

Original Research Article

Influence of the habitat on marine macroalgae toxicity

Abstract

Macroalgae synthesise molecules that may be toxic to other organisms. These molecules are synthesised as a defence strategy against herbivores. It has been proven that the synthesis process is directed by several physiological, chemical and even spatial-temporal variables. The purpose of this study was to determine whether the complexity of the habitat influences the expression of marine macroalgae toxicity. Algae of 31 species (39 samples) were collected in localities with different habitat morphology: a coral reef in the Mexican Caribbean, three myxohaline localities in the Yucatán peninsula and six rocky intertidal localities, four of these in the Mexican Pacific and two in the Gulf of Mexico. Results identified 19 strongly toxic species from the reef, followed by algae collected in the rocky intertidal area, and the least number of toxic species in the myxohaline environments. The results support the hypothesis established by several researchers worldwide regarding the complexity of coral reefs, which promotes the synthesis of toxic substances as a defence against herbivores. These substances have been employed as molecules that are useful in the fight against diseases or as synthesis matrices of other compounds with pharmacological potential.

Keywords: toxicity, ichthyotoxicity, macroalgae, habitat, Mexico

1. Introduction

A great diversity of marine organisms produces toxins as part of their metabolism. Among these are Cyanobacteria and dinoflagellates (Miozoa, Dinophyceae) [1, 2, 3], sponges (Porifera) [4, 5], ascidians (Chordata, Ascideacea) [6, 7, 8] and ahermatypic corals (Cnidaria, Anthozoa) [9, 10, 11]. Secondary metabolites are important in the regulation of a variety of defence mechanisms, and many are considered more potent than a few toxic macromolecules [12, 13, 14]. Macroalgae also produce a series of secondary metabolites that are intermediaries of ecological responses, as occurs in the case of chemical defence against herbivory [15, 16, 17, 18, 19], anti-fouling [20, 21] and those responsible for allelopathic responses [22, 23, 24].

Macroalgae produce a great number of secondary metabolites including terpenes, aromatic compounds, acetogenins, polyphenols and fluorotannins, substances derived from amino acids and various alkaloids [25, 26, 27] that are ecologically useful to algae. Substances synthesised by species of the Dictyotaceae (Ochromytha, Phaeophyceae), like the Dictyoles (dictyol B, dictyol acetate A, B, H and pachydictyol), act against herbivores and are also used as a defence against epiphytes and epibionts that invade algal surfaces [28, 29, 30, 31]. Many members of the Division Rhodophyta synthesise biologically active metabolites that vary from simple brominated aromatic acetones and brominated phenols to mono, diterpene and sesquiterpene complexes [32, 33, 34]. Algae of the Phylum Chlorophyta mainly synthesise di- and sesquiterpenes that have a variety of biological activities including anti herbivory, bactericidal, antifungal and spermicidal, among others [33, 34, 35, 36].

Mexico has more than 10,000 km of coasts that, as a result of the rocks that form them, provide diverse habitats that marine organisms use to establish complex ecological relationships. It is well known that herbivorous fish in coral reefs exert great pressure on algae, favouring an increase in algal diversity, though with lower biomass than that in temperate zones or in rocky intertidal areas, where herbivory pressure is mainly due to invertebrates. However, this pressure causes the algae that survive and prosper to be chemically protected [37, 38].

In Mexico, studies on marine macroalgae toxicity are scarce, despite the high species diversity of these organisms along its coasts [39, 40]. It is thus important to carry out an inventory of the algal species that grow in Mexico and harbour molecules that may potentially be useful in medicine, chemistry and biochemistry. The purpose of this study was to detect the presence of toxicity in marine algae of the Mexican coasts and relate the presence of this biological activity to the habitat where these organisms develop.

2. Material and methods

2.1 Collection of algal material

Algae were collected manually along the intertidal fringe of the described localities (Fig. 1). The algal material was separated from the rock at its base with a spatula, rinsed with seawater, separated by genus, frozen with solid CO₂, and transported to the laboratory for analysis. Ten localities were visited, four in the Yucatán peninsula, four in the Mexican Pacific and two in the Gulf of Mexico. Only one locality was visited in the Mexican Caribbean, the Puerto Morelos reef (20° 50' 08'' N and 86° 55' 04'' W) in the state of Quintana Roo, located north of Playa del Carmen, a Caribbean reef complex. The localities

of the Gulf of Mexico are wetlands formed by the locally-called “aguadas”, which are upwellings of sub-surface water or rivers that flow into the sea. These include Dzilam de Bravo ($21^{\circ} 23' 43''$ N and $88^{\circ} 52' 55''$ W), Chelém ($21^{\circ} 14' 35''$ N and $89^{\circ} 50' 34''$ W) and Celestún ($20^{\circ} 51' 33''$ N and $90^{\circ} 22' 51''$ W) in the state of Yucatán. Champotón ($19^{\circ} 19' 14''$ N and $90^{\circ} 44' 53''$ W), in the state of Campeche, is an artificial breakwater located at the mouth of the river of the same name. The localities in the state of Veracruz were the breakwater of Alvarado harbour ($18^{\circ} 47' 24''$ N and $95^{\circ} 44' 34''$ W), to the south of the lagoon inlet, that provides a fairly complex habitat for algal populations to settle. Boca del Río ($19^{\circ} 9' 53''$ N and $96^{\circ} 6' 16''$ W) is a shallow open beach with artificial breakwaters on which algae grow. Costa de Oro ($19^{\circ} 9' 21''$ N and $96^{\circ} 5' 45''$ W) is a beach with many submerged boulders, ideal for the settlement of large and diverse algal populations, located in an area of a great hotel and commercial growth, in the municipality of Boca del Río. Along the Mexican Pacific, in the state of Michoacán, are Punta San Telmo ($18^{\circ} 37' 26''$ N and $103^{\circ} 41' 7''$ W) to the north of the town of the same name, a rocky shore with a steep slope and difficult access, and Faro de Bucerías ($18^{\circ} 20' 40''$ N and $103^{\circ} 30' 40''$ W), a rocky outcrop where the morphology of the rocky coves generates complex habitats. In the state of Guerrero, Puerto Vicente Guerrero ($17^{\circ} 16' 11''$ N and $101^{\circ} 3' 9''$ W), also called Puerto Escondido or Playa Escondida, was formed by two gigantic artificial breakwaters that provide a fairly complex habitat, and La Barrita ($17^{\circ} 24' 32''$ N and $101^{\circ} 10' 52''$ W), in the municipality of Petatlán, provides a natural rocky area of medium extension where algal populations are seen to grow.

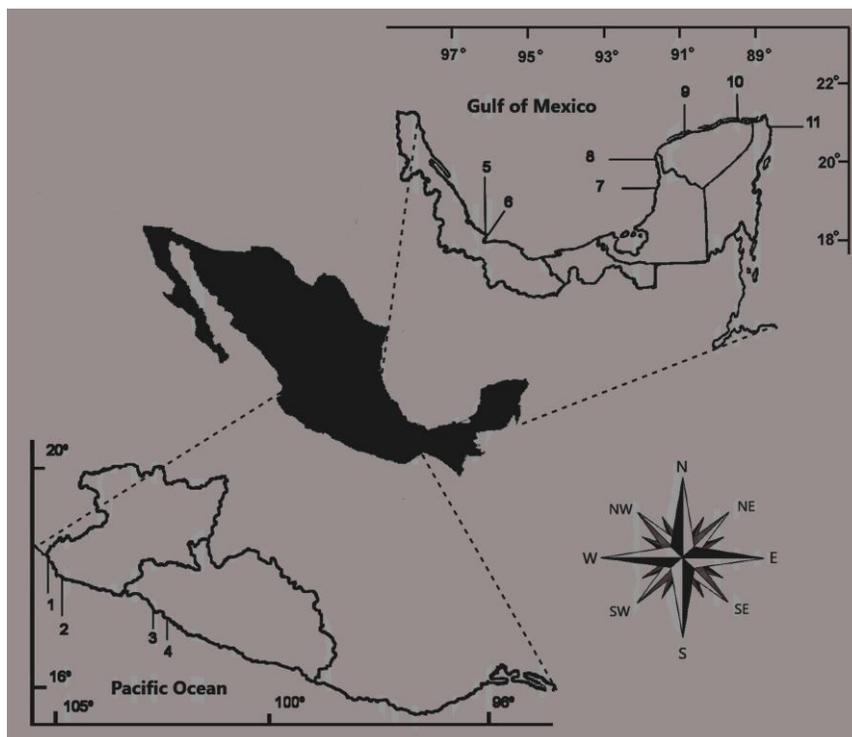


Figure 1. Localities of algal material collection: 1. Punta San Telmo, 2. Faro de Bucerías (Michoacán); 3. La Barrita, 4. Puerto Vicente Guerrero (Guerrero) Pacific Ocean Coast. 5. Boca del Río, 6. Costa de Oro (Veracruz); 7. Champotón, 8. Celestún (Campeche); 9. Chelem, 10. Dzilam (Yucatán) Gulf of Mexico Coast. 11. Puerto Morelos (Quintana Roo) Mexican Caribbean Coast.

2.3 Extract preparation

The samples were unfrozen at room temperature in order to obtain the extracts. One part of each sample was preserved in glycerinated formalin for later identification of the species and preparation of reference specimens for the herbarium of the Laboratorio de Ficología Aplicada. The remaining material was washed under running water and cleaned under a microscope to free it of epiphytes and other impurities that could affect the results.

Algal extracts were prepared to mix 50 g of alga and 150 ml of solvent (alcohol, acetone or distilled water). The mixture was centrifuged at 3,400 rpm for 20 minutes at 6 °C. The supernatant was dried by roto-evaporation and the crystals that were obtained were re-suspended using 0.9 ups saline solution used for the extraction [41, 42, 43].

2.4 Bioassays

The goldfish *Carasius auratus auratus* (Linnaeus, 1758) was used as a test animal in order to detect extract toxicity. Toxicity tests were carried out in triplicate, in 250 ml crystalisers containing 200 ml of aquarium water and 1 ml of the extract to be tested. Controls were prepared by adding 1 ml of the 0.9 ups saline solution. The solvent was evaporated before the experimental test, in order to ensure that the results of possible toxicity were solely the responsibility of unknown substances [44]. The extracts were classified based on the organisms' behaviour as Toxic (T) when the fish died during the test period (2 h), Moderately toxic (MT) when the fish did not die but presented behavioural changes such as fast or slow movements, loss of balance and in some cases paralysis, and recuperated after a time, and Non Toxic (—) when the fish did not react and their behaviour was similar to that of the controls. The surviving fish were placed in clean water for a period of 24 h.

3. Results

Bioassay results are presented in Table 1. Algae of 31 species were collected and included six of Chlorophyta, seven of Phaeophyceae and 18 of Rhodophyta (39 samples). A species was considered highly toxic when toxicity was detected in at least one of its extracts. This occurred in six species of Chlorophyta, four of Phaeophyceae and nine of Rhodophyta

(total 19, 62 %). Only two species of Rhodophyta were considered moderately toxic (total 2, 6.4 %) when moderate toxicity was detected in all their extracts. Three species of Phaeophyceae and seven of Rhodophyta presented no toxicity (total 10, 32 %). The most toxic species were *Caulerpa cupressoides*, *C. racemosa*, *C. paspaloides*, *Chaetomorpha antennina* and *Penicillus capitatus* (Chlorophyta), *Dictyopteris delicatula*, *Padina gymnospora* (formerly *Padina vickersiae*) and *Styopodium zonale* (Phaeophyceae), and *Ceramium nitens*, *Gracilaria tikvahiae*, *Laurencia obtusa* and *Liagora ceranoides* (Rhodophyta). Of special note is the potent activity of *Chondriopsis dasyphylla* f. *pyrifera* (Rhodophyta), of which the three extracts were toxic. With respect to the localities where algae were collected, the 12 species with the greatest toxicity were collected mostly in three localities, the Puerto Morelos reef in the Caribbean, Costa de Oro in the Gulf of Mexico and Faro de Bucerías in the Mexican Pacific. A change in toxicity level was detected in *Caulerpa cupressoides*, *Acantophora spicifera*, *Gracilaria cervicornis*, *Hypnea musciformis* and *Laurencia obtusa* with respect to the date and locality of the collection (Table 1). Regarding the solvent, 24 of the extracts obtained using acetone had toxic activity, 20 toxic extracts were obtained using ethanol, and only the toxic extract, of *Chondriopsis dasyphylla* f. *pyrifera*, was obtained using water.

Table 1. Localities: 1. Yucatán, 2. Quintana Roo, 3. Michoacán, 4. Veracruz, 5. Guerrero, 6. Campeche. Toxicity: — nontoxic extract, MT Moderately toxic extracts, T toxic extract.

Division/Species	Locality	Aqueous	Ethanollic	Acetonic
Chlorophyta				
<i>Anadyomene stellata</i> (Wulfen) C. Agardh	¹ Dzilam	—	MT	T
<i>Caulerpa cupressoides</i> (West) C. Agardh	² Pto. Morelos	—	—	—
<i>Caulerpa cupressoides</i> (West) C. Agardh	² Pto. Morelos	—	T	T
<i>Caulerpa cupressoides</i> (West) C. Agardh	² Pto. Morelos	—	MT	MT
<i>Caulerpa paspaloides</i> (Bory) Greville	¹ Chelem	—	MT	T
<i>Caulerpa racemosa</i> (Forssk.) J. Agardh	³ Faro de Bucerías	—	—	T
<i>Chaetomorpha antennina</i> (Bory) Kütz.	³ Faro de Bucerías	—	—	T
<i>Penicillus capitatus</i> J.V. Lamour.	² Pto. Morelos	—	T	T
Ochrophyta, Phaeophyceae				
<i>Dictyopteris delicatula</i> J.V. Lamour	² Pto. Morelos	—	T	T
<i>Dictyota implexa</i> (as <i>Dictyota linearis</i> (C. Agardh) Greville)	¹ Celestún	—	—	—
<i>Dictyota bartayresiana</i> Lamour.	³ Pta. San Telmo	—	—	—
<i>Lobophora variegata</i> (J.V. Lamour.) Womersley	² Pto. Morelos	—	—	—
<i>Padina gymnospora</i> (as <i>Padina vickersiae</i> Hoyt.)	³ Faro de Bucerías	—	—	T
<i>Sargassum liebmannii</i> J. Agardh.	³ Faro de Bucerías	—	MT	T
<i>Stypodium zonale</i> (J.V. Lamour.) Papenf.	² Pto. Morelos	—	T	T
Rhodophyta				
<i>Acanthophora spicifera</i> (Vahl) Børgesen	⁴ Costa de Oro	—	—	—
<i>Acanthophora spicifera</i> (Vahl) Børgesen	² Pto. Morelos	—	MT	T
<i>Acanthophora spicifera</i> (Vahl) Børgesen	¹ Dzilam	—	MT	MT
<i>Amphiroa beauvoisii</i> (as <i>Amphiroa mexicana</i> W.R.)	³ Faro de Bucerías	—	T	MT

Taylor)

<i>Centroceras clavulatum</i> (C. Agardh) Mont.	⁵ La Barrita	—	T	MT
<i>Ceramium nitens</i> (C. Agardh) J. Agardh.	² Pto. Morelos	—	T	T
<i>Chondria littoralis</i> Harvey	⁴ Costa de Oro	—	—	—
<i>Dermonema virens</i> (C. Agardh) Pedroche & Ávila Ortiz	⁵ Pto. Vicente Gro	—	—	—
<i>Digenea simplex</i> (Wulfen) C. Agardh	² Pto. Morelos	—	—	—
<i>Crassiphycus caudatus</i> (as <i>Gracilaria caudata</i> J. Agardh)	⁴ Costa de Oro	—	—	—
<i>Gracilaria cervicornis</i> (Turner) J. Agardh	⁴ Boca del Río	—	—	MT
<i>Gracilaria cervicornis</i> (Turner) J. Agardh	⁴ Costa de Oro	—	MT	T
<i>Gracilaria tikvahiae</i> McLachlan	⁴ Costa de Oro	—	—	T
<i>Grateloupia filicina</i> (C. Agardh) Lamour.	⁴ Boca del Río	—	—	—
	⁴ Escollera-			
<i>Grateloupia filicina</i> (J.V. Lamour.) C. Agardh	Alvarado	—	—	—
<i>Hypnea musciformis</i> (Wulfen) J.V. Lamour.	⁴ Boca del Río	—	—	—
<i>Hypnea musciformis</i> (Wulfen) J.V. Lamour.	⁴ Costa de Oro	—	MT	MT
<i>Hypnea spinella</i> (Wulfen) J.V. Lamour.	⁵ La Barrita	—	MT	MT
<i>Yuzurua poiteaui</i> var. <i>gemma</i> (Harvey) M.J.Wynne	⁶ Champotón	—	—	—
<i>Chondriopsis dasyphylla</i> f. <i>pyrifera</i> J. Agardh	² Pto. Morelos	T	T	T
<i>Laurencia obtusa</i> (Huds.) J.V. Lamour.	² Pto. Morelos	—	T	T
<i>Laurencia obtusa</i> (Huds.) J.V. Lamour.	⁶ Champotón	—	MT	MT
<i>Liagora ceranooides</i> J.V. Lamour.	² Pto. Morelos	—	T	—
<i>Tayloriella dictyurus</i> (J. Agardh) Kylin	³ Pta. San Telmo	—	—	—

4. Discussion

Variations in the potency of macroalgal toxic activity have been related to ecological actions for protection against herbivory, as was conclusively proven by [29, 45, 37] observed that the complexity of habitats in reef areas increased the ecological process of herbivory, leading macroalgae to develop protection mechanisms such as the formation of hard structures (calcification), or chemical protection through the production of toxic secondary metabolites, or both. In contrast, in algae that grow in other types of habitats, such as rocky shores or coastal lagoons that receive seawater, the development of strong chemical defences is not clear [46]. The results obtained in the present study coincide totally with these findings, since in all cases the algae with potent toxicity in at least one of their extracts were collected in the reef system of the Mexican Caribbean, nine of 10 tested species caused test animals to die when exposed to at least one of their extracts, and only one species was not toxic. The algae collected in freshwater areas with marine influence (myxohaline environments) were recorded with the lowest toxicity in this study (Dzilam, Celestún and Chelém). The algae collected in rocky intertidal localities presented an intermediate toxicity spectrum, with an increase in the number of moderately toxic species. Of note is Faro de Bucerías, in Michoacán, where the great number of tidal pools and spaces carved in the rock by erosion provides a degree of morphological complexity, and all algae were toxic. In contrast, a smaller number of toxic algae and a decrease in toxicity potency were observed in the macroalgae collected in nearby localities like La Barrita and Puerto Vicente Guerrero, which are typical simple rocky areas (Table 1). [45] stated that reefs are complex habitats due to the great number of areas that provide protection, facilitate escape from predators, and sustain a high algal diversity. The coral reef at Puerto Morelos is the second-longest in the world, and it has the characteristics that are required

for algal species to develop potent chemical defences in response to the strong herbivory pressure they are subjected to, as was recorded by the results obtained in this study.

The cases where algae presented a moderate toxicity in one extract and a high toxicity in another (*Caulerpa cupresoides*, *C. paspaloides*, *Sargassum liebmanii*, *Acantophora spicifera*, *Amphiroa mexicana*, *Centroceras clavulatum* and *Gracilaria cervicornis*) may be explained considering that the solvent was not completely effective in extracting the toxic molecule and dissolved only a low concentration of it, resulting in a medium toxicity. This occurs frequently as the solvents that are used have a similar polarity.

It was also observed that some species, when collected at different times, decreased or lost their toxicity. This occurred in samples of *Caulerpa cupressoides* collected in one same locality, with that of June presenting no toxicity and those of other dates being toxic. The presence/absence of toxicity has been well studied in this family (Caulerpaceae) and has been associated with algal age, with the younger plants being more toxic than the older and longer plants [47, 35, 48].

Several species presented a varied pattern related to the locality where they were collected. *Acantophora spicifera* and *Laurencia obtusa* had a variable level of toxicity, with the sample collected in the Puerto Morelos reef being more toxic than the sample collected in a myxohaline environment. In this case, it is clear that this is the result of herbivory pressure, which makes one species toxic and not another, notwithstanding that the sampling dates and localities were close.

In the case of *Gracilaria cervicornis* and *Hypnea musciformis*, these species were collected in Veracruz on the same day from habitats with similar complexity. Variations in toxicity on a geographical scale have been studied for algae of the Division Phaeophyta, with those

that develop in tropical and subtropical areas having greater toxicity than those of temperate areas. However, this phenomenon is not yet quite understood. It is possible that it is related to physiological variables of the algal populations that grow in these areas or to a decrease in nutrients that restricts the production of toxic metabolites [48].

The high number of species that are toxic is an indirect indication of the ecological relationships that are present in the different habitats where macroalgae grow. Toxic molecules may be directly used in the struggle against diseases such as HIV, various viruses and carcinogenic tumours, or as base molecules that, after changing through chemical synthesis, may enhance their spectrum and force of action against these ailments. In Mexico, greater research efforts need to focus on identifying the number and type of toxic molecules present in algae that may be used in medicine, clinical studies, chemistry and biochemistry, among others.

5. Conclusions

It was detected a gradient of toxicity related with the complexity of the habitat, the more toxic macroalgae extracts were founded in reef localities, followed by rocky intertidal ambient and the last were at myxohaline environments. Also was observed a presence/absence of toxicity due to the locality of collection and to the climate data, previously observed in the world. A large number of toxic species can be used in pharmacological research for potential application against HIV, various viruses and carcinogenic tumours or as moulds molecules.

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