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Assessing the Antifouling Effectiveness of the Novel Organic Coating for Adherent Species in the Seawater of the coastal area of Ha Long City, Quang Ninh Province (Vietnam)

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ABSTRACT

This paper presents the results of a 12-month testing period in seawater at Vung Oan, Ha Long City marine area, Quang Ninh Province (Vietnam), assessing the fouling organism's removal effectiveness of polysiloxane (PS)/Ag-Zn zeolite/Cu_2O nanocomposite coatings. This experiment is a substantial base to evaluate impact of Ag-Zn/zeolite and Cu_2O nanoparticles (NPs) on growth of bacteria and macro-fouling organisms compared to a control coating without the above additives. The results indicated that the coating loaded with Ag-Zn/zeolite and Cu_2O NPs exhibited a lower bacterial count than the coating without biocide additives. Specifically, the bacterial count was 9.6×10^5 for the coating with biocide and 2×10^7 for the coating without biocide. Regarding macro-fouling species, the analysis and identification of organisms attached to the coating samples revealed the presence of three fouling species: *Perna viridis*, *Balanus amphitrite*, and *Haliclona cinerea*. *Modiolus barbatus*, *Nereis* sp., and *Xanthidae* were only observed on surface of the control samples, suggesting that Ag-Zn/zeolite and Cu_2O NPs can impede the development of *Modiolus barbatus*, *Nereis* sp., and *Xanthidae* on the coating surface. Furthermore, the average weight of macro-fouling organisms on the coating containing Ag-Zn/zeolite and Cu_2O NPs was significantly lower (90 g/sample) than that of the macro-fouling organisms on the control coating (333 g/sample). Notably, one of the three samples with the Ag-Zn/zeolite and Cu_2O NPs had the lowest weight of macro-fouling organisms, measuring only 35 g. Based on these findings, it can be concluded that polysiloxane/Ag-Zn/zeolite/Cu_2O nanocomposite coatings show promise as antifouling paints for marine work applications.

Keywords: Antifoulants, organic coating, macro-fouling organism, test sample, control sample.

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INTRODUCTION

Marine biofouling poses significant economic challenges and severe problems for maritime industries, underscoring the urgency and importance of our research. For instance, the aquaculture industry incurs direct economic costs of biofouling control, estimated at 5–10% of production costs (equivalent to US $1.5 to 3 billion/year) [1]. Additionally, biofouling has a substantial impact on naval forces. A study analyzing the economic impacts of hull fouling on a mid-sized naval surface ship of the US Navy estimated the associated costs, including hull coating application and removal, hull cleaning, and fuel, to be approximately US $56 million/year [2]. Overall, biofouling losses in the US are predicted to amount to around US $120 billion/year [3]. A biofouling film typically forms in four stages [4]. Initially, bacteria adhere to the material surface, forming extracellular polymeric substances, including proteins, glycoproteins, glycolipids, extracellular DNA, polysaccharides, and other compounds. These substances are crucial in linking different bacterial strains to establish the bacterial membrane. Subsequently, diatoms and algae spores accumulate on the bacterial membrane's surface, utilizing nutrients from the biofilm. Following this, larvae of macro-organisms and invertebrates attach to the algae, contributing to developing a biofouling film [4]. The macro-fouling organisms commonly encountered in biofouling include algae fouling (such as green algae, brown algae, and red algae), hard shell organisms (including barnacles, tubeworms, and spirorbis), grass and bush-type organisms (such as encrusting bryozoans, bryozoans, and hydroids), and spineless organisms (including sponges, ascidians, and tunicates) [5].

Antifouling coatings have garnered significant interest from scientists and manufacturers due to their ability to mitigate marine fouling organisms' impacts and prevent invasive species introduction. Numerous efforts have been made to develop effective antifouling coatings using various polymer matrices, such as polyurethane [6, 7], acrylic copolymer [8, 9], silicone [10], polyvinylpyrrolidone [11], zinc-based acrylate resin [11], and zinc-based polyurethane copolymer [12]. These coatings could act through two main mechanisms: biocide release and self-polishing [4]. However, this field's current research and development focuses on creating environmentally friendly and long-lasting antifouling coatings. In pursuit of these objectives, studies have explored green biocides derived from sources such as butenolide (obtained from marine bacteria) [8, 9], capsaicin [13], as well as biodegradable polymers like poly(lactic acid) [7], and poly(ε-caprolactone) [8]. Chen et al., investigated the effect of butenolide concentration on the antifouling activities of an acrylic copolymer resin-based coating incorporating various pigments like TiO_2, Fe_2O_3, Cu_2O, and ZnO [9]. Their marine field test results indicated that all coating formulations began to lose antifouling performance after three months. In another study, Duan et al., developed a superhydrophobic coating based on polysiloxane and cellulose nanocrystals [10]. The results from a 90-day marine field test showed an 82% reduction in fouling compared to the control sample. However, a biofouling film still formed on the coating's surface after 45 days of immersion, suggesting the need for further improvement in long-term performance. Nguyen Van Chi et al., conducted a study to evaluate the antifouling performance of various commercial antifouling paints on rubber surfaces [14]. The research findings revealed that two antifouling paints, namely Seaquantum Classic by Jotun Group and AF-450 by Hai Au Company, exhibited effective antifouling properties. The investigation involved a 6-month testing period at the Dam Bay sea-testing station in Nha Trang Bay, Vietnam. In another study, researchers examined the antifouling capabilities of six commercial antifouling paints available in the Vietnamese market [15]. The results demonstrated that the adhesion of all tested paints significantly decreased within 35 days of immersion. However, the antifouling paints by Jotun and Hai Au demonstrated the ability to protect the sample surfaces from biofouling organisms after 21 months of immersion.

Research should prioritize understanding the interactions between fouling organisms and different material surfaces. Acquiring comprehensive knowledge in this area is crucial for developing effective antifouling coatings capable of inhibiting the growth of biofouling films. To achieve this, it is essential to classify the bacteria and macro-fouling organisms appeared on the surface of antifouling coatings during marine testing. This classification provides a foundation for selecting suitable antifoulants. The objective of this work is to evaluate the species of bacteria and macro-fouling organisms found on the surface of polysiloxane/Ag-Zn zeolite/Cu_2O nanocomposite coatings over 12 months during a marine field test conducted in coastal area at Vung Oan, Ha Long City, Quang Ninh Province (Vietnam). This research underscores the need for further exploration and development in antifouling coatings, offering a promising avenue for future discoveries and advancements.

EXPERIMENTAL DESIGN

Sample preparation

The coating system consists of three main components: primer coating, intermediate coat, and topcoat, as ref. [16]. The primer coating is prepared using epoxy resin (Epikote™ Resin 1001-X-75, 75% from China), organically modified ZnO nanoparticles (NPs) (99% purity, diameter < 100 nm from Sigma-Aldrich), and ZnO micro-particles from China. The intermediate coat is composed of epoxy resin (Epikote™ Resin 1001-X-75, 75 % from China), rutile TiO_2 nanoparticles (NPs) (99.5% purity, diameter < 100 nm from Sigma Aldrich), and ZrO_2 nanoparticles (NPs) (99% purity, diameter < 50 nm from Sigma Aldrich) modified with a silane coupling agent. The topcoat consists of polysiloxane (SILRES® REN 50, a methyl phenyl silicone with a solid content of 50 wt.% from Wacker Chemical Corporation, USA), TiO_2 nanoparticles (NPs) and ZrO_2 nanoparticles (NPs) modified with a silane coupling agent, a biocide agent as Ag-Zn zeolite (commercial name of Irgaguard B5000 from Basf), and Cu_2O particles (NPs) (99% purity, diameter < 7 µm from Sigma Aldrich).

The samples under investigation were prepared as follows. C45 steel sheet with a size of 100 × 150 × 1 mm was subjected to chemical and physical treatments to remove oil and rust, achieving a cleanliness level of SA 2.0. The coating system was then applied to the steel surface using the following procedure: First, the primer coating formula was mixed with a hardener (Jointmine 9024 from Epochem, China), and the mixture was spray-applied onto the steel surface using an airless spraying method. The application was carried out at an air pressure of 8 atm with a paint consumption of 120 g/m^2, resulting in a dried thickness of approximately 50 µm. Two layers of primer coating were applied. The intermediate coating was applied for after allowing the primer coating surface to dry for about 4 hours. The edges of the samples were covered with primer coating. Lastly, the topcoat was applied over the intermediate coating. The entire coating system had a dried thickness ranging from 200 µm to 250 µm. The same procedure was followed for the control samples, except that the topcoat consisted of polysiloxane without the addition of biocide additives (Irgaguard B5000 and Cu_2O nanoparticles).

Testing of samples under marine conditions

Testing of experiment samples in seawater was conducted at Vung Oan, Ha Long City marine area, province (Vietnam) (107°04′E, 20°56′N) from October 2021 to October 2022. This area hosts numerous clinging organisms throughout the year. During the experiment, the samples were suspended on an inox 304 shelf at a depth of approximately 1 m in seawater. Every 90 days, the samples were carefully removed from the water and gently washed with seawater to remove any accumulated silt. They were inspected and immediately returned to the testing site to continue the evaluation. Table 1 presents the seawater's quality, properties, and characteristics of the seawater at the specific testing location.

Table 1. Quality, properites and characteristics of seawater at testing site

No.	Indexes	Units	Value
1.	pH	-	7.85
2.	Salinity	‰	20.8 ± 0.12
3.	Chemical oxygen demand (COD)	mg O_2/L	106 ± 1.2
4.	Biochemical oxygen demand (BOD)	mg O_2/L	10 ± 0.2
5.	Turbidity	NTU	4 ± 0.01
6.	Suspended solids (SS)	mg/L	7 ± 0.3
7.	Bacteria	CFU/mL	$2.2 \times 10^5 \pm 355$
8.	Number of bacteria strains*	-	3
9.	Actinomycetes	CFU/mL	n.d.
10.	Number of mold strains		n.d.
11.	Yeast	CFU/mL	20 ± 1
12.	Number of yeast strains	-	1

Notes: * Total number of colony types identified in different media; n.d.: not determined.

Determination of microorganisms on coatings surface

To determine the microorganisms that appeared on the polysiloxane (PS) coatings surface after 12 months of immersion in natural seawater, the following procedure was followed: Firstly, a 5 cm^2 section of the PS coating film was carefully peeled off from the steel samples that had been immersed in natural seawater at the testing site. Next, the peeled film was soaked in 10 mL of physiological saline solution (NaCl 0.9%) to extract the microorganisms from the coating. The solution containing microorganisms was then diluted appropriately and cultured on agar plates with different microbiological media. Specifically, Zobell medium was used for bacterial isolation, Yeast Extract-Malt Extract medium for actinomycetes, Potato Dextrose Agar medium for fungi, and Postgate medium for determining the presence of sulfate-reducing bacteria [17]; the media were also used to isolate the microorganisms present on the PS coating film and determine their numbers by the growth observed on the respective agar plates.

The critical dilution determined the number of microorganisms on the tested samples' surface, and the bacteria phenotype was observed using an optical microscopy.

Determination of macro-organisms on coatings surface

Sampling method

Organism sampling: The test sample is removed from the sample holder. Samples were collected from all groups of *Molluscs, Polychaetes, Crustaceans, Sponges,* and *Seaweeds* in the quantification plot until they were no longer found. The samples were then cleaned of mud and soil and stored before analysis on the same day.

Research methods

Density of individual species in the study plots:

$$V = \sum n \Big/ \sum S \times m^2$$

in which: V: number of individuals/m^2; Σn: total number of individuals in the study plots; ΣS: total acreage of study plots.

Identification of species composition: *Molluscs, Polychaetes, Crustaceans, Sponges, Seaweeds* were analyzed by specialized methods for each group of organisms [5].

Cling density: calculation of the number of individual(s)/sample.

Weight: weigh the organism of each tested sample.

Height: measure the height of organisms attached to each sample and calculate the average value.

RESULTS AND DISCUSSION

Investigation of microorganisms on polysiloxane coatings contained m-Cu_2O NPs and Ag-Zn zeolite

Bacteria and other microorganisms play a crucial role in forming biofouling films. Initially, biofouling films are formed by microorganisms. The growth and development of bacteria on material surfaces are greatly influenced by the nature of the material [18]. Therefore, it was essential to identify the bacterial strains that appeared on PS-based coatings applied to steel substrates immersed in natural seawater.

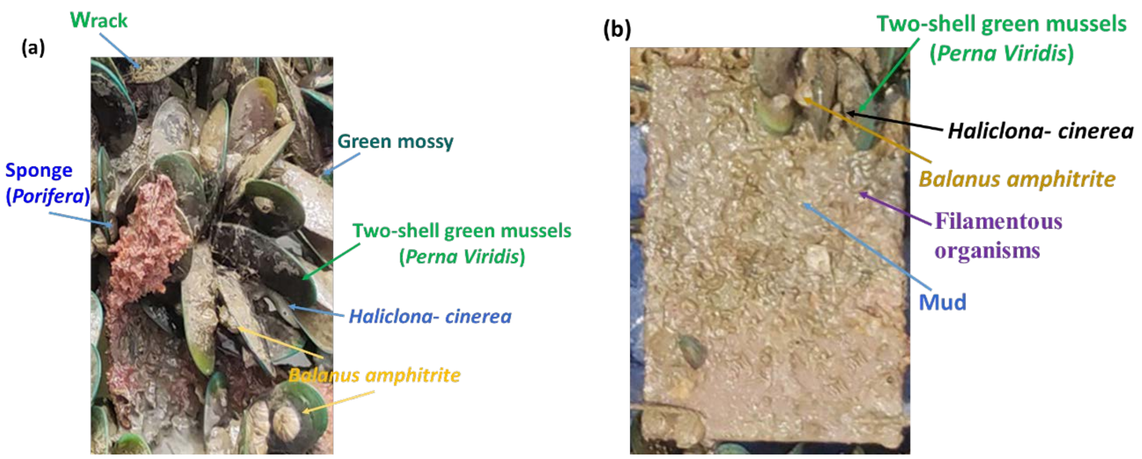

Figure 1. Comparative analysis of steel samples: Polymer coating films with (a) and without (b) nano-antibacterial and nano-antifouling additives exposed to seawater for 12 months

Figure 1 depicts images of different coatings based on PS, both with and without biocide additives, on the steel substrate after 12 months of testing. It is evident that after 12 months of immersion in seawater, various types of biofouling films, microorganisms, and larger organisms have formed on the surface of PS coatings. The control film (without biocide additives) exhibited the attachment of larger organisms, such as barnacles and two-shelled green mussels (*Perna viridis*). In contrast, the surfaces of PS coatings containing Ag-Zn zeolite and m-Cu_2O NPs displayed the presence of fibrous organisms exclusively on the coating surface. The type and quantity of bacteria attached to the immersed samples' surfaces were isolated, determined, and presented in Table 2. The PS coating surface containing Ag-Zn/zeolite and m-Cu_2O NPs harbored three bacterial species with a 9.6 × 10^5 CFU/cm^2 density. Three bacterial species were observed in the testing seawater and on the surface of the control sample. However, the density of bacteria on the control samples' surface (coating without additives) (2 × 10^7 CFU/cm^2) was higher than that on the tested sample's surface. This discrepancy may be attributed to the influence of Ag-Zn/zeolite, which has been reported to exhibit antibacterial activity against marine bacteria strains such as *P. aeruginosa* [19]. On the surface of the control coating, one mold strain (1.2 × 10^2 CFU/cm^2), one yeast strain (40 CFU/cm^2), and on the PS/Ag-Zn zeolite/m-Cu_2O NPs coating surface, one yeast strain (40 CFU/cm^2) and one actinomycete strain (4 × 10^4 CFU/cm^2) were identified. It is worth noting that although mold and actinomycete strains were not detected in the water sample at the testing site, they were still present on the surfaces of the tested samples. This occurrence could be attributed to the growth of microorganisms on PS coating during the formation of antifouling film. Additionally, we speculate that the higher number of microbial species on the coating film/organic polymer in the seawater at the testing site is due to the presence of microorganisms that have a symbiotic relationship with larger organisms such as algae and two-shell green mussels, which were observed on the coating surfaces after the 12-month testing period, as mentioned earlier.

Table 2. Microorganism determination results for PS-based coatings after 12 testing months in natural seawater

Coating sample	Microorganism	Density (CFU/cm^2)	Number of type*
Coating based on PS with biocide additive (testing sample)	Bacteria	$9.6 \times 10^5 \pm 365$	3
	Actinomycetes	$4 \times 10^4 \pm 98$	1
	Mold strain	NI	
	Yeast strain	40 ± 1	1
PS coating without biocide additive (Control sample)	Bacteria	$2 \times 10^7 \pm 563$	3
	Actinomycetes	NI	
	Mold strain	$1.2 \times 10^2 \pm 5$	1
	Yeast strain	40 ± 1	1

Notes: NI: not identified; (*): total number of colony types determined on different media.

Investigation of macroorganisms on polysiloxane coatings contained m-Cu_2O NPs and Ag-Zn zeolite

Species composition attached to the tested sample

After 12 testing months in natural seawater at Vung Oan, Ha Long City marine area, province (Vietnam), seven species were identified adhering to the both organic paint films, with (tested sample) and without (control sample) nano-antibacterial/antifungal additives. These species included green mussel (*Perna viridis*), bearded horse mussel (*Modiolus barbatus*), purple acorn barnacle (*Balanus Amphitrite*), clam worms (*Nereis* sp.), calcareous tubeworm (*Serpula* sp.), Redbeard sponge (*Haliclona cinerea*), and mud crabs (*Xanthidae*) (Table 3). Among these species*, Perna viridis, Balanus amphitrite,* and *Haliclona cinerea* were found in all tested samples, indicating their dominance. *Nereis* sp. was just presented on control samples. *Serpula* sp. appeared two of the three control samples, whereas in the testing sample, it was present in one out of the three samples. The remaining two species, *Modiolus barbatus*, and *Xanthidae*, were encountered only once in control samples. Thus, it is evident that *Perna viridis*, *Balanus amphitrite*, and *Haliclona cinerea* were the prevailing species among those adhering to the tested samples in the seawater at Vung Oan, Ha Long City marine area, province (Vietnam). In a study conducted in Chioggia (Venice, Italy) (lat. 45°14’N, long. 12°17’E), it was reported that eight species were found attached to organic coatings, including two macro-fouling species, namely, red algae and bryozoans, which were present on all tested samples [20]. In contrast, in this study, the predominant species observed on the surface of the samples were *Perna viridis, Balanus amphitrite*, and *Haliclona cinerea*. This difference in species composition may be attributed to the biodiversity of the testing site, suggesting that the macro-fouling species adhering to sample surfaces depend on the specific macro-fouling species in the testing site.

Table 3 reveals that *Nereis* sp. was found in all control samples, whereas no fouling was observed in any testing samples. Similarly, *Serpula sp.* was more common in the control samples, occurring in two out of three, while it was rarely observed in the testing samples. Only one testing sample had an attachment of *Serpula* sp. This discrepancy could be attributed to the influence of Cu_2O and Ag-Zn/zeolite, which act as biocides reducing macro-fouling species attachment [19, 20]. *Modiolus barbatus* and *Xanthidae* were detected on a control sample’s surface, suggesting that macro-fouling species can attach under favorable conditions such as nutrition and surface roughness [20]. However, these observations remain hypotheses that require further investigation.

Table 3. Species composition attached to the tested samples

No.	Samples	*Perna viridis*	*Modiolus barbatus*	*Balanus amphitrite*	*Nereis* sp.	*Serpula* sp.	*Haliclona cinerea*	*Xanthidae*
Control samples								
1	Control-1	x	-	x	x	x	x	-
2	Control-2	x	x	x	x	x	x	-
3	Control-3	x	-	x	x	-	x	x
Frequency (%)		100	33.3	100	100	66.7	100	33.3
Steel samples with polymer coating films								
4	Sample-1	x	-	x	-	-	x	-
5	Sample-2	x	-	x	-	x	x	-
6	Sample-3	x	-	x	-	-	x	-
Frequency (%)		100	0	100	0	33.3	100	0

Density of species attached to tested samples

The study on the attaching density of the 7 species on the tested samples, with the highest density reaching 90 individuals per sample and the lowest density recorded at 19 individuals per sample (Table 4).The samples can be categorized into three levels based on the attached density ranking. The first level includes samples with a high attaching density, exceeding the highest average density of the experimental batch (75.33 individuals per sample). This level comprises two samples, accounting for 33.33% of the total samples. Specifically, Control-1 exhibited a density of 85 individuals, and Control-2 had a density of 90. The second level consists of samples with a medium attaching density. Control-3 demonstrated an average level of attaching density, with 51 individuals per sample, accounting for 16.67% of the samples. The final level encompasses samples with a low attaching density, ranging from 0 to 48.33 individuals per sample (average value). Three samples had low attaching density, accounting for 50% of the total samples: Sample-1 (24 individuals per sample), Sample-2 (21 individuals per sample), and Sample-3 (19 individuals per sample).

Table 4. Number of individual macroorganisms attaching to tested samples after 12 testing months in natural seawater

No.	Samples	*Perna viridis*	*Modiolus barbatus*	*Balanus amphitrite*	*Nereis* sp.	*Serpula* sp.	*Haliclona cinerea*	*Xanthidae*	Total
Control samples									
1	Control-1	18	0	57	2	7	1	0	85
2	Control-2	18	1	65	1	3	2	0	90
3	Control-3	19	0	23	3	3	2	1	51
Average		*18.33*	*0.33*	*48.33*	*2.00*	*4.33*	*1.67*	*0.33*	*75.33*
Steel samples with polymer coating films									
4	Sample-1	9	0	14	0	0	1	0	24
5	Sample-2	5	0	3	0	10	3	0	21
6	Sample-3	7	0	8	0	0	4	0	19
Average		*7*	*0*	*8.33*	*0*	*3.33*	*2.67*	*0*	*21.33*

Considering the attaching density for each experimental batch, the three control samples exhibited a significantly higher average density of 75.33 individuals per sample compared to the tested samples containing antibacterial and antifungal additives, which had an average density of only 21.33 individuals per sample (low-level of attaching density).

A clear distinction between the tested and control samples is evident in terms of their antifouling and anti-adhesive properties. The average density of macro-fouling species on the tested samples was 3.53 times lower than that of the control samples. Furthermore, when comparing the attaching density of the same species on both samples, the tested samples exhibited significantly lower macro-fouling species densities compared than the control samples. For instance, the average density of *Perna viridis* in the control samples was 18.33, whereas in the tested samples, it was only 7. Similarly, the average density of *Balanus amphitrite* per control sample was 48.33 individuals, while the tested samples had an average density of only 8.33 individuals. These findings suggest that nano-antibacterial/antifouling additives significantly prevent fouling during testing [20].

Biomass of species attached to tested samples

The total weight of the species attached to tested samples averaged 90 g/sample, while the control sample exhibited a significantly higher weight of 333.33 g/sample (3.70 times higher) (Figure 2). This evidence supports the investigation that the polysiloxane coating with biocides can effectively reduce fouling microorganisms.

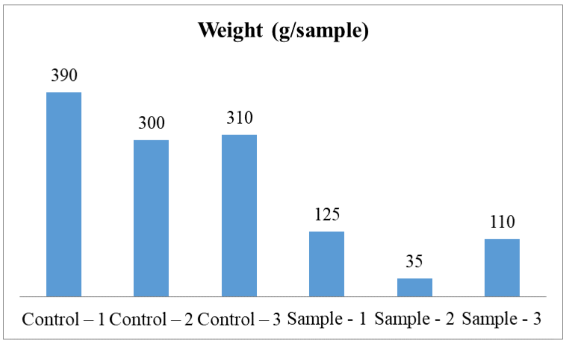

Figure 2. Graph of adherent species weight on tested samples

The results depicted in Figure 2 demonstrate a substantial disparity in the weight of adherent species between the control samples and the tested samples after 12 testing months. As mentioned, the tested samples exhibited a low mass of adherent species, whereas the control samples displayed high and medium adherent species.

Height of adherent species

Figure 3 illustrates the average height of species attached to the tested and control samples. The control samples exhibited an average height of 7.17 cm/sample, while the tested samples had an average height of 5.90 cm/sample.

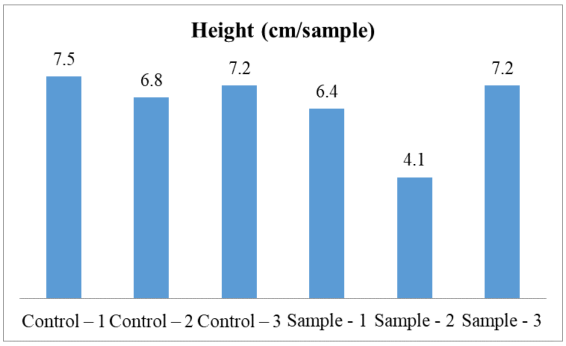

Figure 3. Height of adherent species on tested samples

When comparing the growth rate of adherent species between the tested samples with polymer coating films and the control samples, it is evident that the tested samples exhibited a lower growth rate. Specifically, two tested samples showed a low growth rate, ranging from 4.1 cm/sample to 6.4 cm/sample, suggesting that the presence of the polymer coating films had a deterrent effect on the growth of adherent species.

CONCLUSION

After 12 testing months in natural seawater at Vung Oan, Ha Long City marine area, Quang Ninh province (Vietnam), it was observed that seven species adhered to the surface of steel with the polymer coating film. Notably, *Perna viridis* and *Balanus Amphitrite* exhibited the highest attachment rates among these species. The polymer coating film samples, which included nano-antibacterial and nano-antifouling additives, showed a lower number of adherent species compared to the control samples without these additives. Furthermore, the polymer coating film samples utilizing nano-antibacterial and nano-antifouling additives demonstrated a reduced attachment level, weight, and average height of adherent species compared the control samples. These results signify the effectiveness of antibacterial and antifouling additives in polymer coating films, highlighting their potential application for steel surfaces.

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Data Availability: The data used to support the findings of this study are included within the article.

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