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Microplastics in jellifying algae in the Bay of Biscay. Implications for consumers' health

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ABSTRACT

Microplastics pollution is widespread throughout the world, affecting all marine ecosystems and their organisms. The red alga *Gelidium* sp. is the source of best quality agar and is directly consumed as seafood in many countries. Here we quantified microplastics in *Gelidium corneum* harvested along the Asturias coast (southwest Bay of Biscay) from locations differently affected by factors that influence microplastics concentration like substrate size, ports, rivers, etc. Results showed that *G. corneum* collected near sandy substrates accumulates more microplastics than those from rocks, suggesting that the substrate's dynamics is key to allow microplastics to reach these algae and enter the human diet. Considering their composition, polyethyleneimine and polyester were identified and classified as harmful according to the European Chemicals Agency. The polyethyleneimine is present in the 28,6 % analysed from the algae and may pose a risk to consumers. The amount of microplastics potentially ingested by consumers would be lower than mussels and eels of the same region; but could potentially increase in agar if microplastics are not retained during processing. More research to identify microplastics sources and measures to prevent them in coastal areas are recommended.

1. Introduction

Since the decade of 1950 global plastic production has been increasing [1]. These industrial polymers originate from oil and gas sources and for their chemical stability they are employed for diverse uses worldwide [2]. Microplastics (MP thereafter) are defined as a heterogeneous mixture of plastic materials <5 mm [3]. These emerging pollutants are diverse in colour, shape, composition, and weight, characteristics that determine its dispersion, toxicity, adsorption and absorption capacity of different pollutants, potential bioavailability, and microbial colonisation [4]. Primary MP, fabricated to that size, are commonly used in cosmetics products, facial cleansers, textile fibers, in diet and in medicine; secondary MP are produced in situ from the erosion of plastic surfaces by UV rays, wind, oxidation and other factors [5]. Being produced in land, MP end in the sea. The estimated amount of MP in the ocean is so enormous that marine MP pollution is currently called the *microplastics crisis* [6,7]. Known sources of marine MP are as diverse as those of plastics litter: mismanaged landfills, fishing and aquaculture activities, rivers, urban concentrations –for laundry and domestic washing, and even touristic concentrations that increase the population seasonally so beach littering (e.g., [8]); moreover, smaller substrate sizes (fine sand, typical of touristic beaches) retain more MP than larger substrate sizes like gravel or rocks [9]. MP may even escape wastewater treatment plants (WWTP), that are not always capable of retaining and removing these microscopic materials [10,11].

Due to their small size, MP spread easily and become bioavailable from the simplest organisms to the most complex ones throughout the food chain from primary producers [12] to predatory fish [13] and indeed human consumers (e.g., [14]). In contrast to many publications about MP in animals, studies about MP accumulation in marine macrophytes are relatively scarce and limited to a few taxa; nevertheless, in all of them MP have been reported, highlighting the capacity of algae to retain MP [15–18]. The morphology and the gelatinous surface of some algae allow the easy adhesion of MP to their surface contributing to biofilms [19]. MP attached to macroalgae may be a risk for human consumers in a moment of increased popularity of algae consumption

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[20,21]; also, those contained in products derived from algae widely employed, like jellies [22]. Here we will focus on jellifying algae for their economic importance and widespread use in different sectors, from food to cosmetics or medicine.

Gelidium and *Gracilaria* are the main algae genus contributing to agar production [23]. *Gelidium* was first employed as a source of agar in Japan, but nowadays it is exploited for this purpose throughout the world [24]. Some species like *G. corneum* (formerly *G. sesquipedale*) are high-quality agar producers and a direct food source in many parts of the world [25]. This study will focus on the southwest Bay of Biscay, where *G. corneum* is the most exploited alga of high commercial interest in the area, being a source of agar [26]. Previous studies have shown the need for a regulation of the maximum amount of pollutants in edible seaweeds commercialised for human consumption [27]. Although the presence of MP has been analysed in several species of macrophytes, Li et al. [18] reports the lack of studies about MP content in the genus *Gelidium*, particularly in species like *G. corneum* that has a growing human consumption. This study will fill in that gap providing novel data on this contaminant in this species, taking into account factors that influence microplastics concentration and their composition, and estimating the possible consumption risk for humans.

The objectives of the present study were two-fold. First, to determine the factors that increase the exposure of *G. corneum* to MP in the region of interest comparing samples taken from different locations along the coast; second, to infer the implications of the found MP on consumer's health, estimating MP uptake from algae consumption and comparing the results with those of other species consumed in the region.

2. Materials and methods

2.1. Validation steps

The validation of the method was performed in situ with alga of the genus *Gelidium*. Samples were collected on January 21, 2022, at San Lorenzo beach (Gijón, Asturias). To analyse the recovery rate for polymers by selected method, spikes of two types were added. On the one hand, plastic beads in the form of granules (<5 mm) of three colours were added: dark green, dark purple and gold. On the other hand, plastics spikes in the form of fibers (<5 mm) of two colours were added: dark blue and orange. All samples were subjected to dehydration and digestion processes. Once the samples were digested, flotation and isolation of MP were performed using a vacuum pump. As a last step, a visual analysis of MP was performed by observing the petri dishes using a stereomicroscope. This procedure confirmed the recovery of 100 % of the pellets and 77 % of the fibers in total.

2.2. The species in study

Gelidium corneum is a perennial Rhodophyta distributed along coastal areas at shallow depths in the tidal and subtidal area (0–20 m) and with high exposure to sunlight, commonly attached to a solid substrate [25]. In Asturias region (south Bay of Biscay), the main producer of this alga in Spain, the common name of *G. corneum* is "ocle". In this region, *G. corneum* is harvested hand-plucking underwater during the summer season, and also cast seaweed is collected [24,26]. Here the raw algae are sold to Spanish processing companies. Although more data about cast seaweed collection are needed to ensure a thorough sustainability assessment, hand-plucking harvesting in the region is considered sustainable [26].

2.3. Samples and sampling area

The study region (Asturias, southwest Bay of Biscay, north of Spain) has a mountainous orography and is deeply influenced by the sea. The most densely populated areas are located on or near the coast [28]. Wind direction varies seasonally, but during summer (when *G. corneum* is harvested) sea breezes are stronger. Tides are semi-diurnal, with periods of about 12 h and 20 min [29]. The general orientation of the coastline is W-E. The coastline consists of vertical and in some cases arched cliffs, islets, estuaries, some coves, and sedimentary terraces [30]. The largest cliffs of the entire coastline are found at the steepest cape, Cabo Peñas, one of the sampling areas of this study [29].

Samples of *G. corneum* were collected by divers from the Fisheries Research Centre of Gijón between July and September 2019, at seventeen points off the Asturian coast along 122 km (Fig. 1). Sampling was conducted by fishing boats during commercial harvesting; thus the samples represent the algae product really collected by fishermen. Two-three replicates were taken from each location with 40 × 40 cm grids. Divers collected algae manually and stored them immediately in sealed non-plastic vials, then aliquots for MP analysis were transferred to glass jars carefully closed to prevent contamination during the transport to the laboratory. The algae samples were frozen at −20 °C until processed. Together with algae, 5 L surface water samples were taken from each sampling point using sterile bottles that were closed, labelled, and stored cold until arrival at the laboratory. The results of these water samples have been published in Menendez et al. [31].

2.4. Microplastics extraction

2.4.1. Sample treatment

Samples were previously defrosted before handling. To prepare samples for dehydration process, the algae samples were placed on plates and cleaned with steel tweezers. During cleaning, bryozoans that were attached to the surface of the macroalgae samples were carefully removed. Subsequently, 6 g of wet algae per sample were added to around 600 mL glass jars. The amount of wet algae to be dehydrated was estimated considering the wet/dry weight ratio for *Gelidium* taken from Romero [32]. Jars were covered with aluminium foil and placed in a static oven at 80 °C for 2–3 days until all algae samples were dehydrated. During the dehydration process, data on grams of wet and dry algae were measured. The grams of dry algae data were transformed to kilograms dry algae and the SDs were calculated (Table 1), in order to compare with the data of the gelling alga *Gracilaria lemaneiformis* found by Li et al. [18].

Water was vacuum filtered with a pump through a 0.22 μm pore membrane (Supor® 220 Membrane Disc Filter).

Dehydrated algae were digested following a modification protocol described by Li et al. [17]. Briefly, 10 mg of cellulase (Cellulase R-10 [Onozuka R-10]. PhytoTech Laboratories) were added to a 1 L filtered water solution, and 100 mL of the mixture were added to the sample jars. Manual agitations were carried out to be sure that all algae were covered by the solution. Jars were placed in a static oven at 55 °C for 1 h to take place the enzymatic hydrolysis treatment, until all cell wall of algal cells was dissolved. 50 μL of alcalase (Alcalase®, *Bacillus licheniformes*. EMD Millipore) were added to the solution. The mixture of digested algae, enzymatic solution and alcalase were manually agitated to mix the sample. The liquid solution was placed in a static oven at 55 °C for 1 h to be sure the released protein was digested. Finally, 100 mL of filtered H_2O_2 was added for each jar for oxidative digestion. Before placing them in the oven, all solutions were manually shaken. Samples were left in a static oven at 65 °C for 2 weeks until the organic material was dissolved. During these weeks of incubation, the reaction of each sample was observed and more H_2O_2 was added whenever needed. Once the digestion process was completed, the solution was diluted until 1 L with filtered water to prevent filters to clog. All solutions containing MP were carefully filtered with 0.45 μm pore size PES (polyethersulphone) filters (PALL Corporation) by a vacuum pump. Two PES filters (in some cases three), were finally obtained per sample (around 400 mL per sample).

All filters were immediately stored in glass petri dishes, properly covered, and dried at room temperature for 48 h before counting MP.

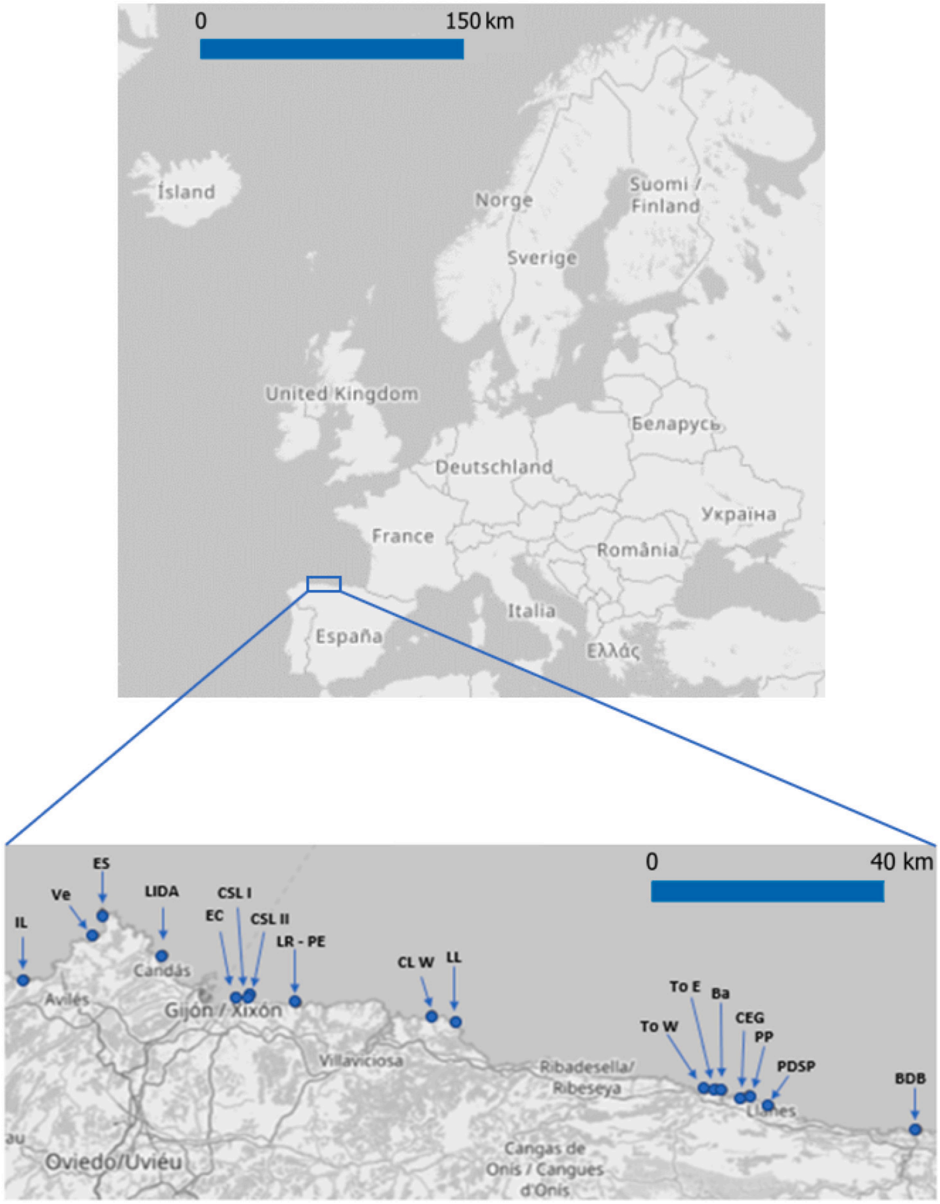

Fig. 1. Sampling area in the Asturian coast. Islote Ladrona (IL), Verdicio (Ve), El Sabín (ES), La Isla de Antromero (LIDA), El Cervigón (EC), Cabo San Lorenzo I (CSL I), Cabo San Lorenzo II (CSL II), Los Regatos – Punta Escalera (LR - PE), Cabo Lastres W (CL W), Les Lastres (LL), Torimbia W (To W), Torimbia E (To E), Barro (Ba), Castro el Gaitero (CEG), Palo Poo (PP), Paseo de San Pedro (PDSP) and Bocana de Bustio (BDB) sampling points.

2.4.2. Quality control

Control measures were taken to avoid samples contamination. All the material (forceps, plates, glass jars, vacuum pump, and petri dishes) was cleaned with distilled water (0.2 µm pore size PES filters) before use. The reagent for the oxidative digestion process, H_2O_2, was also filtered (0.2 µm pore size PES filters). Blanks prepared with the same reagents and distilled water without algae, were processed together with the samples, to control for possible MP contamination during the procedure. All the process took place in a closed laminar flow chamber to avoid airborne MP contamination. Researchers wore non-latex nitrile gloves, cotton laboratory coat and a face mask during the whole experimental process.

2.4.3. Microplastics visualization and composition analysis

MP were identified under a stereomicroscope with 40× magnification. Plastics smaller than 5 mm length were counted and classified according to the classification established by Hidalgo-Ruz et al. [33]: fragments, beads, and fibers.

Visual differentiation of microplastics can lead to erroneous analysis, as it is very difficult to differentiate them from natural organic and inorganic particles that are present in the environment, due to their small size [34]. Once the MP were counted, a 20 % were randomly picked for analysis by Fourier-Transform Infrared spectroscopy (FTIR-Varian 620-IR and Varian 670-IR).

The potential health danger caused by the materials found in MP analysis was identified according to the European Chemical Agency (ECHA) (Source: https://echa.europa.eu/home).

2.5. Risk of MP ingestion from consumption of the considered species

Ingestion of MP was calculated for an estimated serving size of 100 g wet algae, which could be roughly one cup. The mean number of MP per gram was multiplied by 100, and a range was estimated from the standard deviation. The risk of MP ingestion from gelatine was estimated from a conversion of 15 g of *Gelidium corneum* into 1 g of agar.

For comparison with other species of the same region, we did the same calculations with mussels *Mytilus galloprovincialis*, and glass eels

Table 1
Microplastics pollution in algae and water samples in the 17 sampling locations analysed. Results are given as number of microplastics items per gram (MP/g; SD in parentheses for algae). Not determined, nd.

Location	MP/g wet alga (SD)	[MP]/g water
Islote Ladrona	1.57 (1.1)	0.000195
Verdicio	0.852 (0.45)	0.000195
El Sabín	0.371 (0.24)	0
La Isla de Antromero	0.469 (0.09)	0
El Cervigón	1.142 (0.22)	nd
Cabo San Lorenzo I	0.655 (0.38)	nd
Cabo San Lorenzo II	0.757 (0.32)	0.000195
Los Regatos – Punta Escalera	0.123 (0.67)	0.000292
Cabo Lastres W	0.427 (0.09)	0.000195
Les Llastres	0.159 (0.22)	0.000195
Torimbia W	1.312 (0.61)	0.000195
Torimbia E	1.303 (1.26)	0
Barro	0.335 (0.44)	0.000195
Castro El Gaitero	0.813 (0.57)	0.000195
Palo Poo	0.393 (0.33)	0.000195
Paseo de San Pedro	0.326 (0.456)	0.000195
Bocana de Bustio	0.366 (0.23)	0.000195

Anguilla anguilla MP data. These data were taken respectively from Masiá et al. [35] and Menendez et al. [31]. These two species are fished and consumed in the region. Fishing statistics in the region is available at the website of the General Directorate of Marine Fisheries of Asturias and can be consulted at https://pesca.asturias.es/pesca-subastada-en-lonjas/-/document_library/ot0uH304wWwn/view/92847?_com_liferay_document_library_web_portlet_DLPortlet_INSTANCE_ot0uH304wWwn_redirect=%2Fpesca-subastada-en-lonjas%3Fp_p_id%3Dcom_liferay_document_library_web_portlet_DLPortlet_INSTANCE_ot0uH304wWwn%26p_p_lifecycle%3D0%26p_p_state%3Dnormal%26p_p_mode%3Dview%26_com_liferay_document_library_web_portlet_DLPortlet_INSTANCE_ot0uH304wWwn_mvcRenderCommandName%3D%252Fdocument_library%252Fview (accessed on November 2022).

2.6. Data analysis

MP concentration was measured in MP/g in all the samples considered (algae, water, eels, and mussels), to make the results comparable. For organisms it was MP/g of wet tissue.

The following factors that influence MP quantity in a coastal location [8,9] were taken into account:

a) Distance to the closest WWTP, weighted by WWTP size (a map with the location of WWTP can be found on: https://consorcioaa.com/saneamiento/, and characteristics of the WWTP of Asturias can be found on: https://www.miteco.gob.es/es/agua/temas/saneamiento-depuracion/sistemas/edar/default.aspx,accessed in November 2022);
b) Distance to the mouth of the closest river, weighted by its stream order (briefly, number and complexity of tributaries; [36]);
c) Distance to the closest fishing port;
d) Fishing activity in the closest port, measured from catch tonnes during the 2018 (regional catch statistics available at: https://tematico.asturias.es/dgpesca/din/estalonj.php, accessed in November 2022);
e) Sandy substrate in the tidal range of each sampling point (dummy 1 presence/0 absence);
f) Number of inhabitants for the closest population nucleus in 2020 (data available at: https://citypopulation.de/es/spain/localities/asturias/, accessed in November 2022);
g) Touristic occupation in the nearby area during the sampling time. Data were taken from Asturias Regional Government website at https://www.google.com/search?q=Turismo+en+Asturias+en+2019+SITA&rlz=1C1CHBD_esES762ES762&oq=Turismo+en+Asturias+en+2019+SITA&aqs=chrome..69i57j33i160l3.10652j0j4&sourceid=chrome&ie=UTF-8, accessed in November 2022.

MP sources (ports, rivers, WWTP, urban areas) located at the west of each sampling point were considered preferentially because the dominant current in this area goes eastwards [37]. Data for the locations here considered are in the Supplementary Table 1.

Principal component analysis (PCA) was performed to determine the factors contributing principally to the dataset variance, with the following settings: correlation option; variables with $r < 0.8$ pairwise correlation; significant components with >0.7 eigenvalue. C2 was plotted over C1 to visualize the effect of different factors on algae MP concentration. Due to the few number of replicates, analysis of similarities (ANOSIM) with 9999 permutations was preferred over ANOVA, for comparison of algae MP concentration in among all the locations. ANOVA was performed to test for differences between groups of samples (e.g., locations with sandy versus rocky substrates), after checking for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test), for MP concentration. Student's *t*-test (for equal means) and Mann-Whitney test (for equal medians) were carried out with pairwise comparisons. Contingency chi square tests were employed for the comparison between distributions of MP by type and colour, or by plastic chemicals, in algae and water samples.

Multiple regression analysis was performed with the factors influencing MP concentration as independent variables and the MP concentration in algae as dependent variable, to determine which factors predict the quantity of MP in algae. Correlation between the concentration of MP in the algae and in water in the sampling sites was tested using Pearson's r.

Statistical analyses were done with the free software PAST v.2.17 [38].

3. Results

3.1. MP quantity and profile in Gelidium and water

In total 206 MP were found from algae and 14 from water samples in this study. All the MP items identified were microfibers (Fig. 2). Dominance of fibers is usual in marine samples from this region, where they may represent >90 % of MP [31,35], but would stay among the highest ratio of microfibers published.

Table 1 shows MP concentration in water and algae in the sampling locations. The mean number of MP per gram of dry algae was 2.35 (SD 1.85), and in wet algae 0.669 (SD 0.419). In water it was much lower, 0.00016 (SD 0.00007). Differences between locations were evident for algae, between 0.2 MP/g of dry algae in Los Regatos to >6 MP/g in Cabo San Lorenzo and El Cervigón. The data obtained for water were very similar to each other, with zero to two MP items per water sample, and the relative pollution did not coincide with that of algae, being Los Regatos the most polluted location from the water sample (two MP items) and the least polluted from the algae sample (Table 1). No significant correlation between MP concentration in algae and water was found ($r = -0.172$, 13 d.f., n.s.), although this should be taken with caution for very few MP found from water samples.

MP concentration in the blanks (0.007 MP/g) was much lower than algae MP concentration, thus airborne contamination in the laboratory is unlikely to affect the results. The composition of the particles found in the blanks were: 38.46 % cellulose, 30.77 % rayon (modified cellulose), 7,69 % polyethylene terephthalate (PET) and 23.08 % polyethyleneimine (PEI).

Classifying MP visually by colour, black, blue, reddish, and white-transparent were the majority; other colours found from water samples were brown, purple, and yellow, that for analysis purposes were combined in a single class as "Others" (Fig. 2). Dominant colours were white/transparent followed by blue in algae, and black in water. MP profiles of water and algae were significantly different ($p = 0.003$,

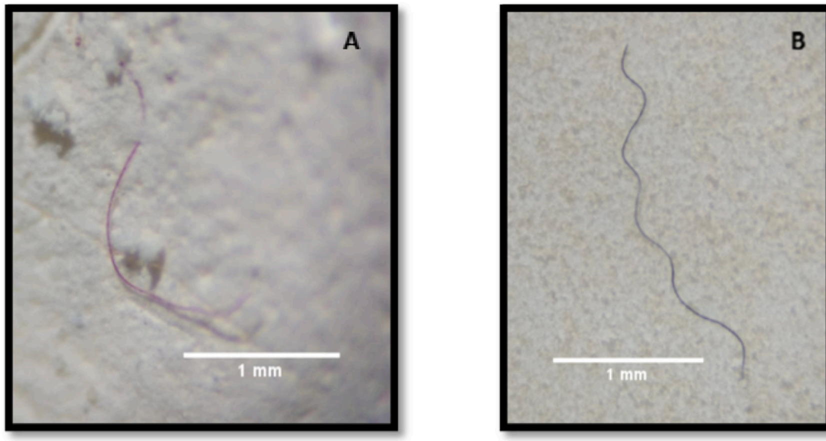

Fig. 2. Examples of the type of microplastics found in the samples of this study. A, red fiber. B, blue fiber. Photographs taken by A. Bilbao. Scale: 1 mm (ImageJ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cramer's V: 0.557) (Fig. 3).

Regarding the chemical composition of the MP found in this study (218 items in total analysed by FTIR, excluding the blanks), 83 % of the particles were artificial and 17 % were alpha-cellulose, one of which was identified as natural cotton. The artificial fibers were: 40 % of rayon (artificially transformed cellulose), 11.4 % of polyester, 28.6 % of polyethyleneimine – PEI and 3 % of polyacrylonitrile - PAN. In water samples 12 artificial fibers were found: 41.7 % rayon, 25 % PE, 16.7 % polyester, 8.3 % vinyl and 8.3 % acrylic. The profile of MP composition was significantly different between algae and water (Chi Square: $p = 0.013$, Cramer's V: 0.591).

3.2. *Spatial distribution of and environmental factors contributing to Gelidium corneum MP pollution*

The concentration of algae MP was only marginally significant among locations (ANOSIM with mean rank within = 536.8, between = 590.9, $R = 0.09$, $p = 0.10$). Only one pairwise comparison was significant: ToW versus LR-PE ($R = 0.39$, $p = 0.046$), that is, one of the most MP-polluted versus the least polluted algae samples (Table 1).

Principal component analysis (PCA) provided a visual interpretation of the underlying patterns of relationships between variables and sampling sites (Fig. 4). The diagonal representing MP concentration in algae was in the second quadrant together with tourism and population size, with three locations in the centre of the sampled area (EC, CSL I and CSL II) and the westernmost point IL. The closest diagonal to MP concentration was the sandy substrate, that was located in the first quadrant with the diagonal corresponding to distance to rivers, together with the points ToW, ToE, Ve, ES and Ba. The diagonal representing the concentration of MP in water was in the third quadrant together with WWTPs and ports, with the sampling points LIDA, CL W, CEG, PP and PDSP. In the fourth quadrant were located the remaining sampling points (LR–PE, LL and BDB).

Pairwise correlations between the considered variables are in

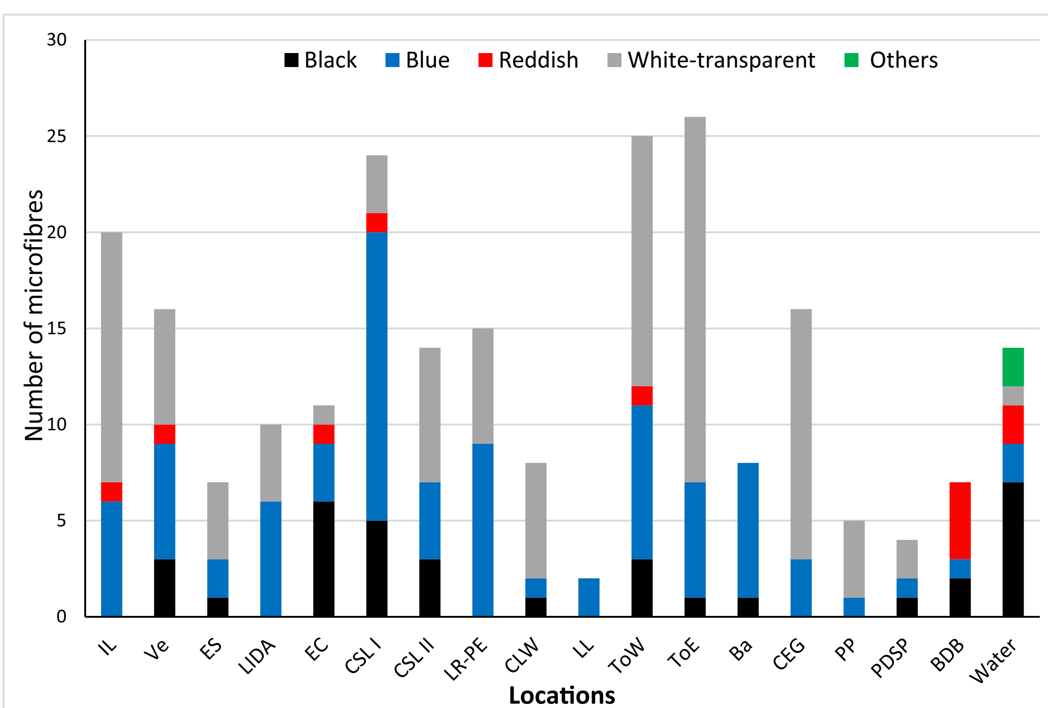

Fig. 3. Profile of MP by colour in samples of algae from each sampling point, and in water samples.

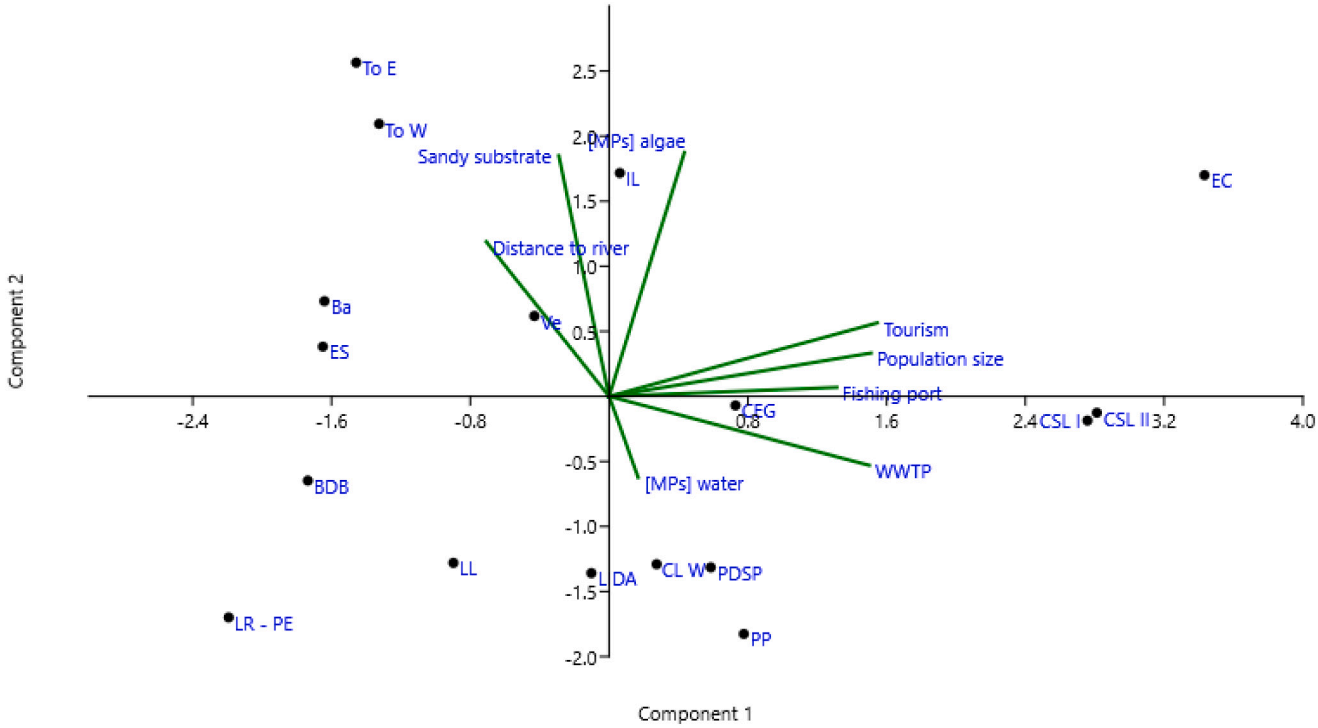

Fig. 4. PCA plot representing the correlation between the different sampling points and the variables considered. Sampling sites: Islote Ladrona (IL), Verdicio (Ve), El Sabín (ES), La Isla de Antromero (LIDA), El Cervigón (EC), Cabo San Lorenzo I (CSL I), Cabo San Lorenzo II (CSL II), Los Regatos – Punta Escalera (LR – PE), Cabo Lastres W (CL W), Les Lastres (LL), Torimbia W (To W), Torimbia E (To E), Barro (Ba), Castro El Gaitero (CEG), Palo Poo (PP), Paseo de San Pedro (PDSP) and Bocana de Bustio (BDB). Concentrations of MP ([MP]) in algae and water are indicated. WWTP, wastewater treatment plants.

Supplementary Table 1. Since population size and tourism were strongly correlated to each other ($r = 0.91$), tourism was discarded for multiple regression analysis. From this analysis the only significant predictor of algae MP concentration was the sandy substrate (Table 2). The group of sampling locations close to a sandy beach exhibited a significantly higher concentration of MP in algae than the group of locations on rocky substrates (mean 0.96 versus 0.41 MP/g with variances 0.2 and 0.04 respectively, ANOVA with F (1.15) = 11.1, $p = 0.004$).

3.3. *Implications of Gelidium MP pollution for consumer's health*

Two types of chemicals identified by FTIR from algae MP are registered as harmful by the ECHA: PEI and Polyester (Table 3 – marked in bold; Supplementary Fig. 1). The most abundant MP was rayon in algae samples, but there is no information available about the evaluation of this type of plastic. The main risk for algae consumers in this region would be posed by PEI, that was found in 28.6 % of the MP found from algae samples. In addition to these two chemicals found from algae, vinyl fibers that are also dangerous for health were found in water.

Although there are studies that predict total MP intake by salt and seafood consumption [39], no studies yet reference seaweed intake. If the latter were directly consumed, the algae found in this study would contribute to the MP load of consumers, with an ingestion varying from 12 to 157 MP/serving (100 g wet algae) depending on the sampling location; these values would correspond to Los Regatos and Islote Ladrona respectively. Regarding agar contamination, according to the data provided by the Fisheries Research Centre of Asturias, around 15 g of *G. corneum* are employed to produce 1 g of agar in the region. From average MP concentration of alga in the study area of 0.669 ± 0.437, each gram of agar produced from *G. corneum* harvested on Asturias coast would carry around 10 microfibers.

The concentration of MP in *G. corneum* was significantly lower than that obtained for other species consumed in the same region harvested from the same areas (Supplementary Table 5). In glass eels (European eel *A. anguilla*) a mean of 2.647 MP/g (SD 0.099) was found [31], and in mussels (*M. galloprovincialis*) the mean was 1.628 MP/g (SD 1.005) [35]. ANOVA analysis showed highly significant differences between species (F (2.25) = 15.2, $p = 0.00006$). Post-hoc test with pairwise comparisons showed significant differences between all the species pairs (Table 4).

Table 2
Multiple regression model with concentration of MP in water ([MP]), beach, wastewater treatment plant (WWTP), river, port and population as independent variables, and concentration of MP in alga ([MP]) as dependent variable. SE, standard error. Significant factors marked in bold.

	Coefficient	SE	t	p	R^2
[MP] algae	−0.0664	0.774	−0.086	0.933	
vs. [MP] water	−263.52	1177.2	−0.224	0.827	0.027
vs. Sandy substrate	**0.556**	**0.195**	**28.529**	**0.017**	**0.425**
vs. WWTP	−0.007	0.171	−0.042	0.967	0.001
vs. River	0.021	0.063	0.337	0.743	0.045
vs. Fishing port	0.071	0.062	1.145	0.279	0.073
vs. Population size	0.007	0.012	0.595	0.565	0.038

Table 3
Composition of MP analysed in this study and their risk for environment by ECHA. In bold harmful substances. PAN, polyacrylonitrile; PE, polyethylene; PEI, Polyethyleneimine; Rayon.
X correspond to the presence of these substances in the algae and/or water.

Substance	Risks from ECHA	Algae	Water
Acrylic	No notified hazards		X
PAN	Pre-registration process	X	
PE	Under evaluation (pre-registered)		X
PEI	**Harmful if swallowed, harmful to aquatic life**	**X**	**X**
Polyester	**Harmful to aquatic life**	**X**	
Rayon	Under evaluation (pre-registered)	X	X
Vinyl	**Harmful if inhaled, respiratory irritation, may cause cancer**		**X**

Table 4
Post-hoc Tukey's test. Values of t and their corresponding *p* are given below and above the diagonal, respectively.

	Algae	Mussels	Eels
Algae		0.045	0.0002
Mussels	3.603		0.033
Eels	7.42	3.82	

4. Discussion

This study provides evidence of MP in *G. corneum* harvested from south Bay of Biscay, principally for agar production. Significantly higher MP concentration in algae collected from locations of sandy substrate suggests that, as in other regions [9], the fine grain of the sand facilitates the accumulation and retention of MP. On the other hand, these MP could come from the shore in sandy coasts. Litter left by beach visitors contributes to the presence of MP on beaches [40]. The action of the tides, waves, currents, and winds against the sand can break up larger plastics and create MP directly on the beach [41]. Surface currents can then move MP from the shore to deeper areas where algae are present [42,43]. Besides, although according to Browne et al. [44] cities are the main sources of MP pollution, in our study no evidence of such influence has been observed. Beaches nearby cities are systematically cleaned in this region [45], thus the production of MP by fragmentation of plastics litter [41] would be reduced here, explaining the lack of the expected effect of population size on MP concentration in algae.

The concentration of MP found in our study was relatively high. As an example, Li et al. [18] found a mean of 1070 ± 690 items/kg dry alga in the gelling *G. lemaneiformis* from the eastern coast of China. In our study the mean concentration expressed in the same units was roughly two-fold: 2349.19 ± 1848.47 items/kg dry alga. Li et al. [18] did also analyse non-gelling algae, observing that there is a large variability among them and with *G. lemaneiformis* in terms of MP concentration; in any case, our data from the Bay of Biscay would be among the most polluted.

The items found in this study were similar to those reported from the same and other marine regions. The only type of MP in the algae samples analysed here were fibers, the most abundant MP particles and common in the marine environment [10,46]. Some of these fibers could come from textile production [47], due to the non-retention of such small particles by WWTPs [11]. In this study, 60 % of the fibers identified were cellulose (20 %) or artificially modified cellulose (rayon) (40 %), which are according to Suaria et al. [48] the materials of most fibers present in the ocean.

The presence of PEI in our samples (28.6 %) could be a matter of concern, because from the ECHA shows that this material is harmful if ingested (see Table 3). Polyimides are a relatively new class of specialty plastic materials. PEI, a modified polyimide, is an amorphous engineering thermoplastic offering exceptional mechanical, thermal and electrical properties. It has been found previously in this same region in eels, and water and sediment [31], so its presence in the study area is not new. In small quantities (11.4 %) polyester, another of synthetic fibers found in the analysed algae samples, is also considered harmful to aquatic life, according to ECHA.

The physical characteristics of MP make them suitable for introduction into the human body by various routes, being the ingestion one of the most common [49,50]. Although the risks to human health caused by the ingestion of MP are not yet well known (i.e., [51]), there are some studies on the possibility of introduction into cells through macrophages, or into endothelial cells of blood vessels [49]. The consequences of this emerging contaminant in humans would vary depending on the concentration to which they have been exposed to MP through food [39]. Regarding the indirect effects of these contaminants, it is known that plasticizer additives alter the endocrine system and can interfere with human reproduction and growth, causing negative effects [49]. Thus, there may be a problem if shellfish and fish contaminated with MP are consumed. In diets with regular intake of macroalgae, MP would be problematic as well, although to a minor extent from our data. Even so, this is a recent area in which much remains to be investigated.

Organisms that make up the lower levels in the trophic chain can uptake or retain MP in a non-selective way, due to the small size of this contaminant. Its presence in the food chain can accumulate throughout the web [52,53], increasing its value at the highest levels of the chain. Consequently, organisms at higher trophic levels, such us seafood products of interest for human consumption, may have high toxicity rates [49]. Transfer between various organisms has confirmed this bioaccumulation up the food chain; for example, from plankton to fur seals [53]. From our calculations up to 160 microfibers could be eaten in a single alga serving. On the other hand, the main use of these algae is agar-agar production. In the absence of a thorough cleanliness control during the production process, MP would be introduced in the multiple derivatives of agar-agar: in food and pharmaceutical industries, in dentistry, in cosmetics, in gels for biotechnological use, in bacteriology, and many others [23].

4.1. Study limitations

A limitation of the present study was the small volume (only 5 L) and lack of replicates of water samples. Although they were taken simultaneously with the algae samples, they are likely not representative of the real MP pollution in algae because they correspond to a single point in time [54]. For example, polyethylene, a material employed in fishing gear that is relatively abundant in this region [45], was found only from water samples but not on algae. The amount of water that touches the algae along their life until the moment of harvesting is enormous due to currents and tides; being collected at depths down to 20 m, these algae are probably more influenced by bottom currents [25]. For future studies, if comparison of algae and water samples is sought it would be better to sample a larger amount of water in multiple moments –at least in different seasons and at different depths.

A technical limitation of our study was the lack of buffer during cellulase treatments. It is possible that the proportion of cellulose fibers in algae was overestimated by incomplete cellulose digestion, due to the lack of pH control for the optimal cellulase function. On the other hand, although the methodology validation showed quite solid results for beads that were fully, recovered at the end of the process, the fiber counts are perhaps not fully accurate. Only 77 % of the fibers were recovered. This may be due to the degradation or loss of colour that they suffer during the validation process, which could cause confusion with small remains of algae and the consequent underestimation of the fibers. Therefore, the estimates of ingestion risk in this study could be considered conservative.

4.2. Management recommendations

MP pollution of algae in this region could be easily prevented. Rayon-Viña et al. [55] and Masiá et al. [56] demonstrated a strong association between the amount of macroplastics and beach services: plastic pollution is higher on beaches with no maintenance and cleaning up. This would be the case of Islote Ladrona, Verdicio, El Sabín, Cervigón, Castro El Gaitero, Torimbia and Barro. It is worth noting that a beach located near Isla Ladrona, Playa de Santa María del Mar, has the European Blue Flag (Spanish beaches with Blue Flag in 2021: https://viajes.nationalgeographic.com.es/a/estas-son-playas-espana-bandera-azul-2021_16818, accessed in November 2022). Surprisingly, Islote Ladrona was the most polluted beach in our study; but MP are not considered as a criterion to obtain the Blue Flag. Perhaps if the authorities took legal measures, MP contamination could be minimized and controlled. In fact, prevention will be much more effective with the collaboration of the public, in this case the visitors to the beach. Perhaps if the authorities took legal measures, MP contamination could be minimized and controlled. In fact, prevention will be much more effective with the collaboration of the public, in this case of beach visitors. To encourage the latter, launching beach clean-up campaigns could be recommended [57].

As a final remark, considering the increasing global demand of algae as seafood for their nutritional properties [58], it is very important to assess all the possible contaminants they may carry, and from our results MP are one of them. Even when the consequences of the ingestion of MP for human health are not totally understood yet [51], by precautionary approach further research on MP in gelling algae is necessary in order to prevent possible harms in the future.

5. Conclusions

This study analyses for the first time the microplastics present in *G. corneum* inhabiting the Asturian coast (Bay of Biscay). The results obtained highlight that the beaches indicate the existence of MP.

Although no correlation was seen in this study between the presence of MP in the algae and in the water, the presence of these pollutants was observed in both analyses. Different studies affirm that anthropogenic and biological factors interfere in the presence of these pollutants on beaches, and consequently in the water. This should be made known to the entities responsible for both beaches and coastal control, so that they can take the corresponding measures.

Knowing the composition of the MP present in the study area, as has been done in this case, can help to identify the sources and risks of these materials and intervene to control them.

As this alga is consumed by animals and humans, it is transferred throughout the food web. In addition, their gelling nature makes them direct food for humans through agar-agar, and therefore of economic interest. For this reason, the presence of microplastics in this organism should be highlighted and research into them should be encouraged in order to find out the consequences that they can have on human health in the long term.

The results obtained in this study represent a significant advance in the field of science due to the novel research conducted on the presence of MP in the species *G. corneum*. Even so, given the scarcity of studies and the growing importance of algae in the human diet, further research on other edible species is recommended.

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CRediT authorship contribution statement

Amaia Bilbao-Kareaga: Data Curation, Formal Analysis, Research, Metodology, Resources, Writing – original draft. Daniel Menendez: Metodology. Paloma Peón: Resources. Alba Ardura: Conceptualization, Supervision, Monitoring, Visualization, Writing – review & editing. Eva Garcia-Vazquez: Conceptualization, Data curation, Formal analysis, Obtaining funding, Project Management, Supervision, Monitoring, Visualization, Writing – review & editing.

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

No data was used for the research described in the article.

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