Thalassia Salentina
Thalassia Sal. 32 (2009), 73-89
ISSN 0563-3745, e-ISSN 1591-0725
DOI 10.1285/i15910725v32p73
http://siba-ese.unisalento.it

ABDEL-HAMID ABDEL-RAHMAN MOHAMED ALI,
YOSRY ABDEL-AZIZ SOLIMAN

National Institute of Oceanography and Fisheries, P. O. Box 182, Ataqa, Suez, Egypt
e-mail: abdelhamid_ama@yahoo.com

ANTIFOULING ACTIVITY OF CRUDE EXTRACTS FROM SOME RED SEA SOFT CORALS

SUMMARY

The antifouling activity of crude extracts of 5 common Red Sea soft corals was examined. The extracts were mixed with a marine paint, applied to PVC panels immersed in the seawater of Suez Bay (Red Sea). The barnacle *Balanus amphitrite* (Crustacea) and tube worms *Hydroides elegans* (Polychaeta) are the dominant fouling organisms in this area. The results demonstrated that all the tested soft coral extracts exhibited significant antifouling activities with varying degrees. Extracts of *Sinularia heterospiculata* and *Sinularia variabilis* showed the highest and potent wide spectrum antifouling activity, particularly in the first 17 days of fouling formation. Extracts of *Sinularia polydactyla* exhibited significant selective inhibition against settlement of barnacle, while the extracts of *Lithophyton arboreum* showed significant antifouling activity against the latter successional stages of tube worms. The results of the current study propose that these soft corals may contain bioactive compounds with antifouling activity. These bioactive molecules can be isolated, purified, identified and chemically synthesized for commercial uses in the development of nontoxic and environmentally acceptable antifouling coatings.

INTRODUCTION

Marine biofouling causes severe problems (such as increases in mass and corrosion of surfaces) for the submerged structures. These problems are of particular consequence in the aquaculture industry, supports of oil drilling platforms, ship hulls (Armstrong *et al*., 2000), and cooling water cycles of large industrial equipments and power plants. Biofouling on ships' hulls for example results in an increase in roughness, which in turn leads to an increase in frictional drag, with a corresponding decrease in speed and

maneuverability (GISP, 2008). Increased fuel consumption and transit time, emissions of harmful compounds, hull cleaning, paint removal and repainting, and associated environmental compliance measures all contributing to the costs of biofouling. Callow and Callow (2002) estimated, for the US Navy only, an annual expense of about 1 billion to face with the biofouling associated problems. Accumulation of biofouling on the ships' hulls leading also to introduction of invasive (non indigenous species) species into the environment.

Fouling in the marine environment is considered to be a successional sequence that consists of macromolecular adsorption, bacterial colonization, and establishment of unicellular and multicellular epibionts on exposed living or nonliving surfaces. Within minutes of immersing a clean surface in water it adsorbs a molecular 'conditioning' film, consisting of dissolved organic material. Bacteria colonize within hours, as may fungi, unicellular algae (mainly diatoms), Cyanobacteria, Protista and other microorganisms (Characklis, 1981; Callow and Callow, 2002). These early small colonizers form a biofilm: an assemblage of attached cells sometimes referred to as 'microfouling' or 'slime'. Settlement of higher organisms such as macroalgae and invertebrates may be moderated by the presence of the biofilm (Davies *et al.*, 1989). However, Maki *et al.* (1988) showed that competent barnacle cypris larvae do not require a biofilm to settle. The macrofouling community consists of either 'soft fouling' or 'hard fouling. Soft fouling comprises algae and invertebrates, such as soft corals, sponges, anemones, tunicates and hydroids. Hard fouling comprises barnacles, mussels and tubeworms. The specific organisms that develop in a fouling community depend on the type of substratum, geographical location, local hydrodynamic regimes, season, and biotic factors such as competition and predation (Terlizzi *et al.*, 2001).

A common method for avoiding settlement of marine fouling organisms has been coating marine structures with antifouling paints. An antifouling coatings based on organotin compounds mostly tributyltin (TBT) or Copper as the active agent, pose a world-wide threat to the marine environment (Stebbing, 1985; Bryan *et al.*, 1987; Volkirs *et al.*, 1987; Oyewo, 1989; Ellis and Pattisina, 1990; IMO, 1990) and are being subjecting to growing restrictions. The toxic doses of TBT for marine organisms such as the molluscs may be as low as 1 ng L^{-1} (Grinwis *et al.*, 1998; Fisher *et al.*, 1999). The use of organotin compounds for biofouling control is prohibited world-wide since 2008 (van Wezel and van Vlaardingen, 2004). The ban of organotins such as TBT and triphenyltin (TPT) and other toxic biocides in marine coatings represent a serious issue for shipping industry and for the producers of coatings. Therefore, environmentally acceptable, safe and effective antifouling substances are needed for incorporation into antifouling coatings, and these may include natural products isolated from certain marine organisms (Clare, 1996).

Incorporation of naturally repellent products into antifouling paints has been tried by some researchers (Armstrong *et al.,* 2000; Peppiatt *et al.,* 2000). Some corals, algae, sponges, and ascidians have been shown to produce antifouling substances which in nature maintain them free from undesirable encrusting organisms (Slattery *et al.*, 1995; Hentschel *et al.,* 2001; Harder *et al.*, 2003; Dobretsov and Qian, 2004; Maréchal, *et al*., 2004; Kelman *et al*., 2006).

Soft corals (Coelentrata: Octocorallia, Alcyonacea) are among the major benthic components occupying space in the tropical Indo-Pacific reefs (Dinesen, 1983; Huston, 1985), as well as in the coral reefs of the Northern Red Sea (Benayahu and Loya, 1977). Their evolutionary success in areas of high levels of predation has been attributed to their production of significant amounts of secondary metabolites (Sammarco and Coll, 1988; 1992). The ability of soft corals to control micro- and macrofouling has been attributed to the excretion of slime and production of secondary metabolites (Slattery *et al*., 1995, 1997; Kelman *et al*., 1998). Alcohol extracts of *Dendronephthya* sp. inhibited the settlement of *Balanus amphitrite* cyprids and byssus production of the bivalve *Perna viridis* (Wilsanand *et al*., 1999b). Furthermore, Wilsanand *et al*. (2001) found that extracts of the gorgonian coral *Echinogorgia complexa* and the soft coral *Dendronephthya* sp. showed 100% growth inhibition against four dominant marine fouling diatoms (*Navicula subinflata*, *Navicula crucicula*, *Amphora* sp. and *Nitzschia* sp.). Kelman *et al*. (2006) examined the antimicrobial activity of extracts of six dominant soft corals from the Northern Red Sea. From the active soft coral species examined, *Xenia macrospiculata* exhibited the highest and most potent antimicrobial activity.

The aim of the current study was to investigate the antifouling activity of extracts of five common soft coral species (different from the already cited) from the coral reefs of the Red Sea coast of Egypt.

MATERIALS AND METHODS

Collection and extraction

Five soft coral (Octocorallia: Alcyonacea) species *Sinularia variabilis*, *S. polydactyla*, *S. heterospiculata*, *Lithophyton arboretum*, and *Sacrophyton trocheliophorum* were collected using SCUBA gear from the coral reefs of the Northern Red Sea (Egypt coast) during May and June 2006 at 2-20 m depth range. These species are among the most abundant soft corals observed in the area. Voucher samples were collected for later identification. All corals collected were identified to species level. 1 kg wet weight was collected from each coral species for the subsequent extractions. After collection, each coral sample was immediately cut into small pieces, placed in a mixture of 1:2 (v/v) methanol: dichloromethane (MeOH: DCM) solution and transferred

to the laboratory for extraction. The quantity (2 l) of solvent used was twice the volume of specimens. After extraction in a 1:2 (v/v) MeOH:DCM for one week at room temperature, the organic extracts were filtered, centrifuged at 3000 rpm for 20 min and the solvent was removed by rotary evaporation under vacuum at 30 °C. The resulting crude extracts were weighed and kept at –20 °C until testing for antifouling activity.

Antifouling test

The crude extracts of soft corals were incorporated into simple paint formulations (inert binder rosin and iron oxide) with concentrations illustrated in Table 1. The concentrations of crude extracts are expressed as percentage. The viscosity of paints was adjusted using blend of solvents.

Table 1 - Concentrations (%) of soft coral extracts in the paint formulation and codes of extract panels.
Extract panels: the panels coated with paints containing soft coral extracts.

Soft coral species	Panel codes	Concentration of crude extract (%)
Sinularia variabilis	AF1	25
Sinularia polydactyla	AF2	26
Sinularia heterospiculata	AF3	26
Lithophyton arboreum	AF4	27
Sarcophyton trocheliophorum	AF5	25

The paint formulations were applied on Polyvinylchloride (PVC) plates (panels) with the dimensions of 10 x 15cm. Eight steel frames were realized to carry the test panels. Six PVC coated panels including control panels (coated with paint formulation free of crude extract) were attached to each frame and to each other by nylon ties. The coral extracts were tested for inhibitory activity against fouling organisms, particularly barnacle *Balanus amphitrite* and tube worms *Hydroides elegans*, the dominant fouling organisms in the Suez Bay (El-Komi, 1980). Suez Bay is a shallow water (maximum depth 17 m) semi-closed protected embayment with elliptic shape occupying the Northern tip of Gulf of Suez (Fig. 1). Its major axis is NE-SW direction, the average length is 13.2 km and the average width along minor axis is 8.8 km. It is situated at the southern entrance of Suez Canal, therefore it acts as a transit and waiting area for ships that cross the Suez Canal.

Suez Bay is considered as one of the heavily-polluted areas in the Red Sea coast of Egypt (EIMP, 2005). The environmental variables in the Bay have been measured and are summarized in Table 2.

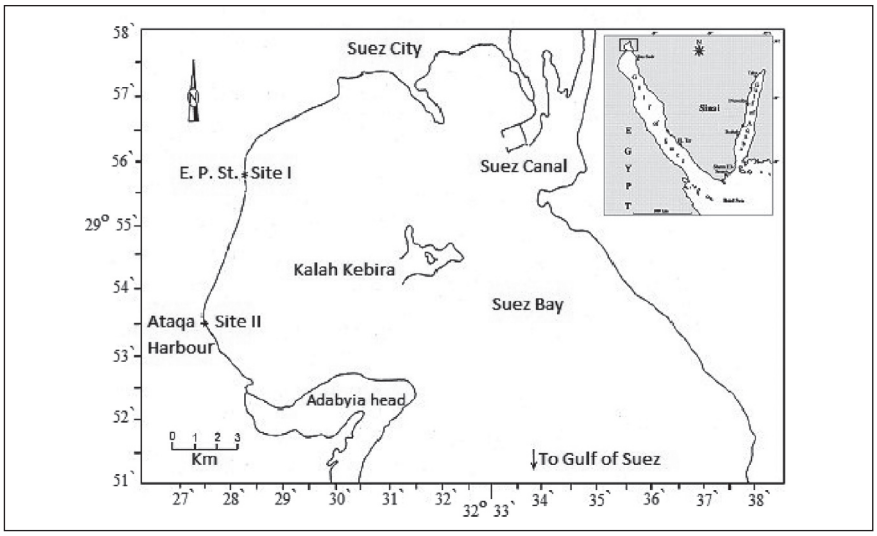

Fig. 1 - Map of Suez Bay showing the location of the immersion sites. Inset shows location of Suez Bay. E. P. St. = Electrical Power Station.

Table 2 - Mean values of temperature, pH, salinity, dissolved oxygen (DO), Oxidizable organic matter (OOM), nitrite (NO_2-N), nitrate (NO_3-N), ammonia (NH_3-N), dissolved inorganic nitrogen (DIN), reactive phosphate (PO_4-P), reactive silicate (SiO_4-Si), chlorophyll *a* (Chl-*a*), total suspended matter (TSM) and transparency (TR) in seawater of Suez Bay during the antifouling test.

Parameter	Dates of measurements during 2006					
	03/09	10/09	20/09	04/10	16/10	04/11
Temperature (°C)	28.30	28.50	27.20	26.10	25.40	24.71
pH	8.12	8.13	8.11	8.14	8.15	8.12
Salinity (ppt)	42.50	42.50	42.53	42.50	42.46	42.31
DO ($mg\ l^{-1}$)	6.57	6.27	6.37	6.74	6.51	6.57
OOM ($mg\ l^{-1}$)	1.81	1.82	1.85	1.42	1.99	1.90
NO_2-N (µM)	2.42	2.28	1.49	1.11	1.46	1.53
NO_3-N (µM)	13.42	12.81	12.61	13.06	12.62	13.42
NH_4-N (µM)	5.12	6.51	6.10	7.11	8.71	10.90
DIN (µM)	20.96	21.60	20.20	21.28	22.79	25.85
PO_4-P (µM)	0.06	0.07	0.06	0.08	0.07	0.05
SiO_4-Si (µM)	2.33	2.16	2.66	2.38	2.03	2.50
Chl-*a* ($\mu g\ l^{-1}$)	5.34	4.80	5.08	5.37	5.23	5.34
TSM ($mg\ l^{-1}$)	17.15	17.10	17.32	18.95	18.40	18.13
TR (meter)	2.30	2.45	2.75	2.75	3.10	2.87

The test frames were immersed in two sites in the Suez Bay (Fig. 1) during 2006 for a period of two months, the first site (Site I) located in the front of Ataqa Electrical Power Station and the second site (Site II) is the Ataqa harbour. Four replicates (4 frames) were placed vertically in each site at depth 1.5 m.

The fouling process was measured by estimating the percentage of the panel surface covered by fouling organisms, and calculating the wet weight of fouling. Percent cover was measured on both sides of each plate using of Dot-grid estimate method (Foster *et al.*, 1991). The outline of a panel was traced onto a clear transparency sheet and the area within was marked in a dot grid with all points 1cm apart. The sheet was then filled over both sides of each plate and the number of points with organisms underneath was recorded. Percentage cover was then calculated by dividing the recorded points by total number of points. To prevent the fouling organisms from drying, the plates were kept in a large container full of seawater during measurements. Fouling organisms were identified to the lowest possible *taxon* level. One-way analysis of variance (ANOVA) was used to detect the significant difference between test panels. The difference between extract panels and controls was estimated using *t*-test for independent samples. The level of significance was set at $P<0.05$.

RESULTS

Antifouling results at site I:

AFTER 7 DAYS OF EXPOSURE

The biological inspection of test panels indicated a high incidence of slime (biofilm) formation on the control panels. Marine bacteria, unicellular algae (mainly diatoms), Cyanobacteria (blue-green algae), and protozoan were the major microfouling organisms constituting the slime. Panels coated with paint treated with crude extracts of soft corals *Sinularia heterospiculata* (AF3), *S. variabilis* (AF1), *S. polydactyla* (AF2) exhibited higher inhibition of slime formation. The extracts of soft corals *Lithophyton arboreum* (AF4) and *Sacrophyton trocheliophorum* (AF5) inhibited at least 70 % of slime formation.

AFTER 17 DAYS OF EXPOSURE

Few taxa of macrofouling organisms were observed on the test panels. Barnacle *Balanus amphitrite* predominate in the test panels, represented 90 % of fouling organisms. The remaining taxa were represented by the tubeworms *Hydroides elegans* (5 %) green algae *Enteromorpha* spp. (3 %) and Bryozoa (2 %).

In terms of percent cover and wet weight of fouling organisms (Fig. 2A, B), the attachment of fouling organisms was significantly (ANOVA: $F = 166.02$, $P<0.0001$ for percent cover; and $F = 114.26$, $P<0.0001$ for wet weight) inhibited by the crude extract of the 5 soft corals examined. The average percent cover (66.03 ± 8.81 %) of fouling organisms (Fig. 2A) in control panels was more than 44-fold of the panels coated with extracts of *S. heterospiculata* (1.48 ± 0.19 %), 40-fold of the panels coated with extracts of *S. variabilis* (1.65 ± 0.39 %) and 11.5-fold of the panels coated with extracts of *S. polydactyla* (5.6 ±1.28 %). Also, the lower average wet weights of fouling organisms (Fig. 2B) were recorded in the extract panels of *S. heterospiculata* (12.38 ± 1.25 g/panel), and *S. variabilis* (12.63 ± 1.11 g/panel), while the greatest value (49.75 ± 4.65 g/panel) was recorded in the control panels. Unfortunately, the frames immersed in this site were lost after three weeks of immersion date. However, the first 17 days of the experiment were considered a critical and important stage of fouling formation. During this stage the cypris larvae of barnacle is selecting the appropriate surface to attach, once attached, dislodging of larvae becomes difficult because they secretes one of the strongest glues known to man to secure it to the surface.

Antifouling results at site II (Ataqa Port):

AFTER 7 DAYS OF EXPOSURE

The biological inspection revealed high rate of biofilm formation as a result of rapid colonization of test panels by marine bacteria and diatoms, which were the dominant microfouling organisms in this site. The surface of panels coated with extracts of *Sinularia heterospiculata* and *S. variabilis* were found to be free of slime. The extracts of these species reflected great resistance to the bacterial and diatom colonization of panel surfaces. Panels coated with extracts of *Sacrophyton trocheliophorum* showed also great inhibitory activities against slime formation.

AFTER 17 DAYS OF EXPOSURE

The most dominant macrofouling organisms in this site were the tubeworms *Hydroides elegans* which constituted 97 % of the fouling, followed by the green algae *Enteromorpha* spp., which constituted 3%. The average percent covers of fouling organisms (Fig. 3) on panels coated with extracts of *Sinularia heterospiculata* (2.3 ± 0.44 %), *S. variabilis* (3.03 ± 0.41 %) and *Sacrophyton trocheliophorum* (25.25 ± 2.5 %) were significantly (t-test: $t = 11.20$, $P<0.0001$ for AF3; $t = 11.02$, $P<0.0001$ for AF1; and $t = 5.27$, $P<0.01$ for AF5) lower than controls (47.5 ± 8.06 %). However, the average percent covers of fouling in the panels coated with extracts of *Sinularia polydactyla* and *Lithophyton arboreum* were slightly higher than the average value of the control panels (Fig. 3).

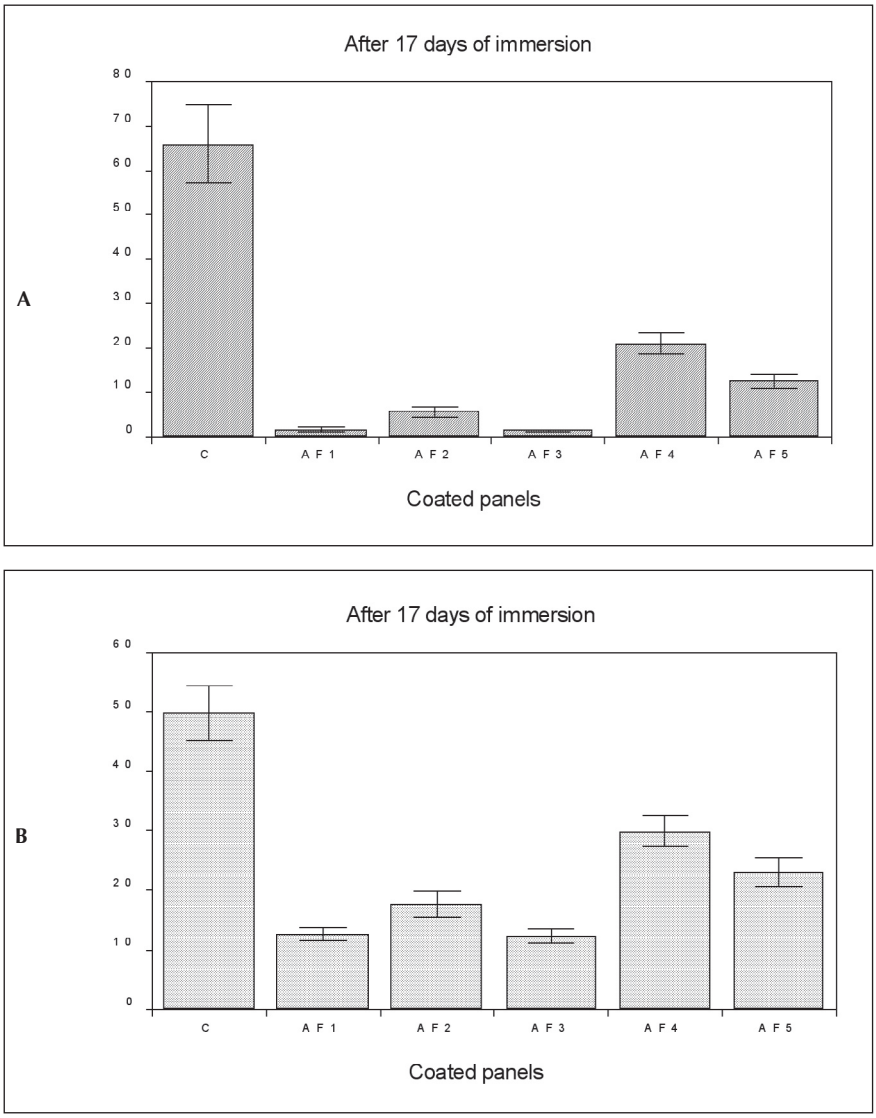

Fig. 2 - Mean percent cover (A) and wet weight (B) of fouling organisms on test panels after 17 days of immersion in site I. Error bars represent the standard deviation (n = 4).
Abbreviations: C = Control, panels coated with crude extract-free paint; AF1 = panels coated with paint containing crude extract of *Sinularia variabilis*; AF2 = panels coated with paint containing crude extract of *Sinularia polydactyla*; AF3 = panels coated with paint containing crude extract of *Sinularia heterospiculata*; AF4 = panels coated with paint containing crude extract of *Lithophyton arboreum*; AF5 = panels coated with paint containing crude extracts of *Sarcophyton trocheliophorum*.

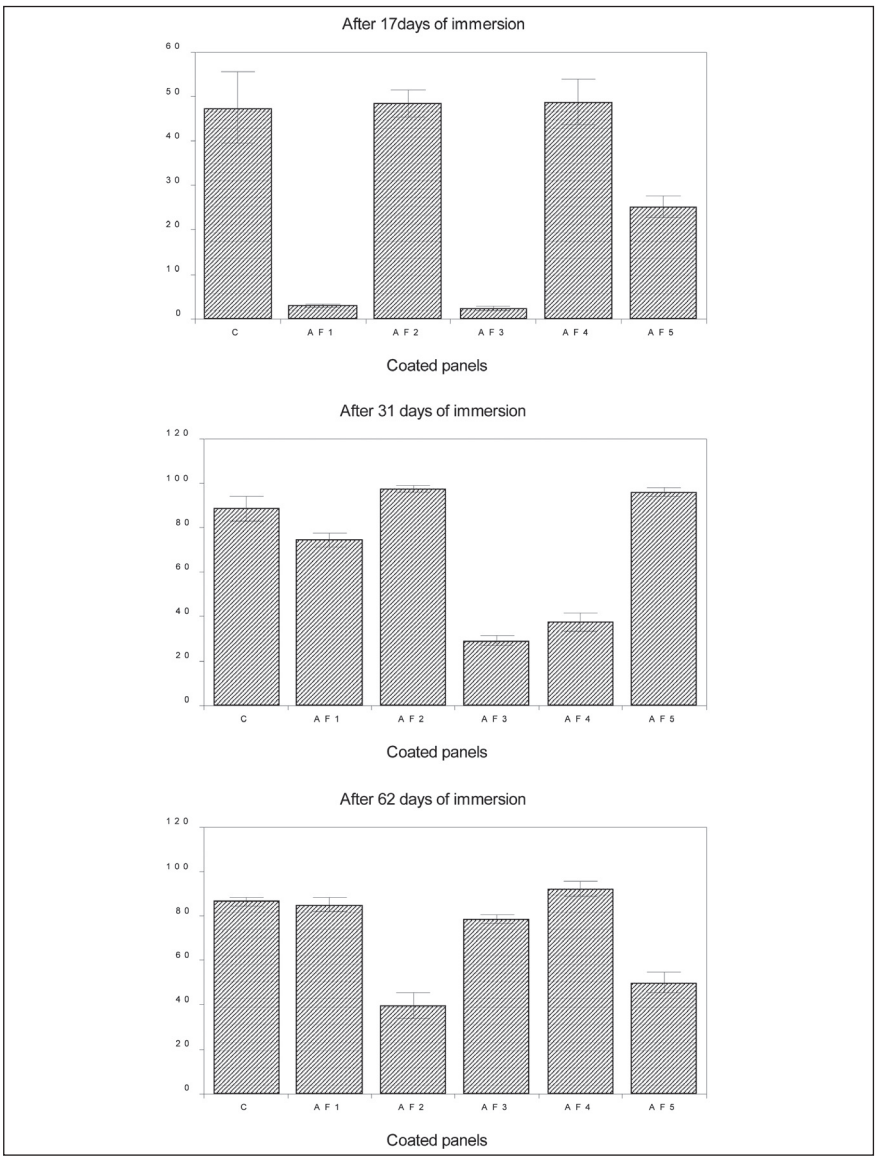

Fig. 3 - Mean percent cover of fouling organisms on test panels at different immersion times in Ataqa harbour (site II). Error bars represent the standard deviation (n = 4).
Abbreviations: C = Control, panels coated with crude extract-free paint; AF1 = panels coated with paint containing crude extract of *Sinularia variabilis*; AF2 = panels coated with paint containing crude extract of *Sinularia polydactyla*; AF3 = panels coated with paint containing crude extract of *Sinularia heterospiculata*; AF4 = panels coated with paint containing crude extract of *Lithophyton arboreum*; AF5 = panels coated with paint containing crude extracts of *Sarcophyton trocheliophorum*.

The average wet weight of fouling organisms was significantly different (ANOVA: F = 49.16, P<0.0001) among the test panels (Fig. 4). The lower average wet weights were recorded on *S. heterospiculata* (12.0 ± 0.91 g/panel) and *Sinularia variabilis* (12.63 ± 1.25 g/panel) panels, whilst the highest value (32.38 ± 3.45 g/panel) was recorded in the control panels.

AFTER 31 DAYS OF EXPOSURE

Passive leaching of paint components from the test panels into seawater resulted in evident weak antifouling activities in the majority of test panels. The majority of test panel surfaces were extensively fouled by tubeworms *Hydroides elegans* which was the most dominant fouling organism in this site. Nevertheless, primary stages of other fouling organisms (mainly barnacle *Balanus amphitrite*) were detected on the free spaces left after fell down of tubeworms from the panel surfaces.

There was an apparent variability in the percent cover (ANOVA: F = 311.52, P<0.0001) and wet weight (ANOVA: F = 85.82, P<0.0001) of fouling organisms between the test panels (Figs. 3 and 4). The lowest average percent cover (29.25 ± 2.22 %) was observed in the panels coated with extracts of *S. heterospiculata* (AF3) which was significantly (t-test: t = 19.72, P<0.0001) lower than that of the control panels (88.76 ± 5.61 %, Fig. 3). The least average wet weight of fouling (60.75 ± 5.06 g/panel) was found in the panels coated with extracts of *Lithophyton arboreum* (AF4), the greatest value (136.0 ± 8.37 g/panel) was found in the controls (Fig. 4). The wet weight of fouling organisms was significantly higher in the control panels than panels coated with extracts (t-test: t = 15.39, P<0.0001 for AF4; t = 14.39, P<0.0001 for AF3; t = 10.46, P<0.0001 for AF1; t = 9.18, P<0.0001 for AF5; t = 5.30, P<0.01 for AF2).

AFTER 62 DAYS OF EXPOSURE

The paint was completely removed from the panels coated with extracts of *S. heterospiculata* and reduced to a very thin layer in the other panels due to the noticed high dissolution rate of binder from the paint formulation into the seawater. It was observed that high percentage of tubeworms fell down from the test panels into the seawater and the resulted free spaces were occupied by barnacle *Balanus amphitrite* and algae. The lowest average percent cover (39.63 ± 5.76 %, Fig. 3) and wet weight (138.5 ± 6.56 g/panel, Fig. 4) of fouling were recorded in the panels coated with extracts of *S. polydactyla* (AF2). This indicated the high inhibitory activity of S. *polydactyla* against the attachment of *B. amphitrite* which replaced the *Hydroides elegans* in the striped space on AF2.

The percent cover of fouling in the control panels was significantly greater than in AF2 (t-test: t = 15.30, P<0.0001), AF5 (t-test: t = 13.98, P<0.0001)

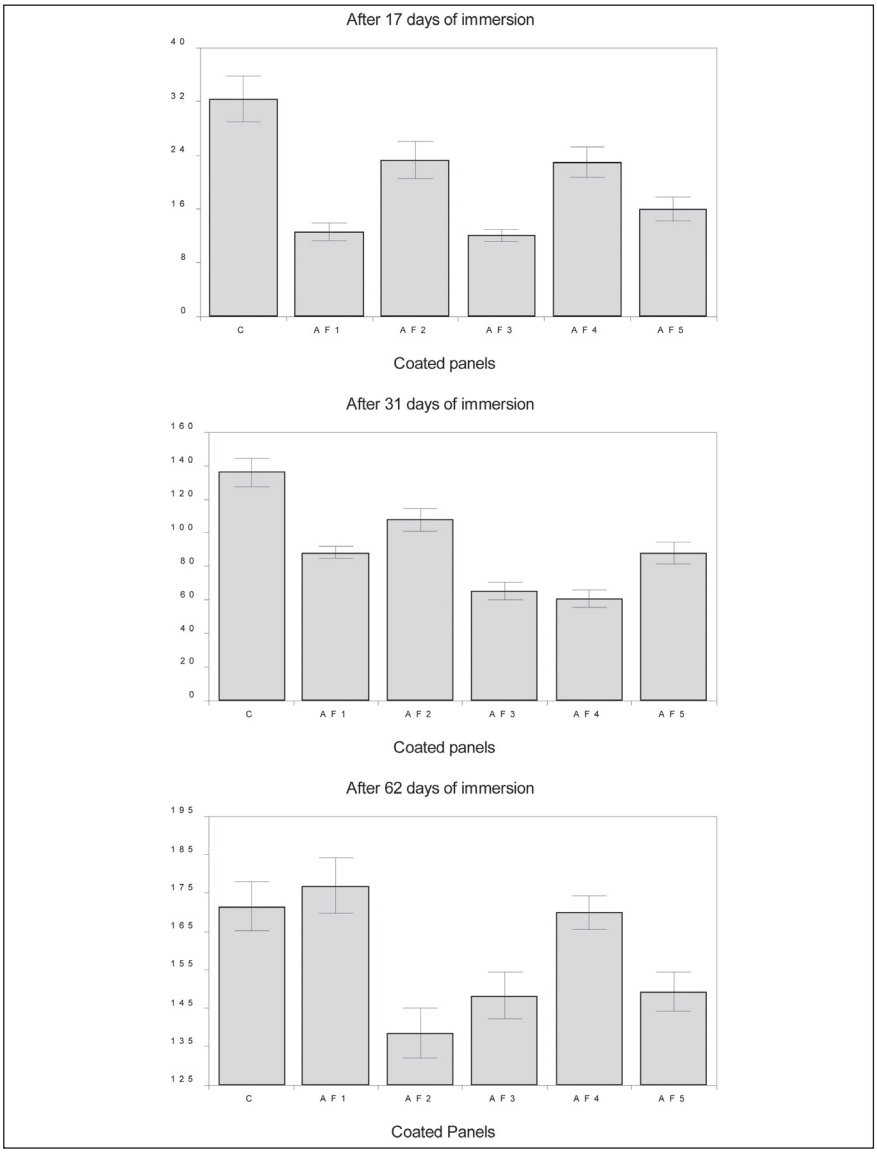

Fig. 4 - Mean wet weight of fouling organisms on test panels at different immersion times in Ataqa harbour (site II). Error bars represent the standard deviation (n = 4).
Abbreviations: C = Control, panels coated with crude extract-free paint; AF1 = panels coated with paint containing crude extract of *Sinularia variabilis*; AF2 = panels coated with paint containing crude extract of *Sinularia polydactyla*; AF3 = panels coated with paint containing crude extract of *Sinularia heterospiculata*; AF4 = panels coated with paint containing crude extract of *Lithophyton arboreum*; AF5 = panels coated with paint containing crude extracts of *Sarcophyton trocheliophorum*.

and AF3 (t-test: t = 5.38, P<0.01). Similarly, the average wet weight of fouling (Fig. 4) showed a significant decrease in AF2 (t-test: t = 7.22, P<0.001), AF5 (t-test: t = 5.42, P<0.01) and AF3 (t-test: t = 5.26, P<0.01) when compared with control panels.

DISCUSSION

The high levels of eutrophication indicators (Oxidizable organic matter, nutrients, Chlorophyll *a*, total suspended matter and transparency) measured in Suez Bay during the experiment (Table 2) explained the high rate of fouling incidence observed in the sites of immersion. The high nutrient load for example is known to promote the massive growth of phytoplankton (Lapointe *et al.*, 1993), which are used as food by barnacles, the worldwide fouling organisms (Matias *et al.*, 2003). The low seawater quality in Suez Bay caused by different sources of pollution e.g. effluents from textile, fertilizers, steel, cement and food oils industries; petroleum refineries disposal; outflow from Ataqa and Adabyia ports; traveling vessels runoff; discharges of domestic sewage; and power plants output, in addition to other projects in the area. The area has no major freshwater rivers to dilute the seawater so that salinity is reasonably stable and water temperature is typically ranged from 24.71 to 28.50 ºC during the bioassay period (Table 2).

It is important for scientists who need quick answers as to the performance of coatings to select the best site that provides a more aggressive year-round fouling environment. By combining the optimum site with aggressive fouling condition for marine exposure testing and a versatile dynamic system, it is now possible to accelerate the evaluation of experimental coatings to create improved and environmentally acceptable marine coatings for the future (Matias *et al.*, 2003). Accordingly, in the present study the coated panels were submerged in a heavily-fouled (Sites 1 and II) marine environment (El-Komi, 1980), and left for a short periods of time to determine the degree of resistance provided by the test paints against attachment of hard and soft fouling.

Previous studies have demonstrated that the soft coral extracts contain bioactive products with antifouling activities against attachment and growth of microfouling organisms such as marine bacteria, diatoms, fungi and protozoa (Davies *et al.*, 1989; Wahl, 1989; Slattery *et al.*, 1995; Wilsanand *et al.*, 1999a,b; Wilsanand *et al.*, 2001; Bhosale *et al.*, 1999; Bhosale *et al.*, 2002; Harder *et al.*, 2003; Kelman *et al.*, 2006), and macrofouling organisms such as seaweeds, barnacles, mussels, tube worms and other invertebrates (Standing *et al.*, 1984; Coll *et al.*, 1987; Devi *et al.*, 2004). Furthermore, the copious amount of mucus produced by soft corals has been shown to play

an important role in impeding bacterial attachment and biofilm formation (Duclow and Mitchell, 1979; Rublee *et al.*, 1980; Krupp, 1985).

The present results demonstrated that, the majority of tested soft coral extracts exhibited appreciable antifouling activity against fouling organisms in the first 17 days of exposure as compared with controls. Many studies (e.g. Davies *et al.*, 1989; Wahl *et al.*, 1994; Abarzua and Jakubowski, 1995; Callow and Callow, 2002) have indicated that the first days of exposure is the important and critical stage of fouling formation because the material immersed in seawater rapidly become conditioned by a series of physico-chemical events that stimulate the attachment of microorganisms to form biofilm of bacteria, fungi, diatoms, protozoa and other microorganisms. Successful settlement and development of macrofouling communities is usually preceeded by establishment of a diverse and abundant biofilm community (Little, 1984; Roberts *et al.*, 1991). So, prevention of surface colonization by early successional stages of biofilm community can inhibit or limit the settlement of higher fouling organisms such as barnacles, tubeworms, ascidians, bryozoans, sponges and macroalgae (Wahl, 1989; Slattery *et al.*, 1995). The results of the current study suggest that the compounds contained in the extracts of the examined soft corals may directly reduce the attachment of fouling organisms by inhibiting the larval settlement. This is probably due to the presence of antifouling compounds and large amount of mucus or indirectly through inhibition of slime formation on the exposed surfaces.

The higher significant ($P<0.0001$) antifouling activities of the extracts of *Sinularia heterospiculata* and *Sinularia variabilis* at sites I and II as compared with controls indicates these species as having a highly potent compounds with a wide spectrum antifouling activities. The observed high potency of *S. heterospiculata* and *S. variabilis* extracts lead us to choose these corals for further purification of target compounds and further attempts for their chemical synthesis.

On the other hand, the extracts of *S. polydactyla* showed selective inhibition against attachment of barnacle *Balanus amphitrite* as indicated by the following observations: 1) significant ($P<0.0001$) lower percent cover and wet weight of fouling organisms reported on the panels coated with extracts of *S. polydactyla* after 17 days of exposure at site I as compared with controls, whereas *Balanus amphitrite* was the most dominant fouling organisms in this site; 2) extracts of *S. polydactyla* exhibited no inhibitory activities against attachment of tubeworms *Hydroides elegans* the major fouling organism at site II (Fig. 3), so that the percent cover of fouling on the extract panels of *S. polydactyla* was slightly higher than controls after 17 days of exposure; 3) the lowest percent cover and wet weight of fouling organisms observed on extract panels of *S. polydactyla* after 62 days of exposure at site II can be ascribed to the inhibitory activities of *S. polydactyla* extracts against

attachment of *Balanus amphitrite* which occupied the panel surfaces after fell down of tube worms into seawater.

The biological activities of Alcyoniide soft corals have been previously reported by number of workers. Bhosalle *et al.* (1999) screened the antifungal activity of crude extracts obtained from 31 species of various marine organisms against food poisoning strains of *Aspergillus*. They found that the extract of soft coral *Sinularia leptocladus* was one of the 5 species exhibited significant (inhibition zone of 3-5 mm) antifungal activity against all strains, while *S. compressa* and *S. maxima* showed moderate (inhibition zone of 2-3 mm) activity against respective strains. Bhosalle *et al.* (2002) found that the extracts of soft corals *S. compressa* were active against all strains of marine bacteria *Bacillus* spp. and *Pseudomonas* spp. Devi *et al.* (2004) indicated that the soft corals *S. numerosa*, *S. compressa* and *Cladiela pachyclados* showed inhibitory action against the settlement of green mussel *Perna viridis* and act as a potential sources of antifoulants.

As compared with controls, *Sacrophyton trocheliophorum* extracts exhibited significant ($P<0.001$) antifouling activities in the first 17 days of exposure at the two sites, but its extract panels extensively fouled by tubeworms after 31 days of exposure. The relatively low average percent cover and wet weight of fouling organisms on the extract panels of *Sacrophyton trocheliophorum* after 62 days (Figs. 3 and 4) when compared with controls may be attributed to the detachment of tubeworms from test panels and beginning of *Balanus amphitrite* takeover.

Although the extracts of *Lithophyton arboretum* exhibited no antifouling activities against tubeworms *Hydroides elegans* after 17 days of exposure at site II when compared with controls, its extract panels showed low percent cover and wet weight of fouling after 31 days of exposure (Figs. 3 and 4). This may indicate that the extract of this corals contain bioactive metabolites may inhibit the later stages of successional fouling communities.

The above results lead us to speculate that the extracts of soft corals investigated in the present study differ in their chemical composition as well as the potency of active metabolites. The extensive fouling observed on the test panels after 30 and 62 days of immersion was mainly due to the high leaching rate of paint layers from the surface of test panels, so that the paint was completely removed from the extract panels of *S. heterospiculata* and reduced to a very thin layer in the other panels after 62 days of immersion. This problem can be overcome by choosing strong binding material for the paint formulation in order to avoid high dissolution of paint layers from the submerged surfaces in the seawater for long time as possible.

REFERENCES

Abarzua S., Jakubowski S., 1995 - Biotechnological investigation for the prevention of biofouling: I. Biological and biochemical principles for the prevention of biofouling. *Mar. Ecol., Prog. Ser*. **123**: 301-312.

Armstrong E., Boyd K. G., Pisacane T., Peppiat C. J., Burgess J. G., 2000 - Marine microbial natural products in antifouling coatings. *Biofouling* **16**(2-4): 215-224.

Benayahu Y., Loya Y., 1977 - Space partitioning by stony corals, soft corals, and benthic algae on the coral reefs of the northern Gulf of Eilat (Red Sea). *Helgol Wiss Meeresunters* **30**: 362-382.

Bhosale S. H., Jagtap T. G., Naik C. G., 1999 - Antifungal activity of some marine organisms from India, against food spoilage *Aspergillus* strains. *Mycopathologia*, **147**(3): 133-138.

Bhosale S. H., Nagle V. L., Jagtap T. G., 2002 - Antifouling potential of some marine organisms from India against species of *Bacillus* and *Pseudomonas*. *Mar. Biotechnol*., **4**(2): 111-118.

Bryan G. W., Gibbs P. E., Burt G. R., Hummerstone L. G., 1987 - The effects of tributyltin (TBT) accumulation on adult dog-whelks, *Nucella lapillus*: long-term field and laboratory experiments. *J. Mar. Biol. Ass. U. K.*, **67**: 525-544.

Callow M. E., Callow J. A., 2002 - Marine biofouling: a sticky problem. *Biologist*, **49**(1): 1-8.

Characklis W., 1981 - Fouling biofilm development: a process analysis. *Biotechnol. Bioeng*., **23**: 1923-1960.

Clare A. S., 1996 - Marine natural product antifoulants: status and potential. *Biofouling*, **9**(3): 211-229.

Coll J. C., Price I. R., Konig G. M., Bowden B. F., 1987 - Algal overgrowth of alcyonacean soft corals. *Mar. Biol*., **96**: 129-135.

Davis A., Targett N., McConnell O., Young C., 1989 - Epibiosis of marine algae and benthic invertebrates: natural products chemistry and other mechanisms inhibiting settlement and overgrowth. *Bioorg. Mar. Chem*., **3**: 86-114.

Dinesen Z. W., 1983 - Patterns in the distribution of soft corals across the central Great Barrier Reef. *Coral Reefs*, **1**: 229-236.

Devi P., Vennam J., Naik C. G., Parameshwaran P. S., Raveendran T. V., Yeshwant K. S., 2004 - Antifouling activity of Indian marine invertebrates against the green mussel *Perna Viridis* L. *J. Mar. Biotechnol*., **6**(4): 229-232.

Dobretsov S., Qian P., 2004 - The role of epibiotic bacteria from the surface of the soft coral *Dendronephtya* sp. in the inhibition of larval settlement. *J. Exp. Mar. Biol. Ecol*., **299**(1): 35-50.

Ducklow H. W., Mitchell R., 1979 - Bacterial populations and adaptations in the mucus layers on living corals. *Limnol. Oceanogr*., **24**: 715-725.

El-Komi M. M., 1980 - Ecological studies on the marine fouling of the southern region of the Suez Canal. M.Sc. Thesis, University of El-Mansoura, Egypt.

Ellis D. V., Pattisina L. A., 1990 - Widespread neogastropod imposex: A biological indicator of global TBT contamination? *Mar. Poll. Bull*. **21**(5): 248-253.

EIMP (Environmental Information and Monitoring Program), 2005 - Annual report of environmental data from coastal areas of the Suez Gulf, Aqaba Gulf and Red Sea proper during 2005. EEAA, DANIDA and NIOF: 164 pp.

Fisher W. S., Oliver L. M., Walker W. W., Manning C. S., Lytle T. F., 1999 - Decreased resistance of eastern oysters (*Crassostrea virginica*) to a protozoan pathogen (*Perkinsus marinus*) after sublethal exposure to tributyltin oxide. *Marine Environmental Research*, **47**(2): 185-201.

Foster D. J., Harrold C., Hardin, D. D., 1991 - Point versus photo quadrat estimates of the cover of sessile marine organisms. *J. Exp. Mar. Bio. Ecol*., **146**: 193-203.

GISP (Global Invasive Species Programme), 2008 - Marine Biofouling: An assessment of risks and management initiatives. Compiled by Lynn Jackson on behalf of the Global Invasive Species Programme and the UNEP Regional Seas Programme, 68pp.

Grinwis G. C. M., Boonstra A., van Den Brandhof E. J., Dormans J. A .M .A., Engelsma M., Kuiper R.V., van Loveren H., Wester P. W., Vaal M. A., Vethaak A. D., Vos J. G., 1998 - Short-term toxicity of bis (tris-*n*-butylin) oxide in flounder (*Platichthys flesus*): Pathology and immune function. *AquaticToxicology*, **42**(1): 15-36.

Harder T., Lau S. C. K., Dobretsov S., Fang T. K., Qian P., 2003 - A distinctive epibiotic bacterial community on the soft coral *Dendronephthya* sp. and antibacterial activity of coral tissue extracts suggest a chemical mechanism against bacterial epibiosis. *FEMS Microbiology Ecology*, **43**(3): 337-347.

Hentschel U., Schmid M., Wagner M., Fieseler L., Gernert C., Hacker J., 2001 - Isolation and phylogenetic analysis of bacteria with antimicrobial activities from Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. *FEMS Microbiology Ecology*, **35**(3): 305-312.

Huston M. A., 1985 - Patterns of species diversity on coral reefs. *Ann. Rev. Ecol. Syst*., **16**: 149-177.

IMO (International Maritime Organization), 1990 - Use of tributyltin compounds in antifouling paints for shipping - environmental impact. Submitted by the Federal Republic of Germany. MEPC 30/20/2, 19 September 1990.

Kelman D., Kushmaro A., Loya Y., Kashman Y., Benayahu Y., 1998 - Antimicrobial activity of a Red Sea soft coral, *Parerythropodium fulvum fulvum*: reproductive and developmental considerations. *Mar. Ecol. Prog. Ser*., **169**: 87-95.

Kelman D., Kashman Y., Rosenberg E., Kushmaro A., Loya Y., 2006 - Antimicrobial activity of Red Sea corals. *Mar. Biol*., **149**: 357-363.

Krupp, D. A., 1985 - An immunochemical study of the mucus from the solitary coral *Fungia scutoria* (Scleractinia: Fungidae). *Bull. Mar. Sci*., **36**: 163-176.

Lapointe B. E., Littler M. M., Littler D. S., 1993 - Modification of benthic community structure by natural eutrophication: The Belize Barrier Reef. Proc. 7th Int. Coral Reef Symp., Guam, **1**: 323-334.

Little B. J., 1984 - *Succession in microfouling*. In Costlow J. D., Tipper R. C. (eds.), Marine Biodeterioration: an interdisciplinary study. E. and F. N. Spon Ltd., London: pp. 63-67.

Maki J. S., Rittschoff D., Costlow J. D., Mitchell R., 1988 - Inhibition of attachment of larval barnacles, *Balanus amhitrite*, by bacterial films. *Mar. Biol.* **97**: 199-206.

Maréchal J. P., Culioli G., Hellio C., Thomas-Guyon H., Callow M. E., Anthony S. Clare A. S., Ortalo-Magne A., 2004 - Seasonal variation in antifouling activity of crude extracts of the brown alga *Bifurcaria bifurcata* (Cystoseiraceae) against cyprids of *Balanus amphitrite* and the marine bacteria *Cobetia marina* and *Pseudoalteromonas haloplanktis*. *J. Exp. Mar. Biol. Ecol*., **313**: 47-62.

Matias J. R., Rabenhorst J., Mary A., Lorilla A. A., 2003 - *Marine biofouling testing of experimental marine paints: Technical considerations on methods, site selection and dynamic tests*. Poseidon, New York: pp. 1-5.

Oyewo E. O., 1989 - Organotin compounds, fouling and the marine environment. *Ocean & Shoreline Management*, **12**: 285-294.

Peppiatt C. J., Armstrong E., Pisacane T., Burguess J. G., 2000 - Antibacterial activity of resin based coatings containing marine microbial extracts. *Biofouling,* **16**(2-4): 225-234.

Roberts D., Rittschoff D., Holm E., Schmidt A. R., 1991 - Factors influencing initial larval settlement: temporal, spatial and surface molecular components, *J. Exp. Mar. Biol. Ecol.*, **150**: 203-211.

Rublee P. A., Lasker H. R., Gottfried M., Roman M. R., 1980 - Production and bacterial colonization of mucus from the soft coral *Briarium asbestinum*. *Bull. Mar. Sci.*, **30**: 888-893.

Sammarco P. W., Coll J. C., 1988 - *The chemical ecology of alcyonarian corals (Coelenterata: Octocorallia)*. In Scheuer P.J. (ed.), Bioorganic marine chemistry, Springer-Verlag, Berlin: pp. 87-116.

Sammarco P. W., Coll J. C., 1992 - Chemical adaptations in the Octocorallia: evolutionary considerations. *Mar. Ecol. Prog. Ser.*, **88**: 93-104.

Slattery M., McClintock J. B., Heine J. N., 1995 - Chemical defenses in Antarctic soft corals: evidence for antifouling compounds. *J. Exp. Mar. Biol. Ecol.*, **190**: 61-77.

Slattery M., Hamann M.T., Perry T. L., Puglisi M. P., Yoshida W.Y., 1997 - Ecological roles for water-borne metabolites from Antarctic soft corals. *Mar. Ecol. Prog. Ser.*, **161**: 133-144.

Standing J. D., Hooper I. R., Costlow J. D., 1984 - Inhibition and induction of barnacle settlement by natural products present in octocorals. *J. Chem. Ecol.*, **10**(6): 823-834.

Stebbing A. R. D., 1985 - Organotins and water quality – some lessons to be learned. *Mar. Poll. Bull.*, **16**(10): 390-393.

Terlizzi A., Fraschetti S., Gianguzza P., Faimali M., Boero F., 2001 - Environmental impact of antifouling technologies: state of art and perspectives. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **11**: 311-317

Van Wezel A.P., van Vlaardingen P., 2004 - Environmental risk limits for antifouling substances. *Aquat. Toxicol.*, **66**(4): 427-444.

Volkirs A. O., Davidson B. M., Seligman P. F., 1987 - Sublethal growth effects and mortality to marine bivalves from long-term exposure to tributyltin. *Chemosphere*, **16** (1): 201-220.

Wahl M., 1989 - Marine epibiosis: I. Fouling and antifouling: some basic aspects. *Mar. Ecol., Prog. Ser.*, **58**(1-2): 175-189.

Wahl M., Jensen P. R., Fenical W., 1994 - Chemical control of bacterial epibiosis on ascidians. *Mar. Ecol. Prog. Ser.*, **110**: 45-57.

Wilsanand V., Wagh A. B., Bapuji M., 1999a - Antibacterial activities of anthozoan corals on some marine microfoulers. *Microbios*, **99**(394): 137-145.

Wilsanand V., Wagh A. B., Bapuji M., 1999b - Antifouling activities of sedentary marine invertebrates on some macrofoulers. *Indian J. Mar. Sci.*, **28**: 280-284.

Wilsanand V., Wagh A. B., Bapuji M., 2001 - Antifouling activities of octocorals on some marine microfoulers. *Microbios*, **104**(409): 131-140.