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Assessment of Leachate Generated by *Sargassum* spp. in the Mexican Caribe: Part 1 Spatial Variations

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Abstract: In this study, we evaluate the degradation by *Sargassum* spp. as a consortium in 2020 and 2021, and by species during 2021, collected at different distances from a coastline and in land deposits. The year 2021 had the largest leachate volume and the offshore site with the highest volume (60 mL/day) among five sites of collection. In relation to species' leachate generation, *S. fluitans* reached 47.67 mL/day as its peak, which is earlier than *S. natans* (41.67 mL/day 14 days after *S. fluitans*). pH shows alkaline behavior and EC reflects the saline condition in the leachate, the consortium and species reaching values of pH 7.5 to 8.3 and 80 to 150 mS/cm of EC; the results do not show significant differences among sites, or between species. Despite a BOD/COD ratio of less than 0.1, the degradation process occurs as evidenced by the presence of leachate. The results confirm the existence of a variability in leachate production and the composition of *Sargassum* under the influence of factors such as the periodicity, site of collection, and proportions of species. Thus, even though these results emphasize leachate generation, knowing the limitations of leachate generation is crucial information for decision making on *Sargassum* storage and environmental management.

Keywords: leachate; *Sargassum* spp.; *S. natans*; *S. fluitans*; Mexican Caribe; waste

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1. Introduction

Since 2011, several points along Mexico's Caribbean coast began receiving atypically large amounts of pelagic *Sargassum* [1]. In 2015, the massive amount of *Sargassum* increased drastically, registering in September a biomass of ≈2360 m^3 of macroalgae per Km, reaching 200 m wide on the coasts of Cancun and Puerto Morelos of the state of Quintana Roo. In July 2019, a volume of more than 10 million Tons of *Sargassum* was recorded, similar to that obtained in July 2015, which was 11 million Tons [2].

The Mexican Ministry of Environment and Natural Resources [3] stipulated the Technical and Management Guidelines, which establish the strategies for the collection of *Sargassum* in containment sites and beaches along the Mexican Caribbean and the Gulf of Mexico.

The economy of the Mexican Caribbean was affected by the large amounts of *Sargassum*, and it accumulates in tourist centers; in addition, removing *Sargassum* from the beaches or preventing it from reaching the beaches is very costly. Faced with this situation, the collection and commercial use of *Sargassum* has been proposed. There are several investigations on its possible implementation as a potential raw material for the production of fertilizers, fuels, and animal feed; however, their variable composition and the possible presence of marine pollutants limit these uses [4]. As a consequence, its valorization at a large scale has not yet been fruitful and the demand as a raw material is low; therefore, *Sargassum* mitigation actions have become almost non-existent, bringing with them its accumulation on the coast and deposit areas.

However, the accumulation of *Sargassum* presents a risk in the alteration of the environment where it is deposited. The process of the degradation and stabilization of organic matter results in the production of contaminant vectors, such as the generation of leachate, which is the liquid generated from the biochemical disintegration of organic waste, surface runoff, and infiltration of rain that by gravity crosses the thickness of the materials, carrying with it compounds in dissolved or suspended form [5,6].

Moreover, there is evidence that the presence and accumulation of large quantities of *Sargassum* at certain points release high concentrations of ammonium and hydrogen sulfide, and hypoxic conditions have been detected in the water where the algae are floating for a long time period. In addition, some macroalgae have a high capacity to absorb several contaminants, including *Sargassum* species. Therefore, it is important to analyze the decomposition processes that could release contaminants contained in *Sargassum* during the exposure media time [7,8].

Leachate is considered highly polluting waste so it has a negative impact on the environment [9]. It is characterized by a high content of organic matter, macro-components, and heavy metals, which cause adverse effects in the ecosystems, like eutrophication, which leads to the trophic alteration of surface and underground water bodies [10–12].

Although there is little information on the impacts and composition of *Sargassum* leachate generated per se, there is a distinction between leachate generated in landfills, given that it comes from a marine biomass. Likewise, there are indications that in the first days of generation, it can influence the development, immobilization, and resilience of corals [13]. On the other hand, it is known that the proportions between species of *S. fluitans* III, *S. natans* I, and *S. natans* VIII are variable; likewise, it has been reported that some of these species of *Sargassum* have a greater capacity for the bioaccumulation of pollutants [2,14].

Furthermore, the continental arrival region is characterized by a karstic system predominantly of carbonate rocks with high porosity and permeability due to the dissolution of the rock matrix, fractures, and the scarcity of soils, allowing for the infiltration of any pollutant fluid or pollutant dissolved in water [15,16]. Thus, karstic systems are highly vulnerable where attenuation processes of pollutants such as retention, mineralization, absorption, etc., are null or inefficient, so pollution is exacerbated [17].

The degradation of *Sargassum* spp. has been a concern since the rise in this biomass in the Mexican Caribe; however, the few studies related to this degradation have focused on the effect of its presence in ecosystems [13] and water quality parameters at the coastline [18].

Thus, the accumulation of fresh and residual amounts of *Sargassum* represents a potential generator of leachate that can affect water quality and thus affect water resources in a highly dependent groundwater area.

The aim of this study was to evaluate the generated leachate and its conditions regarding its freshness in relation to the distance to the coastline and as a dried residual having moved to continental deposits, as a *Sargassum* consortium, and to assess the influence of the species regarding this degradation.

2. Material and Methods

2.1. Study Area

We focused on the collection of *Sargassum* spp. for this study at the north part of the Mexican Caribe specifically at Puerto Morelos Country and Cancun, Quintana Roo, since this area has received an important influx of *Sargassum* since 2015 [19], affecting touristic beaches. Collection occurred in 2020, September 17 to 24, regarding the end of arrival in 2020 [20], and 2021 (as the initial influx of the year 2021—28 April to 3 May [21]).

The samples still floating on water were considered fresh, and the dried remains that accumulated on the beach and inland deposits were considered as residuals (as solid waste).

To assess the spatial variation, a transect of four points based on the distance to the coastline (Figure 1) was defined: (1) external fluxes of *Sargassum* spp. from 6 to 19.7 Km as Altamar (AL); (2) at the contention barriers (usually barriers vary between 300 and 450 m from beach) as Antes de Barrera (AB—before barrier); (3) over the barrier towards the beach as Despues de Barrera (DB—after barrier); the last floating fresh type was collected at the coastal line Playa (PL—beach); and finally the residual at inland deposits (DP), Figure 2.

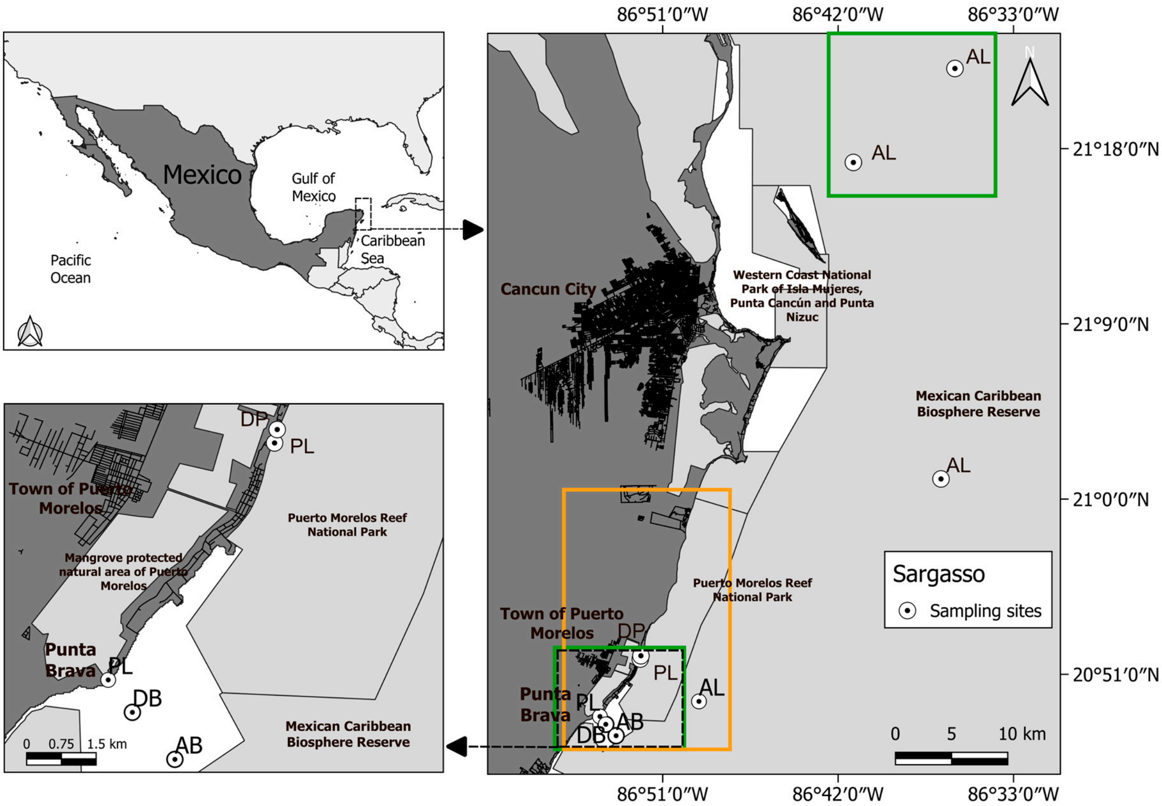

Figure 1. The map shows the location of the study area and the collection points for 2020 (green rectangles) and 2021 (orange rectangles), where AL represents the sites at 6 to 19 Km from the coast, AB before the barrier, and DB after the barrier considering the content of the *Sargassum* barrier located from 350 to 450 m from the coast, and PL refers to the accumulation at the coastline.

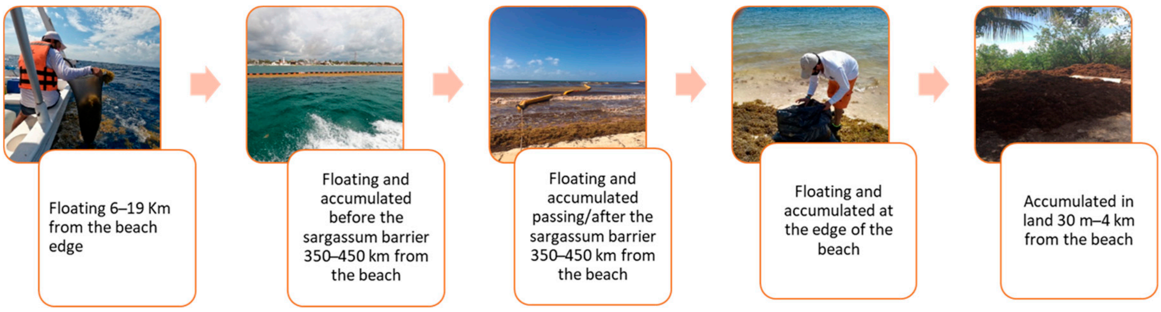

Figure 2. The figure presents the spatial distribution of the sites and description of collection points in relation to distance to the coastal line. Following the arrows from left to right, it starts with the farthest distance to end in a deposit inland. The deposit is considered to represent *Sargassum* as a residual (dry) and the rest of the pictures are where *Sargassum* is fresh.

2.2. Sargassum spp. *Collection*

All samples were collected in black plastic bags, transported to a laboratory, and distributed in the designed containers to evaluate the generation of leachate the same day as collection. Fresh samples (AL, AB, DB, and PL) were collected randomly and the dried samples (residual, DP) were collected based on quartile methodology [22]. Samples from 2020 were analyzed as a consortium; however, for 2021, it was decided to evaluate both *Sargassum* spp. as a consortium as well as species *Sargassum natans* and *Sargassum fluitans* (identified by morphological characterization [23]; the two main identified species in the Mexican Caribe) [24]. Consortiums are defined as the whole collected species of *Sargassum* at each and from all sites.

2.3. Experimental Design

Collectors of transparent polyetilene terephthalate (PTE) (named COLSAR) to evaluate the generated leachate at laboratory conditions at room temperature were adapted to be filled with *Sargassum* spp.; each site was evaluated in triplicate (see Figure 3a–c). During both 2020 and 2021, the leachate uptake was obtained from two different processes: (a) it was generated by the degradation of *Sargassum* spp. (called leachate per se), which is produced by its natural process of dehydration and degradation without adding water, and (b) the obtaining of leachate simulating rainfall (called leachate by percolation), to which an average volume of the recorded precipitation for the Quintana Roo state (of the past 10 years) was added to simulate precipitation. In 2020 and 2021, the first generated leachate was identified as per se, which corresponds only to fresh composite samples as seen in Figure 3a,b.

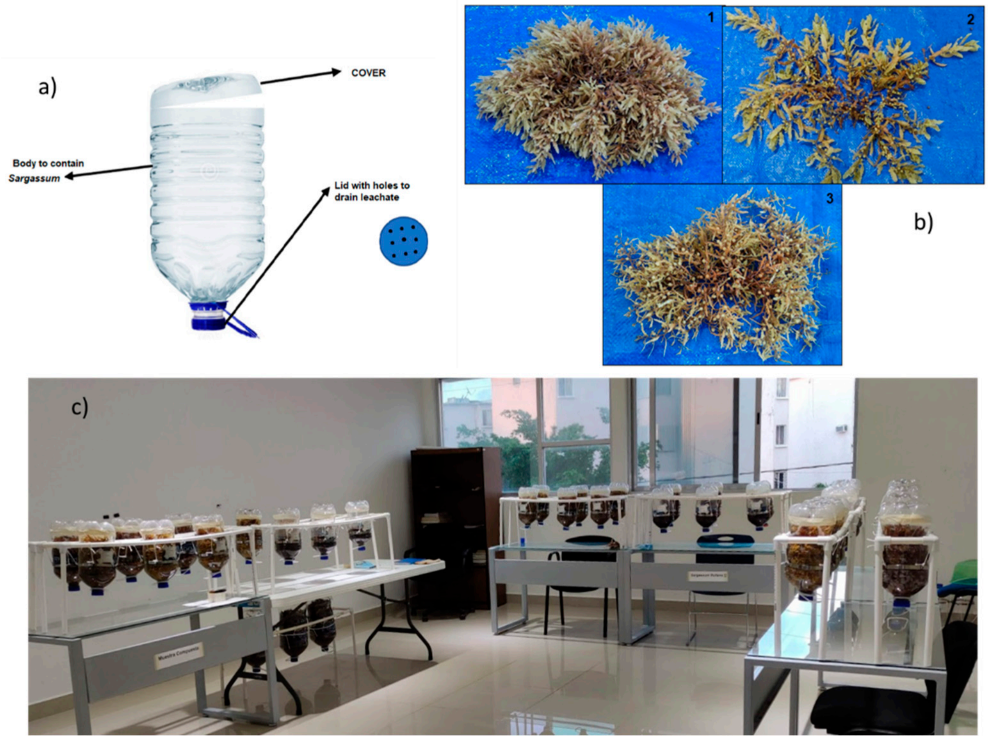

Figure 3. Representation of (**a**) COLSAR design, (**b**) *Sargassum* tissues as consortium and differentiated species (*S. fluitans* (b1) and *S. natans* (b2 and b3)), and (**c**) COLSAR filled with *Sargassum* spp. collected in triplicate per site.

In each COLSAR, one kilogram of *Sargassum* was added as a consortium in both 2020 and 2021; for 2021, besides the assessment of the consortiums, COLSAR with differentiated species (*S. fluitans* and *S. natans*) were evaluated in order to be able to compare the behavior by species. In the case of the DP site, species differentiation was not carried out, because the *Sargassum* had lost its structure due to moisture loss.

For all AL, AB, DB, and PL, the percolation process started when the per se process showed a decrease in the generated leachate volume. Since the *Sargassum* in DP conditions already underwent a natural dehydration process at the deposit sites, it did not present leachate per se; thus, in this case, DP only generated leachate by percolation.

The obtained volume of leachate was collected in a graduated cylinder and stored in high-density polyethylene (HDPE) bottles and refrigerated at 4 °C for a further laboratory analysis. It was decided to record the generated volume every third day due to the small volume of leachate per se obtained per day in each of the samples.

2.4. Differentiation of *Sargassum* spp.

The differentiation of species was carried out in the Water Sciences Unit, with the help of the illustrated guide for the identification of species of the genus *Sargassum* spp. of the application SargaZoom Mobile Application Version 1.1.0 [23]. This provides a gallery of images and morphological descriptions of the different species of *Sargassum* that arrive in the Mexican Caribbean.

For this work, the species of *S. fluitans* and *S. natans* were taken into account, since they are found in greater proportions in the *Sargassum* that reaches the coasts of Quintana Roo.

2.5. Characterization of Sargassum Leachate

As is recommended in the literature for organic waste, we used temperature (T), pH, electrical conductivity (EC), biochemical oxygen demand at day 5° (BOD_5), and chemical oxygen demand (COD) to know the degree of biodegradability of *Sargassum*.

We used a multisonde by Conductronic SA de CV Mexico model PC-18 to obtain T, pH, and EC readings directly in the samples at the moment of leachate collection. BOD_5 was developed following methodology of American Waste Water Association [25] using 30 mL of a sample per each Winkler bottle. Dissolved oxygen was measured by the membrane method by a Fisher Scientific Accumet XL600 Dual Channel DO Meter. COD was developed by commercial kit (LR COD 420 nm) CHEMetrics; a 2 mL vial of a sample was added, which reacts with an acid solution of potassium dichromate in the presence of the catalyst and was digested for 2 h at 150 °C in the digester model HB-1 of the brand Wealtec Corp. The vials were then read on a SMART Spectrophotometer UV-Vis spectrophotometer at the wavelength of 420 nm and the equation COD = (2301) $\times$ $(Abs)_{-3}$ was used to obtain the quantity of milligrams of oxygen consumed per liter of a sample (mg/L of COD).

The compositional analysis of *Sargassum* tissue was performed according to the procedures established by the National Renewable Energy Laboratory [26]. This procedure consists of an acid hydrolysis of the biomass in two phases: Regarding a quantitative acid hydrolysis in the first phase, 500 mg of a sample with 5 mL of sulfuric acid at 72% (v/v) was hydrolyzed in test tubes, constantly stirring for one hour at 35 °C, in a water bath; the mixture was transferred to a 250 mL bottle with an airtight lid and diluted by weight with distilled water to reach 148.7 g. The second phase was carried out in an autoclave, at 121 °C and 1 Kg/cm^2 of pressure for one hour. The solid fraction was recovered using Gooch filters (medium pore, 30 mL), considered as Klason lignin, and whose quantification was determined by cellulose and hemicellulose recovered from the liquid fraction, which was analyzed by HPLC (High-Performance Liquid Chromatography) in Agilent 1260 Infinity II equipment, with a column MetaCarb 87H (300 $\times$ 7.8 mm, Agilent) at 60 °C, flow of 0.7 mL/min with H_2SO_4 (5 mM) as the mobile phase, and refractive index detector, taking into account the concentration obtained from a standard curve of carbohydrates with glucose, cellobiose, xylose, and arabinose as standards.

3. Results

3.1. *Sargassum* Consortium's Leachate by per se Process

In the evaluation of leachate generated by *Sargassum* as a consortium, by the process identified as per se, the variation detected by year of collection and by site stands out, with 2021 being when the collection generated the largest volume and the offshore site (AL) being where this volume was the largest. Likewise, the sites of AB and DB can be identified as the sites where the peak volume is not greater than 20 mL compared to the 60 and 44 mL of peak volume generated by AL and PL; see Figure 4a–d.

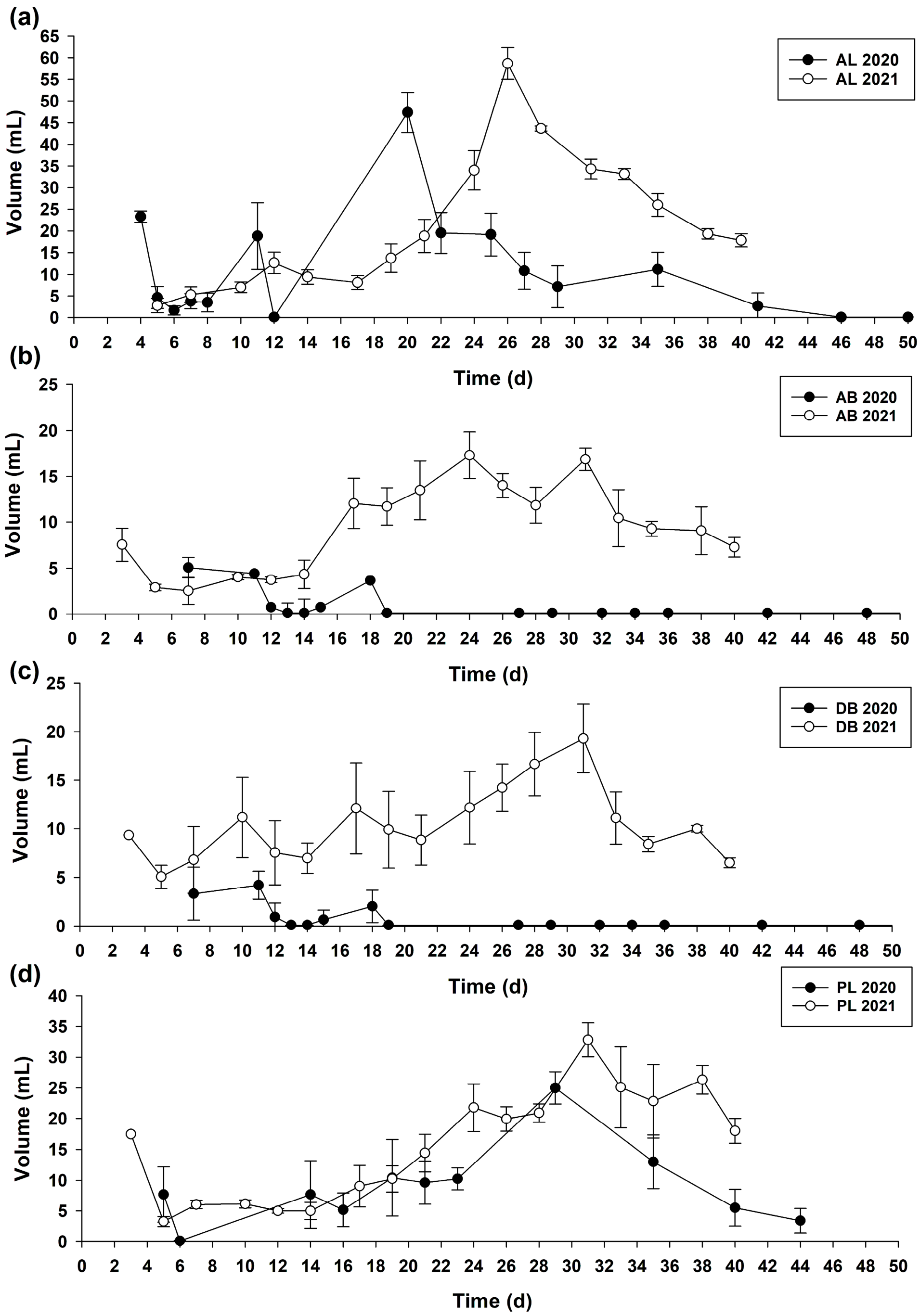

Figure 4. Variation volumes of leachate generated by the *Sargassum* consortium at different sites from 2020 and 2021 collections: (**a**) *Sargassum* collected from sites at 6 to 19 Km from the coast, (**b**) AB represents before the barrier, (**c**) DB represents after the barrier, and (**d**) PL refers to the accumulation at the coastline. Data represent means ± standard deviation of three measurements (n = 3).

In other words, there is a variation per site (*) identified as follows:

AL > PL > AB > DB.

In general, the per se process was performed for 46 days (*) and the following percolation process for 15 days. Although the experiment of the leachate generation of the *Sargassum* consortium dropped to reach the lowest generation at 42–46 days for both collections (2020 and 2021), the peak generation times present a variation that is presented in the following Table 1.

Table 1. Results of volume and peak days of leachate from *Sargassum* (consortium vs. species—2021) at different sites.

	Consortium 2020		**Consortium 2021**		***S. fluitans***		***S.natans***	
Site	**Vol., mL**	**Day**	**Vol., mL**	**Day**	**Vol., mL**	**Day**	**Vol., mL**	**Day**
AL	47.33	20	58.67	26	47.67	26	24.83	40
AB	5.07	4	17.30	24	36.83	17	30.80	31
DB	4.20	6	19.30	31	30.83	17	10.80	38
PL	25.00	20	32.83	31	42.17	17	41.67	38

3.2. Leachate by per se Process: S. fluitans and S. natans

As it was mentioned before, for 2021, we compare the production of leachate of the consortium versus species (*S. natans* and *S. fluitans*); thus, we include the characterization of the *Sargassum* tissue as it is presented in Table 2.

Table 2. Compositional analysis and elemental composition of *Sargassum* spp. tissues.

	Proximal Composition (%)						**Elemental Composition**				**Ref.**
Site	**H**	**VS**	**Ash**	**T.C.**	**Lignin-Like**	**Others**	**C**	**H**	**N**	**S**	
Consortium *Sargassum* spp. Puerto Morelos, Q.Roo 2020											
AL	12.98 ± 4.34	77.78 ± 1.95	22.22 ± 1.95	-	-	-	33.84 ± 1.05	4.71 ± 0.22	1.39 ± 0.22	1.21 ± 0.22	[27]
			21.13 ± 0.25	24.61 ± 0.77	29.52 ± 0.18	-	31.87 ± 1.87	4.92 ± 0.09	1.16 ± 0.02	0.98 ± 0.03	[26]
Consortium *Sargassum* spp., Puerto Morelos, Q.Roo, 2021											
AL	86.15 ± 0.56	77.37 ± 0. 82	22.63 ± 0.82	24.64 ± 0.69	37.96 ± 0.54	14.77	34.15 ± 0.35	3.23 ± 0.20	1.41 ± 0.02	1.70 ± 0.05	This study
AB	85.65 ± 0.50	72.95 ± 1.52	27.05 ± 1.51	18.49 ± 1.31	36.79 ± 0.76	17.67	27.60 ± 0.55	3.50 ± 0.19	1.59 ± 0.05	1.72 ± 0.02	
DB	83.65 ± 0.29	80.46 ± 0.81	19.54 ± 0.81	20.44 ± 1.67	41.24 ± 0.76	18.78	27.94 ± 0.29	3.70 ± 0.10	1.63 ± 0.04	1.59 ± 0.02	
PL	80.55 ± 4.12	78.68 ± 2.01	20.91 ± 0.56	19–53 ± 1.06	40.59 ± 0.02	18.97	28.12 ± 0.13	3.28 ± 0.12	1.50 ± 0.02	1.17 ± 0.02	
***Sargassum* spp., Puerto Morelos, Q.Roo, 2021**											
S. fluitans	12.10 ± 0.73	73.34 ± 0.62	21.63 ± 0.55	34.43 ± 0.01	25.40 ± 1.30	-	28.03 ± 0.11	3.24± 0.12	1.21 ± 0.05	1.39 ± 0.01	[28]
S. natans	11.10 ± 0.39	70.41 ± 0.62	16.63 ± 0.55	45.39 ± 0.12	29.50 ± 2.35	-	29.03 ± 0.01	3.47 ± 0.02	1.19 ± 0.15	1.28 ± 0.01	

Notes: All results are from a dry basis. The values are averages of triplicate samples with standard deviation ($p < 0.05$). T.C. = Total Carbohydrates, H = Humidity, VS = Volatile Solids.

Table 3 presents the percentages by species for 2020 and 2021; however, the evaluation by species was restricted to the collection of 2021, so it is compared only with the consortium of the same year.

Table 3. Identified percentage of *Sargasuum* species *S. fluitans* and *S. natans* from AL, AB, DB, and PL during 2021.

Species	AL	AB	DB	PL
S. fluitans III	78.4%	36.9%	14.9%	48.2%
S. natans	21.7%	63.1%	85.1%	51.8%
	100.0%	**100.0%**	**100.0%**	**100.0%**

With regard to the volumes generated, in both periods (2020 and 2021), the consortium and the species maintain the trend detected by site. However, it is notable that it is the species *S. fluitans* that generates a higher peak overall compared to *S. natans* (see Figure 5a–d).

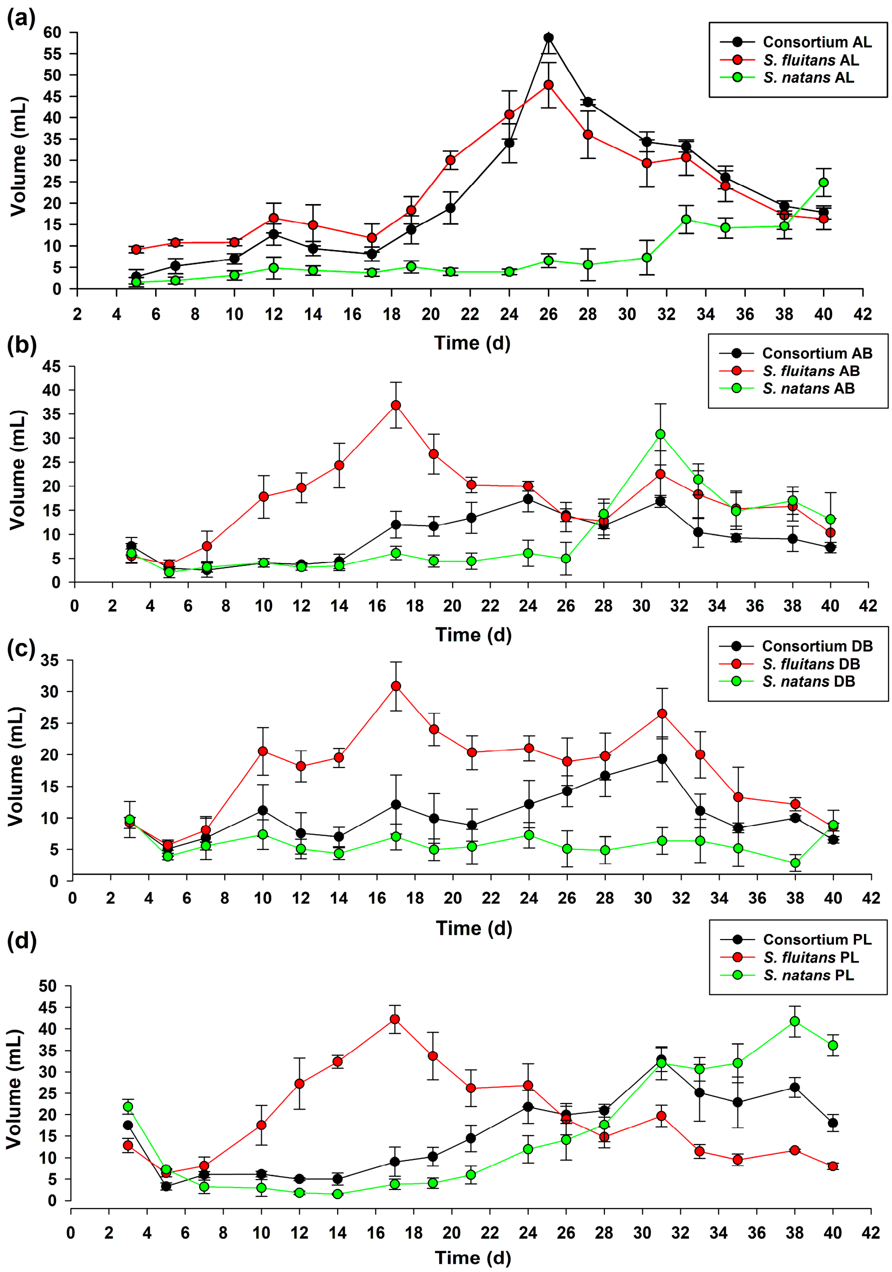

Figure 5. Variation volumes of leachate generated by the *Sargassum* consortium vs. species at different sites from 2021 collections: (**a**) Sargasssum collected from sites at 6 to 19 Km from the coast, (**b**) AB represents before the barrier, (**c**) DB represents after the barrier, and (**d**) PL refers to the accumulation at the coastline. Data represent means ± standard deviation of three measurements (n = 3).

The peak generation times present a significant variation between species with respect to the consortium. Peak times are similar among *S. natans* and the consortium; however, *S. fluitans* presents shorter times for its production peaks (see Figure 5a–d).

Similarly, the AL site continues to maintain the highest volume both in consortia and by species. This suggests that the conditions of *Sargassum* away from the coast maintain not only the characteristics of greater use as some authors have already mentioned, but that it is the one that when degraded will also generate the highest proportion of leachate.

3.3. Leachate Characteristics: pH and EC

Results of pH and EC for the periods of 2020 and 2021 do not show significant variation among sites (see Table 4). Furthermore, the pH results show values in the range of 6.02–6.99, one unit lower than those of 2021 where the range was 7.69–7.97; moreover, for 2021, they show a slightly alkaline trend (7.9). Regarding the EC comparing the two periods, the reported results are lower during 2020 compared to 2021 where the results are a magnitude higher; however, no variation between sites is detected for the consortium results.

Table 4. Average of pH and CE results of the 46 days from per se process of *Sargassum* species *S. fluitans* and *S. natans* from AL, AB, DB, and PL during 2021.

Consortium		Site			
		AL	**AB**	**DB**	**PL**
2020	**pH**	6.98	6.88	6.02	6.99
	EC mS/cm	53.97	74.52	72.54	85.57
2021	**pH**	7.69	7.97	7.92	7.82
	EC mS/cm	106.04	119.44	122.58	123.75
S. natans	**pH**	7.88	7.96	8.00	7.85
	EC mS/cm	106.43	113.19	112.13	120.75
S. fluitans	**pH**	7.32	7.94	7.89	7.80
	EC mS/cm	113.92	124.72	126.49	125.46

Regarding the behavior of pH between species, the results do not show significant differences among sites, or between species (*S. natans* and *S. fluitans*), as present with the consortium. In other words, the behavior between species and as a consortium does not show variations; however, each site has its own tendency as seen in Figure 6.

Considering the development of the EC, the results indicated that 2020 (58.97–85.57 mS/cm) was lower than that reported during 2021 (122.91–147.12 mS/cm), where AL recorded the lowest value and the PL was always with the highest value as the consortium. On the other hand, *S. fluitans* is the species with higher values in comparison with *S. natans*; however, as seen in Figure 7a–d, the consortium integrates both behaviors, and thus *S. fluitans* does not exceed those reported as a consortium. Regarding the variation among sites, the initial values of EC present a range of 80 to 100 mS/cm, which are increasing along the 44 days until they are reaching values of 120 to 150 mS/cm. This is true for AB, DB, and PL sites but not for AL, which presents a behavior where the highest values of EC are decreasing and also coincide with the peaks of generation (see Table 4).

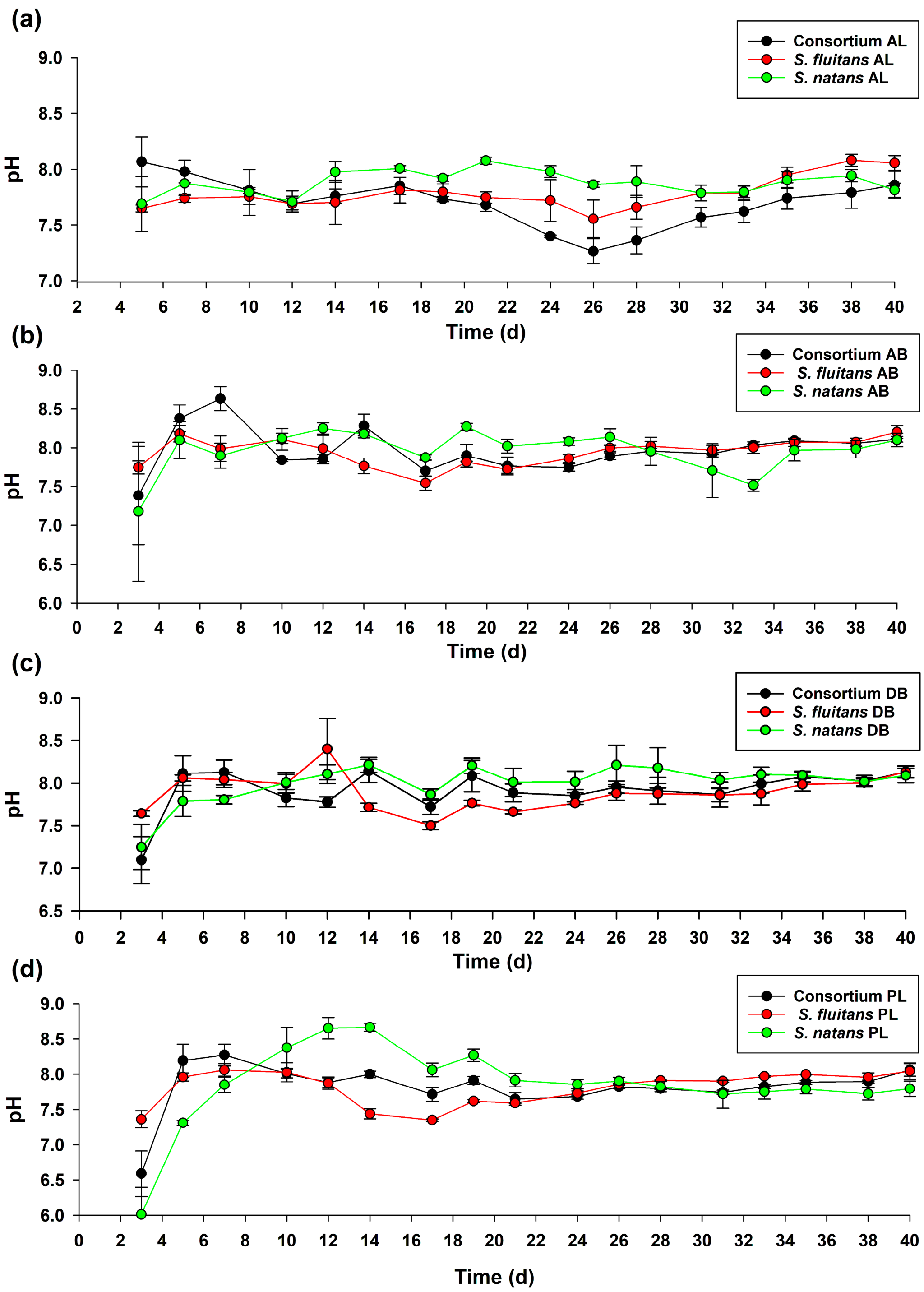

Figure 6. The behavior of the pH variation volume of leachate generated by the *Sargassum* consortium vs. species at different sites from 2021 collections: (**a**) *Sargassum* collected from sites at 6 to 19 Km from the coast, (**b**) AB represents before the barrier, (**c**) DB represents after the barrier, and (**d**) PL refers to the accumulation at the coastline. Data represent means ± standard deviation of three measurements (n = 3).

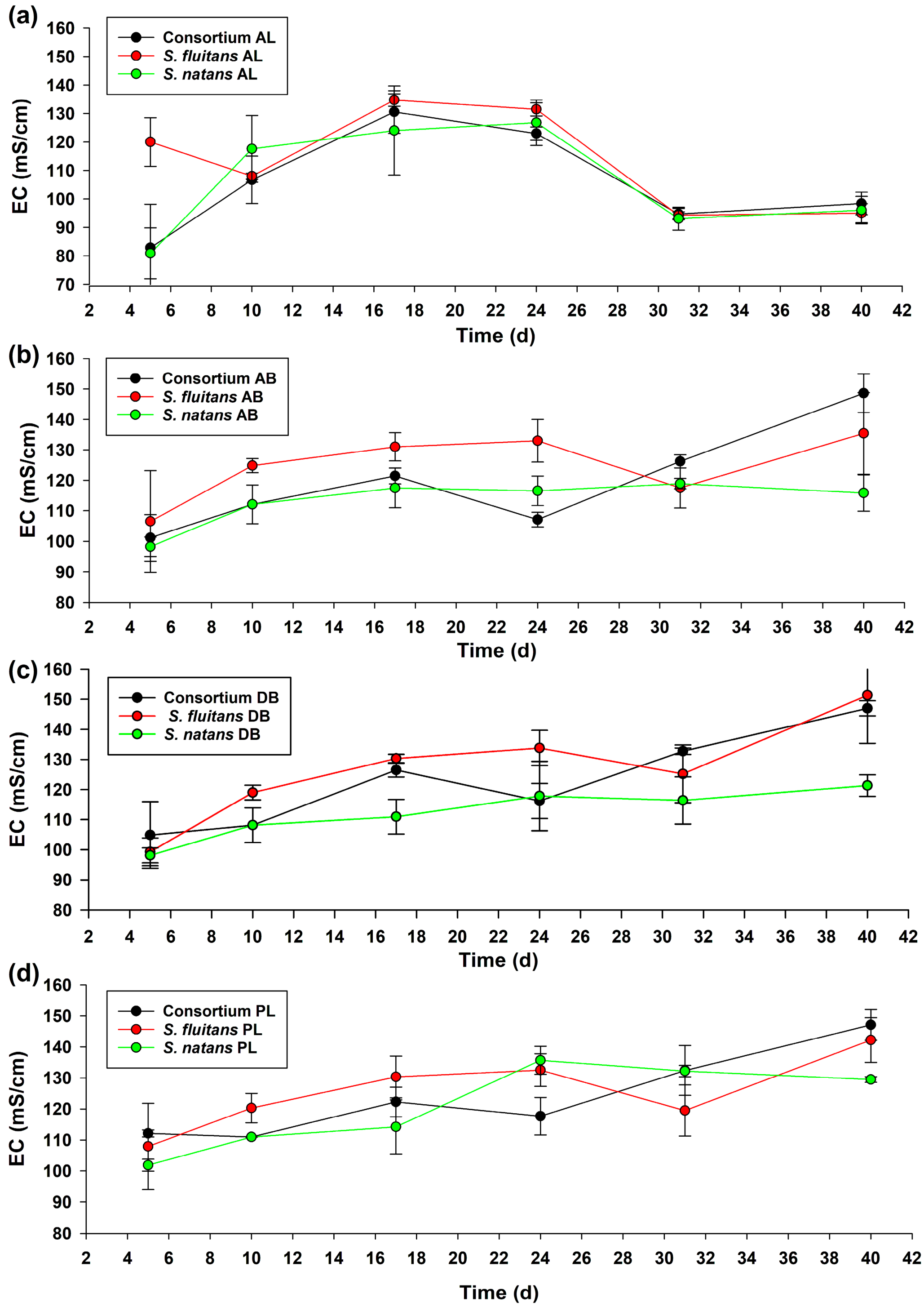

Figure 7. The behavior of the EC variation volume of leachate generated by the *Sargassum* consortium vs. species at different sites from 2021 collections: (**a**) *Sargassum* collected from sites at 6 to 19 Km from the coast, (**b**) AB represents before the barrier, (**c**) DB represents after the barrier, and (**d**) PL refers to the accumulation at the coastline. Data represent means ± standard deviation of three measurements (n = 3).

3.4. Biodegradability of Sargassum: Consortium and Species

For this parameter, we focused on the oxidation of carbon of an organic origin by microorganisms (BOD_5) and the corresponding oxidation as COD of inorganic carbon.

Concerning BOD_5 as the consortium, the results for 2020 (169–234 mg/L) are higher than the results obtained in 2021 (51–88 mg/L). On the other hand, COD results remain similar in both periods: (1717–4267 mg/L) in 2020 and (1483–3190 mg/L) in 2021. This indicates that there is no significant variation in biodegradation per period of collection; see Table 5. Even though the results of the BOD/COD relationship suggest that the biodegradability of *Sargassum* should be low in comparison with ratios of waste reported by [29–31], the obtained results indicate that the biodegradation process not only takes place but also behaves differently, suggesting that not only the factors of carbon proportion, temperature, and humidity affect the process but also the main origin (sea water) with a high concentration of COD and high values of conductivity.

Table 5. Average COD and BOD_5 results of the 46 days from per se process of *Sargassum* from AL, AB, DB, and PL during both 2020 and 2021.

	Site			
Consortium	**AL**	**AB**	**DB**	**PL**
2020				
BOD5, mg/L	233	234	232	169
COD, mg/L	1717	4267	3916	1659
BOD/COD	0.136	0.055	0.059	0.102
2021				
BOD5, mg/L	82	68	88	51
COD, mg/L	3190	1483	2000	3047
BOD/COD	0.026	0.046	0.044	0.017

3.5. Biodegradability by Species: S. natans and S. fluitans

Results determined for these parameters by species indicated in both species that the highest values belong to the AL site and they decreased among the sites getting closer to the coast (AB, DB, and PL), and each parameter range remains in the same magnitude; see Table 6. Regarding the BOD/COD relationship, the results are lower than those reported for the consortium; thus, biodegradability would remain low [2,27].

Table 6. Average COD and BOD_5 results of the 46 days from per se process by species from AL, AB, DB, and PL during both 2020 and 2021.

	Site			
Species	**AL**	**AB**	**DB**	**PL**
S. natans				
BOD5, mg/L	81	61	72	78
COD, mg/L	2807	2833	2097	2497
BOD/COD	0.029	0.022	0.035	0.031
S. fluitans				
BOD5, mg/L	82	19	27	53
COD, mg/L	3270	910	1160	1313
BOD/COD	0.025	0.021	0.023	0.041

The BOD/COD ratio is similar between AL and PL sites, as well as AB and DB, with values less than 0.1 (Tables 5 and 6) for 2020 compared to 2021, for which the same sites have a lower ratio. On the other hand, the biodegradability ratio for 2021, whether as a consortium or species, presents values between 0.02 and 0.04; this indicates that biodegradability can remain in similar ranges between sites, as long as they are from the same collection period. These values are below the results obtained in municipal solid waste leachates. The literature reports that a ratio of 0.4 is necessary when biodegradability conditions are present [29], and according to the results obtained in this study, biodegradability should not occur; nevertheless, *Sargassum* leachate is obtained. Figure 8 shows how the BOD/COD ratio is very low, because BOD_5 values are low compared to high COD values.

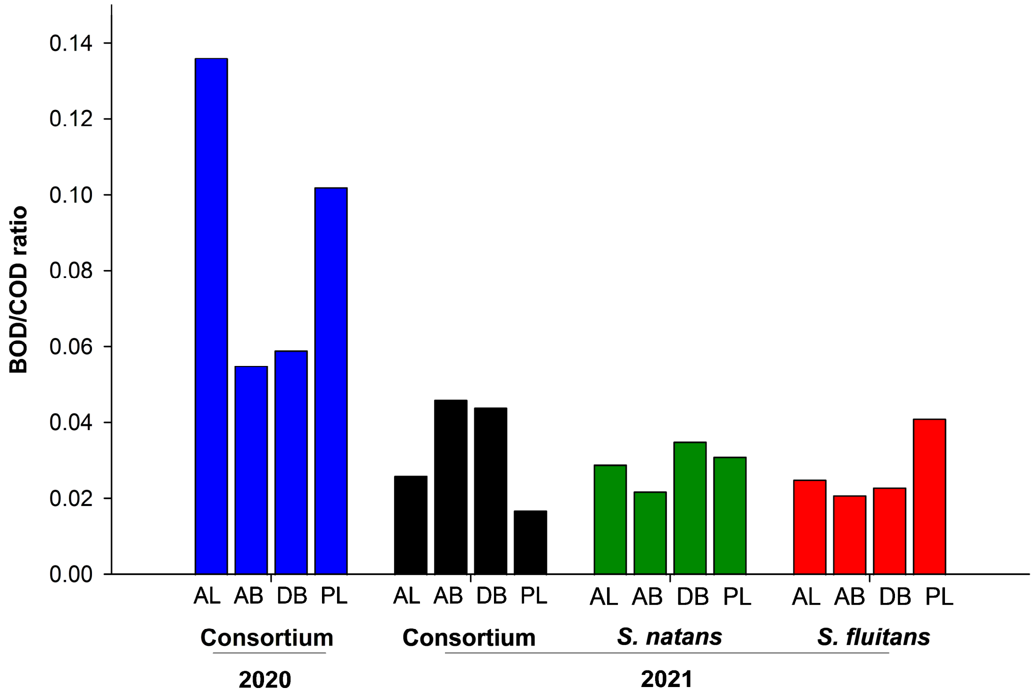

Figure 8. A representation of variability of the biodegradation ratio per site as the consortium vs. species. AL represents the sites at 6 to 19 Km from the coast, AB before the barrier, and DB after the barrier considering the content of the *Sargassum* barrier located from 350 to 450 m from the coast, and PL refers to the accumulation at the coastline.

3.6. Percolation Process

As it was mentioned once, the per se process reached its lowest volume, and the addition of water Type 1 was initiated to replicate the rain (84 mL per day) that would drain through the *Sargassum* in COLSAR.

Results from percolation were very similar regardless of the collection site (including deposits); the leachate drainage had a collectible volume 3 days after the start of water addition, until an outlet volume similar to the added volume was reached. This is indicative that *Sargassum* achieved its rehydration as occurring with other leachates generated in domestic solid waste deposits [32,33]. From this point, the volume generated stabilizes as a function of the aggregate volume, i.e., the percolation leachate depends on the milliliters of rain that can drain.

On the other hand, the pH gradually, from fresh sites (AL, AB, DB, and PL), decreased over time, maintaining an alkaline range of 7.9 to 8. On the contrary, the DP site starts with a pH of 6.7 and reaches a pH of 8.7 at the end of the experiment.

Regarding the EC, the five study sites showed a similar behavior since the values tended to decrease over the time that the leaching lasted, due to the entrainment of salts in the water that percolated through the samples, having an average value of 30.5 mS/L at the end of the process.

However, the DP site had the highest EC values, presenting a value of 154.5 mS/cm at the beginning of leachate generation and 48.3 mS/cm at the end of the process.

3.7. Percolation Leachate by Species

The generation of leachate by percolation by species at the fresh sites showed a similar trend for *S. natans* and *S. fluitans*. There was a sharp rise on the second and third day; however, both species presented a more stable generation at the end of the evaluation, and thus there was not different behavior between both species. The pH showed a narrow range for *S. natans* (7.72 to 8.09); in the case of *S. fluitans*, the pH presented a slight decrease at the end of the evaluation (8.17 to 8.08 except for PL site at 7.66 to 8.04). In the case of EC, this parameter presented a decrease at the end of the evaluation in both species for most of the sites but not the DB site.

Thus, the behavior of the pH and EC in the percolation process is not only related to the dilution (by the percolation of water) but also to the stabilization of degradation towards the final periods of contact with water. During the percolation, the EC tended to gradually decrease at all sites. Yet, these values are above those reported from the per se process reaching a greater magnitude, which may be due to the fact that the passage of water leaching tends to drag or wash more solids that influence the final result.

4. Discussion

4.1. Variation of Volumes per Year and per Site

The leachate production from the *Sargassum* consortium collected in 2020 and 2021 shows variations between one collection and another. Noticeable differences were obtained in the quantities of leachate volumes generated; moreover, late leachate was noticeable in the production dynamics for all cases of *Sargassum* collected in 2021 with respect to the 2020 *Sargassum* samples. Furthermore, the cumulative volumes generated with the samples collected in 2020 were lower compared to the samples collected in 2021, specifically with respect to the collection sites.

Particularly, the *Sargassum* consortium of AB and DB generated smaller volumes compared to sites AL and PL, for both collections. In this sense, this behavior suggests a variation in the content of the *Sargassum* biomass with respect to the sites due to the structural and composition modifications of the *Sargassum* during its journey through the route of origin, North Equatorial Recirculation Region [34].

In addition to the quantities that arrive every certain period, it has been observed that there are changes in the composition of *Sargassum* according to the temporality of the collection, as well as the incidence of one or another species (or subspecies) [2,35–37].

One of the main parameters that influence the leaching of an organic waste is the moisture content, which encourages the microorganisms present during the degradation of the biomass to act in the different stages of digestion [35].

In some species of *Sargassum*, it has been seen that a drying and rehydration process occurs during their stay in the tide and that influences their carbon fixation and the content of the carbon dissolved in the medium; this would justify the influence of the humidity present in the samples of *Sargassum* collected in the different sites, as well as the influence of its degradability [38].

The set of aerobic conditions accelerates the process of the degradation of organic matter, solubilizing it and generating leachate [29]. On the other hand, [31] mentions that, initially, organic substances can be oxidized to CO_2 and H_2O in the presence of oxygen; later, with the reduction of oxygen, they pass through a hydrolysis phase, in which the contained humidity of the waste increases and gives way to a process of the dissolution of organic matter [39]. Moreover, it has been pointed out that in the processes of hydrolysis of seaweed, during the first weeks of the process, there is a large release of soluble organic matter [40], so this would explain the gradual increase in the volume of leachate generated. Similarly, other studies [41,42] found that the solubilization of organic waste components occurs in the early stages of degradation and, as the process stabilizes, leachate generation decreases. In the present experiment, the behavior in the generation of leachate was similar to the results of the aforementioned studies, establishing that at the beginning of the process of degradation of *Sargassum*, there was a rapid degradation of organic matter and, over time, the concentration of biodegradable compounds decreased, as well as the moisture present in the *Sargassum*, which in turn reduced the volume of leachate generated.

As was mentioned before, the production of leachate from a waste is an indication of a biochemical decomposition of organic matter, where parameters such as humidity, pH, and temperature are crucial for the digestion stages to be efficient [35,43], which indicates that the behavior in the dynamic production of leachate is closely related to the microorganisms associated with the decomposition of organic matter [35]. The process of the biochemical degradation of waste occurs in four main phases, hydrolysis, acidogenesis, acetogenesis, and methanogenesis [44], which are carried out by specialized microorganisms, under specific conditions for each stage, and under different biomass origins. There is a difference between the decomposition of a marine and a terrestrial biomass, since they are from different environments; a marine biomass has resistance to salinity, and consequently different microbial ecosystems are involved in the stages of their degradation [45,46]. In this digestion, there is an assimilation of the main sources of proteins, carbohydrates, and fatty acids, where, in addition to generating a liquid fraction with soluble organic matter, a biogas is generated in the last phases, which is a mixture composed of CH_4 and CO_2, and to a lesser extent H_2S, O_2, and H_2 [47]. The composition of the organic matter dissolved in a leachate generated by decomposition, as well as the proportions of the gases generated, may vary depending on the composition of the decomposing organic matter, rate, and microbial activity, as well as the conditions of the environment; in the case of *Sargassum* sp. during its decomposition, it is possible that H_2S may prevail since its main polysaccharides include sulfated carbohydrates [48].

Thus, we hypothesize that the variations in leachate production performances are due to differences in tissue composition found at each collection site, so more detailed analyses were performed on the samples collected in 2021. Table 2 presents the proximal characterization of the *Sargassum* consortium collected in 2021 in the four sites, where in the first instance, the percentages of humidity are gradually reduced, taking in the function of the collection distances, from the high seas (86.15%, AL) to the coast (80.55%, PL), so that the organic contents (volatile solids) are also variable with respect to the collection sites, which coincides with Zhao et al. [38], who mention that the changes caused by dehydration and rehydration during their residence at sea are related to the carbon content. Carbohydrate content is variable in the *Sargassum* consortium according to the collection site; the AL samples contain the highest percentage of total carbohydrates with 24.64% and since in the early stages of a biochemical degradation, hydrolysis of the main carbon sources occurs [44], which in the case of *Sargassum* are the carbohydrate biopolymers in these samples, a greater volume of leachate was obtained (58.67 mL), while with the AB samples containing the lowest percentage of carbohydrates (18.49%), the lowest volume of leachate (17.3 mL) occurred on day 24 of experimentation.

On the other hand, regarding the presence of polyphenolic compounds (lignin-like) in *Sargassum* tissue, it represents a main system of the stress response and recalcitrance to degradation [28], so the high "lignin-like" content in DB samples (41.24%) gives them greater recalcitrance compared to the other samples, thus causing less degradation and low leachate production. Additionally, according to the spatial distribution of the collection sites, variations were presented in the content of these polyphenolic compounds. In the tissue collected in AL, 37.96% was obtained; AB, 36.79%; DB, 41.24%; and the samples collected from the beach, 40.59%. Since these compounds play an important role in the biosorption process of metallic contaminants [14], it is possible to relate the increase with the content of the inorganic material (ash content) with respect to the *Sargassum* path, so that in samples where the "lignin-like" content is higher (AB with 41.24%), there is the lowest percentage of ash content (19.54%) in relation to the other sites. Finally, a percentage of content not yet identified (determined as "other") also shows an increase in spatial distribution from the high seas (14.77%) to the coast (18.97%).

Regarding CHNS elemental analysis results, a percentage of carbon content of 34.15% was obtained in the samples of the *Sargassum* consortium collected offshore (AL), which is similar to those reported by other authors [27,49]. As for the AB, DB, and PL samples, they presented a carbon content of 27–28% similar to that reported by [26]; however, as for the C:N ratio, in AL and PL, a ratio of 24:1 was obtained, which falls in the ideal range for the optimal digestion or fermentation of organic matter [27], which does not happen with samples collected in AB and DB as they have a C:N ratio of less than 20:1.

These variations were presented in the *Sargassum* consortia; however, in the case of the species *S. fluitans* and *S. natans* (which have been reported as the most abundant [50,51]), it is observed in Table 3 that the percentages of *S. natans* are higher in the samples collected in DB and AB with 85.1% and 63.1%, respectively, while the samples collected in AL present a higher percentage proportion of *S. fluitans* (78.4%). As for their part in the PL consortium, 48.2% was registered for *S. fluitans* and 51.8% for *S. natans*, which shows according to the sites where the consortium resides and that the populations of the species are variable. In an evaluation of the same type of collection in 2021, Alzate-Gaviria et al. [28] report that the "lignin-like" content is higher in *S. natans* compared to *S. fluitans*, and since these compounds give it the recalcitrant characteristic, it is possible to assume that by containing a greater presence of *S. natans*, the samples collected in AB and DB caused a greater resistance to biodegradation and therefore a lower volume of leachate, contrary to what happened in the sample collected in AL.

The degradation of species present in the consortium affects the behavior of the generation of leachate, which is evident in the case of *S. fluitans*, since in the consortium where the percentage of this species predominates, the behavior between the leachate generation processes of the species is very similar to the consortium process, Figure 5a, which does not occur when the predominate percentage is that of *S. natans*.

From the results observed in Figure 5b,c, *S. natans* alone presented a variable leachate production while in *S. natans* in AB, 30.8 mL was produced on day 31, and in *S. natans* in DB, only a maximum volume of 10.8 mL was reached in 38 days (Table 1). In the case of *S. fluitans* collected in AB and DB, the highest peak in leachate production was generated on day 17 (Figure 5b,c). This suggests that the generation of leachate is a function of the percentage proportions of the species present in the consortium, as well as the synergy that exists between the microorganisms involved in the decomposition of each species (Figure 5d).

4.2. Leachate Characteristics: pH and Electric Conductivity (EC)

Overall, pH monitoring during leachate generation is indicative of a distinction in the performance of the microbiota associated with the degradation of *Sargassum* as a consortium, and consequently in its degradation by species. The behavior in the results of the pH measurements in the leachate generated from *S. fluitans* in AL indicates a lower pH (7.2) on day 26 of collection (Figure 6a), coinciding with the highest point of leachate volume production at the same site (AL) but as a consortium, due to the largest amount of *S. fluitans*. On the contrary, it is observed that the lowest pH, in the leachate generated with *S. fluitans*, was obtained for the AB, DB, and PL sites (Figure 6b–d), recorded on the days with the highest volume production, because there is an adaptation of the microorganisms involved in the digestion of the biomass to the initial pH conditions; once adapted by acidogenic microorganisms, the pH is gradually reduced, so that it is in a pH range of 6.5–7.5, as reported by [52], and this agrees with when optimal biomass hydrolysis conditions are reached.

Although pH is an important parameter for the development of microorganisms of the different stages of degradation, the nature and chemical composition of a biomass influences which genera and microbial species may be found, so that in a residual biomass with a higher proportion of cellulose, there will be greater diversity of cellulase-producing species [35]; in this sense, given the information on the difference in carbon sources between *S. fluitans* and *S. natans* [28,50], the microorganisms responsible for degrading the tissue of *S. fluitans* may be different from those responsible for the degradation of *S. natans*, so a marked decrease in pH was not observed and that is reflected in the slow production of leachate with *S. natans* with the exception of site AB, where it is observed that after 26 days, there is a more noticeable reduction in pH and increase in the volume of leachate.

In this sense, the microorganisms responsible for degrading the tissue of *S. fluitans* may be different from those responsible for the degradation of *S. natans*, so that a marked decrease in pH was not observed and that is reflected in the slow production of leachate with *S. natans* with the exception of *S. natans* in AB, where it is observed that after 26 days (Figure 6a), there is a drastic reduction in pH and increase in the volume of leachate. In Figure 6d, it is observed that in the leachate of the PL samples starting at pH 6 for *S. natans*, pH 7.2 for *S. fluitans*, and an average pH value of 6.5 in the consortium and that during the degradation of the biomass both by species and in the consortium, the pH of the leachate increased gradually during the first days until about 20 days, and the pH was stably maintained the rest of the days of experimentation, suggesting the difference in carbon sources between *S. fluitans* and *S. natans* [28,50].

In Figure 6d, it is observed that in the leachate of the PL samples starting at pH 6 for *S. natans*, pH 7.2 for *S. fluitans*, and pH 6.5 in the consortium and that during the degradation of the biomass both by species and in the consortium, the pH of the leachate increased gradually during the first days until about 20 days, and the pH remained stable the rest of the days of experimentation. Generally during the first phases of decomposition of the matter, the pH of the leachate is less than 7 due to the acids generated during the stages of hydrolysis and acidogenesis; however, with respect to time, the pH increases due to the consumption of these acids by acetogenic microorganisms, and once the pH range 6.8–7.4 is reached, it is possible to obtain the highest load of dissolved substances, which is optimal for methanogenic microorganisms involved in anaerobic digestion systems [44,53]; consequently, the volume of leachate was reduced by going from an acidic to alkaline pH in coincidence with that reported for *Sargassum* leachate [49] given its bioconversion of the material dissolved in the liquid fraction to biogas during the methanogenic stage.

The electrical conductivity denotes the salinity of the solubilized compounds, providing valuable information as to the maturity of the leachates, that is, if they are suitable for use as a substrate. In this study, the EC results are presented in Figure 7 (data corresponding to the days indicated for leachate collection, i.e., it is not accumulated leachate). The behavior of the EC both for the leachate generated as a consortium and species of the sites AB, DB, and PL is observed as upward behavior coinciding with the times of the greatest production of the leachate volume, followed by a reduction in EC (Figure 7b–d).

The literature indicates that it is common for the EC to present an increase with respect to time, which is related to the increase in the concentration of degradation products of complex organic compounds [54]; in this case, the EC values are higher than in the literature for dump site leachate, in landfills of continental waste, so it is suggested that the EC is higher in this case due to the marine origin of the biomass. On the other hand, for AL, there is a decrease in EC (both consortium and species) during the last days of generation; this coincides with the volume and moreover this can be considered as an accelerated degradation of biomass [41]. In the case of fresh *Sargassum* in AL, this is reflected to a greater extent with leachate production.

4.3. Biodegradability

In general, the leachate shows a low BOD/COD ratio due to the difference in BOD_5 and COD values. In 2020, in the four sites, the BOD/COD ratio is less than 0.3 and therefore the leachate presents low biodegradability conditions; by 2021, the ratio reduces its value even in the leachate generated by species, which do not exceed what was found as a consortium. Given these values, a very low volume of leachate and a slow biodegradation process would be expected, and this relates to the presence of inorganic carbonate where COD values present a very high difference with respect to BOD_5 values, becoming up to 35 times higher. Due to this, the BOD/COD ratio is lower and therefore the biodegradability conditions would be limited if the waste were of a terrestrial origin [44].

High COD values may be attributed to the presence of the intermediates of microbial metabolism, such as proteins and polysaccharides, released during the decomposition of organic material [44,55]. In the degradation of municipal or food waste, the decrease in the concentration of BOD_5 and COD in their effluents indicate an increase in the age of these leachates [56]. Therefore, in effluents where COD is in a range between 5000 and 10,000 mg/L, they are called old leachate when the BOD/COD ratio> 0.5, 0.3–0.5

for a medium age and <0.3 for young leachate [44,55]. Therefore, the results obtained demonstrate an intense microbial activity, which caused high biodegradability, presenting characteristics of young leachate compared to mature compost leachate, more stable and less active [55]. Although the concentrations of organic matter in *Sargassum* leachate are lower than those reported for urban waste that requires treatment before being destined for reuse, other physicochemical parameters, such as pH and EC, indicate that these liquids cannot be disposed of for reuse, so it is important to continue with more robust studies that allow establishing an adequate treatment and correct disposal of these wastes and their leachates.

5. Conclusions

5.1. Per Se

In the evaluation of leachate generated by *Sargassum*, the variation detected by year of collection and by site stands out, with 2021 being when the collection generated the largest volume and with the offshore site (AL) as the consortium. In both collections (2020 and 2021), the consortium and the species maintain the trend detected by site. However, it is notable that *S. fluitans* generates a higher peak overall compared to *S. natans*.

Regarding the peak generation time, it presented a significant variation between species with respect to the consortium. Similarly, the AL site continues to maintain the highest volume both in consortia and by species at its peak time.

As for the consortium, the pH and EC results were lower in 2020 than those of 2021; moreover, for 2021, pH results showed an alkaline trend, and for both parameters, no variation between sites is detected for the consortium results. However, results of pH between species do not show significant differences among sites. *S. fluitans* is the species with higher EC values than those of *S. natans*; however, it does not exceed those reported for the consortium.

For the consortium, the BOD_5/COD ratio is similar among sites, with values less than 0.1 for 2020 compared to 2021. Moreover, as species' BOD_5/COD ratio was lower than those reported for the consortium, biodegradability would remain low. Even though these values are below the results obtained in municipal solid waste leachates, the biodegradation process goes beyond taking place, suggesting that not only the factors of carbon proportion, temperature, and humidity affect the process but also its sea origin as pelagic *Sargassum* with a high concentration of COD and high values of conductivity.

5.2. Percolation

Once the residual *Sargassum* located at deposit DP achieved its rehydration, the volume generated stabilized as a function of the aggregate volume; thus, the percolated leachate is a function of the milliliters of rain that it can drain.

There was not different behavior in between both species and the consortium. Thus, the behavior of the pH and EC in the percolation process is not only related to the dilution by the percolation of water but also to the stabilization of degradation towards the final periods of contact with water. During the generation of leachate by percolation, the EC tended to gradually decrease at all sites. However, these are above those reported for leachate per se to a greater magnitude, which may be due to the fact that the passage of water leaching tends to drag or wash more solids that influence the final result.

Once the peak day of leachate generation has been exceeded, all sites (AL, AB, DB, and PL) show a gradual decrease. We notice that in both periods (2020 and 2021), the decrease to a lower volume of leachate over time was similar in all sites.

Results indicate that *S. natans* is the species at each site that generates the lowest volume over time, while *S. fluitans* presents a greater volume from the beginning of the experiment. This indicates the *S. fluitans* predominance in the generation of leachate in the consortium sample.

The consortium samples in both periods showed variability of pH at the beginning of the process, but in the degradation progress, the pH reaches a more stable level.

This study implies that *Sargassum* varies its per se degradation in relation to the distance from the coast, as seen with its generated volume and peak time and its pH and EC characteristics. The dry residual *Sargassum* could also generate an important leachate volume as it gets rehydrated by rain.

Since the proportion of species plays an important role in the volume and peaks of degradation, it is possible to expect the variation of the degradation of *Sargassum* reflected in the volume and peak time either along the sites of collection or coastal arrival time.

These results intend to assist public managers by defining critical values of leachates; characteristics of *Sargassum* spp. like the degradation and its importance in the prevention of public health risks (water resources and aquatic ecosystems); and the identification of the evolution of the process for better handling control as raw material or waste.

The results confirm the existence of a variability in leachate production and the composition of *Sargassum* under the influence of factors such as the periodicity, site of collection, and proportions of *S. fluitans* and *S. natans* in the consortium.

Given the variables that influence the degradation of *Sargassum* spp., it is relevant to consider a monitoring strategy that allows us to know the limitations represented by the generation of leachates; this becomes crucial information for decision making towards environmental management and storage. Thus, it is important to point out the importance of continuing with future studies for a more robust characterization of possible contaminants present in the leachates derived from the decomposition of *Sargassum*, in order to know the extent of its possible impact on the accumulation sites.

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