Environmental Pollution 349 (2024) 123931

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Are native microalgae consortia able to remove microplastics from wastewater effluents?☆

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A R T I C L E I N F O

Keywords:
Wastewater
Microplastics
Microalgae
Mitigation

A B S T R A C T

Wastewater Treatment Plants (WWTPs) are potential sources of microplastics (MPs) in the aquatic environment. This study aimed to investigate the potential of wastewater-native microalgae consortia to remove MPs from the effluent of two different types of WWTPs as a dual-purpose solution for MPs mitigation and biomass production. For that purpose, the occurrence of MPs from two types of WWTP effluents was analysed over one year. MPs were characterized in terms of morphology (microbead, foam, granule, irregular, filament and film), colour and size. The wastewater characterisation was followed by the removal of MP loads, using native microalgae consortia, pre-adapted to the wastewater effluent. Microalgae consortia evolved naturally through four mitigation assays, adapted to seasonal conditions, such as temperature, photoperiod, and wastewater composition. MPs were present in all the effluent samples, ranging from 52 to 233 MP L^{-1}. The characterisation of MPs indicated a predominance of white and transparent particles, with irregular and filament shapes, mainly under 500 μm in size. The μFTIR analysis revealed that 43% of the selected particles were plastic, with a prevalence of polypropylene (PP) (34%) and polyethylene terephthalate (PET) (30 %). In the mitigation experiments, substantial biomass production was achieved (maximum of 2.6 g L^{-1} (d.w.)), with successful removal of MPs, ranging from 31 ± 25% to 82 ± 13%. These results show that microalgae growth in wastewater effluents efficiently promotes the removal of MPs, reducing this source of contamination in the aquatic environment, while generating valuable biomass. Additionally, the strategy employed, requires minimal control of culture conditions, simplifying the integration of these systems in real-world WWTP facilities for improved wastewater management.

1. Introduction

The escalating global issue of plastic pollution poses a threat to marine and freshwater ecosystems, potentially impacting both wildlife and human health (Hu et al., 2019; Rodrigues et al., 2020; Liu et al., 2022). Microplastics (MPs) are plastic particles with less than 5 mm, originated from the degradation of macro plastic materials (secondary MPs), or fabricated intentionally with small dimensions for diverse purposes, such as incorporated in a variety of personal care products, cleaning agents, and paints (primary MPs) (Hartmann et al., 2019). More than 390 million metric tons of plastic material are produced every year globally, with an estimated annual increase of 4% (Adekomaya and Majozi, 2020). Consequently, anthropogenic activities and the wrong management of plastic waste raise the quantity of MP particles that are introduced and persist in the aquatic environment (Bayo et al., 2021; Constant et al., 2020). MP particles are very heterogeneous, and there are no standardized methods for their detection, quantification, and monitoring in environmental matrixes (Blair et al., 2019). Furthermore, MPs can also absorb other toxic chemicals on their surface, due to their hydrophobic nature, being a vector of these contaminants to the aquatic environment (O'Donovan et al., 2020; Joo et al., 2021). Due to their inert nature, and consequent low rates of degradation, MPs persist in the aquatic environment for a long time (Pequeno et al., 2021). Therefore, identifying potential sources of MPs and solutions for their removal from the aquatic environment is of great importance (Tagg et al., 2020).

Wastewater Treatment Plants (WWTPs) are a significant pathway for

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

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https://doi.org/10.1016/j.envpol.2024.123931
Received 2 January 2024; Received in revised form 1 April 2024; Accepted 4 April 2024
Available online 4 April 2024

the introduction of MPs into the marine environment (Sun et al., 2019; Sol et al., 2021; Do et al., 2022). Plastic particles enter the wastewater systems through the disposal of household and personal care products (PCPs) and of the synthetic laundry wash that generates large amounts of plastic fibres, estimated to represent approximately 35% of all the MPs released into the aquatic environment (Boucher and Friot, 2017; Prata et al., 2020; Sol et al., 2021). Despite the existence of several data regarding MP levels in WWTPs, the lack of methodological standardization for the identification and quantification of MPs makes meaningful comparison of data elusive, and the real scenario of MPs load on wastewater effluents and their impact is still unclear (Sun et al., 2019; Liu et al., 2022; Blair et al., 2019). MP particles are very heterogeneous, and many efforts have been made to develop optimized protocols for MP extractions from the different matrices. Chemical digestion methods, including oxidative treatments with hydrogen peroxide (H_2O_2) or potassium hydroxide (KOH), are commonly employed to dissolve organic matter and release MPs for subsequent analysis (Gao et al., 2023; Prata et al., 2020). These are usually complemented by density separation techniques, such as sedimentation and floatation, commonly used to isolate MPs from organic and inorganic particles in wastewater samples (Blair et al., 2019). Most of the data of MPs load in WWTPs available to date, is based on visual sorting and identification by microscopy, combined in some cases with identification by μFTIR spectroscopy to confirm the nature of the microparticles (Conley et al., 2019; Tagg et al., 2020). The load of MPs in WWTP effluents detected in different parts of the globe (Table 1), confirms the occurrence of relatively high levels of MPs and that they are important point sources of MPs to the marine environment (Lasee et al., 2017; Akarsu et al., 2020; Conley et al., 2019). Wagner et al. (2019) found a positive relationship between high levels of MPs in a river, and the proximity to the discharge of an urban WWTP. Tagg et al. (2020) detected the presence of 1.5 MP L^{-1} in a WWTP effluent in the East Midlands (United Kingdom), and an annual discharge range of 9×10^7 to 4×10^9 MPs particles into the environment was estimated in 12 WWTPs located in Germany (Mintenig et al., 2017). In Portugal, Prata et al. (2020) estimated the release of 1.5×10^{12} MP particles through WWTP discharges in the year 2009. Data available on WWTPs discharge loads of MPs in Portugal is scarce, and data assessing the levels of these particles in wastewater effluents is urgently needed.

Table 1
Microplastic (MP L^{-1}) in the final effluent of WWTPs from different parts of the globe.

WWTP location	Treatment process	Detection method	Microplastics MP L^{-1}	Reference
Madrid, Spain	Primary and secondary	Visual sorting; μFTIR	12.8 ± 6.3	Edo et al., 2019
South Carolina, USA	Secondary	Visual sorting	1–30	Conley et al., 2019
East Midlands, United Kingdom	Secondary	μFTIR	1.5	Tagg et al. (2020)
Netherlands	n.d.	Visual Sorting; FTIR	9–91	Leslie et al., 2017
Mikkeli, Finland	Primary and secondary	Visual sorting; FTIR; μRaman	0.1–3	Lares et al., 2018
Denmark (10 WWTPs)	Secondary (9 WWTPs) Tertiary (1 WWTP)	FPA-FTIR	19–447	Simon et al., 2018
Lower Saxony, Germany (12 WWTPs)	Secondary (8WWTPs) Tertiary (4 WWTPs)	FPA-FTIR	0.1–10.5	Mintenig et al., 2017
Danang, Vietnam	Secondary	Visual sorting; μFTIR	138–340	Do et al. (2022)
Sidney, Australia	Primary (WWTP.A) Secondary (WWTP.B) Tertiary (WWTP.C)	Visual sorting; μFTIR	0.28–1.54	Ziajahromi et al. (2017)
Denisli, Turkey	Secondary	Visual sorting; μFTIR	140	Koyuncuoğlu and Erden (2023)
Republic of Korea	Tertiary	Visual sorting	297	Hidayaturrahman and Lee (2019)

n.d. - not defined.

Considering the input of MPs in the marine environment through WWTPs, there is an urgent need for novel solutions that could help to mitigate this issue. Existing methods to remove MPs in WWTPs are based on membrane filtration techniques, that require large amount of energy, or the use of chemicals to induce flocculation, which creates another problem of water contamination (Cheng and Wang, 2022). The use of microalgae has been explored regarding their potential for wastewater treatment, showing high efficiency in removing common compounds such as nitrogen and phosphorous (Díaz et al., 2022; Maryjoseph and Ketheesan, 2020). More recently, the use of microalgae to remove other types of contaminants, such as pharmaceutical compounds, metals, microplastics and nanoplastics has begun to be explored (Maryjoseph and Ketheesan, 2020; Satya et al., 2023). Cheng and Wang (2022) reported the removal of up to 84% of MPs by the microalga *Scenedesmus abundans*, where hetero aggregation between microplastic particles and microalgae cells was suggested to be the main mechanism responsible for the removal of 70% of MPs. Lagarde et al. (2016), also reported hetero aggregation between microalgae and MPs, in which aggregates were formed when microalgae were exposed to MPs in suspension and composed of 50% microplastics. Furthermore, microalgae growth generates biomass that can be integrated in a biorefinery concept, serving as a valuable feedstock for bioenergy production, and addressing the pressing demand for renewable energy sources (Abomohra and Hanelt, 2022; Díaz et al., 2022). The costs associated with biomass cultivation and its conversion into biofuels pose a major challenge to establish industrial-scale production facilities. The use of wastewater for microalgal cultivation can be a solution to decrease biofuel production costs, improving the sustainability and economic viability of the process, and addressing two environmental challenges simultaneously: reducing marine pollution and energy demand (Abomohra and Hanelt, 2022).

Traditionally, monocultures of microalgae species were employed for wastewater treatment with biomass production. These are difficult to maintain, demanding a high control of the cultivation system to avoid the growth of unwanted microorganisms, and need a tight control on growth conditions, such as temperature, photoperiod, aeration, and pH (Gururani et al., 2022). To overcome these problems, an alternative approach is being explored, where microalgae consortia or microalgae-bacteria cultures are used. In this context, two different strategies are commonly applied: artificially obtained consortia or native microalgae consortia comprising diverse and naturally occurring microalgae species, bacteria, yeasts, and fungi (Foladori et al., 2018; Gururani et al., 2022). Considering the native microalgae consortia, these microorganisms are well adapted to the wastewater environment and the symbiotic interactions that occur between them can positively affect microalgal growth improving biomass production (Abomohra and Hanelt, 2022). By integrating diverse microorganisms into metabolic processes, a robust biological system is formed, capable of functioning effectively across different nutrients and environmental conditions, which poses significant advantages over monocultures, potentially decreasing biomass production costs (Foladori et al., 2018; Gururani et al., 2022). Additionally, these interactions can generate biomass aggregates, mainly improved by the excretion of polysaccharides by bacteria and filamentous fungi. These aggregates are more easily harvested compared to cells in suspension, improving the harvesting process and helping to reduce biomass production costs (Lagarde et al., 2016; Gururani et al., 2022). To this date, the potential of microalgae for MPs removal is still unexplored, and available data is very scarce (Abomohra and Hanelt, 2022; Cheng and Wang, 2022). The existing reports are principally focused on monocultures or artificial microalgae-bacteria cultures and addresses specific types of plastic, that are intentionally chosen and added to cultures for the evaluation of removal efficiencies (Cheng and Wang, 2022).

In the present study, composite wastewater samples were collected from two different types of wastewater treatment plants (WWTPs), located in the South of Portugal, where one of them discharges its effluent into a natural reserve (Ria Formosa lagoon), and the other into the Atlantic Ocean. Composite wastewater samples were collected seasonally over a year. Genuine loads and plastic types present in the wastewater were assessed and treated using native microalgae consortia, aiming to explore their potential for removing MPs. The load and characteristics of MPs present in the WWTPs effluents were assessed, before and after microalgal growth, along with the impact of wastewater composition. This work provides a comprehensive study of the content and characteristics of MPs in wastewater effluents while presenting a viable sustainable solution to reduce MPs input from WWTPs into the marine environment.

2. Materials and methods

2.1. wastewater collection and characterization

Two distinct WWTPs were selected for this study. WWTP-A is in a touristic area, with important seasonal variation. This WWTP was designed to treat a maximum of 31,537 m^3 day^{-1}, with a current flow rate of 10,000 m^3 day^{-1}. The system consists of a biological treatment with two parallel lines, each able to treat 50% of the flow. The first line comprises a trickling filter system, designed to operate only during peak periods. The second line consists of an activated sludge system, which ensures the removal of organic matter, nitrogen, and phosphorus. After treatment, the effluent undergoes UV disinfection before being discharged into an artificial maturation lagoon, which has a residence time of approximately 48 h before being discharged into the marine environment. WWTP-B has a maximum capacity to treat 26,712 m^3 day^{-1}, with a current flow rate of 4000 m^3 day^{-1}, and its system relies on a biological treatment process using activated sludge, operating under extended aeration conditions, in two biological reactors configured as oxidation ditches with surface aerators. After secondary treatment, the wastewater undergoes UV disinfection before being discharged into the receiving waterbody. Four sampling campaigns were carried out over one year (Winter, Spring, Summer, and Autumn). In each campaign, 24h-flow-weighted composite wastewater samples (208 mL collected hourly) were collected into 5L glass jars before the maturation lagoon in WWTP-A (WWTP-A.1), and after the disinfection step in WWTP-B. After the maturation lagoon (WWTP-A.2), grab samples were collected.

2.2. Experimental design

Four mitigation experiments were performed over a year: Winter, Spring, Summer, and Autumn. In each experiment, microalgal growth was carried out in the three wastewater samples: WWTP-A.1, WWTP-A.2 and WWTP-B. In the Winter mitigation experiment, each wastewater sample was inoculated with previously established, native microalgae consortia, composed of naturally present microalgae and microorganisms, pre-adapted to the wastewater environment. In the Spring experiment, the cultures were inoculated with the consortia obtained at the end of the Winter experiment. The Summer samples were inoculated with the consortia obtained at the end of the Spring experiment, and finally, in the Autumn experiment, the cultures were inoculated with the consortia obtained at the end of the Summer experiment. Each condition was performed in triplicate. Cultures were maintained under controlled temperature and photoperiod, adapted according to the season of the year: 15 ± 1 °C and 8/16h (light/dark) in Winter; 18 ± 1 °C and 10/14 (light/dark) in Spring and Autumn, and 25 ± 1 °C and 12/12h (light/dark) in Summer. This strategy intended to replicate the natural evolution of native microalgae consortia throughout different growth cycles when subjected to real variations scenarios in the wastewater composition and environmental conditions. Aeration was kept constant. Microalgal growth was performed in fed-batch mode, with 2 L of initial volume, and 30% of the culture was replaced with fresh wastewater after 15 days of growth. After 30 days of growth, biomass was harvested by centrifugation in an ultracentrifuge (Gyrozene 2236R High-speed ultra-centrifuge, Korea), at 14,000 rpm, for 20 min at 4 °C. The biomass was preserved for further microbial composition analysis, and the supernatant was acidified to pH 2, to prevent microbial growth, and kept at 4 °C for MP extraction and quantification. To validate the impact of microalgae on MP removal, a control experiment was conducted where wastewater lacking microalgae growth underwent the same centrifugation procedure, and the number of microplastics was counted before and after centrifugation.

2.3. Mitigation assays

Growth parameters such as pH, dissolved oxygen (%) and conductivity were regularly measured (data not shown). Microalgal growth was monitored by collecting a 50 mL sample of culture every three days, for dry weight (d.w.) determination and nutrient quantification. Dry weight was determined by filtering 10 mL of the cell suspension through a pre-weighted 1.2 μm fibreglass filter (Whatman, 934-AH), followed by incubation of the filters for 1 h at 103 °C (Venti-line 56-Prime, VWR). After cooling the filters were weighed. The d.w. was determined by calculating the difference between the weight of the filter with and without dry biomass.

2.4. Microplastic extraction and characterisation

The wastewater discharged from the two WWTPs meets strict criteria for wastewater quality to minimize its impact on the environment. Considering that the organic matter and mineral particles present in the wastewater samples are the main constraints during microplastic extraction, to evaluate the right methodology for MP extraction, the wastewater was characterized in terms of its content of organic matter (measured as Chemical Oxygen Demand, COD) and total suspended solids (TSS). Low content of organic matter, (COD ranged from 32.0 ± 2.8 to 85.9 ± 0.4 mg O_2 L^{-1}), and TSS, ranging from 10.7 ± 0.9 to 314.7 ± 51.9 mg L^{-1} were detected (Table S1, supplementary material). The tests performed to recover MPs from these matrices revealed that there was no need for the steps of density separation and the Fenton reaction (data not shown), as suggested by other researchers for wastewater matrices (Lares et al., 2018). Briefly, MPs were extracted from wastewater samples, in triplicate, with and without the steps of Fenton reaction and density separation. It was observed that the number of MPs did not show significant differences when the Fenton reaction and density separation were not used, therefore, the experiments were further carried out using only oxygen peroxide (H_2O_2) digestion.

Microplastics were extracted as described by Cassola et al. (2017), with slight modifications. Briefly, 500 mL of wastewater samples were filtered through a 5 μm nitrocellulose filter (Whatman), in triplicate. The retained fraction, containing the MPs and organic matter, was digested with 50 mL of H_2O_2 at 30% (v/v) (H_2O_2, 30%, VWR) for 1 h at 60 °C. After digestion, the filter was rinsed with ultra-pure water and carefully removed from the glass flask, making sure that all the particles fell into the solution. 50% of H_2O_2 (30% v/v) was added to the remaining solution and left for 48 h at room temperature. The solution was then filtered through a 5 μm nitrocellulose filter (Whatman) and analysed under a microscope for visual detection of the number, size, shape and colour of the microplastics present. Validation of the extraction protocol was assessed by calculating the recovery rates after spiking MilliQ water with known concentrations of PE (125–500 μm), LDPE (125–500 μm), PVC (300 μm), purchased from micro-Powders, inc. (NY-USA) and obtained for the EPHEMARE project, and wastewater with a weathered PVC sample collected from the environment, that was previously washed, dried, and macerated to obtain particles <1 mm. 50 particles of each purchased polymer were carefully counted under the microscope on a black surface and added to 500 mL of MiliQ water, and 10 particles of the PVC to 1 L of wastewater. The extraction of MPs was conducted as described for wastewater samples. Recovery rates for the tested microplastics are depicted in Table S2 from the supplementary materials section.

This protocol was established based on a series of tests conducted to enhance the digestion of organic matter on filters while minimizing filter damage. In brief, three types of filters were examined: fiberglass (Whatman 934-AH), cellulose (Whatman), and nitrocellulose. Various temperatures (25, 50, 60, and 70 °C) and exposure times (7, 5, 2, 1 h) were tested, alongside different concentrations of H_2O_2 (15% and 30% (v/v)). Initial experiments on fibreglass filters (as suggested by Prata et al., 2019) with 15% H_2O_2 (v/v) revealed significant filter degradation and excessive fibre release into the solution, making it difficult to visually sort MPs particles. In contrast, nitrocellulose filters exhibited high resistance to H_2O_2 digestion, even under high temperatures (60 and 70 °C), and with 30% H_2O_2 (v/v), resulting in minimal fibre release. Optimal conditions were determined as follows: nitrocellulose filters, H_2O_2 at 30% (v/v), digestion for 1 h at 60 °C, suitable for wastewater with low organic matter content (COD ranging from 35 to 90 mg O_2 L^{-1}).

The visual sorting of microplastics was carried out considering the approaches described by Akarsu et al. (2020) and Conley et al. (2019). Particle visualisation was carried out under a microscope (Olympus CH-2, CHT) with 5x, 10x and 40x magnifications. The fragments sorted and counted as microplastics were exposed to KOH (10% w/v) or fragmented with a hot needle tip, to confirm the plastic behaviour. The exposure to KOH serves to digest the remaining organic particles. Additionally, when this procedure is conducted on the filter under a microscope, any potential degradation of particles can be observed. In such instances, if a particle shows signs of degradation, it is not counted as plastic. The needle test is particularly valuable for assessing fibre particles, facilitating the differentiation between cellulose fibres and plastic fibres. Cellulose fibres typically degrade instantly upon contact with the needle, whereas plastic fibres exhibit different behaviour, like curling or melting. The particles were counted based on the characteristics described by Hidalgo-Ruz et al. (2012), Fries et al. (2013), Akarsu et al., 2020, Conley et al. (2019) and Kay et al. (2018). The type of particles examined and selected under the microscope were categorized into six different morphologies: film, granule, filament (fibers), foam (particles with a spongy constitution), irregular (fragment) and microbeads (Li et al., 2018; Constant et al., 2019). Moreover, the colour and size were determined. The filters visualized under the microscope with identified particles were photographed and the size of the particles was determined by using the ImageJ FIJI software.

μFTIR analysis was utilized to determine the plastic composition of 109 randomly selected plastic particles within each morphology category. Microplastic particles were examined under a Nicolet iN10MX μFTIR instrument (Thermo Fisher Scientific). Spectral data was collected in the middle infrared range, covering from 4000 to 675 cm^{-1}. The spectra obtained was analysed in the OMNIC 9 software and compared against several databases with plastic and non-plastic materials. All the spectra with a similarity match higher than 70% were considered valid for identification. Only two particles (identified as rayon) had a match below 70% (55% and 69%) but were considered identifiable due to the high similarity between the main peaks of the spectra. The total number of MPs counted by optical microscopy was adjusted accordingly based on the percentage of plastic particles detected from the total number of the visually selected particles (Blair et al., 2019).

2.5. Quality control

To avoid external contamination, all the microplastic extraction and analysis steps were performed with glassware material, and inside a small clean room, where only MP samples were handled. All the materials used during the extraction steps were carefully rinsed with hot water and immediately covered with aluminium foil until use. All the reagents and Ultra-pure water (MilliQ) used were previously filtered through a 0.2 μm glass-fibre filter (Whatman). To minimize the overestimation of MPs quantities, external contamination was assessed by blank extractions, where Ultra-Pure water was subjected to the same protocol as the wastewater and any plastic particles found in the control filters were discounted in the total particle number.

2.6. Statistical analysis

All statistical analyses were performed using GraphPad Prisma (version 9.5.0) Software. Data was tested for normality, which was confirmed by QQ plots. Shapiro-Wilk test was performed to test for variance homogeneity and data distribution. Two-way ANOVA Tukey's posthoc multiple comparison tests were used to assess the significance of the differences between the data, with a confidence interval of 95% ($p <$ 0.05).

3. Results and discussion

3.1. MPs seasonal load

The number of MPs in the three wastewater plant effluents investigated over the course of a year is present in Fig. 1. The abundance of MPs ranged from 70 ± 29 to 163 ± 64 MP L^{-1} in WWTP-A.1, 52 ± 19 to 189 ± 26 MP L^{-1} in WWTP-A.2 and 168 ± 16 to 233 ± 19 MP L^{-1} in WWTP-B. These levels are higher than most of the data reported in final effluents of WWTPs worldwide (ranging from 0 to 13 MP L^{-1}) (Table 1) (Akarsu et al., 2020; Mason et al., 2016). Nonetheless, they are consistent with the data reported by Do et al. (2022) for three different WWTPs with secondary treatments, in Danang (Vietnam), where the MPs load ranged from 138 to 340 MP L^{-1}. Similarly, Leslie et al. (2017), reported loads of seven different WWTP effluents in the Netherlands, ranging from 9 to 91 MP L^{-1} and Simon et al. (2018), found between 19 and 447 MP L^{-1} in 10 different WWTP effluents in Denmark. Numerous factors contribute to the variability of MP loads across different studies. These

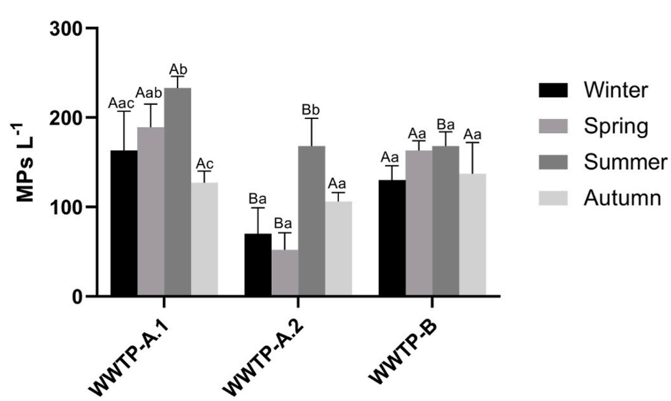

Fig. 1. MPs content (mean ± S.D.) in the effluent of WWTP-A before (WWTP-A.1) and after (WWTP-A.2) the maturation lagoon, and in the WWTP-B effluent. Different upper-case letters denote statistically significant differences between WWTP effluents in the same season, and different lower-case letters denote statistically significant differences between the seasons in the same WWTP sampling point (n = 9, $p <$ 0.05).

include variations in WWTP treatment processes, encompassing primary, secondary, or tertiary treatment steps, as well as the capacity of the facilities and the population size influencing the volume of treated wastewater. Cultural habits and living standards of different populations certainly also influence MP loads. Furthermore, differences in methodologies applied for MP extraction from wastewater sampling, processing, and MP analysis, further contribute to these variations.

In WWTP-A before the maturation lagoon (WWTP-A.1), significant differences were observed in the MPs load in the sample collected during the Summer when compared to the Autumn and Winter seasons ($p < 0.05$). The sample collected in Spring was the only one that was not significantly different from that in the Summer ($p > 0.05$). There were also no significant differences between Winter and Spring and between Winter and Autumn ($p > 0.05$), but there were significant differences between Spring and Autumn ($p < 0.05$). In general, in WWTP-A.1 and WWTP-A.2, the load of MPs was significantly higher in the Summer ($p < 0.05$), and the lowest load was in the Spring in WWTP-A.2. However, like in WWTP-A.1, there are no significant differences in the MPs levels in WWTP-A.2 between Winter, Spring and Autumn ($p > 0.05$). Comparing the two sites before (WWTP-A.1) and after (WWTP-A.2) the maturation lagoon, it is interesting to note a tendency for lower MP levels on WWTP-A.2, (only in the Autumn the differences were not significant ($p > 0.05$)), which is the final effluent of this WWTP, indicating retention of MP particles in the lagoon system. The variation between the MP loads before and after the lagoon (WWTP-A.1 and WWTP-A.2) can be attributed to the effect of an uneven distribution of MPs along the maturation lagoon, where the residence time of the wastewater is approximately 48 h before reaching the point of discharge, and to some retention of MPs in the maturation lagoon of the WWTP. During this period, MPs can settle in the sediments on the bottom of the lagoon, a process which is certainly influenced by MPs density, the prevailing water circulation and temperature patterns.

In the WWTP-B, the highest load was also during Summer (168 ± 16 MP L^{-1}), yet no significant differences were observed between the four samples (Winter, Spring, Summer and Autumn) ($p > 0.05$).

Concerning the two WWTPs final effluents, WWTP-A.2 and WWTP-B, the Winter and Spring samples differed significantly ($p > 0.05$). The number of MPs in the WWTP-B effluent was significantly higher than WWTP-A.2 (WWTP-B: 130 ± 16, Winter and 163 ± 11, Spring; WWP-A.2: 70 ± 29, Winter and 52 ± 19, Spring). However, comparing the number of MPs within WWTP-A.1 and within WWTP-B, all collected after disinfection steps, levels were similar at all seasons except for Summer, where higher loads were found in WWTP-A.1. This disparity could potentially be attributed to a population increase during the touristic season. It is reasonable to assume that the higher influx of tourists during this period contributes to an elevated production and disposal of wastewater flow leading to an increase of MPs. The monthly flow rates of both WWTPs supports this assumption. In WWTP-B the flow rate has lower variation along the year, varying between 104×10^3 m^3 in February and 175×10^3 m^3 in December. In contrast, in WWTP-A the flow more than duplicates during the Summer season (June, July, August and September), varying between 188×10^3 m^3 in February and 441×10^3 m^3 in August (Table S3, supplementary material) (data provided by the local infrastructure managing company). Notably, the seasonal variation was also evident in WWTP-A.2, with the number of MP being significantly higher during the Summer compared to the other three seasons ($p < 0.05$). During Summer, not only the number of MPs influent to the WWTP-A is expected to be higher, but also the higher flow rate poses additional stress on the efficiency of the removal within the WWTP, leading to significantly higher MP levels on the discharged waters in Summer ($p < 0.05$). In contrast, the number of MPs in the effluent of WWTP-B displayed no significant seasonal variation ($p > 0.05$), indicating a relatively lower impact from population variations during the Summer season. These findings further support the hypothesis of a direct relationship between the increase in the population during the touristic season and increased MP levels in the treated wastewater. Similar findings were obtained by Franco et al. (2023), who reported a significant increase of MPs during the Summer season, in the effluent of a WWTP located in a resort area, while no differences were detected in the effluent of a second WWTP situated in an urban area, both WWTPs sited in the Bay of Cadiz (Spain). Overall, these results highlight the complex dynamics influencing the occurrence of MPs in the effluents of wastewater treatment plants. While different treatment steps and daily volumes of treated wastewater contribute to the overall variability in MP levels, population variation, particularly during the touristic season, can significantly influence the levels of microplastic contamination in the aquatic environment.

3.2. MPs characterization

The MPs detected throughout the four seasons were characterized based on their morphology, colour, and size. Fig. 2 illustrates some examples of the different morphology of MPs collected over the course of the year. Fig. 3 illustrates the average distribution of types, shapes, colour and size of MPs detected in each WWTP during the four sampling campaigns (Winter, Spring, Summer, and Autumn). In total, six different shapes (granule, microbead, film, foam, filament, and irregular) were present in all WWTPs effluents. This is consistent with the findings reported by Bayo et al. (2020b) and Ziajahromi et al. (2017), who used similar morphological classifications and detected the presence of these shapes in WWTP effluents with secondary treatment processes, located in Cartagena (Spain) and Sydney (Australia).

The relative abundance of each MP morphology was similar in all three WWTP effluents (Fig. 3A). The irregular and filament shapes were the most abundant particles in all the effluents. In WWTP-A.1, the most abundant MPs were characterized by an irregular morphology, accounting for 31.1 ± 8.1% of the particles, followed by granules (16.9 ± 8.1%). In WWTP-A.2, 30.4 ± 11.4% of the particles were filaments and 22.4 ± 8.5 % were irregular, while in WWTP-B, 34.7 ± 8.1% were irregular, followed by filaments (19.3 ± 7.8%). Most of the available data on MP loads in WWTP effluents indicate that filaments (fibres) are the most abundant shape in WWTPs (Blair et al., 2019; Ziajahromi et al., 2017; Lares et al., 2018; Mintenig et al., 2017). However, in the present case, the number of MPs with irregular shape slightly surpassed that of the filaments, which is consistent with the data reported by Carr et al. (2016), where 90% of MP particles in a secondary treatment WWTP effluent were also irregularly shaped. Nonetheless, Bayo et al. (2020b) divided the shapes into five different categories: fragments, foams, beads, films and filaments, and found the fragment shape to be the most abundant (46%) in the effluent of a secondary treatment WWTP located in Cartagena (Spain), which is similar to the present data. Likewise, the same tendency was observed in Danang (Vietnam) and in Minikel (Finland), in effluents of WWTPs secondary treatment processes, where fragments and filaments have a similar distribution (Do et al., 2022; Lares et al., 2018). Yet, the comparison between data is challenging, due, in part, to the use of different classification systems. MPs with irregular shapes are mainly secondary MPs, originating from the degradation of larger plastic objects and debris, such as plastic bags, bottles, food wraps, and diverse household items, while filaments (fibres) are mainly primary MPs liberated during the washing process of textiles. Granules and microbeads are also primary MPs that are found in diverse abrasion products such as toothpaste, face scrubs, and cleansing agents (Bayo et al., 2020a; Kalčíková et al., 2017).

Regarding the colour of the MPs (Fig. 3B), transparent and white were the most abundant followed by dark. Similar results were obtained by Koyuncuoğlu and Erden (2023) in Denisli (Turkey), Edo et al. (2019) in Madrid (Spain) and Mintenig et al. (2017) in Lower Saxony (Germany), who reported the predominance of transparent and white MPs in wastewater effluents from secondary treatment WWTPs. DO et al. (2022) found dark to be the predominant colour, in a secondary treatment WWTP in Vietnam. Additionally, blue, red and yellow were also present in all of the samples. Green, pink, orange and purple were the

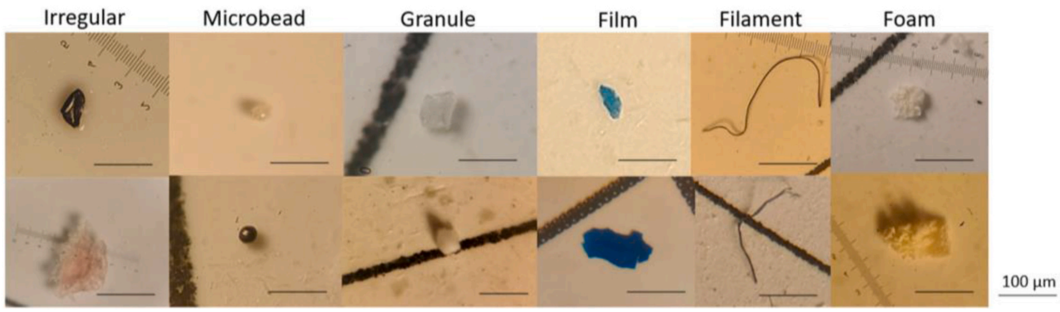

Fig. 2. Example of each morphology of MPs visually sorted under the microscope: Irregular, microbead, granule, film, filament, and foam.

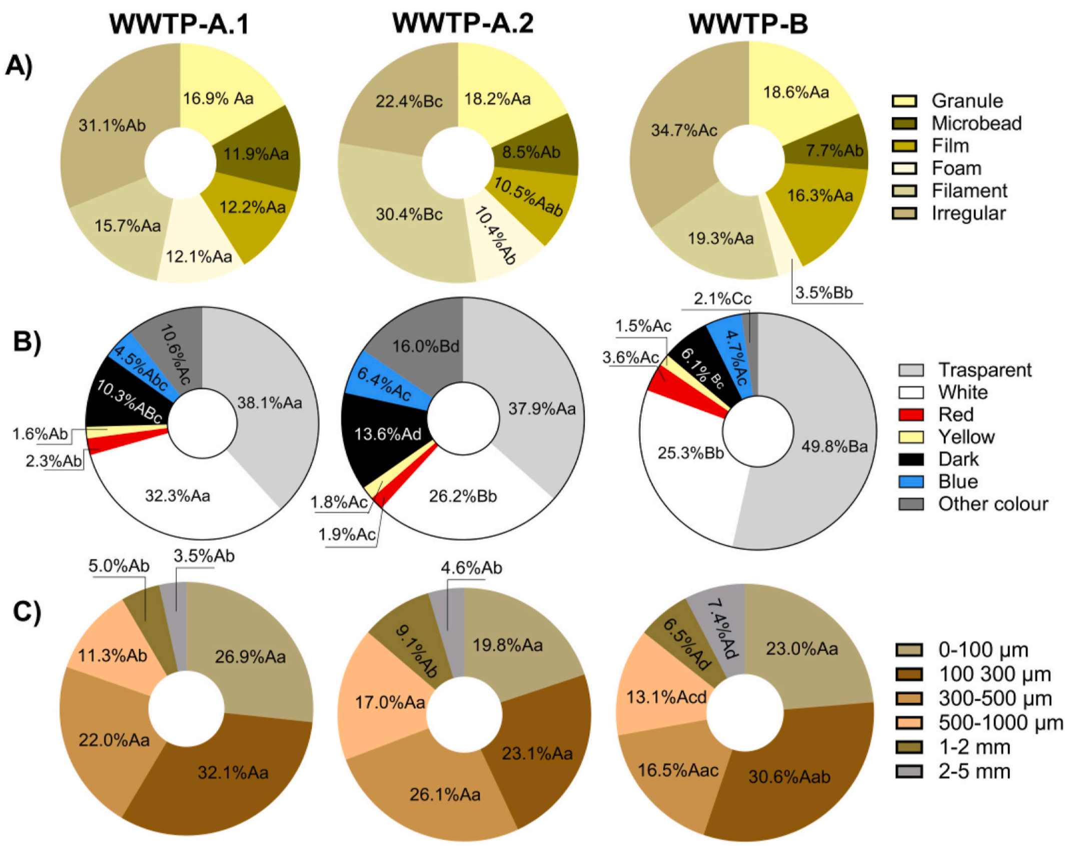

Fig. 3. Average distribution MPs morphology, colour and size for each sampling site: WWTP-A.1; WWTP-A.2 and WWTP-B. Within each category, different upper-case letters denote significant differences between the same type of MP in the WWTPs, and different lower-case letters denote significant differences between each type of MP in the same WWTP. (n = 3; $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

most detected among the "other colours" category. This variety of colours indicates a diversity of the sources that originate MPs that end up in the WWTPs. The same variety of colours was also found in other WWTPs' final effluents, with primary, secondary and tertiary treatments, located in Lower Saxony (Germany) (Mintenig et al., 2017), Cartagena (Spain) (Bayo et al., 2020a; Bayo et al., 2020b), Madrid (Spain) (Edo et al., 2019) and Marsin bay (Turkey) (Akarsu et al., 2020). The prevalence of transparent and white MPs may reflect a predominance in the manufacture of plastic items featuring these colours, which can be attributed to several factors. Most common polymers, naturally exhibit a white or transparent appearance. Furthermore, microbeads and granules that are present in personal care and cosmetic products (face cleansers, body exfoliants and toothpastes), are predominantly made of white or opaque polyethene (Liu et al., 2022). Additionally, transparent, and white plastics are primarily utilized in the production of short-lived disposable items, while coloured plastics are more commonly employed in the manufacture of long-lasting products (Zhang et al., 2018). Any colour of MPs can have a negative impact on the environment. For instance, Zhao et al. (2022) reported an increase in photodegradation in coloured plastics, which accelerates the degradation of macroplastics, increasing the number of MPs in the environment. Additionally, coloured MPs are more easily mistaken for food by aquatic organisms, facilitating their ingestion and entry into the food chain with potential negative effects on living organisms (Zhao et al., 2022; Zhang et al., 2020).

Regarding the size of MPs, they ranged from 0 μm to 5 mm (Fig. 3C). Among these size ranges, MPs between 0 and 500 μm were the most abundant, accounting for 81% of the total MPs in WWTP-A.1, 69% in WWTP-A.2 and 70% in WWTP-B. MPs between 500 μm and 1 mm represent 11% in WWTP-A.1, 17% in WWTP-A.2 and 13% in WWTP-B.

Larger particles, between 1 and 5 mm, were less frequent (8.5% in WWTP-A.1; 14% in WWTP-A.2; 14% in WWTP-B). These results were in line with the data reported by Mintenig et al. (2017) from 12 WWTPs with secondary and tertiary treatments, supporting the notion that the number of MPs increases as their size decreases (Prata et al., 2020; Mintenig et al., 2017; Simon et al., 2018) Similarly, Do et al. (2022), only detected the presence of MPs within a size range between 500 and 5000 μm at the influents, being absent at the effluents of three WWTPs with secondary treatment processes. This author suggests that the bigger particles tend to break down during the wastewater treatment process, increasing the number of smaller particles in the effluent. However, it is also important to note that comparisons between the available data are difficult, as different studies address specific size ranges, depending on the method chosen for particle analysis (Fries et al., 2013).

Plastic identification of randomly selected particles, by μFTIR analysis, revealed that 43% of the particles were confirmed to be plastic, while 47% were composed of cellulose, minerals and salts, and 10% of the particles were impossible to identify. Notably, these non-plastic particles (57%) can be easily misidentified as microplastics, leading to overestimation when relying solely on visual sorting. These findings are in line with those reported by Gies et al. (2018) and Bayo et al. (2020b), who showed that 32% and 63%, respectively, out of the total selected particles, were plastic. Fig. 4 illustrates two examples of μFTIR spectra used to identify the most abundant MPs detected: polyethylene terephthalate (PET) and polypropylene (PP).

Fig. 5 depicts the relative abundance of eight different plastic materials that were identified, where the most abundant were PP (34%), and PET (30%), followed by rayon (21%). PET MPs were predominantly in the form of filaments, while PP particles exhibited irregular and granular shapes. The other plastic types identified were polyvinyl acetate (PVA), ethylene–vinyl acetate (EVA), polynorbornene (PNB), EPON 1004, and acrylic. These results are slightly different from other reports, where polyethylene (PE) is typically the most abundant plastic type, and polystyrene (PS) is commonly present in most samples (Mintenig et al., 2017; Lares et al., 2018); Nevertheless, the prevalence of PP in the wastewater is expected, as it is the second most extensively used plastic polymer in the world, incorporating all kinds of different plastic products, such as packaging materials (i.e. Food packaging, cleaning agents, bottles, first-aids packages, and others), houseware materials (mats, carpets, rugs, brooms and brushes, and others), long-lasting toys, outdoor furniture and many industrial applications including the automotive industry (Mannheim and Simenfalvi, 2020; Zheng et al., 2023). On the other hand, PET, the second most abundant MP detected in the present samples, is also used in most everyday plastic products, such as packaging for food and water, household and personal care products

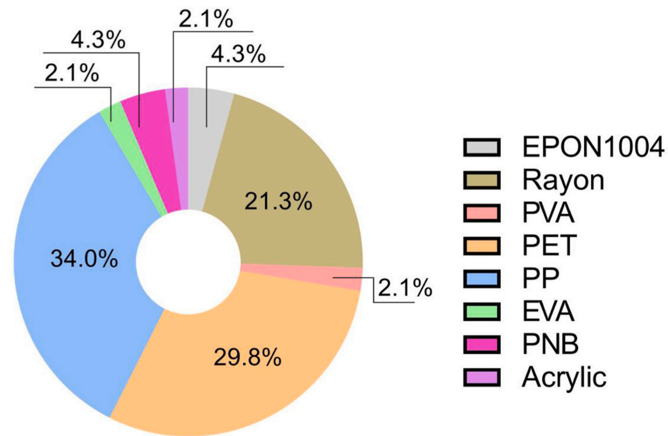

Fig. 5. Relative abundance of the different types of MPs identified by μFTIR analysis.

such as face-scrubs and textiles. Furthermore, PET fibres represent about 50% of the global market of synthetic fibres, and are used along with rayon, cellulose and cotton in clothes manufacture (Park and Kim, 2014). Many of these fibres are released during the washing process of clothes, and end up entering the WWTPs systems, which can explain the present results, with the prevalence of PET fibres in the wastewater effluents. The third most detected polymer type was rayon, a semi-synthetic fibre produced from cellulose, that is not biodegradable, prevailing in the environment with low degradation rates (Woodall et al., 2014). These fibres, are largely used in the textile industry and are also released during the washing of clothes, passing through WWTPs with little removal efficiency. The predominance of the PP polymer, and rayon fibres found in wastewaters is in line with the findings reported in coastal sediments from Southern Portuguese shelf waters, near the location of these WWTPs effluents. Frias et al. (2016) found a predominance of rayon fibres (84%) and PP (16%) and did not find any PE or PS particles. On the other hand, Vital et al. (2021) reported the prevalence of PE, followed by PP particles, in the tissues of marine mussels collected along the South coast of Portugal, where these effluents are discharged.

The other polymers detected such as EVA, PVA, PNB and acrylic, have also been reported to be present in WWTPs effluents and in the marine environment (Franco et al., 2023; Do et al., 2022; Mintenig et al., 2017; Lares et al., 2018). PVA is a water-soluble polymer, that has emerged as an alternative to conventional plastic polymers (Sun et al., 2017). It is widely used in laundry and dish detergent pods and sheets,

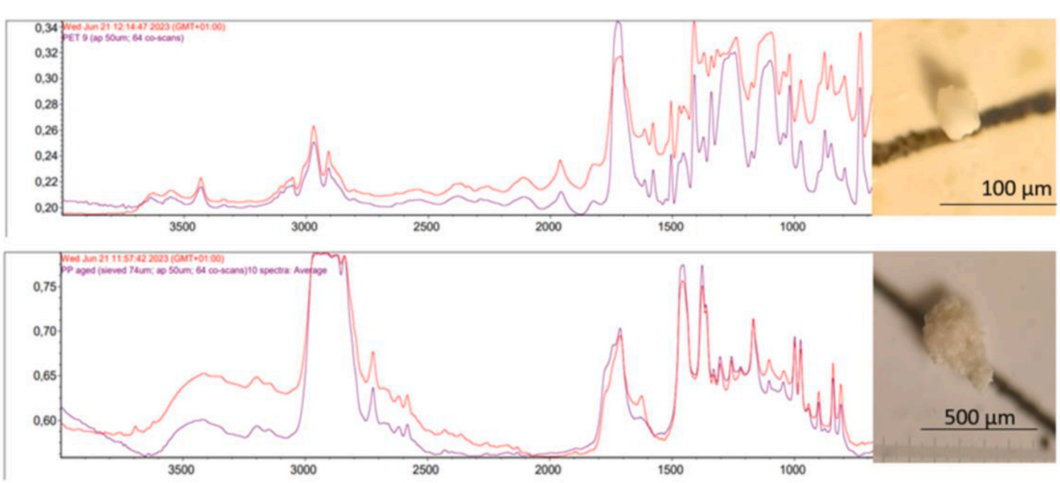

Fig. 4. μFTIR spectra of the two most abundant types of plastic polymers, a) white granule MP identified as PET, (match of 83.31 %; b) White foam MP identified as PP (match of 97.63 %).

hand soaps, toilet cleaner pods, and all sorts of cleaning agents, and has been recognized to be one of the most ubiquitous pollutants in wastewater (Rolsky and Kelkar, 2021). Despite being classified as a biodegradable polymer, biodegradation of PVA can only occur under specific conditions, that are generally not met in WWTPs environment (Chiellini et al., 2003; Sun et al., 2017). Rolsky and Kelkar (2021) highlighted the low degradation reporting that 65% of the PVA that enters the WWTPs and end up trapped in the sludge fraction of the wastewater treatment, and 15.5% reaches the aquatic environment through the wastewater effluent. PVA poses an additional threat to the aquatic environment, due to its potential to absorb metals and other hydrophilic contaminants (Rolsky and Kelkar, 2021).

3.3. Biomass production during mitigation experiments

Mitigation experiments were performed to evaluate the potential of native microalgae consortia to remove MP particles while producing biomass. Furthermore, one of the main goals of this work was to achieve biomass production, with minimal control over the conditions, where each consortium evolved naturally throughout the four experiments (growth cycles). Table 2 shows the biomass production of the three consortia during the four seasons assays, expressed as dry weight (d.w.). High-density cultures were obtained after 30 days of growth, reaching a maximum of 2.63 ± 0.54 g L^{-1} (d.w.) in the WWTP-A.2 consortia during the Autumn experiment. In general, the native microalgae consortia showed a tendency to increase biomass production in each growth cycle ($0.37 \pm 0.00 – 0.72 \pm 0.0$ g L^{-1} in Winter; $1.46 \pm 0.10 – 1.85 \pm 0.20$ g L^{-1} in Spring; $1.70 \pm 0.38 – 2.08 \pm 0.21$ g L^{-1} in Summer and $1.59 \pm 0.98 – 2.63 \pm 0.54$ g L^{-1} in Autumn). This observation may indicate that these consortia evolved to be adapted to the wastewater environment over the different growth cycles. Moreover, the different conditions (temperature, photoperiod and wastewater composition) of each season, directly affect microalgal and microbial growth and consequently, the consortia composition (Bhatia et al., 2022). They can have a positive or negative influence, depending on the optimal growth conditions of the species that are present in the consortia. Although the consortia compositions were not determined, it was evident by the visual analysis of the culture's appearance, during each experiment, that it changed over time. This suggests that the consortia composition evolves differently in each experiment.

Table 2
- Dry weight (g L^{-1}) of the microalgae consortia cultures (average ± standard deviation (n = 9)) during the mitigation experiments, in different wastewater effluents: WWTP-A.1, WWTP-A.2, WWTP-B, at the start of each experiment (day 0), before and after replacement of 30% of the medium (day 15 and 15.5, respectively) and at the end of each assay (day 30). Different upper-case letters denote significant differences between the WWTP effluent for the same day of culture, and lower-case letters denote significant differences between seasons for the same day of culture.

	Time (days)	Winter	Spring	Summer	Autumn
WWTP-A.1	0	0.03 ± 0.00^{Aa}	0.13 ± 0.03^{Aa}	0.06 ± 0.03^{Aa}	0.05 ± 0.02^{Aa}
	15	0.35 ± 0.07^{Aa}	0.86 ± 0.02^{Ab}	0.82 ± 0.06^{Ab}	0.66 ± 0.04^{Ab}
	15.5	0.25 ± 0.00^{Aa}	0.46 ± 0.04^{Aa}	0.34 ± 0.08^{Aa}	0.45 ± 0.04^{Aa}
	30	0.72 ± 0.00^{Aa}	1.85 ± 0.20^{Abc}	2.08 ± 0.21^{Ab}	1.59 ± 0.98^{Ab}
WWTP-A.2	0	0.03 ± 0.00^{Aa}	0.07 ± 0.02^{Aa}	0.06 ± 0.01^{Aa}	0.04 ± 0.03^{Aa}
	15	0.29 ± 0.02^{Ba}	0.67 ± 0.04^{Bb}	0.78 ± 0.06^{Ab}	0.64 ± 0.41^{Ab}
	15.5	0.21 ± 0.00^{Ba}	0.42 ± 0.03^{Aa}	0.31 ± 0.10^{Ba}	0.37 ± 0.12^{Aa}
	30	0.53 ± 0.00^{Ba}	1.78 ± 0.19^{Ab}	1.98 ± 0.15^{Ab}	2.63 ± 0.54^{Bc}
WWTP-B	0	0.03 ± 0.00^{Aa}	0.17 ± 0.01^{Aa}	0.06 ± 0.04^{Aa}	0.06 ± 0.02^{Aa}
	15	0.24 ± 0.01^{Ca}	0.76 ± 0.10^{ABb}	0.27 ± 0.06^{Ba}	0.27 ± 0.09^{Aa}
	15.5	0.17 ± 0.00^{Ca}	0.42 ± 0.03^{Ab}	0.09 ± 0.04^{Aa}	0.26 ± 0.05^{Ab}
	30	0.37 ± 0.00^{Ca}	146 ± 0.08^{Bb}	1.70 ± 0.38^{Bc}	2.48 ± 0.44^{Bd}

The quantity of biomass obtained is significantly higher especially when compared with the data of monoculture systems and synthetic microalgae-microalgae or microalgae-bacteria consortia (Wang et al., 2020; Ferro et al., 2019). Wang et al. (2020) reached 1.3 mg L^{-1} with cultures of a synthetic *Chlorella – Exiguobacterium* consortia, in piggery wastewater, and higher biomass production was obtained by Ferro et al. (2019) using a consortium of *Chlorella vulgaris – Rhizobium* sp. in synthetic domestic wastewater, reaching a growth between 0.6 and 0.84 g L^{-1}. On the other hand, Cai et al. (2019) used native microalgae consortia to treat synthetic domestic wastewater and obtained a maximum biomass production of 2.7 g L^{-1}. These results are similar to the present ones and show a tendency to improve biomass production when naturally occurring microalgae consortia are used. Overall, these results show the potential of cultivating native microalgae consortia in wastewater effluents for biomass production, with minimal control and no supplementation.

3.4. Removal of MPs by native microalgae consortia

To our knowledge, the data available to this date on the removal of MPs by microalgae is solely based on controlled experiments, where known types and quantities of MPs are intentionally added to microalgae culture to assess their potential for MPs removal (Cheng and Wang, 2022; Cunha et al., 2019). However, the present study pursued a more realistic approach, providing a pioneering insight into the potential of microalgae consortia to effectively remove real loads of MPs from wastewater samples.

After microalgae cultivation and harvesting, the treated wastewater was analysed, to assess the potential of the native microalgae consortia in removing the MPs present in the wastewater. Fig. 6 illustrates the removal efficiencies achieved in each effluent, in each season. The removal efficiencies ranged from $31.1 \pm 24.5\%$ in WWTP-A.2 during Spring, to $82.4 \pm 13.2\%$ in WWTP-B during Spring. All the removal efficiencies were significantly higher than the control, except for the WWTP-A.2 during Spring ($p < 0.05$). WWTP-A.1 and WWTP-B show similar seasonal variations, with no significant differences between seasons ($p > 0.05$). WWTP-A.2, however, shows significant differences between Summer and the other seasons ($p < 0.05$). This indicate that the consortium of WWTP-A.2 have larger seasonal variations in its

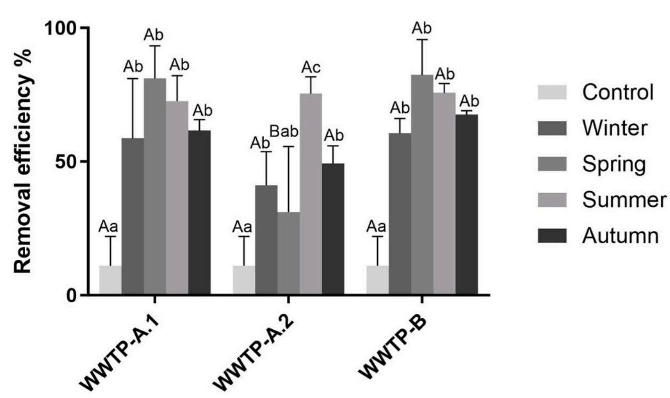

Fig. 6. Removal efficiencies of MPs by native microalgae consortia. Different lower-case letters indicate significant differences in the seasons within each sampling point, and different upper-case letters denote significant differences between the same seasons in the different sampling points (n = 9; p < 0.05).

characteristics, than the consortia of WWTP-A.1 and WWTP-B.

It is important to note, that each bar represents a different microalgae consortium, composed of different microorganisms that have an influence on the removal capacity of the cultures. For instance, cultures composed of filamentous algae and/or fungi, may potentiate the formation of aggregates, whereas a consortium with microalgae in suspension may not be so effective in aggregate formation. Visual examination of the cultures revealed that all the consortia of WWTP-A.2 displayed homogeneity, in contrast to other cultures where filamentous algae and fungi were visible and tended to aggregate, resulting in heterogeneous cultures, which was more obvious in WWTP-B cultures. Notably, the cultures derived from WWTP-A.2 exhibited a tendency for lower removal efficiencies, although significant differences were observed only on WWTP-A.2 cultures during Spring ($p < 0.05$), which displayed a significantly lower removal efficiency when compared to the other experiments. Fig. 7, shows the visual differences between WWTP-A.2 and WWTP-B Spring cultures, with significant differences in the MPs removal: 31.1 ± 24.5% in the WWTP-A.2 and 82.4 ± 13.2% in WWTP-B. The results obtained are in line with those reported by Cheng and Wang (2022), where the removal of PS, polylactide (PLA) and poly (methyl methacrylate) (PMMA) particles, by the microalgae *Scenedesmus abundans*, ranged from 31% to 98%.

Considering the size of MPs present (≈ 50 μm – 5 mm), the main mechanism by which MPs are removed is suggested to be the formation of hetero-aggregates, as reported by Cheng and Wang (2022), Cunha et al. (2019) and Lagarde et al. (2016). These aggregates consist of agglomerates composed of microorganisms, exopolysaccharides (EPS), proteins, microplastics and other organic matter that may be present in the wastewater effluent. These are formed by the electrochemical interactions between the surface of MPs and the molecules present on the cell walls, leading to the adsorption (or entrapment within the matrix) of MPs to the microorganisms, and the adhesion of the exopolysaccharides (EPS) excreted by the microorganisms to the surface of the MPs. Different factors can contribute to the enhancement of the aggregate formation, such as the roughness of the particle surface, the chemical composition of the polymer and the additives present in the MPs (Cunha et al., 2019; Lagarde et al., 2016; Cheng and Wang, 2022). The presence of filamentous fungi and algae further enhances this aggregation, where MPs end up trapped in the biomass and are consequently removed from wastewater during microalgae harvesting. Although hetero-aggregation is suggested here as the main mechanism by which microalgae remove MP particles, it is important to consider other possible mechanisms such as the biodegradation of the smaller and older particles. Therefore, more research is needed to confirm the hetero-aggregation mechanism.

Overall, this data indicates that microalgae growth in wastewater effluents presents a promising approach to mitigate microplastic discharge from wastewater treatment plants (WWTPs), effectively preventing environmental contamination. Moreover, the strategy employed, with minimal control of culture conditions and the natural development of microalgae consortia across various growth cycles, simplifies the integration and operation of such systems within real-world WWTP facilities. Nonetheless, in the present study, centrifugation was used to harvest the biomass, which could present some limitations when applied on a larger scale. This method may hardly be applicable in real-world scenarios, where gravity settling is appropriate. However, in this case, the hetero-aggregates could facilitate the sedimentation of the biomass due to the increased density and size of the agglomerates formed (Cheng and Wang, 2022; Cunha et al., 2019). Additionally, the control experiments conducted to assess the impact of centrifugation on microplastic removal, depicted in Fig. 6, showed that centrifugation alone had a negligible effect on MPs reduction. However, the possibility of the biomass dragging the MPs to the bottom during centrifugation should not be neglected and must be considered in further research. Nonetheless, data reported in the literature demonstrates that hetero-aggregates of microalgae and MPs, accelerate the sedimentation of the biomass, which presents an advantage when considering scaled-up processes in real-world scenarios (Cheng and Wang, 2022). Another question that can be a limitation in this process, is the introduction of MP particles retained in the biomass from the previous experiments when inoculating the new experiment. MPs trapped in the biomass will inevitably be introduced in the new experiment when inoculating it with the consortia. Nevertheless, only a fraction of the biomass is used as inoculum, resulting in negligible contamination. Consequently, in the new cultures, the total number of MPs does not significantly increase. Notably, there is no reduction of the removal efficiencies in the subsequent experiments, which would be expected if a significant quantity of MPs were introduced with the inoculum. Thus, it is safe to assume that it does not affect the removal efficiencies.

4. Conclusions

In this study, significant quantities of MPs were detected in all wastewater samples analysed, with a maximum of 233 MP L^{-1} in the wastewater from WWTP-A.2 during Summer, highlighting the role of WWTPs in the release of these pollutants into the aquatic environment. The characterisation of the MPs revealed the tendency for a prevalence of irregular and filament shapes, with white and transparent particles, mostly under 500 μm in size. The use of native microalgae consortia for the removal of MPs from these wastewaters is proposed as a mitigation strategy. Microalgal growth was successfully achieved, reaching high yields of biomass production (2.6 g L^{-1}). Moreover, the growth and harvesting of native microalgae consortia in these wastewaters demonstrated the effective removal of MPs up to 83%. The utilization of native microalgae consortia offers a promising solution, not only for the

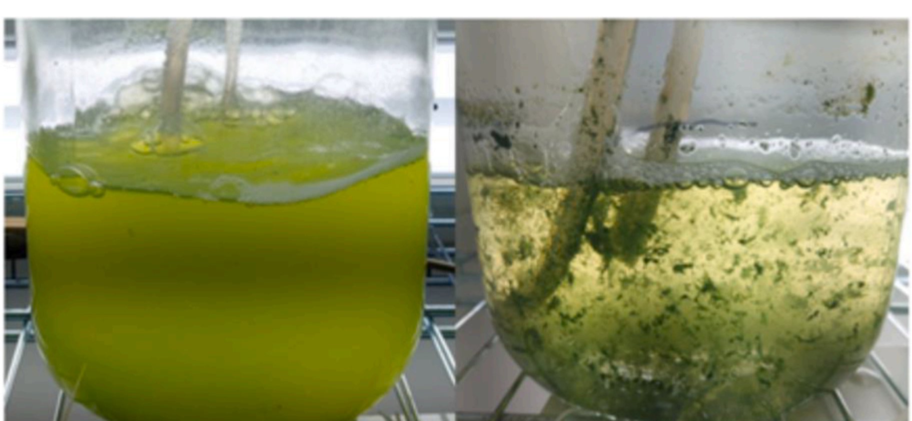

Fig. 7. Differences in cultures homogeneity during the Spring experiments, in WWTP-A.2 (left) and WWTP-B (right).

effective removal of MPs but also for biomass generation. Considering the potential contamination of this biomass by the MPs and other contaminants present in the wastewater effluents, and consequent limitations of its applications in other industries, this biomass is a potential feedstock for bioenergy production. The conversion of the biomass into biofuels relies on several processing steps that can degrade the contaminant molecules. The use of microalgal biomass growth in wastewater effluents presents a promising avenue for sustainable energy generation and management. The present findings contribute to advancing the understanding of microplastic removal by microalgae and emphasize the significance of adopting more realistic approaches in assessing the potential of microalgae consortia for real-world applications in wastewater treatment systems.

CRediT authorship contribution statement

Valdemira Afonso: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rodrigo Borges:** Validation, Resources. **Brígida Rodrigues:** Validation, Resources. **Raúl Barros:** Writing – review & editing, Supervision, Resources, Conceptualization. **Maria João Bebianno:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Sara Raposo:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Authors acknowledge the support of FCT - Portuguese Foundation for Science and Technology, for the PhD grant UI/BD/150774/2020, and for the projects LA/P/0069/2020 awarded to the Associate Laboratory ARNET (https://doi.org/10.54499/LA/P/0069/2020), UIDB/00350/2020 (https://doi.org/10.54499/UIDB/00350/2020) and UIDP/00350/2020 (https://doi.org/10.54499/UIDP/00350/2020) awarded to CIMA of the University of the Algarve.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.123931.

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