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Antifouling efficacy of a controlled depletion paint formulation with acetophenone

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Summary: Biofouling is an inevitable problem that occurs continually on marine fishing vessels and other small crafts. The nature of the antifouling (AF) coatings used to prevent biofouling on these small vessels is of great environmental concern. Therefore, the efficacy of a non-toxic AF candidate, acetophenone, was evaluated in preliminary laboratory assays using marine bacteria, diatom and *Ulva* spores. At a low concentration of 100 µg cm^{-2} of acetophenone, spore attachment of a green fouling alga was significantly reduced (p<0.01). Similarly, 40% acetophenone coatings significantly inhibited diatom attachment. This new non-toxic AF agent was incorporated into controlled depletion paint (CDP). Fouling coverage (%), biomass, and fouling resistance (%) were estimated. On CDP coatings made with acetophenone (40%), a significant decrease in fouling biomass was estimated (p<0.01).

Keywords: *Ulva* spores; antifouling; acetophenone; fouling biomass; fouling resistance; controlled depletion paint (CDP).

Eficacia antiincrustante de una formulación de pintura de reducción controlada con acetofenona

Resumen: El biofouling es un problema inevitable que ocurre continuamente en los buques de pesca marina y en las pequeñas embarcaciones. La naturaleza de los recubrimientos antiincrustantes (AF) usados para prevenir el bioincrustado en estos pequeños buques tiene gran preocupación ambiental. Por lo tanto, la eficacia de un candidato AF no tóxico, la acetofenona, se evaluó en ensayos preliminares de laboratorio usando bacterias marinas, diatomeas y esporas de *Ulva*. A una concentración baja de 100 µg cm^{-2} de acetofenona, la adherencia de esporas de una alga incrustante verde se redujo significativamente (p<0.01). Del mismo modo, el revestimiento de acetofenona a un nivel del 40% inhibieró significativamente la adherencia de diatomeas. Además, esta nueva acetofenona AF no tóxica se incorporó a la pintura de reducción controlada (CDP). La cobertura de las incrustaciones (%), la biomasa y la resistencia a la incrustación (%) fueron estimadas. En recubrimientos de CDP donde se incorporó la acetofenona (40%), se estimó una disminución significativa de la biomasa incrustante (p<0.01).

Palabras clave: esporas de *Ulva*; antiincrustante; acetofenona; abordaje biomasa; resistencia a la incrustación; pintura de depleción controlada (CDP).

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INTRODUCTION

Marine biofouling is one of the most important problems faced by marine technologies (Yebra et al. 2004). In the marine environment any solid surface submerged in seawater will become covered by a complex layer consisting of an organic conditioning film and microfouling and macrofouling organisms such as marine bacteria, algae, protozoa, barnacles, mussels and tube worms (Mieszkin et al. 2013, Dobretsov et al. 2013). Macrofoulers such as barnacles, mussels and algae constitute a large source of fouling biomass. These organisms attach to the ship's hull, which increases the friction of the boat in the water. Subsequently, fouling on the hull lowers speed, impairs manoeuvrability and ultimately increases fuel consumption (Karlsson and Eklund 2004), thereby causing additional CO_2 emissions and maintenance costs (Schultz et al. 2011). The increase in fuel consumption can be up to 40% and in an overall voyage costs are as much as 77% more. In addition to the shipping industry it also affects aqua farming industries (Guardiola et al. 2012).

Several physical, chemical and biological methods for preventing marine biofouling have been tested in the last four decades. To prevent the attachment of fouling organisms, the boat hulls are protected with anticorrosive and antifouling (AF) paints. Earlier forms of AF paints are based on the leakage of toxic substances such as copper, tributyltin (TBT) and Irgarol, which prevent the settlement and growth of marine organisms (Soroldoni et al. 2017). These paints are very effective but they also significantly affect other non-target organisms. Potential harmful effects of AF marine paints containing organotin compounds, copper and Irgarol 1051 on coral reef communities are reported (Owen et al. 2001). Among these, TBT, Irgarol 1051 and diuron were banned in 2008. TBT is an endocrine disruptor due to its induction of imposex (imposition of male sexual organs on females) in mollusc species (Horiguchi et al. 1997). Periodic sediment quality surveys carried out around the world indicate persistence of high copper concentrations in marinas and harbours (Schiff et al. 2007). Dissolved trace metals in measured water columns frequently exceed the levels of concern in marinas because of leaching from ship hulls (Hall et al. 1988).

Most of the AF paint formulations manufactured to replace organotin-based paints contain predominantly copper and toxic organic biocides. At elevated concentrations copper has a capacity to cause reproductive disorders in a variety of aquatic organisms (Adeleye et al. 2016). Therefore, the paint industries have been urged to develop tin-free alternatives with the same economic benefits but without harmful effects on the environment and marine life. The toxic effects of zinc pyrithione (ZPT), which is used as a booster biocide in antifouling paints, are well documented (Karlsson and Eklund 2004). However, ZPT is the most prevalently used organic biocide in paints, owing to its shorter half-life and photodegradation properties (Kim et al. 2015). Environmentally acceptable solutions are still needed to prevent fouling organisms. Natural products could provide alternatives as antifouling agents, but their incorporation into a working AF coating is challenging (Chambers et al. 2011).

In this study, the organic compound acetophenone (AP) was formulated with controlled depletion paint (CDP). As an aromatic ketone, AP occurs naturally in many foods such as apples, cheese, apricots, bananas, beef, cauliflowers and castoreum (Müller-Schwarze and Houlihan 1991). Its derivatives are widely used in the food, perfume, pharmaceutical and polymer industries. To this end, the AF efficiency of AP was screened with the marine bacterium *Bacillus macrolides*, the diatom *Navicula incerta* and the macroalgal spore *Ulva pertusa*. Newly prepared AF coatings were tested in various field conditions using test panels. The AF performance of AP was assessed against fouling organisms of the east coast of Korea.

MATERIALS AND METHODS

Antibacterial activity

Antibacterial assays were conducted with the AF candidate AP and other reference biocides [tributyltin chloride (TBTCl) and ZPT]. A marine bacterium, *Bacillus macroides* (strain KORDI-13724), was used. Assays were conducted using the paper disc method. Aliquots of test chemicals were loaded onto a paper disc and dried at room temperature for about 12 h. *B. macroides* culture was raised in marine agar broth (DIFCO, Detroit, MI, USA). At the log phase of growth, it was inoculated onto an agar plate (prepared with marine agar (DIFCO, Detroit, MI, USA) and spread uniformly to make a bacterial lawn. Then experimental paper discs were placed on the bacterial lawn and the agar plates were incubated for 48 h at 28°C. At the end of the experiments, the inhibition zone formed was measured. Three replicates were used and the results were expressed as mean±sd.

Diatom attachment

A biofilm-forming diatom species, *Navicula incerta* (KMMCC no. B001), was used for this assay. *N. incerta* cultures were raised in f/2 medium and during their exponential growth stage diatom cells which settled down at the bottom of the flask were carefully collected without disturbing the attached cells. Then the culture was repeatedly washed with sterile seawater (0.45 μm filtered) to obtain a cell suspension with single cells. The initial cell density was measured using an improved Neubauer haemocytometer (Paul Marienfeld, GmbH and Co., KG, Germany) and was then adjusted to 1.0×10^5 ml^{-1} using sterile seawater. Standard microscopic slides (7.5×2.5 cm) were brush-coated with about 0.5 mL of experimental coatings (AP-CDP 10-40%, AP and ZPT 10:3 and 20:3 levels and acid-washed glass slides served as control). These slides were subjected to a diatom assay by the method given elsewhere (Pettitt et al. 2004), with a slight modification of microscopic cell enumeration. Attached cells were carefully removed into 1 mL of sterile seawater and the number of cells was examined under a microscope. The cells were counted in 20 random fields of view (Muthukrishnan et al. 2017). Results are expressed as diatom cells attached per mm^2 of experimental coating surface.

Ulva spore attachment and germination

Spore attachment and germination experiments were conducted in 24 well plates (Corning Co. USA) coated with different concentrations of AF candidates [dissolved in methanol (MeOH)], as outlined by Hattori and Shizuri (1996). *Ulva pertusa* samples were collected from the west coast of the Republic of Korea. In the laboratory, debris and epiphytes were removed by washing twice in seawater (0.45 μm filtered). Spore release was facilitated after a desiccation treatment (Fletcher 1989). Initial spore density was determined using a hemocytometer under a light microscope (Olympus CK2, Japan). Spore density in the inoculation suspension was adjusted to 2×10^5 ml^{-1} by addition of seawater (0.45 μm filtered). In each coated well, 1 mL of spore suspension containing 2×10^5 spores was inoculated along with 1 ml of filtered seawater and 10 μl of ampicillin (100 mg l^{-1}). Well plates were then incubated in a darkroom for 6 h. Thereafter, the spore suspension in the well plate was discarded and the wells were gently washed twice with sterile seawater (0.45 μm) to remove the loosely attached or unattached spores. Spore attachment was examined under microscope (200×). Six microscopic fields were counted per well along the central region. Later, the wells were refilled with two mL of Provasoli's Enriched Seawater medium and the well plates were incubated in a growth chamber under continuous light (60 μmol m^{-2} s^{-1}). Five days later, the culture medium was pipetted out from each well and successfully germinated spores were counted in 20 fields of view under microscope (200×) (Muthukrishnan et al. 2017). Results were expressed as a mean number of spore mm^{-2}.

Formulation of CDP and coating

In the preparation of CDP formulations, vinyl resin (DOW Co., South Korea), wood rosin, AP (2',4' –dihydroxyacetophenone, Acros chemical Co., USA), AP (1-Hydroxypyridine-2-thione zinc salt, Acros chemical Co., USA) and TBTCl (Sigma-Aldrich Co., USA), methyl isobutyl ketone (MIBK) and methyl ethyl ketone (Samchun Co., South Korea) and other pigments and additives (industrial grade) were used.

In order to evaluate the performance of AP in field conditions, typical CDP formulations were made using MIL-P-15931B formula 121/63 (D3623-78a: ASTM 2004), and are given in Tables 1 and 2. Using the above formulation, the AP- and TBT-CDPs were prepared. The TBT-CDP was used as a positive control. The required quantity of xylene and MIBK was taken in a container and homogenized with the help of a high-dispersion mechanical stirrer. An absorbent (zeolite) was added, followed by a dispersant, and it was stirred well to get a homogenous mixture. Rosin and vinyl resin binders were added and the mixture was again stirred for 20 to 40 min. The other ingredients, AF agent, pigment, iron oxide (extender) and zinc oxide (anticorrosive), were added and stirred well (1300 rpm) for 20 minutes. Finally, a booster biocide, a plasticizer, an anti-settling agent and other additives were added and the mixture was stirred well for 20 minutes. The homogeneous mixture was transferred to a glass bead mill to obtain a fine dispersion (Motor mill, Hwa Sung Industrial CO., Korea). Particle size was fixed at 50 μm. The fineness and viscosity of the prepared paint were determined by a Hegman-type gauge and a Ford cup (#3, #4 and #5), respectively. For comparison, two copper-based commercial AF paints with rosin matrix, Waterways and Cruiser (Future Series, International paint Co., UK), were also used.

Table 1. – Formulations of controlled depletion paints with acetophenone and zinc pyrithione. Based on ASTM standard formula (Mil-p-15931B, 121/63) (D3623-a: ASTM 2004). AF agent, acetophenone; booster biocide, zinc pyrithione; MIBK, methyl isobutyl ketone.

Recipe	Acetophenone (%)				Acetophenone: booster biocide (%)			
	10	20	30	40	10:3	20:3	30:3	40:3
AF agent	10	20	30	40	10	20	30	40
Booster biocide	-	-	-	-	3	3	3	3
Vinyl resin	5	6	6	6	5	6	6	6
Wood rosin	8	8	8	8	8	8	8	8
Iron oxide	9	6	5	5	9	6	5	5
Zinc oxide	20	14	12	9	17	11	9	6
Thickener	2	2	1	1	2	2	1	1
Anti-settling agent	5	3	3	1	5	3	3	1
Dispersant	1	1	1	1	1	1	1	1
Tricresyl phosphate	7	5	4	4	7	5	4	4
Absorbent	2	4	1	1	2	4	1	1
Xylene	23	23	21	18	23	23	21	18
MIBK	8	8	8	6	8	8	8	6
Total	100	100	100	100	100	100	100	100

Table 2. – Formulations of controlled depletion paints with tributyltin chloride (TBT) and zinc pyrithione (ZPT) based on ASTM standard formula (MIL-P-15931B, 121/63) (ASTM 2004). AF agent, TBTCl; booster biocide, ZPT; MIBK, methyl isobutyl ketone.

Recipe	TBT 30 (%)	TBT:ZPT 30:3 (%)
AF agent	30	30
Booster biocide	-	3
Vinyl resin	6	6
Wood rosin	8	8
Iron oxide	5	5
Zinc oxide	12	9
Thickener	1	1
Anti-settling agent	3	3
Dispersant	1	1
Tricresyl phosphate	4	4
Absorbent	1	1
Xylene	21	21
MIBK	8	8
Total	100	100

Test panel coatings and evaluation

Test panel studies were conducted with PVC panels (10×10×0.5 cm). Before coating, the test panel surface was roughened with emery paper (#1000). The prepared paint was sprayed three times on the panels with a spray gun (W-2000, nozzle size: 1.3 mm, Hyup Sung.

Co., Korea) with compressed air (0.7 MPa) and a spray distance of 150-200 mm. The wet thickness of the coatings was measured by wet comb gauge (Elcometer 2400 viscosity disc). The coated panels were then air dried at room temperature for 24 hours.

A standard test method of adhesion by knife (D6677-1: ASTM 2001) was determined by making an "X" cut into the coating film to the substrate and by lifting the coating with a knife. Adhesion was evaluated qualitatively on a 0 to 10 scale. The degree of blistering of paint film was evaluated by comparison with the photographic references of standards (D714-87: ASTM 2000). The fineness of dispersion of the pigment-vehicle system was evaluated using a single-path Hegman-type gauge (D1210-96: ASTM 1988). The prepared CDP was spread by a scraper on a machine-tapered path. At some point in this path, particles or agglomerates, or both, became visible. A direct reading from the graduated scale was then made at the point where the particles formed a definite pattern.

Field experiments

The coated panels were tied in a random order to PVC frames with nylon ties. The prepared panel setup was immersed in Ayajin harbour at a minimum depth level of 0.3 m for 3 months. After being exposed to Ayajin harbour waters, test panel sets were taken out and digital images of every individual panel were recorded with a five-megapixel camera (C-4000, Olympus, Japan). For the purpose of identification and quantification, a 25-square mesh (10×10 cm) was used to examine the fouling assemblages. Each small square was equal to 4% of the whole mesh. Later, from every individual panel, fouling assemblage on a section of area was scraped and preserved in 4% formalin. In the laboratory, both digital images and preserved samples were analysed. From digital images and microscopic observations, micro- and macroscopic algae and animals were identified (Meinkoth 1990). Their fouling coverage and biomass were also determined as per ASTM (2004). For data analysis, comparison between means of control and treatment was performed using SPSS 10.0. A one-way ANOVA was carried out to find significant differences at $p<0.05$ or $p<0.01$.

RESULTS

The viscosity and fineness of dispersion of prepared CDP formulations were found to be consistent with the spray coatings (Table 3). Moderate viscosity levels were obtained for AP-CDPs (Ford cup #4: <132 cSt) except the AP:ZPT (10:3) formulation, whereas commercial coatings (copper-based Waterways and Cruiser) exhibited high viscosities (Ford cup #4: 112-122 cSt). A fine dispersion of 40 µm was achieved for all the AP-CDP combinations. The physicochemical properties of prepared CDP coatings—adhesion, hardness, appearance of surface blister and wettability determined as per standard ASTM methods—are given in Table 4. The AP-CDP formulations exhibited moderate physical properties when compared with commercial AF paints. CDP coatings prepared with TBT (30%) and TBT:ZPT (30%:3%) exhibited low hardness with high surface blisters.

The antibacterial activities of the AF candidate,

Table 3. – Viscosity (cSt) and dispersion (µm) of prepared AP-CDP formulations. [a] Fineness of dispersion by Hegman gauge; AP, acetophenone; ZPT, zinc pyrithione; TBT, tributyltin chloride; Comm., commercial formulations ([b] and [c], Copper-based Future series, International paint Co., Ltd.); [d], viscosity (cSt) of oil standards at 25°C.

Groups	CDP formulation	Ford cup (Viscosity cSt) # 3	# 4	# 5	Dispersion [a] (µm)
AP	10%	80	122	472	40
	20%	68	106	411	40
	30%	59	92	363	40
	40%	47	75	303	40
AP:ZPT	10:3%	86	132	508	40
	20:3%	63	99	387	40
	30:3%	56	89	351	40
	40:3%	43	69	278	40
TBT:ZPT	30:0%	43	69	278	40
	30:3%	68	106	411	40
Comm.	Waterways [b]	73	112	436	80
	Cruiser [c]	80	122	472	80
Con	control paint	84	129	496	40
Std	Oil standard	49-220 (120)[d]	70-370 (120)[d]	200-1200 (460)[d]	

Table 4. – Physical properties of prepared CDP coatings. AP, acetophenone; ZPT, zinc pyrithione; TBT, tributyltin chloride; Comm.: commercial formulations (Copper-based Future series, International paint Co., Ltd.). Surface blisters: F, few; M, medium; MD, medium dense; D, dense. Wettability: G, Goo. Adhesion: strength of adhesion is indicated in a 0-10 point scale.

Groups	CDP Coating	Adhesion	Hardness	Surface blister	Wettability
AP	10%	2	0.165	M	G
	20%	2	0.128	F	G
	30%	2	0.109	F	G
	40%	4	0.081	F	G
AP:ZPT	10:3%	4	0.151	M	G
	20:3%	2	0.156	M	G
	30:3%	4	0.065	F	G
	40:3%	2	0.118	F	G
TBT:ZPT	30:0%	4	0.003	M	G
	30:3%	8	0.009	D	G
Comm.	Waterways	2	0.332	MD	G
	Cruiser	2	0.413	MD	G
Con	control paint	2	0.491	M	G

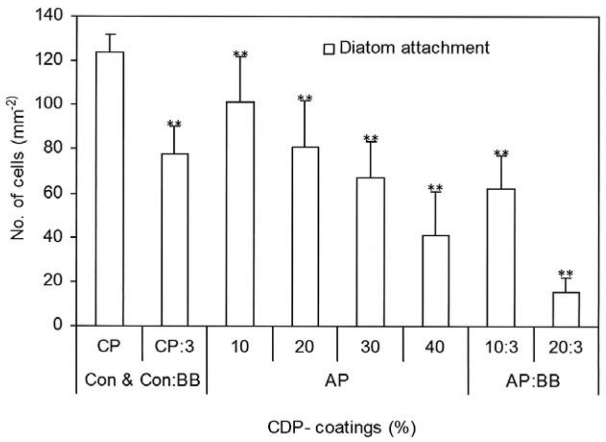

Fig. 1. – Diatom (*Navicula incerta)* attachment on CDP coatings prepared with acetophenone (AP) and zinc pyrithione (BB). Means are compared with control by one-way ANOVA followed by Dunnett's test (*$p<0.05$; **$p<0.01$).

Table 5. – Antibacterial activity of AF agents and booster biocide used in this study against *Bacillus macroides* (Mean inhibition zone mm±SD, n=3). AP, acetophenone; ZPT, zinc pyrithione; TBT, tributyltin chloride; DMSO, dimethyl sulfoxide.

Test chemicals	Concentration (µg disc^{-1}) 0	50	100	500	1000
Control	0				
DMSO	0	0	1.00±0.00	2.00±1.00	-
AP	0	2.00±0.05	5.00±1.00	7.00±0.50	9.50±0.50
ZPT	0	4.00±1.00	6.50±1.80	10.00±1.00	14.00±2.00
TBT	0	8.50±1.00	14.50±1.32	20.00±3.00	27.00±1.32

Table 6. – Effect of AP on attachment and germination of spores of the fouling alga, *Ulva pertusa* (means are compared with control by one-way ANOVA followed by Dunnett's test: *p<0.05; **p<0.01). AP, acetophenone; MeOH, methanol; ZPT, zinc pyrithione; TBT, tributyltin chloride; Cu, copper sulfate.

Test chemicals	Conc. (µg cm^{-2})	No. of *Ulva pertusa* spores mm^{-2} Attached	Germinated
Control		27.70±4.33	20.65±6.05
MeOH	50	28.30±5.59	17.75±5.48
AP	50	25.25±5.20	18.65±6.33
AP	100	21.85±6.37**	14.20±6.70**
AP	250	13.90±3.75**	11.45±5.41**
AP	500	11.25±4.04**	7.95±3.47**
AP	1000	8.20±1.85**	4.30±2.58**
ZPT	100	9.50±3.19**	4.05±1.96**
TBT	100	9.20±2.61**	5.85±1.57**
Cu	100	15.10±3.97**	10.75±3.16**

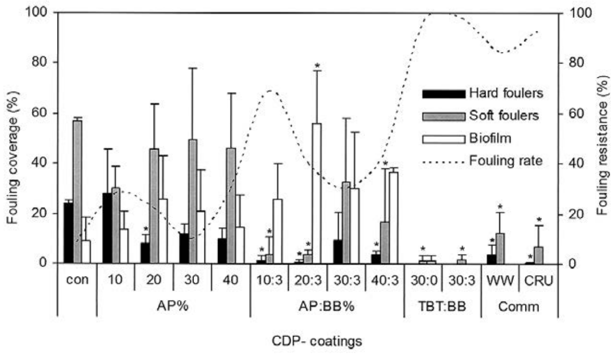

Fig. 2. – Comparison of coverage (%) of major components of fouling assemblages on CDP-coated panels exposed for three months to waters of Ayajin harbour, South Korea. Means are compared with control by one-way ANOVA followed by Dunnett's test (*p<0.05).

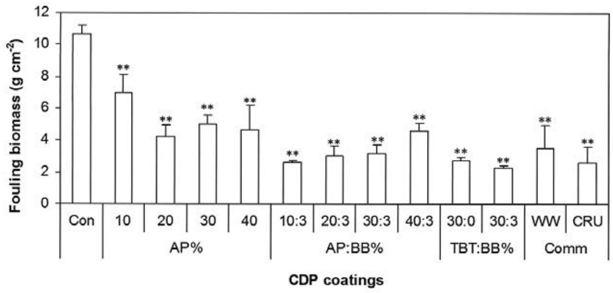

Fig. 3. – Comparison of total fouling biomass estimated on CDP-coated panels exposed for three months to waters of Ayajin harbour, South Korea. Means are compared with control by one-way ANOVA followed by Dunnett's test (*p<0.05; **p<0.01).

AP, and biocides against the marine biofilm-forming bacterium *B. macroides* are shown in Table 5. The antibacterial efficiency was found to be in the following order: TBTCl>ZPT>AP > control. In all the concentrations tested, a moderate antibacterial activity was observed for AP at 1000 µg disc^{-1} with 9.50±0.50 mm of inhibition zone, whereas TBTCl (positive control) exhibited the highest inhibitory zones due to its toxic nature. Attachment of the diatom *N. incerta* to CDP coatings made with series of AP and ZPT concentrations is shown in Figure 1. A substantial decrease in diatom attachment was observed on AP (40%) and a combination of AP:ZPT (20%:3%) coatings (67% and 87% of controls, respectively, p<0.01).

Effects of AP and other biocides on attachment and germination of spores of *U. pertusa* are shown in Table 6. AF activity against *Ulva* spores increased with increasing concentrations of AP. On experimental AP coatings (1000 µg cm^{-2}), a maximum of 70% and 79% reduction in *Ulva* spore attachment and germination were observed, respectively, compared with control. On the other hand, at a low concentration of 100 µg cm^{-2} of toxic biocides such as TBTCl and ZPT, more than a 70% reduction in *Ulva* spore germination was observed (p<0.01). For non-toxic AP coatings at 250 µg cm^{-2}, both spore attachment and germination were significantly reduced to ≤50% levels (p<0.01).

In the fouling assemblages observed on untreated control panels, six major components were observed: macroalgae (*Ulva pertusa*, *Ectocarpus* sp., *Ceramium* sp., *Porphyra tenera* and *Gelidium* sp.), barnacles (*Balanus* sp.), tube worms (*Spirobis* sp.), sponges (*Cliona* sp., *Halichondria* sp.), tunicates (*Botrylloides* sp.) and microalgal biofilm consisting of diatoms. Based on standard fouling ratings (ASTM 2004), data obtained on antifouling characteristics of different CDP coatings prepared in this study were assessed. The antifouling properties of AP and AP and ZPT coatings were comparable to the commercial paints, which contain copper (Figs 2 and 3). The order of reduction in fouling coverage (%) on the experimental panels was found to be TBT>Cu>AP and ZPT>AP>control. The assessments of fouling coverage of all the experimental panels indicate the predominance of soft foulers. AP- and TBT-CDP coatings prepared in combination with ZPT (the most commonly used booster biocide) effectively inhibit the recruitment of soft foulers (Fig. 2). There was a twofold decrease in coverage of hard foulers in AP-CDP coatings (at 20% level, p<0.05), whereas the addition of ZPT (3%) further prevented the growth of hard foulers, with a 3-4 fold decrease (AP (20%):ZPT(3%), p<0.05), compared with control.

Fouling resistance was determined for the panels coated with AP and AP and ZPT experimental paints according to the ASTM (2004) procedure (Fig. 3). AF performance of prepared AP-CDP formulation increased with increasing concentrations. Similarly, with ZPT booster biocide, the prepared AP-CDP formulations showed higher fouling resistance against fouling organisms of the Ayajin harbour waters. AP-CDP (10%-40%) coatings showed 10%-30% fouling resistance against both soft and hard fouling communities. Addition of ZPT (3%) increased the fouling resistance to a maximum of 69% (Fig. 2). Very high fouling resistance equal to that of commercial AF paints (Waterways and Cruiser) was observed from TBT and

TBT-ZPT coatings ($p<0.05$), due to their toxic biocidal properties.

The total fouling biomass observed from experimental panels (g cm^{-2}) is shown in Figure 3. On AP-CDP coatings (10-40% levels), a 35%-60% reduction in fouling biomass was observed ($p<0.05$). More than 50% reduction in fouling biomass buildup was seen in all the AP- and TBT-SPC coatings in which ZPT was used as a booster biocide ($p<0.05$). Their AF efficiencies were comparable to the two commercial reference coatings tested.

DISCUSSION

The new antifouling candidate AP was tested for antimicrobial activity against the marine bacterium *B. macrolides* in this study. As a result, the concentration of AP should increase up to two-fold to achieve the same activity as commercial AF agent ZPT (positive control). Yeast cells maintained 25% viability when the AP was used at 17 mM (Rogers et al. 1999). Lin et al. (2011) reported that a paper disc assay using AP concentration at 200 μg $disc^{-1}$ had antibacterial activity against *Staphylococcus epidermidis* similar to that of natural products. AF activity against microalgae *N. incerta* increased with increasing concentrations of AP from 10% to 40% in paint. In a previous study, the antifouling agent Cu_2O and TBT were found to be effective at 150 μg ml^{-1} (Hellio et al. 2002). Coatings containing TBT, copper and silicon were evaluated for AF activity against diatom (Cassé and Swain 2006) in static condition, and TBT and copper coatings were not significantly different.

The most common macrofouling algae are species of *Enteromorpha*, *Ulva*, *Chaetomorpha*, and *Cladophora* (green algae); *Ectocarpus* (brown algae); and *Polysiphonia*, *Ceramium* (red algae) occurring along the coastal regions with high density (Callow 1996). Many of these fouling organisms are opportunistic and able to colonize on a variety of hard substrata in the marine environment. Colonization of these fouling organisms drastically decreases the speed of vessels because of the high frictional resistance (Adkins et al. 1996). The speed loss is proportional to fuel consumption. It is reported that the increased fuel consumption can be 40%-70% of the overall cost of voyage (Champ 2001). The species of *Enteromorpha* and *Ulva* are cosmopolitan in distribution and are considered to be one of the dominant shipfouling algae. Biofouling is a site-specific phenomenon in which environmental factors play a decisive role. Especially in harbours, which are enclosed and thus have restricted tidal movement, both algal and animal fouling organisms undergo prolific growth. In the Ayajin harbour, on the east coast of Korea, soft fouling is mainly due to *U. pertusa*, as this alga grows in high densities. In an AF test panel study conducted from October 2000 to March 2001, *U. pertusa* occurred on test panels with high fouling coverage (Sidharthan et al. 2004). The very high abundance of *U. pertusa* prevailing on the Korean coast frequently causes green tides. Over the last 10 years, the occurrence of green tide has been recognized as a major environmental problem in Korea (Choi et al. 2001). A seasonal peak with very high biomass (2.2 kg fwt. m^2) of *U. pertusa was* reported from the Korean coast for the month of May (Kim et al. 2004). However, the seasonal variation was found to be less in Ayajin harbour waters as the prevailing high nutrient influx is conducive to the exorbitant growth of *U. pertusa*. It is therefore all the more important to control the recruitment and growth of this monospecific algal fouler. During the present study *U. pertusa* was also found in high densities. The non-toxic AP coatings screened in this study exhibited effective AF activity against *U. pertusa*. The effective concentration from 250 μg cm^{-1} AP affected macroalgae of attachment and germination compared with control and MeOH. However, the AP exhibited two times less AF activity than the commercial agent ZPT.

To know the species involved in biofouling and their assemblage pattern, test panel studies were undertaken. A typical biofouling experiment was conducted by ASTM (2004) with 25×10 cm coated panels. Similarly, in the present study at Ayajin harbour, South Korea, soft foulers such as macroalgae, sponges and tunicates were found with high fouling coverage.

The CDP type of coatings incorporated with non-toxic AF agents such as sodium benzoate and three different tannins are shown to inhibit the attachment of nauplii of *Balanus amphitrite* (Stupak et al. 2003). Later, in field trials conducted for 4 months at the Club de Motonautica, Argentina, these AF paints effectively inhibited major fouling organisms such as *Balanus amphitrite*, *Polydora ligni*, *Enteromorpha* and *Ectocarpus*. In view of known environmental fate and cost-effectiveness, these types of non-toxic organic chemicals are recognized as an alternative to inorganic and toxic AF chemicals. In the present attempt such an AF CDP coating formulated with AP was found to effectively prevent hard and soft foulers compared with controls in Ayajin harbour along the east coast of Korea.

AF paints are complex materials with regard to the large number of compounds introduced in their formulation. The optimization of the protective activity of paints is an economic and ecological challenge which requires the understanding of the precise function of each compound (Thouvenin et al. 2002). The paint forms a single layer on the substrate, which has antifouling chemical attributes. Though it is a chemical, it is not toxic. Additives are important to preserve the physical and chemical properties of paints.

Soluble matrix types of paint have been developed in order to avoid the loss of AF efficiency with time by incorporating a binder (rosin) which dissolves slowly in seawater. These kinds of paint contain high proportions of rosin, which occurs naturally from the exudation of pine and fir trees (Rascio et al. 1988). Rosin may not prevent seawater from penetrating into the polymer matrix through hydrophobic interactions (Yebra et al. 2004). In the resulting, leached layer was >50 μm in thickness due to the continuous dissolution of the copper(I) oxide pigments (Yebra et al. 2004). However, the main drawback of rosin-based coating is that erosion of the paints increases with increasing vessel speed (Anderson and Hunter 2000).

Since 1986, the traditional antifouling coatings have been modified with reinforcing resins and are now generally referred to as CDP. To improve the physical properties, plasticizers are used as desiccants and dispersants (Chung 1994). In the present study, wood rosin used in CDP formulation with AP served as a soluble matrix in order to increase the polishing rate of coating.

High rosin content with a co-binder and plasticizer can give good mechanical properties (Rascio et al. 1990). On the other hand, addition of vinyl resin used at optimum levels tends to control the rapid solubilization of the rosin-based matrix and prevent excessive release of the incorporated AF agent and biocides (Lejars et al. 2012).

A smooth paint surface without extensive fouling (i.e. macrofouling and thick slimes) is necessary to avoid friction during sailing. The modelling of AF systems is based on different physical and chemical reaction mechanisms.

Coating technology is fundamentally dependent upon good adhesion between the coating and the substrate, and in many cases adhesion is the limiting factor for the wider application of the technology (Aldrich-Smith et al. 2005). A phenomenon peculiar to painted surfaces is the formation of blisters which can weaken the coatings. A test method called D714-87 (ASTM 2004) describes the size and density of the blister so that comparisons of severity can be made. In the present study, compared with moderately dense blisters on commercial coatings (Cu based), few blisters were observed on the new CDP coatings containing AP.

An ideal paint film thickness can only be achieved if the coating has even upper and lower surfaces and a defined density. In practice, neither the surface of the coating nor that of the substrate is even. The influence of surface irregularities and density on the results of each coating is well documented. Film thickness of CDP coatings made with AP ranged from 70 to 286 µm. The film thickness of the coatings can be increased further to give a desired leaching rate with a better coating life.

In a previous study conducted in Ayajin harbour with a seaweed-based (*Ishige okamurae*) soluble matrix type of AF coating, over an 80% decrease in coverage of a macrofouling alga, *Ulva pertusa*, is reported. Non-toxic hybrid CDP coatings prepared with ethyl heptanoate (10-40%) in acrylic resin matrix showed more than 75% fouling resistance for four months in Ayajin harbour waters (Sidharthan et al. 2006). Test panels of these hybrid CDP coatings in combination with 4-12% of ZPT provided more than 60% fouling resistance over a period of one year. Similarly, AP-CDP coatings were effective against *U. pertusa* and the addition of a co-biocide, ZPT, at 3% substantially reduced the recruitment of hard foulers on test panels exposed to Ayajin harbour waters.

These studies clearly indicate the importance of compatibility of binders with AF agents and biocides in formulating coatings with better leaching properties. Similar observations were seen in AP-CDP coating (10%) in which comparatively less fouling coverage was observed, which may be due to insufficient concentration of AP leached in the coating-seawater interface. As suggested by Del Amo et al. (1989), with proper designing of the CDP-matrix using compatible co-binders, plasticizers and pigments, a much improved leaching effect can be achieved.

Independent laboratory bioassays carried out in this study with micro- and macrofouling organisms revealed the AF potential of AP. In particular, the results showed effective control of a fouling alga, *U. pertusa*, by AP incorporated in CDP coatings. In combination with AP, the new AF formulation effectively prevented both algal and animal macrofoulers. The performance of these AF coatings can be comparable to toxic copper- and TBT-based coatings. However, further studies are needed to reveal the AF mechanism of AP in order to use it more effectively in environmentally acceptable AF coatings.

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