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PLASTIC INGESTION IN GIANT TORTOISES AS A NOVEL ANTHROPOGENIC IMPACT FOR GALAPAGOS WILDLIFE

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A las Tortugas Gigantes de Galápagos

INDEX

LIST OF TABLES V

LIST OF FIGURES VI

MANUSCRIPT 8

Abstract 9

1. Introduction 10

2. Materials and Methods 12

2.1. *Study site* 12

2.2. *Sampling design and sample collection* 13

2.2.1. *Quantification of human waste within tortoise dung* 13

2.2.2. *Characterization of environmental garbage availability* 14

2.2.3. *Giant tortoises' distribution and abundance* 14

2.3. *Statistical analysis* 15

3. Results 16

3.1. *Quantification of human waste within tortoise dung* 16

3.2. *Characterization of environmental garbage availability* 16

3.3. *Giant tortoises' distribution* 17

4. Discussion 19

5. Acknowledgments 23

6. References 24

7. Table captions 33

8. Figure captions 33

LIST OF TABLES

Table 1. Human waste fragments found within Santa Cruz giant tortoise' feces *(Chelonoidis porteri)* classified by type of material. *33*

Table 2. Giant tortoise abundance clustered by study area and land-use type for the Galapagos tortoise' plastic ingestion study in Santa Cruz Island..... 33

LIST OF FIGURES

Figure 1. Sampling locations of giant tortoise' plastic ingestion study in Santa Cruz Island according to its predominant land-use type: protected, agriculture, rural road, industrial, main road, and urban area..... 34

Figure 2. Comparison of the percentage of types of waste items found within Galapagos giant tortoises' feces to types of waste items found in human-modified areas on Santa Cruz Island. 34

Figure 3. Comparison of the percentage of human waste grouped by color found in tortoise feces and in the environment within the human modified areas of Santa Cruz Island..... 35

Figure 4. Average Santa Cruz tortoise recapture rate clustered by land-use type. Notice a significantly tortoise recapture rate in the industrial area when compared to the other sampling areas. 35

Figure 5. Galapagos tortoise (*Chelonoidis porteri*) health threats associated to human impacts and documented during the giant tortoise' plastic ingestion study in Santa Cruz Island. 36

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Full Paper

PLASTIC INGESTION IN GIANT TORTOISES AS A NOVEL ANTHROPOGENIC IMPACT FOR GALAPAGOS WILDLIFE

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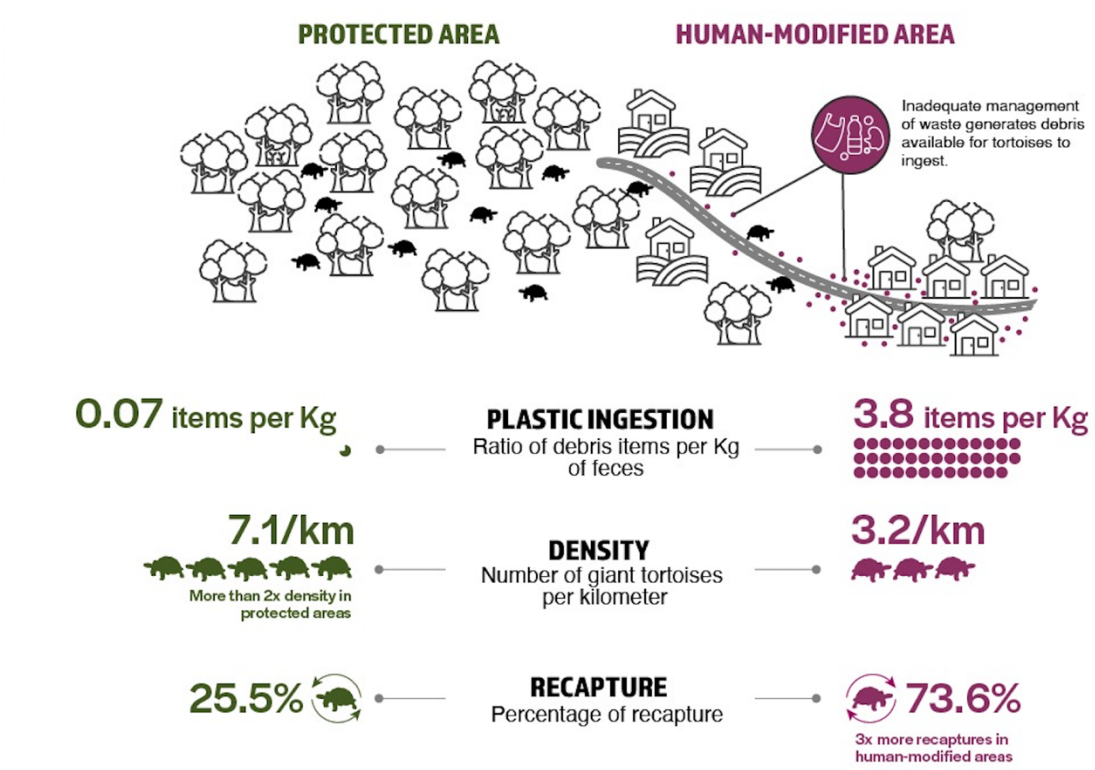
Abstract

The human population of Galapagos has rapidly increased in the last decades accelerating the anthropogenic pressures on the archipelago's natural resources. The growing human footprint, including the associated inadequate management of waste, may lead to conservation conflicts. We conducted this study to determine to what extent giant tortoises were ingesting human waste in Santa Cruz Island, and which human-modified areas are most affected by this novel threat to environmental health. Additionally, we characterized the environmental debris available for tortoises to ingest and quantified tortoise abundance. We collected and processed a total of 6,629 tortoise feces along a gradient of anthropogenic disturbance based on human debris presence. We found 590 pieces of debris within human-modified areas (3.8 items/kg of feces) and only two pieces in the protected area (0.08 items/kg of feces). Plastic waste was the predominant garbage category we found into feces in the anthropic area (86.3%; n = 511), followed by cloth, metal, paper, synthetic rubber, construction materials, and glass. The composition of waste items found in the environment differed to the trash found within the feces, with plastics more abundant in tortoise feces than the environment. Tortoise abundance was higher in the protected area when compared to the human-modified, but proportion of tortoise' recaptures was triple in the anthropic area than in the National Park. Additional tortoise health threats were observed during this study, including dog bite lesions, tortoise entanglement, and car accidents. Our results indicate that plastics are a frequent item in the diet of tortoises in anthropic areas of western Santa Cruz. More research is needed to understand the negative health impacts associated with plastics and other pollutants for Galapagos terrestrial species. We encourage local stakeholders to limit human-waste from entering the Galapagos environment to minimize threats to human and animal health.

Keywords (5-6 words)

Chelonoidis, pollution, One Health, plastics, Anthropocene, wildlife surveillance

Graphical abstract



Main Text

Introduction

The Anthropocene is the current geological epoch characterized by the growing human footprint leading to global impacts that affect planetary health (Crutzen, 2002; Keys et al., 2019; Zalasiewicz et al., 2020). Earth is increasingly threatened by climate change, the extinction of species, land-use changes, the exploitation of organisms, pollution, and invasive alien species (Deem et al., 2019). Among these challenges, anthropogenic pollution is one of the greatest threats, and is leading to the loss of biodiversity and harming animal, human, and environmental health (Myers et al., 2013). Plastic is among the most prevalent global pollutants (Diaz et al., 2019; Marques et al., 2021) and it is estimated that the input of plastics into ecosystems will triple within 20 years unless immediate action is taken to reduce their production and limit their use (da Costa, 2021).

The ubiquity of plastics, both macro and micro, represents a major threat to the health of wildlife species, as plastics often lead to ingestion, injuries, and entanglement (Gall & Thompson 2015; Nicastro et al., 2018; Lima et al., 2019). Furthermore, microscopic plastic particles may cause endocrine disruption, and more recently have been shown to act as vectors of invasive species, infectious diseases, and even antimicrobial resistance (Bhandari et al., 2015; Pahm et al., 2021; Leslie et al., 2022). Recent studies have documented plastic waste in at least 1,565 species, of which 277 are terrestrial and freshwater and the remainder belong to marine ecosystems (Santos et al., 2021). Terrestrial species might be underrepresented due to a lack of studies for land compared to marine ecosystems (Staffieri et al., 2018; Blettler & Mitchell, 2021; Kutralam-Muniasamy et al., 2021; Gamarra-Toledo et al., 2023).

The Galapagos Islands is a global conservation icon and one of the most emblematic places for unique marine and terrestrial flora and fauna (Reck, 2014). However, the archipelago is not exempt from anthropogenic environmental degradation, including habitat loss, pollution, invasive species, and climate change (Sachs & Ladd, 2011; Toral-Granda et al., 2017; Alfaro-Núñez et al., 2021). Plastic pollution has been recently documented in the Galapagos marine reserve, with up to 52% of the analyzed marine invertebrates ingesting microplastics (Jones et al, 2021). Additional impacts have been observed, including flightless cormorants (*Phalacrocorax harrisi*) building their nest with plastic bags, and sea turtles and sea lions mistaking plastics with food or getting trapped or entangled (Zambrano-Monserrate & Ruano, 2020). Modelling approaches have identified continental inputs as a major source of incoming plastic contamination to Galapagos coasts, mostly from southern Ecuador and northern Peru (van Sebille et al., 2019). However, an increasing amount of human waste and plastics can be observed on land for all human-populated islands, but its magnitude and potential impact to wildlife health has not been documented. The impact of plastics in terrestrial Galapagos species has only been documented by Harvey et al. (2021), reporting a mortality of up to 18% in Darwin's finches due to anthropogenic debris used to build their nests, and leading to hatchling entanglement and strangulation.

Galapagos tortoises (*Chelonoidis* spp.) are one of most emblematic species of the archipelago and can act as bio sentinels of ecosystem health (Nieto-Claudin, 2022). Giant tortoises play key roles maintaining healthy ecosystems in the archipelago as well as supporting local economies based on tourism (Blake et al., 2021; Frazier, 2021). Santa Cruz is the most human-populated island and represents 61.5% (21,214 inhabitants) of the entire Galapagos population (INEC, 2021). Additionally, this island supports two critically endangered giant tortoise species (*Chelonoidis porteri* and *Chelonoidis donfaustoi*) (Cayot et al., 2017a,b). The Western Santa Cruz tortoise (*C. porteri)* has an estimated population of more than 6,000 individuals, and it is the species that overlaps the most with anthropogenically disturbed areas (Nieto-Claudin et al., 2021; Pike et al., 2022). During their annual migrations, tortoises in Santa Cruz leave the protection of the National Park areas and enter private land (i.e., agricultural, livestock, urban, and rural areas), where they may be exposed to numerous threats including vehicle strikes, introduced species, and exposure to agrochemicals and resistant bacteria (Flanagan, 2021; Nieto-Claudin et al., 2021; Nieto-Claudin et al., Unpublished data).

In recent years, we have observed increased local plastic pollution in the environment and encountered plastics in tortoise feces within anthropic areas, prompting the current study. The lack of data on free-living giant tortoises and their exposure to, and potential ingestion of human waste led us to design a study to describe and characterize anthropogenic waste in the feces of *C. porteri* within areas with varying levels of anthropogenic disturbance. Additionally, we characterized the environmental debris available for tortoises to ingest and quantified tortoise abundance.

Materials and Methods

Study site

We collected data from two areas of Santa Cruz: (1) a protected area within the National Park called “La Torta,” and (2) a human-modified area that included farmland, industrial, and urban sites (Figure 1).

We sampled on a 4.6 km transect within “La Torta” that extends from 65 m above sea level to 130 m, covering a total area of 0.02 km^2. La Torta is a protected area with little to no human impact, and regularly monitored by the Galapagos National Park rangers to assess tortoise nesting sites. No human activities are permitted within the protected area, except for hunting of invasive species (e.g., wild boars, goats) and scientific research. The eastern end of the first transect was followed by the second transect (7.7 km; 0.06 km^2) which traversed several zones under anthropogenic disturbance with an elevation gradient between 26 m to 130 m above sea level. Land-use types along the transect were agriculture and livestock, industrial, main and secondary roads, and urban areas.

The human-modified area was classified according to its predominant land-use type, into five categories: (1) agriculture: crop and livestock farms where rural houses with no sewage systems are present in small numbers; (2) rural road: recently opened unpaved road mainly used to connect the agriculture and industrial areas, (3) industrial: locally called “Artisanal Park” where environmental and acoustic pollution exists from industrial and artisanal activities including carpentry, ship building, block construction, and automobile mechanics, (4) main road: paved Baltra highway which is the only access to the city of Puerto Ayora, and (5) urban: highly populated neighborhood of "El Mirador" added to the town of Puerto Ayora in 2012.

Sampling design and sample collection

Quantification of human waste within tortoise dung

Fecal samples were collected weekly in the anthropogenic area and monthly in the protected area, from April to November 2021. We walked the whole transects during morning hours (6h30 to 13h00), from northwest to southeast in the anthropogenic area and from southwest to northeast in the protected area. Fecal samples were collected by hand using sterile gloves. The number of feces was recorded per transect, and all samples from the same transect were preserved together within labeled Ziploc bags until analyses. In the anthropic area, we removed all feces from the trail one week before starting the study. Consequently, only feces defecated

since the previous sampling effort were collected, since the trail was covered every Friday for 34 consecutive weeks.

Within 6 hours of collection, fecal samples were transported to the laboratories of the Charles Darwin Research Station (CDRS) and frozen (-20 °C) for 24-48 hours to kill invertebrates. Following the thawing of the feces, all samples were dehydrated for 7 days at a constant temperature of 45°C by using an artisanal plant dryer made with nine lamps (200 WATT). Dry samples from each transect were weighed with an analytical balance (BOECO, BWL51), followed by a close examination using gloves and tweezers to manually disaggregate each sample and identify anthropogenic residues. We identified and quantified all items that were observable to the naked eye (≥ 1 mm). In addition, we used a magnifying glass and a stereoscope to verify their characteristics. For every item, we recorded size (length and width), color, state of degradation (ranging from 0-3, 0 no degradation and 3 highly degraded), and type of material using the categories shown in Table 1. Plastics were further classified into microplastics (1 ≤ 5 mm) and macroplastics (> 5 mm). Those plastic particles smaller than 1 mm were recorded as observed but not counted.

Characterization of environmental garbage availability

We quantified the amount of human waste available for tortoise ingestion in both sampling areas and within both seasons of the year (dry and wet). This work was performed at separate times from the tortoise counts and fecal sample collection. In May (wet season) and September (dry season) 2021, two 20 m^2 quadrants were randomly selected for each transect. All anthropogenic garbage was quantified and characterized per quadrant, and a photo with a 150 cm flexible measuring tape was taken. All waste was returned to the collection sites until the completion of the study. Garbage items were characterized and classified using the same parameters shown in Table 1.

Giant tortoises' distribution and abundance

We estimated tortoise location and abundance using a capture-recapture technique at the sampling sites concurrent with fecal sample collection. Tortoises encountered

for the first time were classified as “captured” and marked with unique numbers using nail polish on the dorsal-caudal scutes of the carapace. Consecutive numbers were added with each captured individual. A GPS location for each tortoise was recorded as well as curved carapace length (CCL), dorsal photo, sex and age (based on tail length and CCL as described in Nieto-Claudin et al., 2021), activity (i.e., sleeping, resting, walking, eating, copulating, drinking), and any signs of injury or disease (based on a visual exam). All marked tortoises found on future surveys were considered as “recaptured.” To assess individual movements, a GPS location for all recaptures was noted as well as a dorsal photo, activity, and a visual exam.

Statistical analysis

Tests of equal proportions were used to compare the number of garbage items and the number of feces collected per zone area. Pearson’s Chi-squared test was used to test the proportion of garbage types against a uniform distribution, and to test the number of garbage items by season. We used the non-parametric Kruskal-Wallis test to compare the weights and sizes of garbage in feces and in the environment between land-use zones. Mann-Whitney U tests were used to compare garbage size and garbage weight in feces by season. Fisher’s exact test was used to determine if there was a significant association between land-use type and recapture status.

We performed descriptive statistics of tortoise capture and recapture events clustered by sampling areas, land-use type, mean elevation per transect, sexes, and ages. To compare differences in mean tortoise size between land-use zones, we used a one-way ANOVA and a post-hoc Tukey’s HSD for pairwise comparisons if a significant difference was found. Two-proportion z-tests were used to compare differences in age and sex proportions by season, as well as differences in the proportion of garbage types and colors in feces and the environment. All statistical analyses were undertaken in RStudio Version 4.1.1

Results

Quantification of human waste within tortoise dung

A total of 1,039 tortoise feces (26.1 kg) were collected in 8 surveys within the protected area (1.2 kg/1000 m^2), whereas 5,590 feces (156.1 kg) were collected in 34 surveys within the anthropogenic area (2.4 kg/1000 m^2). A significant difference was found between land-use types within the anthropic area ($p < 0.001$), with a higher density of feces within the main road (6.2 kg/1000 m^2) and urban area (5.1 kg/1000 m^2) compared to the other sampling areas ($p < 0.001$).

Only two fragments of hard plastics were found in tortoise feces in the protected area (0.076 items/kg of feces). In contrast, 590 pieces of debris were found in tortoise feces within the human-modified area (3.8 items/kg of feces). This difference was statistically significant ($p < 0.001$). Plastic waste was the predominant garbage category in the anthropic area (86.3%; n = 511), followed by cloth (8.4%; n = 50), metal (2%; n = 12), paper (1.7%; n = 10), synthetic rubber (0.7%; n = 4), construction materials (0.5%; n = 3), and glass (0.3%; n = 2). Classified by land-use type, a higher ratio of trash items per kg of feces was found in the main road and urban area (6.7 items/kg and 4.6 items/kg, respectively) compared to the other three areas ($p < 0.001$). Seasonality influenced the ratio and weight of trash items within the anthropic area, with more (chi-squared test $p < 0.001$) and heavier (Mann Whitney U test, $p = 0.04$) debris recorded during the wet season. By contrast, debris collected in the dry season were longer on average than on the wet season (Mann Whitney U test, $p = 0.04$). Predominant trash colors included transparent, green, and white, followed by light blue and black.

Characterization of environmental garbage availability

We covered a total of 28 quadrants of 20 m^2 (560 m^2) within the protected area and 32 quadrants (640 m^2) within the anthropogenic area in two sampling efforts (wet and dry season). No anthropogenic garbage was found within the protected area whereas a total of 462 items were found within the human-modified area with an average of 0.72 items/m^2. Clustering by land-use type, more debris were quantified

within the industrial zone (1.1 items/m^2), followed by the main road (0.98 items/m^2), agriculture (0.42 items/m^2), urban (0.38 items/m^2), and rural road (0.3 items/m^2) ($p < 0.001$). The most frequent category were plastics (65.4%; n = 302) ($p < 0.001$), followed by paper (9.7%; n = 45), cloth (8.0%; n = 37), glass (5.6%; n = 26), metal (5.4%; n = 25), synthetic rubber (3.7%; n = 17), and construction materials (2.2%; n = 10). There were no significant differences on item length ($p = 0.29$) and width ($p = 0.16$) by land-use area.

The composition of waste items found in the environment differ to the trash found within the feces. Plastics were more abundant in feces than within the environment ($p < 0.001$) whereas material constructions ($p = 0.03$), rubber ($p = 0.001$), paper ($p < 0.001$), metal ($p = 0.005$), and glass ($p < 0.001$) were more abundant in the environment when compared to feces (Figure 2).

Overall, more garbage items were found during the wet season (n = 289) than during the dry season (n = 173) ($p < 0.001$). Clustered by land-use type this difference was significant for the industrial ($p < 0.001$) and the agricultural areas. Items were also bigger during the wet season (244 cm^2 on average) compared to the dry season (73.4 cm^2) ($p < 0.001$).

Garbage colors found in the environment were predominantly transparent, white, and black, followed by green, blues, greys, and yellows. Statistical differences were found when comparing color composition between fecal items and the environment; pink, light blue, and green garbage were significantly more abundant within tortoise feces and transparent, orange, grey, and silver were significantly higher in the environment (Figure 3).

Giant tortoises' distribution

We recorded a total of 153 tortoises in 8 transect surveys (19.1 tortoises/survey) within the protected area, and 208 tortoises in 34 transect surveys (6.1 tortoises/survey) within the anthropic area. Tortoise abundance was higher in the protected area (7.1 captured tortoises/1000 m^2) when compared to the human modified area (3.2 tortoises/1000 m^2). In the anthropic area, tortoise abundance was

278 higher in the agriculture (6 tortoises/1000 m^2) and urban areas (4.4 tortoises/1000
279 m^2), and at the main road (4.5 tortoises/1000 m^2). Tortoises were significantly bigger
280 in the agricultural area and rural road when compared to the other areas ($p < 0.001$).
281 Tortoises observed more often tend to be smaller in both protected and anthropic
282 areas (Table 2).

283 Tortoise recapture events were common in both the protected area (80 recapture
284 events) and the anthropic area (563 recapture events). In the protected area 25.5%
285 of marked tortoises were observed at least two times, and the majority of tortoises
286 marked in the anthropic area (73.6%) were observed twice or more. The industrial
287 zone had a significantly higher proportion of recaptured tortoises (7.6 recaptures to
288 captures ratio) ($p < 0.001$) when compared to all other sampling areas. In the
289 protected area the recapture to capture ratio was 0.5. Only one adult male tortoise
290 initially captured in the protected area was recaptured in the anthropic area
291 (agricultural zone).

292 The proportion of sex (male, female, undetermined) and age (adult, subadult, or
293 juvenile) differed across elevation (bigger and more mature animals at higher
294 elevations), and land-use type. More subadults were observed during the wet
295 season, compared to the dry season ($p = 0.02$).

296 During the course of this study, additional tortoise health threats were observed
297 within the anthropized areas. These included an adult male tortoise (CCL = 152)
298 entangled by a rope within a pool in the agriculture area, a subadult female (CCL =
299 71.2) recaptured six-times on the industrial area was observed the last time with an
300 injury on the cranial carapace, most likely due to a car impact, and a dead subadult
301 tortoise with multiple fractures of the carapace was found by the edge of the road in
302 the agriculture area (Figure 5). In addition, 13% (20/153; 95% CI 0.09-0.19) of the
303 tortoises from the protected area and 7% (14/208; 95% CI 0.04-0.11) from the
304 anthropized area presented lesions on the edges of the carapace consistent with
305 dog attacks.

306

Discussion

In this study we describe the ingestion of human waste by a critically endangered species of Galapagos giant tortoise. To the authors' knowledge this is the first report of plastic and other human waste in the diet of land tortoises in the Galapagos archipelago. Tortoises within human-modified landscapes had a significantly higher frequency of plastics and other human debris compared to tortoises in natural protected areas.

Our results indicate that plastics are a frequent item in the diet of tortoises in anthropic areas in Santa Cruz Island. However, there is no information on the potential effect of plastics and microplastics in giant tortoise health. Endocrine disruptors (EDCs) have been described as omnipresent chemicals that can be found within medicines, pesticides, some products for human consumption, and all types of plastic materials (Encarnacão et al., 2019). Of mounting concern are Bisphenol A (BPA) and 17α-ethynylestradiol (EE2) the most widespread in marine and terrestrial habitats, negatively influencing the health and reproductive fitness of animals and humans (Bhandari et al., 2015; Deem & Holliday, 2022). The impacts of EDCs have been studied in some reptiles (Jandegian et al., 2015; Tubbs &McDonough, 2018) and main negative effects include malformation of gonads, altered plasma hormone concentrations and liver function (Milnes & Guillette, 2008), feminization of aquatic turtles, and behavioral changes in turtle hatchlings (Jandegian et al., 2015; Deem & Holliday, 2022). In fact, studies on EDCs have shown that painted turtles and alligators could act as sentinel species of ecosystem health (Guillete et al., 2000; Deem & Holliday, 2022). The concern of how endocrine disruptors might affect the health and reproductive physiology of reptiles is greatest for critically endangered species (Tubbs & McDonough, 2018), such as the giant Galapagos tortoises. Since juveniles remain longer within the low anthropic areas and near town, the effect of EDCs in non-sexually mature animals may possess an even greater risk for the reproductive fitness of these individuals and consequently for the long-term survival of this species.

A higher prevalence of tortoises with lesions on the carapace edges were found within the protected area. The characteristic of these lesions supports the negative impact that feral dogs have on tortoises within the National Park areas. The additional threats observed during this study (tortoise entanglement and deadly car encounters) indicate that tortoises within human-populated islands are facing a number of anthropogenic threats that are not only limited to the human-modified areas.

The different composition of garbage items found in tortoise feces and the environment can be explained due to the degradability of some materials (such as paper, that might be more difficult to find in feces) and the size and hardness of some others (rubber, material constructions, metals, and glasses) that can be difficult for tortoises to ingest. A higher number of plastics in feces when compared to the environment is significant and can be explained because plastics are easy to ingest and sometimes mistaken for food. Considering the digesta retention time observed in Galapagos tortoises of up to 28 days (Sadeghayobi et al., 2011), the accumulation of these materials within the intestinal track of tortoises must also be considered, which could favor absorbance of chemical components such as EDCs (Deem & Holliday, 2022). These results are of major concern for tortoise health and long-term wellbeing and conservation of the species inhabiting human-modified areas.

Tortoises might have a color preference for some items. Despite transparent plastics were the most abundant in the field, feces showed a higher number of green items. This can be explained by tortoises mistaking green plastics with grasses and could also indicate that tortoises are able to distinguish even transparent plastics, and potentially avoid them. It should be noted that smell may also play a role in plastic ingestion, as our team has directly observed tortoises trying to ingest silver containers and plastic bags with human food leftovers.

The amount of garbage in the environment was higher in the industrial and main road areas, compared to the other anthropic areas. However, the highest ratio of trash per feces was found within the urban and main road area. On the other hand, tortoise density was higher in the urban and main road areas than in the industrial

area, which could explain the higher amount of feces in those areas. The urban and main road sampling areas were very narrow and limited by thick vegetation, which might force tortoises using that areas to remain in a relatively small feeding area, having less opportunities to find feeding areas with less human pollution. By contrast, the agriculture, industrial, and rural road sampling areas are wider, located at higher elevations (more humid) where more pastures are available in the surroundings, which may allow tortoises to find close food resources with less human pollutants.

Tortoise density, doubled in the protected area when compared to the anthropic zone, highlighting the importance of habitat protection within the National Park areas. Within the human-modified areas, tortoises were more abundant in the agriculture, urban, and main road areas. During their annual migration, at least 69% of the adult tortoises use the agriculture zone, remaining there for 150 days on average, at which time they return to the lowlands in the protected area (Pike et al, 2021). In recent years, more tortoises have been observed foraging and resting near the northern limits of Puerto Ayora, and an increased number of car encounters have been reported, some of them with fatal consequences for tortoises. We hypothesize that these recently urbanized areas (such as El Mirador) offer several resources for tortoises all year around (i.e., water, fresh plants that grow near the road, food waste) that together with habitat degradation and new roads and trail openings have favored tortoise access from the protected areas to the edges of the town.

Tortoise philopatry to farm areas has been described by Pike et al. (2021). In the current study, tortoise recaptures within anthropic areas were triple than in the National Park, with more than 10% of the individuals recaptured four or more consecutive months. The higher proportion of recaptures in the human-modified areas could be explained due to the higher sampling effort conducted (weekly vs. monthly). However, the recapture frequency indicated that tortoises remain in polluted environments for long periods of time and do not only use those areas in transit to protected and less polluted habitats. Tortoises most recaptured in lower areas corresponded to juveniles and subadult, which is also in agreement with the movement studies describing the direct correlation between migration and tortoise

size (Blake et al., 2013). The proportion of males and females increased with elevation in both protected and human-modified areas, whereas the proportion of juveniles and subadults decreased with elevation. Both results are in agreement with migration patterns, having smaller and/or non-migratory individuals at lower elevations during the dry season (Blake et al., 2013; Bastille-Rousseau et al, 2017).

The ratio of feces to tortoises was lower within the protected and agricultural areas, despite tortoise abundance being higher there. These differences may be explained due to sampling bias. In the protected area, tortoises were highly dispersed, the transect very narrow, and the surrounding vegetation dense and thick; all of which may decrease our ability to find fecal samples. In the agricultural area, we primarily covered the paved road since vegetation in the edges was too thick. Terrain characteristics may have led to an underestimation of feces within some of the sampling areas. In addition, some unexpected habitat changes took place during the study period (vegetation and garbage cleaning events conducted by the municipality and local organizations) which reduced the number of feces sampled right after this event and increased the number of tortoises that we were able to find due to vegetation clear ups.

Here we describe a new threat to the conservation of Galapagos tortoises. The finding of human waste, especially plastics, in feces of free-living tortoises highlights the importance of taking actions that minimize human waste within the fragile environments of the Galapagos. As the local population and tourism in the archipelago increases, the amount of garbage generated, and disposed of, on the islands will increase by the same amount, putting at risk the health and wellbeing of both animals and humans. Plastic pollution is currently being studied and addressed by several institutions within the Galapagos marine environments (Mestanza et al., 2019; Zambrano-Monserrate & Ruano, 2020; Alfaro-Núñez et al., 2021; Jones et al, 2021; Jones et al., 2022), but no previous studies are currently being conducted on terrestrial ecosystems and the impacts of human waste produced *in situ*. This study highlights some of the garbage hotspots near Puerto Ayora town where management actions should be prioritized to protect tortoise health and its foraging

areas. More research is needed to understand to what extend plastic pollution are impacting the health of tortoises and other wildlife in the Galapagos. We urge local stakeholders, including the community at large, to take immediate action to limit human-waste from entering the Galapagos environment which should include long-term education campaigns to promote behavioral changes within the local population and visitors to the iconic Galapagos archipelago.

Conflict of Interest Statement

Karina Ramón-Gómez, Santiago Ron, Sharon L. Deem, Colton Stevens, Juan Carlos Izurieta, and Ainoa Nieto-Claudín declare there is no conflict of interest.

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685 **Table captions**

686 Table 1. Human waste fragments found within Santa Cruz giant tortoise' feces
687 (*Chelonoidis porteri*) classified by type of material.

688 Table 2. Giant tortoise abundance clustered by study area and land-use type for the
689 Galapagos tortoise' plastic ingestion study in Santa Cruz Island.

690 **Figure captions**

691 Figure 1. Sampling locations of giant tortoise' plastic ingestion study in Santa Cruz
692 Island according to its predominant land-use type: protected, agriculture, rural road,
693 industrial, main road, and urban area.

694 Figure 2. Comparison of the percentage of types of waste items found within
695 Galapagos giant tortoises' feces to types of waste items found in human-modified
696 areas on Santa Cruz Island.

697 Figure 3. Comparison of the percentage of human waste grouped by color found in
698 tortoise feces and in the environment within the human modified areas of Santa Cruz
699 Island.

700 Figure 4. Average Santa Cruz tortoise recapture rate clustered by land-use type.
701 Notice a significantly tortoise recapture rate in the industrial area when compared to
702 the other sampling areas.

703 Figure 5. Galapagos tortoise (*Chelonoidis porteri*) health threats associated to
704 human impacts and documented during the giant tortoise' plastic ingestion study in
705 Santa Cruz Island. A) Tortoise with tearing injuries in the marginal scutes of the
706 frontal carapace caused by dog attacks; B) dead tortoise found with multiple trauma
707 by the road in the agricultural zone; and C) recaptured tortoise in the agriculture area
708 found with injures on the frontal marginal scutes potentially due to a car impact.

709

710 Table 1.

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Type of material	Items (entire or fragments)
Plastics	bags, plastic wraps, containers, bottles, ropes, foam, scotch tape, label, waterproof, nylon, tube, broom sow, dish.
Cloth	clothing, thread, string, sandpaper cloth, shoes, wiper or cleaning cloth
Metal	wrapper, wire, metal mesh, bottle cap
Synthetic rubber	tube, bracelet, balloon
Paper/cardboard	cigarette butt, newspaper
Glass	Bier and soft drinks bottles
Construction material	Roof and floor tile fragments

712

713 Table 2.

Study Area	Land-Use Type	Elevation (m)	Zone length (m)	Area (m^2)	Tortoises Abundance	
					# per 1000 m^2	Average size (CCL)
Galapagos National Park Area	Protected	65-130	4,573	21,433.8	7.1	76.4
Human-Modified Area	Agriculture	130-142	1,890.7	20,641.1	6.0	99.2
	Rural road	142	717.3	5,083.6	1.4	94.2
	Industrial	142-177	1,590.5	25,821.1	0.8	84.5
	Main road	117-73	1,587.3	3,518.9	4.5	65.7
	Urban	73-26	1,797	9,644	4.4	72.22

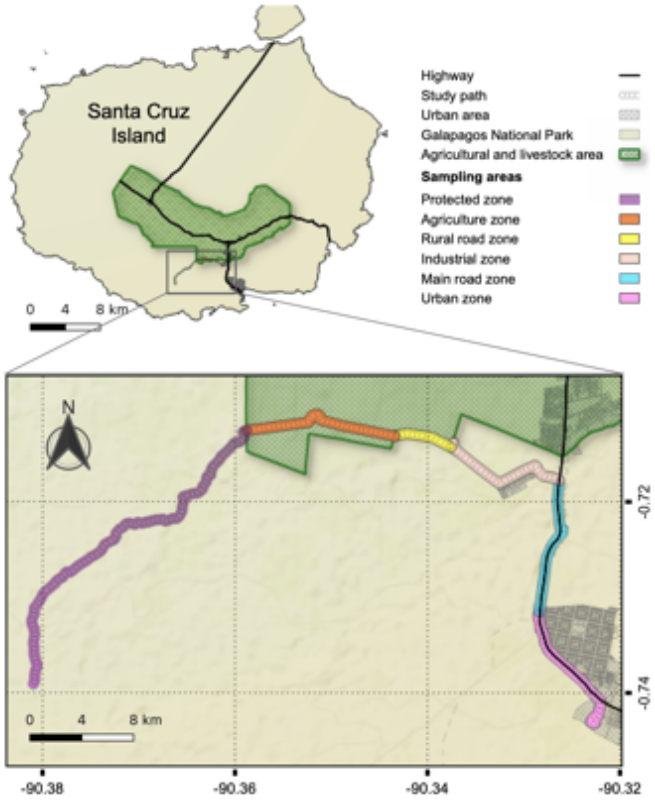
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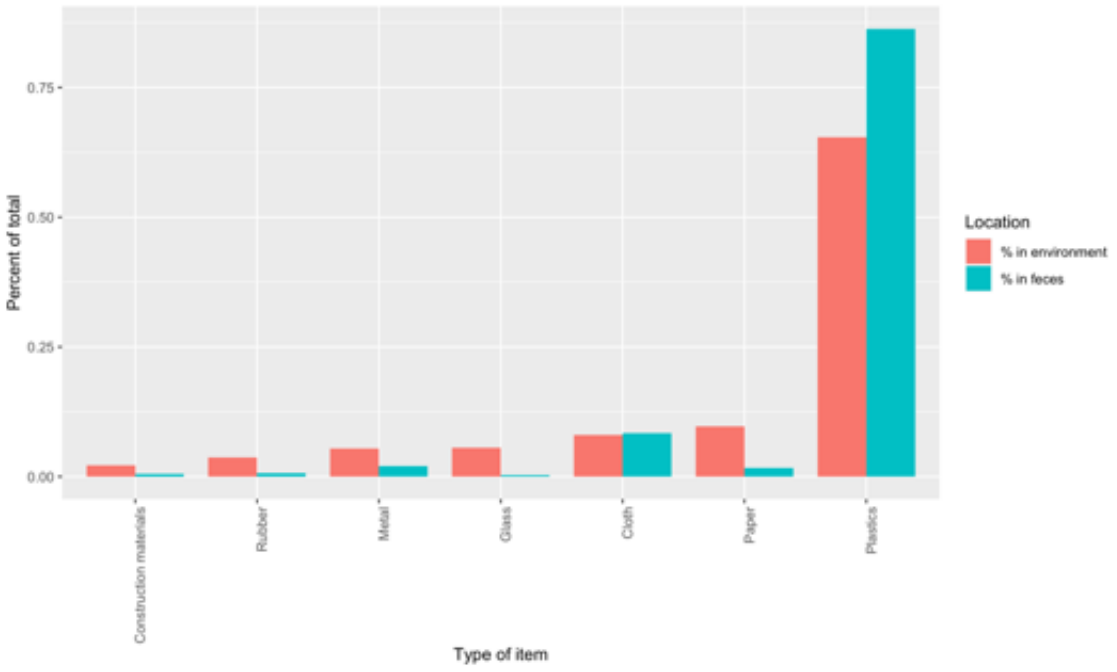
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718 Figure 1.



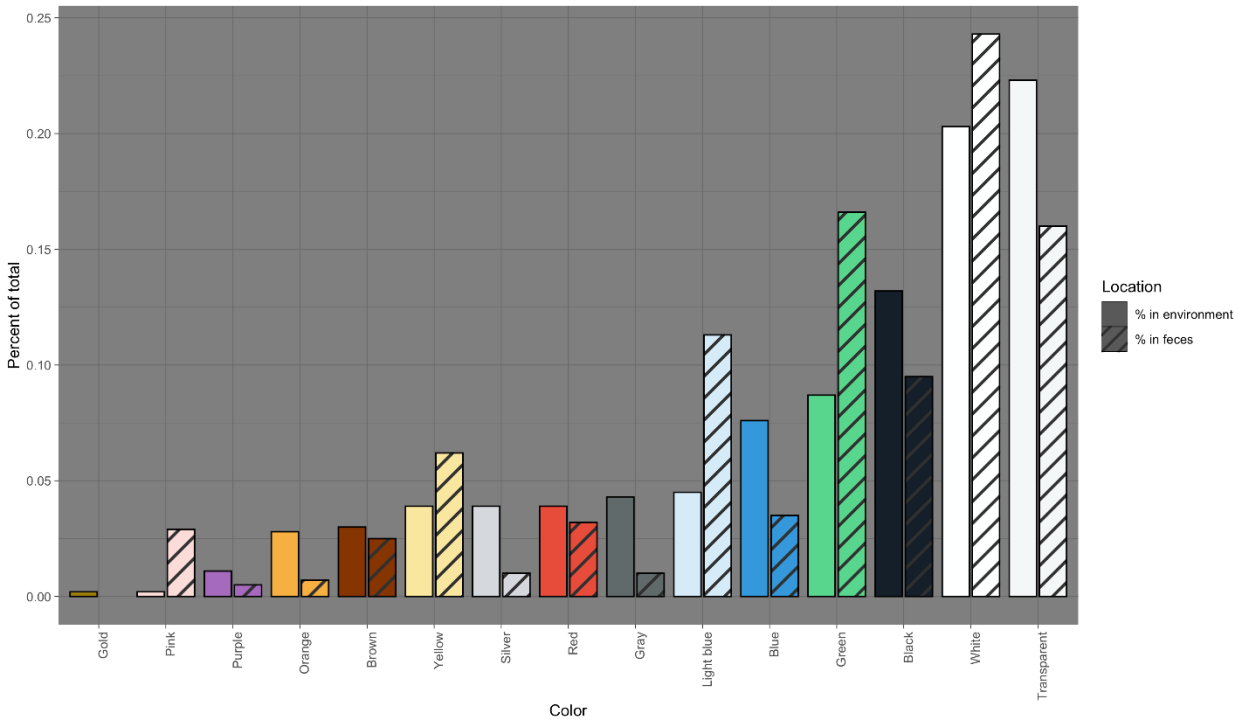
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720 Figure 2.



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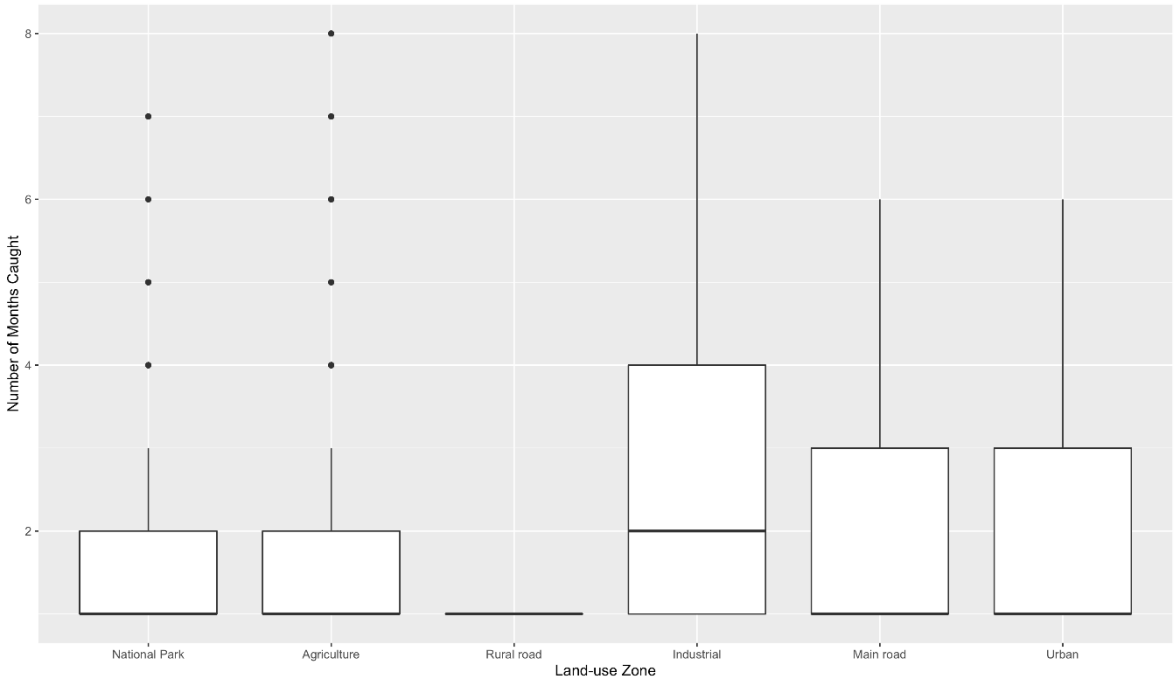
722 Figure 3.



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724 Figure 4.

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