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Microplastic accumulation in snake-eyed lizard (*Ophisops elegans* Menetries, 1832) after long-term monitoring: habitats matter, not years

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Abstract

Microplastics (MPs) have become pervasive environmental pollutants with significant impacts on ecosystems, particularly aquatic environments. As these particles infiltrate various habitats, they are ingested by a wide range of organisms, from plankton to large marine mammals. The ingestion of MPs disrupts the food web, causing physical and chemical harm to animals at multiple trophic levels. Here, we studied the accumulation of MPs in the gastrointestinal tracts (GITs) of a terrestrial lizard species after long-term monitoring using museum specimens in the collection of the Fauna and Flora Research and Application Center at Dokuz Eylül University from decades ago. These museum samples were from 1986 to 2013, but not consecutive years. GITs from 300 individuals were analyzed and MPs were detected in the GITs of only 25 individuals. In 25 individuals, the most dominant form of microplastic was fiber. The highest number of MPs was detected in 2001, followed by 1995. It is thought that this accumulation is caused by human activities in the lizard's environment and that it enters the food web indirectly because it lives in areas with high human interaction. Overall, this study shows that MPs have been present in the past, entering the food web of terrestrial species, and that MPs can inherently transfer to other living things. It is understood that MPs will pose significant threats to biodiversity and ecosystem health as they are transferred through the food chain.

Keywords Ectotherm, Food web, Polymer, Pollution, Reptile, Terrestrial

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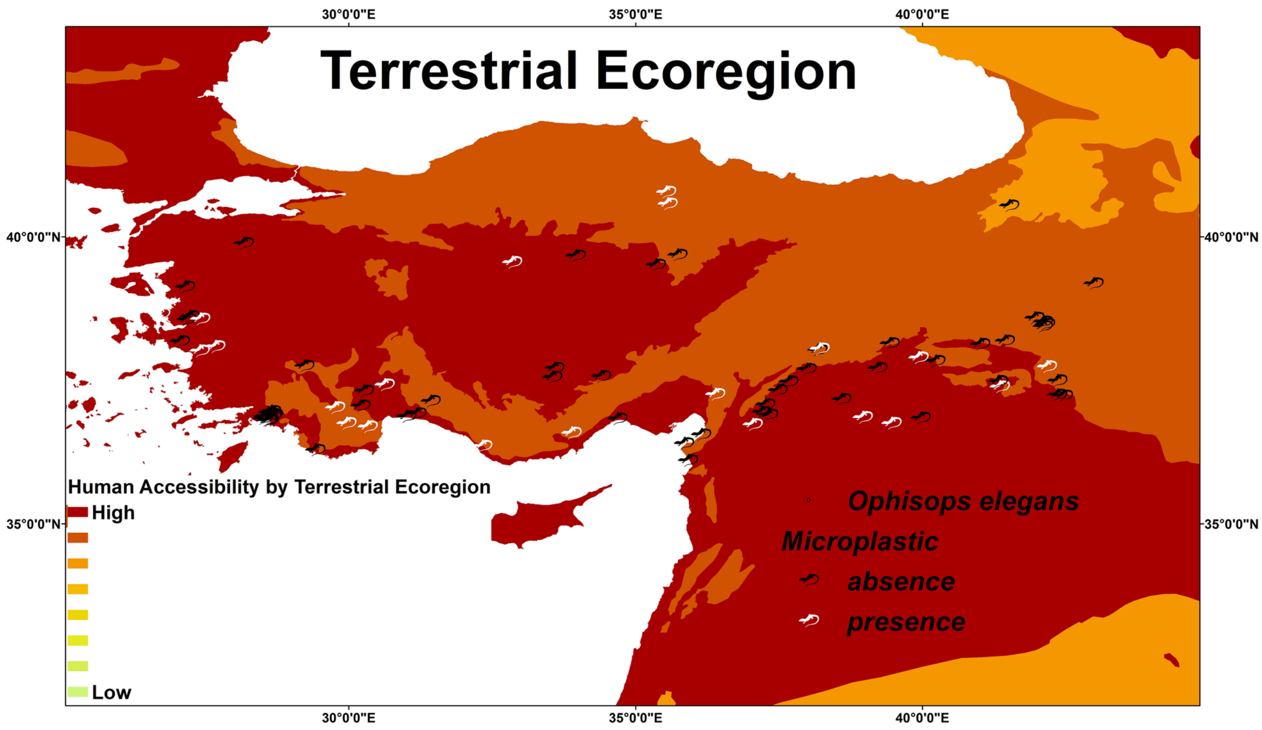
Graphical Abstract



Background

Plastic pollution is a worldwide issue that demands immediate attention because of its long-lasting presence in the environment and its harmful effects on ecosystems, infrastructure, society, and the economy [1]. Just 5 years ago, 353 million tons of plastic waste was generated globally, and this figure is projected to triple to over one billion tons by 2060 [2]. The global average plastic consumption per person is 45 kg annually. In contrast, Western Europe (which excludes Central Europe and the Commonwealth of Independent States) consumes roughly three times that amount, with each person using about 136 kg of plastic per year [3]. Plastics can gradually break down into smaller fragments, which are typically classified based on their size: macroplastics (larger than 2 cm), mesoplastics (ranging from 5 mm to 2 cm), MPs (from 1 micron to 5 mm), and nanoplastics (smaller than 1 µm) [4]. MPs are plastic particles smaller than 5 mm, typically classified into two main types [5, 6]. Primary MPs are deliberately manufactured for particular uses, like microbeads in cosmetics, microfibers in fabrics, or resin pellets used in industry [7, 8, 64]. On the other hand, secondary MPs result from the degradation of larger plastic items, such as bottles, bags, and packaging, which break down over time under the influence of environmental factors like sunlight and heat [7, 9, 64].

MPs are widespread in almost every environment in the world we live in [10]. In recent years, their presence has been identified in many living (biotic) and non-living (abiotic) environments. For example, in many invertebrates [11–14], vertebrates, such as fish [15–17]; amphibians [6, 18–23]; reptiles [23–28]; birds [23, 28–30]; mammals [23, 28, 31, 32]), freshwater sediments [33, 34]. MPs can impact environmental quality and biological health due to their physical and chemical characteristics, as well as their interactions with chemicals and microorganisms present in the surrounding environment (10). Furthermore, living organisms can ingest MPs, which may lead to alterations in feeding and reproductive behaviors, as well as higher mortality rates [65]. These toxic effects primarily arise from the release of harmful chemicals contained within the plastic and from pollutants that accumulate on the surface of floating particles [35].

The habitat is crucial for terrestrial ectothermic organisms, especially in the context of pollution. Ectotherms, such as reptiles and amphibians, rely on their surroundings to regulate their body temperature and metabolic processes. Habitat pollution, including chemical contamination, heavy metals, and MPs, can disrupt their physiological functions, reproduction, and survival. Pollutants can be absorbed through soil, water, and food sources, leading to bioaccumulation and detrimental effects [36]. Lizards can serve as valuable environmental indicators due to their sensitivity to changes in ecosystems and exposure to contaminants. Research shows that lizards are useful for biomonitoring environmental health, particularly because of their role in food chains and their direct exposure to soil, water, and other environmental factors [37, 38]. Lizards also show physiological and behavioral changes in response to land use changes, pesticide exposure, and habitat degradation and also, they can reflect the ecological impact of human activities [39]. In this study, we aimed to determine the biological accumulation of MPs in their bodies and to characterize MPs by using a lizard species with high human interaction as a model organism. Using museum collections in order to see the past microplastic accumulation and to shed light on the present makes important contributions to such studies. Therefore, we also emphasized whether years or habitat are important by observing long-term data for the past.

Material and methods

Fieldwork

A total of 300 adult individuals, collected from various regions of Türkiye between 1986 and 2014, were stored at the Fauna and Flora Research and Application Center at Dokuz Eylül University (Fig. 1). The samples were preserved in glass jars with 96% ethanol. The body weight of each museum specimen was measured with a scale accurate to 0.01 g, and body length was recorded using a digital caliper with a precision of 0.1 mm. To examine the presence of MPs, the GITs of each individual were carefully removed using a scalpel, starting from the upper esophagus and continuing to the anal opening. The extracted samples were then preserved in glass jars containing 96% ethanol for future analysis.

Studied species

Ophisops elegans, a small lacertid lizard, is characterized by fused eyelids that form a transparent capsule over the eyes, similar to those of a snake. This terrestrial species typically inhabits open, arid plains with sparse vegetation, favoring rocky or sandy substrates and steppe ecosystems. Its frequent occurrence in agricultural landscapes makes it particularly vulnerable to anthropogenic pressures [66]. The snake-eyed lizard is a diurnal insectivore, preying on insects, larvae, spiders, and small crustaceans [67]. Its geographical range extends from the southern Balkan Peninsula and the Aegean and Mediterranean islands to Southwest Asia, with an altitudinal distribution reaching up to 2000 m [66]. In Türkiye, it is widely distributed across suitable habitats, with the exception of the eastern Black Sea region [40].

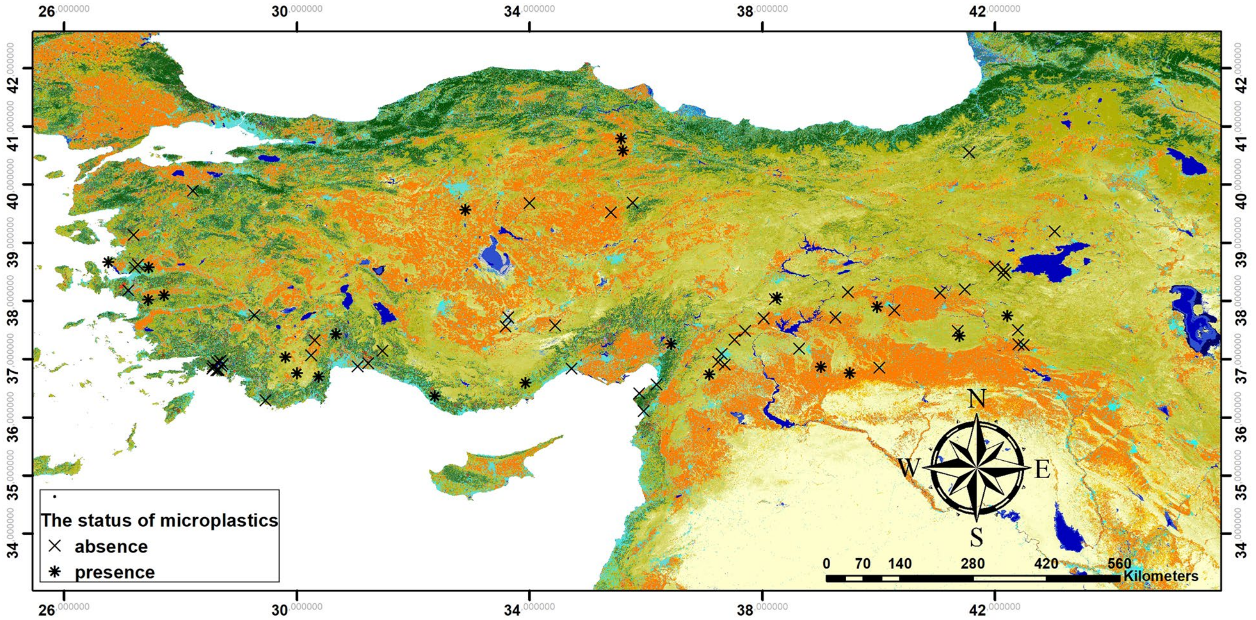


Fig. 1 The sampling locations of *Ophisops elegans* throughout Türkiye. Colors in the map present changes of land cover and land use between 2000 and 2020 (see [63] for color legend)

Digestion process and spectroscopic analysis samples

GITs from *O. elegans* were weighed individually with an analytical balance. Each gastrointestinal track sample was placed in 100 mL borosilicate tubes, and then 5 mL 10% aqueous KOH solution per 0.2 g samples were loaded into the tubes [41]. The tubes were sealed with a sheet of aluminum foil, and were placed in a water bath operated at 65 °C. After 24 h digestion, residues were collected on 47 mm diameter Whatman grade 4 qualitative filter papers and stored in glass Petri dishes. To reduce contamination risk, filtration processes were operated in a fume hood and cotton laboratory coats and single-use latex gloves were used during sample handling. Visual characterization of the particles by type, size, and color were analyzed with a Leica S6D® stereomicroscope, and all the suspected particles (145) were further analyzed with a Fourier-transform infrared spectrophotometer (PerkinElmer Spectrum 100) equipped with attenuated total reflectance apparatus (ATR/FTIR). FTIR analysis of samples were performed with number of 12 scans, and with resolution of 4 cm^{-1}, and over a range of 4000–650 cm^{-1}. Particles were transferred on to ATR crystal by using an insulin syringe with 5 mm needle length and 0.23 mm external diameter. In order to observe transferred particles, an 8 × magnifying glass with light was placed on to ATR plate. Obtained FTIR spectra were compared with reference data and spectra with similarity score higher than 70% were considered as plastics. Also, the size of MPs was determined by analyzing images captured under a stereomicroscope. This analysis was performed using the ImageJ software, with ruler calibration set to 1 mm at 4 × magnification, following the method outlined by Aragón-Sánchez et al. [42, 43].

Data analysis

Descriptive statistics were calculated for all continuous variables. The measurements of MPs were assigned to size categories following Chen [44]: small = 26.97–500 µm; medium = 500–1000 µm; large = 1000–5000 µm. The relationships between microplastic size, body length, body weight and GIT weight were investigated using correlation and regression analyses. To demonstrate the frequency of subcategories indicating the features of identified MPs and to compare the proportional contributions across microplastic characteristics, bar and pie-donut charts were set up based on plastic types. An alluvial diagram was produced to show the flow among microplastic characteristics. Lastly, the Chi-square test was used to interpret the difference between MPs type-size, shape, color and year. All analyses were executed in R Programming Language v4.1.2 [45].

Results

In the content of this study, 37 MPs were found in GITs of 25 different samples. The number of MPs per sample is 1.48 with determined specimens which are 0.12 for all samples (N = 300). All the suspected particles that are the materials of the false positives were organic materials and cellulose. The percentage of MP determined individuals represents 8.33% of all data. The dataset is presented in Supplementary Table S1. Plastic shape was mainly found as fiber (N = 36, 97.29%), but one fragment (N = 1, 2.71%). In total, 7 different types of MPs were unveiled in investigated samples (Fig. 2). Polyethylene terephthalate (PET) was mostly observed plastic type (N = 20, 54.06%) followed by polyacrylonitrile (PAN) (N = 11, 29.73%), Nylon (N = 2, 5.41%), styrene/butadiene rubber (SBR) (N = 1, 2.70%), nitrile butadiene rubber (NBR) (N = 1, 2.70%), ethylene vinyl acetate (EVA) (N = 1, 2.70%) and polypropylene (PP) (N = 1, 2.70%). The main color was determined as blue (N = 18, 48.65%) followed by red (N = 9, 24.33%), transparent (N = 4, 10.81%), pink (N = 4, 10.81%), black (N = 1, 2.70%) and yellow (N = 1, 2.70%). Plastics were mainly small (N = 32, 86.49%; mean length = 178.48 µm), as well as four medium (10.81%; mean length = 709.85 µm) and one large size (2.70%, mean length = 2015.69 µm). As for the year, a total of 8 MP was found in 2001 (21.62%) and 6 MPs in 1995 (16.21%) as maximum. The connection between all these categorical variables was demonstrated by alluvial plot in Fig. 3. Taking the plastic type into consideration as basic category, all plastics were fiber, but PP was observed by fragment shape. The large plastic was NBR while medium sized plastics were found in PET and PAN. A total of four different colors were observed in PET and PAN. Black and yellow MPs were seen in Nylon and NBR, respectively. Years were diversified in PET and PAN types associated with the number of determined MPs. The number of each subcategory and their percentages in data were visualized with bar and pie-donut graphics in Fig. 4. According to the Chi-square test results, there was a significant difference in terms of plastic type among years ($\chi2 = 98.16$; df = 72; $p < 0.05$), color ($\chi2 = 65.64$; df = 30; $p < 0.001$) size ($\chi2 = 41.83$; df = 12; $p < 0.001$) and shape ($\chi2 = 37$; df = 6; $p < 0.001$).

The average MP length was calculated 285.59 ± 57.72 µm (min: 26.97 µm; max: 2015.69 µm) for 37 plastics, body length was 47.74 ± 0.48 cm (min: 42.48 cm; max: 54.46 cm), body weight was 3.38 ± 0.14 g (min: 2.00 g; max: 5.10 g) and GIT weight 0.23 ± 0.02 g (min: 0.08 g; max: 0.40 g) for 25 samples. Correlation test yielded that there was not a significant relationship between body length–MP length ($r = -0.020$; $p > 0.05$), body length–GIT weight ($r = 0.035$; $p > 0.05$), body weight–MP length ($r = -0.127$; $p > 0.05$), GIT

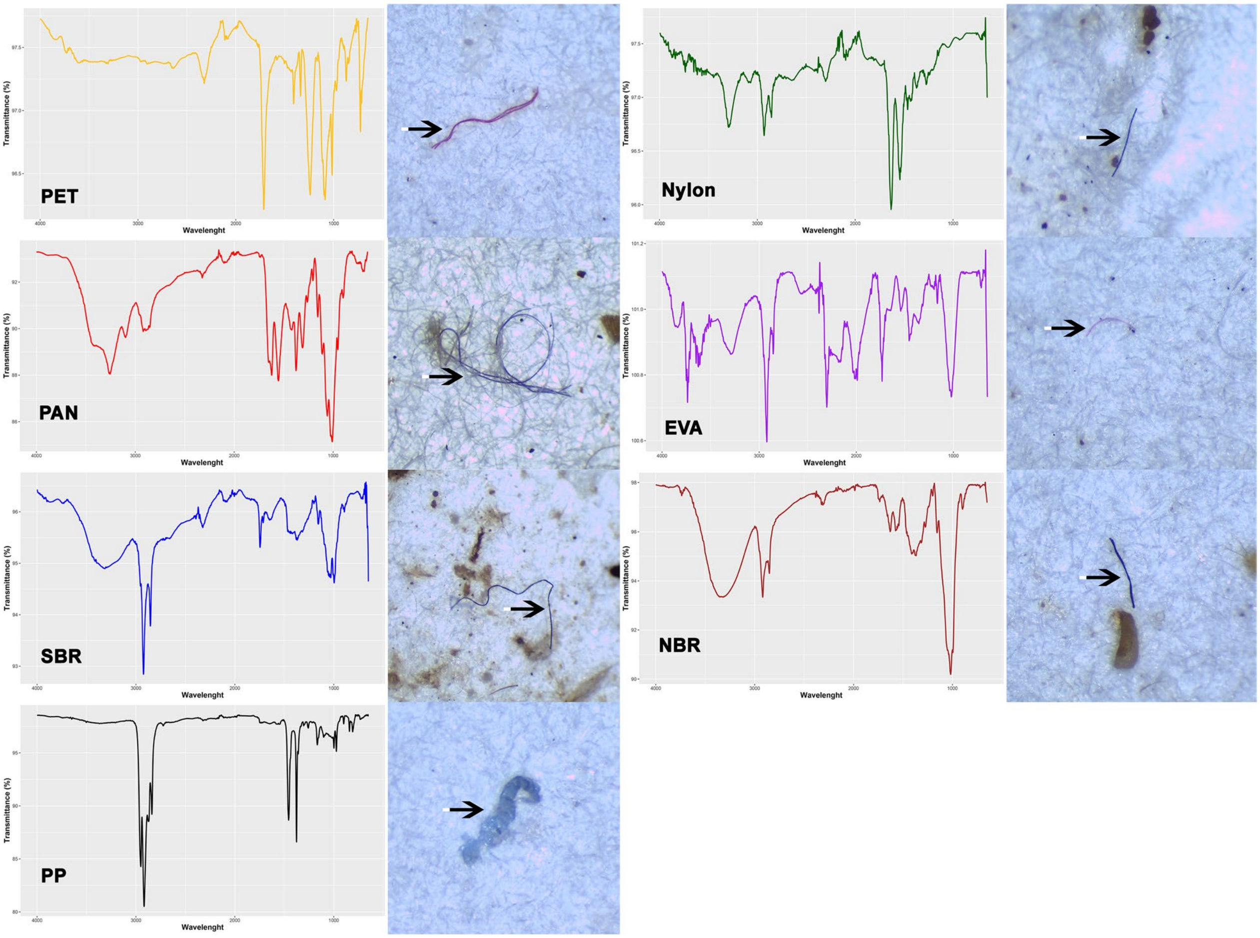


Fig. 2 FTIR spectra of microplastics detected in sampled lizards with representative photographs

weight–MP length (r = − 0.017; $p > 0.05$). However, a statistically significant relationship was determined between body weight–body length (r = 0.672; $p < 0.01$); and body weight–GIT weight (r = 0.363; $p < 0.05$). Linear regression models were also validated correlation results for body weight–body length (F: 28.88; R^2: 0.43; $p < 0.001$) and body weight–GIT weight (F: 5.32; R^2: 0.10; $p < 0.05$).

Discussion

Although it is mainly reported from aquatic environments, microplastic has infiltrated terrestrial habitats, as well. MPs can enter habitats from water bodies, soil, contaminated food or anthropogenic debris [68–70]. From that aspect reptiles have been paid less interest compared to marine animals and amphibians. However, it is vital to report the effect of microplastic presence in reptiles to develop conversation strategies and keep measurements against environmental degradation.

According to the results, MPs in GITs were found that approximately 9% of investigated individuals (N = 300) which is 0.12 per sample but 1.48 with MP determined samples. Recently, Mackenzie and Vladimirova [46] handled MP presence in terrestrial herpetofauna from South Paraguay using feces. They determined MPs 12.03% of 133 samples for the gecko *Hemidactylus mabouia*, and 6.00% of 50 samples for the lizard *Tropidurus torquatus*. The number of MPs per sample was 0.12 and 0.06, respectively, as similar to the findings in this study. Lu et al. [47] investigated the prevalence of MPs in terrestrial environments and reported the number of MP items per individual as 4.13 for *Gekko subpalmatus* species which is larger than observed items for *O. elegans* (1.48 items per determined samples). However, Teampanpong and Duengkae [23], reported MPs in 5 of 6 Distinct lizard species (71.43%) at the western Thailand and the average MP items were 1.29 per individual. The authors also noted the presence of MPs in the feces of Butterfly lizard *Leiolepis belliana* as 1.33 per sample [28] supporting the mean plastic number found in this study.

The main plastic type dominated the observed MPs were fiber with the rate of 97.29% while only one fragment was found among 37 MPs. Gül et al. [27] monitored

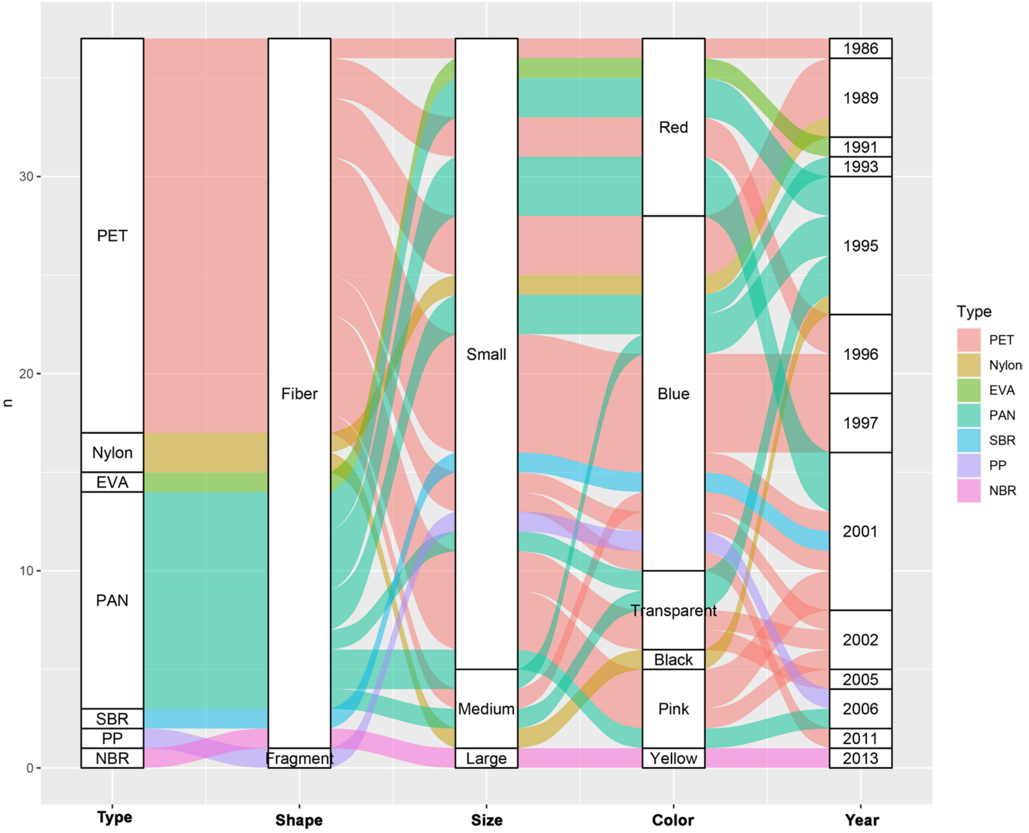


Fig. 3 Microplastic characteristics at five different categories with flow diagram

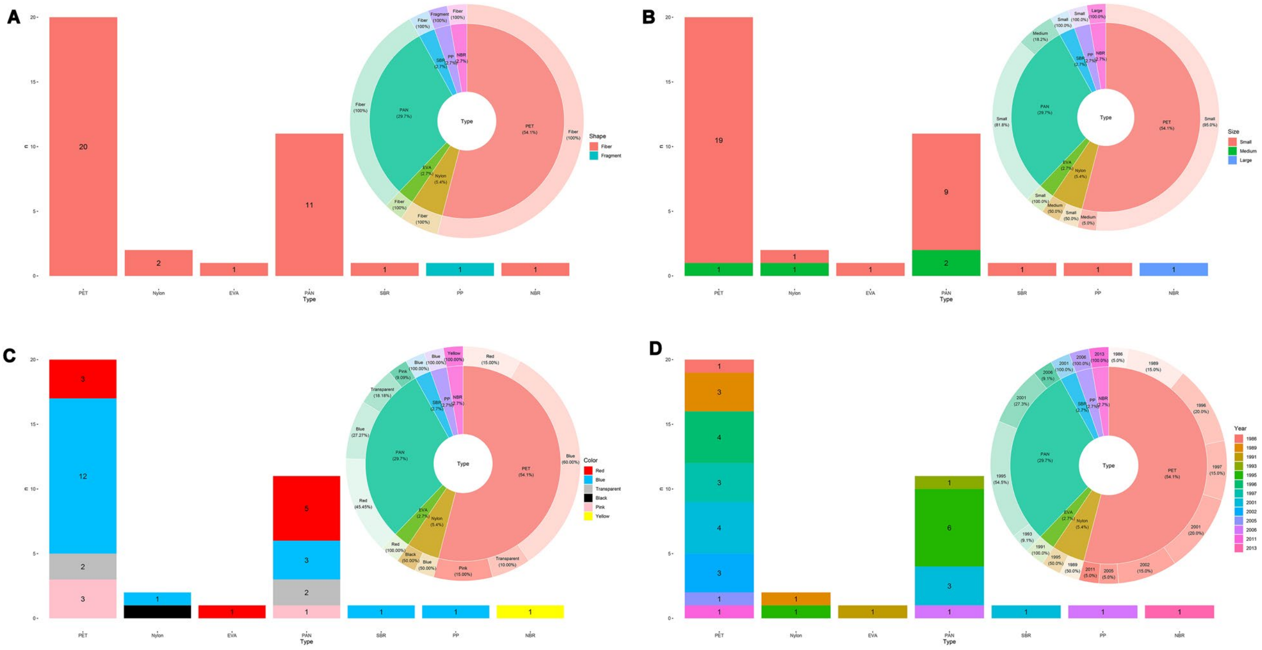


Fig. 4 Microplastic characteristics based on types. **A** Type-shape; **B** Type-size; **C** Type-color; **D** Type-year. The numbers within the bars show the count of and within pie-donuts show the percentage of each subcategory

MPs in two different reptiles namely *Natrix natrix* (Grass snake) and *Natrix tessellata* (Dice snake). They determined MPs in 22 of 41 examined individuals. Moreover, the fiber was the most observed plastic shape with the rate of 94.70% for *N. natrix*, and 87.90% for *N. tessellata* samples. Mackenzie and Vladimirova [46] also recorded 112 fibers among 132 MPs for terrestrial reptiles. Meaza et al. [48] observed MPs in seven different turtle species, and they indicated that fiber was the most predominant plastic shape in this organism group. Besides reptiles, fiber is also reported as prevalent plastic shape in amphibians which are the other herpetofauna group. For example, Hu et al. [49] showed the highest fiber content in different four different anuran tadpoles (*Bufo gargarizans*, *Microhyla ornata*, *Rana limnochari* and *Pelophylax nigromaculatus*). Szkudlarek et al. [6] studied the similarity of microplastic characteristics between amphibian larvae including toads, frons and newts from 23 different sampling sites and fiber was the most abundant microplastic. Kuranova et al. [50] also pointed out that fiber was the most observed shape between accumulated MPs in gastrointestinal tract of the Siberian wood frog *Rana amurensis*. Fiber-shaped MPs are very common in environments due to being an important source of synthetic textile industry and especially during washing process they can penetrate waterbodies [51]. These materials are highly durable and become smaller in degradation rather than disappearing. Therefore, the material tends to accumulate in ecosystems and is easily observed in different animal groups because of prey dietary or water requirements [52–54]. For instance, Lu et al. [71] evaluated terrestrial MP pollution in various animal groups, and they found that 84.68% of detected MPs were microfibers. Sherlock et al. [72] monitored MPs in terrestrial environments using insectivores as model organism, and they found that microfibers were most prevalent type in GITs and fecal sacs. Considering these characteristics, it is reasonable to find fibers as prevalent material in *O. elegans*, as well.

The results revealed that PET was the most abundant type in dataset with the rate of 54.06% in all MPs. PET (polyethylene terephthalate) is prevalent in animals because it is the main material in packaging such as food containers, plastic bottles and synthetic textile products. The material is highly durable against degradation and can stay in the environment for long periods. The material tends to break down into microfibers due to external factors such as physical weathering (mechanical abrasion), UV degradation, and releasing tiny particles in degradation processing [78]. Therefore, smaller pieces are easier to ingest by animals and can be transferred among different groups by bioaccumulation [55]. The second predominant plastic type was found as PAN which is used in textiles, filters, and as a precursor for carbon fibers [79, 80]. Considering *O. elegans* is a terrestrial species, it is logical to observe these MPs due to anthropogenic effects on environmental pollution. Lu et al. [47] reported that 40.45% of MPs investigated terrestrial animals were PET including reptiles. Prata et al. [56] characterized MPs in internal tissues of animals from urban environments and they reported the presence of PET as diagnosed type. However, it is not a certain trend and can be changed by the organism and its habitat associated with it. For example, Pastorino et al. [57] searched for MP occurrence in European Common Frog (*Rana temporaria*) from Cottian Alps and PET was the fourth abundant material with the rate of 20% after PA and PE. As a study on reptiles, Zhang et al. [58] monitored MP pollution in China Sea via the organism *Chelonia mydas*, and they found that PE, PP and PS were shaped polymer composition. Therefore, it can be assumed that plastic type is more associated with the local pollutant factors such as plastic industry and manufacturing, tourism activities, road and vehicle wears and agricultural practices [73–75].

Another characteristic, six different colors were observed in MPs and blue was the most prevalent color among them (N = 18, 48.65%) followed by red (N = 9, 24.33%) and transparent (N = 4, 10.81%). Gül et al. [27] reported the dominance of blue MPs in *Natrix* snakes (52.60%). Mackenzie and Vladimirova [46] also reported transparent and blue MP particles as most observed colors in Southwestern Paraguay herpetofauna. Banaee et al. [25] also noted that MPs were mainly blue observed for the pond turtle *Emys orbicularis*. Besides, the dominancy of these colors were reported in different amphibian species [6, 32, 57, 59, 53% blue]. Blue and transparent MP particles were mostly ingested by animals because they are mostly used in packaging such as water bottles. These types of MPs also do not sufficiently absorb UV light, they spoil faster under the sunlight, therefore the highest observation is possible due to the material characteristic [60]. In addition, blue plastic materials degrade slower compared to lighter materials due to pigment structure such as phthalocyanine which is resistant to UV radiation, or chemical characteristics such as used stabilizers in dying process providing higher thermal capacity [76, 77].

As for size of MPs, most of them were small, which is less than 500 µm (N = 32, 86.49%) and only one large MP (1000 µm) was found in all particles in which the mean size calculated as 285.59 µm. Small-sized MPs are widespread in different environments such as oceans, rivers and soil [49]. Besides, the particles are easier to ingest by animals and they resemble small prey items [61]. They can also be transferred through different trophic levels via bioaccumulation [62]. Therefore, microplastic presence in the gastrointestinal tract of *O. elegance* can also be evaluated as the transition of MPs in with regard to trophic levels because numerous insects were observed in digestive system.

Lastly, the data were presented for a long-term period as 27 years. In these years, PET was observed on a large scale followed by PAN. Even though PAN is not one of the most commonly detected plastics in environmental monitoring, agricultural and textile products, like fertilizers pesticides and water-resistant clothes, can contain the material. Given the habitat of *O. elegans* contains agricultural sides and the materials have been in industrial usage since the past, it makes sense to observe these materials comparing to others such as SBR, PP and NBR which were more recent. Most of the detected MPs was in small size and the dominant color was blue. Dursun et al. [22] handled retrospective patterns of MPs in *Pelophylax ridibundus* and reported the data from 37 years (1984–2021). They also found similar plastic characteristics in this study such as blue and transparent domination in color, mostly small and medium size MPs and fiber shape. Gül et al. [27] also presented data collected in 30 years characterized by PET domination with blue and transparent colors probably due to plastic bottle waste. Kankanige and Babel [81] investigated small MP contamination in Thailand derived from the PET-bottles, they reported that blue and transparent colors were frequently observed. Lim et al. [82] monitored MP pollution of corals in Taiwan, and they found that the most observed color was blue followed by transparent while the dominant type is PET. Although the results were supported by the literature, it must be taken into consideration that data distribution cannot be homogenous because of the large sampling area in different time periods.

To conclude, the data based on the long-term monitoring period were presented for the first time in literature for *O. elegans* species. Besides, MPs were characterized in terms of color, shape, type and size. Further studies can handle more terrestrial lizard species to monitor the presence and impact of MPs, allowing for a comprehensive analysis of their accumulation, distribution, and effects on different species. By comparing interspecific differences, researchers can better understand how various species might be differently affected by MPs, which can depend on factors such as diet, habitat, and behavior. Additionally, expanding the research to include a wider range of habitats and geographical locations could reveal patterns and hotspots of contamination, contributing valuable data for environmental management and conservation efforts.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-024-01042-0.

Additional file 1.

Author contributions

C.D. investigation, methodology, formal analysis, visualization, writing-original draft. K.K.: methodology, formal analysis, writing-original draft. K.C., E.Y.C., Ç.I., and Y.K..: resources, writing-original draft. S.G.: conceptualization, methodology, writing-original draft, review and editing, supervision, funding acquisition. All authors read and approved the final version of the manuscript.

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Availability of data and materials

No datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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