QUANTIFICATION OF MACRO AND MICROPLASTICS ON A DESERT ISLAND, SANTA LUZIA, CABO VERDE ARCHIPELAGO, NORTH EAST ATLANTIC OCEAN

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SUMMARY

The main goal of this externship was to gain experience in the field of environmental toxicology and pathology. Therefore, I had the opportunity of analyzing samples in the Laboratory of Ecotoxicology, Department of Populations Studies of the Institute of Biomedical Sciences of Abel Salazar of the University of Porto. Simultaneously, data analyses were performed and a baseline article was written, using data from field work previously done by me. Therefore, this report will be divided into two different chapters: the first one with the submitted article, and the second one with laboratory work.

Previously to my curricular externship, I did an extracurricular externship in a non-governamental organization “Biosfera 1”, located in São Vicente, Cabo Verde. While there, I collected 55 samples of fish from a desert island (Santa Luzia, Cabo Verde), for posterior analysis in the ECOTOX laboratory. The aim was to find microplastics in fish tissues, organs and systems. Additionally, to complement my environmental study, I performed two cleaning campaigns on the Santa Luzia, and the resulting data was kept for posterior analysis.

During the curricular externship, this data was organized and used to write a baseline article about the quantification and characterization of marine debris in Santa Luzia Desert Island, here presented in the first chapter.

In the ECOTOX laboratory, I analyzed three different tissues (muscle, digestive tract and gills) of 55 fishes. These fishes were from three different species, chosen because of their commercial importance in Cabo Verde Archipelago. I had the opportunity of learning the methodology of microplastics extraction in biologic tissues and applying it to fish samples. A primary visual identification was performed to identify the microplastics, and they were characterized according to their color, size and shape. This primary visual identification will be completed with Fourier Transform Infrared (FTIR) spectrometry, in order to confirm the results.
ACKNOWLEDGMENTS

I would first like to thank my thesis advisor Professor Augusto Faustino for his availability, for all the ideas and motivation, for the patience and attention during the meetings, and for the friendship. Without him none of this would have been possible.

I would like to thank Professor Lúcia Guilhermino for the all her support and advices as a knowledgeable person in the scientific community and an excellent professional. Thank you for encouraging my critical spirit and helping me throughout this work.

I would like to say thank you to the ECOTOX personnel at the time I was present for welcoming me and for making the time fly in the laboratory.

To the NGOs Biosfera Cabo Verde and SPEA as well as their workers, for helping me during my extracurricular externship, which was essential for the present work. They made my time in Cabo Verde pass by quickly with so much fun, even on a desert island.

To the “bananas” Carla, Carolina, Joana, Raquel and Rita. You’re the greatest friends I could have made. Thank you for being so true to yourselves, so spontaneous and random. The time we spent together and all the craziness we shared will be among the best times of my live.

To Bárbara for the crazy adventures, the laughter, for all the honesty, and support.

To Inês, because to me she’s family and I know our friendship is timeless.

To Arielson, for all the support, kindness and patience, and to say that I’m very happy you walked into my life.

Finally, I would like to thank my family for the unconditional love and support, for believing in me and in my abilities, even when I don’t. To my parents, thank you for being such great people and passing your values to me. To my sister, thank you for growing up with me and being such a great companion. To the others, thank you for being such good friends and making me feel at home when I’m with all of you.
LIST OF ABBREVIATIONS

FTIR - Fourier Transform Infrared Spectrometry
IOC - Intergovernmental Oceanographic Commission
NGO – Non-governmental Organization
POPs – Persistent Organic Pollutants
SPEA – Portuguese Society for the Study of Birds
UNEP - United Nations Environment Programme
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I. INTRODUCTION, OBJECTIVES AND OUTLINE OF THE REPORT

Toxicology is the study of how chemical compounds affect the normal function of a biological system. Toxicology growth stood out simultaneous with the chemical industry, especially after World War II, when thousands of new compounds were produced, creating a need to understand their possible hazardous effects (Gerba, 2019). As the world population increased without precedent, so have human activities. Unfortunately, these activities resulted in the discard of thousands of contaminants in both aquatic and terrestrial ecosystems worldwide (Alimba & Faggio, 2019). A new branch of toxicology gained importance, Environmental Toxicology, which focuses on the effects of chemical contaminants on several ecological systems, either large or small (Gerba, 2019).

Plastic is one of the synthetic substances which is currently threatening ecosystems worldwide. Plastic includes at least one polymer with mixtures of mainly synthetic organic materials, which are linked together and molded during polymerization (PlasticsEurope, 2017). The first plastic was created in 1839 by Eduard Simon, and since then plastics use and production have been rising at high rates, with the global plastic production reaching 335 million tons in 2016 (PlasticsEurope, 2017). Jambeck et al. (2015) reported 4.8 to 12.7 million metric tons of plastic waste entering the oceans in 2010, only from land-based sources. The plastic debris accumulation in the environment constitutes a threat to ecosystems, and has toxicological impacts on biota (Gall & Thompson, 2015; Alimba & Faggio, 2019).

In the last years, the problem of marine plastic pollution and its biological effects have been investigated. However, considering the wide dimension of the marine environment and its high biodiversity, and the relative small number of species investigated, more knowledge is needed, especially in areas of high conservational interest, such as desert islands used for reproduction of marine species. Thus, the main goal of the present study was to investigate the plastic and microplastic contamination on Santa Luzia, a desert island of Cabo Verde Archipelago (sub-tropical North-East Atlantic Ocean) and its marine fauna. This island is under the influence of the Canary current, associated to the North Atlantic Gyre, which is known to accumulate large amounts of plastic debris (Law et al., 2010). This island is of high conservational interest since it is uninhabited, it is a nature reserve, hosts threatened species (Rendall et al., 2015), and has a rich marine environment which is exposed to the hazardous effects of marine pollution.

This report is divided in four Chapters: I, II, III and IV, and includes also two Annexes. Chapter I corresponds to the introduction, objectives and structure of the report. Chapter II is the
result of the first study performed, namely resulting of the first activities that took place were marine debris surveys on two Santa Luzia beaches. The information gathered was then organized and analyzed in order to write a baseline article, which will be in soon submitted for publication in an international indexed scientific journal and is included here in that form. Chapter III is the study performed on 55 fishes collected in Santa Luzia with the help of local fisherman and the NGO Biosfera 1 and further analyzed for their contamination by microplastics in the Laboratory of Ecotoxicology of ICBAS-UP. Chapter IV is the general discussion and Chapter V is the list of references. Annex I contains the protocol followed to isolate the microplastics and Annex II has the report of the activities performed in Cabo Verde during the extracurricular externship in the NGOs Biosfera 1 and SPEA

II. PLASTIC AND OTHER MARINE DEBRIS IN A DESERT ISLAND, AND ITS IMPEDIMENT TO SEA TURTLE HATCHLINGS REACHING THE SEAWATER (CABO VERDE, NORTH EAST ATLANTIC OCEAN)

Abstract

Marine debris is found worldwide, even in remote regions and desert islands. The main goal of this study was to quantify sea-originated pollution by plastic and other debris in Santa Luzia (Cabo Verde Archipelago, North East Atlantic Ocean), a desert island with high conservational interest mainly in relation to sea turtles and birds. Two surveys were carried out on two beaches: one directly exposed to the Canary Current (Praia dos Achados) and the other approximately on the opposite side of the island (Praia Palmo Tostão). Large ropes and fish nets partially buried and unidentifiable objects were not quantified. Regarding large debris (> 2.5 cm), in the first survey on Praia dos Achados (~ 1 year after beach cleaning), 203 debris weighting 92.5 Kg were collected in a 500 m² area (density: 0.406 items/m², 185 g/m²), from which 61 % was plastic waste. The amount of debris estimated for the whole beach was 17,441 Kg. In the second survey, carried out 2 months after, a total of 134 large debris weighting 21 Kg were collected, in a density of 0.268 items/m² (42g /m²), from which 58 % was plastic. The total amount of debris accumulated on all the beach for 2 months was 3,959 Kg. The density of small debris (0.12-2.5 cm) was 102 items/m². In both surveys carried out on Praia Palmo Tostão, no debris was found. In Praia dos Achados, loggerhead turtle (Caretta caretta) hatchlings were found dead tangled in debris. This study provides quantitative baseline information on Santa Luzia island pollution by marine debris, suggesting that the Northern side of the island acts as a sink for debris carried by the Canary
Current, among other potential sources. Such pollution impedes turtle hatchlings of reaching seawater, thus being a threat to loggerhead turtle population sustainability.

**KEYWORDS:** Marine debris, Plastics, Cabo Verde islands, North Atlantic Ocean, loggerhead turtle, Caretta caretta

1. Introduction

Marine pollution by plastics, microplastics and other debris is a global problem of high concern. Plastics and other debris have been found along shorelines (e.g. Martins & Sobral, 2011; Chang *et al.*, 2018), in seawater (e.g. Eriksen *et al.*, 2014; Sá *et al.*, 2016; Urban-Malinga *et al.*, 2018), marine sediments (Claessens *et al.*, 2011) and marine biota (Boerger *et al.*, 2010; Poon *et al.*, 2017; Basto *et al.*, 2019), including in remote regions (e.g. Munari *et al.*, 2017; Morgana *et al.*, 2018) and beaches of desert islands (e.g. Barnes, 2005; Lavers & Bond, 2017; Pavés *et al.*, 2017; Andrades *et al.*, 2018). Generally, the majority of marine debris is plastic items, such as identifiable objects and materials, fragments and fibers of different sizes, among several others (Duhec *et al.*, 2015; Honorato-zimmer *et al.*, 2019). The most part of marine litter comes from continental based sources, entering the marine environment through rivers, urban and industrial effluents, and other routes (Gold *et al.*, 2014). However, some debris are introduced directly during fishery, shipping, and other maritime activities (e.g. nets, ropes, plastic objects), through the deposition of air particles (Jang *et al.*, 2014; Rech *et al.*, 2014; Isobe *et al.*, 2016; Allen *et al.*, 2019), and by other ways.

Studies carried out in the last years provided some data regarding plastic and microplastic pollution in the North Atlantic Ocean (Lusher *et al.*, 2014). However, the studies on the subtropical part of the North Atlantic Ocean are still scarce (Law *et al.*, 2010). This area is of particular interest due to main currents associated with the North Atlantic gyre and the inputs of main rivers from both East and West sides of the Atlantic Ocean likely transporting considerable amounts of debris.

The Cabo Verde Archipelago is located in subtropical North East (NE) Atlantic Ocean, about 570 Km far from the West African coast (Fig. 1). Coastal pollution is considered an environmental management problem of the country (Ministry of Environment, Agriculture and Fisheries, 2004). However, to the best of our knowledge, quantitative studies on marine litter in Cabo Verde coastal areas do not exist. Cabo Verde islands have rich marine habitats that provide main ecosystem services, including fishery of several species with high economic value (Ministry of Environment, Agriculture and Fisheries, 2004). They also host the third most important loggerhead turtle (*Caretta caretta*) nesting rookery in the world (Marco *et al.*, 2011). *C. caretta* is a threatened
species and its global population is decreasing, along with five other sea turtle species (IUCN, 2019). Sea turtles are considerably affected by entanglement (Duncan et al., 2017) and ingestion (Tomás et al., 2002; Domènech et al., 2019) of plastic debris. Furthermore, the pollution of shores and beaches by large debris has a negative impact on sea turtle nesting behavior (Fujisaki & Lamont, 2016) and decreases the success of hatchlings reaching the ocean (Triessnig et al., 2012).

The main goal of the present study was to quantify large marine debris, including plastics, on two beaches of Santa Luzia island (desert island of Cabo Verde Archipelago) with distinct exposure to the Canary Current. Moreover, loggerhead turtle hatchlings found dead on surveyed areas were also recorded.

2. Material and Methods

2.1. Sampling areas

Santa Luzia is a desert island located of Cabo Verde archipelago. It is under influence of the Canary Current, one of the marine currents surrounding the subtropical North Atlantic Gyre (Hernandez-Guerra et al., 2002) (Fig.1A). It has a surface of 35 km² and is currently classified as a nature reserve (Rendall et al., 2015). The closest human settlement is located in São Vicente, another island of Cabo Verde archipelago, located about 8.4 Km way. In the Atlantic Ocean, Santa Luzia island is a significant breeding site for the loggerhead turtle (Rendall et al., 2015). The local NGO “Biosfera Cabo Verde” has a monitoring programme on the population of C. caretta with summer surveys in Santa Luzia. Since 2011, 11,022 nests were recorded (NGO Biosfera, 2019, personal communication).
Marine litter beach surveys were carried out in Santa Luzia island. Beach survey was used in the present study because is a cost-effective approach to quantify debris in beaches (Bergmann et al., 2015; Andrades et al., 2016). Surveys were carried out on two beaches (Fig. 1B): Praia dos Achados (the name is curious as it indicates the beach where lost things are found), located on the North coast of the island and directly exposed to the Canary current; and Praia Palmo Tostão, located on the South West (SW) of the island and not directly exposed to the Canary current.

Figure 1 (A) Location of Santa Luzia island on the North East Atlantic Ocean, with the Canary Current represented (gray arrow). (B) Santa Luzia Island and the location of the beach debris transects (rectangles), as well as the hatchlings survey transect (dotted line). Beach debris transects took place on the northern side, in Achados Beach (16°45'16.11"N, 24°42'18.41"W) and on the southern side, in Palmo Tostão Beach (16°45'21.09"N, 24°45'17.69"W).
These particular beaches were selected for the present study because of their location in relation to the Canary current and their relevance for loggerhead turtle nesting.

Praia dos Achados is the largest beach on the North coast of the island (~ 94,276 m²). It is an important nesting location for the loggerhead turtle (Rendall et al., 2015), with 4688 nests recorded since 2011 (NGO Biosfera, 2019, personal communication). Praia Palmo Tostão has about 294,820 m², with fewer loggerhead turtle nests, and hence the total number isn’t monitored by the local NGO. According to the information provided by the NGO “Biosfera Cabo Verde”, Praia dos Achados was cleaned in the Summer of 2017, previous to this study, and no cleaning was ever performed in Palmo Tostão beach.

2.2. Debris sampling

In both Praia dos Achados and Palmo Tostão, two surveys were made: one in August 2018 and the other in November 2018. In the Praia dos Achados, the first survey collected the debris accumulated during ~ 1 year, and the second during ~ 2 months.

In Praia dos Achados, large debris (> 20 mm) were collected in 500 m² (50 m along the low tide water line x 10 m from this line up to backshore). In Praia Palmo Tostão, large debris (> 20 mm) were collected in 10,000 m² (200 m along the low tide water line x 50 m from this line up to backshore). In Praia Palmo Tostão, the area was higher because of the scarcity of marine debris, as recommended in the “UNEP / IOC Guidelines on Survey and Monitoring of Marine Litter” by Cheshire and Adler, 2009. Very large debris that was not possible to weight (due to their dimensions or because they were partially buried deeply in the sand) were quantified and their position was GPS recorded (to avoid double counting in the second survey). Large debris (> 20 mm) were divided into five major categories (plastic, wood, glass, metal, and others) hereafter indicated as material type. According to Cheshire and Adler (2009), in each material type, items were further sorted according their pre-disposal use (fishing nets, plastic bottles, etc.). Fishing nets and ropes, and items that was not possible to separate individually were weighted together. All the other items were counted. Pre-disposal groups of more than 5 items were weighted separately. The others were weighted together (separated by material type).

To sample small debris (0.12 – 2.5 cm), in each of the previous selected sampling areas, two squares (50 x 50 cm) were considered. Following Jang et al. (2018), the squares were located between the lower and upper tidal lines. In each square, all the most superficial sand (5 cm deep) was sieved through a 1.2 mesh and the retained fraction was collected. In the laboratory, each fraction was sorted according to the material type (plastic, wood, glass, metal, and others) using a
magnifying glass. Plastic items were further categorized by type (fiber, fragment, pallet as in Doyle et al., 2011) and color (white, blue, yellow and red).

2.3. Loggerhead turtle hatchlings

The second debris survey was performed during the loggerhead turtle hatching season in Cabo Verde archipelago. In each beach, loggerhead turtle hatchlings survey was carried out once, in the morning, along a 1.3 Km transept parallel to the low tide water line and about 6 m from it (Fig. 2). Walking on the transept, all the hatchlings observed within 1 m of each side of the transept were recorded.

3. Results and Discussion

The results from the first and second surveys is presented in Table 1 and Table 2, respectively. During the first survey on the Praia dos Achados, 92.5 kg of marine debris were collected in the investigated area (500 m$^2$) corresponding to a density of 0.406 items/m$^2$ (185 g/m$^2$). The amount of debris estimated for the total area of the beach (~ 94 276 m$^2$) accumulated in about one year was 17,441 kg, excluding items with large dimensions or buried in the sand that were not quantified. Moreover, high amounts of litter were observed in the dunes which were not included in this study. The most part of the debris was plastic items (60.9 %), followed by manufactured wood items (36.8 %), glass (1.1 %), metal (0.3 %) and other types of material (0.8 %) (Table 1). Among plastic items, the most abundant were parts of fishing nets and ropes (69.1%) and plastic buoys (5.3%) (Table 1). In the second survey on Praia dos Achados, 21 kg of debris were collected (500 m$^2$) corresponding to a density of 0.268 items/m$^2$ (42 g/m$^2$), and an estimated amount of 3,959 Kg for the total area of the beach. The amount accumulated in two months corresponds to 22.5 % of the total accumulated in one year.
Table 1 Results from the first survey on Achados Beach

<table>
<thead>
<tr>
<th>Material type</th>
<th>Pre-disposal use</th>
<th>Number of items</th>
<th>Weight (kg)</th>
<th>Percentage (by weight)</th>
<th>Within material type</th>
<th>Total material type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>Fishing nets and rope</td>
<td>-</td>
<td>39</td>
<td>69.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plastic buoys</td>
<td>8</td>
<td>3</td>
<td>5.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plastic bottles &lt;2L</td>
<td>51</td>
<td>2.5</td>
<td>4.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plastic bottles, drums, buckets &gt;2L</td>
<td>11</td>
<td>1.9</td>
<td>3.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bottle caps and lids</td>
<td>94</td>
<td>0.5</td>
<td>0.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plastic bags</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Food packaging</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cutlery (knives handles)</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Household cleaning</td>
<td>1</td>
<td></td>
<td></td>
<td>60.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toys</td>
<td>1</td>
<td>1.7</td>
<td>3.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cigarette lighters</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pens, stationary items</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plastic tape</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Foam buoys</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Footwear(thongs)</td>
<td>7</td>
<td>1.2</td>
<td>2.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unidentifiable objects (fragments, fibers, etc.)</td>
<td>-</td>
<td>6.1</td>
<td>10.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Foam fragments</td>
<td>10</td>
<td>0.5</td>
<td>0.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood</td>
<td>Processed timber, pallet crates, fragments</td>
<td>-</td>
<td>34</td>
<td>-</td>
<td>36.8%</td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td>Fluorescent light tubes</td>
<td>1</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Light bulbs (intact)</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>1.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glass jars and bottles</td>
<td>3</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Metal</td>
<td>Cans (hairspray and glue)</td>
<td>2</td>
<td>0.3</td>
<td>-</td>
<td>0.3%</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>The back of a computer (plastic and metal merged)</td>
<td>1</td>
<td>0.8</td>
<td>-</td>
<td>0.9%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>203</td>
<td>92.5</td>
<td>-</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Results from the second survey on Achados Beach

<table>
<thead>
<tr>
<th>Material type</th>
<th>Pre-disposal use</th>
<th>Number of items</th>
<th>Weight (kg)</th>
<th>Percentage (by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>Fishing nets and rope</td>
<td>-</td>
<td>6.2</td>
<td>50.8%</td>
</tr>
<tr>
<td></td>
<td>Plastic bottles &lt;2L</td>
<td>15</td>
<td>0.7</td>
<td>5.7%</td>
</tr>
<tr>
<td></td>
<td>Bottle caps and lids</td>
<td>67</td>
<td>0.4</td>
<td>3.3%</td>
</tr>
<tr>
<td></td>
<td>Plastic bottles, drums,</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>buckets &gt;2L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Food packaging</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disposable cutlery</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Household cleaning</td>
<td>1</td>
<td>2.9</td>
<td>23.8%</td>
</tr>
<tr>
<td></td>
<td>Syringe</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plumbing pipe</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Footwear(thongs)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mesh bags</td>
<td>6</td>
<td>0.3</td>
<td>2.5%</td>
</tr>
<tr>
<td></td>
<td>Ring carriers</td>
<td>6</td>
<td>0.2</td>
<td>1.6%</td>
</tr>
<tr>
<td></td>
<td>Unidentifiable objects</td>
<td>-</td>
<td>1</td>
<td>8.2%</td>
</tr>
<tr>
<td></td>
<td>(fragments, fibers, etc.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Foam fragments</td>
<td>6</td>
<td>0.5</td>
<td>4.1%</td>
</tr>
<tr>
<td>Wood</td>
<td>Processed timber, pallet</td>
<td>18</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>crates, fragments</td>
<td></td>
<td></td>
<td>38.1%</td>
</tr>
<tr>
<td>Glass</td>
<td>Glass jars and bottles</td>
<td>5</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.8%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21</td>
<td>-</td>
<td>100%</td>
</tr>
</tbody>
</table>

Several factors affect the amount of debris reaching the beach, including marine currents, winds and others that show variations along the year. As in the first survey, most of the debris...
were plastic items (58.1%, Table 1) mostly related to fishery (parts of fishing nets and ropes, 50.8 %) (Table 1).

Regarding small debris (0.12 – 2.5 cm) quantified in the second survey, the number of items found in 0.50 m² was 51, corresponding to a density of 102 items/m². Most of the debris was plastic items (98 %), mainly fragments (58 %), followed by fibers (28 %) and pellets (14 %) (Fig.2). In both surveys, no debris (large or small) were found in Praia Palmo Tostão.

Because Praia dos Achados (where a large amount of debris was found) is directly exposed to the Canary current and Praia Tostão (where no debris were found) is not, the findings of the present study suggest a major role of the Canary current in the transport of the debris arriving to Praia dos Achados. In both surveys carried out in Praia dos Achados, Octopus traps and their fragments (Fig. 3C) were found in considerable amounts (included in the pre-disposal use group indicated as Plastic B >2L in Table 1). Such traps are used in Octopus fisheries on the Atlantic and Mediterranean coasts of the Iberian Peninsula (Portugal and Spain), and of African countries, such as Mauritania. Furthermore, in this study, the most abundant plastic type was fisheries related items, which is similar to other findings on the Portuguese coast, where besides cords and strings, Octopus traps were also found (Zhukov, 2017). These findings support the hypothesis of a major contribution of the Canary current as an important transport route for marine debris arriving and accumulating in Praia dos Achados.

Large debris density on Praia dos Achados (0.406 items/m²) is similar to the density found in other Atlantic desert islands such as the Trindade islands ( 0.5 items/m², Andrades et al., 2018) and it’s comparable with north-western Mediterranean beaches (0.14 - 0.57 items/m², Munari et al., 2016) and beaches from the Spanish coast (0.05 - 0.373 items/m², Anfuso & Williams, 2019). In Portugal, some beaches have recorded significantly higher debris densities, (28.6 to 392.8 items/m², Martins & Sobral, 2011). However, it is worth noting that the debris density calculated by weight (185 g/m²) is similar to some highly polluted beaches such as some Sri Lanka beaches (175 ± 538 g/m², Jang et al., 2018), even though the debris density calculated by items (0.406 items/m²) is considerably lower (4.1 items/m², Jang et al., 2018). This is possibly explained by the existence of large and heavy items in Praia dos Achados, particularly parts of nets and ropes (Fig.3). Furthermore, the total amount of debris estimated on Praia dos Achados (17,441 kg) is comparable to the total amount of debris estimated in the whole Henderson Island (17,601 kg) which recorded one of the highest densities of debris in the world (347.3 items/m², Lavers & Bond, 2017). In both surveys on Praia dos Achados, plastic was the most abundant material type, and a
large proportion consisted of fishery related items (parts of nets and ropes); these findings are similar to other reports in desert islands (Lavers & Bond, 2017; Pavés et al., 2017).

During the second survey on the Praia dos Achados, 20 corpses of loggerhead turtle hatchlings were found, either trapped in plastic items, entangled in fishing nets or near a plastic obstacle between them and the sea. These animals likely died because they could not reach seawater in due time (Fig. 3). It should be highlighted that only one transect of 1.3 Km was investigated, therefore the total number of turtle hatchlings dying because of the debris accumulated on the beach is likely much higher. Several studies reported negative impacts of beach

![Figure 3. (A, B) Loggerhead hatchlings' corpses. (A) Loggerhead hatchling (pink arrow) entangled in fishing nets and ropes fragments; (B) Loggerhead hatchling dead under a fishing net, pulled by ghost crabs; (C) Marine debris on Achados beach, with visibly large numbers of octopus traps (white arrows)]](image-url)

11
debris on turtle hatchlings. Debris on the beach may prevent hatchlings from reaching the ocean (Triessnig et al., 2012; Aguilera et al., 2018). They can also increase the crawling time of hatchlings on the beach making them more vulnerable to terrestrial predators because they are more time on the beach and less able to escape, and also to marine predators because they have less energy to escape due to additional crawling time (Tomillo et al., 2010; Triessnig et al., 2012 Burger & Gochfeld, 2014). Also, beach debris may act as a barrier in the sand column impeding hatchlings from leaving the egg chamber (Nelms et al., 2016) and change hatchling sex ratios by altering nesting properties (Carson et al., 2011; Beckwith & Fuentes, 2018). Considering that only 1 in 1000 sea turtle eggs is estimated to hatch and reach maturity (Frazer, 1986), the additional negative impacts of beach debris in nestling areas is a serious threat to the sustainability of sea turtles populations.

4. Conclusion

The findings of the surveys carried out on Praia dos Achados showed high amounts of plastic and other debris on Praia dos Achados, in the desert island of Santa Luzia (subtropical NE Atlantic Ocean), likely arriving mainly through the Canary current. This island is a natural reserve of most importance for the loggerhead sea turtle (Caretta caretta) nesting. Its pollution by marine debris together with similar situations in other important reproduction areas is a threat to C. caretta population sustainability. Furthermore, the contamination of shorelines of Cabo Verde islands by marine debris is also a challenge for marine live conservation, fishery and related activities, as well as human health and wellbeing.

III. FROM THE OCEAN TO OUR PLATES: MICROPLASTICS FOUND IN COMMERCIAL FISHES FROM THE SURROUNDING WATERS OF A DESERT ISLAND LOCATED IN SUBTROPICAL NORTH ATLANTIC

1. Introduction

Plastic presence in our oceans is extremely worrying, due to its persistence and ubiquity. Currently is estimated that there are more than 5 trillion plastic items floating in the oceans (Eriksen et al., 2014). Furthermore, the concern with micro-sized plastic pieces, called microplastics, has risen due to their ubiquitous presence in the marine environment, accentuated over the last decades (UNEP, 2016).
Microplastics are solid synthetic polymers and even though it is still debated, they’re commonly accepted as plastic particles with less than 5mm (Arthur et al., 2009; Shim et al., 2018). They can be divided into two different categories: a) primary microplastics and b) secondary microplastics. Primary microplastics a) are manufactured as small-sized particles for industrial use, including resin pellets, microbeads in cosmetics, toothpaste, micro-sized powders for textile coating, etc. (Shim et al., 2018). Secondary microplastics b) are the result of fragmentation or degradation of larger plastics caused by mechanical abrasion and photochemical oxidation in the environment (Andrady, 2011). They can enter the ocean through the direct introduction with runoff or the degradation and breakdown of meso and macroplastics, like previously mentioned (Andrady, 2011). Once microplastics reach the sea, they undergo a process of biofouling, which can make their density increase, and give them neutral or negative buoyancy (Chubarenko et al., 2016). Posteriorly, they suffer defouling in the water column, which can decrease its density causing the debris to return back to the surface (Andrady & Song, 1991). As a result, they may be found not only on the water surface, but also in the water column and in the sea bed (e.g. Dai et al., 2018; Näkki et al., 2019). In fact, microplastics presence has been found in every marine environment: in coastal or deep-sea sediments (e.g. Bergmann et al., 2017; Antunes et al., 2018), in the water column (Dai et al., 2018), in the deep sea (Courtene-jones et al., 2017), in the ocean surface (Khamarzadeh et al., 2019), and even in the most remote habitats such as Antarctica and Arctic polar waters (Lusher et al., 2015b; Cincinelli et al., 2017).

Microplastics affect a wide range of marine biota, and their ingestion has been reported in invertebrates, fishes, birds and marine mammals (e.g. Neves et al., 2015; Nelms et al., 2016; Sun et al., 2016; Perez-venegas et al., 2018). An increasing number of studies show that microplastics have been ingested by a wide variety of fish species, whether demersal, pelagic, estuarine and freshwater, some of which have commercial importance (review in Herrera et al., 2019). In the North Pacific, a study estimated the ingestion rate of plastic debris by mesopelagic fishes to be from 12000 to 24000 tons per year (Davison & Asch, 2011).

Besides the direct physical impacts of ingestion, which include laceration, inflammation and starvation, there are also less established chemical effects (Carbery et al., 2018). These microscopic particles can be a potential source of toxic chemicals added during manufacturing. Furthermore, their high surface area to volume ratio, along with non-polar surface, facilitates the sorption of organic and inorganic contaminants from the surrounding water column to its surface. Once under gut conditions, the desorption of these chemicals is enhanced, and so they can be released into the organism and potentially transferred to body tissues (Bakir et al., 2014). Plastics also represent a
risk because of the release of the constituent monomers themselves, some of which have mutagenic and/or carcinogetic potential (Lithner et al., 2011).

Recent studies have proven that trophic transfer of microplastics occur (Setälä et al., 2014; Carbery et al., 2018; Nelms et al., 2018). However, the role of microplastics potentially ingested by humans through the food chain is still unclear (da Costa et al., 2016).

The aim of this study was to find out if the three analyzed species (Sparisoma cretense, Cephalopholis taeniops and Diplodus prayensis) from Santa Luzia desert island were contaminated by microplastics. These species were selected because they are demersal fishes of important commercial value in Cabo Verde islands and are consumed as food by the human population.

2. Material and Methods

2.1. Sampling

The samples were collected in the waters of Santa Luzia nature reserve, Cabo Verde, North East Atlantic Ocean, from August 2018 to January 2019. Three species were chosen because of their high intake by Cabo Verde’s population: Sparisoma cretense (n=20), Cephalopholis taeniops (n=20) and Diplodus prayensis (n=15) (Fig.4). Sparisoma cretense, is an herbivorous fish associated with coral reefs, in shallow waters to about 50 m. Cephalopholis taeniops is a demersal fish found mainly on sandy or rocky bottoms in depths of 20 to 200 m, feeding on small crustaceans and fish. Diplodus prayensis is a demersal fish which inhabits rocky bottoms up to 100m deep, feeding mainly on invertebrates and seaweeds. The 55 fishes were collected by São Vicente local fisherman, using artisanal fishing techniques. Upon collection, the fishes were wrapped in aluminum sheets, to prevent plastic contamination, and stored at -20ºC. Once in the laboratory, the fishes were thawed at room temperature. Each fish was weighed (g) and the body length was measured (cm), before dissection.

Gastrointestinal tracts were removed through a cut in the ventral surface of the fish, from the top of the esophagus and cut away at the vent. Gills were hold with forceps and cut with a scissor. Muscle was removed from the right dorsal half of the fish, after removing the skin and scales. To prevent contamination, work surfaces were cleaned with distilled water and alcohol, and a filter was placed on the balcony as an air contamination control. Gloves (nitrile) were worn during the dissection and the instruments used were cleaned with distilled water after every specimen. To minimize the risk of contamination, each different tissue was immediately placed in a petri dish and stored at -20ºC until digestion.
2.2. Microplastics isolation

One of the biggest problems when comparing field studies is the diversity of approaches used to isolate and characterize the plastic particles from the fishes. Therefore, in this study, the IPMA Microplastic Extraction Protocol was followed (Annex I), with only the necessary modifications.

The visual direct identification of particles in the tissues fails to identify a large amount of microplastics, particularly with smaller sizes. Hence, studies have developed protocols to improve the efficiency of their extraction, either with enzymes or chemicals (e.g. H₂O₂, KOH, etc.) (e.g. Foekema et al., 2013; Avio et al., 2015) which dissolve the biological tissues. However, some compounds can also alter and destroy some plastic polymers, underestimating the total amount of plastics present (Karami et al., 2017). In more recent studies, potassium hydroxide (KOH) has been identified as the most suitable reagent to isolate plastics in marine organisms (e.g. Karami et al., 2017; Kühn et al., 2017). Because of this, KOH was the chemical used in this study to dissolve the biomass of the samples.

Figure 4 - The investigated species: A - Sparisoma cretense; B - Diplodus prayensis; C - Cephalopholis taeniops
Each sample was transferred to a glass jar and filled with a 10% KOH prepared previously, with ultrapure water. The solution added was at least 3 times the volume of the biological material (maximum 150 ml). Gills were incubated at 40ºC for 3 days and muscle was incubated at 60ºC for 24 hours. The digestive tracts incubated at 60ºC for 24 hours, however 11 digestive tracts took longer to digest than the remaining, incubating at 60ºC for 3 days.

After incubation, the resultant liquid in each jar was vacuum filtered through 1.2 μm Munktell Filters. Several filters were needed per sample. Some digestive tracts had too much sediment to be filtered, so the supernatant was filtered, and the sediment was displaced in a Petri dish. Each filter was sealed in a Petri dish and dried at 40ºC, before analyzing with a SMZ800N Nikon stereomicroscope.

To avoid any contamination, laboratory material used during the sample processing was cleaned with distilled water. The usage of plastic material was avoided whenever possible, and laboratory coats were worn during the all procedures. The procedures associated with the incubation and filtration were performed inside the laminar flow cabinet, in order to prevent airborne contamination. Furthermore, the color of the clothes during the processing were recorded, and two blanks were placed inside the laminar flow cabinet, in order to assess if there was any airborne contamination during the filtration.

2.3. Visual identification

A primary identification was performed in a total of 342 filters, with a SMZ800N Nikon stereomicroscope. Following Barboza et al. (2019), particles were classified and categorized according to their shape: fibers (elongated), fragments (angular and irregular pieces), films (thin and transparent) and pellets (cylinder and round edges), and color. All particles were measured at their largest cross section and categorized according to their size class (<100μm, 100-500, 500-1000, 1000-1500,1500-3000).
3. Results

3.1. Microplastics occurrence in fish

94.5% of the studied fishes had microplastics at least in one of the tissues (52 out of 55 fishes) and the average number of microplastics was $4.8 \pm 4$ plastics/fish. The variability ranged from 0 – 16 microplastics per fish. In *Sparisoma cretense* individuals, 19 out of 20 had microplastic at least in one tissue. Most of the microplastics were found in the digestive tract (76.5%, Fig.5), and it was the species with least microplastics present in the muscle (8 out of 20). In *Cephalopholis taeniops*, 19 out of 20 individuals had microplastics at least in one tissue. The distribution of the microplastics was relatively similar in the three tissues, but the highest concentration was found in the gills (38.6%, Fig. 5). *Diplodus prayensis* individuals had microplastics in 14 out of 15 individuals. The majority of the microplastics was found on the gills (54.6%).

![Graph showing relative percentage of microplastics in different tissues of each species.](image)

Figure 5 Relative percentage of microplastics in different tissues of each species.

The total concentration of the microplastics in the muscle was $0.031 \pm 0.036$ microplastic items per g of muscle (MP items/g). In *S.cretense*, the concentration was $0.030 \pm 0.044$ items/g. Similarly, in *C. taeniops* the concentration was $0.031 \pm 0.033$ items/g of muscle. Finally, in *D. prayensis*, the concentration was $0.033 \pm 0.030$ items/g.
3.2 Microplastics characterization

Concerning microplastics’ shape, the majority was represented by fragments (56%), followed by fibers (32%), films (6%) and pellets (6%) (Fig. 6).

![Figure 6](attachment:figure6.png)  
**Figure 6** Relative percentage of each microplastics’ type in the three different tissues (digestive tract, gills and muscle)

Regarding the microplastics’ type distribution, in the muscle and digestive tract the most abundant type was fragments (Fig. 7). On the contrary, the gills showed a higher abundance of fibers (48.9%) compared to fragments (41.1%) (Fig.7). Films and pellets represented a smaller proportion of the microplastics in all tissues, and no pellets were found in the gills.

Concerning the microplastics color, in all the three tissues, there was a higher abundance of blackish (23%), translucent (19%) and whitish (19%) microplastics, even though there was a wide variety of colors found (Fig. 8). Blue microplastics were also found at a relative high abundance.
*Diplodus prayensis* showed a higher percentage of whitish and blackish microplastics (24.8% and 26.7%, respectively). *Cephalopholis taeniops* presented more translucent and bluish microplastics (35.0% and 20.0%, respectively). *Sparisoma cretense* had a more homogeneous distribution of color, and the most prevalent microplastics were blackish (24.5%).

Most of the microplastics found in all three tissues had a size comprised between 100 and 500 µm (Fig. 10). In the muscle, microplastics with less than 100 µm represented 22.4% of the total, more than in the other two tissues. In the digestive tract, 24.6% of the microplastics ranged from 500-1000 µm. Since the blanks of the processing of the muscle had no contamination, the occurrence of microplastics bigger than 1000 µm in the muscle is most likely explained by
microplastics that were transferred from the digestive tract or gills from the same fish, while they were being dissected, carried in the dissection tools. While on the visualization on the stereoscope, there were some particles difficult to identify or distinguish between plastic or other materials (e.g. sand particles, minerals). Consistency of the particles was assessed with the tip of an insulin needle of 25G to help identifying the microplastics. Even still, in every tissue there were particles which were not possible to identify, hence referred to as non-identified microparticles (Fig. 11). These particles were not accounted in the analysis and its composition will be posteriorly analyzed with Fourier Transform Infrared (FTIR) spectrometry. From a total of 55 fishes analyzed, 143 non-identified microparticles were found. These particles may be unidentified plastics, as well as sand particles from the sediment found in the guts of all the three species.
4. Discussion

4.1. Occurrence of microplastics in fish

Marine fishes ingesting microplastics has been reported worldwide (e.g. Boerger et al., 2010; Lusher et al., 2013; Neves et al., 2015). Some studies suggest that microplastics can be ingested when confused with fishes’ prey, since many of them have similar shapes and colors (Boerger et al., 2010; Ora et al., 2017) A study found that the microplastics’ odor induced food search behavior on anchovies (Savoca et al., 2017). Additionally, they can be ingested from prey which already contain plastic pieces (Lusher et al., 2015a).

In this study, the findings on this demersal species revealed a higher microplastic prevalence (94.5%) than reports on other fish species across the world, such as demersal fishes in the Spanish coast (17.5%, Bellas et al., 2016), mesopelagic fish in the North Atlantic (11%, (Lusher et al., 2015a), coastal fish in the Northeast Atlantic (47.7%, Murphy et al., 2017), and commercial fishes from the Portuguese coast (19.8%, Neves et al., 2015). The average of microplastics found per fish was 4.8 ± 4 plastics/fish, and in the entire digestive tract was 2.3 ± 3.0 plastics/digestive tract, which was superior to other findings on marine fish species. Lusher et al., reported a mean of 0.13 items per individual in all the fish sampled, Murphy et al., 2017 found a mean of 0.6 ± 1.3 items/fish and Neves et al., 2015 about 0.27 ± 0.63 items/fish.

There are several factors which affect the bioavailability of microplastics such as microplastics’ size, density, color and abundance. An increased abundance in the marine environment increases the chance of an organism to encounter microplastics (Wright et al., 2013),
and thus, possibly ingest it. The high occurrence of microplastics in fish reported in this study is possibly explained by the microplastics bioavailability in the fishes’ habitat. The three studied species were demersal, meaning they live and feed close to the seabed, which acts may act as a sink for the plastic in the marine environment (Bellas et al., 2016). Some studies have reported a higher prevalence of microplastics in demersal fish when compared to pelagic and mesopelagic fish (Neves et al., 2015; Bellas et al., 2016; Jabeen et al., 2017). However, some other studies have reported the opposite, with higher microplastic occurrence in pelagic species when compared with benthic or benthopelagic (Lusher et al., 2013; Bessa et al., 2018), and the correlation between the two is still unclear. Nonetheless microplastics are present in sedimentary habitats, meaning that species close to the seabed are exposed to their presence (Wright et al., 2013). Additionally to all of this, the nature reserve of Santa Luzia is under the influence of the Canary Current and near the North Atlantic Gyre, which is known to accumulate large amounts of debris. Poulain et al., 2018 estimated between 60 million and 4.5 billion microplastics/km² in the North Atlantic subtropical gyre and reported that their abundance is about 5 to 171 times higher than for macroplastics. A previous study had concluded that the highest concentration of plastic in the North Atlantic was recorded in subtropical latitudes and connected with the convergence of surface currents (Law et al., 2010). However, it’s important to bear in mind that we searched for plastics on three different tissues, whereas most studies only focused on digestive tract. Additionally, it is worth noting that the identification of the microplastics in this study was solely based on their visualization under the stereoscope, a method that has propensity to overestimate the total amount of microplastics (Hidalgo-Ruz et al., 2012; Rocha-santos & Duarte, 2015). The microplastics analysis with FTIR is essential to estimate the occurrence of microplastics in the studied fish with more accuracy.

4.2. Characterization of microplastics

In this study, the majority of microplastics was represented by fragments (56%), with fibers only representing 32%. This result contrasts with the findings from several studies, such as Neves et al., 2015, which reported 65.8% of fibers and 34.2% of fragments in commercial fish from the Portuguese coast. However, Poulain et al., (2018) reported higher amounts of fragments than fibers in surveys of microplastics conducted on the North Atlantic sub-tropical Gyre waters.

Concerning their color, blackish (23%), translucid (19%) and whitish (19%) microplastics were the most abundant in the three different tissues. These findings are in line with some other findings such as Murphy et al., 2017, who reported black (43.0%) as the predominant color, followed by clear (21.9%) microplastics and Bellas et al., 2016, who reported 51% of black
microplastics in demersal fishes from the Spanish coast. Some other studies reported higher proportions of blue microplastics (e.g. Ora et al., 2017; Bessa et al., 2018), whereas in this study, blue microplastics only represented 12% of the total.

Most of the microplastics ranged from 100 to 500 µm, and the maximum size recorded was 3200 µm. Lusher et al., 2015a reported microplastics ranging from 0.5 to 11.7 mm (median: 1.9 mm) in mesopelagic fish from the North Atlantic. In the Spanish coast, microplastics found in demersal fishes had between 0.38 to 3.1 mm, which is similar to our findings.

4.3. The risks of microplastics

In this study, the impacts of the microplastics on the fishes were not accounted. However, it important to understand the risks that these particles represent to the marine organisms, and possibly to the human health.

Once ingested the microplastics are either egested or retained. They may block the digestive tract, causing pseudo-satiation and interfering with feeding (Jovanovic, 2017). Since many fragments are irregular and often with sharp edges, they may pierce the intestinal lining of the fish and cause injuries (Jovanovic, 2017). Furthermore, they may interfere with predatory efficiency (de Sá et al., 2015), delay growth rates and increase mortality (Mazurais et al., 2015).

Plastics are composed of monomers, additives and other ingredients, which are not degraded by enzymes present in the organisms. It is commonly considered biochemically inert and not a threat to aquatic animals or humans, because of its large molecular size. However, many of the polymer have dangerous monomers, additives or chemical byproducts in their composition, and since the polymerization reactions during plastic production are often uncompleted, residual monomers are left in the polymer, several of which are a hazard to the human health and the environment (Lithner et al., 2011). In fact, Lithner et al., 2011 reported that 31 out of 55 polymers studied were made of monomers associated with carcinogenicity, germ cell mutagenicity, reproductive toxicity, acute toxicity, etc. Furthermore, other polymerization impurities besides unreacted monomers may remain present in the plastic, such as oligomers, polymer fragments with low molecular weight, catalysts, solvents and a wide range of additives (Bergmann, et al., 2015). Due to their low molecular weight, these components may migrate from the plastic to the surrounding environment or organisms (as cited in Bergmann et al., 2015).

The threats that plastics represent, don’t come only from their material, but also from pollutants that are sorbed in their surfaces. Harmful pollutants such as Persistant Organic Pollutants (POPs) accumulates in plastics due to their hydrophobicity, and their concentration is increased by orders of magnitude higher than in the seawater (Andrady, 2011) Posteriorly, these
plastics are ingested by several kinds of biota, and may represent an exposure pathway to these pollutants. Rochman et al., 2013, reported higher concentrations of POPs on fish fed with marine plastic debris than with virgin plastic, suggesting that plastic in the marine environment serves as a vector to these pollutants for the fauna. Furthermore, it has been demonstrated that POPs are released from plastic debris under simulated physiological conditions (Bakir et al., 2014). POPs are known for their toxicity and persistence, which together with a high lipophilic capacity, are responsible for their bioaccumulation and biomagnification both in the wildlife and humans (Ontiveros-Cuadras et al., 2019). To human health concern the daily interaction with plastic items allows oral, dermal and inhalation exposure to its associated chemicals (Bergmann et al., 2015). Additionally, as this study suggest, they might be already present in the food chain, possibly being ingested with some frequency. In the muscle, an average of 0.031 ±0.036 microplastics/g was found. Considering that these fishes have high commercial importance, this result is troubling. Even if a small proportion of the microplastics found in the muscle came from the fishes’ digestive tract contamination, it shows that it is difficult to prevent the contamination of the edible part of the fish. However, the harm that microplastics from environmental origin or from the food chain represent to the human health is still understudied.

IV. GENERAL DISCUSSION

The aim of this work was to investigate the plastic pollution in Santa Luzia desert island, both on its coasts, with marine debris surveys, or in its fauna, searching for microplastics in the 55 fishes collected. The aims of the study were accomplished, and the findings are in line with the reports across the world, showing that even protected areas without the human presence suffer from anthropogenic debris, and in this study, with an alarming dimension.

This externship was a positive experience that allowed me to gain experience in the laboratory. I learned to be more rigorous and systematic in my approach, I improved my technical skills (e.g. handling reagents, preparing solutions, preparing the necessary material, avoiding contaminations, etc.) and improved my attention to detail while observing the filters. Additionally, I developed a stronger critical sense and improved my scientific writing. I enhanced my knowledge in the area of environmental toxicology, particularly concerning the problematic of plastic pollution and its impacts on the environment, fauna, human health and economy. The written baseline article is soon to be submitted, providing information about marine debris in an island where there aren’t any studies about plastic pollution. Our findings show that the northern side of this island is heavily polluted, probably due to the Canary Current, which transports marine debris, accumulating on Santa Luzia island. We also found a display of
the hazardous effects of marine debris in the marine fauna, with loggerhead hatchling corpses entangled or trapped in plastic. Regarding microplastics, my findings were alarming, with 94.5% of the analyzed fish having the presence of these particles in one of their tissues. Besides the higher amounts of microplastics found in this study when compared to others, we also found microplastics present in the muscle, the edible part of the fish. This suggests that microplastics may already be in our daily meals. However, microplastics found couldn’t go through the FTIR analysis, due to the short time of the externship and the long waiting list for this analysis.

I did consider the externship to be too short, since I wouldn’t be able to accomplish all of this work if I hadn’t collected all the information and samples previously, in my extracurricular externship. Nonetheless, it was a good experience that enriched my academic course.

V. REFERENCES


ANNEX I: MICROPLASTIC EXTRACTION PROTOCOL

3- PROTOCOLO DE EXTRAÇÃO DE MICROPLÁSTICOS DE MATERIAL BIOLÓGICO

Este método foi o que obteve os melhores resultados, tendo em consideração o custo-tempo-eficácia, num estudo comparativo de várias metodologias de extração dos microplásticos de material biológico. De entre 15 polímeros testados, permitiu uma digestão eficiente do material biológico sem degradação significativa para 14 polímeros (HDPE, LDPE, PA-12, PA-6, PC, PET, PMMA, PP, PS, PSXL, PTFE, PUR uPVC e ePS) com exceção do acetato de celulose (CA) [1]. Este protocolo é uma adaptação de uma metodologia já existente [2].

1- Pesar e medir o comprimento do peixe (comprimento à furca). Remover o tubo digestivo completo (esófago, estômago e intestinos), registar o peso e congelar a -20ºC.

2- Descongelar as amostras à temperatura ambiente e colocá-las em recipientes de vidro (erlenmeyers).

3- Usar um volume de uma solução de KOH a 10% pelo menos 3 vezes superior ao peso do material biológico, de forma a imergir todo o material biológico (registrar o volume).

4- Incubar as amostras em recipientes fechados de forma a impedir a evaporação da solução, durante 24h a 60ºC até a matéria orgânica ser completamente digerida. Não agitar o material biológico ou agitar a um máximo de 300 rpm.

5- Filtrar a amostra com recurso a um sistema de filtração a vácuo. Usar filtros com poro de 20 µm, preferencialmente de nitrato de celulose. Caso a amostra tenha muita gordura ou muito sedimento, usar mais do que um filtro para que estes não colmatem.

6- Analisar os filtros com recurso ao microscópio ótico/lupa binocular. Contabilizar o número de microplásticos e catalogar por cor, tamanho e forma.

Para prevenir a contaminação por fibras, aconselha-se a fazer todo o processamento da amostra numa câmara de fluxo laminar, bem como o uso de filtros controlo. Deve manter-se a caixa de Petri com o filtro controlo aberta enquanto se trabalha e no final contabilizar o número de fibras e a cor existentes no filtro. Evitar usar material de laboratório de plástico e antes de cada utilização lavar com água MilliQ. Evitar o uso de roupa sintética e registar a cor da roupa que se utiliza no dia do processamento.


ANNEX II: ACTIVITY REPORT OF EXTRACURRICULAR EXTERNSHIP IN BIOSFERA 1/SPEA, CABO VERDE

The externship at Biosfera 1 and SPEA, in Cabo Verde, took place between July 2018 and December 2018. Biosfera 1, associated with SPEA, is an NGO located in São Vicente, Cabo Verde, which deals mainly with the protection of native and endangered species in Santa Luzia natural reserve. In São Vicente, they focus on sensibilization of the population and work with several schools for that purpose. Additionally, they set up a monitoring camp during the loggerhead turtle nesting and hatching season in Santa Luzia, and a monitoring camp to monitor seabirds and geckos in Raso. I integrated MAVA’s project of the recovery of Santa Luzia’s natural reserve, in Cabo Verde, which, among other things, aims to eradicate the invasive mammal present in Santa Luzia island, *Felis silvestris catus*.

In that sense, I learned methodology and strategic approaches to the eradication of an invasive species as well as the most ethical ways of doing it, with two international specialists in the eradication of invasive mammals, Paulo Oliveira and Félix Medina. In Santa Luzia desert island, during one week, I learned the basics on prospecting and searching traces of activity, on how to approach the problem on different stages of the invasive species population, the diversity of materials and methods used during the different stages of eradication, and developed strategic thought. Afterwards, I led for two weeks a small group in Francisca camp to prospect and distribute poison baits with PAPP, in order to increase the efficacy of the eradication. During this time, I improved my skills to follow traces and footsteps of invasive mammals, how to properly record information with the aid of GPS devices, how to elaborate information sheets which are practical on the field, and how to work with traps and poison baits. Furthermore, I experienced and learned how to work on the field, in remote and desert places, and how to cooperate in a camp. Once in Biosfera 1 office, located in São Vicente, Cabo Verde, I had access to the project information, in order to understand how it functions and how to elaborate a project. I organized databases already made with information of samples took from the mammal invasive species. Additionally, I elaborated databases with the information from the poison baits, and learned the basics of geographic software, using the programs QGIS and Google Earth. I travelled to Santa Luzia island to follow the advances made in the eradication, and to help the field personnel. I performed a necropsy on a feral cat in the island, in order to assess its condition and to collect samples for further analysis.

In São Vicente, I helped in sensibilization campaigns with the locals, regarding native species of the archipelago and particularly of Santa Luzia natural reserve. I organized the samples
from the invasive mammals, dealt with the necessary papers in order to send them to University of Porto. Additionally, I wrote protocols to investigate the presence of plastic in the marine birds of Raso island and elaborated a protocol for first aid of injured sea turtles found at Santa Luzia monitoring camp. I performed three bird census, learning the basics of this activity and also about the endemic species in Cabo Verde.

I collected 20 rectal swabs from domestic cats, cooperating with the locals, for the doctoral thesis of Amanda Bolt Botnen from the Centre for GeoGenetics, Section for Evolutionary Genomics in Copenhagen, Denmark. Furthermore, working with the local fishermen, I managed to collect 55 fishes in specific conditions to avoid plastics contamination, for posterior analysis in the University of Porto, with the aim of searching the presence of microplastics in their tissues.

Overall this externship provided me with great experience in field work, marine debris surveys, invasive mammals eradication strategies, sample collection, and data treatment. It also gave me experience and basic notions in birds’ identification and census and sensibilization campaigns. Additionally, I learned how to deal with the local population and how to communicate in order to gain their trust and cooperation.

Figure 1. Collecting rectal swabs from domestic cats, with the help of locals

Figure 2. Dinner in the camp of Francisca, in Santa Luzia desert island