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Assessment of anthropogenic pollution by UV filters using macrophytes as bioindicators

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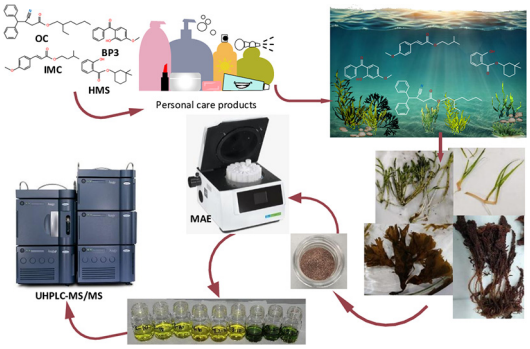
HIGHLIGHTS

- An analytical method for eight organic UV filters was optimized for macrophytes.
- All target compounds have been detected in different frequencies.
- Octocrylene was detected in all samples and it was found in the highest concentration.
- First monitoring of organic UV filters and bioconcentration assessment on macrophytes
- Detection of target compounds in macrophytes indicates their potential as bioindicators.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine environment pollution has increased in recent decades as a result of anthropogenic activities. Macrophytes can assimilate the compounds dissolved in the water and respond to changes in surround conditions, for that, they can be used as bioindicators of pollution in aquatic environments.

Currently organic ultraviolet (UV) filters have shown ever-increasing in pollution levels in marine ecosystems. The anthropogenic pollution produced by eight organic ultraviolet (UV) filters in coastal macrophytes was studied. A microwave-assisted extraction (MAE), followed by ultrahigh-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) was applied to 76 macrophyte (seaweeds and seagrass) samples from three different beaches on the Gran Canaria Island (Spain), collected for 6 months. All studied UV filters were found with different detection frequencies from 16% to 100% in macrophyte samples. Octocrylene (OC) was detected in all the analysed samples throughout the sampling period. The highest concentration, 19,369 $ng \cdot g^{-1}$ dry weight (dw), was for this compound in the seagrass *Cymodocea nodosa*.

The bioconcentration ratio was determined for several seaweed groups (red, brown, green). Different bioconcentration grades were obtained. Those above 1000 indicated significant accumulation, which increases the possibility of chronic effects on seaweed and at upper tropic levels.

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1. Introduction

Seaweeds and seagrass are altogether known as macrophytes, which are multicellular photoautotrophic organisms with a wide geographical distribution. Seaweeds comprise the Genus Rhodophyta (red seaweed), Ochrophyta (brown seaweed) and Chlorophyta (green seaweed), and seagrass is a grass-like marine plant. They are deemed a useful tool for environmental monitoring as bioindicators of pollution given their sessile nature in a dynamic habitat where pollutants settle or resuspend due to tidal action. (Ruiz Chancho et al., 2010; Chaudhuri et al., 2007).

Seaweeds are used for identifying heavy metal pollution in marine environments because they are easy to identify, and also for their availability in some areas (Chakraborty et al., 2014). Seaweeds can be employed to monitor organic pollution because their diversity is strongly impacted by the presence of organic compounds (Sabri et al., 2020). As seaweeds are also the basis of several marine food webs, understanding their pollution by anthropogenic compounds is essential for their environmental importance.

Several pollutants constantly enter marine ecosystems, of which organic ultraviolet (UV) filters have increased in the last few decades. These compounds are used in different products, such as personal care products (PCPs), including sunscreen, soaps, makeup, lotions and toothpaste, to protect skin from harmful UV radiation effects (Díaz-Cruz and Barceló, 2015). The maximum concentration of each compound in cosmetics is controlled in the European Union by Regulation no. 1223/2009.

Organic UV filters reach the environment both directly (washed off skin and clothes) and indirectly (treated wastewater, industrial discharges, runoff) (Molins-Delgado et al., 2014). Given their extensive use, hundreds of tonnes of these compounds are released to the environment (Danovaro et al., 2008) and are considered a new pollutant type (Emmanouil et al., 2019). PCPs generally do not undergo structural changes and, consequently, unaltered compounds are released to the environment (Brausch and Rand, 2011).

The occurrence of organic UV filters in the environment may have negative effects on the aquatic biota (marine and fresh waters) because of their accumulation or long-term exposure (Carve et al., 2021). In fact some organic UV filters like benzophenone-3 (BP3), 4-methylbenzylidene camphor (4MBC) and octocrylene (OC) produce coral bleaching, impaired reproduction, malformation and increased mortality for some marine organisms (Danovaro et al., 2008; Schmitt et al., 2008; Araújo et al., 2018).

Their presence has been globally reported in several matrices, such as wastewater (Ramos et al., 2016), seawater, marine sediments, marine organisms (Cadena-Aizaga et al., 2020), lakes and rivers (Ramos et al., 2015). These environmental pollutants can affect coastal waters' ecological integrity. Therefore, biological indicators like seaweeds can be globally used to assess water pollution. However, to the best of our knowledge, only one work has previously reported the occurrence of such compounds in seaweeds (Pacheco-Juárez et al., 2019).

Coastal tourism is one of the main reasons for visiting the Canary Islands (Spain) and, as such, it is one of the mainstays of its economy. Beaches there are used almost all year long, which can have a marked impact on the aquatic ecosystem. This makes the Gran Canaria Island coast a suitable scenario for carrying out studies about the presence of organic UV filters in different marine matrices.

Hence the aim of this work is to assess the use of macrophytes as bioindicators of UV filters pollution. To that end, an analytical approach based on microwave-assisted extraction (MAE), followed by ultra-high-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS), was applied to determine the presence of eight commonly used organic UV filters in coastal macrophytes. One of the main advantages of the MAE technique is that it requires small volumes and amounts of solvent and sample. It is also is easy to perform and many samples can be extracted at the same time.

Three beaches on the Gran Canaria Island (Spain) were monitored for 6 months (May – October 2019). Seventy-six macrophytes (seaweeds and seagrass) samples from 14 species were analysed to demonstrate if they were suitable indicators of pollution by emerging pollutants like organic UV filters. As far as we are aware, only one of the studied compounds has been reported in seaweed samples by Pacheco et al. (Pacheco-Juárez et al., 2019) in the Canary Islands. The bioconcentration ratio was also calculated for the different seaweed groups (red, brown, green) and for seagrass which is based on the results obtained for in seawater in the same beaches and periods (Cadena-Aizaga et al., 2021).

2. Materials and methods

2.1. Reagents

Eight organic UV filters, namely homosalate (HMS), 4MBC, BP3, drometrizole trisiloxane (DTS), octocrylene (OC), butyl methoxydibenzoylmethane (BMDBM), isoamyl p-methoxicinnamate (IMC) and methylene bis-benzotriazolyltetramethylbutylphenol (MBP) of analytical grade (purity ≥99%), were purchased from Sigma-Aldrich (Madrid, Spain). Methanol (MeOH), acetone, acetonitrile (ACN), hexane (Hex), water and formic acid, of LC-MS grade, were supplied by Panreac Química (Barcelona, Spain). Main characteristics of the target organic UV filters analysed in macrophytes are summarised in the Supplementary Material (Table S1).

Stock solution (250 $mg{\cdot}L^{-1}$) was prepared in acetone and stored in amber glass bottles in a freezer until used. Working solutions were prepared daily in MeOH.

2.2. Characteristics of sampling sites, sample collection and pre-treatment

The Gran Canaria Island was selected as a study site because of the many tourists that arrive there throughout the year. Three beaches with different tourism pressures and characteristics were compared (Fig. 1). The geographic coordinates of the sampled beaches are reported in the Table 1.

The Las Canteras beach is located in the northeast part of the island and is characterised by the presence of a natural barrier that runs parallel to its coast. This implies a lower renovation ratio at low tide due to almost null wave action (Perez-Torrado and Mangas, 1994). Ochrophyta seaweed dominates as a rocky-sandy substratum is present on this beach (Tabraue et al., 2009). Due to low water renovation, the long residence time of pollutants can affect the local fauna. This beach is used mainly by locals and moderately by foreigners all year round, where the maximum activity takes place in summer.

The Arinaga beach is located southeast of the Gran Canaria Island and its principal characteristic is the intense influence of wind and swell due to trade winds and the Canary Current (Alonso et al., 2001), which make water renewal easy. It is an open beach employed principally by locals, but barely by international tourists. Here, like the Las Canteras beach, a rocky-sandy substratum is present, where Rhodophyta and Ochrophyta seaweed dominate (Tabraue et al., 2009).

The Playa del Inglés beach lies to the south of the Gran Canaria Island and presents artificial barriers. The effect of trade winds and the Canary Current is milder (Alonso et al., 2001), which creates a calm zone with a light swell. In this case a sandy substratum is found, where seagrass meadows are typical and *Cymodocea nodosa* is one of the main phanerogams present (Tabraue et al., 2009). This open beach is used all year long by numerous international tourists, essentially northern Europeans, according to the Gran Canaria Tourism Agency (G.C.P. de Turismo Estadísticas - Web Oficial de Turismo de Gran Canaria, n.d.). National tourists also prevail in summer.

Macrophyte samples of both seaweed and seagrass species were taken monthly from May to October 2019. The sampling time was selected due to the tourist affluence in this period. Number of the collected samples per beach and month are presented in Table S2. They were collected at low tide along the beach and only the seaweeds washed ashore were picked up. For this reason, different species were taken during each sampling. The seaweed species grouped according to type (red, brown, green) and the seagrass are presented in Table 1. Some species were repeatedly collected. For the Las Canteras beach, *Cymopolia barbata* (green seaweed), *Lobophora*

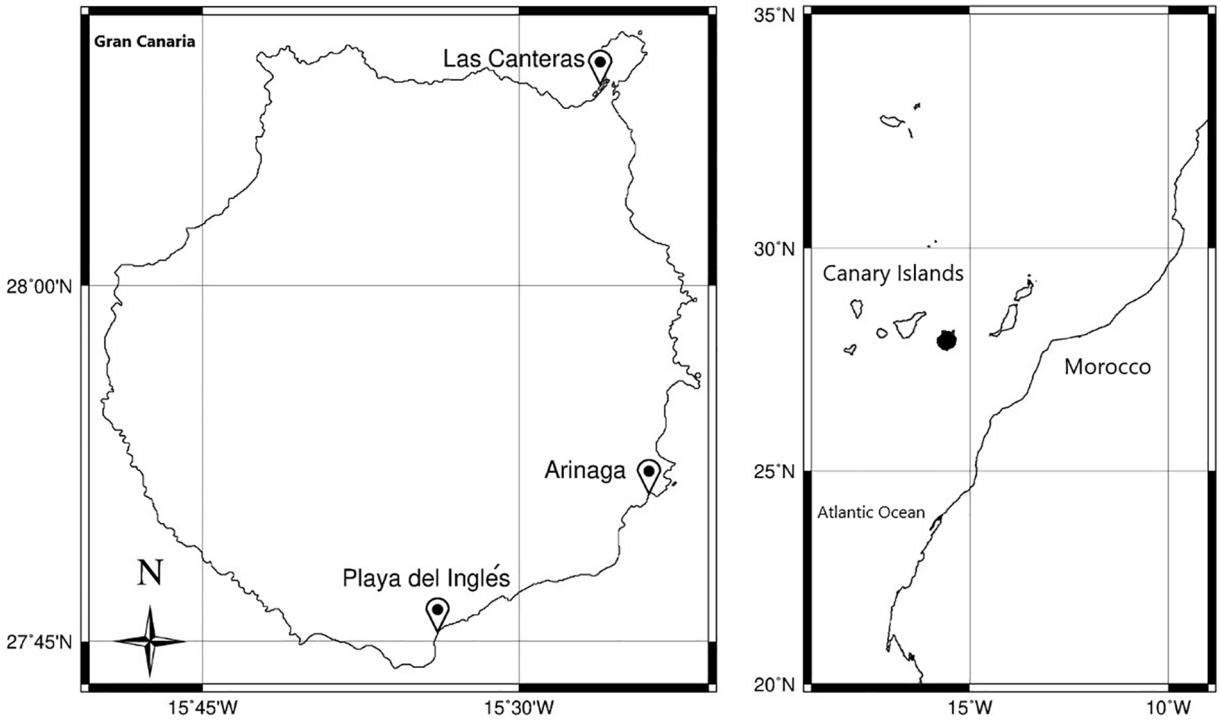

Fig. 1. Geographical location of the Canary Islands, Gran Canaria Island and the sampling sites.

variegata and *Dictyota dichotoma* (brown seaweed) were present during each sampling. On the Arinaga and Playa del Ingles beaches, only *Asparagopsis taxiformis* (red seaweed) and *Cymodocea nodosa* (seagrass) were respectively found during all the samplings. After collection, samples were transported to the laboratory in glass bottles in a portable fridge, where they were rinsed with deionised water to remove sand and salt. Then clean seaweeds were identified and frozen at −20 °C to be subjected to freeze-drying. To obtain a homogenous sample, whole tissues of each species were sifted through a < 300 μm particle size and stored in the dark in a fridge until analysed.

Table 1
Macrophytes species collected in the three beaches.

Sampling Place	Phylum	Scientific name
Las Canteras beach (28°8′27.982″N, 15°26′8.237″W)	Rhodophyta	*Asparagopsis taxiformis*
		Corallina elongata
		Laurencia sp.
		Liagora sp.
		Lophocladia trichoclados
	Ochrophyta	*Lobophora variegata*
		Sporochnus pedunculatus
		Dictyota dichotoma
		Sargassum sp.
		Stypocaulon scoparium
	Chlorophyta	*Cymopolia barbata*
Arinaga beach (28°8′27.982″N, 15°26′8.237″W)	Rhodophyta	*Asparagopsis taxiformis*
		Corallina elongata
		Laurencia sp.
		Liagora sp.
		Lophocladia trichoclados
	Ochrophyta	*Taonia atomaria*
		Stypocaulon scoparium
		Dictyota dichotoma
	Chlorophyta	*Codium decorticatum*
Playa del Inglés beach (27°45′23.579″N, 15°33′51.2809″W)	Rhodophyta	*Corallina elongata*
		Liagora sp.
		Lophocladia trichoclados
	Ochrophyta	*Stypocaulon scoparium*
		Dictyota dichotoma
	Chlorophyta	*Cymopolia barbata*
	Tracheophyta	*Cymodocea nodosa*

2.3. Sample preparation and extraction

In order to obtain a representative sample, a mixture of seaweeds from the Genus Ochrophyta, Chlorophyta and Rhodophyta was employed for extraction optimisation purposes. It was spiked with the target compounds, stirred and air-dried at room temperature in the dark for 24 h to obtain a homogeneous dry sample.

Organic UV filter extraction was carried out in a Titan MPS microwave oven equipped with 16 TFM vessels (Perkin Elmer, Madrid, Spain). The MAE procedure was performed using a factorial design strategy in order to achieve the most suitable combination of the tested values for the different variables that affect the extraction procedure. The factorial design was conducted in two different stages, and time and volume were that variables that have a greater influence on the extraction efficient. In the optimized conditions, one hundred milligrams of the spiked mixture were transferred to the MAE vessels and 2 mL of acetone were added to the mixture. Then vessels were closed and subjected to the optimized MAE process, which consisted in applying 50 °C for 5 min. Once extraction was done, the extract was carefully filtered through a 0.2 μm syringe filter. Then another extraction process was performed with the same sample under the same conditions. The second extract was also filtered, and both were combined to be dried under a nitrogen stream and reconstituted in 2 mL of MeOH.

2.4. Instrumental analysis

Determination was performed by an ACQUITY UHPLC system equipped with a binary solvent manager, a thermostated autosampler, a BEH C18 column (50 × 2.1 mm, 1.7 μm particle size) and a tandem triple quadrupole mass spectrometer detector (MS/MS) with electrospray ionization (ESI). All the components were controlled by the MassLynx Mass Spectrometry software (Waters Chromatography, Barcelona, Spain). The mobile phase consisted of MeOH (A) and water (B), of LC-MS grade, with 0.1% (*v*/*v*) formic acid, and each one at a flow rate of 0.3 mL·min^{-1}. The following

gradient was employed for analytes separation: starting with 25% A: 75% B, which was kept for 3 min, then decreased to 0% of A for 2 min and held for 1 min. Finally, A was increased to 25% in 1 min and held for 1 min for the next injection. The injected extract volume was 10 μL. The MS/MS conditions were previously established (Cadena-Aizaga et al., 2021) and are summarised in Table S1. In brief, the ESI parameters were fixed as follows: capillary voltage at 4 kV, cone voltage 15 V, source temperature at 120 °C, desolvation temperature at 450 °C, and desolvation gas at 500 L h^{-1}. Nitrogen and argon gases were used for desolvation and collision, respectively.

2.5. Statistical analysis

As most seaweed species were not present in all the samplings, they were grouped per type (red, brown, green) to perform the statistical analysis. On the Playa del Inglés beach, the phanerogam *Cymodocea nodosa* was subjected to a statistical analysis alone because it is a seagrass and cannot be grouped with seaweeds.

For the statistical analysis, concentrations were compared by the combined effect of beach for each seaweed type and compound (the Las Canteras, Arinaga and Playa del Inglés beaches) and seasonal period: pre-summer (May and June), summer (July and August) and post-summer (September and October). Given lack of normality within groups, the Kruskal-Wallis test was run to assess the significance of the differences associated with beach-period, for which differences with *p*-values (p) below 0.05 are considered significant. When this test was not significant for the combined effect, the same test was used to look for individual effects. When an individual test was significant, *post hoc* comparisons were made by the Conover test. This test was utilised because it establishes the exact groups within which significant differences can be found, whereas the Kruskall-Wallis test only allows the presence or lack of significant differences to be evaluated. Version 4.1.1 of the statistical R software was used for this purpose (R.C. Team, 2021).

2.6. Bioconcentration ratio (BCR)

Seaweeds and seagrass are potential biomonitors due to limited mobility, the potential to absorb organic substances (Pavoni et al., 2003) and abundance in marine environments. Bioconcentration is the process by which the aquatic organism absorbs a pollutant from the environment *via* non-dietary uptake (Ismail and Ismail, 2017), and is a quantitative measure of its accumulative capacity (Jahan and Strezov, 2019). In order to assess the ability of the studied macrophytes as organic UV filters bioindicators, the bioconcentration ratio (BCR) was determined. BCR is the concentration (accumulation) of a pollutant in an aquatic organism in relation to this pollutant in the surrounding environment. The BCR is calculated by the following formula (Arnot and Gobas, 2006):

$$BCR = \frac{C_{Macrophyte}}{C_{water}}$$

where $C_{Macrophyte}$ is the concentration of a pollutant in seaweed or seagrass (expressed as mg·kg^{-1}) and C_{water} is the concentration of the same pollutant in water (Table S3) (Cadena-Aizaga et al., 2021) expressed as mg·L^{-1}). When the BCR is above 1, the bioaccumulation process is considered to take place. A BCR higher than 1000 indicates significant bioaccumulation (Jahan and Strezov, 2019).

3. Results and discussion

3.1. MAE optimisation

The variables that can affect extraction efficiency in the MAE technique were optimized. A 2^4 experimental design was built with the MiniTab software as the first approach. This consisted in four variables at two levels: temperature (50 °C and 60 °C); extraction time (3 and 6 min); extractant volume (2.5 and 5 mL); solvent type (MeOH and ACN). With the results, a Pareto Chart analysis was performed to see which variables most affected extraction. They are denoted in blue in the Supplementary Material in Fig. S1 for 4MBC. Any correlations between the variables were also analysed, where 0 means no influence, −1 is a maximum negative effect and 1 represents a maximum positive effect. The variables showing the strongest effect were extractant volume and solvent type. A marked combined effect was noted between them (Fig. S1). MeOH performed better recoveries than ACN for each volume, temperature and extraction time. Regarding correlations, the extractant volume obtained the highest values for all the compounds, but was negative (−0.84 to −0.96), while the extraction time presented a correlation between −0.01 and 0.07. The lowest correlation was for temperature (0.01–0.02). The strong negative effect on the extractant volume means that the higher the volume, the more negative the influence on recoveries (Fig. S1). For extraction time, similar results were obtained at 3 min and 6 min, but slightly better results were obtained at 3 min. Regarding the temperature results, this parameter had no significant influence (similar results were obtained at 50 °C and 60 °C). For this reason, temperature was set at 50 °C. Therefore, extractant type (MeOH) and temperature (50 °C) were fixed, while extraction time and extractant volume were analysed in more depth.

In a second stage, 3^2 (two variables at three levels), a factorial design was applied with the aforementioned fixed variables. The two target variables extractant volume and extraction time were analysed at 2, 3 and 4 mL, and at 3, 4 and 5 min, respectively. The OC surface response obtained from this design appears in Fig. S2. The best recoveries were obtained at 2 mL of extractant and for a 5-min extraction time, which were observed for all the compounds.

Later other organic solvents were tested to give the best recoveries. The employed organic solvents were acetone, MeOH:acetone (1:1, *v*,*v*) and hexane (Fig. S3). Similar results were obtained using all the extractants. However, acetone gave generally better recoveries for most compounds. This was why acetone was chosen for the extraction as a compromise in a multicompound analysis.

Finally, a second sample extraction was implemented using another lot of 2 mL of acetone, which resulted in significantly better recoveries. Thus, the optimal conditions were two extractions lasting 5 min at 50 °C with 2 mL of acetone. Both extracts were combined and dried under a nitrogen stream and reconstituted in the 2 mL of MeOH to be injected into the UHPLC-MS/MS system.

3.2. Quality assurance

The linearity, recovery, precision, limits of detection (LODs) and limits of quantification (LOQs) were evaluated under the optimum extraction conditions for the mixture of seaweeds. Each value corresponded to the mean of three replicates.

An external calibration curve was built in MeOH within the 100 ng·L^{-1} to 250 μg·L^{-1} range. Satisfactory linear range coefficients (>0.99) were obtained for each compound.

The instrumental limits of detection (ILODs) and quantification (ILOQs) were calculated from the signal to noise (S/N) of each compound by assuming a minimum detectable limit of 3- and 10-fold the S/N, respectively. The ILODs ranged between 6.84 ng·L^{-1} and 140.34 ng·L^{-1}, while the ILOQs went from 22.79 ng·L^{-1} to 467.81 ng·L^{-1}.

The extraction efficiencies and precision of the method for each compound were calculated considering the application of the whole method. For that, 100 mg of dry sample were spiked to a final concentration of 5, 2000, 10,000 ng·g^{-1} of the mixture of target compounds, which were extracted with 2 mL of MeOH, resulting in final concentrations of 250 ng·L^{-1}, 100 μg·L^{-1} and 500 μg·L^{-1}, respectively.

Extraction efficiencies for each compound were calculated by comparing the signal obtained after applying optimized extraction method to the spiked samples. The obtained recoveries range was 39.8–98.3% (Table 2).

The method's repeatability (intraday precision, $n = 3$) and reproducibility (interday precision, $k = 3$) were expressed as relative standard

Table 2
Analytical parameters: recoveries, intra, inter-day at three levels of concentration (expressed in dw), and ILODs and ILOQs for the developed MAE-UHPLC-MS/MS method.

Compounds	Recoveries (%)[a]			Intra-day precision (%)[a]			Inter-day precision (%)[b]			ILODs[c]	ILOQs[d]
	5 ng·g^{-1}	2000 ng·g^{-1}	10,000 ng·g^{-1}	5 ng·g^{-1}	2000 ng·g^{-1}	10,000 ng·g^{-1}	5 ng·g^{-1}	2000 ng·g^{-1}	10,000 ng·g^{-1}	ng·L^{-1}	ng·L^{-1}
4MBC	–	85.4	89.4	–	3.25	2.42	–	7.00	3.53	51.41	171.35
BP3	51.8	60.7	65.4	8.91	2.66	7.31	10.44	4.22	7.23	22.81	76.05
HMS	39.8	44.0	44.2	0.65	6.55	6.31	11.87	8.91	4.93	11.39	37.97
DTS	44.8	50.6	52.3	4.93	3.57	3.86	8.39	8.24	5.09	6.84	22.79
OC	45.6	78.8	83.2	8.03	2.40	5.56	8.46	6.50	4.31	20.78	69.25
BMDBM	55.6	59.2	65.9	–	11.73	8.44	–	7.47	6.93	40.43	134.77
IMC	56.0	65.4	67.6	7.88	3.91	6.26	8.42	4.20	6.42	16.30	54.35
MBP	–	92.8	98.3	–	2.78	4.76	–	5.59	7.31	140.34	467.81

[a] Mean of three replicates ($n = 3$).
[b] Mean of three replicates performed for three days ($k = 3$).
[c] Calculated from the signal to noise (S/N) assuming a minimum detectable limit of three times the S/N.
[d] Calculated from the S/N assuming a minimum detectable limit of ten times the S/N.

deviation. Intraday precision ranged from 0.65% to 11.73%, and interday precision between 3.53% and 11.87% (Table 2).

3.3. Environmental occurrence of organic UV filters in macrophytes

The MAE-UHPLC-MS/MS method was applied to determine the target analytes in 76 macrophyte samples taken from the Gran Canaria Island. They were collected from three different beaches for 6 months (May – October 2019). The detailed concentrations and detection frequencies for all the organic UV filters analysed in macrophytes are summarised in the Supplementary Material (Table S2).

All the target compounds were found, with different detection frequencies ranging between 16% and 100% in the macrophyte samples. The sum of the measured concentrations in the different species for each compound is represented for seasonal period and beach in Fig. 2. OC was detected in all the analysed samples, while HMS was present in 91% of them (Table S2). The highest concentration corresponded to OC (19,369 ng·g^{-1} dw) in the seagrass *Cymodocea nodosa* on the Playa del Inglés beach in October, and in the green seaweed *Cympolia barbata* (8128 ng·g^{-1} dw) on the Las Canteras beach in September.

OC was the most frequently found compound and the most concentrated one (107–19,369 ng·g^{-1} dw). Its high Log K_{ow} (>6) and low solubility (<0.02) might explain it being highly detected in such a matrix. This may also be related to the fact that this compound is widely used in PCPs formulations and allowed in all countries (Fivenson et al., 2020; Al-Jamal et al., 2014). Conversely, 4MBC, IMC and BP3 presented the lowest frequencies (16–25%), which can be explained by these compounds presenting low Log K_{ow} (<5).

Regarding concentration per beach, 37 seaweed samples were examined at Las Canteras, and the target compounds were detected several times. All the analysed seaweeds presented OC within a concentration range of 126–6372 ng·g^{-1} dw. HMS was present in 97% of the samples, with a concentration range between 4.64 and 8128 ng·g^{-1} dw (Table S2). Three seaweed species were found during all the samplings on this beach: *Cympolia barbata* (green seaweed), *Lobophora variegata, Dictyota dichotoma* (both brown seaweeds). All the target compounds were detected in *Cympolia barbata*. HMS and OC were present throughout the sampling period and had the highest concentrations (8128 ng·g^{-1} dw and 6372 ng·g^{-1} dw, respectively, in September). In *Lobophora variegata,* BMDBM and OC were detected in all the samples (maximum concentrations of 3663 and 2077 ng·g^{-1} dw, respectively). Lastly in *Dictyota dichotoma,* HMS and OC were detected in all the samples, and OC showed the highest concentration (5971 ng·g^{-1} dw) in August. Two of these species (*Dictyota dichotoma* and *Lobophora variegate*), along with *Asparagopsis taxiformis*, have been reported to contain MBP (also called UV 360) on the Las Canteras beach in another study (Pacheco-Juárez et al., 2019), with concentrations between 42.5 and 115 ng·g^{-1} dw were found. They are in the same order as those measured in our study (29.69–78.19 ng·g^{-1} dw).

On the Arinaga beach, 26 seaweed samples were analysed, in which all the compounds were detected at least once. All the samples presented OC at concentrations between 107 and 7163 ng·g^{-1} dw, and its maximum concentration appeared in the green seaweed *Codium decorticatum* in June. Only one species was present throughout the sampling period, the red seaweed *Asparagopsis taxiformis*. In this species, OC, DTS and HMS were present at more than 67% of the samples during the whole period.

On the Playa del Inglés beach, all the compounds were detected at least once in the 13 macrophyte samples. OC and HMS were detected in 100% samples, while BMDBM was found in 85% of them. The highest concentration corresponded to OC (19,369 ng·g^{-1} dw) in the seagrass *Cymodocea nodosa* in October. Only this species was picked up during all the samplings. The frequency of detecting the target compounds was lower in the other analysed species (Table S2).

The results revealed that although the collected green seaweeds were less common, they contained the highest concentrations (Table S2). In this context, the studied green seaweeds suggest being an excellent indicator of organic UV filters pollution. The results of the three beaches suggest a different seasonal behaviour for each species. Some studies indicate that seasonal variation can be explained by solar radiation effects on seaweed growth because metabolic rates slow down in winter due to less light and lower temperatures (Villares et al., 2002). Nevertheless on the Gran Canaria Island, solar radiation on the three beaches is almost the same during the three analysed periods (20–30 MJ·m^{-2}·day^{-1}) (Stackhouse, 2020). The high levels of organic UV filters accumulated in macrophytes reflects the bioavailability of the pollutants in the studied area and the capacity of them to take the pollutants from the surrounding.

Detection frequencies were compared using the data obtained with the seawater analysis from the same beaches during the same period (Cadena-Aizaga et al., 2021). According to the overall frequency in seawater and macrophytes, a tendency was observed: BP3 and IMC were the most frequently found compounds in seawater (>78%), but they were detected in less than 25% of the samples in macrophytes. This can be explained by them presenting the highest solubility and lowest Log K_{ow} of all the target compounds, so they can be more reliably found in seawater. With HMS and OC, they were detected in more than 91% of the analysed samples, while the detection frequency in seawater went below 17%. This can be explained by their high Log K_{ow} (>6.16) and poor solubility (<0.02), so they tend to accumulate in solids. MBP and DTS were barely found in seawater (<28%) and were reported in macrophytes at more than 37%. This relation can be explained by these compounds having the highest Log K_{ow} and lowest solubility of all the compounds. Hence despite their low availability in seawater, they tend to accumulate in solids. 4MBC was scarcely detected in both matrices, which can be justified by this compound having the lowest allowed maximum concentration (4%) for cosmetics in the European Union (EC, 2009).

In addition, by-products (transformation products, metabolites, photodegradation products and conjugates) should be taken into consideration. For example, BP3 (which was reported herein just in the 25% of the

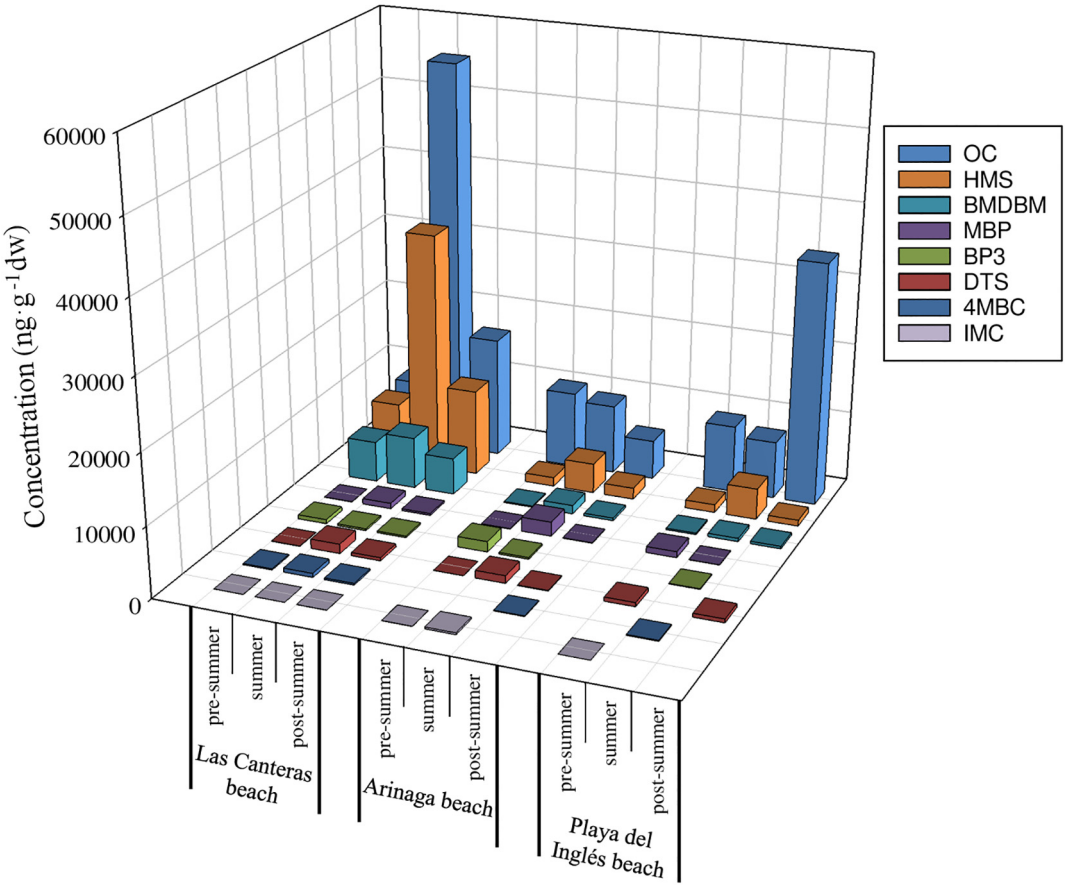

Fig. 2. Organic UV filters detected in the analysed macrophytes.

samples) was reported biodegraded by organic matter in seawater (Li et al., 2016). In fact, a recent study reported BP3 metabolites in seaweed samples (Chiriac et al., 2021). Therefore, for a more reliable approach of the target compounds concentration, their metabolites should be estimated as well as the parents.

3.4. Statistical study

A statistical analysis was performed of the grouped species (red, brown and green seaweed) collected on each beach to see the possible variation in the concentration of the target compounds depending on beach or season (pre-summer, summer, post-summer), or given the combined beach-period effect. The combined beach-period effect per seaweed type was firstly analysed. The obtained results are presented in Table S4 for the red seaweeds and in Table S5 for the brown seaweeds. The individual beach-period effects appear in Table S6A and Table S6B, respectively. Each table contains the median value of the total data, the first quartile (Q1, which is the value under which 25% of data were found) and the median of the third quartile (Q3, which is the value under which 75% of data were found).

For the red seaweeds, differences were significant for the combined beach-period effect on the concentration in BP3 ($p = 0.0155$), IMC ($p = 0.0254$) and OC ($p = 0.0378$), (Table S4). Therefore, the statistical analysis demonstrated that the concentration of the target compounds depended on beach and period. When no significance was observed in the combined beach-period effect, it meant that the statistical test did not have enough sensitivity to define differences between periods for beach or between beaches during the same period. Hence there was no evidence for any beach-period interaction. However, each factor on its own (beach or period) can show significant differences. This occurred with HMS (Table S6A), whose concentration on the Las Canteras beach was always higher than at other locations, and was also higher in summer for all the beaches.

Following the brown seaweeds statistical results, in the beach-period interaction compounds DTS ($p = 0.0046$), HMS ($p = 0.0465$) and OC ($p = 0.0048$) were significantly different (Table S5). For the brown seaweeds, the 4MBC concentration significantly differed for only period (Table S6B) and was detected only in summer. Detailed information about the *p*-values of each comparison are provided in the Supplementary Material. The present results suggest that the compound concentration mainly depended on the combined beach-period effect. This agrees with the fact that the three beaches presented different tourism pressures depending on the period, water removal rates and geomorphological characteristics. For these reasons, the three beaches did not display the same behaviour during the analysed periods. For example, mainly local tourists use the Las Canteras beach in summer (Sánchez Rodríguez et al., 2015), while the Playa del Inglés beach has two tourism peaks: summer with local tourists and post-summer with international tourists. According to these reasons, this agrees with the fact that compounds' concentration depends mainly on the combined beach-period effect as these factors interact.

The green seaweeds showed no significance in any compound. This can be explained by lack of data to not identify significant differences.

Given the Playa del Inglés results, and as seagrass was collected throughout the sampling period, a Kruskal-Wallis test was done with only this species. Only DTS, whose highest concentration was found in post-summer (135 $ng \cdot g^{-1}$ dw), showed a significant difference in relation to the other periods ($p < 0.0223$).

3.5. Assessment of bioconcentration ratios

The BCRs for the target pollutants were calculated using the concentrations herein obtained (expressed as $mg{\cdot}kg^{-1}$) and the data acquired for the same compounds in seawater (expressed as $mg{\cdot}L^{-1}$) (Cadena-Aizaga et al., 2021) for each seaweed and seagrass group at the same locations and times. The minimum and maximum values measured in the seawater samples were used for the BCR estimations (Table 3). The higher the BCR, the higher the concentration in seaweed in relation to seawater. The obtained BCR values indicated the grade of bioconcentration, thus is used to prove the reliability of macrophytes as bioindicators (Ismail and Ismail, 2017) (values over 1 are considered accumulation and those over 1000 indicate significant accumulation).

All the target compounds were detected in macrophytes, but some were absent in seawater. This can be explained by macrophyte uptake from their surroundings because they are exposed to the pollutant while they grew.

The maximum and minimum BCR values for the grouped seaweeds (red, brown, green) per beach are presented in Table 3. All the target compounds showed different grades of bioconcentration in all the seaweed types. This reflects that, although their availability is low in seawater, they are bioconcentrated.

Taking into account seaweed type, the BCR followed this order: green>red>brown. All the target compounds accumulated in the three analysed seaweed types, which indicates their availability in the aquatic phase.

When considering the BRC per beach, the highest values corresponded to BP3 on the Arinaga beach in the green seaweed, followed by OC on the Playa del Inglés beach and BMDBM on the Las Canteras beach in the brown seaweed (Table 3). In contrast, the highest BCR per seaweed type corresponded to BMDBM in the red seaweeds, to OC in the brown ones and BP3 in the green ones.

For the average BCR of all the seaweed types and beaches per compound, the highest values were for OC and the lowest for MBP. However, the highest BCR was calculated for OC in the seagrass *Cymodocea nodosa* on the Playa del Inglés beach.

The obtained results suggest that seaweeds greatly accumulate on all the studied beaches because the generally obtained BCRs were higher than 1000, which could increase the possibility of chronic effects on marine organisms due to biomagnification through the whole food web at the highest tropic levels (Jahan and Strezov, 2019). There was no specific pattern for organic UV filters bioconcentration, since the obtained BCR values varies according to compound, macrophyte and study area.

Seaweeds form an underwater forest that provides habitats and breeding areas for several organisms. They are also an important food source for organisms like sea urchins and gastropods. Nevertheless, the degraded biomass and released spores from seaweeds feed detritivore organisms like filter feeders and zooplankton. Hence seaweed bioaccumulation not only affects direct consumers, but also other organisms, which spells ecological concern (Wiencke and Bischof, 2012).

4. Conclusion

Macrophytes have been used as bioindicators for anthropogenic pollution in the marine environment (both organic and inorganic) because other than

Table 3
Bioconcentration ratios for the grouped seaweeds (red, brown and green algae) and seagrass in the three beaches.[c]

Sampling place	BCR						
	Compounds	Red seaweeds		Brown seaweeds		Green seaweeds	
		min[a]	max[b]	min[a]	max[b]	min[a]	max[b]
Las Canteras beach	4MBC	–	2,954,277	–	5,091,942	–	13,549,300
	BP3	<0.0001	<0.0001	18,686,463	15,585,306	161,214,946	11,894,887
	HMS	–	70,918,586	–	90,636,320	–	204,234,061
	DTS	20,328,206	15,754,142	10,415,351	10,020,673	35,403,056	9,580,505
	OC	108,655,838	28,525,247	22,371,274	34,792,732	46,241,998	37,129,302
	BMDBM	322,935,014	4,600,285	515,874,305	41,208,303	518,339,458	4,104,916
	IMC	8,229,375	828,440	7,897,248	795,005	29,706,937	4,720,496
	MBP	–	–	–	–	–	–
Arinaga beach	4MBC	–	29,932,490	–	<0.0001	–	<0.0001
	BP3	197,072,700	117,624,342	169,868,443	72,925,641	7,207,019,289	743,950,378
	HMS	–	–	–	–	–	–
	DTS	12,331,795	907,788,921	<0.0001	<0.0001	<0.0001	<0.0001
	OC	–	–	–	–	–	–
	BMDBM		3,759,494,178		859,718,048		727,870,984
	IMC	28,957,348	395,798,243	92,954,408	56,311,111	<0.0001	<0.0001
	MBP	–	6,635,749	–	1,132,012	–	<0.0001
Playa del Inglés beach	4MBC	–	–	–	–	–	–
	BP3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	10,673,247
	HMS	–	41,003,641		432,568,947	–	889,739,510
	DTS	–	–	–	–	–	–
	OC	–	2,187,817,967	–	2,274,016,027	–	967,550,768
	BMDBM	134,126,688	9,957,330	251,294,055	41,058,878	<0.0001	<0.0001
	IMC	<0.0001	<0.0001	60,361,334	2,261,972	<0.0001	<0.0001
	MBP	–	–	–	–	–	–
	Compounds	Seagrass					
		min[a]				max[b]	
	4MBC	–				–	
	BP3	<0.0001				<0.0001	
	HMS	–				224,728,489	
	DTS	–				–	
	OC	–				7,686,738,250	
	BMDBM	173,530,188				29,446,372	
	IMC	<0.0001				<0.0001	
	MBP	–				–	

[a] Calculated using the minimum value obtained in seaweed and seawater.
[b] Calculated using the maximum value obtained in seaweed and seawater.
[c] Indicated just for one specie in Playa del Inglés beach (*Cymopolia barbata*). The hyphen indicates that the compound was not detected in seawater; hence was not possible to calculate the ratio.

biomonitoring, they provide an approach to study the indirect effects of pollutants on the complete food web. Of all the different pollutants in the marine environment, organic UV filters are becoming a cause of emerging concern as they are widely used in a variety of personal care products. Hence they are constantly released to the environment, which renders them persistent and they accumulate. Despite these pollutants having been reported in several matrices, they have not been profoundly studied in macrophytes to date.

Therefore, this study presents the assessment of using macrophytes as bioindicators of UV filter pollution. Eight widely used organic UV filters were detected at least once among the 76 studied samples, and belonged to 14 macrophyte species (both seaweeds and seagrass) on three beaches. OC was found in all the samples throughout the sampling period all the three studied beaches. The highest concentration (19,369 $ng \cdot g^{-1}$ dw) was for the *Cymodocea nodosa* seagrass species.

Seasonal variation was detected, despite the beaches on the Gran Canaria Island being used almost all year round. However, this variation very much depended on the seaweed species.

The detection of all the target compounds in all seaweed types (red, brow, green) suggests that they can be used as bioindicators to monitor organic UV filter pollution over time.

BP3, OC and BMDBM were related to the highest bioconcentration ratios. Although different bioconcentration ranges were found, they were generally above 1000, which indicates a significant possibility of causing chronic effects on seaweed and other organisms at upper trophic levels.

CRediT authorship contribution statement

M. Isabel Cadena-Aizaga:: Experimental part, research, writing original draft

Sarah Montesdeoca-Esponda: Reviewing, discussion of results and editing

Ángelo Santana-Del Pino: Statistical analysis, Validation

Zoraida Sosa-Ferrera: Conceptualization, supervision, discussion of results, reviewing and editing; Funding adquisition

José Juan Santana-Rodríguez: Conceptualization, supervision, discussion of results, reviewing and editing; Funding adquisition

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Zoraida Sosa-Ferrea reports financial support was provided by University of Las Palmas de Gran Canaria. Sarah Montesdeoca-Esponda reports financial support was provided by University of Las Palmas de Gran Canaria. M. Isabel Cadena-Aizaga reports financial support was provided by University of Las Palmas de Gran Canaria. Angelo Santana-Del Pino reports financial support was provided by University of Las Palmas de Gran Canaria. Jose Juan Santana-Rodriguez reports financial support was provided by University of Las Palmas de Gran Canaria.

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Appendix A. Supplementary data

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