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Microplastic Pollution in Portuguese Saltworks

Ana Sofia Soares, Carlos Pinheiro, Uirá Oliveira
and Maria Natividade Vieira

Abstract

Currently, microplastics are dispersed everywhere; from our oceans to our rivers, sediments, organisms, air, and even food resources. Therefore, this study aimed to assess the degree of contamination present in the Portuguese traditional table salts depending on their origin and type of salt. Fourteen samples were selected: seven from *fleur de sel* and seven from coarse salts, corresponding to seven distinct regions of the Portuguese territory. The concentration of microplastics, depending on salts' origin, ranged between 595 and 5090 MPs/kg, in sea salt, and in Rio Maior's well salt it varied from 3325 to 6430 MPs/kg. By salt type, the concentration of microplastics in the *fleur de sel* was 2320–6430 MPs/kg and in the coarse salt was 595–3985 MPs/kg. In the analyzed table salt, the most abundant anthropogenic particles were fibers (64%) and fragments (35%). The most predominant colors were transparent, blue, and black. The concentration of microplastics did not vary significantly ($p > 0.05$) between *fleur de sel* samples within different regions. However, statistically significant differences were found between coarse salt samples from the various regions. The results, gathered from this study, demonstrate the high contamination within artisanal Portuguese table salts, thus, becoming crucial to develop more future research, leading to a better understanding of the health risks associated with salt consumption.

Keywords: contamination, food security, microplastic, Portugal, table salt

1. Introduction

Plastics have played a fundamental role in man's daily life since the invention of Bakelite, the first synthetic polymer created by the chemist Leo Baekeland in 1907. Newly synthetic polymers were developed in the following years. These were capable of resisting higher temperatures without degrading, thus allowing the creation of a revolutionary era, within all commercial sectors (mainly industrial, health, and domestic), that shaped the habits of the future generations [1, 2]. The mass production of plastic began in the 1950s and has since evolved exponentially. For instance, in 2017, worldwide plastic production surpassed 348 million tons [3, 4]. Nevertheless, plastics have become persistent pollutants in the most diverse environments (atmospheric air, sources of freshwater, brackish, and saltwater, and soils) mainly due to their industrial characteristics (durability, hydrophobic composition, and plasticity) but also due to their improper handling over the years [1, 2, 5].

There are several ways in which plastic debris can reach the oceans, such as: (1) terrestrial sources (e.g., rivers and estuaries), (2) several industrial sectors,

(3) treated and untreated urban effluents, (4) and human activities (e.g., fishing) [6]. For example, our oceans receive, annually, a total of 4.8 to 12.7 million tons of plastic, from which 1.15 to 2.41 million tons come directly from freshwater streams [5, 7, 8]. Several studies have been carried out over the last two decades regarding the problem of plastics and their degradation into smaller particles. Since the 1970s, many scientists have suspected the harmful effects of these anthropogenic particles on the environment [9–12]. However, it was not until the 1990s that microplastics were considered and recognized as emerging pollutants. Therefore, scientists have been developing identification methods to better understand this problem's magnitude [13].

Microplastics (MPs) are characterized as plastic materials or fragments of small proportions, with measurements inferior to 5 mm [14]. MPs existing in aquatic environments may originate from primary sources (where they are designed and produced for a specific purpose) or from secondary sources (resulting from the degradation of larger plastic debris) [15]. In the oceans, the most prevalent synthetic polymers are polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) [16]. Today we are often surrounded by the presence of microplastics as they are everywhere; from our oceans, rivers, sediments, living organisms, atmospheric air and even food resources [14, 17]. These synthetic micropolymers have the ability to accumulate in our food chain, thus reaching various types of organisms. Several studies have been conducted in recent years to understand the potential impacts of these anthropogenic compounds on human health [6, 18].

In Portugal, the population is culturally linked to the sea and its resources, thus becoming more exposed to pollution present in the Atlantic Ocean. In recent years, several studies have been published about the accumulation of microplastics on beaches [19–21], estuaries [22, 23] and aquatic organisms [22, 24, 25] in Portugal. Moreover, many of these MPs had high concentrations of polychlorinated biphenyls (PCBs), pesticides (e.g., DDTs), polycyclic aromatic hydrocarbons (PAHs), and other persistent contaminants adsorbed to their surface [20, 26–28]. Additionally, all these chemical compounds are capable of causing problems in various aquatic ecosystems (such as marine, estuarine, lotic and lentic ecological communities). Hence, Yang et al. [14] hypothesized that saltwater-based commercial table salts were possibly contaminated, since oceans are polluted by plastic debris. In their work, the presence of MPs was analyzed in numerous types of Chinese sea salt brands, where it was found 7–681 MPs/kg of sea salt. After this first study was published, six others were able to characterize and identify (with relevant results) the presence of MPs in commercial table salts, with their concentrations varying mostly between sites [3, 5, 8, 29–31].

Considering that sodium (Na^+) is an essential element for our well-being [3] and the above information, it can be assumed that MP intake may vary depending on the country's gastronomic culture and environmental pollution. For example, WHO suggests a maximum daily intake of 5 g of salt, however, the Portuguese population consumes, on average, 3 g more than the recommended [32]. We can then assume that the Portuguese people are subject to a greater exposure of microplastics via table salt consumption. Therefore, this study aims to analyze the available Portuguese table salts and determine the degree of microplastic contamination, depending on their origin and type of salt.

2. Methods and materials

2.1. Study area and sample collection

A total of seven geographically distinct saltworks were previously identified and selected for this study: Aveiro (Av), Figueira da Foz (FF), Rio Maior (RM), Tejo (Tj), Olhão (Ol), Tavira (Tv) and Castro Marim (CM) (**Figure 1**). In each region,

samples of *fleur de sel* and artisanal coarse salt were collected ($n_t = 14$), in triplicate, between February–April 2019 under the same brand.

Depending on their origin, salts can have different designations. In all the selected saltworks, except for RM, both *fleur de sel* and coarse salt are produced through the solar evaporation of brackish and/or marine waters. Thus, salt originated from these solar saltworks is commonly named sea salt. However, in RM's saltworks the salt is produced from the storm- and groundwater that leaches the halite deposits, present in the region, to form a brine. This one is then collected in open solar saltworks and undergoes the same evaporation processes as the remaining. Therefore, this type of salt is designated as “well salt” [33].

The packages obtained from *fleur de sel* and artisanal coarse salt were sold in plastic bags of 200–250 and 1000–1500 g, respectively. Previous studies have already shown that plastic packaging does not influence the concentration of MPs in the final results [5, 8, 31].

2.2. Preventive methods of contamination

A protocol, based on published studies [5, 14, 29], was developed to prevent microplastic contamination throughout the study. Protective gloves and white coats were implemented when handling any material or reagent, since numerous plastic fibers can be found in our hands and clothing, which can have in its composition synthetic fibers (e.g., polyester, nylon...) [34]. Moreover, all equipment was cleaned with previously filtered deionized water and 70% (v/v) ethanol. Finally, materials were protected and/or sealed [29].



Figure 1.
Location of Portugal's main saltworks and respective sampling sites.

The deionized water used during the study was previously filtered with the aid of a vacuum pump using a 1 µm porosity cellulose nitrate membrane (Whatman®; CAT: 7190-002), thus, removing possible microplastic contamination. This filtered water was used to clean the equipment and dissolve the tested table salts, thus, preventing any contamination from external sources via microplastics [14].

2.3. Microplastic extraction and identification

The microplastic extraction from both salt types was performed according to Iñiguez et al. [5], with some modifications. Briefly, 200 g of salt (*fleur de sel* or coarse salt) was homogeneously dissolved in 1 L of previously filtered deionized water. Next, the solution was placed in a centrifuge for 1 h at 1900 rpm in order to isolate the denser (in)organic material from the supernatant [5]. The supernatant was subsequently collected and filtered with the aid of a vacuum pump using a 5 µm porosity cellulose nitrate filter (Whatman®; CAT: 7195-004). According to Branca [35], the plastic particles are expected to be suspended in the supernatant due to density differences. Nevertheless, preliminary tests were performed to assess whether certain microplastics were “trapped”, or not, in the precipitate. No microplastics were found in all of the precipitates. After filtration, the membranes were carefully collected, preserved and sealed in Petri dishes and allowed to dry at room temperature. Each Petri dish was previously brushed with petroleum jelly to fix the membrane and avoid, consequently, the plastic microparticles displacement during microscopic or stereoscopic manipulations. All filtrations and hand-manipulations were performed inside a fume chamber, hence, avoiding any kind of atmospheric contamination [29].

The visual identification of microplastics was performed using a stereoscope (Carl Zeiss Stemi DV4 CLS120X) and a camera. A total of 59 membrane filters were recorded since some replicas required more than one membrane due to clogging. The open software ImageJ (v1.80) was used to analyze each collected picture. The classification of microplastics was made according to their shape and color [3] (Table 1).

Shape	Color
<ul style="list-style-type: none">• Fibers, thin plastics and often cylindrical in shape.• Films, thin and flat plastics.• Fragments, microplastics with an irregular shape and/or surface.• Microspheres, perfectly round plastic particles.• Styrofoam, a lightweight polymer with a sponge-like texture.	<ul style="list-style-type: none">• Beige• Black• Blue• Brown• Gray• Green• Multicolor• Orange• Pink• Red• Transparent• Violet• White• Yellow

Table 1. Microplastics classification according to their shape and color as used in this study (adapted from [3]).

2.4. Data analysis

A one-way ANOVA, followed by Tukey’s HSD multiple comparison test, was performed in order to discover significant statistical differences between the abundance of microplastics of each region, for the same salt type (*fleur de sel* and coarse salt). Homogeneity and normality tests were applied to the data to validate the tests. Additionally, several independent sample *t*-student tests were employed to understand the differences between salt types, in each saltworks region. All analyses were performed with a significance level of 0.05. All statistical tests were performed using the SPSS v25 software.

3. Results

3.1. Presence of microplastics in Portuguese salt

In this study, all samples ($n_t = 14$) of artisanal *fleur de sel* and coarse salt were contaminated by microplastics. Overall, 23,175 anthropogenic plastic microparticles were analyzed, of which approximately 64% corresponded to fibers, 35% to irregular fragments, and only <1% to films (Figure 2A). Indeed, fibers were the

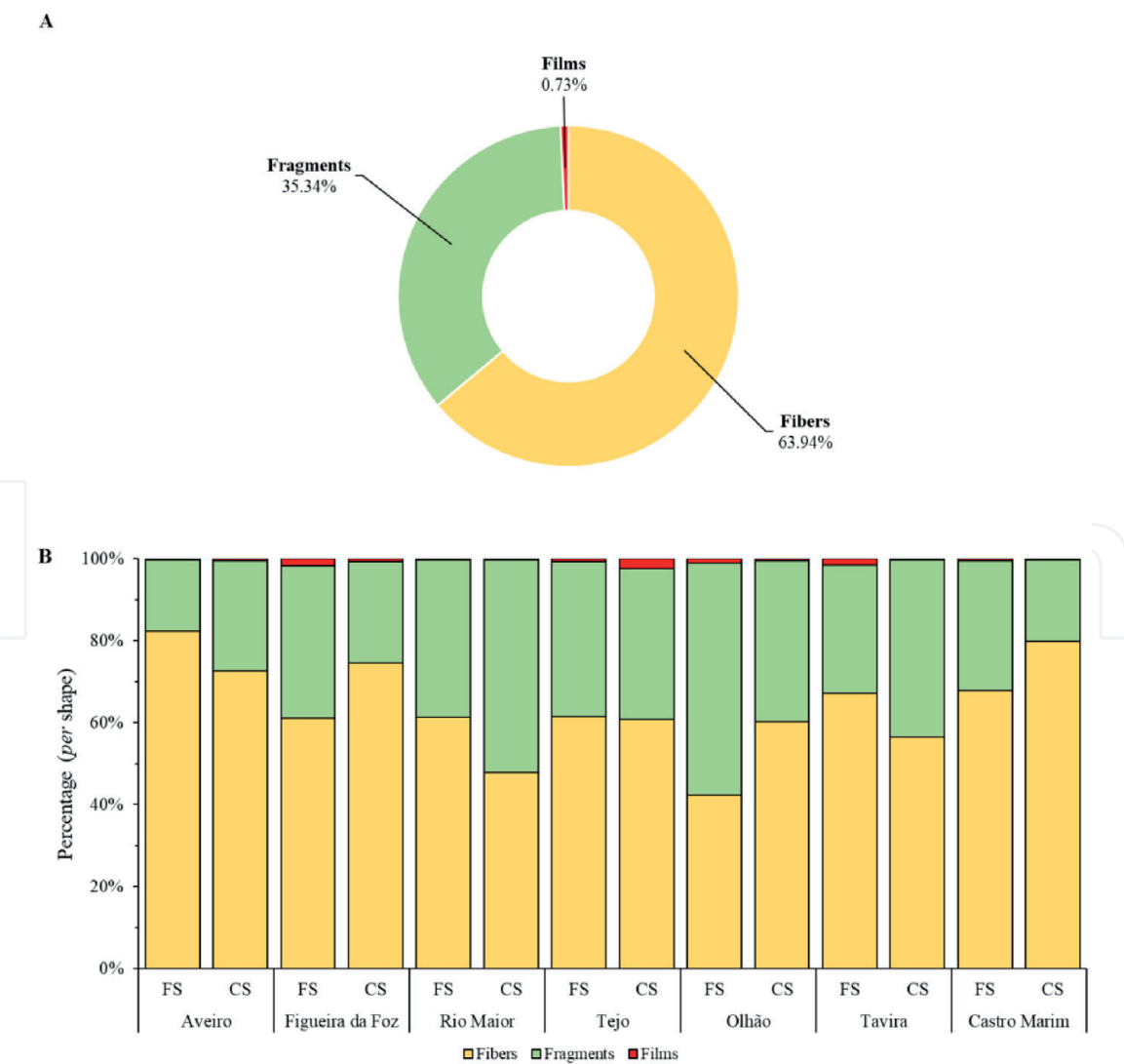


Figure 2. Percentage of the different shapes of microplastics identified (A) throughout the study and (B) in each saltworks region. Microspheres and Styrofoam microparticles were not found. FS—Fleur de sel; CS—Coarse salt.

most predominant type of microplastic (>50%) present in all the studied regions, except in Rio Maior and Olhão (**Figure 2B**). Here, a higher percentage of fragments (51.8 and 56.5%, respectively) was found in coarse salt and *fleur de sel*, respectively. Films were also present in all samples, although in lower percentages (<2.5%) when compared to the other microplastic shapes (**Figure 2B**). No spherical and Styrofoam microparticles were identified in all samples.

The most common colors found were transparent (54.9%), blue (15.3%), and black (14.6%), corresponding to roughly 85% of the analyzed microplastic polymers (**Figure 3A**). Gray and green accounted for about 4.6 and 3.2%, respectively. The remaining 7.5% includes microplastics with the colors designated as yellow, white, beige, brown, orange, pink, red, and violet (**Figure 3A**).

According to **Figure 3B**, transparent was the most predominant color (>40%) throughout all sampled regions. From the remaining colors, blue and black proved to be the most prevalent in all regions when compared to the remaining colors (**Figure 3B**). No multicolored microplastics were found in this study.

Moreover, it was possible to observe a high amount of lesser dense organic matter throughout the membrane filter analysis (e.g., small invertebrates, *Artemia* sp.

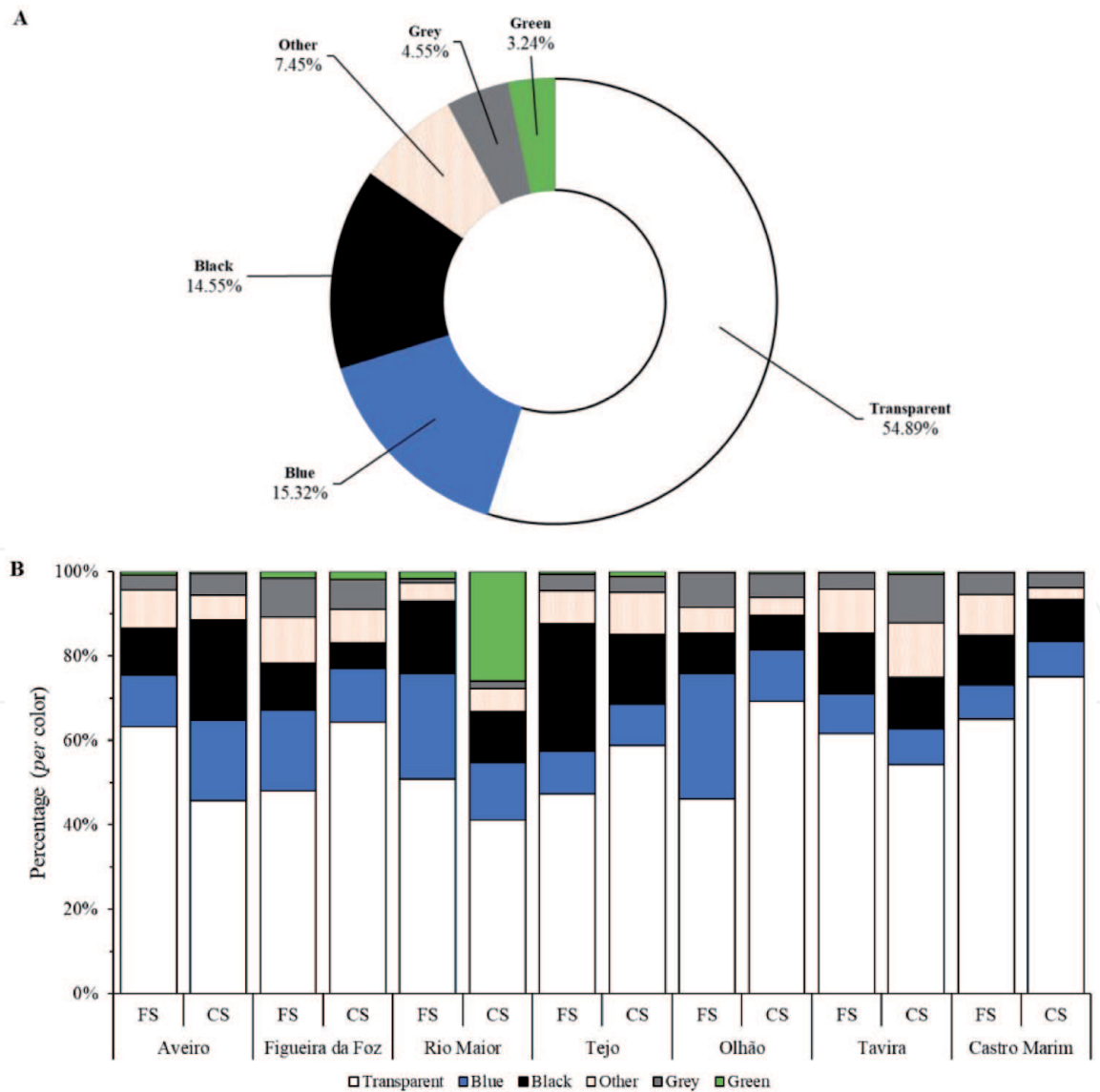


Figure 3. Percentage of the different colors of microplastics identified (A) throughout the study and (B) in each saltworks region. “Other” represents the sum between the following colors: beige, brown, orange, pink, red, violet, yellow, and white. No multicolor microplastics were identified. FS—Fleur de sel; CS—Coarse salt.

cysts, feathers, vegetal organic matter...; **Figure 4**), which demonstrates the degree of impurities present in these types of salt.

3.2. *Fleur de sel* vs. coarse salt

Figure 5 shows the significant differences between the different types of salt (artisanal *fleur de sel* and coarse salt) in each saltworks' region. According to **Figure 5**, significantly higher values ($p < 0.05$) of microplastics in the *fleur de sel* were found in all studied regions, when compared to coarse salt ($Av - t_{(4)} = 2.816, p = 0.048$; $FF - t_{(4)} = 4.000, p = 0.016$; $Tj - t_{(4)} = 7.376, p = 0.002$; $Ol - t_{(4)} = 2.990, p = 0.040$; $Tv - t_{(4)} = 5.249, p = 0.006$), except for Rio Maior's ($t_{(4)} = 1.457, p = 0.219$) and Castro Marim's ($t_{(4)} = 2.152, p = 0.098$) saltworks (see **Table 2** for mean and standard deviation values). Moreover, regarding artisanal coarse salt, statistically significant differences were detected between regions according to the one-way ANOVA ($F_{(6,14)} = 18.752, p = .000$). Tukey's *post hoc* test revealed that Rio Maior artisanal coarse salt contains a significantly higher amount of microplastics than the coarse salt from the other regions studied ($Av, p = 0.012$; $FF, p = 0.000$; $Tj, p = 0.000$; $Ol, p = 0.000$; $Tv, p = 0.000$; $CM, p = 0.001$). However, there was no statistically significant differences ($p > 0.05$) between the different *fleur de sel* samples from the various studied saltworks ($p = 0.170$).

Additionally, the region who presented the highest quantities of microplastics in artisanal *fleur de sel* and coarse salt was Rio Maior (4830.0 ± 1408.0 and 3611.7 ± 338.4 , respectively). The lowest amount of *fleur de sel* and coarse salt was found in the two most southeastern saltworks of Portugal: Castro Marim (2798.3 ± 595.3) and Tavira (666.7 ± 72.5), respectively (**Table 2**).

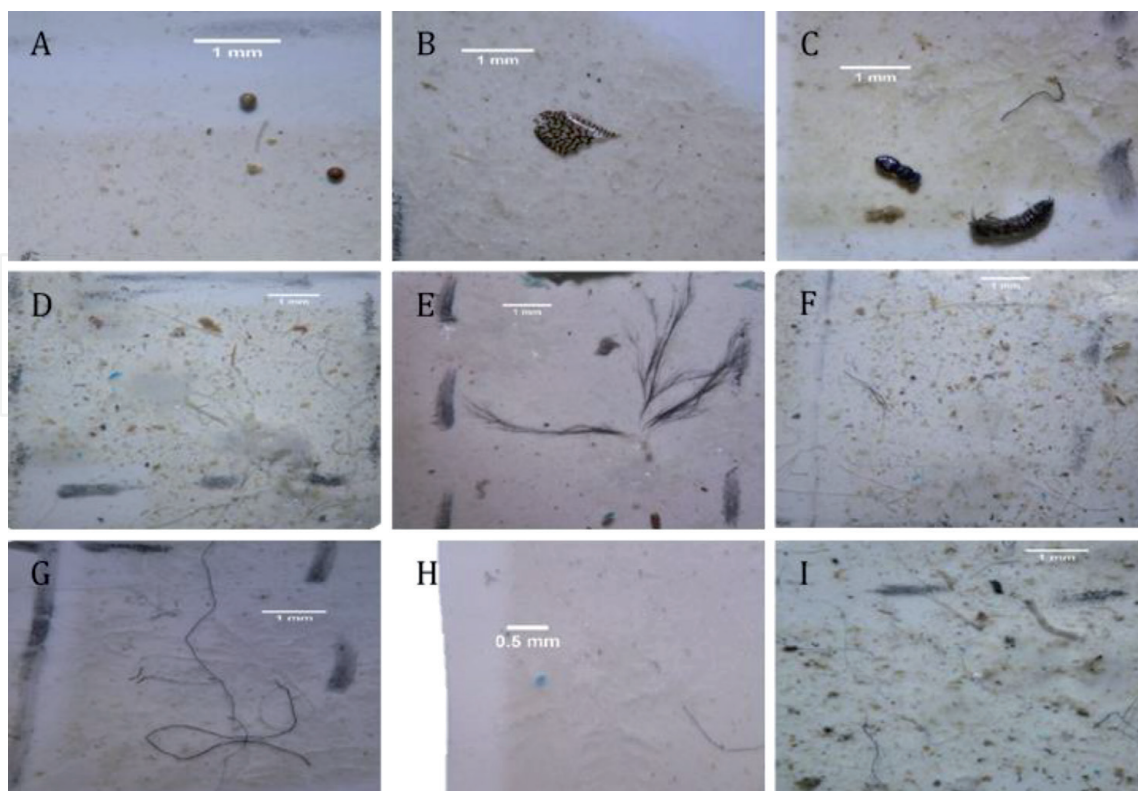


Figure 4.
 Different types of organic and inorganic particles found in artisanal table salts: (A) *Artemia* sp. cysts, present in most of *fleur de sel* samples; (B) an invertebrates' chitin; (C) insects body; (D, F, I) overview of a membrane filter being analyzed; (E) birds' feather; (G) a mesoplastic, with more than 5 mm; (H) blue microplastic fragment, identified in most samples across regions.

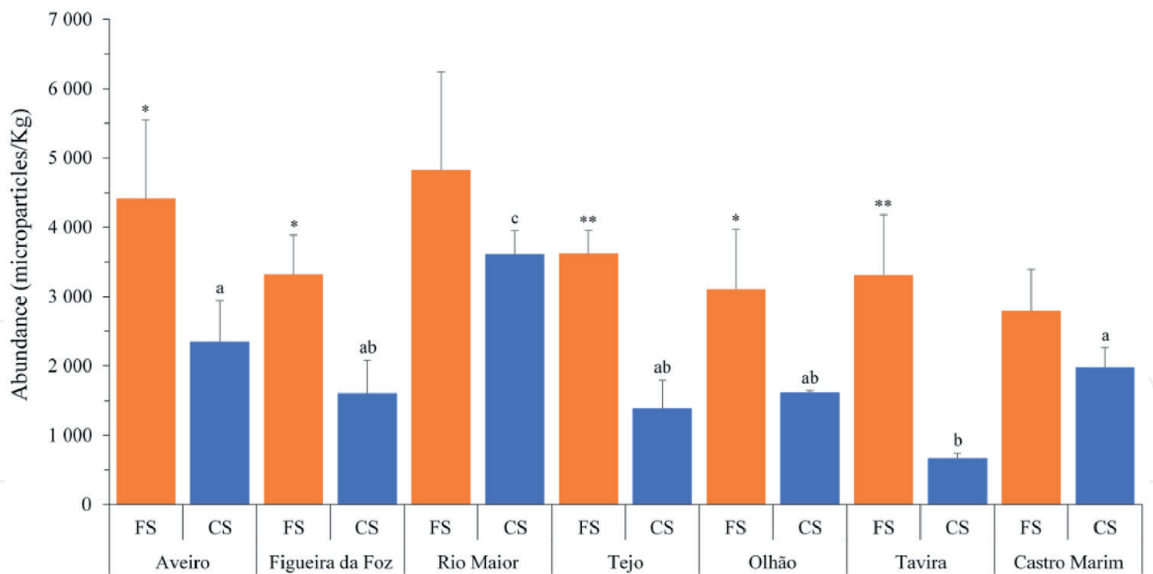


Figure 5. Comparison between the abundance of plastic microparticles present in the artisanal fleur de sel (FS) and coarse salt (CS) from the various saltworks selected for this study. Each value represents the mean \pm standard deviation. Asterisks represent statistically significant differences between fleur de sel and coarse salt according to Student's t-test. *— $p < 0.05$; **— $p < 0.01$. Different letters represent statistically significant differences ($p < 0.05$) between samples of artisanal coarse salt across the various regions studied. There are no statistically significant differences ($p > 0.05$) between the samples of fleur de sel.

Microplastic particles per kilogram (Kg)								
Fleur de sel					Coarse salt			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Av	3120	5050	4420.0	1126.0	1670	2735	2351.7	591.9
FF	2675	3730	3320.0	565.4	1085	2040	1603.3	482.7
RM	3780	6430	4830.0	1408.0	3985	3900	3611.7	338.4
Tj	3255	3900	3621.7	331.5	1030	1830	1390.0	406.0
Ol	2395	4070	3108.3	864.7	1585	1630	1615.0	21.2
Tv	2510	4235	3310.0	869.3	595	740	666.7	72.5
CM	2320	3465	2798.3	595.3	1700	2270	1978.3	285.2

Table 2. Minimum (min) and maximum (max) values of the number of microplastic particles per kilogram (kg), and respective mean and standard deviation (SD) for the different types of artisanal table salt (fleur de sel and coarse salt) collected in all regions.

4. Discussion

The main purpose of this study was to assess the level of anthropogenic contamination via microplastic particles of two types of table salts present in the Portuguese territory. Seven different locations were selected, where both artisanal *fleur de sel* and coarse salt were obtained for analysis, from the same brand.

In Portugal, the vast majority of the salt production starts by capturing sea-water, due to tidal changes, into several successive ponds with different widths and heights. Next, it undergoes through various evaporation processes, due to the wind and solar actions, improving the salt crystals' precipitation. The coarse

salt is then collected, roughly washed, and packaged. On the other hand, *fleur de sel* is only the first surface layer of formed salt produced in saltworks. This type of table salt is collected and immediately packaged without being previously cleaned [36]. Nevertheless, some types of salts may undergo sanitization, as well as a refining process, before packaging [8]. Therefore, to understand the level of contamination existing in the Portuguese saltworks, all the salts acquired for this study were of artisanal origin, i.e., no refinement or industrial treatment was applied.

Once samples were analyzed, it was evident the presence of a high MP concentration compared to other studies already carried out in several countries (Table 3). These values may be a direct proof of the lack of refinement or treatment processes that undergo in Portuguese saltworks, thus, reflecting the traditional methods still used nowadays. Most studies related to the presence of microplastics in table salt samples either used refined, treated salts and/or salts from which there is no information on their treatment [5, 8, 14, 30, 31]. Hence, it becomes important to study and understand how refining processes influence the abundance of microplastics in table salts.

Overall, *fleur de sel* presented always higher contamination values of MPs than those found in coarse salts. These salt “scales” are formed at the crystal-lizers’ surface and, as such, greater air contamination of plastic particles is expected [2]. Also, since it does not undergo any cleaning process up to its packaging, it is expected a higher concentration of MPs, when compared to artisanal coarse salt.

Moreover, laboratory errors should be considered while manipulating samples. For instance, MPs were only analyzed using a stereoscope, with most of them measuring less than 500 µm. However, several studies indicate that from this size

Abundance of MPs per Kg(<i>n</i> _{samples})			Predominant		References
Sea salt	Well salt	<i>Fleur de sel</i>	Shapes	Colors	
550–681 (5)	7–204 (5)	—	Fr/Fb	BLa/G/BLu/W	[14]
50–280 (16)	115–185 (5)	—	Fb	BLa/G/ BLu/W	[5]
0–10 (17)	0 (5)	—	Fr/Fb/Fl	ND	[3]
16–84 (5)	—	—	Fb/Fr/Fl	ND	[29]
46.7–806 (11)	113–367 (1)	—	Fb/Fr	BLu/G/R/T	[30]
22–19,800 (12)	—	—	Fr/Fb	BLa/T/G/BLu	[31]
0–13,629 (28)	0–148 (9)	—	Fr/Fb	W/T/BLa/BLu	[8]
595–5090 (6)	3325–6430 (1)	2320–6430 (7)	Fb/Fr	T/BLu/BLa	This study

The microplastic particles are ordered from most to least predominant.
Fr—Fragments; Fb—Fibers; Fl—Films; BLa—Black; BLu—Blue; G—Green; R—Red; T—Transparent; W—White; ND—No data.

Table 3.
Comparison between the concentrations of MPs found in several published articles and the current study.

an underestimation of the real value may occur. Hidalgo-Ruiz et al. [37], stated that the visual identification of MPs is a valid method for dimensions superior to 500 μm , while MPs smaller than this threshold need to be analyzed using stricter methodologies. A visual identification-only approach can lead to underestimations ranging from 20% [38] to 70% [37], with that percentage increasing inversely with MPs' size [31].

Among the studies conducted so far, two presented similar results to those found in the Portuguese table salts: Renzi and Blašković [31] analyzed sea salt from Italy and Croatia and found between 22 and 19,800 MPs/Kg; and Kim et al. [8], that discovered 0 to 13,629 MPs/Kg and 0 to 148 MPs/Kg in sea salt and well salt, respectively, from worldwide salt samples. In this study, values ranged from 595 to 5090 MPs/Kg in sea salt and from 3325 to 6430 MPs/kg in Rio Maior's well salt.

Regarding *fleur de sel*, values ranged from 2320 to 6430 MPs/Kg and, for coarse salt, between 595 and 3985 MPs/Kg. Until now, only Portuguese salt has had a higher concentration of MPs in well salt [33], when compared to sea salt. Unfortunately, there is a lack of studies in other regions with similar characteristics to Rio Maior's saltworks, which do not allow for further data analysis. Concerning the MPs classification (shape and color), this study presented similar results to Gündoğdu [29], Iñiguez et al. [5], and Kosuth et al. [30], with fibers and fragments being the most predominant overall, as well as, black, blue, and transparent microplastics.

Therefore, we can argue that regardless of its source, microplastic contamination in table salts is an emerging concern, mainly due to their public health implications and environmental pollution. Indeed, microplastics are consumed not only through table salts but also via tap water, beer, honey [30], atmospheric air [2] and a wide variety of seafood. In fact, bivalves are the most studied group of animals since most species are filterers and become easily contaminated [39–41]. Microplastics can be hazardous already by themselves in the environment, however, they also function as transporters/emitters of persistent organic pollutants (e.g., bisphenol A, organochlorides) due to their adsorption ability. Hence, it is important to investigate and evaluate possible transmission risks in which microplastics may affect public health [5].

The World Health Organization recommends a maximum daily salt (Na^+) intake of 5.0 g/day. Nevertheless, the Portuguese population generally consumes 8.0 g/day, due to a strong and rooted gastronomic culture. Therefore, with the values obtained in this study, Portuguese people would consume, on average, approximately 7443 or 12,325 MPs/year depending on the table salts origin (sea salt and well salt, respectively). If we calculate by the salt type, an average Portuguese person can consume 5551 or 10,769 MPs/year if they consume only coarse salt or *fleur de sel*, respectively. However, these values are only theoretical and that in reality the amount MPs ingested is relatively smaller, since WHO also takes into account the salt found in food additives and processed food for the maximum daily salt intake threshold [3].

5. Conclusions

The present study was the first to assess the presence/absence of microplastic polymers in *fleur de sel*, a type of salt formed at the surface of saltworks' crystal-lizers. Overall, microplastics were found in all samples of Portuguese table salt, regardless of their origin and type, with higher contamination values being found in Rio Maior's "well salt".

Moreover, more studies are needed, since salt is a very common and well-rooted ingredient in Portuguese gastronomic culture. In fact, there are other available table salts to the population that were not studied yet. Additionally, new management tools need to be applied to decrease the concentration of impurities (e.g., insects' feathers, exoskeletons, etc.,...) and contaminants, such as microplastics.

Nowadays, research needs to be increasingly strengthened regarding microplastics contamination so that better regulations can exist together with a broader understanding. Also, these regulations need to be supported by government agencies in order to implement actions that reduce the emission of plastics into the environment and, thus, preventing environmental and human impairments.

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Conflict of interest

The authors declare no conflict of interest.

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