Hidrobiológica 2025, 35 (1): 1-12

UNIVERSIDAD AUTÓNOMA METROPOLITANA
Casa abierta al tiempo
Unidad Iztapalapa

HIDROBIOLÓGICA
http:/hidrobiologica.izt.uam.mx
ISSN: 2448-7333

OPEN ACCESS
Research Article
April, 2025

Estimation of the specific surface area in marine macroalgae using Langmuir isotherms as an alternative technique for studies of epibenthic assemblages

Estimación del área superficial específica de macroalgas marinas mediante isotermas de Langmuir como una alternativa para estudios de ensamblajes epibentónicos

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Recibido: 23 de abril de 2024. *Aceptado:* 29 de agosto de 2024. *Publicado:* abril de 2025.

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To quote as:
Estrada-Vargas, L., H. Hernández-García & Y. B. Okolodkov. 2025. Estimation of the specific surface area in marine macroalgae using Langmuir isotherms as an alternative technique for studies of epibenthic assemblages. *Hidrobiológica* 35 (1): 1-12.

DOI:10.24275/CLYF7157

ABSTRACT

Background: Benthic macroalgae offer a suitable habitat for the development of different epibenthic species. Knowing the macroalgal surface area allows the study of epibenthic assemblages, as well as the evaluation of interactions between host and epibiont. **Goals:** The aim of this research was to estimate the specific surface area of benthic macroalgae collected at two coastal sites in Veracruz, southwestern Gulf of Mexico. **Methods:** From August 2016 to July 2017, 12 monthly collections were made. The formation of the Methylene Blue monolayer on the macroalgal surface was verified using the Langmuir isotherms. The biomass of brown algae was chemically treated to cause the formation of the monolayer. **Results:** For all examined algal species (11 Rhodophyta, 6 Chlorophyta and 4 Phaeophyceae from four morpho-functional groups) the adsorption equilibrium point was reached with a high correspondence between the experimental and calculated data (R^2>0.96). The formation of the monolayer allowed estimation of the macroalgal specific surface area, which varied significantly among species, from 24 to 387 $m^2\ g^{-1}$. The corticated algae exhibited the greatest specific surface area (143-222 $m^2\ g^{-1}$), and the articulated calcareous forms had the least area (63-104 $m^2\ g^{-1}$). However, no correlation between the specific surface area and the morpho-functional groups was found ($P<0.05$). In most algae, significant differences in specific surface area were observed in thalli of the same species ($P<0.05$). **Conclusions:** The results could be related to infraspecific variability in morphological characteristics of the thallus that occur during ontogenesis under environmental conditions. The Methylene Blue adsorption technique is suitable for determination of the surface area and allows the comparison of macroalgae of different morpho-functional groups, thus minimizing the uncertainty associated with species-specific characteristics.

Keywords: epibenthos, Langmuir adsorption model, macroalgae, Methylene Blue, specific surface area

RESUMEN

Antecedentes: Las macroalgas bentónicas ofrecen un hábitat adecuado para el desarrollo de diferentes especies epibentónicas. Determinar el área superficial específica de las macroalgas permite el estudio de las asociaciones epibentónicas, así como, la evaluación de las interacciones entre el hospedero y el epibionte. **Objetivos:** El objetivo de esta investigación fue estimar el área superficial específica de las macroalgas bentónicas, recolectadas en dos sitios del suroeste del Golfo de México. **Metodología:** De agosto de 2016 a julio de 2017 se realizaron 12 recolectas mensuales. Mediante isotermas de Langmuir se verificó la formación de una monocapa de azul de metileno sobre la superficie de las macroalgas. Con la finalidad de promover la formación de la monocapa, a la biomasa de las algas pardas se le aplicó un tratamiento químico. **Resultados:** Se observó una alta correspondencia entre los datos experimentales y calculados (R^2>0.96) en el punto de equilibrio de adsorción para todas las algas estudiadas (11 especies de Rhodophyta, 6 de Chlorophyta y 4 de Phaeophyceae de cuatro grupos morfo-funcionales). La formación de la monocapa permitió la estimación del área superficial específica de las macroalgas, la cual varió significativamente entre especies, desde 24 hasta 387 $m^2\ g^{-1}$. Los resultados mostraron que las algas corticadas prestaron la mayor área superficial especí-

fica (143-222 m^2 g^{-1}), y que las calcáreas articuladas tenían la menor área (63-104 m^2 g^{-1}). Sin embargo, no se encontró correlación entre el área superficial específica y los grupos morfofuncionales ($P<0.05$). En la mayoría de las algas, se observaron diferencias significativas en el área superficial específica en talos de la misma especie ($P<0.05$). **Conclusiones:** Los resultados pueden estar relacionados con la variabilidad intraespecífica en las características morfológicas del talo que ocurren durante la ontogenia bajo condiciones ambientales. La técnica de adsorción de azul de metileno es adecuada para la determinación del área superficial específica y permite la comparación de macroalgas de diferentes grupos morfofuncionales, minimizando la incertidumbre asociada a las características específicas de especie.

Palabras clave: Epibentos, modelo de absorción de Langmuir, macroalgas, azul de metileno, área superficial

INTRODUCTION

The surface of marine macroalgae provides a fitting habitat for animal and plant species development and provides food and shelter (Ryland, 1974; Chemello & Milazzo, 2002). The relationship between the epibionts and their host has been studied by several authors (Taylor & Cole, 1994; Parsons & Preskitt, 2007; Bates, 2009). Macroalgal morphology-based studies have explained the probable interactions (Taylor & Cole, 1994; Chemello & Milazzo, 2002; Bates, 2009; Torres *et al*., 2015)

To explain the association between the epifauna and their host, Bates (2009) used the functional classification by Steneck & Dethier (1994). In contrast, Parsons & Preskitt (2007) classified macroalgae according to the structure of their thalli; they found that epibenthic dinoflagellates prefer microfilamentous macroalgal species, perhaps because of the surface area provided by the latter. Taylor & Cole (1994) found correlations between macroalgal morphology and epifauna and suggested that the more complex macroalgal forms resulted in higher epifaunal diversity.

Macroalgae classification based on their thallus morphology allows the assessment of the association between them and the epibionts. However, although the results are usually expressed as the number of epibiont individuals per gram of the host biomass, the estimation is inaccurate because the relation between weight and specific surface area in aquatic plants differs between species (Sher-Kaul *et al*., 1995; Armstrong *et al*., 2003). Therefore, Lobel *et al.* (1988) and Bomber *et al.* (1989) suggested that in comparative studies it is necessary to know the specific surface area of macroalgae to allow standardization of the abundance of epibionts in cells/individuals per area.

Different methodologies have been proposed to estimate the macroalgal specific surface area (S_m). Lobel *et al.* (1988) and Armitage & Sjøtun (2016) made these estimations for *Galaxaura* sp., *Dictyota* sp., *Codium fragile* (Suringar) Hariot and *Fucus serratus* Linnaeus based on geometrical forms; however, their technique assumes a flat surface. Lobel *et al.* (1988) and Bomber (1989) estimated the S_m by the weight difference between dry weight before and after immersion of the algae in a surfactant solution. For aquatic plants, Cattaneo & Carignan (1983) and Armstrong *et al.* (2003) estimated the specific surface area by using a mixture of detergent and different dyes. These techniques are promising; however, they need to be modified to obtain consistent and reliable data (Lobel *et al.,* 1988). The chemical adsorption methods improved the specific surface area estimation because both texture and roughness were taken into account (Bergey & Getty, 2006).

It is important to mention that to compare the superficial area between different macroalgal species, it is necessary to standardize the methodology, and it must be suitable for all species, regardless of their morphological complexity and chemical composition. It has been reported that using geometric or mathematical techniques has low accuracy in algal species with complex structures (Harrod & Hall, 1962). In addition, the geometrical technique can neglect the algal surface microstructure (Lobel *et al*., 1988), which may be relevant for epibenthic assemblages. On the other hand, the specific surface area determination per weight depends on the morphological complexity, composition, and surface characteristics of the algal species (Rubín *et al*., 2010). The quantities of water and salts affect the gravimetric determination of dry weight (Zhu & Lee, 1997). Therefore, it is necessary to homogenize the samples and eliminate water, organic and inorganic substances. Arredondo-Vega & Voltolina-Lobina (2007) suggest elimination of water by drying the microalgal biomass at temperatures between 60 and 70°C until a constant weight at a constant temperature is obtained.

Nevertheless, when using the adsorption methods, it is important to guarantee that the monolayer is formed on the surface of the adsorbent material for the accurate estimation of surface area. In the case of weight differences, as mentioned by Lobel *et al.* (1988) and Bomber *et al.* (1989) in their investigations, the monolayer formation by adsorption of the surfactant is not guaranteed. One way to ensure the monolayer formation is by applying the Langmuir model to the adsorption model (Sandoval-Ibarra *et al*., 2015).

Methylthionine chloride ($C_{16}H_{18}ClN_3S$: Methylene Blue (MB) is one of the adsorbates used to evaluate the monolayer formation. This organic dye has been used to estimate the specific surface area of different materials such as bentonite (Pinzón-Bello, 1997), cotton fiber (Kaewprasit *et al*., 1998) as well as terrestrial and aquatic plants (Vilar *et al*., 2007; Bestani *et al*., 2008; Rubín *et al*., 2010). Rubín *et al.* (2005) and Pratiwi *et al*. (2019) evaluated the adsorption capacity of MB in *Sargassum muticum* (Yendo) Fensholt and *Ulva lactuca* Linnaeus. They showed that there is affinity between the MB and the algal biomass and that this adsorption fits that of the Langmuir type. Rubín *et al.* (2010) estimated the S_m of *S. muticum* according to the postulates of this model; the authors calculated an average specific surface area (S_m) of 242-747 m^2 g^{-1}, suggesting that these variations may be related to the pretreatment of the studied thalli. Therefore, the aim of this study was to estimate the S_m of different macroalgal species that belong to different morpho-functional groups (corticated, articulated calcareous, corticated foliose and coriaceous) by means of the MB adsorption technique, according to the Langmuir adsorption model.

MATERIAL AND METHODS

Sampling. From August 2016 to July 2017, 12 monthly collections of macroalgae were made, which comprised corticated, articulated calcareous, corticated foliose and coriaceous algae, also referred to as functional-form groups, functional groups (Littler & Arnold, 1982; Littler *et al*., 1983; Hanisak *et al*., 1988; Steneck & Dethier, 1994; Phillips *et al*., 1997; Padilla & Allen, 2000; Airoldi, 2001; Biber *et al*., 2004; Collado-Vides *et al*., 2005), morphological functional or morpho-functional groups (Balata *et al*., 2011; the latter is used herein) at two coastal sites

in Veracruz, Mexico, Chachalacas and Villa Rica, in the southwestern Gulf of Mexico (Fig. 1). The climate in this region is sub-humid warm (Aw2) with three seasons: rainy (July to September), dry (April to May) and "nortes" (the northern winds beginning in October with the incursion of strong northern winds that persist until the end of winter). Their temporality and occurrence vary according to weather conditions (Tunnell Jr, 1992; Salas-Pérez & Granados-Barba, 2008). The sampling sites are characterized by mixed coasts with beaches, dunes, marshlands and coastal lagoons (Sánchez-Rodriguez, 1980; Geissert-Kientz, 1999).

At Chachalacas, samples were taken at the reef plain of Primera Laja (19°27.791'N, 96°18.370'W) 700 m from the coastline. Sandy-rocky substrate and coral fragments characterize this area, with depths from 0.5 to 2.5 m (Estrada-Vargas *et al*., 2019). At Villa Rica (19°27.850'N, 96°18.521'W), characterized by coastal dunes and a rocky massif (García-López *et al*., 2017), macroalgae were sampled in the intertidal zone with site depths that varied with the tide (<1.5 m) from a sandy-rocky substrate with sparse coral fragments.

Random samplings were performed at each site, where three specimens from each species were collected by free diving. Underwater, these samples were placed in 500 ml polypropylene bottles with the surrounding water and sealed. The collected material was kept at 4°C during transportation. All the samples were processed the same day they were collected. The species identification was based on morphological features, reproductive structures and cell arrangement (Littler & Littler, 2000; Guiry & Guiry 2024). The species classification by morpho-functional groups was made according to Steneck & Dethier (1994).

Preparation of the macroalgal biomass to estimate surface area. Benthic macroalgae (171 specimens) were used for specific surface area estimation. To standardize the specific surface area estimation methodology and to minimize the error due to the amount of water, organic and inorganic substances, the material was prepared according to Rubín *et al.* (2010). To remove organic and inorganic particles from the macroalgal surface, the thalli were brushed and washed individually with filtered seawater (pore size 11 µm). The cleaned material was first dried at ambient temperature (72 h) and then at 60° C for 24 h and passed through a plastic sieve; the size of dry biomass was homogenized at 1.0 mm. The dried and homogenized material was stored in polypropylene airtight bags in desiccators.

Figure 1. Map of the southwestern Gulf of Mexico indicating the two sampling sites (marked by stars) on the coast of Veracruz, Mexico.

Methylene Blue safety use considerations. MB has several medical uses in both human health and veterinary care, for therapeutic and diagnostic procedures. It is also used as a stain in bacteriology and as a redox coloring agent. Some common applications are for treating overexposure to certain drugs, industrial chemicals, or environmental poisons, such as excessive nitrate or cyanide compounds. In humans, a high dose of it (>500 mg) when injected has been reported to cause nausea, abdominal and chest pain, cyanosis, methemoglobinemia, sweating, dizziness, headache and confusion (Harvey, 1980). However, neither the Occupational Safety and Health Administration, the National Institute for Occupational Safety and Health, nor the American Conference of Governmental Industrial Hygienists have established permissible exposure limits for MB (NTP, 2008; NOAA, 2024). Nevertheless, it is recommended to follow the safety standards in the use of chemical reagents.

Methylene Blue adsorption by macroalgae biomass experiment. The test was based on Rubín *et al.* (2010), with a few modifications. MB (C.I. 52015, Merck, dye content 82 %) was the dye used to form the monolayer (Kaewprasit *et al.*, 1998; Rubín *et al.*, 2010). MB was dried for 2 h at 60° C; a 1000 mg l^{-1} standard solution was then prepared.

In determining the Langmuir type adsorption isotherms, 0.05 g of dry biomass samples were put into Erlenmeyer flasks with 50, 100, 200 and 500 mg l^{-1} MB dilutions. The mixtures were shaken (150 rpm) at a controlled temperature ($\overline{X}$=24° C) for 2 h. Subsequently, absorbance was read in a Hach DR 5000 spectrophotometer at a wavelength of 665 nm. The samples were diluted to obtain a concentration within the Lambert-Beer law range for the dye used. All experiments were performed in duplicate, and the analytical determinations were in triplicate.

Chemical modifications for macroalgal biomass that did not show monolayer formation during the Methylene Blue adsorption experiments. Chemical modifications of the biomass were performed on those species that did not show the Langmuir isotherm during the first set of MB adsorption experiments. The carboxyl group was modified with acidic methanol solution only; however, satisfactory determination coefficients were not obtained (R^2<0.6). The same scenario was observed with lipid extraction with methanol alone for some species of marine macroalgae. Therefore, lipid extraction was attempted after decarboxylation, achieving R^2>0.9. Consequently, the thalli underwent carboxylic acid esterification followed by extraction of the lipid fraction. Esterification was performed by suspending 1.5 g of dry biomass in 100 ml of methanol and 0.9 ml of HCl concentrate. The mixture was shaken for 24 h at 150 rpm, then washed with distilled water, decanted, and dried at 60° C for 24 h. The lipid extraction was performed by suspending 0.5 g of dry biomass in 50 ml of methanol 50% (v/v) and shaking (150 rpm) for 24 h. Finally, the biomass was washed with distilled water, decanted, and dried at 60° C for 24 h. Both processes were carried out according to Rubín *et al.* (2010).

The determination of isotherms was performed by placing 0.05 g of the treated biomass in Erlenmeyer flasks and adding 100, 200, 300 and 500 mg l^{-1} of MB dilutions. Each subsample was shaken for 12 h at 150 rpm at a controlled temperature (24° C) and pH (7.1±0.21). At a wavelength of 665 nm, absorbance readings were performed by using a UV spectrophotometer to assess the equilibrium time.

Determining maximum adsorption capacity of the monolayer for macroalgal biomass. The value of the maximum adsorption capacity of the monolayer for macroalgal biomass (X_m) was estimated by using the Langmuir adsorption model (Eq. 1), which satisfactorily describes the adsorption equilibria of both aquatic and terrestrial vegetable biomass (Vilar *et al.*, 2007; Rubín *et al.*, 2010).

$$X = \frac{X_m K C_f}{1+KC_f} \quad \text{Eq. 1}$$

This equation can be expressed linearly as:

$$\frac{C_f}{X} = \frac{C_f}{X_m} + \frac{1}{X_m K} \quad \text{Eq. 2}$$

Where C_f represents the solute final concentration or equilibrium (mg_{MB} l^{-1}), X is the amount of adsorbed solute per milligram of adsorbent (mg_{MB} mg_{alga}^{-1}), and K is a constant depending on adsorption and desorption.

Estimating the macroalgal biomass surface area. The macroalgal biomass surface area (S_m) was calculated by using the equation: $S_m = X_m N_o a$, which in the specific case of the MB can be expressed as (Sharma & Forster 1994) (Eq. 3):

$$S_m = \left(\frac{X_m \cdot N \cdot A_m}{MW}\right) \cdot 10^{-20} \quad \text{Eq. 3}$$

where S_m is the surface area (m^2 g_{alga}^{-1}), X_m is the maximum adsorption capacity of the monolayer (mg_{MB} g_{alga}^{-1}), N represents the Avogadro constant (6.02 x 10^{23}) and A_m and MW represent the cross section in Armstrong ($Å^2$) and the molecular mass (mg $mmol^{-1}$) of the MB, respectively. The transversal area used by an MB molecule varies from 66 to 125 $Å^2$ according to the observations on which its adsorption is supported (Hähner *et al.*, 1996). However, aiming to standardize and perform an appropriate comparison of the results, this study employed the value Rubín *et al.* (2010) proposed for determining the S_m of macroalgae (108 $Å^2$).

RESULTS

Macroalgal species composition. During the study period at Chachalacas and Villa Rica the best represented taxonomic group was Rhodophyta (11 species), followed by Chlorophyta (6) and Phaeophyceae (4). *Digenea simplex* (Wulfen) C. Agardh, *Laurencia* sp., *Alsidium triquetrum* (S. G. Gmelin) Trevisan, *Caulerpa sertularioides* (S. G. Gmelin) M. Howe, *C. racemosa* (Forsskål) J. Agardh, *C. mexicana* Sonder ex Kützing, *Cymopolia barbata* (Linnaeus) J. V. Lamouroux, *Padina* sp. and *Dictyota* sp. were present and collected from both sites. The only species collected at Chachalacas throughout 12 samplings was *Halimeda scabra* M. Howe, whereas at Villa Rica the recurrent species were *Alsidium triquetrum, Haliptilon subulatum* H. W. Johansen, *Caulerpa sertularioides, Cymopolia barbata, Padina* sp., *Dictyota* sp. and *Sargassum vulgare* C. Agardh. The species were classified into four morpho-functional groups: nine species were placed in the corticated group, eight in the articulated calcareous, three in the corticated foliose and one in the coriaceous group (Table 1).

Chemical modification effects applied to the macroalgal biomass that did not show monolayer formation in the first set of adsorption experiments. In brown algal species, the non-chemically treated adsorption experiments did not show a linear correlation between the final MB concentration and the algal biomass. In contrast, the treated biomass adsorption results showed that the sterilization and lipid extraction processes caused the dye to form a monolayer on the surface of *Padina* sp., *Dictyota* sp., *Dictyopteris delicatula* J. V. Lamouroux and *Sargassum vulgare* (Fig. 2).

Tests for the Methylene Blue monolayer formation in macroalgae. The MB adsorption dynamics in the three studied major taxonomic algal groups showed that the dye amount adsorbed by algae increased with increasing dye concentration in the solution. However, once the saturation point was reached, the change of the MB concentration had no effect on the solute amount adsorbed per gram of algal biomass (Fig. 3). In all studied species, the adsorption equilibrium point was reached; it was associated with the MB monolayer formation on the algal surface. Correlation between the experimental data and values calculated by the Langmuir isotherm model showed a high correspondence for all the tests (R^2>0.96).

Estimating the surface area of macroalgae. Once all examined algal species showed a Langmuir type adsorption isotherm, it was assumed that the MB amount corresponded to that required for the monolayer formation; therefore, it was possible to estimate the surface area of each macroalga using equation 3.

Table 1. List of species, their affiliation with a morpho-functional group, sampling sites in the SW Gulf of Mexico and months of sampling in 2016–2017. CH – Chachalacas, VR – Villa Rica.

Taxa	Morpho-functional group	Sampling site and month
RHODOPHYTA		
Digenea simplex (Wulfen) C. Agardh 1822	Corticated	CH: Aug
		VR: Nov
Hypnea spinella (C. Agardh) Kützing 1847	Corticated	VR: Apr, May
Laurencia sp.	Corticated	CH: Sep, Oct
		VR: Dec-Mar
Alsidium triquetrum (S. G. Gmelin) Trevisan 1845	Corticated	CH: Aug, Sep, Nov, Mar
		VR: Aug-Jul
Liagora sp. 1	Corticated	CH: Mar, Apr, Jun
Liagora sp. 2	Corticated	CH: Apr-Jun
Haliptilon subulatum H. W. Johansen 1970	Articulated calcareous	VR: Aug-Jul
Jania cf. *adhaerens* J. V. Lamouroux 1816	Articulated calcareous	VR: Aug-Sep, Jun, Jul
Amphiroa sp.	Articulated calcareous	CH: Oct
Tricleocarpa cylindrica Huisman et Borowitzka 1990	Articulated calcareous	CH: Sep, Dec, Jul
Galaxaura sp.	Articulated calcareous	CH: Sep, Jan
CHLOROPHYTA		
Caulerpa sertularioides (S. G. Gmelin) M. Howe 1905	Corticated	CH: Aug-Mar
		VR: Aug-Jul
C. racemosa (Forsskål) J. Agardh 1873	Corticated	CH: Sep-Nov, Jan, Feb, Apr, May
		VR: Aug, Nov, Jan, May, Jun
C. mexicana Sonder ex Kützing 1849	Corticated	CH: Dec-Mar
		VR: Dec-Apr
Halimeda scabra M. Howe 1905	Articulated calcareous	CH: Aug-Jul
Cymopolia barbata (Linnaeus) J. V. Lamouroux 1816	Articulated calcareous	CH: Aug, Sep, Mar, May-Jul
		VR: Aug-Jul
Rhipocephalus phoenix (J. Ellis et Solander) Kützing, 1843	Articulated calcareous	CH: Jul
PHAEOPHYCEAE		
Padina sp.	Corticated foliose	CH: Aug-Oct, Dec, Jan, Mar-Jul
		VR: Aug-Jul
Dictyota sp.	Corticated foliose	CH: Aug, Sep, Mar-Jul
		VR: Aug-Jul
Dictyopteris delicatula J. V. Lamouroux 1809	Corticated foliose	CH: Sep, Nov-Feb, Apr, Jun, Jul
Sargassum vulgare C. Agardh 1820	Coriaceous	VR: Aug-Jul

Within the four morpho-functional groups studied, the coriaceous group was represented only by the *Sargassum vulgare* collected in Villa Rica, with 190-333 $m^2 g^{-1}$, whereas the corticated, corticated foliose and calcareous groups were represented by the species found at both sampling sites. The statistical analysis showed that 50% of the specimens in the corticated group in both Chachalacas and Villa Rica presented the largest surface area: 119-198 $m^2 g^{-1}$ and 150-233 $m^2 g^{-1}$, respectively, followed by the corticated foliose group: 112-157 $m^2 g^{-1}$ for Chachalacas and 132-182 $m^2 g^{-1}$ for Villa Rica. The articulated calcareous group showed the smallest surface area, 69-128 $m^2 g^{-1}$ for Chachalacas and 59-94 $m^2 g^{-1}$ for Villa Rica (Fig. 4). The statistical analysis did not show any correlation between morpho-functional group and surface area ($P<0.05$).

Macroalgal surface area varied significantly (tenfold) among species, from 24 $m^2 g^{-1}$ to 387 $m^2 g^{-1}$. At Chachalacas, the species with the higher surface area were *Alsidium triquetrum* and *Caulerpa mexicana*, and those with the smallest surface area were *Amphiroa* sp. and *Halimeda scabra*. At Villa Rica, *Hypnea spinella* (C. Agardh) Kützing and *Caulerpa mexicana* showed the higher surface area, whereas the smallest surface area corresponded to *Jania* cf. *adhaerens* J. V. Lamouroux and *Haliptilon subulatum* (Table 2).

In contrast, significant differences were observed in most cases when comparing the surface area of different thalli of the same species, except for *Tricleocarpa cylindrica* Huisman et Borowitzka (112±23 $m^2 g^{-1}$, $P=0.05$), *Laurencia* sp. 1 (226±4 $m^2 g^{-1}$, $P=0.1$) and *Liagora* sp. 2 (126±18 $m^2 g^{-1}$, $P=0.07$) from Chachalacas. These differences were also observed when comparing thalli from the same species between the two sites for the same sampling period. Significant differences were not observed in *C. sertulariodes* collected in September at both sites (159±41 $m^2 g^{-1}$, $P=0.08$); *Cymopolia barbata* collected in June (125±45 $m^2 g^{-1}$, $P=0.05$); *Alsidium triquetrum* collected in November (173±44 $m^2 g^{-1}$, $P=0.67$) and *Padina* sp. collected in August and May (149±29 $m^2 g^{-1}$, $P=0.07$; 162±41 $m^2 g^{-1}$, $P=0.18$, respectively).

DISCUSSION

During our tests, the MB adsorption by macroalgal thalli was satisfactorily described by the Langmuir isotherms (Kaewprasit *et al.*, 1998). Hence, it was assumed that the change in the MB concentration was due to the monolayer formation on the macroalgal surface. A high degree of accuracy in the estimation of the macroalgal surface area is thus indicated.

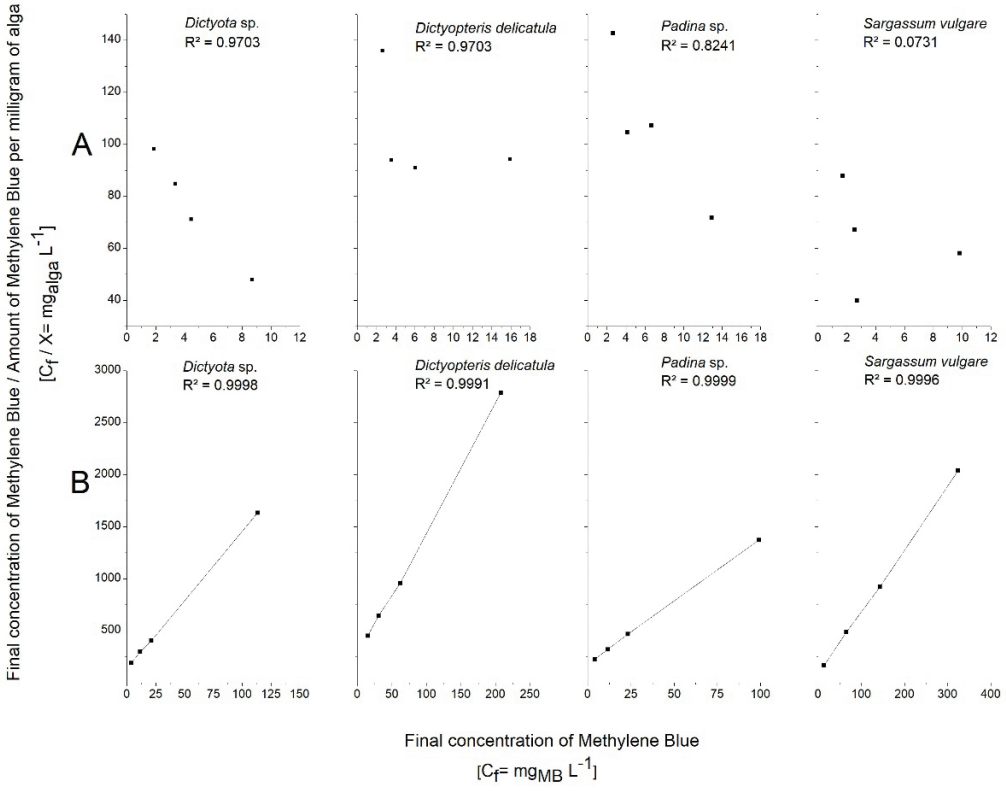

Figure 2. Methylene Blue adsorption dynamics for the brown algae *Dictyota* sp., *Dictyopteris delicatula, Padina* sp. and *Sargassum vulgare* with chemical treatment (A) and without it (B).

Table 2. Surface area of the examined macroalgal species in the SW Gulf of Mexico in 2016–2017. C – Chlorophyta, P – Phaeophyceae, R – Rhodophyta; nf – not found.

Taxa	Major taxonomic group	Surface area, $m^2\ g^{-1}$	
		Villa Rica (VR)	Chachalacas (CH)
Corticated			
Digenea simplex	R	103-109	179-199
Hypnea spinella	R	348-387	nf
Laurencia sp.	R	203-313	223-229
Alsidium triquetrum	R	112-256	106-268
Liagora sp. 1	R	nf	87-191
Liagora sp. 2	R	nf	105-143
Caulerpa sertularioides	C	129-220	77-172
C. racemosa	C	158-263	150-226
C. mexicana	C	221-350	168-281
Articulated calcareous			
Haliptilon subulatum	R	44-94	nf
Jania cf. *adhaerens*	R	24-58	nf
Amphiroa sp.	R	nf	51-57
Tricleocarpa cylindrica	R	nf	69-133
Galaxaura sp.	R	nf	89-162
Halimeda scabra	C	nf	50-134
Cymopolia barbata	C	61-127	84-178
Rhipocephalus phoenix	C	nf	106-113
Corticated foliose			
Padina sp.	P	99-200	109-294
Dictyota sp.	P	nf	104-148
Dictyopteris delicatula	P	nf	78-165
Coriaceous			
Sargassum vulgare	P	190-333	nf

For brown algal species, it was necessary to apply a chemical treatment to achieve the monolayer formation, which resulted in monomolecular MB adsorption due to the modification of available sites. However, it is important to stress that the modification is specific for each type of chemical treatment; consequently, different chemicals may modify the results of the area estimation (Lodeiro *et al*., 2004; Rubín *et al*., 2005, 2010; Vilar *et al*., 2007). Despite this, the technique applied in this study can be used to compare macroalgae (specimens or species) analyzed under the same conditions (Rubín *et al*., 2005, 2010; Vilar *et al*., 2007). Accordingly, it can be argued that the differences found on the macroalgal surface area may be due to species-specific morphological features (Chemello & Milazzo, 2002; Bates, 2009).

Some morphology-based studies noted that the surface area is in direct proportion to the macroalgae structural complexity, with which they tended to show a higher diversity of epibionts (Chemello & Milazzo, 2002; Bates, 2009). The results of the present study agree with the above-mentioned ones. Less morphologically complex species such as articulated calcareous algae presented a smaller surface area; more complex species presented a larger surface area. However, in general, no correlation between the surface area and the morpho-functional groups was found ($P<0.05$). In contrast, other studies reported that less complex species, such as filamentous algae, showed larger surface areas (Parsons & Preskitt, 2007). Bates (2009) suggested that a greater morphological complexity is not necessarily related to a larger surface area, but the surface may be a function of ramifications and specific structures located along the thallus. For example, Taylor & Cole (1994) mentioned that the brown alga *Carpophyllum plumosum* (A. Richard) J. Agardh, characterized by fine structures on the frond, has a larger surface area than thick-frond species. In our study, the species with more developed ramification and fine thalli showed a greater surface area (*Hypnea spinella* and *Laurencia* sp.).

Another factor that could explain the differences is the surface area variation of the thallus components. The frond section for most of the species in this study was analyzed, except for *Sargassum vulgare* (for which samples containing both frond and stipe were analyzed) and *Caulerpa* species thalli (both frond and stolon were included). Christie *et*

al. (2003) reported that the frond, the stipe and the holdfast of *Laminaria hyperborea* (Gunnerus) Foslie present different surface areas. Sher-Kaul *et al.* (1995) concluded that for stoloniferous plants the stolon and leaves modify the surface area. Armstrong *et al.* (2003) suggest that in determining the surface area of a species with a stolon it is important to consider the leaf to stolon ratio because the leaves provide a greater surface area than that of the stolon.

In articulated calcareous species, the differences observed in the stems of the same species could be related to both the cellular arrangement and the amount of $CaCO_3$ (Wefer, 1980; Lee & Carpenter, 2001; Hatt & Collado-Vides, 2019). Anderson *et al.* (2006) noticed that for coenocytic species calcification between segments and filament adhesion to the surface influenced the surface area. Lee & Carpenter (2001) stated that the produced inorganic carbonate amount depended on the morphology of each species and on the calcification mechanisms during ontogenesis.

In this study, the specimens were collected over a year; consequently, the specimens may have presented different development stages. For example, conceptacles (specialized cavities that contain the reproductive organs) with rough ramuli (spherical branchlets) were observed in some *Jania* cf. *adhaerens* thalli; only smooth ramuli were observed in other species (absence of conceptacles). In our study, development of either conceptacles or tetrasporangia (sporangia containing four asexual spores), or both, were also observed in *Haliptilon* cf. *subulatum* and *Amphiroa* sp. According to Rosas-Alquicira *et al.* (2013) and Rover *et al.* (2015), tetrasporangia and conceptacle formation modify macroalgal surfaces. Vesk & Borowitzka (1984) reported that the formation of conceptacles is evident on the surface of *Haliptilon cuvieri* (J. V. Lamouroux) H. W. Johansen et P. C. Silva during the reproductive stage. Rosas-Alquicira *et al.* (2013) found that the formation of conceptacles in *Amphiroa* J. V. Lamouroux species allows observation of different cell growth stages on surfaces such as the elongation of the terminal layer cells and the formation of cell layers on the surface. This suggests that the increase in cell layers during the formation of reproductive structures may affect the surface area.

Regarding the differences found between the sampling sites, at each site environmental conditions could have modified the species morphology (Shaughnessy *et al.* 1996; Anderson *et al.*, 2006; Parsons & Preskitt, 2007) and, therefore, the surface area estimation. Anderson *et al.* (2006) mentioned that the surface area varies when water movements increase in speed. Shaughnessy *et al.* (1996) suggested that the surface area and the thallus morphology are modified according to hydrodynamics. In the present study, Chachalacas was considered a high energy site, whereas Villa Rica was one of medium energy (Tindall & Morton, 1998).

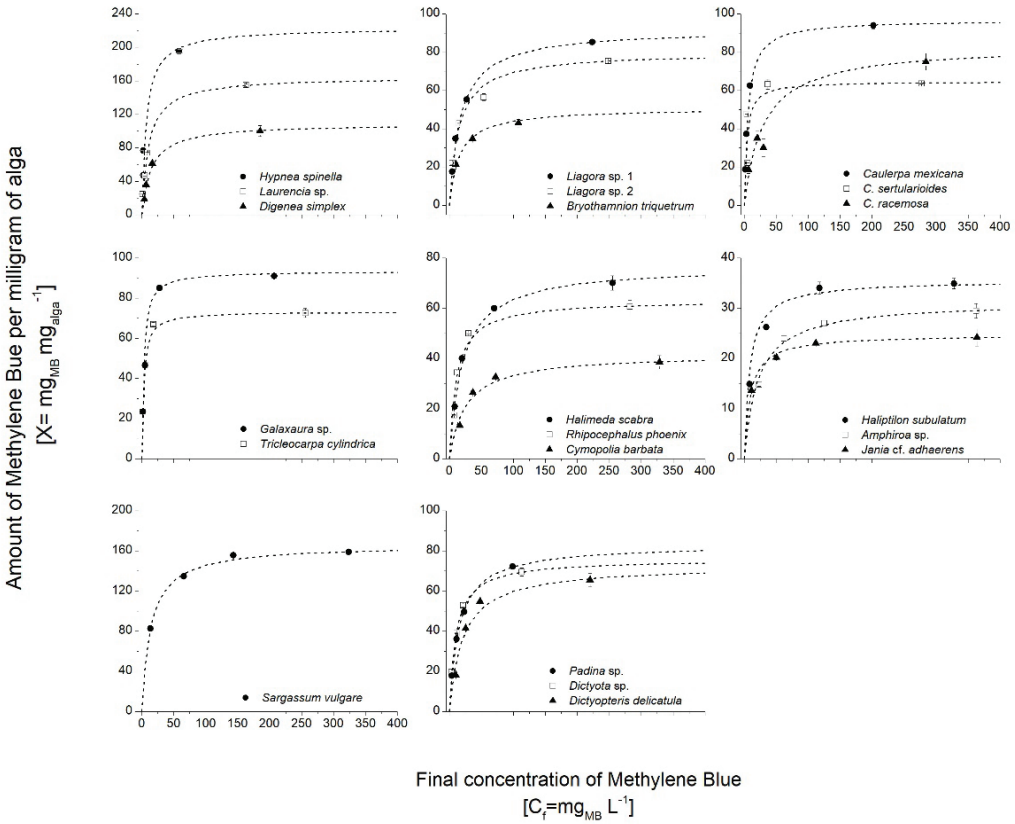

Figure 3. Methylene Blue adsorption isotherms for the studied macroalgal species. Points correspond to the experimental data, and dotted lines correspond to the adjusted Langmuir isotherms.

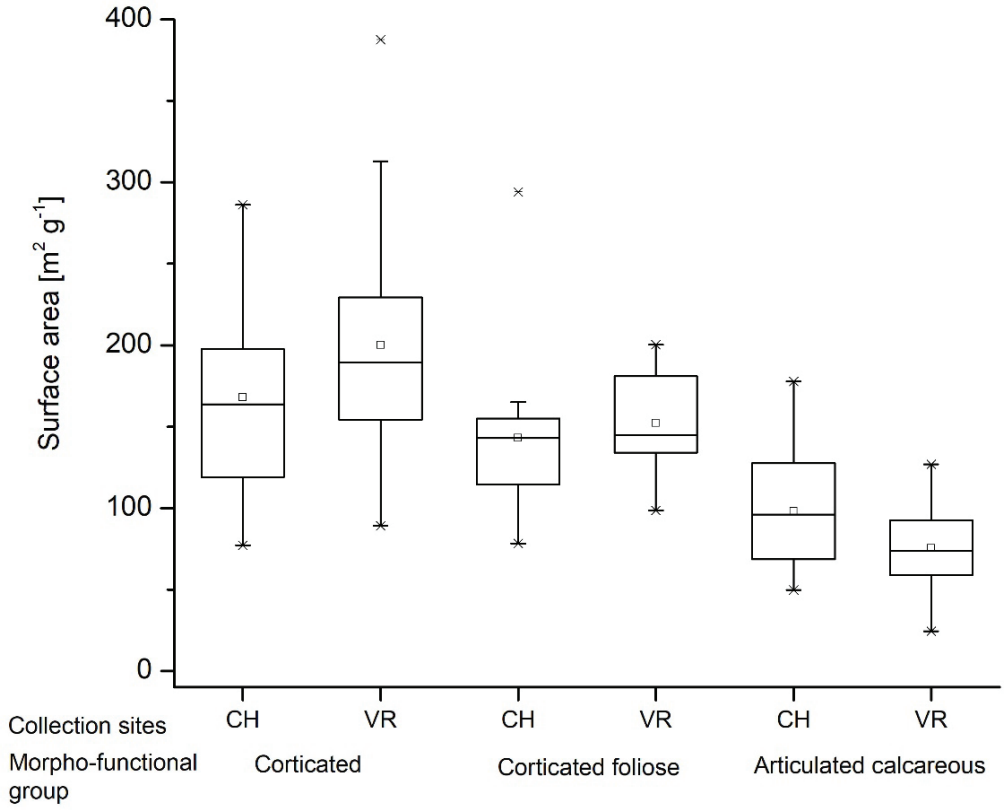

Figure 4. Surface area per morpho-functional group of macroalgae at Chachalacas (CH) and Villa Rica (VR). The coriaceous type is not included due to the occurrence of only one species (*Sargassum vulgare*) at one sampling site (see Table 2).

Finally, the present study showed that the MB adsorption technique is suitable for estimating the macroalgal surface area. The differences in this morphological (relief) characteristic may be explained by: 1) species-specific morphological features, 2) the structural components that constitute the thallus, 3) the morphological changes that occur during ontogenesis, and 4) the ecosystem hydrodynamics.

There is indeed a difference in size between MB and epibionts (macro *vs.* micro); however, the accuracy of the proposed methodology to approximate the surface area could be a good option for studies of epibenthic assemblages because it minimizes the effect of structural differences, chemical composition and surface/relief characteristics of the host, thus allowing a comparison among different algal species. Further investigations should focus on the availability of the surface area of host macroalgae for unicellular epiphytic species that may be much less than that estimated in the present study.

ACKNOWLEDGMENTS

LEV is grateful to the National Council of Science and Technology (Consejo Nacional de Ciencia y Tecnología – CONACyT, Mexico) for the PhD scholarship (registration No 174749). We thank Yolanda Cocotle-Ronzón for laboratory support, Citlalli Galicia-García for her help in the identification of macroalgae, Abraham Hernández-García for the map design, Patricia A. Tester for valuable advice, and Marcia M. Gowing for improving the English style. The study was partially funded by the CONACyT as a stipend (No. 174749) awarded to LEV in 2016-2021.

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