 2022; Schwarz et al. 2023). Moreover, plastics contain chemical compounds added during its production or adsorbed from the environment, which have the potential to bioaccumulate and are potentially toxic to wildlife (Bucci et al. 2020). However, some bird species are able to incorporate plastic and other anthropogenic materials into their activities, such as in the nest construction (Suárez-Rodríguez et al. 2017; Reynolds et al. 2019; Lopes et al. 2020; Jagiello et al. 2022).

The number of studies that reported anthropogenic materials in bird nests has increased considerably (e.g., Jagiello et al. 2019). Among the most commonly materials found in bird's nests are strings, papers, textiles, and pieces of plastic (Jagiello et al. 2019, 2023; Potvin et al. 2021). Several potential explanations for the incorporation of these materials in birds' nests have been advanced (Jagiello et al. 2023). This behaviour may occur when anthropogenic debris increases and natural materials decrease in the surrounding environment when constructing the nests – birds may include these materials because of their similarity to preferred natural materials like moss or hair/others (Jagiello et al. 2019). The incorporation of anthropogenic materials in nests may also be associated with age and experience, with older individuals being expected to carry a greater number of these materials into their nests (Sergio et al. 2011; Reynolds et al. 2019 but see Jagiello et al. 2023). Anthropogenic materials may be beneficial for birds when present in nests, as they can reinforce the nest structures or repel some nest-dwelling ectoparasites due to their toxicity (e.g., cigarette butts) (Jagiello et al. 2019). Nevertheless, the use of these materials as nest components may also have detrimental consequences, as they could result in the entanglement of nestlings, thereby increasing mortality (Votier et al. 2011; Janic et al. 2023), in addition to the toxicity of leached chemicals. For example, Kwieciński et al. (2006) identified the presence of plastic strings in nests as one of the main causes for leg bone atrophy in white stork (*Ciconia ciconia*) chicks ultimately leading to a lower post-fledging survival, because their ability to move on land was impaired. Additionally, this may expose nestlings to higher levels of toxic compounds, such as those from cigarette butts (Suárez-Rodríguez et al. 2017).

Many studies reporting the use of anthropogenic materials in nests focused on seabird species, and there is little information for terrestrial bird species, particularly for small-sized birds (Jagiello et al. 2019; Potvin et al. 2021). In line with this, it is also unclear whether the proximity to potential contamination sources may influence the

presence of anthropogenic materials in nests of birds breeding within and around urban areas. Tits (Paridae) are small, terrestrial passerines that nest in natural cavities, such as tree cavities, which protects them from unfavourable environmental conditions and predators (Alabrudzińska et al. 2003; Maziarz et al. 2017). Tits are widely studied across Europe due to their affinity to breed in nest boxes in a wide range of habitats, including urban and non-urban areas. Therefore, tits provide an excellent model to study the effects of urbanization, human disturbance and solid waste pollution on reproduction (e.g. Hanmer et al. 2017; Jagiello et al. 2022).

Cavity-nesting birds, such as tits, build their nests primarily using hay, fur, feathers, moss and aromatic plants (Potvin et al. 2021; Deeming 2023). However, the availability of plastic and other persistent anthropogenic materials in the surrounding environment, in addition to their attractive colours and appearance, may lead birds to transport these unnatural materials to their nests. The use of anthropogenic materials in tit nests has been previously described by Hanmer et al. (2017) and Jagiello et al. (2022) for both great tit (*Parus major*) and blue tit (*Cyanistes caeruleus*) and by Reynolds et al. (2016) for the blue tit, but the relationships between the amount of anthropogenic materials in the nests and the distance to pollution sources needs further study.

The goal of this study was to describe and compare the use of anthropogenic materials, including the use of chemical signatures to identify their synthetic origin, in both great tit and blue tit nests and relate it with breeding success and distance to main contamination sources. These two species were studied in a suburban forest in Portugal over two consecutive years (2020 and 2021). We related this use with the breeding season in which the nest was built and the intensity of anthropogenic activities, as pandemic restrictions were progressively lifted during the period of the study, and also to the distance of possible sources of contamination in the surroundings of the nest boxes. According to previous studies (e.g., Hanmer et al. 2017), it is expected that great tits will place a greater amount of anthropogenic materials in their nests compared to blue tits. During 2020, due to the restrictive measures imposed in Portugal in face of the Covid-19 pandemic, there was a decrease in the movement of people, namely in parks and gardens, which likely reduced the availability of anthropogenic materials during that year. Therefore, we hypothesize that the amount of anthropogenic materials in the nests will increase in 2021 in comparison with 2020. Finally, it is also expected that there will be a higher use of anthropogenic materials for nest construction in those areas closer to contamination hotspots, i.e. with higher human activity.

Materials and methods

Great and blue tit sampling

This study was conducted in Mata Nacional do Choupal, a suburban mixed forest located in Coimbra, Central Portugal, with ca. 79 hectares, flanked to the south by the Mondego River and with several ditches crossing it. During the breeding seasons of 2020 and 2021, the reproduction of great and blue tits was monitored (in 99 nest boxes in 2020 and in 114 nest boxes in 2021), in order to record the presence and building state of nests, and the presence and number of eggs and nestlings. Relevant reproductive dates, such as the laying date, hatching date and fledging date were also recorded. Mean average mass was obtained before incubation onset by weighting 2–3 random eggs in each clutch to the nearest 0.01 g with a Pesola. At day 14 after hatching (hatching date = 0) the nestlings were ringed and their body mass (to the nearest 0.1 g) was obtained using a Pesola.

Once all the nestlings had left their nest boxes, nests where at least one nestling successfully fledged were carefully removed and placed inside sealed plastic bags, identified with the nest box's number and the collection date, and then transported to the laboratory where they were frozen. In total, we analysed 26 nests from the 2020 breeding season (14 from great tits and 12 from blue tits) and 41 nests from the 2021 breeding season (26 from great tits and 15 from blue tits). Those nests were equally distributed throughout the area of the Mata Nacional do Choupal in order to reflect different distances to possible sources of pollution (Fig. S1), and also covered clutches initiated throughout the entire reproductive season.

Laboratory nest analysis

Nests were placed in an oven at 40 °C during 48 h to obtain their dry weight to the nearest of 10 mg using a precision balance. Afterwards, each nest was sorted out to remove anthropogenic materials. For the purpose of this study, anthropogenic materials were considered any materials that do not exist naturally in the form or appearance they were found in the nests, i.e., anthropogenically fabricated or processed materials, inclusively by techniques that make them more persistent, such as plastics and textiles.

The sorting of materials was made visually by naked eye and, therefore, only macroscopic materials were considered. Single fibres were not included in the analysis. All materials that appeared to be non-natural were collected with the help of tweezers and placed in Petri dishes, duly identified with the nest number. The materials were then observed under a stereomicroscope, with a magnification of 16.5 ×, to facilitate their classification. Non-natural materials were

classified into three categories according to: (a) their shape as fibre yarns, filaments or fragments. For each category, materials were classified by (b) their colour as uncoloured or coloured, and by (c) their composition as plastic, such as rope and parts of plastic bags, or other (including tangles of mixed fibres of unidentified composition, see below). In this last category we included small proportions of other materials found in nests, such as paper and metal.

In all nests, the presence of a material constituting of uncoloured tangles of mixed fibres (undyed fibres that resembled natural fibres such as fur or plant material; e.g., plumed seeds) was detected (Fig. S2). However, it was not possible to obtain a homogeneous and representative sample of this material, as it was greatly altered (fragmented and mixed) during the period in which it was collected, incorporated and kept inside the nest. Because its composition could not be reliably classified and it could not be efficiently separated, this material of unknown origin was considered of unidentified composition. Because uncoloured tangles of mixed fibres of unidentified composition were not collected from the nests of the 2020 breeding season, when we compared data between years (2020 versus 2021) we excluded this category. We also classified as “other material” the coloured filaments and tangles of mixed fibres that appeared to belong mostly to pieces of clothing, but whose composition was not confirmed with further specific chemical analysis.

To assess the incorporation of synthetic materials in the category “tangles of mixed fibres of unidentified composition”, we randomly selected 25 nests for chemical analyses. From each nest, three random points were selected within the tangle of fibres and were placed directly in the FTIR device. The systematic random point sampling allowed for a representative selection of materials from various areas within each nest. This approach ensures that the sampled points are not biased and provide a comprehensive overview of the nest composition. The chemical composition of these materials was evaluated by Fourier transform infrared spectroscopy (FTIR), in the mid-IR interval (400–4000 cm^{-1}), at the vibrational spectroscopy laboratory of the “Molecular Physical-Chemistry” R&D Unit (QFM-UC, Coimbra, Portugal), using a Bruker Optics Vertex 70 FTIR spectrometer purged by CO_2 -free dry air. FTIR-ATR spectra were acquired using a Bruker Platinum ATR single reflection diamond accessory and a Ge on KBr substrate beamsplitter with a liquid nitrogen-cooled wide band mercury cadmium telluride (MCT) detector. Each spectrum was the sum of 128 scans, at 2 cm^{-1} resolution, and the 3-term Blackman-Harris apodization function was applied. Under these conditions, the wavenumber accuracy was better than 1 cm^{-1} . The spectra were corrected for the frequency dependence of the penetration depth of the electric

field in ATR (considering a mean refraction index of 1.25). The Bruker OPUS Spectroscopy Software (8.1 version) was used to process the spectra (baseline correction, ATR correction and normalisation relative to the most intense band, for each sample). Spectra were then processed using the OMNIC software and compared with a commercial spectral library (Hummel Polymer Spectral Library, Thermo Fisher Scientific Inc.) and the BASEMAN library developed by Primpke et al. (2018). Only particles with a match higher than or equal to 70% were accepted and classified as “Synthetic polymer”, considering the component with the highest agreement value (Cowger et al. 2020). The proportion of nests containing one, two or three synthetic materials in the selected points was evaluated.

The amount of anthropogenic material present in each nest was assessed based on its weight rather than its size or number. For each nest, all material categories included in the classification were weighed separately to the nearest of 0.1 mg in a precision balance. The percentage of each anthropogenic material relative to the total dry weight of the nest was also calculated.

Statistical analysis

A Mann-Whitney test was used to compare: (a) the overall percentage of anthropogenic materials between great tit and blue tit nests collected in 2021, (b) the percentage of anthropogenic materials in great tit and blue tit nests between 2020 and 2021, and (c) the percentage of coloured filaments of unidentified composition in great tit nests between 2020 and 2021.

We used Generalized Linear Models without Poisson distribution to evaluate the relationship between the distance from the nest box to the different potential sources of contamination, and the percentage of anthropogenic materials in great tit and blue tit nests in separate models by tit species, using data from 2021. We considered as possible sources of anthropogenic materials those areas with a greater intensity of human activities, namely the edge of the forest closest to

the nest box, the Choupal’s bar/tennis court and the wastewater treatment plant (WWTP). The distance from the nest box to the potential sources of contamination was measured using Google Earth’ measurement tool. For the bar/tennis court we considered the intermediate point between these two places, as they are adjacent.

We used Generalized Linear Models to assess the relationship between the use of anthropogenic materials in nests with reproductive metrics, separately by tit species, in 2021. The reproductive metrics included laying date, incubation duration in days, clutch size, mean egg mass, brood size, number of fledglings, hatching and fledging success. The models were fitted with Poisson distribution except for laying date, incubation duration, mean egg mass and mean fledging mass which were fitted with normal distribution. Laying date and hatching date were converted to Julian date. This date refers to the number of days since January 1st (which is considered as day 1).

All data is presented as mean \pm standard error (SE). All statistical analysis were performed in the JMP Pro 17 software.

Results

Description of nest anthropogenic materials

Considering the three classes of materials regarding shape, tangles of mixed fibres corresponded to 93% of the anthropogenic materials’ weight, largely due to the highly significant amount of those uncoloured (which were present in all nests), whose composition visually was not possible to ascertain (Fig. 1a; Fig. S2). These materials constituted 78% of the total weight of anthropogenic materials, while the second most abundant materials, which were coloured tangles of mixed fibres of unidentified composition, constituted 15% of the total weight of anthropogenic materials. Fragments corresponded to only 2% of the total weight of anthropogenic materials in nests being, therefore, the

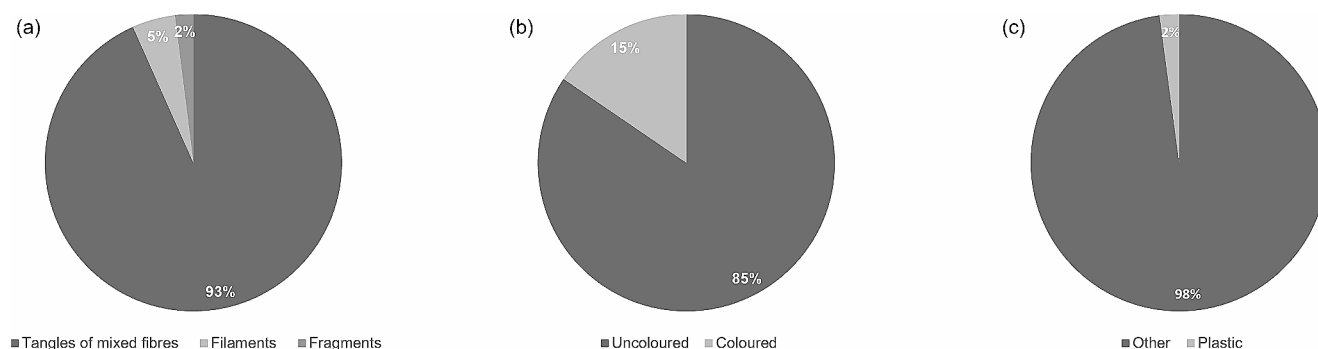


Fig. 1 Weight percentage of anthropogenic materials according to their (a) shape, (b) colour and (c) composition, in relation to the total weight of anthropogenic materials collected from great tit nests in 2021

shape of materials less frequently detected (Fig. 1a). Within this category, we highlight the presence of surgical mask fragments in three nests collected in 2021 and fragments of cigarette butts collected in nests from both 2020 and 2021 (Fig. S3). Coloured materials accounted for 15% of the total weight of the anthropogenic materials (Fig. 1b).

As for the composition, plastic materials represented only a small percentage of the total anthropogenic materials (about 2% of the weight) and appeared mostly in the form of coloured filaments. Fragments of plastic bags and filaments of rope were also found (Fig. S3). A significant part of the materials was included in the “other” category because it was not possible to determine their composition by naked eye (Fig. 1c). Among the materials classified as “other”, we collected white filaments of dental floss, coloured metal fragments belonging to steel scrubber or paper fragments, among others (Fig. S3). Most of the tangles of mixed fibres and coloured filaments appeared to belong to textiles but were classified as “other” because their composition could not be confirmed.

The chemical composition of the mixed materials defined as “tangles of mixed fibres of unidentified composition” revealed that 56% (14 out of 25) of the nests showed

evidence of synthetic materials in these mixtures (at least one of the three random points and fibres selected was confirmed to be from synthetic origin) (Fig. 2).

From the total fibres analysed ($n=75$) by FTIR from these mixtures, 74% were confirmed to be from natural sources (dog fur, linen and/or other compounds) and 25% of the randomly selected fibres were classified as synthetic ($n=19$). The main polymer types identified were Polyester (32%), Polypropylene (26%), High-density polyethylene (21%), Polyacrylamide (11%), and 10% of these particles presented a match lower than 70%, being defined as “not assigned”, but their composition was confirmed as synthetic (Fig. 2).

Comparison of anthropogenic materials between tit species and years

Percentage of anthropogenic materials in great tit and blue tit nests

In 2021, the weight percentage of anthropogenic materials was significantly higher in great tit nests ($9.71\% \pm 1.21$) than in blue tit nests ($2.37\% \pm 0.37$) ($U=28.00$, $p<0.001$) (Fig. 3).

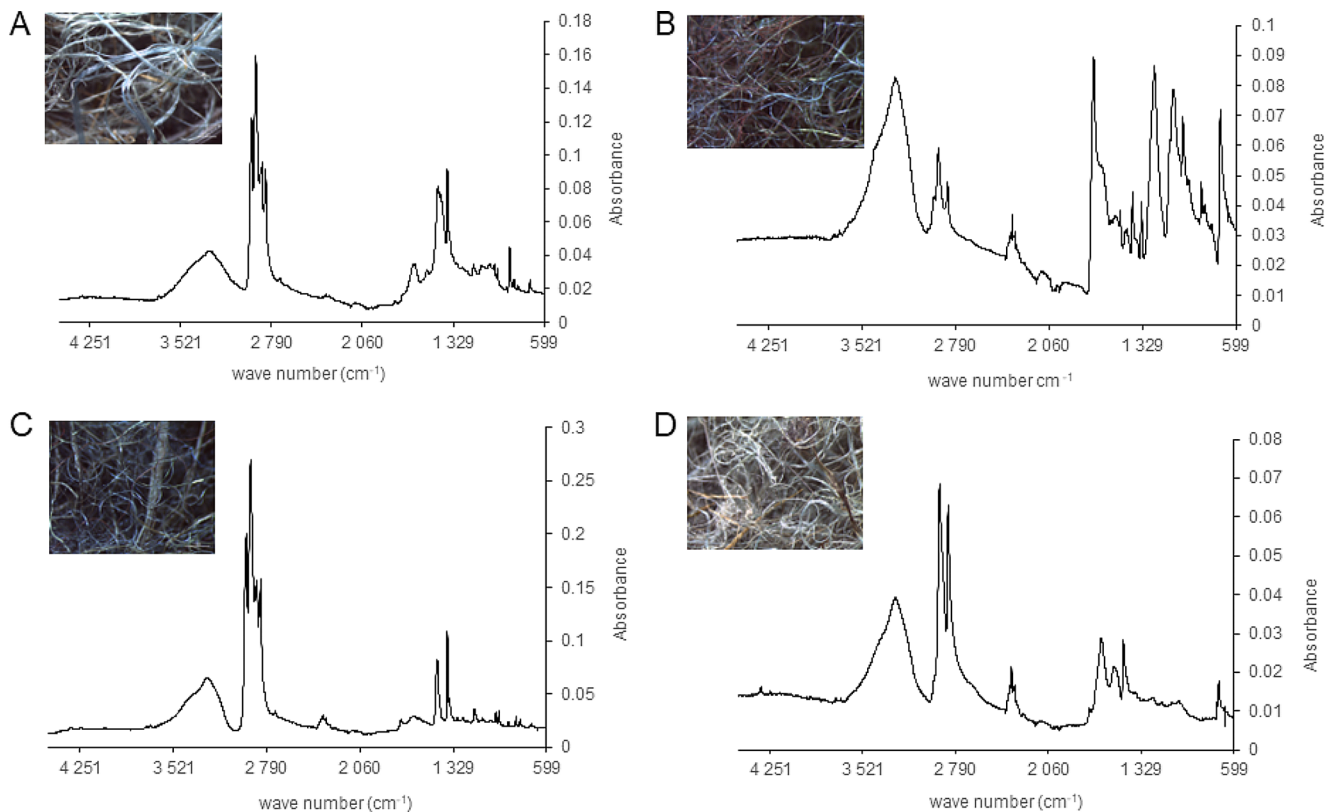


Fig. 2 Examples of synthetic particles found in random sample points from the mixtures from the nests classified as “tangles of mixed fibres of unidentified composition” and their infrared spectra: **(a)** Polypro-

pylene fibre; **(b)** Polyester fibre; **(c)** Polypropylene fibre; **(d)** High-density polyethylene fibre

Fig. 3 Weight percentage (mean \pm standard error) of anthropogenic materials (relative to total dry weight of the nest) in great tit and blue tit nests from 2021

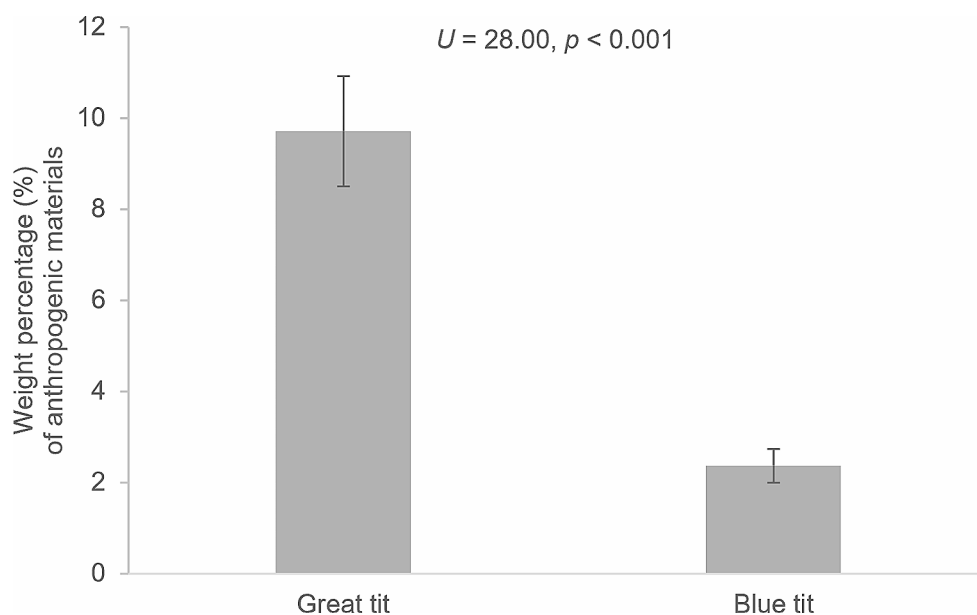
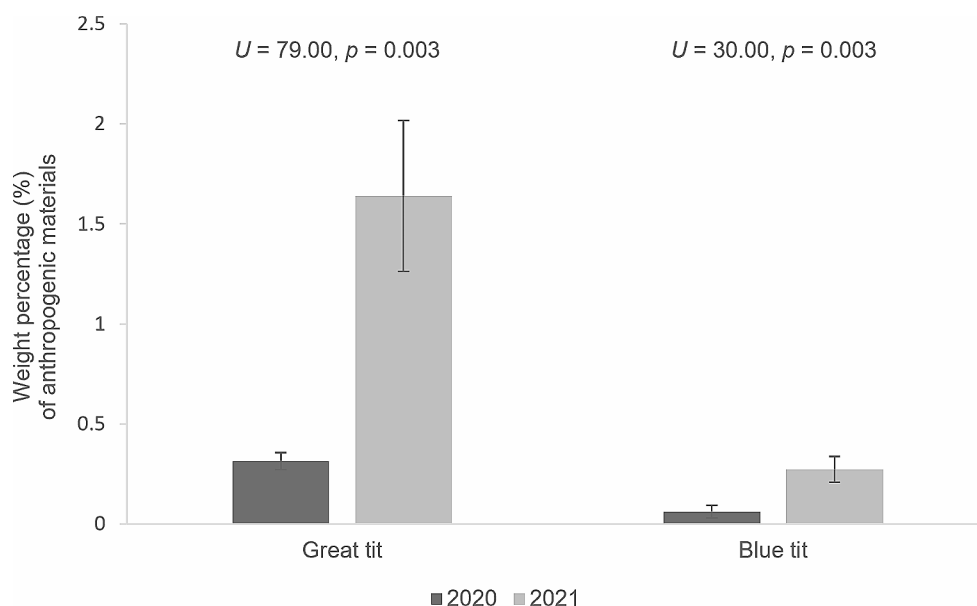


Fig. 4 Weight percentage (mean \pm standard error) of anthropogenic materials (relative to total dry weight of the nest) found in 2020 and 2021, for both great tit and blue tit nests, excluding the uncoloured tangles of mixed fibres of unidentified composition



Percentage of anthropogenic materials in 2020 and 2021 nests

When excluding uncoloured tangles of mixed fibres of unidentified composition, the weight percentage of anthropogenic materials in nests of both species was significantly higher in 2021 (great tit: 1.64% \pm 0.38; blue tit: 0.27% \pm 0.07) than in 2020 (great tit: 0.32% \pm 0.04; blue tit: 0.06% \pm 0.03) (great tit: $U = 79.00, p = 0.003$; blue tit: $U = 30.00, p = 0.003$) (Fig. 4). The weight percentage of coloured filaments of unidentified composition in nests did not differ between the two years (2020: 0.13% \pm 0.03; 2021: 0.16% \pm 0.06; $U = 142.00, p = 0.257$).

Influence of the distance to contamination sources on the percentage of anthropogenic materials in great tit and blue tit nests

For the blue tit, we did not find a significant relationship between the weight percentage of anthropogenic materials in the nests collected in 2021 and the distance from the nest box to all three potential contamination sources (bar/tennis court, WWTP and forest edge: linear regressions, $p > 0.05$ in all cases).

Although the relationship between the weight percentage of anthropogenic materials in great tit nests from 2021 and the distance from the nest box to the nearest edge of the forest was not significant ($\chi^2_{1,24} = 0.0006, p > 0.05$), there was

a significant decrease in the weight percentage of anthropogenic materials present in great tit nests collected in 2021 with both the distance to the bar/tennis court (estimate = -0.0058 ± 0.0001 , $\chi^2_{1, 24} = 16.06$, $p < 0.0001$) (Fig. 5a), and the distance to the WWTP (estimate = -0.0001 ± 0.0002 , $\chi^2_{1, 24} = 18.28$, $p < 0.0001$) (Fig. 5b).

Relationship between the percentage of anthropogenic materials in great tit and blue tit nests and reproductive parameters

In the great tit there was a tendency for clutches laid later in the season to have a higher percentage of anthropogenic materials using the data from 2021 (estimate = 1.29 ± 0.7 , $\chi^2_{1, 13} = 3.19$, $p = 0.07$).

In neither the blue tit or the great tit there were significant relationships between the percentage of anthropogenic materials and any of the reproductive parameters – egg mass, incubation duration, clutch and brood size, number of fledglings, fledging mass, and hatching and fledging success ($p > 0.05$ in all cases).

Discussion

Our study provides new information about the presence of plastic and other anthropogenic materials in tit nests, the influence of the intensity of human activities (related here to the lifting of pandemic restrictions), and the distance to anthropogenic contamination sources on the percentage of these materials used by birds in the construction of their nests. The spectroscopy analyses used in this study to characterise mixed fibres enabled us to identify the synthetic composition of fibres integrated into the nests. This newly acquired information is particularly crucial, as plastic and other synthetic materials may contain chemical combinations that result in extremely slow degradation, making them

highly persistent in the environment. This persistence may provide additional threats to these species, as some chemical additives in plastics (e.g., flame retardants, phthalates and other compounds) are known to accumulate in several wild species (Yamashita et al. 2021). The use of plastics in nesting material may be associated with the availability and abundance of anthropogenic material in urban environments (Lato et al. 2021).

We found that the percentage of anthropogenic materials in nests was higher in great tit nests than in blue tit nests for both 2020 and 2021. Similarly, Hanmer et al. (2017) found a higher percentage of anthropogenic materials in great tit nests, suggesting that blue tits are more selective when constructing their nests and prefer to use natural materials instead of anthropogenic ones (Surgey et al. 2012; Hanmer et al. 2017). However, the percentages of anthropogenic materials found in nests by Hanmer et al. (2017) were much higher (24% of the total nest weight in great tit nests, 2–16% in blue tit nests) than those in our study ($9.71\% \pm 1.78$ in great tit nests, $2.37\% \pm 0.37$ in blue tit nests; data from 2021). This difference could potentially be explained by the fact that the study of Hanmer et al. (2017) was carried out in Reading, England, a more densely populated city than Coimbra.

On the other hand, Jagiello et al. (2022) did not find significant differences in the relative amount of anthropogenic materials between great tit and blue tit nests, but significant differences were detected when considering the quantity of natural materials, because blue tit nests had a greater amount of moss and feathers. In our study population, observations during the fieldwork also suggest that blue tits used more feathers in the construction of their nests than great tits (pers. obs.).

The presence of anthropogenic materials in avian nests has been described for other terrestrial birds. For example, Townsend and Barker (2014) found that 85.2% (46 out of 54) of American crow (*Corvus brachyrhynchos*) nests along

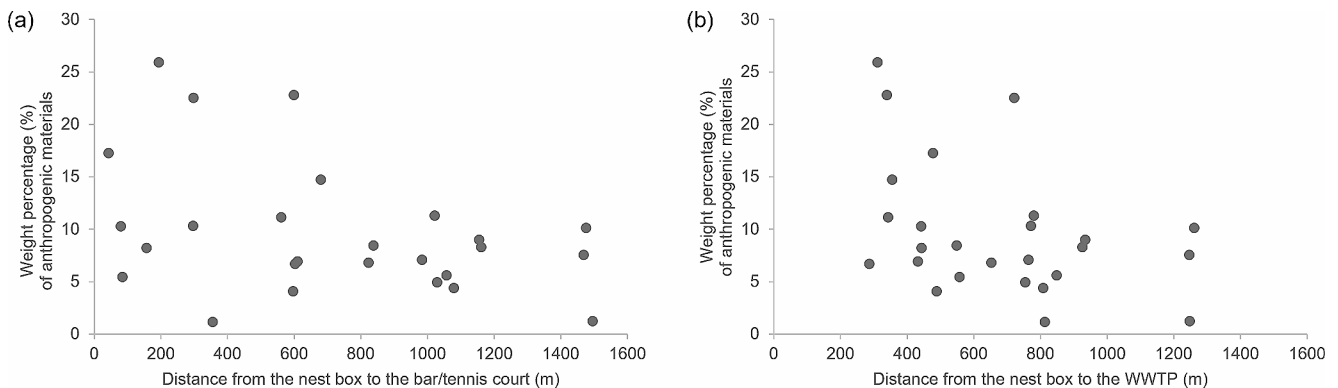


Fig. 5 Relationship between the weight percentage (%) of anthropogenic materials (relative to total dry weight of the nest) in the great tit nests collected in 2021 and the distance, in meters (m), from the

nest box to (a) the bar/tennis court (estimate = -0.0058 ± 0.0001 , $\chi^2_{1, 24} = 16.06$, $p < 0.0001$) and to (b) the wastewater treatment plant (WWTP) (estimate = -0.0001 ± 0.0002 , $\chi^2_{1, 24} = 18.28$, $p < 0.0001$)

an urban to agricultural gradient contained anthropogenic materials, including strings, strips of plastic or cloth, tapes and wires. Radhamany et al. (2016) reported the presence of anthropogenic materials, including plastic pieces and fine rope, in 10–22% of house sparrow (*Passer domesticus*) nests along an urban to rural gradient. However, the use of anthropogenic materials in avian nests, and the reason as to why birds use these materials when constructing their nests are still recent and poorly explored research topics, with only a few studies tackling these questions (Reynolds et al. 2019; Jagiello et al. 2022).

We found that, regardless of the species (great or blue tits), the percentage of anthropogenic materials in nests was higher in 2021 than in 2020. A potential explanation for this difference is the fact that, in 2020, people's movements and outdoor activities were conditioned since mid-March, due to the restrictive measures implemented to control the Covid-19 pandemic (Diário da República Portuguesa DL 2-A/2020). Although these materials can be persistent and occur in the environment even during absent or reduced human activities, their abundance was probably lower during that spring. In 2021, we detected fragments of surgical masks inside tit nests for the first time. Overall, the percentage of these materials that could be compared between years in our study (i.e. excluding the uncoloured tangles of mixed fibres of unidentified composition) was very low in nests, but was consistent with sporadic encounters with materials left behind by visitors to the study area. This contrasts with the uncoloured tangles of mixed fibres of unidentified composition that seem to be derived from a persistent source (see below).

In Jagiello et al. (2022), a positive relationship was observed between the intensity of human presence and the amount of anthropogenic materials in great tit nests. Also, Janic et al. 2023 found that nests of black storks *Ciconia nigra* closer to the forest edge had higher probability of containing plastic fragments. In our study, the data also pointed out to a positive relationship between the percentage of anthropogenic materials in tit nests and the distance to potential contamination sources, namely the bar/tennis courts and the WWTP. Nests closer to the bar/tennis court and WWTP had a greater percentage of these materials, in terms of their total dry weight. The effect of forest edge, which here includes low traffic roads and the river as potential sources of anthropogenic waste to tits, was not significant in our study.

Data from 2021 revealed a strong presence of uncoloured tangles of mixed fibres, whose composition could not be determined. This material constituted 78% of the total weight of anthropogenic materials, while coloured tangles of mixed fibres of unidentified composition, the second most abundant materials, constituted only 15%. In

fact, these uncoloured tangles of mixed fibres seem to be a mixture of materials from different origins, which are highly altered and mixed during the period of collection, incorporation and permanence in the nest. Future studies should be conducted in order to identify the chemical composition of these materials, due to the important fraction of their weight relatively to the total dry weight of nests, but also focus on their effects on breeding parameters, egg and nestling temperatures and nest arthropod communities.

Regarding the colour, we found that tits often chose less coloured materials, suggesting that these birds may have a preference for uncoloured materials that can be mistaken with natural fibres or animals' fur. Surmacki and Zduniak (2022) artificially placed acrylic fibres of different colours (blue, white, red and yellow) in the same quantity around nest boxes and assessed the females' preferences when selecting such colours. In addition to white materials, great tit females showed a preference for red coloured artificial materials, while they transported less blue and yellow coloured materials to their nests. Contrastingly, Surgey et al. 2012 revealed that four tit species showed no preference for colour when wool-like artificial cushion stuffing was artificially provided to the birds in dispersers throughout the breeding area. In our study, it was not possible to identify the composition of most of the anthropogenic materials, which were, therefore, classified as "other materials". In Jagiello et al. (2022), the most commonly found anthropogenic materials in nests consisted of cloth insulation materials, fabric threads and plastic strings, which may indicate that tits selected these materials due to their function (insulation, structure) as these could be especially important in Warsaw, Poland, the study area of these authors', located at a higher latitude and exposed to relatively cold springs.

Nevertheless, there is still no agreement as to why birds transport these materials to their nests, and several hypotheses have been proposed to explain such behaviour (Jagiello et al. 2019). Jagiello et al. (2022) showed a negative relationship between the amount of anthropogenic materials and natural (animal-derived: feathers and fur) materials within nests. This finding suggests that birds would seek non-natural materials in those environments where natural materials (animal-derived materials in the case of tits) used for insulation are typically scarce, such as urbanized areas. However, although the percentage of anthropogenic materials (including the tangles of mixed fibres) was not exceptionally high (averaging 2–10%) in Mata Nacional do Choupal, natural materials were abundant (pers. obs.) making this explanation unlikely.

An interesting finding was the presence of cigarette butts in nests collected both in 2020 and 2021. There are several studies reporting the effects of the presence of these materials in the nests of various bird species. Suárez-Rodríguez et

al. (2013), for example, showed that the presence of cigarette butts in house sparrow and house finch (*Carpodacus mexicanus*) nests reduced the number of nest-dwelling ectoparasites, thus indirectly benefiting nestling's growth (Merino and Potti 1995). This antiparasitic function is hypothesised to be similar to that attributed to some aromatic plants, often used by tits in the construction of their nests (Suárez-Rodríguez et al. 2013). However, the toxicity of some chemical compounds present in cigarettes could potentially outweigh any benefits resulting from their antiparasitic effect (Suárez-Rodríguez and Macías Garcia 2014; Suárez-Rodríguez et al. 2017).

Despite the essential role played by nests in the reproduction of birds, the knowledge of the consequences of using anthropogenic materials in their construction is very limited (Reynolds et al. 2019; Potvin et al. 2021). In addition to the inherent toxicity of many of these materials, Hanmer et al. (2017) suggested that, in blue tit nests, the use of more natural materials in comparison to materials of anthropogenic origin can potentially contribute to a lower abundance of ectoparasites such as fleas, because natural materials support a more diverse arthropod community which may include predators of ectoparasites, eggs and larvae. The presence of anthropogenic materials may be associated with an increase in brood mortality due to entanglement or strangulation (Townsend and Barker 2014). In fact, during nest box monitoring in 2021, a great tit chick was injured in the leg due to entanglement. Another study also related the presence of anthropogenic materials with a decrease in the reproductive success of the blue tit (Jagiello et al. 2022). However, in our study we did not find any significant relationships between reproductive parameters and abundance of anthropogenic materials in nests in neither tit species.

This work showed that anthropogenic materials, in particular plastics, constitute a considerable fraction of the total weight of tit nests, particularly great tit nests, and that the use of these materials could be related to the intensity of anthropogenic activities and proximity to potential contamination sources. In addition, synthetic fibres can be incorporated in the mixtures of the nests and the exposure to these persistent materials and their potential effects to these species needs to be better assessed. Quantifying the presence of plastic and other anthropogenic materials in the nests of these species over time can also serve as a useful indicator of plastic pollution in the environment and should be closely monitored. Therefore, in an increasing urbanization scenario, it becomes essential to raise public awareness on the potential consequences of the presence of anthropogenic waste in the environment. In addition, we encourage future studies focusing on the causes and consequences of the incorporation of anthropogenic materials in avian nests, with special emphasis on potential adverse consequences of their use.

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Author contributions ACN and JAR contributed to the study conception and design. Material preparation, data collection and analysis were performed by JG, FB, CSB and JGB. FB, MPMM, LAEBdC assessed the chemical signatures of particles. The first draft of the manuscript was written by JG and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Data generated or analysed during this study is available upon request to the corresponding author.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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