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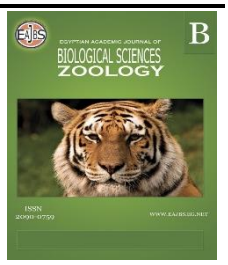

Marine Environmentally Friendly Antifouling Coatings in Eastern Harbor, Alexandria, Egypt

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ABSTRACT

This research aimed to reduce reliance on chemical antifouling agents; it was conducted at depths of 0.5 and 1.5 meters in the Eastern Harbor, at Alexandria, Mediterranean Sea. This study delves into the realm of environmentally sustainable antifouling solutions by exploring the efficacy of natural crude extracts from two Red Sea sponge species, *Acanthella acuta* (Extract A) and *Carteriospongia* sp. (Extract B).

The results revealed a rich diversity of fouling organisms, encompassing six phyla, seven classes, eleven orders, twenty-two families, and thirty-three species. Noteworthy absences were noted at specific depths, indicating depth-dependent variations in fouling communities. The application of extract A at 0.5 meters depth demonstrated significant reductions in fouling biomass and shifts in dominant species. Treatment 3 stood out with the lowest biomass (9.69 ± 2.83 g/m^2) and the fewest species (only 2 species). At 1.5 meters depth, extract B exhibited consistent trends with treatment 6 displaying the lowest biomass (20.85 ± 23.35 g/m^2) and also species diversity (only 2 species). Meanwhile, Extract B showcased compelling antifouling potential. At 0.5 meters depth, treatment 4 exhibited notable reductions in fouling biomass, dominated by *Balanus amphitrite*, *Balanus eburneus*, and *Balanus sp*. At 1.5 meters depth, treatment 6 emerged as the most effective, with the lowest biomass (249.3 ± 120.4 g/m^2) and a streamlined species composition.

This study underscores the promise of natural crude extracts from the Red Sea sponges as potent alternatives for environmentally friendly antifouling strategies. The observed variations in fouling biomass and species composition offer critical insights for the development and application of these extracts in combating fouling organisms, presenting a significant stride toward sustainable marine ecosystem conservation.

INTRODUCTION

Marine biological fouling, usually termed marine biofouling, can be defined as the undesirable accumulation of microorganisms, plants, and animals on artificial surfaces

immersed in seawater. In the case of ships, the adverse effects caused by this biological settlement are well known. Due to generated roughness, high frictional resistance leads to increased weight and subsequent potential speed reduction and loss of maneuverability. To compensate for this, higher fuel consumption is needed, which causes increased emissions of harmful compounds (Tadros *et al*., 2022). It may also be a need for heavier and less energetically efficient machinery. The increase in fuel consumption can be up to 40 % and in voyage overall costs, as much as 77 % (Schultz *et al*., 2010). Many toxic wastes are also generated during this process (Abbott *et al.,* 2000). Deterioration of the coating so that corrosion, discoloration, and alteration of the material's electrical conductivity (Cooney & Tang, 1999).

Most biofouling research has concentrated on investigating methods of control (Brown & Eaton, 2001). Previously, toxic biocides such as copper, chrome and arsenic and tributyltin (TBT) have been used for antifouling (Brown *et al*., 2003; and Railkin, 2004). Among all the proposed solutions, tributyltin copolymer paints (TBT-SPC paints) have been the most successful antifouling on ships. The widespread use of these paints has led to important economic benefits (Abbott *et al*., 2000 and Champ, 2000). Unfortunately, the TBT-SPC systems adversely affect the environment. As an example, it has been shown that extremely low concentrations of tributyltin moiety (TBT) cause defective shell growth in the oyster *Crassostrea gigas* (Evans et al., 1995; and Swain, 1998).

Malformations have been observed in many other species, and the International Maritime Organization (IMO) also reported accumulation in mammals and debilitation of the immunology in fishes. These facts forced the development of national regulations in countries all over the world (Champ, 2000). Due to the toxicity of these biocides on the environment, the European Unit has recognized the difficulties that biofouling poses for the aquaculture industry in Europe by funding Collective Research on Aquaculture Biofouling to produce a pan-European baseline for biofouling pressure and provide recommendations on the management strategies that are both economic and non-toxic (CRAB, 2006; and Fragoso & Icely, 2009).

Some research on antifouling is focused on producing environmentally friendly antifouling paint. These studies resulted in derivations of non-toxic foul-release coatings (FRCs); some of which are commercially available, especially for the shipping industry (Akesso *et al*., 2009; Fang *et al*., 2010; Xie *et al*., 2011; and Carl *et al*., 2012). Also, Nurafni *et al*. (2021) reported that some marine organisms, such as mangroves and seagrasses have antifouling activity.

Therefore, the goal of this study is to mitigate the harmful effects of chemical antifouling agents, traditionally used in ship paints, by exploring and evaluating the effect of natural crude extracts from two Red Sea sponge species (*Acanthella acuta* and *Carteriospongia sp*.) as environmentally friendly antifouling paint.

MATERIALS AND METHODS

Study Area

This study was conducted in front of the National Institute of Oceanography and Fisheries (NIOF), Alexandria. Located at the Eastern Harbor of Alexandria, it is a relatively shallow semi-circular bay, surrounded by the city except for its northern side, which opened to the sea through two outlets (Fig. 1).

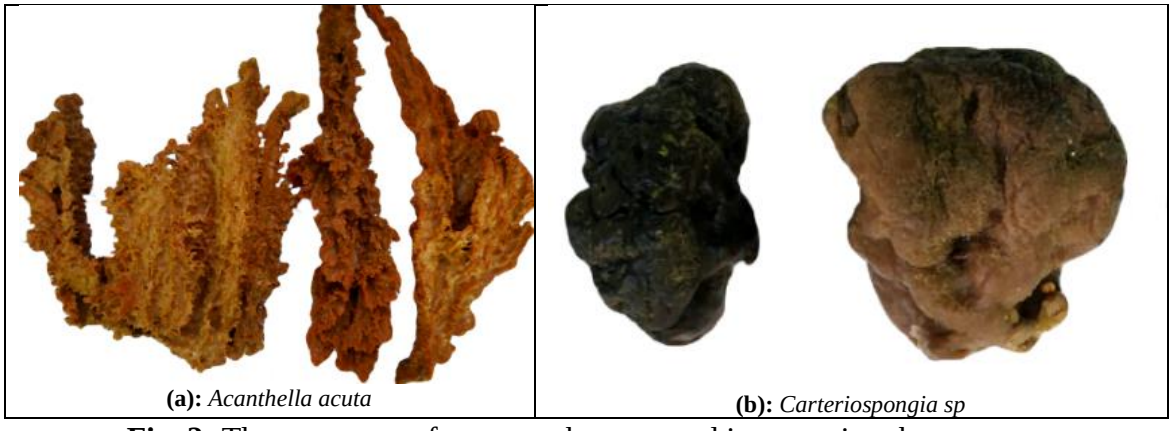
Fig. 1: Location map. (A). The geographical location of Alexandria at the Egyptian Mediterranean Sea coast. (B). Enlarged map showing the sampling area.

Sponge Sampling and Extraction Procedure:

Two species (Fig. 2) of Red Sea sponges, *Acanthella acuta* (Extract A) and *Carteriospongia sp.* (Extract B) were chosen for the present study. The crude bioactive materials were isolated, and their antifouling property had to be tested. The samples of these sponge species were collected from the Red Sea and taken to the laboratory of Zoology Department, Faculty of Science, Al-Azhar University, Cairo for immediate processing.

Preparation Of Sponge Crude Extract:

One hundred grams of marine sponge specimens were macerated with 200 ml of 70% aqueous ethanol. After soaking for a week gently shaking, they were filtered through Whatman 542 filter paper. The solvent was evaporated using a rotary evaporator to obtain extract (Ballantine, 1987).



(a): *Acanthella acuta* **(b):** *Carteriospongia sp*

Fig. 2: The two types of sponges that are used in preparing the extracts.

The Preparation of Polyvinyl Butyral (PVB) Abietic Acid (Ab) Blend:

Sigma Aldrich Co. Rosin supplied the Polyvinyl Butyral (PVB) consisting mainly of abietic acid supplied from HAB. Co, India.

In a beaker of 100 ml, 12 g of abietic acid was dissolved in 80 ml of isopropanol alcohol at room temperature. In another beaker, 8 g of Polyvinyl butyral (PVB) was dissolved in 100 ml of isopropanol alcohol at room temperature. Then the two mixtures were mixed at a stirrer of 200 RPM until obtained of homogenous solution. The extract (3g) was added to the mixture with continuous stirring until it completely dissolved of all extract. The (PVB/Ab) blend was loaded with extract and coated on wood samples to investigate the antifouling property.

The extracts were mixed with a simple paint formulation consisting of a (vinyl chloride–vinyl acetate) copolymer using tricresyl phosphate as a plasticizer for paint preparation. The viscosity of paints was adjusted using a blend of solvents consisting of methyl isobutyl ketone and toluene. Different concentrations from the three types of sponges were mixed with the paint formulation of non-toxic vinyl resin, according to Van London, (1973), to test their antifouling activity. The paint formulations were prepared using a porcelain ball mill and a one-liter porcelain jar. The milling process continued over a period of two weeks. The paint formulations were applied on three Wood substrates with 10 x 15 cm dimensions hung to a steel frame.

Antifouling:

The experiment was conducted by using an iron frame of 100 x 60 cm, bearing 36 square test panels (10 x 15 cm) made of impact-resistant polystyrene that served as fouling collectors. The frame was suspended vertically under the buoy of each station at depths of 0.5 m and 1.5 m below the seawater surface (Fig. 3).

Fig. 3 : Steel frame with different plates.

Antifouling Experimental Design:

Two cm thick sheets of wood were cut to panel plates with dimensions of 15x10 cm. The edges were capote, and the surface was smoothed with sandpaper to get suitable roughness. The plates were coated delicately in front and back with two successive coats of the prepared paints, allowing an interval of 12 hours between each coat. The coated panels were connected to the testing cages with nylon threads through a nail board in the panel.

A simulation was made of what happens in the manufacture of boats from the preparation of the panels before coating them with the extract and these plates were divided as follows:

C 1: Negative control of wood plate only

C 2: Negative control of wood plate + primer paint (putty)

C3: Positive control of wood plate + primer paint (putty) + boat paint with chemical antifouling

Then, the same plates were over-painted by the extracts of *Acanthella acuta* (Extraction A) and *Carteriospongia sp*. (Extraction B) to test the validation of using sponge extract as an antifouling paint replacement. The plates were then immersed in two different depths (0.5 and 1.5 meters). These treatment plates were divided as follows:

T 1: Negative control of wood plate only + extraction A

T 2: Negative control of wood plate + primer paint (putty) + extraction A

T 3: Positive control of wood plate + primer paint (putty) + boat paint with chemical antifouling + extraction A

T 4: Negative control of wood plate only + extraction B

T 5: Negative control of wood plate + primer paint (putty) + extraction B

T 6: Positive control of wood plate + primer paint (putty) + boat paint with chemical antifouling + extraction B

Fouling Identification:

In the present work, fouling organisms were identified according to relevant literature, which was consulted for identification (e.g., Balavoine, 1959 and Ryland *et al*., 2009).

Statistical Analysis:

Data was coded and entered using the statistical package SPSS V.22. Data were tested for satisfying assumptions of parametric tests, and continuous variables were subjected to Shapiro-Wilk and Kolmogorov-Smirnov test for normality. Data were presented as mean and standard deviation. ANOVA analyses were done; post-hoc analysis was evaluated using Tukey pairwise comparison; P-values were considered significant at<0.05. Two-way heatmap for fouling species abundance were visualized using PAST software V 4.12. Data when possible, using R studio V 2022.02.4.

RESULTS

Table (1) represents the fouling species observed during the present study at both depths (0.5 and 1.5 m). A total of 6 phylum; 7 classes; 11 orders; 22 families and 33 species of fouling organisms were observed during the present study with special reference to the absence of any observation of *Watersipor subovoidea,* and *Sphaeroma serratum* at 0.5 m depth; and the absence of *Nereis diversicolor, Balanus trigonus, Ericthonius brasiliensis,* and *Alacoma atra*at at 1.5 m of depth.

Table 1: List of the fouling invertebrate species recorded on the experimental plates at different depths of the Eastern Harbor, Alexandria, during the study period.

Phylum	Class	Order	Family	Scientific name of species	Depths (m)	
					0.5	1.5
Cnidaria	Hydrozoa	Leptothecata	1. Campanulariidae	1. *Obelia geniculate* (Linnaeus, 1758)	+	+
Bryozoa	Gymnolaemata	Cheilostomatida	2. Bugulidae	2. *Bugula neritina* (Linnaeus, 1758)	+	+
			3. Lepraliellidae	3. *Celleporaria sp.*	+	+
			4. Schizoporellidae	4. *Schizoporella sp.*	+	+
			5. Cryptosulidae	5. *Cryptosula pallasiana* (Moll, 1803)	+	+
			6. Watersiporidae	6. *Watersipor subovoidea* (d'Orbigny, 1852)	--	+
			7. Electridae	7. *Conopeum reticulum* (Linnaeus, 1767)	+	+
Annelida	Polychaeta	Phyllodocida	8. Nereididae	8. *Nereis diversicolor* Müller, 1776	+	--
		Sabellida	9. Serpulidae	9. *Hydroides elegans* (Haswell, 1883)	+	+
				10. *Spirobranchus tetraceros* (Schmarda, 1861)	+	+
				11. *Spirorbis sp.*	+	+
Mollusca	Bivalvia	Mytilida	10. Mytilidae	12. *Modiolus barbatus* (Linnaeus, 1758)	+	+
Crustacea	Thecostraca	Balanomorpha	11. Balanidae	13. *Balanus amphitrite* Darwin, 1854	+	+
				14. *Balanus eburneus* Gould, 1841	+	+
				15. *Balanus perforates* Bruguière, 1789	+	+
				16. *Balanus trigonus* Darwin, 1854	+	--
				17. *Balanus sp.*	+	+
	Malacostraca	Tanaidacea	12. Tanaididae	18. *Tanais dulongii* (Audouin, 1826)	+	+
			13. Leptocheliidae	19. *Leptochelia savignyi* (Krøyer, 1842)	+	+
		Isopoda	14. Cirolanidae	20. *Cirolana bovina* Barnard, 1940	+	+
			15. Sphaeromatidae	21. *Sphaeroma walkeri* Stebbing, 1905	+	+
				22. *Sphaeroma serratum*	--	+
				23. *Paradella dianae* (Menzies, 1962)	+	+
				24. *Cymodoce truncata* Leach, 1814	+	+
		Amphipoda	16. Corophiidae	25. *Corophium acutum* Chevreux, 1908	+	+
				26. *Corophium sextonae* Crawford, 1937	+	+
			17. Maeridae	27. *Elasmopus pectenicrus* (Spence Bate, 1862)	+	+
			18. Podoceridae	28. *Podocerus variegatus* Leach, 1814	+	+
			19. Stenothoidae	29. *Stenothoe gallensis* Walker, 1904	+	+
			20. Ischyroceridae	30. *Ericthonius brasiliensis* (Dana, 1853)	+	--
				31. Amphipoda tubes	+	+
		Decapoda	21. Polybiidae	32. *Liocarcinus depurator* (Linnaeus, 1758)	+	+
Chordata	Ascidiacea	Phlebobranchia	22. Ascidiidae	33. *Alacoma atra*	+	--

Regarding extract A at 0.5m depth, we found that control 1 covered by 5503.2 ± 3464.9 g/m^2 of total fouling biomass (18 species); the dominant species were *Nereis diversicolor* (2013.3 ± 1509.9 g/m^2), *Hydroides eleganes* (1656.2 ± 880.7 g/m^2) and *Balanus perforatus* (501.1 ± 501.13 g/m^2). In comparison, treatment 1 was covered by 171.5 ± 746.7 g/m^2 (9 species); the dominant species were *Balanus amphitrite* (409.5 ± 282.76 g/m^2), *Balanus* sp. (223.2 ± 123.97 g/m^2) and *Spirobranchus tetraceros* (172.49 ± 83.27

g/m^2). Where control 2 was covered by 8283.7 ± 5845.4 g/m^2 of total fouling biomass (24 species); the dominant species were *Hydroides eleganes* (3875.8 ± 4063.8 g/m^2), *Balanus eburneus* (1891.04 ± 772.6 g/m^2) and *Balanus Amphitrite* (1637.9 ± 1862.6 g/m^2). In comparison, treatment 2 was covered by 686.5 ± 335.4 g/m^2 (4 species); the dominant species was *Balanus amphitrite* (674.73 ± 165.4 g/m^2). Finally, control 3 was covered by 58.71 ± 13.81 g/m^2 of total biomass of fouling (only 3 species); the dominant species was *Modiolus barbatus* (31.8 ± 18.54 g/m^2). In comparison, treatment 3 was covered by 9.69 ± 2.83 g/m^2 of total biomass of fouling (only 2 species), the dominant species was *Spirorbis sp.* (5.64 ± 9.77 g/m^2) (Table, 2 and Figures 4&5).

Regarding biofouling biomass (g) and species numbers, there was significant variation ($p < 0.05$) between control 1 vs. treatment 1, control 2 vs. treatment 2 and control 3 vs. treatment 3. Treatment (3) was the lowest biomass (9.69 ± 2.83 g/m^2) covered by the lowest number of species (2).

Table (2): Effect of extract A on the biomass (g) of fouling invertebrate species during 2020-2021 season at 0.5 m depth

No.	Fouling species		Controls			Treatments		
			C1	C2	C3	T1	T2	T3
1	*Obelia geniculate*		9.02 ± 15.62	39.08 ± 67.7				
2	*Bugula neritina*		2.47 ± 0.53	1.85 ± 0.92		3.71 ± 5.64	0.92 ± 0.92	
3	*Celleporaria sp.*			1.24 ± 1.42				
4	*Schizoporella sp.*			25.34 ± 25.34				
5	*Cryptosula pallasiana*			29.85 ± 27.85		9.26 ± 6.17		
6	*Conopeum reticulum*		25.06 ± 7.17	3.13 ± 5.42				
7	*Nereis diversicolor*		2013.3 ± 1509.9					
8	*Hydroides eleganes*		1656.2 ± 880.7	3875.8 ± 4063.8		47.51 ± 31.15		
9	*Spirobranchus tetraceros*		354.06 ± 54.47	9.07 ± 15.72		172.49 ± 83.27		
10	*Spirorbis sp.*			84.64 ± 105.72				5.6 ± 9.77
11	*Modiolus barbatus*			190.65 ± 95.32	31.8 ± 18.54	127.11 ± 145.6		
12	*Balanus amphitrite*		283.83 ± 181.6	1637.9 ± 1862.6		409.5 ± 282.76	674.73 ± 165.4	
13	*Balanus eburneus*		459.5 ± 200.73	1891.04 ± 772.6		53.2 ± 53.2		
14	*Balanus perforatus*		501.1 ± 501.13					
15	*Balanus trigonus*		4.53 ± 7.84					
16	*Balanus* sp.				4.61 ± 2.39	223.2 ± 123.97		
17	*Tanais dulongii*		172.73 ± 81.54	19.74 ± 22.61				
18	*Leptochelia savignyi*				22.28 ± 28.72			4.05 ± 3.5
19	*Cirolana bovina*			3.74 ± 3.24				
20	*Sphaeroma walkeri*		10.91 ± 18.9	7.27 ± 12.6				
21	*Paradella dianae*		4.05 ± 2.02					
22	*Cymodoce truncata*			0.62 ± 1.08		25.75 ± 15.23	10.04 ± 5.43	
23	*Corophium acutum*			56.01 ± 49.05				
24	*Corophium sextonae*		3.12 ± 0					
25	*Elasmopus pectenicrus*		0.35 ± 0.61	14.61 ± 25.3				
26	*Podocerus variegatus*		1.65 ± 1.14	4.3 ± 7.45				
27	*Stenothoe gallensis*			22.91 ± 39.69				
28	*Ericthonius brasiliensis*		1.01 ± 0.43	0.25 ± 0.43				
29	Amphipoda tubes		0.25 ± 0.44	13.97 ± 11.96			0.76 ± 0.76	
30	*Liocarcinus depurator*			2.41 ± 4.17				
31	*Alacoma atra*			348.2 ± 603.07				
Total		**Biomass**	**5503.2 ± 3464.9**	**8283.7 ± 5845.4**	**58.71 ± 13.81**	**171.5 ± 746.7**	**686.45± 335.44**	**9.69 ± 2.83**
		Species	**18**	**24**	**3**	**9**	**4**	**2**

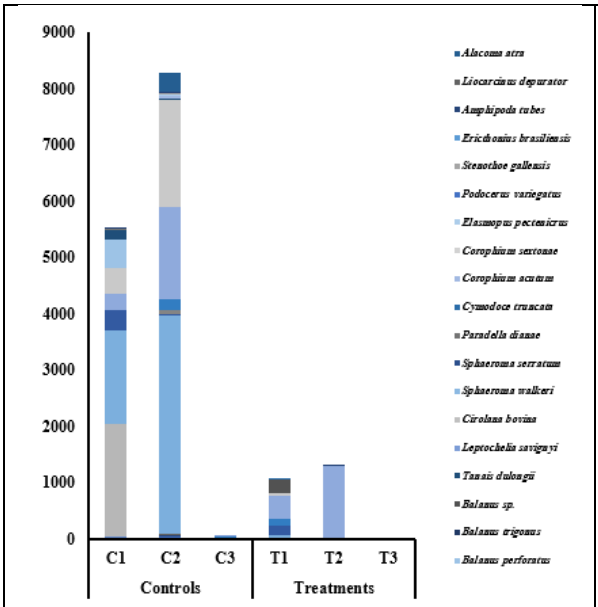


Fig. 4: Species composition of biofouling mass (g/m^2) for experimental group (extract A at 0.5 m depth) during the study period.

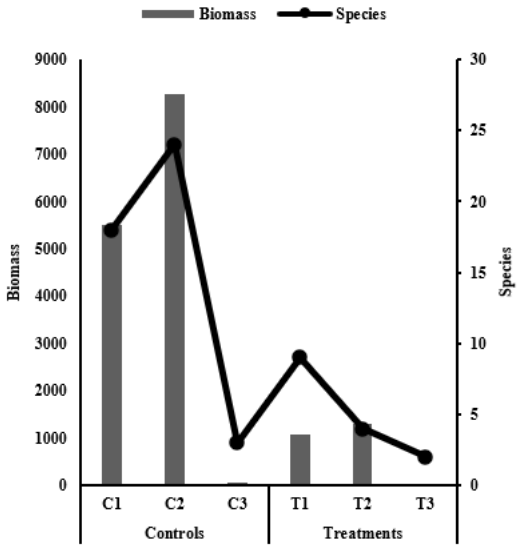


Fig. (5): Number of biofouling species and total biomass (g/m^2) for experimental groups (extract A at 0.5 m depth) during the study period

When applied the same extract at 1.5 m the control group labeled as Control 1 exhibited a total fouling biomass coverage of 13091.7 ± 8449.9 g/m^2, consisting of 20 different species. The dominant species within this group were *Balanus perforatus* with a mass of 6774.6 ± 4955.8 g/m^2, followed by *Hydroides eleganes* with a mass of 4921.1 ± 2741.8 g/m^2, and *Balanus eburneus* with a mass of 512.5 ± 200.7 g/m^2. In contrast, treatment (1) displayed a total fouling biomass of 952.6 ± 641.7 g/m^2, encompassing 8 species. The dominant species in this treatment were Balanus sp. with a biomass of 316.15 ± 214.4 g/m^2, Schizoporella sp. with a biomass of 219.6 ± 162.9 g/m^2, and *Balanus eburneus* with a biomass of 212.08 ± 91.8 g/m^2. Furthermore, control (2) demonstrated a total fouling biomass of 5421.04 ± 3669.7 g/m^2, comprising 19 species. The dominant species in this group were *Balanus eburneus* with a biomass of 2120.8 ± 1180.8 g/m^2, *Hydroides eleganes* with a biomass of 1934.5 ± 3141.4 g/m^2, and *Balanus amphitrite* with a biomass of 502.52 ± 41.87 g/m^2. On the other hand, treatment (2) showed a total fouling biomass of 730.3 ± 308.6 g/m^2, involving 5 species. The dominant species within this treatment were *Balanus amphitrite* with a biomass of 698.0 ± 244.6 g/m^2 and *Cryptosula pallasiana* with a biomass of 18.53 ± 6.17 g/m^2. Lastly, control (3) presented a total fouling biomass of 245.6 ± 356.01 g/m^2, consisting of 7 species. The dominant species in this group were Balanus sp. with a coverage of 139.48 ± 241.6 g/m^2, *Balanus amphitrite* with a biomass of 37.22 ± 21.32 g/m^2, and *Leptochelia savignyi* with a biomass of 32.41 ± 33.47 g/m^2. In comparison, treatment (3) displayed a total fouling biomass of 19.4 ± 22.3 g/m^2, comprising 2 species, with the dominant species being *Cymodoce truncate* with a biomass of 15.07 ± 18.55 g/m^2 (Table, 3 and Figs. 6 &7).

There were significant variations ($p < 0.05$) observed in terms of biofouling biomass and species numbers between control (1) vs treatment (1), control (2) vs treatment (2), and control (3) vs treatment (3). Treatment (3) exhibited the lowest biomass (19.4 ± 22.3 g/m^2) and was characterized by the lowest number of species (2).

Table 3: Effect of extract A on the biomass (g) of fouling invertebrate species during the winter season at 1.5 m depth.

No.	Fouling species	Controls			Treatments		
		C1	C2	C3	T1	T2	T3
1	*Obelia geniculate*		69.15± 10.4				
2	*Bugula neritina*	136.47± 41.5	5.26± 0.53	3.1± 2.33	1.9± 2.45	9.9± 1.41	4.3 ± 3.75
3	*Celleporaria sp.*	0.93± 0.93					
4	*Schizoporella sp.*	25.34± 25.3			219.6± 162.9		
5	*Cryptosula pallasiana*	2.05± 1.78	21.62± 27.45		23.7± 11.7	18.5± 6.17	
6	*Watersipor subovoidea*	7.65± 7.65					
7	*Conopeum reticulum*	21.92± 9.78					
8	*Hydroides eleganes*	4921± 2742	1935± 3141.4		20.4± 35.3		
9	*Spirobranchus tetraceros*	72.62± 83.2					
10	*Spirorbis sp.*	118.5± 58.64	22.57± 25.85				
11	*Modiolus barbatus*		317.8± 334.8	31.8± 55.03			
12	*Balanus amphitrite*	158.2± 65.96	502.5± 41.87	37.2± 21.32	158.2± 121.95	698.0± 244.6	
13	*Balanus eburneus*	512.5± 200.7	2121± 1180.8		212.1± 91.8		
14	*Balanus perforatus*	6775 ± 4956	389.8± 347.7				
15	*Balanus* sp.			139.5± 241.6	316.2± 214.4		
16	*Tanais dulongii*	83.9± 74.52	9.87± 17.09				
17	*Leptochelia savignyi*	20.3± 9.28		32.4± 33.47			
18	*Cirolana bovina*		0.2± 0.36				
19	*Sphaeroma walkeri*		3.63± 6.3				
20	*Sphaeroma serratum*		1.94± 2.43				
21	*Paradella dianae*	2.02± 3.51	4.05± 7.03				
22	*Cymodoce truncata*		3.76± 6.52	0.62± 1.08	0.62± 1.08	3.14± 2.87	15.1 ± 18.55
23	*Corophium acutum*		3.24± 2.53				
24	*Corophium sextonae*	1.39± 0.6					
25	*Elasmopus pectenicrus*	1.78± 1.63	0.35± 0.61				
26	*Podocerus variegatus*	6.95± 1.98	0.33± 0.57				
27	*Stenothoe gallensis*	83.47± 70.72					
28	Amphipoda tubes		9.65± 13.55	1.01± 1.16		0.76± 1.32	
29	*Liocarcinus depurator*	139.93± 94.46					
Total	**Biomass**	**13092± 8450**	**5421± 3669.7**	**245.6±356.01**	**952.6± 641.7**	**730.3± 308.6**	**19.4 ± 22.3**
	Species	**20**	**19**	**7**	**8**	**5**	**2**

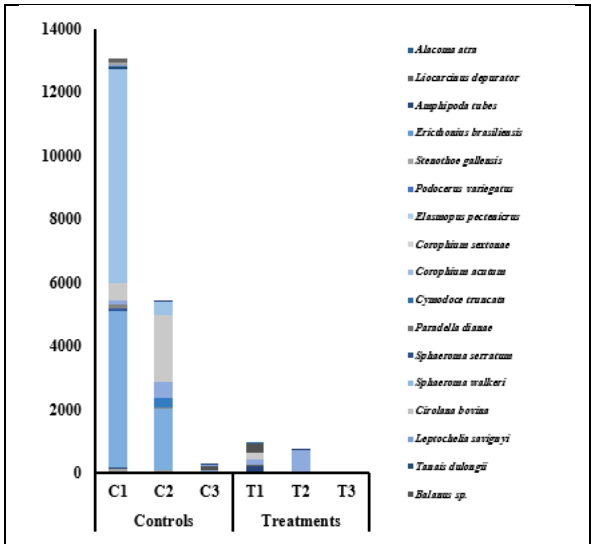


Fig. 6: Species composition of biofouling mass (g/m^2) for experimental group (extract A at 1.5 m depth) during study period.

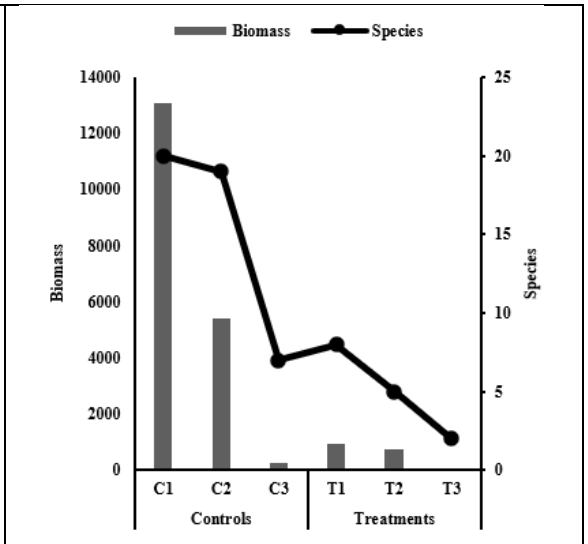


Fig. 7: Number of biofouling species and biomass (g/m^2) for experimental groups (extract A at 1.5 m depth) during study period.

For extract B effect at 0.5 m, control 1 exhibited a total fouling biomass of 5503.2 ± 3464.9 g/m^2, encompassing 18 different species. The dominant species within this group were *Nereis diversicolor* with a biomass of 2013.3 ± 1509.9 g/m^2, *Hydroides eleganes* with a biomass of 1656.2 ± 880.7 g/m^2 and *Balanus perforatus* with a biomass of 501.13 ± 501.1 g/m^2. In contrast, treatment 4 displayed a total fouling biomass of 568.6 ± 439.3 g/m^2, involving 8 species. The dominant species in this treatment were *Balanus amphitrite* with a biomass of 279.18 ± 205.6 g/m^2, *Balanus eburneus* with a biomass of 123.71 ± 61.22 g/m^2, and *Balanus sp*. with a biomass of 116.2 ± 105.6 g/m^2. Furthermore, control 2 demonstrated a total fouling biomass of 8283.7 ± 5845.4 g/m^2, comprising 24 species. The dominant species in this group were *Balanus eburneus* with a biomass of 1891± 772.6 g/m^2, *Balanus amphitrite* with a biomass of 1637.9 ± 1862.6 g/m^2, and *Hydroides eleganes* with a biomass of 3875.8 ± 4063.8 g/m^2. On the other hand, treatment 5 showed a total fouling biomass of 364.5 ± 335.98 g/m2, involving 3 species. The dominant species within this treatment was *Balanus amphitrite* with a biomass of 358.3 ± 330.7 g/m^2. Lastly, control 3 presented a total fouling biomass of 58.71 ± 13.81 g/m^2, consisting of 3 species. The dominant species in this group were *Modiolus barbatus* with a biomass of 31.8 ± 18.5 g/m^2 and *Leptochelia savignyi* with a biomass of 22.28 ± 28.72 g/m^2. In comparison, treatment 6 displayed a total fouling biomass of 20.85 ± 23.35 g/m^2, comprising only 2 species, with the dominant species being *Leptochelia savignyi* with coverage of 16.2 ± 15.29 g/m^2.

Table 4: Effect of extract B on the biomass (g/m^2) of fouling invertebrate species during the winter season at 0.5 m depth

No.	Fouling species		Controls				Treatments	
			C1	C2	C3	T4	T5	T6
1	*Obelia geniculate*		9.02± 15.62	39.1± 67.7				
2	*Bugula neritina*		2.47± 0.53	1.85± 0.92		2.2± 0.53		
3	*Celleporaria sp.*			1.24± 1.42				
4	*Schizoporella sp.*			25.3± 25.3				
5	*Cryptosula pallasiana*			29.9± 27.85		6.2± 8.17	3.08± 3.08	
6	*Conopeum reticulum*		25.06± 7.17	3.13± 5.42				
7	*Nereis diversicolor*		2013± 1509.9					
8	*Hydroides eleganes*		1656± 880.7	3876± 4064				
9	*Spirobranchus tetraceros*		354.1± 54.47	9.07± 15.72				
10	*Spirorbis sp.*			84.6± 105.72				
11	*Modiolus barbatus*			190.7± 95.32	31.8± 18.5	31.8± 55.03		
12	*Balanus amphitrite*		283.8± 181.6	1638± 1863		279.2± 205.6	358.3± 330.7	
13	*Balanus eburneus*		459.5± 200.73	1891± 772.6		123.7± 61.22		
14	*Balanus perforatus*		501.1± 501.1					
15	*Balanus trigonus*		4.53± 7.84					
16	*Balanus* sp.				4.6± 2.39	116.2± 105.6		4.64± 8.05
17	*Tanais dulongii*		172.7± 81.54	19.74± 22.61				
18	*Leptochelia savignyi*				22.3± 28.72			16.2± 15.29
19	*Cirolana bovina*			3.74± 3.24				
20	*Sphaeroma walkeri*		10.91± 18.9	7.27± 12.6				
21	*Paradella dianae*		4.05± 2.02					
22	*Cymodoce truncata*			0.62± 1.08		7.5± 1.88	3.14± 2.17	
23	*Corophium acutum*			56.0± 49.05				
24	*Corophium sextonae*		3.12± 0					
25	*Elasmopus pectenicrus*		0.35± 0.61	14.61± 25.3				
26	*Podocerus variegatus*		1.65± 1.14	4.3± 7.45				
27	*Stenothoe gallensis*			22.91± 39.69				
28	*Ericthonius brasiliensis*		1.01± 0.43	0.25± 0.43				
29	Amphipoda tubes		0.25± 0.44	13.97± 11.96		1.77± 1.16		
30	*Liocarcinus depurator*			2.41± 4.17				
31	*Alacoma atra*			348.2± 603.1				
Total		**Biomass**	**5503 ± 3464.9**	**8284± 5845.4**	**58.71± 13.81**	**568.6± 439.3**	**364.5± 335.98**	**20.9± 23.35**
		Species	**18**	**24**	**3**	**8**	**3**	**2**

Based on the statistical analysis, there were significant variations ($p < 0.05$) observed in terms of biofouling biomass and species numbers between control 1 vs. treatment 4, control 2 vs. treatment 5, and control 3 vs. treatment 6. Also, treatment 6 exhibited the lowest biomass (20.85 ± 23.35 g/m^2) and was characterized by the lowest number of species (2) (Table, 4 and Figs. 8&9).

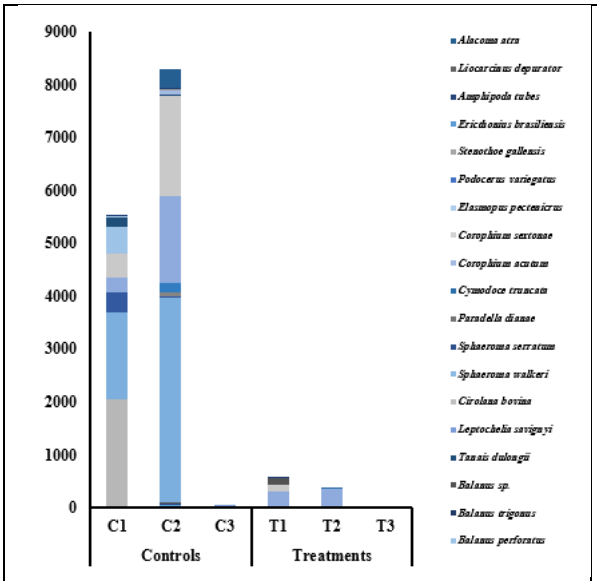


Fig. 8: Species composition of biofouling mass (g/m^2) for experimental group (extract B at 0.5 m depth) during study period.

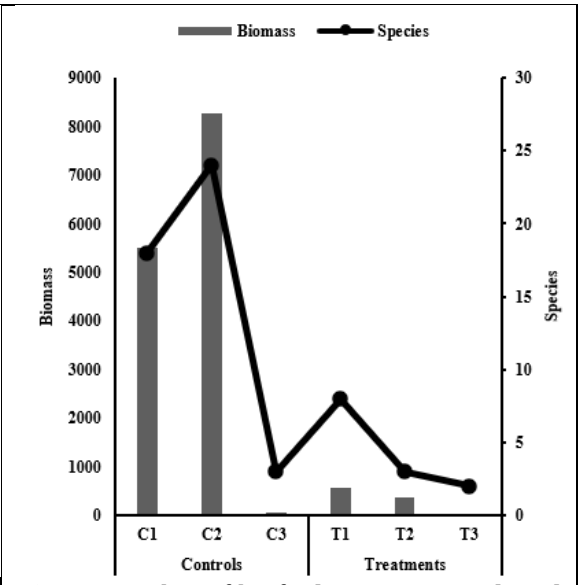


Fig. 9: Number of biofouling species and total biomass (g/m^2) for experimental groups (extract B at 0.5 m depth) during study period.

Finally, extract B at 1.5 m depth, control 1 was characterized by a total fouling biomass of 13091.7 ± 8449.9 g/m^2, encompassing 20 different species. The dominant species within this group were *Balanus perforatus* with a biomass of 6774.6 ± 4955.8 g/m^2, *Hydroides eleganes* with a biomass of 4921.1 ± 2741.8 g/m^2, and *Balanus eburneus* with a biomass of 512.5 ± 200.7 g/m^2. In comparison, treatment 4 exhibited a total fouling biomass of 2991.4 ± 1266.8 g/m^2, involving 7 species. The dominant species in this treatment were *Balanus amphitrite* with a biomass of 130.28 ± 56.41 g/m^2 and *Balanus sp*. with a biomass of 2380.5 ± 878.9 g/m^2. Furthermore, control 2 displayed a total fouling biomass of 5421.04 ± 3669.8 g/m^2, consisting of 19 species. The dominant species in this group were *Balanus eburneus* with a biomass of 2120.8 ± 1180.8 g/m^2, *Hydroides eleganes* with a biomass of 1934.5 ± 3141.4 g/m^2, and *Balanus amphitrite* with a biomass of 502.5 ± 41.87 g/m^2. On the other hand, treatment 5 demonstrated a total fouling biomass of 1208.7 ± 537.2 g/m^2, involving 7 species. The dominant species within this treatment were *Balanus sp*. with a biomass of 836.9 ± 350.9 g/m^2 and *Balanus amphitrite* with a biomass of 88.4 ± 21.32 g/m^2. Lastly, control 3 presented a total fouling biomass of 245.6 ± 356.01 g/m^2, consisting of 7 species. The dominant species in this group were *Balanus sp*. with a biomass of 139.5 ± 241.6 g/m^2, *Balanus amphitrite* with a biomass of 37.22 ± 21.32 g/m^2, and *Leptochelia savignyi* with a biomass of 32.41 ± 33.47 g/m^2. In comparison, treatment 6 displayed a total fouling biomass of 249.3 ± 120.4 g/m^2, comprising 6 species, with the dominant species being *Balanus sp*. with a biomass of 213.9 ± 81.72 g/m^2 and *Balanus amphitrite* with a biomass of 18.61 ± 21.32 g/m^2 (Table, 5 and Figs. 10&11).

Based on the present analysis, there were significant variations ($p < 0.05$) observed in terms of biofouling biomass and species numbers between control 1 vs. treatment 4; control 2 vs. treatment 5; and control 3 vs. treatment 6. Treatment 6 exhibited the lowest biomass (249.3 ± 120.4 g/m^2) and was characterized by the lowest number of species.

Table 5: Effect of extract B on the biomass (g) of fouling invertebrate species during 2020/2021 season at 1.5 m depth**.**

No.	Fouling species	Controls			Treatments		
		C1	C2	C3	T1	T2	T3
1	*Obelia geniculate*		69.15± 10.41				
2	*Bugula neritina*	136.5± 41.47	5.26± 0.53	3.09 ± 2.33	15.47± 2.83	2.47± 0.53	4.02± 2.98
3	*Celleporaria sp.*	0.93± 0.93					
4	*Schizoporella sp.*	25.3± 25.34			388.6± 268.6	261.9± 152.8	
5	*Cryptosula pallasiana*	2.05± 1.78	21.62± 27.45		22.7± 6.42	15.4± 5.34	7.2± 4.71
6	*Watersipor subovoidea*	7.65± 7.65					
7	*Conopeum reticulum*	21.9± 9.78					
8	*Hydroides eleganes*	4921± 2741.8	1935± 3141.4		47.5± 31.1		
9	*Spirobranchus tetraceros*	72.62± 83.2					
10	*Spirorbis sp.*	118.5± 58.64	22.57± 25.85	\			
11	*Modiolus barbatus*		317.8± 334.8	31.8± 55.03			
12	*Balanus amphitrite*	158.2± 65.96	502.5± 41.87	37.2± 21.32	130.3± 56.41	88.4± 21.32	18.6± 21.32
13	*Balanus eburneus*	512.5± 200.7	2121 ± 1180.8				
14	*Balanus perforatus*	6775± 4956	389.8± 347.7				
15	*Balanus* sp.			139.5± 241.6	2381± 878.9	836.9± 350.9	213.9± 81.72
16	*Tanais dulongii*	83.9± 74.52	9.87± 17.09				4.93± 8.54
17	*Leptochelia savignyi*	20.26± 9.28		32.4± 33.47			
18	*Cirolana bovina*		0.2± 0.36				
19	*Sphaeroma walkeri*		3.63± 6.3			3.63± 6.3	
20	*Sphaeroma serratum*		1.94± 2.43				
21	*Paradella dianae*	2.02± 3.51	4.05± 7.03				
22	*Cymodoce truncata*		3.76± 6.52	0.62± 1.08	6.28± 5.75		0.62± 1.08
23	*Corophium acutum*		3.24± 2.53				
24	*Corophium sextonae*	1.39± 0.6					
25	*Elasmopus pectenicrus*	1.78± 1.63	0.35± 0.61				
26	*Podocerus variegatus*	6.95± 1.98	0.33± 0.57				
27	*Stenothoe gallensis*	83.47± 70.72					
28	Amphipoda tubes		9.65± 13.55	1.01± 1.16			
29	*Liocarcinus depurator*	139.9± 94.46					
Total	**Biomass**	**13092± 8449.9**	**5421± 3669.8**	**245.6± 356.01**	**2991± 1267**	**1209± 537.2**	**249.3± 120.4**
	Species	**20**	**19**	**7**	**7**	**7**	**6**

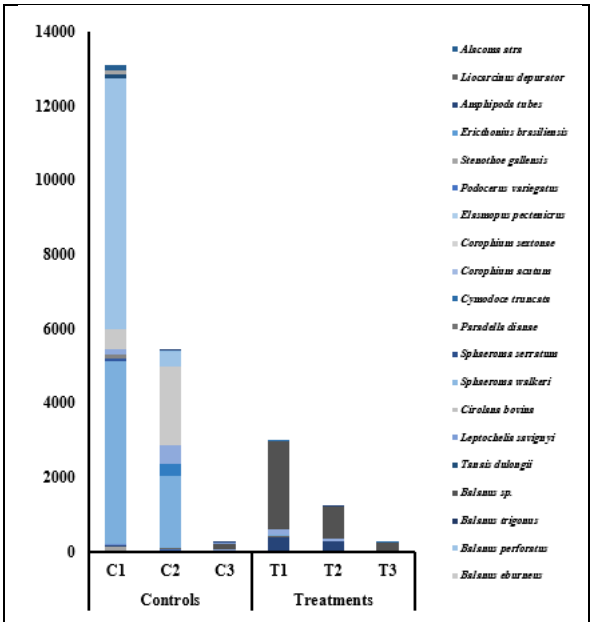


Fig. 10: Species composition of biofouling mass (g/m^2) for experimental group (extract B at 1.5 m depth) during study period.

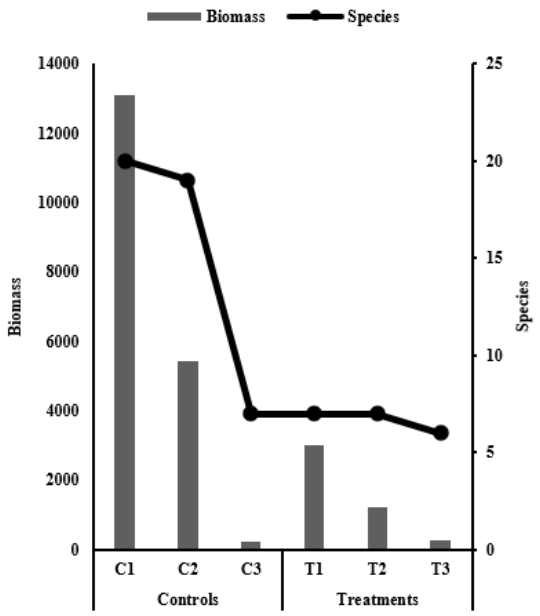


Fig. 11: Number of biofouling species and total biomass (g/m^2) for experimental groups (extract B at 1.5 m depth) during study period.

DISCUSSION

One of the main concerns about the use of natural chemical compounds is the impact that they can have on invertebrate organisms (Hasson *et al*. 2011). The activity of most commercial antifouling products corresponds to international activity criteria: thus, it is required to have a broad-spectrum activity, and on specific target species such as *amphitrite*. The same applies to toxicity tests, which are carried out on a precise list of species. Nevertheless, when a paint formulation is developed for use in a limited geographical area, such tests are sometimes very unrepresentative of the ecosystem and therefore of local fouling pressure (Maréchal & Hellio, 2011); we then find ourselves with poorly adapted formulations and this can lead to a decrease in efficiency and an increased risk of environmental toxicity. As part of our research aimed at the discovery of new antifoulants from marine invertebrates, we explore sponge extract as a powerful replacement for commercial antifouling paints to reduce its toxic activity in the ecosystem.

The relationship between reduced settlement and development inhibition in sessile organisms generated by sponge extracts, through the alteration of neurotransmitter enzyme activity, has been previously demonstrated. The field experiments gave the durability of the coated plates under service conditions that include wind, chemical erosion due to polluted water and biological attack on marine life. So, our study was applied in the field using submerged wooden plates. The main fouling organisms that are present are barnacles, tube worms, ascidians, bryozoans, and algae which are some of our main targets. Our same work approach was reported from other works of paint formulations incorporating characterized natural products (Shin & Smith, 2001). Paint formulations incorporating extracts of sponges were also active in barnacle settling assays (Jin *et al*., 2022) and in a separate study, extracts of sponges and a gorgonian were shown to be active against tube worms when mixed with abietic acid and coated onto panels (Bakus *et al*., 1994).

Sponges from various regions have been evaluated as a source of metabolites with antifouling activity. It was concluded that these organisms represent good sources of antifoulants due to the presence of a great diversity of chemical compounds with diverse and novel structures. These compounds have an ecological function as a chemical defense against other organisms to avoid the settlement of bacteria and epibiont organisms (Sánchez-Lozano *et al*., 2019). In this type of research program, it may seem repetitive to evaluate a genus again, such as *Laurencia*, from which fractions and compounds have been reported that inhibit the growth of biofouling-forming bacteria and the settlement of barnacles (König & Wright, 1997). However, a recent study has demonstrated that results from laboratory assays did not fully concur with the antifouling activity of the paints in the field trial, and because of the lack of field tests, the results of in vitro bioassays are not enough to be critically discussed (Bressy *et al*., 2010). Furthermore, the production of specific compounds by organisms in their ecological context can potentially have a better efficacy on target species when sampling and testing organisms from the same environment (Maréchal & Hellio, 2011). In this sense, the aim of this study is to evaluate the antifouling potential of marine benthic resources from Eastern Harbor (Alexandria) in a more integrated manner, both with various laboratory bioassays with native biofouling-forming bacteria, as well as in field tests against macro-fouling.

The environmental impacts of commercial antifouling paints demonstrate the need to develop new strategies that are truly non-toxic and effective. The International Maritime Organization (IMO) prohibited the use of TBT in antifouling paints due to them causing serious damage in the marine environment, but in the case of cuprous oxide, there is still use despite its toxicity and negative effect on the development of some marine organisms (Trepos *et al*., 2014). So, our findings are in the same manner as IMO guides to develop more friendly nontoxic replacements for chemical antifouling products.

The formulated paint remained in good condition on the plate over time and thus it was determined that it represents a good base to evaluate the extracts. In seawater, any unprotected immersed substratum is rapidly colonized by marine organisms (Bressy *et al*., 2010). When using extracts in paint formulation, the advantage is that mixtures of molecules are incorporated, thus allowing synergistic activities. This type of approach makes it possible to copy the natural strategies of organisms and to obtain the totality of the diversity of defense molecules produced by an organism.

In the search for antifouling agents, it is important to perform assays in the field because only a few extracts or compounds are incorporated in paint and put into marine experiments (Acevedo *et al*., 2013), and the results in laboratory assays do not always provide sufficient information with respect to the field performance of chemically active paints (Bressy *et al*., 2010). So, we follow the filed assay for better testing of our extracts.

For this research, when comparing the results observed in the case of extracts of *Acanthella acuta* (Extraction: A) and *Carteriospongia sp*. (Extraction: B) results were promising. In the field test, the extracts are a good resource to inhibit the fouling attachment. and it is necessary to evaluate in future studies the different factors (biotic and abiotic) that help explain this finding.

The use of extracts incorporated in non-toxic paint could represent a more economical and viable option if the organism's abundance is enough to supply the resource. The use in the formulation of antifouling paints may be possible on a large scale, but it is necessary to study economic feasibility to determine a commercial development.

Conclusion

In this study, we have explored the potential of natural crude extracts from two Red Sea sponge species, *Acanthella acuta* (Extract A) and *Carteriospongia* sp. (Extract B), as environmentally friendly antifouling agents in the Mediterranean Sea. Our findings reveal compelling evidence of their effectiveness in reducing fouling biomass and altering fouling species composition, thereby indicating their promise as alternatives to chemical antifouling solutions. The diversity of fouling organisms observed in this study highlights the complex dynamics of fouling communities at different depths, underscoring the importance of considering depth-dependent variations in antifouling strategies. Extract A, and B when applied at both 0.5- and 1.5-meters depth, demonstrated consistent reductions in fouling biomass and shifts in dominant species, with treatments 3 and 6 emerging as the most effective in their respective depths. Extract B at treatment 4 led to significant reductions in fouling biomass, dominated by *Balanus amphitrite*, *Balanus eburneus*, and *Balanus* sp. At 1.5 meters depth, treatment 6 demonstrated remarkable efficacy, achieving the lowest biomass and a streamlined species composition.

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