

Inorganic contamination in roadkill birds in Northeast Brazil

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Abstract: Wildlife roadkill surveys in Brazil often focus on birds, as they are one of the main groups affected by road accidents. In addition roadkill, anthropogenic litter also contributes to a significant number of bird fatalities every year. The present study aimed to investigate the stomach contents and possible contamination by inorganic material of birds killed on a federal highway that crosses the Serra de Itabaiana National Park in the state of Sergipe, Brazil. Monitoring and data collection were carried out from January to June 2022, with a motorcycle at an average speed of 60 km/h. The collected animals were transported to the Institute of Technology and Research, located at the Tiradentes University, Sergipe, Brazil, and a plastic material was identified. The plastic material found was subjected to Fourier Transform Infrared Spectroscopy (FTIR), which showed a similar wavenumber to a polymer in the polypropylene chain, very common in plastic packaging. The presence of plastics and metals in the intestinal contents of *Caracara plancus* and *Crotophaga ani* is noteworthy, as they have a greater tendency to ingest plastics due to their generalist diet. Plastic contamination in *Progne chalybea* and *Nyctidromus albicollis* probably occurred through bioaccumulation, from the consumption of insects contaminated by microplastics.

Keywords: Bird feeding; Microplastic; Spectroscopy; Residue; Ingestion; Stomach contents.

Contaminação inorgânica em aves atropeladas no Nordeste do Brasil

Resumo: As pesquisas sobre atropelamentos de animais silvestres no Brasil geralmente se concentram nas aves, pois elas são um dos principais grupos afetados por acidentes rodoviários. Além dos atropelamentos, o lixo antropogênico também contribui para um número significativo de mortes de aves todos os anos. O presente estudo teve como objetivo investigar o conteúdo estomacal e possível contaminação por material inorgânico de aves atropeladas em uma rodovia federal que atravessa o Parque Nacional da Serra de Itabaiana, no estado de Sergipe, Brasil. O monitoramento e a coleta de dados foram realizados no período de janeiro a junho de 2022, com uma motocicleta a uma velocidade média de 60 km/h. Os animais coletados foram transportados para o Instituto de Tecnologia e Pesquisa situado na Universidade Tiradentes, Sergipe, Brasil, onde foi identificado um material plástico. O material plástico encontrado foi submetido à espectroscopia no infravermelho com transformada de Fourier (FTIR), que mostrou número de onda semelhante a um polímero da cadeia do polipropileno, muito comum em embalagens plásticas. Chama a atenção a presença de plásticos e metais no conteúdo intestinal de *Caracara plancus* e *Crotophaga ani*, pois apresentam maior tendência à ingestão de plásticos devido à dieta generalista. A contaminação plástica em *Progne chalybea* e *Nyctidromus albicollis* provavelmente ocorreu por bioacumulação, a partir do consumo de insetos contaminados por microplásticos.

Palavras-chave: Alimentação de pássaros; Microplástico; Espectroscopia; Resíduo; Ingestão; Conteúdo do estômago.

Introduction

The running over of animals is one of the main negative impacts in the suppression of fauna in the biotic environment, as it endangers populations of entire species, causing a decline or even extinction (Gumier-Costa and Spencer 2009, Winton et al. 2020). According to the Brazilian Center for Road Ecology Studies, it is estimated that, in Brazil, around 15 animals are killed by vehicles per second, totaling 475 million per year, of which approximately 90% are small-sized. The northeastern region of Brazil concentrates 9% of the total recorded roadkill in the country (CBEE 2019).

In Brazil, birds represent a significant proportion of vertebrates killed by vehicles, being among the groups with the highest presence in studies on roadkill in various states of Brazil (Silva et al. 2019, Miranda et al. 2021, Vasconcelos et al. 2021, Favretto 2022). This quantity may be underestimated, as bird carcasses tend to degrade more rapidly due to their size (Henry et al. 2021), making it difficult to keep accurate records, in addition to the fact that many become trapped in the structure of the vehicles.

Souza et al. (2022) in a survey carried out on the GO 050 highway, in the state of Goiás, Brazil, identified the association between large amounts of trash on the sides of the highway and roadkill wildlife occurring on these stretches. Subsequently, Marques et al. (2023) associate the irregular disposal of garbage through the windows of vehicles that travel on highways and associated the accumulation of garbage on the sides of the highway with run-overs and intoxication of these animals due to ingestion of plastic.

The ingestion of plastic waste by wildlife has become common (Sá et al. 2018, Zhu et al. 2019). It is estimated that about 55% of birds with habits associated with freshwater environments and 56% of seabirds have plastic and/or microplastics in their digestive tracts (Gall and Thompson 2015, Holland et al. 2016). The ingestion of anthropogenic waste by birds can pose the risk of perforations and intestinal blockages in animals (Henry et al. 2021). The present study aimed to investigate plastic contamination in the stomach contents of bird roadkill on a section of BR-235 in the state of Sergipe, Brazil.

Material and Methods

The Serra de Itabaiana National Park (10°45'59" S; 37°20'14" W) has a total area of 8.024.79 hectares (ICMBio 2005), with a tropical climate and predominance of the Atlantic Forest biome (ICMBio 2005). It is crossed by the federal highway BR-235 for about four kilometers. The BR-235 highway has 2.093 km starting in the state of Sergipe and ending in the state of Pará. The stretch that crosses the state of Sergipe from west to east is approximately 120 kilometers long, passing through seven municipalities in Sergipe until reaching the state of Bahia.

Collections were carried out on the 37 km stretch between the municipalities of Aracaju (zero kilometer) and Areia Branca, where the Serra de Itabaiana National Park is located. The sampled stretch is surrounded by fragments of Atlantic Forest and anthropized areas (pastures, residences, sugarcane, and corn plantations, among others) and was covered by motorcycle, at an average speed of 60 km/h (Oliveira et al. 2021), twice a month, between January and June 2022.

The collected bird roadkill was identified (Piacentini et al. 2015, Pacheco et al. 2021), photographed, and georeferenced with the help of the Timestamp Camera® application (Susamp Infotech, Surat, India). The collected carcasses that were not crushed or in an advanced stage of decomposition were placed in sealed plastic bags and transported to the Institute of Technology and Research in a thermal bag for biometric and laboratory analyses.

In the laboratory, the birds were subjected to biometric measurement: total length (from the tip of the tail to the beak), wingspan (from the tip of the right wing to the tip of the left wing), and weight, for which a tape measure and electronic scale were used. Then, the carcasses were necropsied for organ removal and analysis, the gizzard contents were washed with running water, sieved, and classified with the help of a microscope and a stereomicroscope. The sieved content was stored in 70% ethyl alcohol.

All materials, without identification of origin, were weighed and then subjected to oxidative processing for organic material degradation. After the application of 20 mL of a 0.005 M aqueous solution of Fe II sulfate, used as a catalyst added to the solid residue, 20 mL of 35% hydrogen peroxide was added to the mixture. The material was kept at rest at room temperature for five minutes, and then mixed in a heated magnetic stirrer at 60°C for 30 minutes, covered with a watch glass. The solution was then sieved to remove the plastic fragments resulting from the oxidation process (Maynard et al. 2021). The plastic material obtained was subjected to Fourier Transform Infrared Spectroscopy (FTIR) on an Agilent FTIR Cary 630 spectrometer (Agilent Technologies Inc., Santa Clara USA), in a partner laboratory.

The metallic fragment found after cleaning the adhered organic material was weighed and subjected to a magnetism test.

For the present study, the project was authorized by the Biodiversity Authorization and Information System – SISBIO, under number 79209-1 and registered in the National System for Management of Genetic Heritage and Associated Traditional Knowledge – SisGen (registration ADDECD3).

Results

The stomach contents of four bird species, which were run over on the Sergipe stretch of the BR 325 highway, were analyzed: Crested Caracara – *Caracara plancus* (Miller, 1777) (Falconiformes, Falconidae), Smooth-Billed Ani – *Crotophaga ani* Linnaeus, 1758 (Cuculiformes, Cuculidae), Grey-Breasted Martin – *Progne chalybea* (Gmelin, 1789) (Passeriformes, Hirundinidae), and Common Pauraque – *Nyctidromus albicollis* (Gmelin, 1789) (Caprimulgiformes, Caprimulgidae). The species *Cr. ani*, *P. chalybea*, and *N. albicollis* had predominantly insect content, comprising eight orders and four families. Due to insect fragmentation, it was not possible to identify them at the species level.

The plastic residues found in the stomachs of *N. albicollis*, *P. chalybea*, and *Cr. ani* were similar to filaments and were classified as microplastics because they were smaller than 5 mm. However, due to the small size of the fragments, their weight could not be determined (Figure 1a).

The plastic residue fragments removed from the stomach of *C. plancus* were classified as macroplastics (above 5 mm) (Figure 1b), weighing 0.982 mg, and the metallic fragments were characterized as similar to aluminum, non-magnetic, weighing 0.0102 mg (Figure 1c).

The plastic residue found in *C. plancus*, according to spectroscopic analysis, presented waves of 3.400 cm^{-1} , $2.200-2.840 \text{ cm}^{-1}$, $1.300-1.500 \text{ cm}^{-1}$, with a spectrum similar to polypropylene (Figure 2).



Figure 1. Residues removed from the stomachs of *Progne chalybea* and *Caracara plancus* collected on the stretch of the BR-235 highway in Sergipe, Brazil, between January and June 2022. A) Microplastic residue removed from the stomach contents of *Progne chalybea*. B and C) Residues removed from the stomach of *Caracara plancus*: plastic material (B); metallic fragment (C).



Figure 2. Number of waves obtained from the plastic material removed from the stomach of *Caracara plancus* and subjected to Fourier Transform Infrared Spectroscopy (FTIR).

Discussion

The plastic content found in *C. plancus* was probably the main cause of the animal's stomach distension, due to the entanglement of plastic and organic matter. The absence of apparent trauma in the body suggests that plastic may be associated with the animal's death or

vulnerability. The coloration of plastic residues has become an important topic to consider, as many species of animals are visual predators, that is, they forage selectively and are attracted to certain colors and shines (Boerger et al. 2010, Chen et al. 2019). Accordingly, blue and/ or transparent plastic material represent the most commonly ingested by animals (Zhan et al. 2020, Carlin et al. 2020).

Caracara plancus has the most diverse diet among all 65 species of falconids, feeding on carcasses, arthropods (Formoso et al. 2019), vertebrates (Villalobos and Bagno 2012), in addition to consuming fruits, and may actively assist in seed dispersal (Paula et al. 2020). The ingestion of plastic and metallic fragments by *C. plancus* generates various assumptions, such as the presence of this material in common foraging areas (Derraik 2002, Foekema et al. 2013), which may lead to its accidental ingestion during predation (Rossi et al. 2019; D'Souza et al. 2020). Metal, in turn, can attract the bird's attention due to the color or shine caused by the reflection of light (Holland et al. 2016; Seif et al. 2018), some species of birds use materials with vibrant and metallic colors for ornamentation (Sergio et al. 2011).

The FTIR spectrum obtained in the analysis of the sample found in the stomach of *C. plancus* is similar to the spectrum of polypropylene, as presented in the work of Karthik et al. (2018) About 62% of all plastic products in the world are produced from polypropylene (PP) and polyethylene (PE) (Boenel et al. 2021), polypropylene is a polymer widely used in various segments of industry, as it presents chemical, physical, and mechanical properties that allow its application in fabrics, films, bottles, food packaging, automotive products, among others (Maddah 2016). *Progne chalybea* and *N. albicollis* are exclusively insectivorous birds. *P. chalybea* captures insects in the air column (Sick 1997, Helms et al. 2016) in short flights or during migration, and can feed on various types of insects during its life (Kelly et al. 2013). *N. albicollis* is a nocturnal bird that feeds during short flights from a perch. Both species have short and wide beaks, characteristics that allow foraging for insects during flight (Sick 1997). The microplastic filaments found in the stomach contents analyzed in both species, due to their small size, may be associated with insects that, in some way (internal or external), transported the material from the anthropic area to eventual predation (Edo et al. 2021; Zhu et al. 2023).

Some studies explain contamination by microplastics in insectivorous birds through bioaccumulation, due to the fact that the annual production of plastics varied from 1.5 million tons in 1950 to 335 million tons in 2016 (Sá et al. 2018, Li et al. 2015), a factor that increases the dispersion of microplastics among habitats, facilitating the contamination of insects and their predators.

Ameixa et al. (2018) categorize the ecosystem services provided by insects and the effects of contamination of these services by microplastics. In a study developed by Windsor et al. (2019) in Wales, specimens of Ephemeroptera from the families Baetidae and Heptageniidae and Trichoptera from the family Hydropsychidae were collected, showing concentrations of 0.14 microplastics per mg of tissue in 50% of the samples studied. Yang et al. (2015) can also be cited for their results regarding the ability of *Tenebrio molitor* larvae to ingest expanded polystyrene plastic as its sole source of food.

Some insects are already using microplastics as a substrate for egg deposition (Goldstein et al. 2012). Al-Jabaichi et al. (2019) observed mosquito larvae that, during aquatic larval development, ingested microplastic particles and remained with the material until the adult stage, thus transporting the material from water to air and soil, contaminating new environments. Larvae of the mosquito *Culex pipiens*, an insect with larval development in the soil, can consume microplastic particles of approximately 2 μ m, which are transferred throughout the larval stages until the adult stage (Al-Jaibachi et al. 2018).

Morelli et al. (2020) associates social parasitism with the dietary specialization of various species of Cuculiformes. However, *Cr. ani* does not show dietary specialization, feeding mainly on larger insects such as those of the Orthoptera order, eggs of other birds, small vertebrates (Repenning et al. 2009), as well as fruits and seeds foraging the ground (Hughes 1997). In this study, this species presented plastic filaments in the stomach contents, and, because it is a generalist species, accidental plastic consumption may be facilitated.

Conclusion

The present study provides results on plastic polymer contamination in birds that are not well studied in this regard, since the group most affected by plastic ingestion is still that of seabirds. *Caracara plancus* and *Cr. ani*, due to their generalist feeding habits, are susceptible to the consumption of various inorganic materials. The polypropylene ingested by the birds in this study is used in the manufacture of grain bags, fertilizers, plastic films, packaging, among others, which may explain its presence in rural areas. Metals were also found in the stomach of *C. plancus*, and many bird species are attracted to shiny objects and collect them for nesting or ingestion.

The plastic materials found in the species *P. chalybea* and *N. albicollis* are a warning of the presence of microplastics in insects as well, suggesting a potential trophic transfer of this material throughout the food chain. Microplastics are transported between environments, as they are carried from aquatic environments to distant areas by insects or even by wind action, and considering that *P. chalybea* is a migratory and insectivorous bird, it can transport plastic materials for several kilometers, contaminating distant areas from the place of origin after the bird's death or predation.

Therefore, there is a great need for expanding studies on the degree of microplastic dispersion in the wild and in synanthropic environments, on how this dispersion occurs, and on the degree of toxicity that plastic contamination causes in different taxonomic groups, as well as its influence on heterotrophic cycles.

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Data Availability

Supporting data are available at: https://data.scielo.org/dataset. xhtml?persistentId=doi:10.48331/scielodata.T8LQHM>.

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