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Fouling Control by New Egyptian Natural Sources in Marine Aquaculture

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Abstract: In spite of success in using antifouling coatings as the most widely accepted method to control and prevent biofouling, efficient antifouling paints based on copper and tin biocides release toxic compounds causing adverse environmental effects. This work aims to use marine algae and land plant resources as naturally active and environmentally friendly biocides in antifouling paints. *Grateloupia filicina*, *Corallina mediterranea*, Mangrove leaves (*Avecinia marina*), Pepper (black pepper seeds) and *Ulva lactuca* were used in marine paint formulation by 10% concentration by weight. These marine paints' formulations were applied on unprimed steel panels. The steel frame containing the coated unprimed steels with different biocides was hanged in the Eastern Harbor seawater in front of NIOF on 25 May 2017. Obtained results showed that all these new paint formulations recorded an activity against micro-and macro-fouling except barnacles and *Calypsotheca alexandrines* (a new species recorded in 2017). The unprimed coated steel panel containing *grateloupia filicina* showed the best results followed by *Corallina mediterranea* and extended for 140 days. This period (140 days) of study was during spring, summer and autumn seasons which are considered as the highest accumulation seasons for fouling organisms in Eastern Harbour of Alexandria area.

Keywords: Antifouling coatings; physicochemical parameters; Eastern Harbour; Natural biocides; antibacterial activity, Gc-Ms.

1. INTRODUCTION

One of the most serious problems that marine technology is currently facing is the control of biofouling on man-made structures. Marine biofouling is a result of the accumulation of bacterial growth, algae and sessile invertebrates on both natural and man-made submerged surfaces¹. In the last few decades, the use of natural antifouling compounds had been considered as the most effective method to prevent bacterial attachment to submerged surfaces². Several authors tried to use the macroalgae as antifouling agents against zooplankton fouling organisms; they found that *Ulva reticula* bioactive substances inhibited the settlement of the polychaete *Hydroids elegance*³. Active antifouling agents are found in bacteria, algae, corals, sponges and seaweed beside land plants. Studies are now under way into the mechanisms by which they can be combined with the polymeric matrices of antifouling paints in order to allow the development, on the coating surfaces obtained with them, of a sufficient action to prevent the attachment of marine organisms, without leaching out the compounds and without contaminating the environment⁴.

Different concentrations of green algae (*Ulva lactuca*) and *Ulva lactuca* free lipids (2%, 5%, and 10%) were incorporated with the binder material and applied on glass panels. Their effect on the characteristics of their seawater media were recorded after different interval times. The coated surface with 2% algae in the dry paint film showed the lowest epiphytes growth on its surface, also no primary stages of macro algae was detected⁵. These prepared coated glass panels were tested to suppress the adhered and the planktonic *S. aureus* cells in comparison with the effect of the binder coated glass panels. The results showed that, the glass coated surface with vinyl copolymer contains 5% *Ulva lactuca* free lipid, in the dry paint film, led to a significant suppressive effect for both the adhered (82%) and the planktonic (85%) *S. aureus* cells. The results indicated that the used *Ulva lactuca* free lipids, as alternative natural products, in controlling the biofilm problems caused by such bacterium may be promising⁶.

The goal of this work is to use marine and land natural resources (five types of biocides; *Grateloupia filicina*, *Corallina mediterranea*, Mangrove leaves (*Avecinia marina*), *Pepper* (black pepper seeds) and *Ulva lactuca*) to create active and environmentally safe biocides against fouling problems in marine environment. This was carried out through evaluating the effect of unprimed steel coated surfaces with different types of biocides mixed in marine paint formulation based on oil binder material, iron oxide, zinc oxide, complementary pigment in presence of solvent (free from any hazardous materials). Comparative study between the prepared antifouling paints and commercial antifouling products (Sipes Transocean Coatings Optima Antifouling 2.36 and International Interswift 6600 TBT Free Antifouling) was carried out. The effect of the leached components of the prepared coated surfaces on some physicochemical parameters of natural seawater in the area of immersion including: temperature, pH, salinity, transparency, alkalinity, dissolved oxygen, oxidizable organic matter, nutrient salts (nitrite, nitrate, ammonia, phosphate and silicate) besides sulphate, was investigated. Antibacterial and Gas Chromatography-Mass Spectra (GC-MS) studies were done for the most potent biocides (*Grateloupia filicina* and *Corallina mediterranea*).

2. MATERIALS AND METHODS

The steel panel immersion test method was chosen to study the effect of the prepared new formulations as antifoulant and anticorrosion. Only one marine area was selected to immerse the coated panels in front of the National Institute of Oceanography and Fisheries (NIOF) in the Eastern Harbour (E H), Alexandria-Egypt (**Fig. 1**).

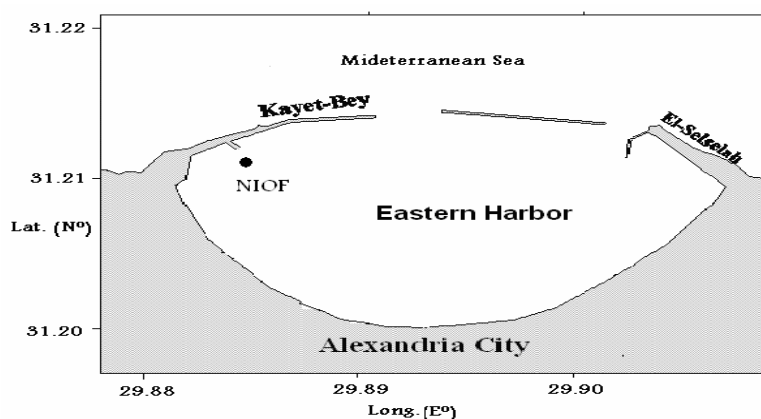


Fig. 1: Location of panels' immersion in the Eastern Harbour (E H) of Alexandria

2.1.Preparation of active material (biocides): Three marine algae (*Grateloupia filicina*, *Corallina mediterranea* and *Ulva lactuca*) were collected from the coast of Alexandria (Egypt), leaves of Mangrove plant (*Avecinia marina*) were collected from Safaga region in the Red Sea and pepper (black pepper seeds) were brought from the market. These biocides were washed with tap water, distilled water and then dried on air followed by drying in oven at 40°C. Finally, each type was grinded till mesh size 40 micron.

Grateloupia filicina and *Corallina mediterranea* that showed antifouling activity in E H seawater were extracted using distilled water (DW). Their bioactive effect on the inhibition of bacterial film formed by reference bacteria and bacterial community as a whole in seawater; *E. coli*, *S. aureus*, *P. aeruginosa*, *S. feacalis* and *V. Damsel* was determined. For extraction, 2 g of powdered algae was transferred into a 150 ml beaker containing 20 ml double distilled water, mixed well on rotary shaker for 1 h and then boiled for 15 min. The obtained extract was filtered ⁷.

2.2. Composition of the most potent biocides: Gas Chromatography–Mass Spectrophotometer GC Ms (Trace DSQii MS) was used to identify the chemical composition of both *Grateloupia filicina* and *Corallina mediterranea* as they showed the most potent biocides through this experiment (140 days).

2.3. The Antibacterial activity of the most potent biocides: The antibacterial activity was measured using the pouring technique according to Chythanya and Karunasagar ⁸. *Grateloupia filicina* and *Corallina mediterranea* were screened for antibacterial activity against indicator strains and marine bacterial community using well-cut diffusion technique.

2.4. Preparation of steel panels: The composition of the used steel is C: 0.21, Mn: 2.5, P: 0.04, S: 0.04 and Si: 0.35. Steel panels with dimensions 5cm×7cm×0.2cm were used. Their surfaces were polished using different grades of emery papers till finesse grade and cleaned using xylene.

2.5. Marine paints preparation: Marine paint formulation based on 25 g of oil binder material, 10 g of iron oxide, 24 g of zinc oxide, 13 g of complementary pigment and 38 g of xylene was prepared through mixing these paint ingredients using ball mill. Each type of the prepared biocides was mixed solely by 10% by weight with the prepared paint formulation forming five paint compositions. Then the mixing of the five biocides was continued for all the ingredients using ball mill till complete mixing.

2.6. Paints applications: The five paint compositions, blank (antifouling paint formulation without adding any biocide) and the two commercial products (Sipes Transocean Coatings Optima

Antifouling 2.36 and International Interswift 6600 TBT Free Antifouling) were applied on eight unprimed steel panels with two successive coats by brush, allowing sufficient time after each coat to allow the surfaces to get dry. The weights of the paint films on steel panels are shown in **Table 1**.

Table 1: Paint film weight (in gm) on each steel panel.

Steel panels coated with	weight before painting	weight after painting	weight of paint film
<i>Grateloupia filicina</i>	79.978	80.819	0.841
<i>Corallina mediterranea</i>	80.82	82.557	1.737
Mangrove leaves	78.833	79.486	0.653
<i>Pepper</i>	78.321	79.506	1.185
<i>Ulva lactuca</i>	77.835	78.884	1.049
Control	79.238	79.917	0.6679
Sipes Transocean Coatings Optima Antifouling 2.36	78.691	79.935	1.244
International Interswift 6600 TBT Free Antifouling	78.999	79.681	0.682

2.7. Panel's immersion test: All the coated unprimed steel panels were hanged in steel frame with dimension 80cmx60cm using nylon thread. The dry coated steel panels were hanged to the steel frame in two rows. Each row contains the coated panels with the five paint compositions, followed by the blank then the two marine products already found in the market (Sipes Transocean Coatings Optima Antifouling 2.36 and International Interswift 6600 TBT Free Antifouling) respectively. The frame contains the coated panels was immersed in E H water of Alexandria-Egypt on 25 May 2017. After immersion of the frame containing the coated panels in seawater, the surfaces were followed visually and by photographic inspection for fouling succession and corrosion over different interval times. The immersion of the panels in seawater was extended up to 140 days (till 10 October 2017).

2.8. Physical parameters measurements of seawater: Water temperature was measured using an inductive portable thermometer. Salinity was measured using Salinometer model Beckman RS-10-X3 range to about 0.1 units. Water transparency was measured using Secchi disc. The clearance of water was measured in cm.

2.9. Chemical parameters measurements of Seawater: The pH-value of water samples was measured to about 0.1 unit in situ by using a portable pH-meter (Orion Research model 210 digital pH-meters) after necessary precautions in sampling and standardization processes. For dissolved oxygen (DO) determination, water samples were collected in 125 ml glass bottles and fixed with 1 ml manganous sulphate followed by 1ml iodide solution until the analysis in the laboratory. DO was determined according to the classical Winkler's method modified by FAO 1975. The amount of dissolved oxygen in each sample was calculated by applying the following equation⁹:

$$mLO_2/L = \frac{N \times V \times 32000/4}{4B \times 1.43}$$

N=Normality of sodium thiosulphate,

V=Volume of sodium thiosulphate

B=Volume of oxygen bottle

The alkalinity is determined according to standard methods¹⁰. The sample (10 ml) was titrated against 0.02 N HCl designated pH value (pH 4.5). Alkalinity is calculated from the following equation:

$$\text{Total alkalinity} = \frac{\text{ml of HCl} \times 1000 \times N_{\text{HCl}}}{\text{ml of sample}}$$

Oxidizable organic matter (OOM) was determined by permanganate oxidation method ¹¹ and calculated from the following equation:

$$\text{Mg O}_2/\text{L (OOM)} = \frac{(V_{\text{blank}} - V_{\text{sample}}) \times 8 \times 1000 \times N_{\text{Na}_2\text{S}_2\text{O}_3}}{V \text{ of sample}}$$

The most important nutrient salts which are the dissolved inorganic forms of nitrogen (NO_2^- , NO_3^- and NH_3), phosphate (PO_4^{3-}) and silicate (SiO_3^-) were determined colourimetrically according to the methods described by Parsons *et al.* ¹². Their absorbance developed colour was measured by using double-beam spectrophotometer model Shimadzu UV-150-02 and the values were expressed as μM .

Sulphate (SO_4^{2-}) was precipitated as barium sulphate and measured turbidimetrically ¹³.

3. RESULTS AND DISCUSSION

Algae were used as they are available near the sea coasts. They were collected from marine areas so they don't cause any pollution to the marine environment. Each type of them was used solely as active ingredient in the marine paint composition applied to unprimed steel surface. The performance of prepared coatings was evaluated during their exposure time in seawater (140 days). From the results obtained; it was noticed that *Grateloupia filicina* and *Corallina mediterranea* were the most potent biocides. They resist most fouling organisms except the barnacles which were observed on the edges and the upper hole by 2% beside the appearance of new species called *Calypsotheca alexandriensis* ¹⁴. According to these results, Gas Chromatography-Mass spectra (GC-MS) and antibacterial activity were measured to determine the most effective components in *Grateloupia filicina* and *Corallina mediterranea*.

3.1. Gas Chromatography-Mass Spectra (GC-MS) of the most potent biocides: The chemical composition of *Grateloupia filicina* and *Corallina mediterranea* are shown in **Tables (2 and 3)** and **Figs. (2 and 3)**.

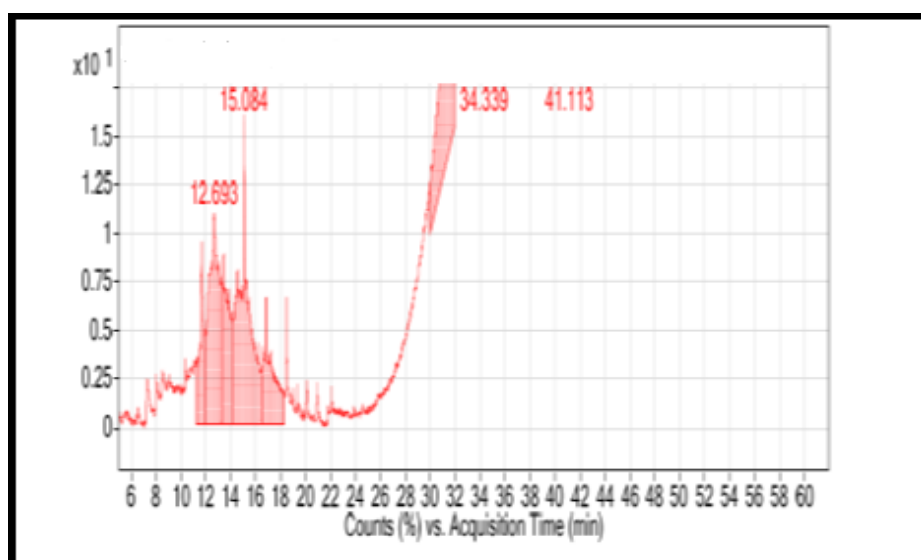


Fig. 2: GC-MS of the major compound from *Grateloupia filicina* extract by distilled water

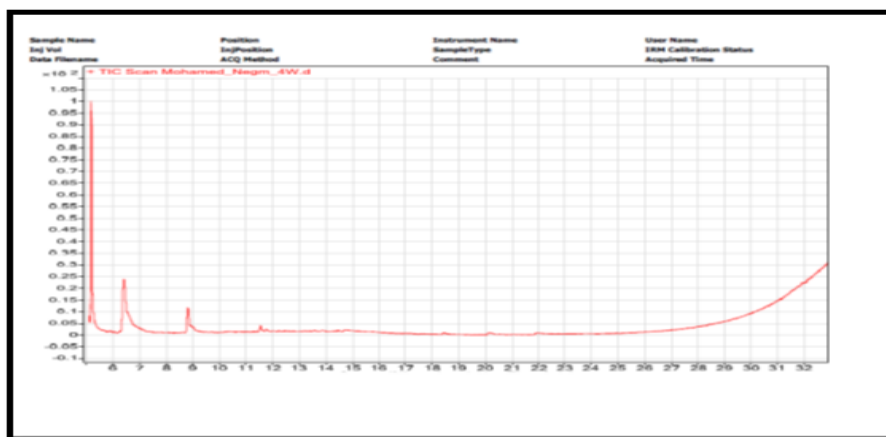


Fig. 3: GC-MS of the major compound from *Corallina mediterranea* extract by distilled water

Table 2: GC-MS of *Grateloupia filicina* extracted by distilled water.

No	Compound	Formula	M.Wt
1	5,6,7,8,9,10-Hexahydro-9-methyl-spiro[2H-1,3-benzoxazine-4,1'-cyclohexane]-2-thione	C ₁₄ H ₂₃ NOS	253
2	<i>Ethyl iso-allocholate</i>	C ₂₆ H ₄₄ O ₅	436
3	<i>17-Pentatriacontene</i>	C ₃₅ H ₇₀	490
4	<i>α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1,4)-β-D-fructofuranosyl</i>	C ₁₈ H ₃₂ O ₁₆	504
5	(5β)Pregnane-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	C ₂₈ H ₄₃ NO ₆	489
6	5-Octadecenal	C ₁₈ H ₃₄ O	266
7	Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-	C ₃₉ H ₈₀ O ₂	580
8	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254
9	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy]methyl]ethyl ester	C ₂₇ H ₅₂ O ₄ Si ₂	496
10	Cyclopropanebutanoic acid, 2-[[2-[[2-(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-,methyl ester	C ₂₅ H ₄₂ O ₂	374

Table 3: GC-MS of *Corallina mediterranea* extracted by distilled water.

No.	Compound	Formula	M.Wt
1	Acetic acid	C ₂ H ₄ O ₂	60
2	Cyclotrisiloxane, hexamethyl	C ₆ H ₁₈ O ₃ Si ₃	222
3	Cyclotetrasiloxane, octamethyl	C ₈ H ₂₄ O ₄ Si ₄	296

3.2.The Antibacterial activity of the most potent biocides: The data in Table 4 revealed that, the antibacterial activity ranged between 19.6 AU and ND. In addition, the DW extract of *Grateloupia filicina* exhibited a broad spectrum of antibacterial activity and recorded the highest AU value (19.6) against *S. aureus*. The DW extract of *Corallina mediterranea* showed antibacterial activity against *S. aureus* (AU= 18.3), *E.coli* (AU= 13.3) and *P. aeruginosa* (AU= 12.8).

Table 4: Screening the antibacterial activity of the most potent natural extracts, in distilled-water expressed as absolute unit (AU).

Marine substrate	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. feacalis</i>	<i>V. damsela</i>	Bacterial community
<i>Grateloupia filicina</i>	18.0	19.6	17.2	16.0	2.6	4.3
<i>Corallina Mediterranea</i>	13.3	18.3	12.8	ND	ND	ND

ND: Not detected.

From the field immersion test during 140 days of immersion; it was noticed that *Grateloupia filicina* and *Corallina mediterranea* gave the best results against different antifouling organisms rather than other biocides. Though their best activities were further studied against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. feacalis* and *V. damsel*.

3.3. Field test results: In this work, trials have been carried out to reach the proper marine paint composition including the five biocides; *Grateloupia filicina*, *Corallina mediterranea*, Mangrove leaves, *Pepper* and *Ulva lactuca* to get the coated surfaces with high performance against fouling. It also aims to evaluate and compare the antifouling properties of these biocides through their addition in the same paint composition with the same percent by weight in the dry paint film. Then assess the coated paint surfaces containing them under the same environmental conditions at the same time. Besides, the antifouling properties of these biocides were compared with the two commercial products (Sipes Transocean Coatings Optima Antifouling 2.36 and International Interswift 6600 TBT Free Antifouling). It was found that the prepared paint compositions were characterized by good adhesion to the unprimed steel surface, durability and storage time.

The eight unprimed steel coated panels: the five coated panels together with their blank (paint free from any biocide) and the two panels coated with the commercial products were hanged in a steel frame in two similar rows and immersed in E H seawater of Alexandria on 25 May 2017. The photographic inspection of the coated panels was taken at different interval times (4th June, 18th September 2017 and 10th October 2017).

Visual inspection showed that after 10 days of immersion there were no slime film formation on all the tested unprimed coated steel panels. Slight amount of green algae was scattered on the unprimed steel frame (**Fig. 4**).



Fig. 4: Field test results on 4 June 2017 (after 10 days of immersion)

After 117 days of immersion; it was noticed that the two panels coated with the paint formulations containing *Grateloupia filicina* and *Corallina mediterranea* respectively recorded the best results. They showed good adhesion to the substrate and their surfaces were kept nearly free from fouling attachment. The two panels didn't accumulate any fouling organisms on their surfaces although their nylon threads were fouled by ascidians. Slight barnacles (2%) were observed on the unprimed coated steel panel containing Mangrove leaves. Barnacles were observed on the unprimed steel coated panel containing *Pepper* (Black Pepper Seeds) as biocide by 20%.

The two unprimed steel coated panels containing *Ulva lactuca* hanged in the upper and lower rows of the steel frame showed different accumulation of fouling organisms. The upper panel was fouled by 40% of barnacles while the lower one was only fouled by 5% (**Fig. 5**). These results may be due to the effect of light and transparency. The highest percentage of barnacles (50%) and the appearance of *Calypsotheca alexandriensis* (5%) were observed on the control panel. The unprimed steel coated panel containing Sipes Transocean Coatings Optima Antifouling 2.36 showed 5-7% barnacles. The last unprimed steel coated panel containing International Interswift 6600 TBT Free Antifouling showed 5% barnacles on the edges only. Ascidians were found on the steel frame and showing no appearance on all the coated unprimed steel panels, i.e. these marine paints formulations and biocides can resist the ascidians formation on the steel coated panels (**Fig. 5**).

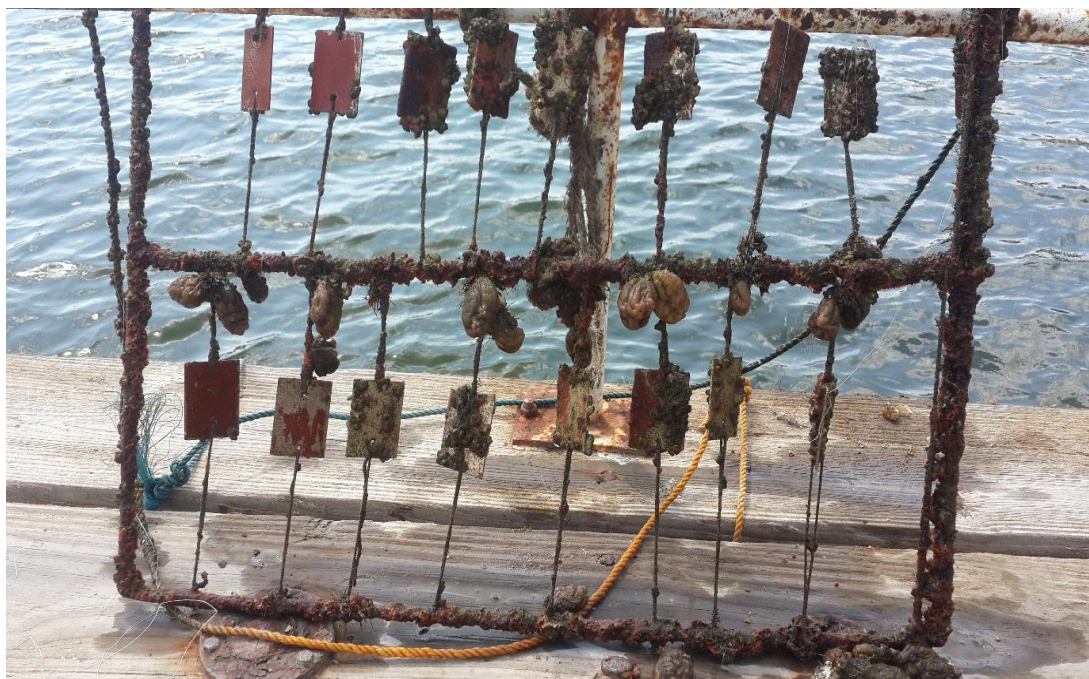


Fig. 5: Field test results on 18 September (after 117 days of immersion)

After 140 days of immersion; photographic inspection (**Fig. 6a**, **Fig. 6b**) showed that the uncoated steel frame (the panels' hanger) was heavily fouled by barnacles, ascidians and *Calyptotheca alexandriensis*. The unprimed steel coated panel containing *Grateloupia filicina* gave the best results in the E H seawater of Alexandria (**Figs. 6a and 6c**). The unprimed steel coated panel containing *Corallina mediterranea* showed barnacles on the edges and the upper hole by 2% (**Fig. 6d**), beside few green algae on its surface (10%). Barnacles (5%) and *Calyptotheca alexandriensis* (50%) were observed on the unprimed steel coated panel with Mangrove leaves and 40% barnacles were observed on the unprimed steel coated panel with *Pepper* as biocide. Nearly the same observation 50% barnacles were observed on the unprimed steel coated panel containing *Ulva lactuca* as biocide. The highest percentage of barnacles (70%) and *Calyptotheca alexandriensis* (10%) were observed on the control panel. The unprimed steel coated panel containing Sipes Transocean Coatings Optima Antifouling 2.36 showed 10-15% barnacles and 30% *Calyptotheca alexandriensis*. The last unprimed steel coated panel containing International Interswift 6600 TBT Free Antifouling showed 30% barnacles (**Fig. 6a**).

It was noticed that the prepared paint formulation showed anticorrosion effect on all coated unprimed steel panels till 140 days of immersion in marine E H water. This agrees with the results obtained by Aida and Hermine ¹⁵ concerning that, the paint compositions play important role for the efficiency of the steel surfaces coated with paints containing tubeworms and sepia officinalis shell against fouling and marine corrosion control.



Fig. 6a: Field test results on 10 October (after 140 days of immersion)



Fig. 6b: The uncoated steel frame on 10 October (after 140 days of immersion)



Fig. 6c: Steel panel treated with paint formulation containing *Grateloupia filicina*



Fig. 6d: Steel panel treated with paint formulation containing *Corallina mediterranea*

3.4. Antifouling property: The prepared antifouling paint showed good adhesion, durability and long lasting along the period of experiment (140 days). The antifouling characteristics of the unprimed steel panels' coated with the prepared marine paint formulation using different biocides were immersed in seawater of the E H were inspected and recorded till 140 days.

The attachment of fouling organisms (barnacles and *Calypsotheca alexandriensis*) increased on the coated surfaces by time. The approximate coverage of the fouled layer during the immersion period showed that the unprimed steel coated panel containing *Grateloupia filicina* had the best results followed by *Corallina Mediterranea* along the immersion period (140 days) in E H seawater of Alexandria-Egypt.

3.5. Physicochemical parameters: This study is important as it appears the relation between these parameters and the existence of fouling organisms on the unprimed steel coated panels used in this study. The physico-chemical parameters of the E H seawater are presented in **Table 5**.

Table 5: Physico-chemical parameters of the Eastern Harbour seawater during steel panels' immersion

Date	Temp.	pH	Salinity	Transparency	Alkalinity	DO	OOM	NO ₂ ⁻	NO ₃ ⁻	NH ₃	PO ₄ ³⁻	SiO ₃ ⁻	SO ₄ ²⁻
	°C		PSU/ppt	cm	meq/L	ml O ₂ /L	mg O ₂ /L	μM					g/L
25-05-17	25.4	8.59	37.40	147	3.65	1.60	1.312	1.35	16.49	0.13	3.50	5.47	2.509
04-06-17	26.1	8.13	37.80	94	3.45	2.88	2.418	0.94	14.32	0.88	3.21	5.31	2.489
18-09-17	27.1	8.01	37.90	10	3.25	1.89	5.482	1.38	9.34	3.99	0.55	4.52	3.554
11-10-17	21.2	8.17	37.60	25	3.15	2.56	4.396	1.45	10.22	0.22	2.10	4.23	2.689
Max	27.1	8.59	37.90	147	3.65	2.88	5.482	1.45	16.49	3.99	3.50	5.47	3.554
Min	21.2	8.01	37.40	10	3.15	1.60	1.312	0.94	9.34	0.13	0.55	4.23	2.489
Average	25.0	8.23	37.70	69	3.357	2.20	3.400	1.28	12.60	1.30	2.34	4.88	2.810
St dev.	2.6	0.25	0.22	63.58	0.22	0.60	1.880	0.23	3.39	1.83	1.34	0.60	0.504

The seawater temperature of the E H differed between 27.1 and 21.2 °C with an average of 25±2.6 °C. The pH ranged between 8.59 and 8.01 showing that it is normal as it lay in the alkaline side. Salinity fluctuated between 37.9 and 37.4 PSU/ppt with an average of 37.7±0.22 PSU/ppt. Transparency was mostly unclear because of the appearance of an oil layer after the immersion of the steel frame. The maximum transparency was 147 cm on the immersion date (25/5/2017) while the minimum value was 10 cm on 18/9/2017 (after 117 days of immersion) with an average value of 69.00±63.58 cm. With respect to the alkalinity it differed between 3.65 and 3.15 meq/L with an average of 3.357±0.22 meq/L. DO ranged between 2.88 and 1.60 mL O₂/L with an average of 2.20±0.60 mL O₂/L indicating the deficiency of oxygen during the studied period which may be due to the presence of the oil layer beside the eutrophic conditions of E H seawater¹⁶. OOM concentrations fluctuated between 5.482 and 1.312 mg O₂/L with an average of 3.400±1.880 mg O₂/L. Nitrite (NO₂⁻) concentrations varied between 1.45 and 0.94 µM with an average of 1.28±0.23 µM. Nitrates (NO₃⁻) differed between 16.49 and 9.34 µM with an average of 12.60±3.39 µM. Ammonia (NH₃) concentrations fluctuated between 3.99 and 0.13 µM with an average of 1.30±1.83 µM. Phosphate (PO₄³⁻) concentrations varied between 3.50 and 0.55 µM with an average of 2.34±1.34 µM. Silicate (SiO₃⁻) concentrations fluctuated between 5.47 and 4.23 µM with an average of 4.88±0.60 µM and Finally, sulphate (SO₄²⁻) concentrations varied between 3.554 and 2.489 g/L with an average of 2.810±0.504 g/L.

From the obtained physico-chemical results; it could be concluded that the highest salinity, OOM, NH₃ and sulphate value beside the lowest values of transparency, NO₃⁻ and PO₄³⁻ were observed on 18/9/2017 (after 117 days of immersion). These results explain the higher concentration of barnacles in the upper row of coated steel panels beside the appearance of the *Calypsotheca alexandriensis* rather than the bottom row especially for the coated panel with *Ulva lactuca* on 18 September (Fig. 5). The eutrophic condition accelerates fouling as described by Ramadan et al.¹⁷ An extreme degree of eutrophication, on the other hand, can lower biomass, favouring opportunistic species and decreasing the total number of fouling organisms¹⁸.

4. CONCLUSION

- In spite of eutrophication condition in the Eastern Harbour during the studied period; it was noticed that the prepared paint formulations gave good results against fouling.
- The paint compositions play important role for the efficiency of the steel surfaces. The surfaces coated with antifouling paint formulation containing *Grateloupia filicina* and *Corallina mediterranea* recorded the best antifouling property and good film performance within the prepared series of the marine paint formulations. They showed a broad spectrum range of antifouling property against most plant and animal fouling organisms present in the study area (Eastern Harbour) over 140 days.
- The prepared paint formulations showed anticorrosion effect on all coated unprimed steel panels till 140 days of immersion in marine E H water.

REFERENCES

1. E. Armstrong, K.G. Boyd, J.G. Burgess, Prevention of marine biofouling using natural compounds from marine organisms. *Biotech. Annu. Rev.*, 2000, 6: 221-241. (ISSN: 1387-2656).

2. H.D. Bhattarai, B. Paudel, N.S. Park, S.L. Kwang, H.W. Shin, Evaluation of antifouling activity of eight commercially available organic chemicals against the early foulers marine bacteria and *Ulva* spores. *J. Environ. Biol*, 2007, 28: 857-863.
3. S.V. Dobretsov, P.Y. Qian, Effect of bacteria associated with the green alga *Ulva* reticulate on marine micro- and macrofouling. *Biofoul*, 2002, 18: 217-228. (ISSN: 0892-7014)
4. A. Elisabete, C.D. Teresa, and Orlando deS, Marine paints: The particular case of antifouling paints. *Progress in Organic Coatings*, 2007, 59: 2–20.
5. B.T. Aida, A.E.R.Z. Fatma, R.Z.T. Hermine, A.E. Abaas, M.M. Kandeel, Impact of coatings containing Algae on some seawater parameters and Epiphytes plankton. *Egyptian Journal of Aquatic Research*, 2006, 32: 24-37.
6. B.T. Aida, H.A. Ibrahim, R.Z.T. Hermine, M.M. El-Naggar, A.E. Abaas, Suppressive effect of coated surfaces contain *Ulva lactuca* free lipid on *Staphylococcus aureus* ATCC6538. *Egyptian Journal of Aquatic Research*, 2009, 35(4): 405-412.
7. Sh.H.S. Shekhar, C. Fleming, C. Selby, J.R. Rao, T. Martin, Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *J. Appl. Phycol*, 2014, 26: 465-490. [http:// dx.doi.org /10.1007/s10811-013-0101-9](http://dx.doi.org/10.1007/s10811-013-0101-9).
8. R. Chythanya, I. Karunasagar, Inhibition of shrimp pathogenic vibrios by a marine *Pseudomonas* I-2 strain, *Aquaculture*, 2002, 208: 1-10.
9. K.Grasshoff, Methods of seawater analysis. Verlage Chemie Weinheim, NewYork.1976.
10. APHA, Standard Methods for the examination of water and wastewater by American public Health Association. Broadway, New York, 1989.
11. FAO, Permanganate value of organic matter in natural waters. Fisheries Technical paper, 1975, 137: 169-171.
12. T.R. Parsons, Y. Maita, C.M. Lalli, A manual of Chemical and Biological Methods for Seawater Analysis. Fish, Res. Bd. Canada Bull, 1984, 311p.
13. J.M. Bather, J.P. Riley, The chemistry of the Irish Sea. Part1. The sulphate-chlorinity ratio. *J. Cons. Perm. Int. Explor. Mer*. 1954, 20 (2): 145–152.
14. M.A. Khaled, D.T. Paul, M.D. Mohamed, A new species of *Calyptotheca* (Bryozoa: Cheilostomata) from Alexandria, Egypt, southeastern Mediterranean. *Zootaxa*, 2017, 4276 (4): 582–590. <http://www.mapress.com/j/zt/>
15. B.T. Aida, R.Z.T. Hermine, Studies on tubeworms and sepia shell containing paints using steel surface. Global Climate Change, Biodiversity and Sustainability: Challenges and Opportunities. International Conference, April 15-18, 15 p. <http://gccbs2013.aast.edu/newgcc/index.php/download-published-papers>.
16. M.F. Hayat, R.Z.T. Hermine, M.A. Okbah, Nutrient salts and chlorophyll-a during short term scale in the Eastern Harbor, Alexandria (Egypt). *Egyptian Journal of Aquatic Research*, 2009, 35(3): 243-250.

17. Sh.E Ramadan, A.M. Kheirallah, Kh.M. Abdel-Salam, Marine fouling community in the Eastern harbour of Alexandria, Egypt compared with four decades of previous studies. *Mediterranea Marine Science*, 2006, 7 (2): 19-29.
18. M. Mayer-Pinto, M.S. Viana, H.P. Lavrado, S.H.G. Silva, Epibiosis on barnacles at Angra dos Reis, RJ: *Eutrophication effects. Nauplius*, 2000, 8 (1): 55-61.

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