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What governs marine fouling assemblages on chemically-active antifouling coatings?

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Abstract

From an environmental perspective, there is a need to reduce the amount of biocides in chemically-active antifouling (AF) coatings, as these biocides can cause severe damage to marine life. In this study, six active molecules known as booster biocides were added at 3% wt. in solvent and aqueous-based acrylic paints. DCOIT, dichlofluanid, zinc pyrithione, copper pyrithione, tralopyril and zineb were used alone and mixed with a lower amount of copper (I) oxide and copper thiocyanate (10 wt.%) than is currently used or with zinc oxide fillers. Field immersion tests were carried out in the Atlantic Ocean (Ste Anne du Portzic) and the Mediterranean Sea (Toulon), with contrasting physicochemical properties of water and macrofoulers' biodiversity. Microscopic analyses showed that AF coatings reduced the bacterial density differently for short immersion times at Ste Anne du Portzic. After four/five months of immersion, results, including multidimensional analyses, showed first specific patterns in the macrofoulers' assemblages depending on the site. In addition, the structure of macrofoulers' assemblages was affected by the biocide itself and by the combination of the biocide and the binder. When efficient, AF coatings appeared to decrease the richness with a strongest effect at Toulon. DCOIT was the most performing booster biocides whatever the immersion site and the polymer binder

used. DCOIT was found to be efficient against marine bacteria and macrofoulers at Ste Anne du Portzic. The combination of zinc pyrithione with ZnO fillers in an aqueous-based AF paints exhibited the highest activity in the Mediterranean Sea over 16 months of immersion.

Keywords: antifouling coating, biocides, marine bacteria, macrofouling, field immersion

1. Introduction

Chemically-active antifouling coatings are currently designed to protect ship hulls from the settlement of marine organisms [1-3]. In Norway, most farmers use copper-based antifouling (AF) coatings to prevent settlement and reduce marine fouling growth on their fish nets [4]. The efficiency of these coatings relies on the release of active /biocidal molecules from the coating surface and thereby creating a hostile environment for the targeted marine organisms. These coatings contain pigments, fillers, and biocides, all of them embedded in a polymer binder. The mechanism of action of chemically-active coatings is based on the diffusion of water into the coating [5, 6]. The seawater-soluble pigments/biocides dissolve near the surface of the coating, creating voids in the upper coating layer. The distance from this dissolving front to the surface of the coating is termed as leached layer or depleted layer. Dissolved species diffuse out of the coating through the seawater-filled leached layer. The voids, filled of water, also play a role in the kinetics of solubilization of the polymer binder as the water/binder interface area increases with time [7]. Depending on its hydrophilic character, the polymer binder can be solubilized by water leading to a renewal of a surface erosion by moving seawater [8-10].

Following the ban of organotin-based paints, tin-free alternatives have oversupplied the antifouling paint market, with paints formulated with copper (I) oxide (Cu_2O) as the main biocides. In Europe, biocides used on vessels, aquaculture equipment or other

structures in water, are under the biocidal product regulation (BPR, 528/2012). All biocidal products require an authorization before being placed on the market, and the active substances contained in this biocidal product must be previously approved [11]. Although Cu₂O is allowed in Europe, it is prohibited for pleasure boats in some marinas in Sweden [12]. Co-biocides are called booster biocides as they enhance the AF efficiency and improve the activity spectrum of the coating against a wide variety of targeted marine organisms [2, 4, 13, 14]. Booster biocides are usually added from 0.1 to 10% in paint formulations and are continuously leached into the surrounding water [11, 15]. They are toxic to target marine organisms, and often to non-target organisms as well [16-22]. Key initiatives in chemically-active paints are mainly focused on the adjustment of the concentration of biocides or booster biocides and the chemistry of the polymer binder used to optimize the released amount of biocides into the marine environment [2, 4, 23].

In this study, two types of acrylic binder, a solvent-based and an aqueous-based polymer solution, were used. The solvent-based acrylic binder is a rosin-containing ablative resin system. Six booster biocides were studied: DCOIT (Sea-Nine™ 211N and Rocima™ 200), dichlofluanid (Preventol® A4-S), zinc pyrithione, copper pyrithione, tralopyril (Econea®) and zineb. All these molecules have been approved by the European BPR with the exception of zinc pyrithione which is still in the approval process. Paint formulations were prepared by mixing 3 wt.% of each biocide and additional fillers or pigments with the polymer binder. Paints with a low amount of Cu₂O or copper thiocyanate (CuSCN) (10 wt.%) with 3 wt.% of booster biocides were prepared. ZnO (10 wt.%) -based paints were also prepared to highlight the effect of ZnO fillers on the AF efficiency of coatings. Field immersion tests were performed at two sites different in terms of biodiversity and water physicochemical conditions (Atlantic

Ocean at Ste Anne du Porzic and Mediterranean Sea at Toulon). Bacterial density was assessed after a short immersion time only at Ste Anne du Portzic using scanning electron microscopy (SEM) and epifluorescence microscopy. Multidimensional analyses were performed after visual analysis to demonstrate any significant variation in the macrofoulers' assemblages which settled on the coating surfaces as a result of the two sites of immersion, the biocide and the binder used.

2. Materials and methods

2.1. Materials

PRIMALTM SF-021 is an ambient curing aqueous-based acrylic polymer solution, formulated without coalescent and without any solvent (ROHM and HAAS, France).

METAMARE B-175 is a biocide-free binder solution in xylene, suitable for the production of antifouling coatings with ablative characteristics (SIRI Spa, Italy).

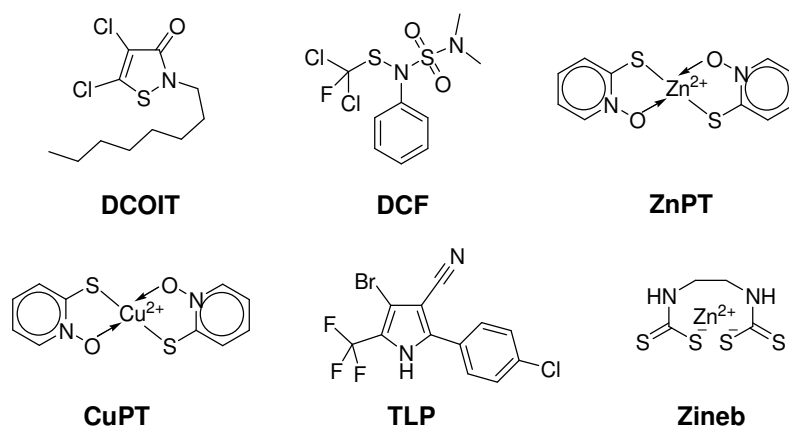
Characteristics of polymer binders are summarized in Table 1. IS360 (Intersmooth® 360 SPC, Akzo Nobel containing 25-50% of Cu₂O and 2.5-10% of zinc pyrrhione as booster biocide was used as an efficient antifouling reference.

Table 1. Characteristics of polymer binders PRIMALTM SF-021 and METAMARE B-175.

Binder	Type	Solid content (%) ^a	Acid number (mgKOH/g) ^b	pH ^a	MFFT ^{a,*} (°C)
METAMARE B-175	Acrylic + 12.5-15wt.% of rosin	60	41	-	-
PRIMAL TM SF-021	Acrylic	45	15	8.0-9.0	0

^aData obtained from the furnishers. ^bAcid number value assessed by titration with KOH 0.1M in ethanol. *MFFT: minimal film formation temperature

4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) (Sea-Nine™ 211N in xylene (30 wt.%) and Rocima™ 200 in water-based solution (20 wt.%) were purchased from ROHM and HAAS and used as received. *N*-(Dichlorofluoromethylthio)-*N,N'*-dimethyl-*N*-phenylsulfamide (dichlofluanid, DCF, or Preventol® A4-S, BAYER), Bis(1-hydroxy-2(1H)-pyridinthionato-O, S) zinc (97%) (zinc pyriothione, ZnPT, JANSSEN PMP), Bis(1-hydroxy-1H-pyridine-2-thionato- O,S)copper (copper pyriothione, CuPT, Arch Chemicals Inc., currently LONZA), 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-trifluoromethyl (tralopyril, TLP, Econea®, JANSSEN PMP) and zinc ethylenebis(dithiocarbamate) (polymeric) (Zineb, AGRIA SA) were used as received. Zinc oxide (ZnO Extra R, SILAR s.a.s), copper (I) oxide or dicopper oxide (Cu₂O) (NORDOX, Aqua grade for aqueous-based paints and Paint grade for the solvent-based paints), and copper thiocyanate (CuSCN) (Aldrich) were used as received. The chemical structures of biocides are summarized in Scheme 1.



Scheme 1. Chemical structures of booster biocides

Coapur™ 830 W (Arkema Group), BYK®-024 and DISPERBYK®-190 (BYK) were used as received for water-based paints. BYK®-024 is a foam destroying

polysiloxanes and hydrophobic solids in polyglycol (BYK, France). DISPERBYK®-190 is a wetting and dispersing additive for aqueous systems and pigment concentrates.

DISPERBYK®-180 (BYK), AEROSIL® 200 (Evonik Industries) were used for solvent-based paints. Rutile titanium dioxide (TiO₂, Kronos), calcium carbonate (CaCO₃, Solvay) and Talc 0 (Mg₃Si₄O₁₀(OH)₂, Luzenac) were used without further purification in aqueous and solvent-based paints.

2.2. Formulation of paints

Solvent (SL)-based and aqueous (PAQ)-based paints were prepared by mixing the polymer binder solution, the biocide and additional fillers and pigments with a Dispermat® AE1-C high-speed laboratory disperser (Brant Industrie). The weight ratio of TiO₂ and Talc 0 was kept constant and was adjusted to achieve a given pigment volume concentration (PVC) of 30% for both types of paints. For example, aqueous- and solvent-based paint recipes containing 3 wt.% of a booster biocide are given in Table 2 and Table 3. The name of each biocide is ZnPT, CuPT, TLP, DCOIT, Zineb and DCF for zinc pyriithione, copper pyriithione, tralopyril, DCOIT, zineb and dichlofluanid, respectively.

Some biocides as CuPT, ZnPT, zineb, tralopyril and Cu₂O do not dissolve or do not be emulsified in water or solvent we use. Then, depending on the type of booster biocides, the composition of the paint is modified to adjust the PVC at 30%.

A solid content (SC) around 50% and 60% was obtained for PAQ- and SL-based paints, respectively. Paints were named as follows: PAQ-biocide or SL-biocide with PAQ for water-based coatings, SL for solvent-based coatings. PAQ-Ref and SL-Ref are designated aqueous- and solvent-based reference coatings without any biocide.

Table 2. Composition in wt.% of the PRIMAL™ SF-021 aqueous-based paints (PAQ) containing 3 wt.% of solid booster biocide.

Component	Type	PAQ-biocide paint composition (%)	PAQ-Ref paint composition (%)
Polymer solution	Binder	45	41
Biocide	Biocide	3	-
TiO ₂	Pigment	7	8
Talc 0	Filler	14	16
DISPERBYK®-190	Dispersant	1	1
BYK®-024	Antifoam	3	3
Coapur™ 830 W	Thickener	1	1
Water	Solvent	26	30
Total		100	100
PVC (%)		30	30
SC (%)		50	49
% of biocide in dry paint		5	0

Aqueous-based paints containing 10 wt.% of Cu₂O or CuSCN as main biocides were prepared using the same procedure (Table S1). Booster biocides were added at 3 wt.% to point out any synergetic effect of the mixture on the activity spectrum of the AF coatings in field.

ZnO (10 wt.%)-based paints were prepared to highlight the effect of ZnO fillers on the AF efficiency of coatings (Table S2). 3 wt.% of booster biocide were added.

Table 3. Composition in wt.% of the METAMARE B-175 solvent-based paints (SL) containing 3 wt.% of solid booster biocide.

Component	Type	SL-biocide paint composition (%)	SL-Ref paint composition (%)
Polymer solution	Binder	40	40
Biocide	Biocide	3	-
TiO ₂	Pigment	10	11
Talc 0	Filler	20	22
DISPERBYK®-180	Dispersant	1	1
AEROSIL® 200	Thickener	0.5	0.5
Methoxypropanol	Solvent	17	17
Butyl acetate	Solvent	8.5	8.5
Total		100	100
PVC (%)		30	30
SC (%)		60	60
% of biocide in dry paint		5	0

2.3. Preparation of coatings

Paints were applied on sandblasted poly(vinyl chloride) (PVC) panels (A6 format) with a 300µm-thick barcoater. The coatings were dried at room temperature for at least 48 h before field immersion. Duplicates were prepared for each test site (Ste Anne du Portzic and Toulon). Then, coatings were named as follows: PAQ-biocide_X or SL- biocide_X with PAQ for water-based coatings, SL for solvent-based coatings and X the immersion site (X = T for Toulon and X = B for Ste Anne du Portzic). PAQ-Ref_X and SL-Ref_X are designated aqueous- and solvent-based reference coatings without any biocide.

2.4. Immersion sites

Two contrasting French sites were chosen for immersion. The first one is in the Rade of Toulon (Mediterranean Sea) in a semi-closed pond of the military harbour (43°06'25''N-5°55'41''E). A static permanent raft allows us to immerse numerous coupons from different size at different depths (from waterline to 2m deep) for long periods if necessary. The second one is in open sea in the Atlantic Ocean (Ste Anne du Portzic, France, 48°21'29''N 4°33'05''W) at the entrance of the Bay, near the Ifremer campus; the tidal currents are very strong (mean of 1.5 to 2 knots, maximum of 4 knots) and instrumented buoys carry out continuous recording of physicochemical parameters in this area. The coated panels were fully immersed in a vertical position at 1 m depth for 4 months at Toulon (T) and from 1 to 5 meters depth depending on the tidal for 5 months at Ste Anne du Portzic (B) (from August and July 2010, respectively). The panels were not caged to eliminate fish from grazing on the fouling. Longer immersion times, up to 18 months, were investigated at Toulon. The antifouling (AF) performances in static conditions were visually inspected every month in accordance with the updated NF T 34-552 standard which describes the practice for evaluating the antifouling efficiency (N) of chemically-active antifouling coatings [24].

Sandblasted and non-coated PVC panels were used as negative controls. The annual variation in seawater temperature and salinity for the two sites during the immersion period was T=10-27 °C and salinity=36-38 for Toulon [25] and T=7-18 °C and salinity=34-35 for Ste Anne du Portzic [26].

Two replicates of each formulation were inspected, and both the percentage of fouling coverage (Intensity factor, IF) and the type of fouling (severity factor, SF) were assessed (Tables 4 and 5). N values were calculated as follows: $N = \sum(IF \times SF)$.

Table 4. Evaluation of the fouling coverage (IF).

% coverage	Intensity factor IF
No fouling	0
$0 \leq \% \leq 10$	1
$10 \leq \% \leq 20$	2
$20 \leq \% \leq 40$	3
$40 \leq \% \leq 60$	4
$60 \leq \% \leq 100$	5

Table 5. Evaluation of the type of fouling (SF).

Fouling type	Severity factor (SF)
Biofilm	1
Algae (brown, red, green)	3
Non-encrusting taxa (hydrozoa, sponges, ascidians ...)	4
Encrusting taxa (barnacles, tubeworms, spirorbid worms, bryozoans, shells...)	6

2.5. Bacterial density determination after short time immersion at Ste Anne du Portzic

Nine replicates per coating were prepared and immersed from April, 27th to May, 4th in two tanks with circulating natural seawater (Ste Anne du Portzic, France, Figure S1).

The seawater temperature at the surface was 12-13°C. After withdrawals at 24h, 72h and 7 days of immersion, samples were rinsed with sterile artificial seawater, fixed by a formol artificial seawater solution (2.5%) and stored at 4°C.

2.5.1. Epifluorescence microscopic investigations

Withdrawn samples were rinsed and desalinated in successive baths containing different proportions of artificial seawater: distilled water (50:50; 75:25; 0:100). Then, samples were stained with DAPI (4',6'-diamidino-2-phenylindole) and placed between glass slides. The number of marine bacteria were counted by microscopy (Continuum,

thermo-nicolet) (magnification x1000). When bound to double-stranded DNA, DAPI has an absorption maximum at a wavelength of 358 nm (ultraviolet) and its emission maximum is at 461 nm (blue).

2.5.2. SEM investigations

Samples were rinsed, desalinated and dried. A gold layer was deposited on the samples to be conductive and samples were analyzed using a Hitachi S-3200N scanning electron microscope at UBO University of Western Brittany Brest. A magnification of 10 000 for the counting of marine bacteria was used.

2.6. Statistical analysis

One way ANOVA was used to show significant differences for the bacterial abundance, ($p < 0.05$, GraphPad Prism[®] 5). Relative abundances of macrofoulers were used to perform multidimensional scale analyses (NMDS) with the dissimilarity index of Bray & Curtis using the XLSTAT Software Package (2007.5 version).

3. Results and discussion

3.1. PAQ- and SL-based coatings containing 3wt.% of booster biocides

First, the antibacterial activity of biocidal PAQ-based coatings was studied in field at Ste Anne du Portzic (Atlantic Ocean). A total of six aqueous (PAQ)-based coatings was investigated. The efficiency of these aqueous (PAQ)-based coatings was also evaluated against macrofouling for a 4/5-month immersion at Ste Anne du Portzic (Atlantic Ocean) and Toulon (Mediterranean Sea). In parallel, six solvent (SL)-based coatings containing the same type and content of biocides were also immersed at Toulon for up to 18 months. . The percentage of macrofoulers and the antifouling efficiency N were assessed on each immersion site. Reference PAQ- and SL-based

coatings without any biocides were immersed in field to better show the AF activity of each biocide embedded in the coating. PVC panels were used as blank panels.

3.1.1. Bacterial density determination after short-term immersion at Ste Anne du Portzic

SEM and epifluorescence microscopy investigations were performed at early stages of immersion to assess the of marine bacteria density on coatings. The study was performed only on coatings immersed at Ste Anne du Portzic. Nine replicates were immersed in tanks filled with circulating natural seawater system. At different immersion time (24h, 72h and 7 days), samples were withdrawn, and bacterial densities were assessed. Longer periods of immersion were not investigated taking into consideration that biofilm formation and especially exopolymeric substances development prevent to count individual bacteria with time [27]. Overall, densities obtained using SEM and epifluorescence were correlated ($R^2=0.640$, $p<0.0001$, $n=21$). However, taking time per time, only data at 24h were not significantly correlated ($p>0.05$). Whatever the microscopic approach, high densities around 10^{-6} bacteria/cm² were found after 24h on all the substrates including AF coatings (Figure 1), as also observed at Toulon on PVC using a flow cytometry approach [28]. A continuous increase in densities with time was then noticed on most of the substrates, but with significant differences between them. A similar range of densities was already reported on AF coatings in the Oman sea after 10 days of immersion and using fluorescence microscopy [29].

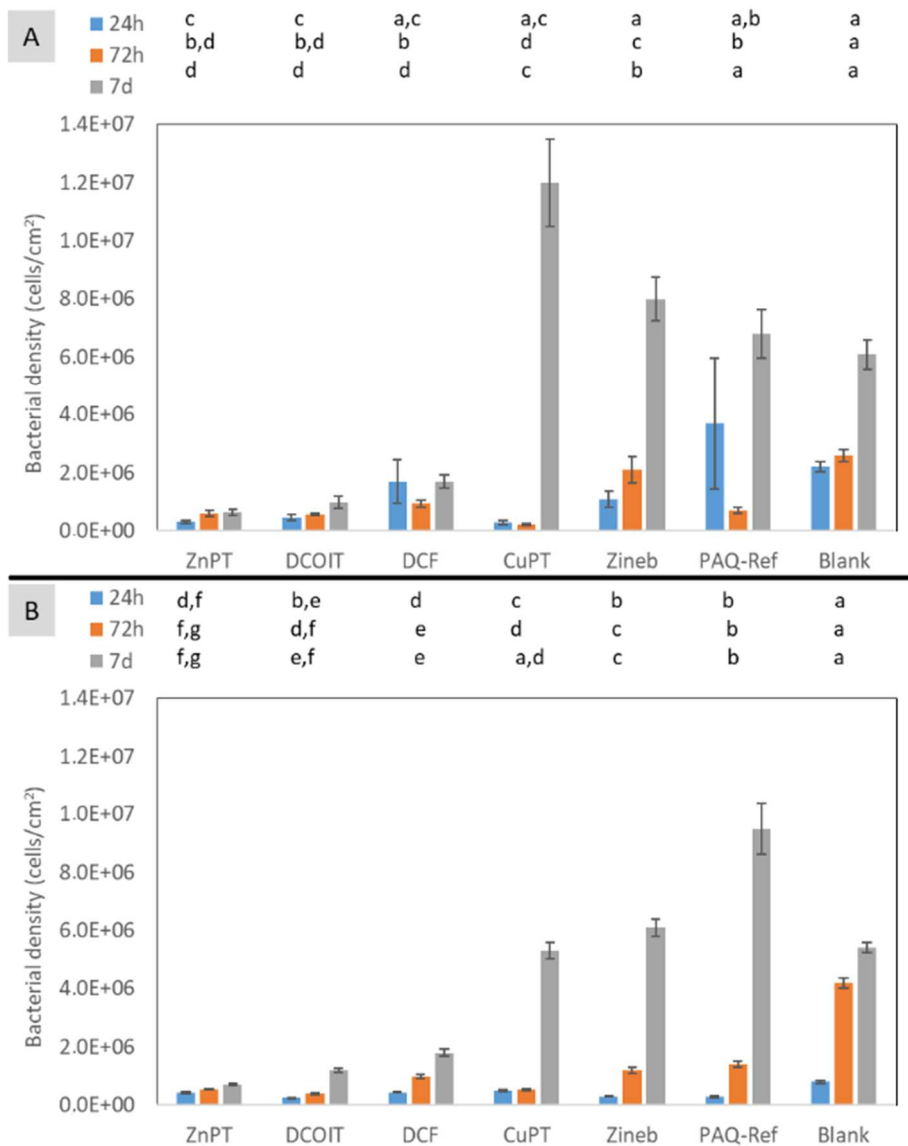


Figure 1. Bacterial density determined using SEM (A) or epifluorescence microscopy (B) at Ste Anne du Portzic on PAQ coatings and a PVC blank panel, after three times of immersion (24h, 72h and 7d). Letters correspond to significant differences between substrates after ANOVA and post-hoc Tukey tests ($p > 0.05$).

The most efficient coatings against marine bacteria after 7 days were those containing ZnPT, DCOIT and DCF as booster biocides ($p < 0.05$). Unfortunately, the efficiency of TLP on marine bacteria was not assessed. DCOIT and ZnPT are known to prevent the growth and the settlement of marine bacteria both using *in vitro* bioassays [30-32] and field immersion [24]. DCF seems to have a broad spectrum of activity too on marine

bacteria [33, 34]. Surprisingly, CuPT at 3 wt.% in a PAQ-based coating did not provide any efficiency against marine bacteria after 7 days of immersion. Indeed, CuPT was found to be effective against marine bacteria after a one-month immersion in the Mediterranean Sea and the Atlantic Ocean when incorporated at 8.3 wt.% in a solvent-based acrylic binder. In this latter work, the amount of CuPT was higher than that studied here, and it should be noted that its efficiency was also reported as seasonal dependent [25]. Zineb at 3 wt.% also did not provide efficiency against marine bacteria after 7 days of immersion. The poor antibacterial efficiency of Zineb, however, has already been reported in a previous work done by our research group on solvent-based paints containing 8.3 wt. % (in dry paint) of biocide and immersed in Mediterranean Sea and Atlantic Ocean for one month [25].

3.1.2 Beta-diversity of the macrofoulers' assemblage and antifouling efficiency of coatings after medium-term immersion at two test sites

Figure 2 was plotted with all groups of macrofoulers found on coatings. The macrofoulers' groups are as listed in Table 5. The size of the symbols represents the AF efficiency factor N. Figure 2 shows that there is one macrofoulers' assemblage clustering for PAQ-based coatings that depends only on the immersion site whatever biocide is used. This observation has already been pointed out in a previous work [35]. At Toulon, there was no cluster based on the polymer binder itself, but the biocide/binder interaction led to significant differences in AF efficiency. From the size of the symbols representing the AF efficiency factor N in Figure 2, we note that both at Toulon and St Anne de Portzic, all coatings containing 3 wt.% of biocides are more efficient against macrofoulers than the reference one (PAQ-REF_T and SL-REF_T, except PAQ-TLP_T).

From the smallest size of the symbol representing N in Figure 2, DCOIT and DCF were the most promising biocides in PAQ-based coatings at Toulon, while DCOIT and TLP were the most efficient biocides in PAQ-based coatings at Ste Anne du Portzic. Thus, DCOIT-based PAQ coatings were the most effective coatings at all sites.

The PAQ-TLP coating was more efficient at St Anne du Portzic than at Toulon. On the contrary, Zineb and CuPT-based coatings were more effective at Toulon than at St Anne du Portzic when incorporated in PAQ-based coatings. Nevertheless, TLP-, CuPT- and Zineb-based coatings showed an intermediate AF efficiency in terms of macrofouling intensity at Ste Anne du Portzic and Toulon, regardless of the polymer binder. DCF-based coatings were more efficient at Toulon than at Ste Anne du Portzic, whatever the polymer binder.

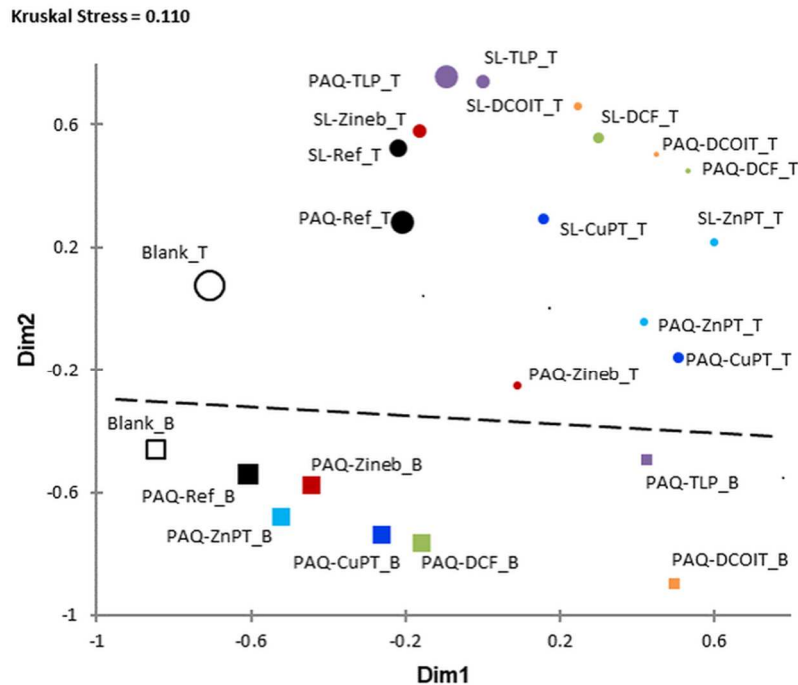


Figure 2. Non-Metric Multidimensional Scaling (NMDS) of macrofouling assemblages using the dissimilarity index of Bray & Curtis calculated with the percentage of

macrofoulers' coverage on coatings, references, and PVC blank panels at both immersion sites (T = Toulon (●) for 4 months and B= Ste Anne du Portzic (□) for 5 months of immersion). Each color stands for a biocide and the size of symbols depends on the efficiency value (N), the higher size corresponds to a higher value of N and then a lower AF efficiency. The two arbitrary dimensions (Dim1 and 2) that best represented the dissimilarity between assemblages are used.

The higher AF efficiency (the lower N value) was then globally found on coatings immersed at Toulon probably due to a shorter immersion time, a lower fouling pressure, a distinct biodiversity or a difference in hydrodynamics. In solvent-based paints, the best ingredients to guarantee AF performances of coatings at Toulon were DCOIT and ZnPT. At Toulon, ZnPT-based coatings with only 3 wt.% of ZnPT were efficient whatever the polymer binder. This biocide has already shown good AF performances at Toulon [35].

All results demonstrate that both the immersion site and the paint composition could affect the coating activity in field. These latter parameters are well-known to affect the release of biocides from chemically-active coatings, as they play a role in the exchange of soluble species between the surrounding liquid water and the coating [36].

3.1.3 Specific effect of the coatings on the macrofoulers' assemblages

Figure 3 shows the difference in macrofouler taxa from one immersion site to the other. The uncoated PVC blank panels (Blank_T and Blank_B) were fully covered by macrofoulers. At Toulon, encrusting bryozoans, spirorbid worms and tubeworms were the main hard macrofoulers on Blank_T panels. At Ste Anne du Portzic, hydroids and tufted bryozoans were relevant to the immersion site on Blank_B panels. Overall, the richness of macrofouler groups, i.e., their number observed without any percent consideration, was significantly correlated with the AF efficiency N ($R^2=0.684$,

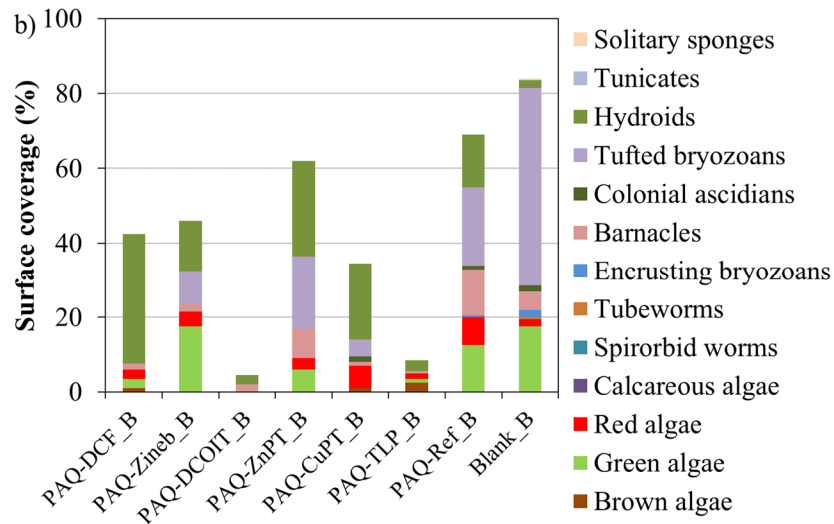


Figure 3. Variation in the macrofouler groups depending on the site of immersion a) Toulon (4 months of immersion), b) Ste Anne du Portzic (5 months of immersion), and their relative abundance depending on the biocide and the polymer binder.

Dichlofluanid (DCF) has a broad spectrum of activity which includes algae, diatoms, and other fouling organisms [34]. However, its toxicity might be caused by its degradation products, since dichlofluanid rapidly undergoes hydrolysis in water [39], even when incorporated in coatings [14]. At Toulon, DCF disrupted the settlement of spirorbid worms and encrusting bryozoans and significantly decreased the percentage of algae on both aqueous- and solvent-based coatings (Figure 3a). At Ste Anne du Portzic, no tufted bryozoans were found on DCF-based coatings (Figure 3b).

Zineb is currently used at 4.5 wt.% for professional and amateur applications and at 10 wt.% for professional application only. It is proposed for use against a range of fouling organisms including red and green algae (e.g. *Chorda filum*; *Fucus vesiculosus*; *Furcellaria lumbricalis*; *Polysiphonia*; *Enteromorpha intestinalis*; *Cladophora rupestris*; *Hildenbrandia rubra*; *Monostroma grevillei*), diatoms (slimes, e.g. *Acanthes* and *Amphora species*) and invertebrate fouling organisms (e.g. *Mytilus edulis*; *Crustacea*; *Balanus improvisus*; *Eliminus modestus* and *Semibalanus balanoides*) [40].

Zineb was reported to have a very limited and qualitative impact on the marine biofilm communities in the Mediterranean Sea and Atlantic Ocean when mixed in a methacrylic solvent-based paint at 8.3 wt.% (in dry paint) [35]. As reported in the EU report, zineb acts as a general inhibitor of metabolic pathways through interactions with thiol groups (-SH) within metabolically active proteins [40]. This effect is evidenced by a reduction in the growth rate of young individuals (pre-settlement stage, e.g. cyprid larvae of barnacles) of common fouling species.

Less abundance of barnacles was found on PAQ-Zineb_B and less abundance of algae was found on PAQ-Zineb_T. No spirorbid worms and encrusting bryozoans were settled on this latter coating (Figure 3a). On the contrary, no effect of Zineb on algae was pointed out on the solvent-based coating SL-Zineb_T suggesting that 3 wt.% or its concentration release was not sufficient to maintain AF performances.

DCOIT is a broad-spectrum antifouling agent that prevents the growth and settlement of soft fouling such as marine bacteria [31], fungi and algae [41]. DCOIT undergoes rapid degradation in natural seawater and binds strongly to sediments, reducing its bioavailability and hence its potential to bioaccumulate [15]. It also prevents hard fouling organisms (e.g. barnacles) from settling on submerged surfaces. It is efficient at low concentrations in paints (1-3%) [41] and mainly used as a control to further develop alternative polymer binders for marine AF paints [42]. Its broad spectrum was confirmed by the lowest percentage of surface coverage found at Ste Anne du Portzic among other coatings with no barnacles on it. At Toulon, PAQ-DCOIT_T and SL-DCOIT_T coatings were mainly covered with brown algae (Figure 3a). Although not measured, the released DCOIT seemed to be enough to inhibit the fouling in all types of paints.

The pyrithione salts, such as zinc pyrithione (ZnPT) and copper pyrithione (CuPT), are

known to inhibit several cellular processes, such as ATP levels, membrane transport and protein synthesis [13]. In addition, CuPT has been proposed for use as co-biocide against algae [43]. ZnPT is generally toxic to a wide range of marine organisms, including algae, bivalves, sea urchins, polychaetes, crustaceans, and fish, typically at $\mu\text{g/L}$ levels [44]. These pyridithione salts exhibited a low water solubility and a high degradability making them suitable for formulation in marine antifouling paints [45]. ZnPT and CuPT were reported to be efficient alone against macrofoulers, as opposed to Zineb, when incorporated in a solvent-based paints at 8.3 wt. % (in dry paint) and immersed in the Mediterranean Sea and Atlantic Ocean [35].

In this study, there is no significant effect on the richness of macrofoulers for pyridithione-based coatings compared to the reference coating at Ste Anne du Portzic (Figure 3b). On the contrary, there was an effect on the abundance of tubeworms, encrusting bryozoans and spirorbid worms which decreased at Toulon (Figure 3a).

CuPT-based coatings only showed a higher efficiency against barnacles at Toulon and Ste Anne du Portzic when compared to ZnPT-based coatings.

Tralopyril (TLP) is an arylpyrrole compound with a broad-spectrum control of hard fouling organisms [46, 47]. It has a low water solubility, making it resistant to leaching, but it can be rapidly hydrolyzed in seawater (half-life of 3 hours at 25°C) [47]. Its efficiency against tufted bryozoans and barnacles is clearly demonstrated at Ste Anne du Portzic, where PAQ-TLP_B was among the most efficient coatings. At Toulon, a lower diversity was observed on TLP-based coatings with most algae on them (percentage of coverage surface above 60%, Figure 3a) mainly driven by the chemistry of the respective reference coatings.

3.1.4. Antifouling efficiency of coatings after 18-month immersion at Toulon

At Toulon, PAQ- and SL-based coatings were immersed for up to 18 months and

compared to a commercially available biocidal coating IS360 (Intersmooth® 360 SPC, Akzo Nobel containing 25-50% of Cu₂O and 2.5-10% of zinc pyrithione as booster biocide) (Figures S2 and S3). IS360 is classified as a self-polishing coating (SPC) with a controlled release rate of biocides with time [2, 3]. Its efficiency against microfoulers such as marine diatoms in tropical and temperate Australia [27] and marine bacteria in the Mediterranean Sea [48, 49] has been already reported. The settlement of macrofoulers such as encrusting bryozoans, spirorbis and tube worms, hydroids, colonial ascidians, and algae is affected by IS360 in Mediterranean Sea as none of them settled on coated panels over 16 months of immersion (Figure S2). Figure 4a shows that the AF efficiency of PAQ-CuPT_T and PAQ-Zineb_T declined after 4 months of immersion. PAQ-ZnPT_T loses its AF efficiency after 7 months of immersion. PAQ-DCF_T and PAQ-DCOIT_T coatings exhibited a decrease in their AF performances from 6 and 7 months of immersion, respectively. As soon as encrusting biofoulers start to settle on, the release rate of biocides seems to be affected by a physical blocking mechanism [50] and the AF performances then collapse. Therefore, these aqueous-based paints might be more suitable for small sized boats operating during the summer season in Mediterranean Sea.

Figure 4b shows that N values for all SL-based coatings are lower than the PAQ-based coatings after 18 months of immersion (Figure S3). Among them, the best performing booster biocides were ZnPT and DCOIT. SL-ZnPT_T declined after 8 months of immersion. SL-DCOIT_T exhibited similar behavior to the commercial IS360 paint after 16 months of immersion.

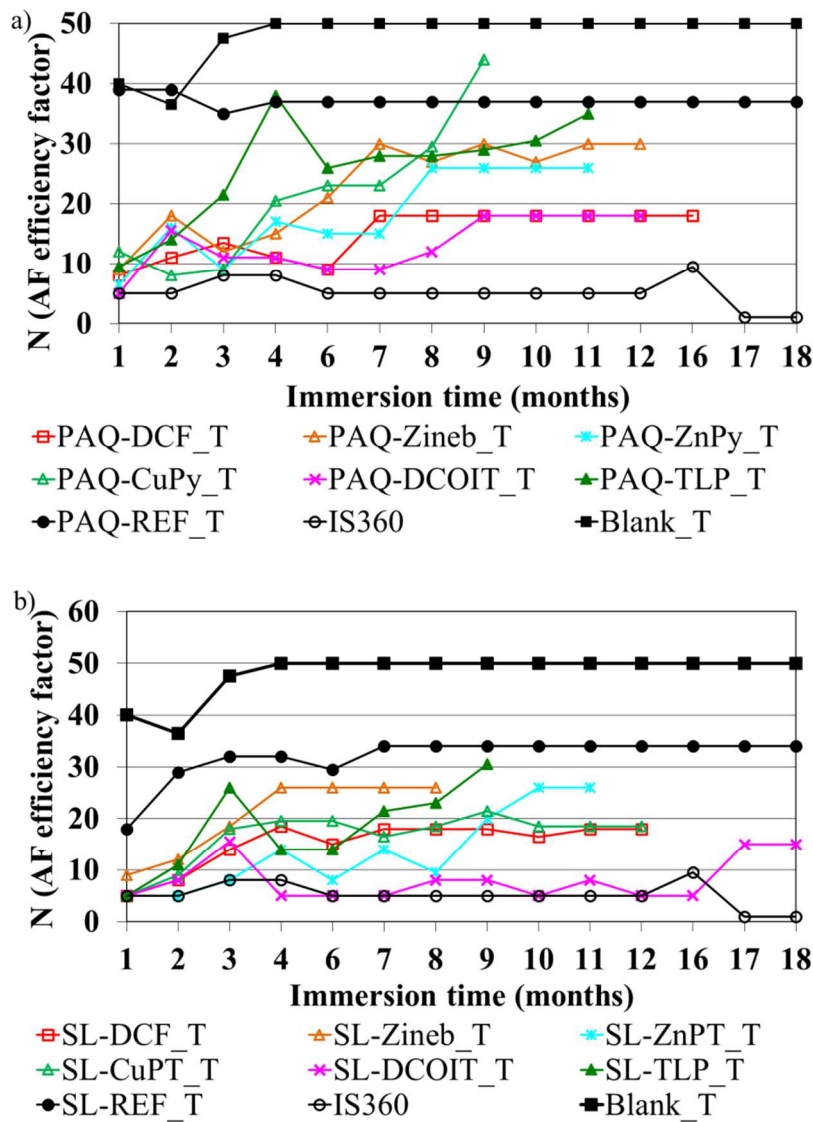


Figure 4. Evolution of the AF efficiency (N) with immersion time for (a) PAQ-based coatings and (b) SL-based coatings at Toulon. IS360 was used as a positive SPC coating control.

The METAMARE B-175 binder used for SL-based paints seems to be suitable to release the minimum concentration of DCOIT required to maintain AF performances with time. As mentioned by the supplier, METAMARE B-175 contains rosin which is used to increase the water absorption of the coating and then the release of the biocides [1,7]. In addition, METAMARE B-175 is reported to have a modest ablation or erosion. Therefore, the good AF performances of SL-DCOIT_T in static conditions make it a suitable paint for medium-sized boats or yachts with intermediate time efficiency.

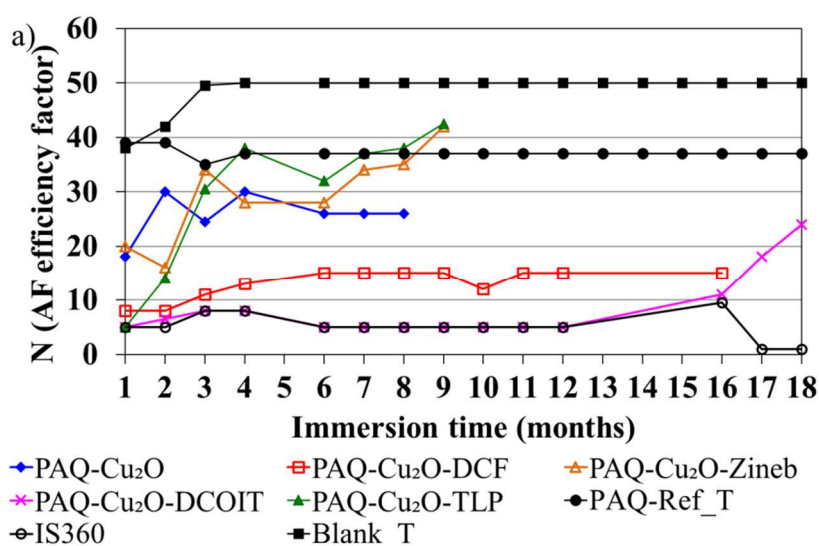
3.2. PAQ- and SL-based coatings containing 10 wt.% of copper-based biocides and 3 wt.% of booster biocides

Most of chemically-active paints on the market contain Cu_2O or CuSCN as main biocide [1, 51]. The amount of these metallic-based compounds could vary from 7 to 75 wt.% in wet paints [23]. When copper from metallic copper, copper thiocyanate or cuprous oxide is leached into seawater in presence of oxygen, the predominant form of the copper is the active substance, the cupric ion, Cu^{2+} . The cupric ion retards the settlement of microscopic larvae of fouling organisms via two mechanisms [52]:

- (1) the ion retards organism's vital processes by inactivating enzymes;
- (2) the ion acts more directly by precipitating cytoplasmic proteins in the form of metallic proteinates.

Bloecher and Floerl [4] reported that the commercial copper coating prevented the settlement of most organisms, with the exception of hydroids and some algal species in Norway sea, which are known to have a high tolerance to copper-based AF coatings. Lindgren et al. reported that low concentrations of copper are needed in various metabolic processes [23]. However, high rates of copper release from AF paints lead to adverse effects on the marine environment. Cyanobacteria, embryos/larvae of mussels, oysters and sea urchins are sensitive to high concentrations [53]. However, chemically-active paints are still the most widely used and most effective. To limit their impact on the marine environment, coatings with lower contents of Cu_2O or CuSCN than IS360 were prepared. Booster biocides were added at 3 wt.%. To go even further in the search for more environmentally-friendly paints, only water-based paints were investigated. Figure 5 shows that the PAQ- Cu_2O and PAQ- CuSCN were not efficient over longer times, indicating that the amount of copper-based biocide and the copper release from these coatings were not sufficient to have an AF effect in the Mediterranean Sea. 10 wt.% of CuSCN had an AF effect of 1-2 months and 10 wt.% of Cu_2O lost its AF

efficiency within the first month of immersion, compared to the copper-free PAQ-Ref. Therefore, copper content of 10 wt.% was not sufficient. The images in Figures S4 and S5 highlight this rapid loss of performances of copper-based coatings compared to coatings containing only 3 wt.% of booster biocides. The addition of a booster biocide to copper-based biocide such as Zineb, TLP, ZnPT and CuPT did not improve the overall AF performances (N values) whatever the copper-based biocide used. The combination of DCF and copper-based biocides showed a significant increase in AF performances compared to the combination with other booster biocides. The best performing coatings were PAQ-Cu₂O-DCOIT and PAQ-CuSCN-DCOIT with good AF properties over 16 and 10 months of immersion, respectively. These AF performances seemed to be mainly brought by DCOIT. However, the addition of Cu₂O to 3 wt.% of DCOIT was a good alternative to extend the AF service life of these aqueous-based coatings from 7 to 16 months.



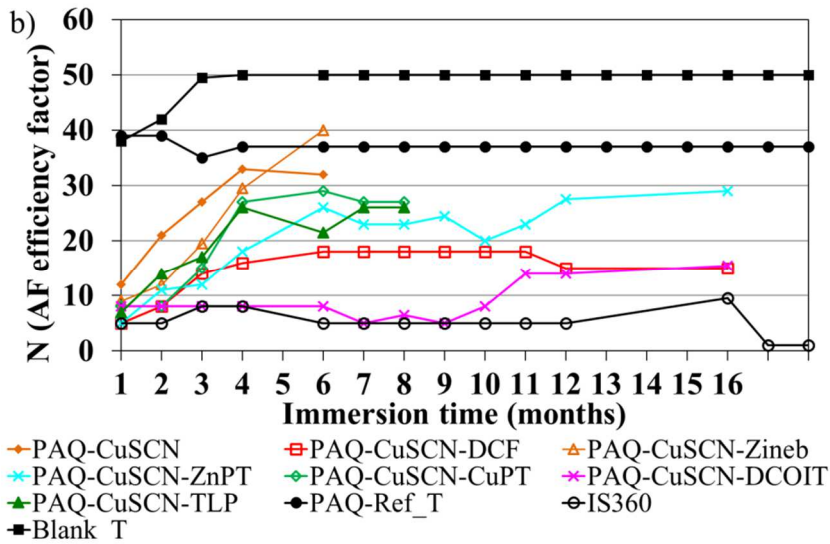


Figure 5. Evolution of the AF efficiency (N) with immersion time for PAQ-based coatings containing (a) Cu_2O and (b) CuSCN as main copper-based biocides. IS360 was used as a positive SPC coating.

Besides these results, the diversity of macrofoulers was affected by the copper-based coatings in comparison with the copper-free coatings for 4 months of immersion at Toulon. Figure 6 clearly shows that DCF, DCOIT and ZnPT broadened the activity spectrum of copper-based coatings, as these coatings were clustered far away from CuSCN- and Cu_2O -based coatings. The comparison of macrofouler groups depicted in Figure S6 and Figure 4a shows that the addition of Cu_2O and CuSCN mainly affects the surface coverage by algae except for the CuSCN/CuPT, CuSCN/Zineb and CuSCN/TLP combinations. Thus, DCF, DCOIT and ZnPT seemed to drive the overall spectrum activity, in contrast with TLP and Zineb.

Kruskal stress = 0.129

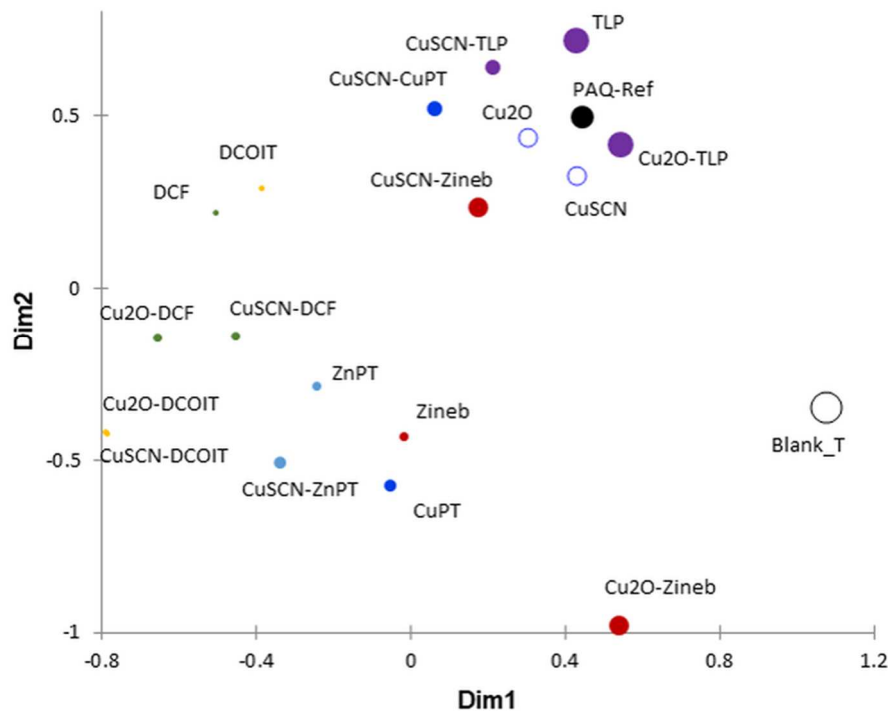


Figure 6. Non-metric multidimensional scaling (NMDS) of macrofoulers' assemblages using the dissimilarity index of Bray & Curtis calculated with the percentage of macrofoulers' coverage on PAQ coatings, including a reference and PVC blank panels at Toulon for 4 months of immersion. Each color stands for a biocide and the size of symbols depends on the efficiency value (N). The two arbitrary dimensions (Dim1 and 2) that best represented the dissimilarity between assemblages are used.

3.3. PAQ- and SL-based coatings containing 10wt.% of ZnO and 3wt.% of booster biocides

Zinc oxide (ZnO) is used as a pigment in AF coatings and is not classified as a biocide according to the European regulation although some recent papers tend to show a toxicity effect of Zn^{2+} on several marine species such as microalga and marine bacteria which are more sensitive than brine shrimps [54, 55]. The biofoulers' community was similar on PAQ-ZnO and PAQ-Ref at 4 months of immersion (Figure S7) except for the percentage of coverage of encrusting bryozoans which was

significantly lower on PAQ-ZnO than PAQ-Ref after 4 months of immersion. Lindgren et al. also reported that zinc oxide alone in the paint matrix had an AF effect against barnacles and bryozoans for approximately a month in the Swedish west coast [23]. Contrary to the booster biocide alone (Figure 4a), the combination of ZnO with ZnPT, DCF, Zineb and CuPT in PAQ-based coatings led to their lowest surface coverage by macrofoulers during 4 months of immersion (Figure S7).

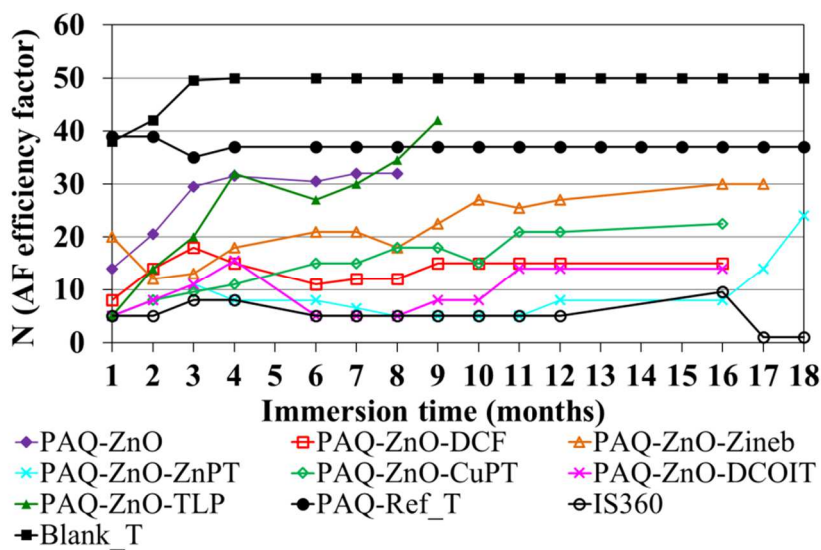


Figure 7. Evolution of the AF efficiency (N) with immersion time for PAQ-based coatings containing ZnO as fillers. IS360 was used as a positive SPC coating.

Figure 7 and Figure S8 highlight the evolution of AF performances over longer periods of immersion. Figure 7 shows that PAQ-ZnO deterred the settlement of macrofoulers compared to the reference PAQ-Ref coating without ZnO during the first 2 months of immersion. After 2 months of immersion, N values increased drastically with time, showing that ZnO, by itself, did not have an effective level of biocidal activity against macrofoulers. N values below 20 were obtained over 10 months of immersion for most of the booster biocide-based ZnO coatings, with the exception of PAQ-ZnO-TLP and PAQ-ZnO-Zineb. PAQ-ZnO-DCF exhibits an intermediate AF

efficiency as reported for PAQ-DCF. It is worth noting that the AF efficiency time of CuPT- and ZnPT- containing coatings was extended from 3 and 7 months to 10 and 16 months, respectively, in the absence and presence of ZnO. The DCOIT-containing PAQ coatings were still efficient with an extended efficiency time from 8 to 16 months when ZnO was added to DCOIT.

PAQ-ZnO-ZnPT was the best performing ZnO-based PAQ coating with a 16-months efficiency in static immersion by maintaining a N value less than 10 (see images in Figure S8). From these results, it can be stated that ZnO is not an inert filler and improves the overall AF efficiency and the duration of efficiency of the biocidal PAQ-based coatings. Lindgren et al. demonstrated that the release rate of copper of copper-based coatings increases by 20% when at least 10 wt.% of ZnO particles are added, between 14 days and 56 days of immersion on the Swedish coast [23]. Measurements of the release rate of booster biocide from coatings in the presence and absence of ZnO were outside the scope of this study. Therefore, it would be interesting to investigate the interactions between the polymer binder and biocides and the biocide release rates.

4. Conclusion

More environmentally friendly AF paints were developed by formulating aqueous-based and solvent-based polymer binder with a reduced amount of copper-based biocides and booster biocides. In field, macrofoulers' assemblages were mainly clustered by immersion site whatever the biocide used. At 4 months of immersion, the AF efficiency was independent of the polymer binder in Toulon (Mediterranean Sea). When efficient, biocidal AF coatings seem to decrease the richness of macrofoulers with a stronger effect in the Mediterranean Sea. In the Atlantic Ocean, the most efficient coatings against marine bacteria after 7 days of immersion were those containing ZnPT, DCOIT and DCF as booster biocides. Only DCOIT was efficient against both marine

bacteria and macrofoulers in the Atlantic Ocean. DCOIT also had the highest activity in the Mediterranean Sea whatever the polymer binder. AF performances of 16 months were then obtained for solvent-based coatings containing 3 wt.% of DCOIT. The AF performances of aqueous-based paints were improved by adding 10 wt.% of copper-based biocides to DCOIT, ZnPT or DCF-based coatings. The aqueous-based antifouling coating containing DCOIT (3 wt.%)-Cu₂O (10 wt.%) was able to prevent marine biofouling for 16 months with similar performances to an established commercial copper-based SPC coating. Replacing copper-based biocides by ZnO fillers led to higher AF efficiencies for most aqueous-based coatings. This study highlighted one new aqueous-based antifouling coating containing ZnPT (3 wt.%)-ZnO (10 wt.%) as a promising copper-free coating for pleasure boats.

Sample CRediT author statement

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Declaration of competing interest

The authors declare no conflict of interest.

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