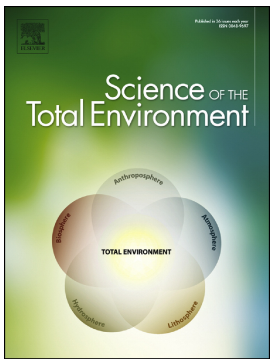

Effects of spatial and seasonal factors on the characteristics and carbonyl index of (micro)plastics in a sandy beach in Aveiro, Portugal

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Effects of spatial and seasonal factors on the characteristics and carbonyl index of (micro)plastics in a sandy beach in Aveiro, Portugal.

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Abstract

Coastal environments are highly contaminated with plastics of various sizes. In order to understand the distribution and factors influencing (micro)plastics contamination in the environment, sampling of a sandy beach in Costa Nova, Aveiro, Portugal, was conducted by collecting plastic particles and sediments for density separation in transects from the mean low tide line to the dunes, during wet and dry seasons. For surface collection, microplastics comprised 69.4% of plastics, presenting concentrations of 3 – 6 items m^{-2} in the wet season, mostly polyethylene pellets carried ashore by storms, and <1 item m^{-2} for dry season, lower due to less backwashing, were found. Collection of infrared spectra of these particles allowed characterization by polymer type and carbonyl index of all particles. Variations in carbonyl index were found to be related to season, site and particle color. Density separated microplastics, mostly fibers, presented 23 times higher concentrations than surface collection (22 microplastics kg^{-1}, 280 microplastics m^{-2}), due to the identification of smaller sizes, and with higher concentrations in dry seasons, likely from accumulation in sediment and bathing season. Overall, different sampling methods allowed identification of different particle types and sizes, which may vary according to seasonal and spatial factors.

Keywords: plastic debris; plastic litter; microplastics; carbonyl index; FTIR-ATR

1. Introduction

In 2017, the global production of plastics reached 348 million tonnes (PlasticsEurope 2018). Due to human activities, including waste mismanagement, littering, and industrial activities, more than 5 trillion plastic particles are estimated to be floating in the oceans (Eriksen et al. 2014).When these plastics are <5 mm, resulting from intentional production (primary) or degradation and fragmentation in the environment

(secondary), they are called microplastics (NOOA 2015; Rocha-Santos and Duarte 2015). Plastics in the environment are exposed to biotic and abiotic factors that induce degradation and fragmentation, due to continued exposure to ultraviolet (UV) radiation, mechanical action, and biological activity (UNEP 2015). In the environment, UV radiation (100-400 nm) is responsible for acceleration of polymer degradation though photolytic, photo-oxidative, and thermo-oxidative reactions by breaking polymer chains, producing radicals and reducing molecular weight, causing loss of mechanical properties, discoloration and yellowing, diminished gloss and gradual deterioration (Shah et al. 2008). Furthermore, mechanical action by wave forces and collision between fragments cause erosion and fragmentation of particles (Resmeriță et al. 2018). These processes could be coupled with biotic degradation, where microorganisms, such as bacteria and fungi, produce a variety of enzymes capable of degrading natural and synthetic polymers (Gardette et al. 2013). Besides causing fragmentation into smaller pieces, such as microplastics, these biotic and abiotic processes cause chemical changes in the polymer structure with the formation of carbonyl functional groups (Rodrigues et al. 2018).

In areas of high concentrations, microplastics interact with organisms possibly being carried through the food chain and having impacts on metabolism, oxidative stress, DNA damage and neurological damage, directly or indirectly reducing survival and reproduction (Carrasco et al. 2019; Prokić et al. 2019). Furthermore, microplastics are known to release plastic monomers and additives as well as chemicals adsorbed from the environment, such as persistent organic pollutants, potentially delivering them in concentrated form to organisms (Rodrigues et al. 2019). Additionally, the presence of plastics may alter important abiotic properties of the habitat, such as increasing water evaporation and cracking of soils (Wan et al. 2019). Thus, there is a need to clarify the

distribution and factors influencing the presence of microplastics in the environment in order to be able to estimate its impacts on ecosystems.

The objective of this work was to study the seasonal and spatial distribution on the abundance and characteristics of (micro)plastics in a sandy beach in Costa Nova, Aveiro, Portugal. Collection of plastics with tweezers was conducted during two seasons in the same sandy beach, in eleven areas marked between the sea and dunes. Collected plastics were characterized in terms of size, shape, color. Chemical identification and carbonyl index were accomplished though the collection of infrared spectra by Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance (FTIR-ATR). Simultaneously, sand was collected to identify smaller microplastics by density separation. This study provides insights on the contamination of the beach, as well as seasonal and spatial factors influencing abundance and characteristics of microplastics in beaches, as well as the use of different sampling methods.

2. Materials and methods

2.1. Sampling location

Costa Nova, Ílhavo, Aveiro District, Portugal (40.619327; -8.752923), an Atlantic sandy beach, was chosen as sampling location (**Figure S-1, Supplementary Material, S.M.**). Aveiro District, in the central region of Portugal, is characterized by its industrial zones and relatively high population density (Sousa 2003), namely of 714,000 inhabitants, with a density of 248.4 inhabitants km^{-2} (City Population 2019). Costa Nova is a stretch of land locked by the Aveiro lagoon, in the east, and by the Atlantic Ocean, in the west, with a population of 1,165 inhabitants (City Population 2019). Aveiro lagoon is a shallow coastal lagoon forming a mesotidal wetland area characterized by a temperate climate, and mostly bordered by areas of agriculture,

agroforestry and nature (Lillebø et al. 2015). The maritime coast of Aveiro is characterized by sediment grain sizes of 0.352 – 1.373 mm, increasing in size in winter and near the intertidal zone, and by intense wave climates, one of the most energetic in the country, especially during the months of November, January and February (Narra et al. 2015). Wave direction often follows a north-west pattern (IPMA 2019). Costa Nova climate is characterized by an average temperature of 14.8°C, reaching 18.8°C in August and dropping to 10.1°C in January, and an average annual rainfall of 1019 mm, with July as the driest month (11 mm) and January with the highest precipitation (141 mm) (Climate Data 2018). In terms of occupation, Costa Nova is dominated by low density urban areas and agricultural or natural vegetation areas (Copernicus 2018) and used for recreative and touristic activities, due to its sandy beaches and plazas, residential purposes, and by small-scale fisheries.

2.2. Sampling strategy

A new sampling strategy was developed in order to allow characterization of (micro)plastics in from water line to dunes, across seasons. Using the stone breakwater as a reference, sampling was conducted south to this structure spawning from near the water line (site 1) to near the dunes (site 11) in the low tide (**Figure 1**). Sampling was performed in subdivided 11 parallel sites 5.45 m long and 1 m wide separated by 2 m intervals, sampling an overall of 59.95 m^2. Suspected plastic samples were collected with metal tweezers from the surface of the sand, stored in aluminum foil pockets correctly identified, and transported to the laboratory for further characterization.

Three squares of 50x50 cm quadrats were marked in each of the eleventh sites (adapted from Naji et al. 2017). In these quadrats, no surface collection of particles was conducted. Inside each of these larger squares, five smaller squares of 10x10 cm were drawn in the corners and center, from each the top 2 cm layer of sand was collected with

metal scoops. One aluminum foil container was used for each 50x50 cm square, containing a pool of sand from the 10x10 cm squares inside. Aluminum containers were capped with paper lids, identified and transported to the laboratory for processing.

Sampling was carried out in four different periods in order to represent both wet, namely 21st of February 2018 (w1) and 13th of February 2019 (w2), and dry seasons, namely 10th of October 2018 (d1) and 27th of June 2018 (d2). Sand was collected only in w1, d1, and d2.

2.3 Sample processing

For collected sand, density separation was conducted in glass beakers containing pre-weighted sand (average 0.63 kg) by adding, in double the volume of the sand, 0.3 g ml^{-1} NaCl (Sigma-Aldrich, Germany) solution, stirring and sitting for an hour (adapted from Clunies-Ross et al. 2016). After settling, the supernatant was filtered through 0.45 µm mixed cellulose esters filters (Gridded GN-6 White, GelmanSciences, U.S.A.) using a glass vacuum system. Filters containing samples were kept in glass Petri dishes.

2.4. Characterization of plastic particles

In the laboratory, suspected plastics collected from the sand surface were classified by size (<5 mm, >5 mm), by shape (fragment, pellet, cigarette filter) and color (transparent, white, yellow, orange, red, pink, blue, green, gray, black). All collected plastics, except those separated by density from collected sand due to their small dimensions, were characterized by ATR-FTIR (Perkin Elmer (USA) Spectrum BX FTIR instrument, 32 scans, 4 cm^{-1} and 5000-550 cm^{-1}). Photographs of samples were captured in a digital stereoscope (Leica DMS300). Microplastics separated by density were identified under the stereomicroscope based on their morphology, confirmed by the hot needle test (Campbell and Williamson 2017), and classified by shape into fibers and fragment (no spheres were observed).

2.5. Carbonyl Index

Obtained spectra allowed for the identification of polymer type by comparison to a database of virgin polymer spectra. Calculation of carbonyl index was determined based on the absorbance at 1635 – 1650 cm^{-1} for carbonyl groups and at 1648, 1635, 1452 cm^{-1} for reference peaks of polyethylene (PE), polypropylene (PP) and polystyrene (PS), respectively (adapted from Rodrigues et al. 2018). Carbonyl index was calculated for each particle (Rodrigues et al. 2018), depending of plastic types, as:

$$\text{Carbonyl Index (CI)} = \frac{\text{Absorbance carbonyl peak}}{\text{Absorbance reference peak}}$$

2.6. Data analysis

Concentrations of microplastics in samples collected from the surface of beach sediment were recorded as microplastics m^{-2} (MP m^{-2}) and from density-separated microplastics as particle kg^{-1} (p kg^{-1}) and particles m^{-2} (p m^{-2}). Excel 2016 was used for recording data (**Table S-1, S.M.**) and producing graphics. IBM SPSS version 26 was used for descriptive statistics and Kruskal Wallis tests followed by pairwise comparisons, due to the failure to comply with ANOVA assumptions. An α=0.05 was considered.

2.7. Measures to prevent sample contamination

Cross contamination in the field was avoided by wearing cotton lab coats, collecting samples in aluminum foil and using all metal equipment (scoops, tweezers). In the laboratory, cross contamination was avoided by working in a clean workspace, wearing cotton lab coats, covering samples with aluminum foil, using glass material previously washed with acid and distilled water, and opening samples only when strictly necessary.

3. Results

3.1. (Micro)plastics collected from the sediment surface

The total amount of particles collected from the sediment surface and characterized to using ATR-FTIR (for polymer identification and carbonyl index) was 733 (**Figure 2,**

Figure S-3, S.M.). More particles were collected during wet seasons (464 and 232) than dry seasons (19 and 18). Higher number of particles were collected closer to the sea line (site 1 – 7), possibly concentrated near tide lines, than closer to the dunes (site 8 – 11). In terms of concentration, the average concentration was 8 and 4 plastics m^{-2} for the first and second wet season, and <1 plastics m^{-2} for both dry seasons. During the wet season, concentrations of microplastics were 6 and 3 plastics m^{-2}, and of meso- and macroplastics of 2 plastics m^{-2}. **Figure 3** illustrates the variability observed in the shape and color of the collected plastic particles.

3.1.1. Size of the sampled plastic particles

Sampled plastic particles were classified by size as <5 mm (microplastics) and >5mm (meso- and macroplastics). Overall, microplastics were the most abundant class, constituting a total of 557 particles (69.4%), while meso and macroplastics constituted only a total of 246 particles (**Figure 4**). Regarding season, microplastics were more abundant during wet seasons, constituting to 79.1 and 63.4% of total number of collected plastics, but not during the dry season, constituting only 30.8 and 33.3% of the plastics found. Regarding distance to the sea, microplastics constituted between 57.7 – 87.5% of plastic particles in sites 1 to 7, closer to the sea line, while meso and macroplastics were more abundant in sites 8 to 11, closer to the dunes.

3.1.2. Shape of the sampled plastic particles

Pellets were the most abundant shape of plastics found in Costa Nova, constituting 72.2% of the total plastics (**Figure 4**). However, shape distribution seems to be highly influenced by season. For instance, during wet seasons, pellets were more abundant, constituting 77.6 and 68.5% of plastics. On the other hand, fragments made up 52.6 and

72.2% of the plastics in the dry season. Since more plastics were collected during the wet seasons, pellets were overall more abundant when considering the total number of particles. Pellets were also more prevalent closer to the sea line, in sites 1 to 7, comprising 58.6 – 81.9% of particles. Fragments were more abundant closer to the dunes, in sites 8 to 11, constituting 56.3 – 83.3% of plastics in these areas.

3.1.3. Polymer type of the sampled plastic particles

PE was the most frequent polymer type (68.6%), followed by PP (25.5%), and finally PS (2.4%) and other polymer types (1.7%) (**Figure 3 and 4**). During the wet season, PE was more abundant, corresponding to 69.2 and 75.8% of the particles found. On the other hand, the abundance of PE (36.8 and 12.0%) and PP (31.6 and 20.0%) are relatively similar in dry seasons. This results from the abundance of pellets found in wet seasons, composed mostly of PE, and fragments in dry seasons, composed by PP. Relative to the total, PS particles were especially prevalent during dry seasons (12.0 – 31.6%) compared to wet seasons (1.1 – 1.6%). However, the absolute values observed were generally the same in the dry (3 - 6) and wet (4 - 5) seasons. The same influence on the abundance of pellets was noted for the distribution by site, with higher abundance of PE closer to the sea line, in sites 1 to 7 (52.6 to 93.1%). In sites 8 to 11, closer to the dunes, neither PE (30.8 – 42.9%) nor PP (15.4 – 38.9%) were dominant. PS (5.4 – 23.1%) and other polymer types (5.5 – 27.0%) appeared to be found closer to the dunes, between sites 6 to 11.

3.1.4. Color of sampled plastic particles

White was the most abundant color (62.3%) in the collected particles, followed by transparent (11.4%) and yellow (8.8%), probably due to the prevalence of pellets

(**Figure 4**). White was highly prevalent across seasons (62.9 – 63.1%) except for the dry season d2 (21.4%), where green (28.6%) was more common. Less plastic particles were found during dry seasons, thus percentages can be highly influenced by the small number of particles. White was also prevalent across sites (56.3 – 64.3%), with the exception of site 10, closer to the dunes, where green (40.0%) was dominant, likely also due to the small number of particles found.

3.1.5. Carbonyl index of sampled plastic particles

The carbonyl index is often used as an indicator of the presence of carbonyl groups, which may result from polymer degradation (Rodrigues et al. 2018). Carbonyl index found for plastic particles collected from Costa Nova are presented in **Figure 3 and 5 (details on Figure S-2, S.M.)**. For PE, there was a significant difference in average carbonyl index determined from samples collected from different sites (p<0.001), namely site 6 and site 3 (p=0.004), site 4 (p=0.040), site 7 (p=0.040), site 10 (p=0.040) and site 11 (p=0.020)(**Table S-2, S.M.**). Site 6 showed the highest average carbonyl index for PE, 0.478, even though fragments only constituted 37.5% of the plastics found in this area. For PP, a significant difference between sites was identified by Kruskal Wallis test (p=0.018), but no significant differences were found in pairwise comparisons after Bonferroni correction (**Table S-2, S.M.**). This is likely a result of the Bonferroni correction process, due to the high number of groups tested. For PS, no significant difference was identified between sampling sites (p=0.231). Differences between seasons were found for PE (p<0.001) and PP (p=0.006), namely between wet seasons (p<0.001 for PE, p=0.003 for PP; **Table S-3, S.M.**). No difference between seasons was identified for PS (p=0.173).

3.2. Smaller microplastics separated from sand samples by density separation

Sand samples were collected and microplastics separated by density using a NaCl solution, aiming to compare the effects of season and site, analyzed for sediment weight (p kg^{-1}) and sediment surface area (p m^{-2}) (**Figure 6**; **Table S-8 and Figure S-4, S.M.**). For sediment weight, fibers were the most abundant class of microplastics found, with a median 21 p kg^{-1} (0 - 384 p kg^{-1}). On the other hand, fragments were rare, with a median of 0 p kg^{-1} (0 - 44 p kg^{-1}), leading to a concentration of total microplastics of 22 p kg^{-1} (0 - 384 p kg^{-1}). Significant differences were found between seasons for fibers (p<0.001) and total microplastics (p<0.001), but not for fragments (p=0.072). In the case of fibers, no differences were found between the wet and dry season d1 (p=0.517) but such differences were found between the wet season and dry season d2 (p<0.001) and between dry seasons (p<0.001). The same trend was observed for total microplastics, no significant difference for the wet and dry season d1 (p=0.431) but significant for the wet and dry season d2 (p<0.001) and between dry seasons (p<0.001). The total number of microplastics was higher during dry seasons, namely of 20 and 43 p kg^{-1} for dry seasons d1 and d2, when compared to the wet season, on which only a median of 11 p kg^{-1} was found. Highest concentrations of fibers and total microplastics were found in site 4, with concentrations of 43 and 43 p kg^{-1}, and site 8, with 35 and 38 p kg^{-1}. Lowest concentration was found in site 6, with a concentration of 9 p kg^{-1} for fibers and 10 p kg^{-1} for total microplastics. However, no differences between sites have been found for fibers (p=0.144), fragments (p=0.128) and total microplastics (p=0.143), possibly due to the large variation of results.

For sediment surface area, the median concentrations of microplastics were 280 p m^{-2} (0 - 3160 p m^{-2}), with a median 260 p m^{-2} (0 – 3160 p m^{-2}) for fibers and 0 p m^{-2} (0 – 400 p m^{-2}) for fragments (Table S-8,, S.M.). Significant differences were found for total

suspected microplastics (p<0.001) and fibers (p<0.001) between sampling seasons, but not for fragments (p=0.067). Pairwise comparisons reveal significant differences between wet and dry season d2 for fibers (p<0.001) and total microplastics (p<0.001), corresponding a concentrations of 120 p m^{-2} for both fibers and total microplastics in the wet season and 400 p m^{-2} for both fibers and total microplastics in the dry season d2. It is worth noting that concentrations for dry season d2 are likely underestimated when calculating by area (p m^{-2}), since some sample was used for other analysis methods that were unsuccessful. Significant differences were also found between sampling sites for fibers (p=0.021) and total microplastics (p=0.033), but not for fragments (p=0.093). However, significant differences are lost in pairwise comparison due to Bonferroni correction (**Table S-7, S.M.**). Highest concentrations, of 540 p m^{-2}, were found in site 8 for both fibers and total microplastics, followed by site 4 for both fibers and total microplastics, of 460 p m^{-2}. Lowest concentrations of fibers and total microplastics, of 160 p m^{-2}, were found in site 1. Fragments were only found in sites 6, 8, and 10, in concentrations of 20 p m^{-2}.

These findings reveal differences in results and data analysis from different methods of calculating concentration for microplastics separated by density from the top sediment. Both methods find differences between the wet season and dry season d2, with highest concentrations during dry seasons, with one of the highest concentrations being found in sites 4 and 8. On the other hand, differences between dry seasons were only found when results are expressed in p kg^{-2}, and between sampling sites when expressed by p m^{-2}. The advantage of expressing results in both units is their wider application, improving comparison with other studies and allowing its use in ecotoxicity testing.

4. Discussion

4.1. Concentrations of (micro)plastics in Aveiro, Portugal

Concentrations of microplastics collected in Costa Nova, Aveiro, varied from <1 to 6 MP m^{-2} for microplastics collected from the surface of sand stretches. These concentrations are in the low range of what has been observed for the Portuguese coast, namely 2 to 1,964 microplastics m^{-2} (Antunes et al. 2018). These values are also low when compared to microplastics concentrations registered in other countries (**Table 1**), such as 4 to 167 pellets m^{-2} in Malta (Turner and Holmes 2011), 0 to 12,869 MP m^{-2} in the Canary Islands in Spain (Herrera et al. 2018), and 7 to 5,560 MP m^{-2} in Baltic beaches in Russia (Esiukova 2017). These differences could be a result from land occupation in Costa Nova, mostly comprised of vegetation and residential areas of low demographic and industrial density, or from the geomorphology and dynamics of the sampled beach. Moreover, sampling strategies vary between studies even when using similar units, including results from the collection of the top layer of sand for density separation (Antunes et al. 2018; Esiukova 2017; Hererra et al. 2018), which could be responsible for the higher concentrations reported. Indeed, these concentrations are more similar to density separated microplastics presently found for Aveiro, where concentrations range from 0 to 3,160 p m^{-2}. A higher amount of microplastics were found by density separation than by collection with tweezers on site due to the small dimensions of these particles, which were mostly comprised of fibers that can be easily neglected in the field collection. In the case of calculating by sediment weight, a median concentration of microplastics of 22 p kg^{-1} was found for density separated samples, mostly comprised of fibers. This is in the range of what was found for subtidal sediment samples in the south of Portugal, where concentrations ranged from 0 to 263 p kg^{-1} (Frias et al. 2016), for European countries, where concentrations varied from 72 to 1512

p kg^{-1} (Lots et al. 2017), and for Po River Delta, in Italy, with concentrations ranging from 3 to 23 p kg^{-1} (Piehl et al. 2019).

4.2. Characteristics of (micro)plastics collected from the sediment surface

In Costa Nova, microplastics (<5 mm) were the most abundant size of plastic particles, comprising 69.4% of all collected plastics. This is in good accordance with previous studies in the Portuguese coast, in which 68% (Antunes et al. 2018) to 72% (Martins and Sobral 2011) of the total number of collected plastics constituted microplastics. However, this fraction may vary, depending on sampling location, with approximately similar values found in Slovenia, of 75% (Laglbauer et al. 2014), but not in South China, where microplastics comprise 98% of plastics found in beaches (Fok et al. 2017).

Pellets used by the manufacturing industry were the most abundant plastic shape found in Costa Nova (72.2%), also responsible for the high quantities of white (62.3%) and PE (68.6%) pieces. In a previous study on Portuguese beaches, pellets comprised 79% of microplastics (Antunes et al. 2018). In wet seasons, a higher number of plastics was found in the beach (464 and 232 items *vs*. 19 to 18 items in dry seasons), mostly comprised of pellets (360 and 159 particles; 68.5 and 77.6% of plastics). Higher accumulation of marine litter in beaches, especially of pellets, have been associated to strong winds and waves during the wet season due to backwashing events (Antunes et al. 2018; Herrera et al. 2018). Moreover, pellets are usually found in regions related to industrial activities (Tziourrou et al. 2019), meaning that this type of plastic could result from Aveiro's district industrial zones. Fragments are also more numerous in the wet season than in the dry season. However, in the dry season, fragments become the dominant plastic type (52.6 to 72.2% of plastics) possibly due to degradation processes caused on shore and due to the lower backwashing of plastics from the sea. It is also

worth noting that beach cleaning may take place during bathing season, which coincides with dry season.

PE and PP comprised most of the total particles collected, respectively 68.6% and 25.5% of plastics. Indeed, PP and PE were the most produced polymer types in Europe (PlasticsEurope 2018), which results in higher prevalence in the natural environment due to pellet losses, littering and mismanaged waste. In previous studies, these two plastic types were also identified as the most common in Portuguese (Frias et al. 2010) and European beaches (Lots et al. 2017). In the wet season, there was a predominance of PE (69.2 and 75.8%) since this was the main type of polymer pellets collected. In the dry season, PE and PP were found in similar quantities due to the mixed presence of pellets and fragments. In fact, most pellets were comprised of PE (83.0%) followed by PP (16.6%) and PS (0.4%), and mostly were white (71.8%), transparent (10.4%) or yellow (11.5%) in color. Conversely, most fragments were PP (52.0%) followed by PE (38.5%) and PS (8.0%), and mostly of white (37.0%), blue (15.0%) or transparent (14.0%) colors. The predominance of pellets in wet season (77.6 and 68.5%) and of fragments in the dry season (52.6 and 72.2%) in part justifies the observed fluctuations in polymer type and color.

4.3. Spatial and seasonal distribution of microplastics

Higher accumulation of microplastics near the dunes was observed in previous studies, identifying similar distribution in buried particles and high temporal variability (Moreira et al. 2016; Turra et al. 2014). In another study, no significant difference was identified between two drift lines, leading to the conclusion that microplastics were homogenously distributed in the 500 m area parallel to the shoreline (Dekiff et al. 2014). In the case of Costa Nova, it seems plastics, collected by both methods, were mostly concentrated in sites 2 to 5, closer to the sea line, which likely corresponds to the

dynamic shifting of the drift line. Indeed, drift lines are areas of accumulation of plastics in beaches (Heo et al. 2013). Costa Nova is a beach where the drift line is farther away from dunes while, in previous studies, drift lines were closer to dunes leading to a higher concentration of plastics in this area. This could justify why plastics were mostly found near the sea in Costa Nova and near the dunes in previously studied beaches. Furthermore, this observation leads to the conclusion that choices of sampling areas in a beach can highly influence estimated concentrations.

Seasonal distribution is likely more related to specific plastic types than to the overall plastic concentration. For instance, microplastics collected from the sediment surface were more abundant during wet seasons due to storm events that caused backwashing and accumulation of pellets on shore. On the other hand, microplastics from density separation were more abundant during the dry season and mostly comprised of fibers (93%). The prevalence of fibers is not a surprise, as other studies have found them to be the prevalent plastic type in sediments (Filho and Monteiro 2019; Laglbauer et al. 2014; Graca et al. 2017). The abundance of fibers in beach sediments during the dry season likely results from its accumulation, due to the lack of removal by storm events, or release from activities conducted during the bathing season, such sunbathing using beach towels that can be a source of fibers. Thus, seasonal variations in microplastic concentration can be highly influenced by the plastic types being studied, as these vary in sources and distribution, mostly dependent on their individual characteristics.

4.4 Comparison between surface collected and density separated microplastics

To the best of the authors knowledge, this was the first study comparing the collection of (micro)plastics from the sediment surface with the concentration found for density separated microplastics in the same area. Different sampling methods produced different results, which greatly influence the conclusions taken from the study. For

instance, the mean number of microplastics found by density separation was 23 times higher than that found by collection from the sediment surface, 280 and 12 MP m^{-2} respectively. While pellets were the predominant type in sediment surface, fibers were the predominant type in density separation comprising 93% of the microplastics found. This difference is likely the results of the size detection limit of each method, with smaller microplastics being identified under the stereomicroscope after density separation. The effect of size on the concentrations of microplastics have also been explored for water samples, with 30 times more microplastics being found when using an 80 µm plankton net than with a 330 µm manta trawl (Dris et al. 2015). Thus, the simultaneous use of both methods is recommended, allowing identification of different type of particles.

4.5. Carbonyl index of (micro)plastics collected from the sediment surface

When considering the total of particles collected from the sediment surface for each polymer type, no differences in average carbonyl index were identified between colors (**Table S-6, S.M.**). Differences between season have been identified for PE, namely between wet seasons (**Table S-4, S.M.**). Differences between shape were found only for PP, with pellets presenting higher carbonyl index (**Table S-5, S.M.**). For PE, differences were found for site 6, in which particles analyzed exhibited higher carbonyl index values. This could result from the central position of this site, between sea and dunes, which could expose particles to higher solar radiation and oxygen concentrations, while also suffering the abrasive action of the waves during high tides.

Besides polymer type, particles were also categorized into shapes, in order to uncover differences between particle color, season and sites. Differences were found in carbonyl index when running Kruskal Wallis considering the factors polymer type and shape (**Table S-7, S.M.**). In a previous study, there was a possible relationship between pellet

color and carbonyl index, with yellow and brown colors presenting higher values (Turner and Holmes 2011). In Costa Nova, differences in carbonyl index between colors were found for PP fragments (p=0.014), namely higher values of blue compared to transparent particles (0.2986 *vs.* 0.1559, p=0.027), and for PP pellets (p=0.041), likely due to differences between black and yellow colors (0.0900 *vs.* 0.3640, p=0.096). The relationship between carbonyl index and color could be justified by changes in tone due to degradation events or due to the protective nature of certain pigments used in plastic. For instance, black pigments are often made of carbon black that, besides providing a dark color, protect polymers from degradation due to the absorption of UV light and trapping of radicals, acting as an antioxidant (Christie 1984).

Differences between seasons were found for PE fragments (p=0.015), PE pellets (p<0.001), PP fragments (p=0.041) and PS fragments (p=0.028), namely between wet seasons. In all cases, wet season w1 presented higher carbonyl index values than wet season w2. In Goa, India, differences in carbonyl index have been found between monsoon periods, with January presenting lower values than June (Veerasingam et al. 2016). Carbonyl index could be highly influenced by a number of factors, including weather conditions before sampling. For instance, strong storm events could carry more degraded plastics to beaches while higher solar intensities could potentiate photodegradation. The differences in carbonyl index between seasons are a good demonstration of seasonal variability in the characterization of microplastics.

Differences between areas were found only for PE fragments (p=0.020), likely between site 4 and 5 (p=0.074, lack of significance likely produced by Bonferroni correction), and for PE pellets (p<0.001), namely between sites 3 with sites 5 (p=0.002) and 6 (p=0.009), and site 4 with sites 5 (p=0.026) and 6 (p=0.037). In the case of fragments, site 4 presented higher carbonyl index than site 5 (0.3979 vs. 0.1564). In the case of

pellets, carbonyl index increased with distance to the sea line (from 0.2127, 0.2267, 0.3044, 0.4433 between sites 3 to 6). An increase in carbonyl index with proximity to the dunes could be justified by longer residence and higher exposure to UV radiation and oxygen, less influenced by seawater. However, results from PE pellets and fragments present opposite patterns: the carbonyl index is higher closer to dunes for pellets and higher closer to the sea line for fragments. Thus, this question should be further explored in future works.

5. Conclusion

Microplastics in Costa Nova were found in concentrations of 3 – 6 MP m^{-2} in wet seasons and <1 MP m^{-2} in dry seasons. On the contrary, a median of 22 particles kg^{-1} or 280 particles m^{-2} were found for microplastics separated by density and highest concentrations were found during dry seasons. For surface collection, the most frequent shapes were pellets and fragments of PE, PP, and PS. Seasons caused variation in polymer type, shape and carbonyl index. Pellets of PE were more abundant during wet seasons likely due to backwashing in storm events, while fragments and pellets of both PE and PP characterized dry seasons. On the contrary, density separated microplastics were mostly comprised by fibers, with higher concentrations found during dry seasons, likely from their accumulation and beach use during bathing season. It is likely that different plastic types, varying in characteristics and sources, present different seasonal and spatial distribution, requiring detailed analysis. Higher accumulation of particles was observed for drift line areas in sites closer to the sea line, probably due to the large extension of the sandy beach. Carbonyl index of the surface collected particles, representing the presence of carbonyl groups associated with polymer degradation, revealed to vary with season, site and particle color, requiring more research in order to

clarify the impact of these factors. Overall, the density separation method reveals concentrations 23 times higher than the surface collection method, likely resulting from the identification of smaller particles. Thus, for future studies, a combination of surface collection and density separation is recommended to reliably sample different particle types and sizes present in beach sediments, considering also seasonal and spatial variability in the experimental design.

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Figure 1. Sampling strategy used in Aveiro, Portugal, comprised of 11 sites of 5.45x1 m ranging from dunes to the seawater line, spaced 2 m between each other, and each containing three smaller quadrants of 0.5x0.5 m, each containing five smaller squares of 0.1x0.1m from which sediment was collected.

Figure 2. Number of plastic particles collected from the sediment surface during sampling by total, season and site.

Figure 3. Examples of (micro)plastics collected in Costa Nova, Aveiro, Portugal during the second wet season (w2). Images A-D under 1 mm scale, E under 2 mm scale and F 5 mm scale. Examples of infrared spectra for polyethylene, polypropylene and polystyrene from samples collected in Aveiro, Portugal, with different degradation profiles.

Figure 4. Classification by size (A), shape (B), polymer type (C), and color (D) of plastic particles collected from the sediment surface during sampling by total, season and site. PE: polyethylene, PP: polypropylene, PS: polystyrene, Other: polyethylene terephthalate, polyvinyl chloride, cellulose acetate, polyurethane, polyoxymethylene.

Figure 5. Average carbonyl index of polyethylene (PE), polypropylene (PP), and polystyrene (PS) of particles collected during sampling by total, season and site. Error bars refer to the standard deviation.

Figure 6. Median concentration of fibers and fragments found in sand samples in Costa Nova, Aveiro by season and site.

Table 1. Concentrations of microplastics found in beach sediment across the world in concentrations of microplastics m^{-2} (MP m^{-2}).

Location	Concentrations (MP m^{-2})	Method	Reference
Aveiro, Portugal	<1 – 6	Surface collection	Present work
Aveiro, Portugal	0 – 3,160	Density separation	Present work
Portuguese coast	2 – 1,964	Density separation	Antunes et al. 2018
Canary Islands, Spain	0 – 12,869	Density separation	Herrera et al. 2018
Baltic coast, Russia	7 – 5,560	Density separation	Esiukova 2017
Maltese coast	4 – 167	Surface collection	Turner and Holmes 2011

Conflict of interest:
The authors declare no conflict of interest.

Highlights

- Microplastics comprised 69.4% of plastics found in Costa Nova, Aveiro, Portugal.
- Concentrations of 3 – 6 and <1 item m^{-2} were found for wet and dry seasons, respectively.
- Density separation revealed a median of 22 microplastics kg^{-1} of sand.
- Differences were found for carbonyl index between shape, color, site and season.

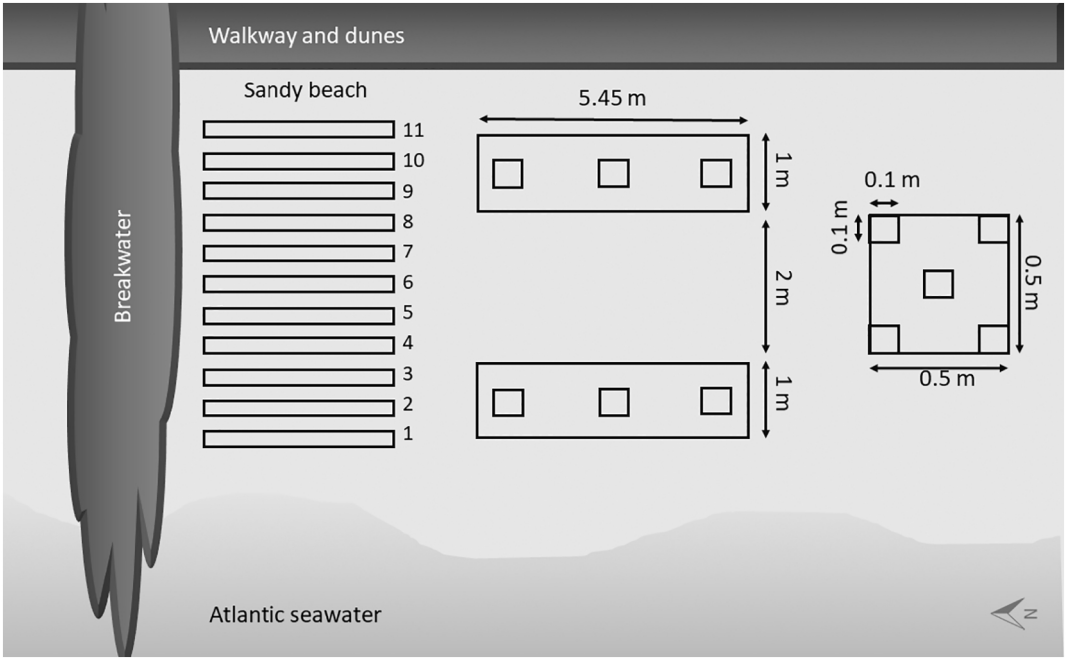
Walkway and dunes
Sandy beach
Breakwater
11
10
9
8
7
6
5
4
3
2
1
5.45 m
1 m
2 m
1 m
0.1 m
0.1 m
0.5 m
0.5 m
Atlantic seawater
N

Figure 1

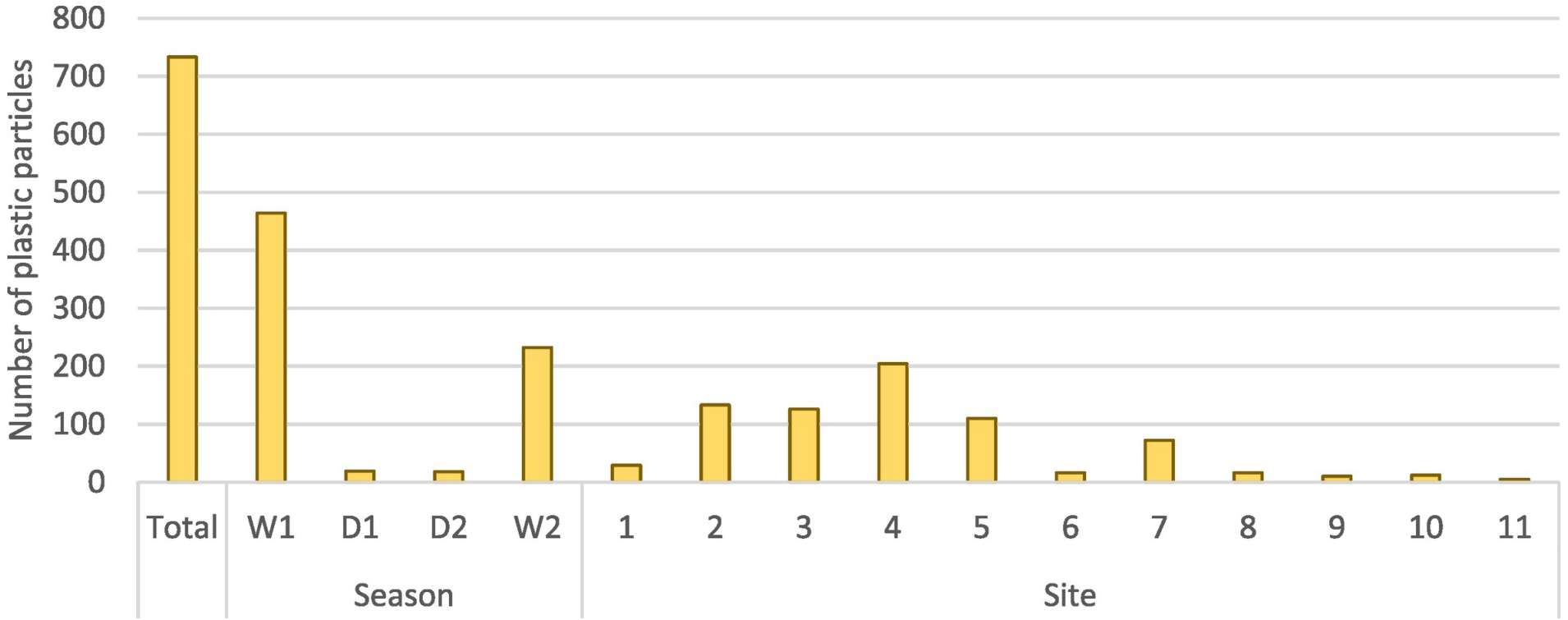
800
700
600
500
400
300
200
100
0
Number of plastic particles
Total
W1
D1
D2
W2
1
2
3
4
5
6
7
8
9
10
11
Season
Site

Figure 2

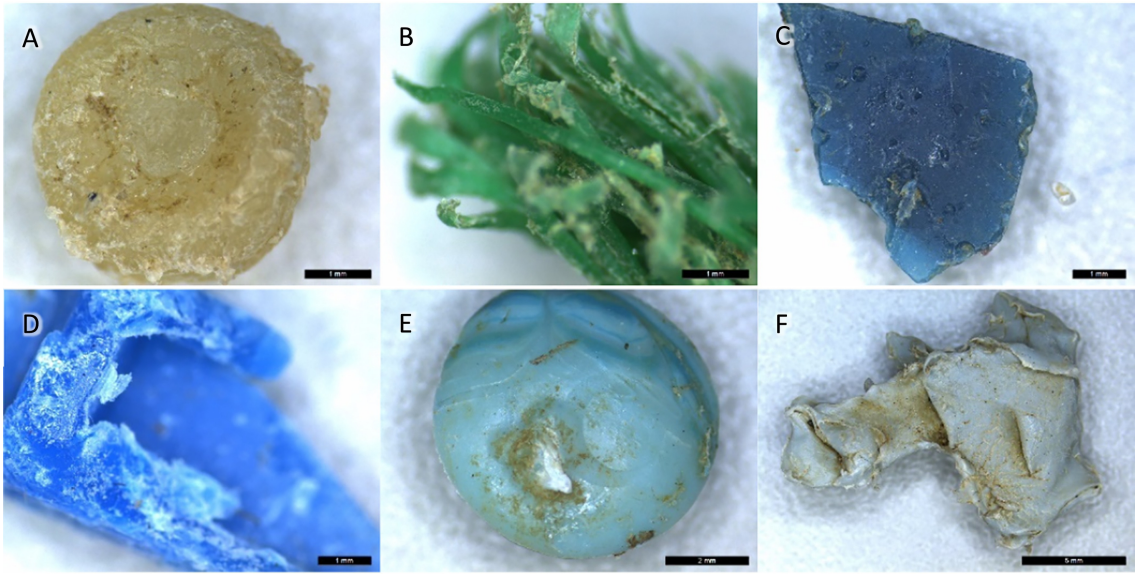
A
B
C
D
E
F
Polyethylene
Sample 2
Sample 1
Reference
600
1600
2600
3600
Polypropylene
Sample 2
Sample 1
Reference
600
1600
2600
3600
Polystyrene
Sample 2
Sample 1
Referenc
600
1600
2600
3600

Figure 3

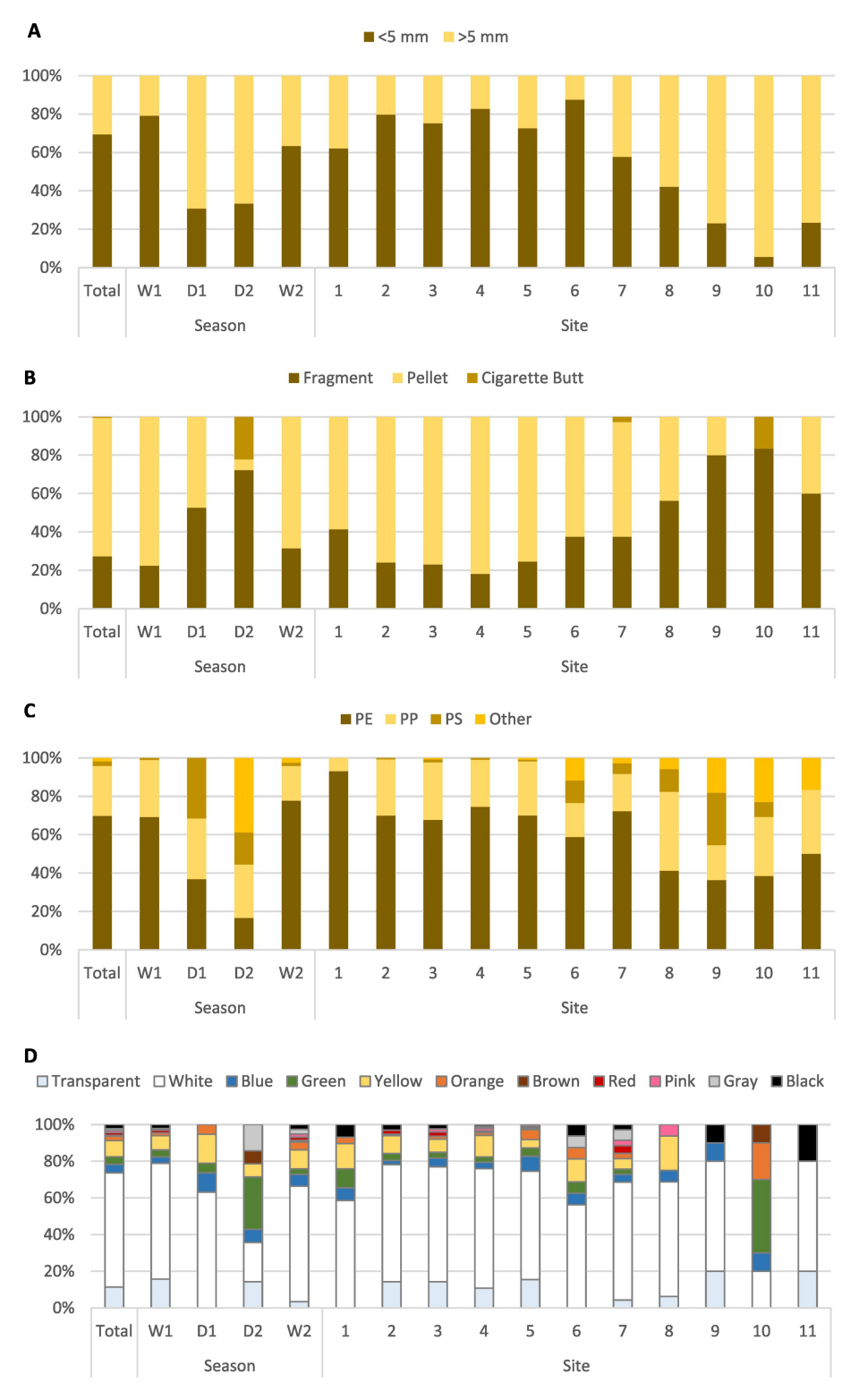
A
<5 mm
>5 mm
100%
80%
60%
40%
20%
0%
Total
W1
D1
D2
W2
Season
1
2
3
4
5
6
7
8
9
10
11
Site
B
Fragment
Pellet
Cigarette Butt
C
PE
PP
PS
Other
D
Transparent
White
Blue
Green
Yellow
Orange
Brown
Red
Pink
Gray
Black

Figure 4

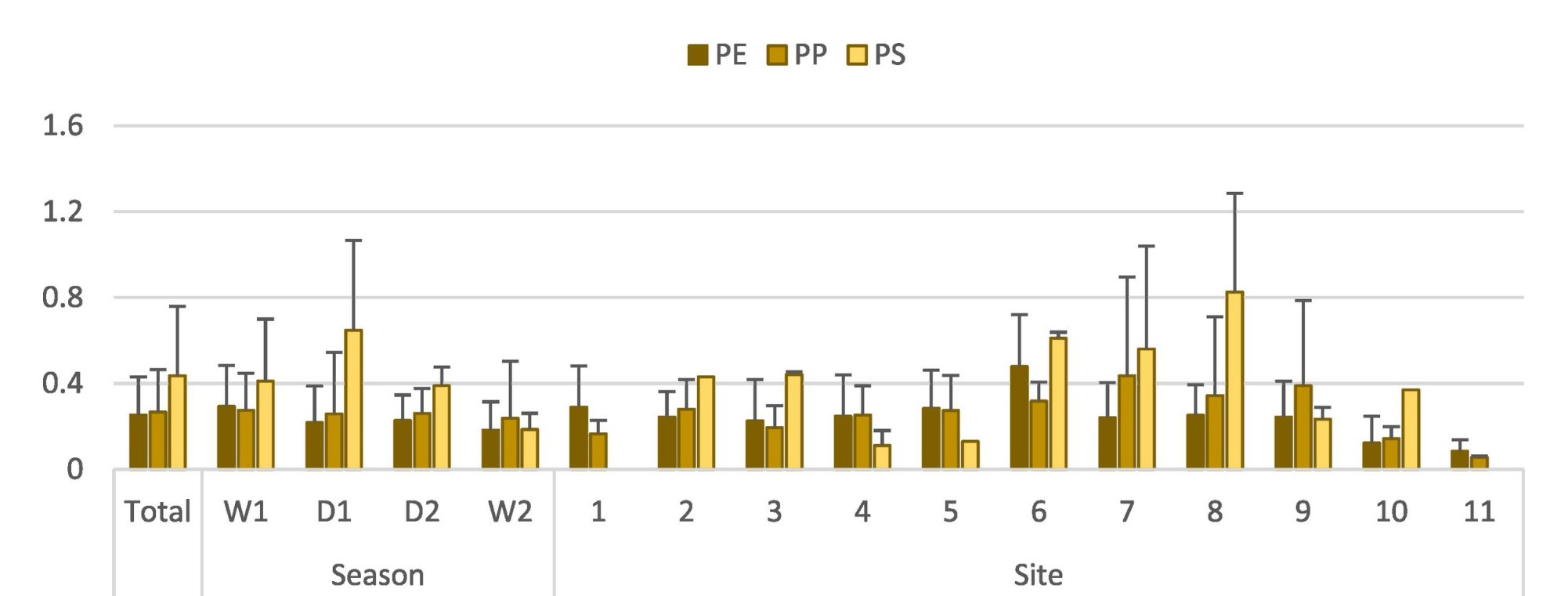
PE
PP
PS
1.6
1.2
0.8
0.4
0
Total
W1
D1
D2
W2
1
2
3
4
5
6
7
8
9
10
11
Season
Site

Figure 5

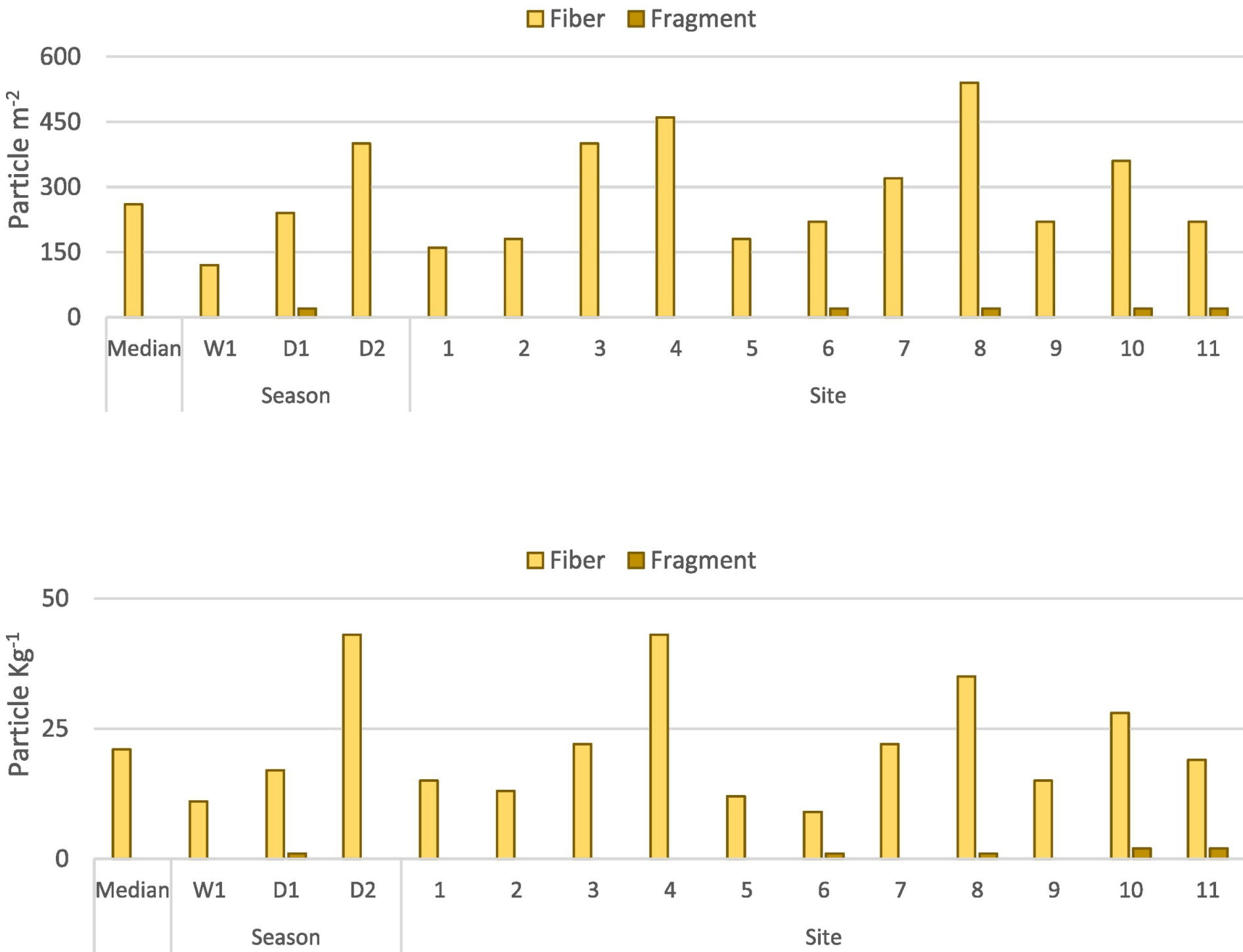
Fiber
Fragment
600
450
300
150
0
Particle m-2
Median
W1
D1
D2
Season
1
2
3
4
5
6
7
8
9
10
11
Site
Fiber
Fragment
50
25
0
Particle Kg-1
Median
W1
D1
D2
Season
1
2
3
4
5
6
7
8
9
10
11
Site

Figure 6