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Wazirotus Sakinah

Naval Architecture Study Program, Engineering Faculty, Jember University, Indonesia

Nugroho Jarot Dwi Ardiansah

Naval Architecture Study Program, Engineering Faculty, Jember University, Indonesia

Saifurridzal

Civil Engineering, Engineering Faculty, Jember University, Indonesia

Pratama Yuli Arianto

Naval Architecture Study Program, Engineering Faculty, Jember University, Indonesia

他

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A Spearman-Based Assessment of Oceanographic Parameters Influences on Biofouling Mitigation through *Averrhoa bilimbi* Leaf Extract

Wazirotus Sakinah^{1,*}, Nugroho Jarot Dwi Ardiansah¹, Saifurridzal²,
Pratama Yuli Arianto¹, Muammar Kadhafi³, Sumarji¹,
R. Puranggo Ganjar Widityo¹

¹Naval Architecture Study Program, Engineering Faculty, Jember University, Indonesia

²Civil Engineering, Engineering Faculty, Jember University, Indonesia

³Department of Mechanical Engineering, Kunsan National University, Republic of Korea

*Author to whom correspondence should be addressed:

E-mail: wazirotus.sakinah@unej.ac.id

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Abstract: This study evaluates the antifouling potential of *Averrhoa bilimbi* leaf extract (ABLE) and its correlation with oceanographic parameters under field conditions. Steel specimens treated with ABLE-oil paint mixtures (ratios 1:1, 3:7, 7:3) were compared to control and commercial paint groups. The 1:1 treatment showed the lowest biofouling growth rate (0.049 g/day). Spearman correlation revealed temperature and current significantly influenced biofouling in control specimens, while treated specimens showed weaker associations. These findings suggest antifouling performance is more dependent on chemical composition than environmental factors, supporting ABLE's potential as a sustainable antifouling agent.

Keywords: anti-fouling; *Averrhoa bilimbi* leaf extract; biofouling growth rate; oceanographic parameters

1. Introduction

Biofouling attachment is one of the most serious problems in shipping due to the losses caused by biofouling attachment on a ship's hull surface. Biofouling can create roughness on all waterlogged hull surfaces, which can affect fluid flow and cause velocity reduction in the turbulence boundary layer caused by increased shear stress and frictional resistance¹. Biofouling attachment is usually overcome using commercial paint containing TBT (Tributyltin). TBT is a common biocide found in ship hull antifouling coatings. However, even at extremely low doses, this substance is highly poisonous to a variety of aquatic non-target creatures². In the non-commercial dogwhelk, *Nucella* sp., on the south coast of England, TBT concentrations as low as 1 ng/L resulted in imposex, or the development of male sexual traits in female animals^{3,4}. In October 2001, IMO prohibited TBT in commercial paints used on ships and established a mechanism to prevent the potential use of other harmful substances in antifouling systems⁵. Following the ban on TBT use in boats under 25 meters, ports and shipyards are the primary sources of pollution in coastal marine areas. A significant amount of waste will be produced close to ports and shipyards

because of the impending phase-out of TBT. However, there is a possibility that TBT will continue to be used in commercial paints in some parts of the world⁴.

Averrhoa bilimbi leaf extract (ABLE) contains phytochemical compounds consisting of saponins, tannins, steroids, flavonoids, and alkaloids. ABLE has anti-inflammatory activity and a high percentage value of hemolysis inhibition⁶. ABLE has natural antioxidant properties⁷. Since ancient times, the plant has been a significant source of medicine⁸. ABLE can prevent the corrosion of carbon steel in a hydrochloric acid solution. If the concentration of ABLE increases, the corrosion rate decreases, and the inhibition efficiency increases⁹. ABLE as an anti-microbial in the health world has been widely utilized, but its effectiveness as an anti-bacterial in the prevention of biofouling has not yet been proven.

Several factors, including operating area, hull painting method, service speed, docking frequency, and the ratio of berthing and sailing time, affect the growth rate of biofouling on ships¹⁰. Light intensity, salinity, tides, temperature, sedimentation, sea depth, currents, and waves are additional components that influence biofouling development¹¹. Several studies on the relationship of

several oceanographic parameters to biofouling growth have been conducted, but these studies were conducted in the laboratory, such as the impact of temperature with a laboratory heat exchanger module ¹²⁾, simulated sunlight ¹³⁾, relative humidity with laboratory approaches also done ¹⁴⁾. Similar research conducted under actual conditions at sea is rarely studied.

Field experiments, while offering a realistic representation of marine environmental conditions, present several limitations and challenges that may affect the validity and reliability of the findings. The natural variability of oceanographic parameters such as tide, current velocity, salinity, and temperature—fluctuating temporally and spatially—introduces uncertainty in measurement and interpretation ¹⁵⁾. Moreover, the lack of control over external factors, including human activity at the research site, sedimentation processes, and weather fluctuations, can directly influence biofouling rates and the effectiveness of antifouling treatments applied. Periodic data sampling and limited precision of field instruments further complicate data acquisition and analysis. Despite these challenges, in situ studies remain essential to capture the complexities of open-water conditions that laboratory simulations often fail to replicate ^{12,13)}. Consequently, beyond evaluating the antifouling potential of *Averrhoa bilimbi* leaf extract, this study also aims to investigate whether oceanographic parameters still exert a significant influence on biofouling development under uncontrolled marine conditions.

2. Research Method

2.1. Research Procedures

Experimental study in field was used on this study.

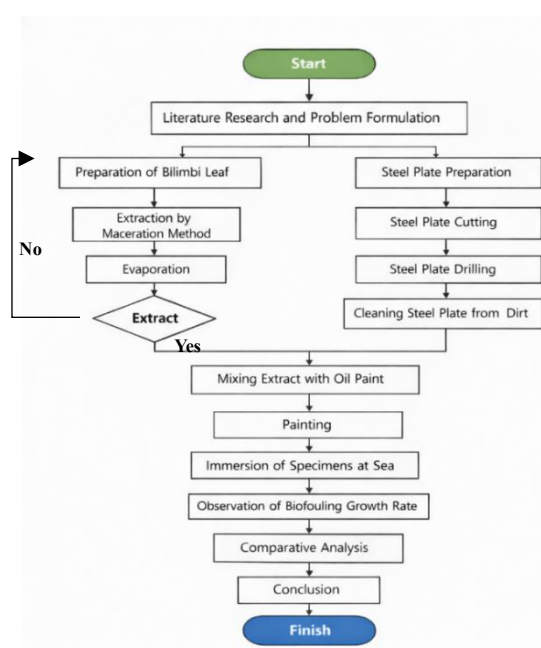


Fig. 1: Research Flowchart

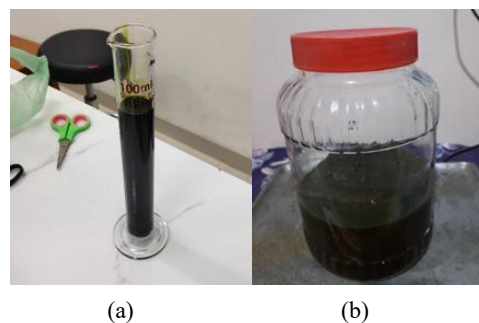


Fig. 2: (a) Maceration process, (b) ABE

The process of working on this research can be described as a flowchart in Figure 1.

2.2. Research Tools and Materials

The tools used in this research are as follows:

- a. Weight Scale
- b. Blender
- c. Sieve
- d. Basin
- e. Glass Jar
- f. Measuring Cylinder
- g. Filter Paper
- h. Measuring Flask
- i. Rotari evaporator
- j. Hand Grinder
- k. Drill
- l. Spray gun
- m. Pipe Assembly
- n. Hawser

2.2.1. Extraction of ABE

The obtained bilimbi leaves were dried by aeration for two weeks and then mashed using a blender. At room temperature, 200 grams of bilimbi leaf flour was macerated using 600 grams of methanol solvent (1:3) for 72 hours. The macerated bilimbi leaves were filtered using Whatman Grade 1 filter paper. This paper is a cellulose filter used for qualitative analytical methods to identify and determine materials ¹⁶⁾. To get a thick extract, the results of the maceration process were evaporated with a rotary evaporator at a temperature of 40°C and a speed of 50 rpm. The maceration process and the obtained of ABE are shown in Figure 2.

2.2.2. Antifouling Treatment

This experiment involved five types of antifouling treatment; each applied to three replicate specimens. The treatment groups were as follows:

- (1) Control – specimens without any coating.
- (2) Commercial antifouling paint – used as a benchmark to assess whether the ABE formulation is comparable to commercial products.
- (3) Treatment 1:1 – a mixture comprising 50% oil-based paint and 50% ABE.
- (4) Treatment 7:3 – a mixture comprising 70% ABE and

30% oil-based paint.

(5) Treatment 3:7 – a mixture comprising 30% ABLE and 70% oil-based paint.

2.3. Specimens Manufacturing Process

Specimens were prepared by cutting them using a grinding wheel, which is in the form of a steel plate with a thickness of 3mm. This test procedure follows the American Society for Testing and Materials ASTM 3623. This standard for antifouling coating systems requires a low carbon steel plate with a plate thickness of 3 mm, a width of 150- 250 mm, length of 250-300 mm¹⁷⁾. Therefore, in this study, the plate specimen has a length of 100 mm and a width of 50 mm with a specimen thickness of 3mm

2.4. Sampling and Data Collection

Boom Beach in Banyuwangi was the site of this study, and the specimen was submerged beneath the dock at coordinates 8o12'33"S 114o22'55"E (Figure 3). A wire was used to hang the specimens from the buoy, which was subsequently secured to the pier. To check for biofouling growth, all specimens were soaked for 84 days and lifted every 7 days. The spores of protozoa, fungi, and macroalgae create bacterial biofilms within a week. Invertebrate larvae can occasionally settle after a few hours of immersion, and seaweed motile spores can settle within minutes¹⁸⁾. Data collection was the next stage. Documentation of fouling buildup and periodic mass measurements of the material, with the primary data sources.

The growth rate of biofouling (marine growth) in this study was calculated using the daily growth rate formula¹⁹⁾ with the following equation:

$$DGR = \frac{W_t - W_0}{T} \quad (1)$$

Where:

DGR: Daily growth rate (g/day)

W_t : Final weight of research (gram)

W_0 : Initial weight (gram)

T: Observation time (day)

2.5. Oceanography Parameters Measurement

Instruments were used to measure oceanographic parameters at each station, which were lowered to a depth of 50 centimeters; however, in this study, only surface data were used. The type of environmental parameters that can be measured among others: temperature (°C) with thermometer; salinity (‰) with refractometer; current velocity (m/s) with current meter; tides (m) with tide ruler.

2.6. Data Analysis

The statistical approach employed in this study was correlation-based analysis, specifically utilizing Spearman's rank correlation. This non-parametric method was selected due to the non-normal distribution of the dataset²⁰⁾. This study employs correlation analysis to examine the relationship between biofouling growth rates and oceanographic parameters. The growth rate data were derived from biofouling observed on control specimens and the most effective treatment specimen, selected based on its significantly different growth rate. The results obtained from the correlation analysis yield a numerical value known as the correlation coefficient²¹⁾. The correlation value can indicate either a positive or negative relationship between variables²²⁾.

Regression analysis serves as a statistical approach to evaluate the potential influence among variables,

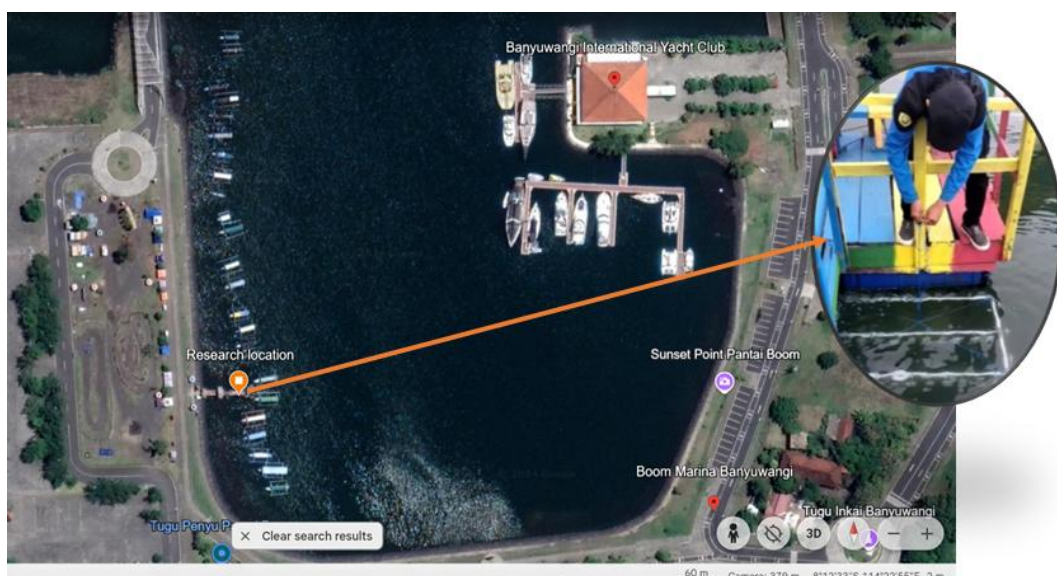


Fig. 3: Study Location

formulated through mathematical expressions (Sari and Jaya, 2019). In this study, it is employed to assess the degree to which oceanographic parameters—serving as independent variables—affect the daily biofouling growth rate, which is designated as the dependent variable. A multiple linear regression model is applied to depict the linear association between two or more independent variables ($X_1, X_2, \dots, X_n$) and the dependent variable (Y)²¹.

3. Results and Discussion

3.1. Daily Growth Rate (DGR) All Specimens

The results of the biofouling growth rate value on each steel plate after 3 months immersion will be obtained using the daily growth rate (DGR) formula shown in Figure 4²³. The Figure shows the results of the average biofouling growth rate on the test specimen obtained through the daily growth rate (DGR) equation. In the treatment variations 1:1, 3:7, and 7:3, in the early weeks has a higher rate value this is due to the maturation stage of the late-stage biofilm colonization, which occurs 24 hours to 1 week after immersion²⁴. Whereas in commercial paints, this colonization maturation stage occurs more slowly because this paint contains Cuprous Oxide (Cu_2O), which is more effective in inhibiting the growth rate of biofouling, and in paints without treatment, only the corrosion process occurs on the surface of the paint so that biofilm colonization has not formed.

The first week of control specimens has a biofouling growth rate value of 0.040 g/day. In this first week, it has a small biofouling growth rate value because only biofilm attachment occurs, and the time of biofouling that sticks continues to increase in the 12th week has a biofouling growth rate of 0.088 g/day; this is because there are several types of biofouling attached to the specimen.

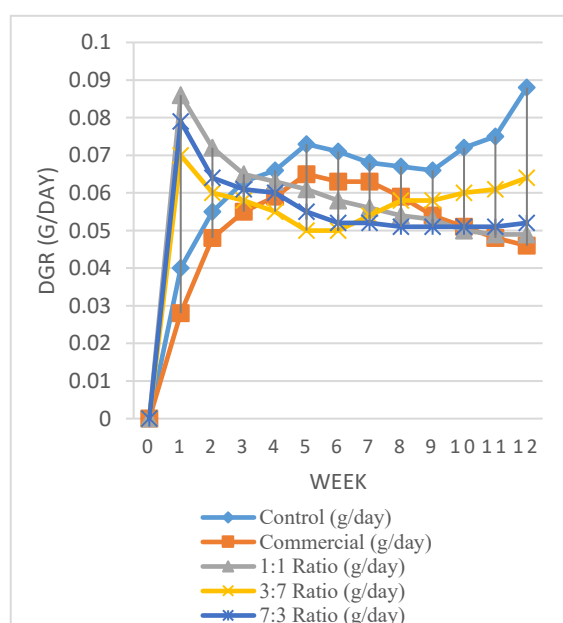


Fig. 4: DGR of Biofouling

While the smallest biofouling growth rate occurs in the commercial paint treatment, the commercial paint used in this study contains Cuprous Oxide (Cu_2O), which functions to prevent the attachment of biofouling on a macro and micro scale²⁵. In the first week, the biofouling growth rate value was 0.028 g/day; until the fifth week, it increased to a value of 0.065 g/day; this occurred due to an increase in biofilm attachment to the surface of the specimen. In the following week, the growth rate decreased. In the 12th week, it had a growth rate value of 0.046 g/day.

The lowest growth rate of the three treatments of adding oil paint with bilimbi leaf extract is in the 1:1 treatment because when viewed on the graph, the 1:1 treatment has a lower graph fluctuation than the others. This is due to the presence of antifouling content in the treatment variation and the balanced ratio between extract and oil paint so that the paint adheres perfectly. In the first week, the 1:1 treatment had a biofouling growth rate of 0.086 g/day. The increasing time, the growth rate in the 1:1 treatment decreased, so the 12th week observation was 0.049 g/day. The growth rate in the first week is quite high because biofilm growth occurs during a period of rapid development in the first week to 2nd week, and after that, biofilm development stabilizes²⁶.

The 7:3 treatment variation in the first week had a biofouling growth rate of 0.079 g/day, the increasing time, the rate in this variation decreased, so in the 12th week, it had a biofouling growth rate value of 0.052 g/day, which is a greater rate than the 1:1 treatment. This is because, in this treatment, the paint is easy to peel off, causing a rough surface on the surface of the specimen, which causes easy attachment of biofilm and microfouling.

While the 3:7 treatment variation in the first week had a biofouling growth rate of 0.070 g/day, in 7th week, the 3:7 treatment variation experienced an increase in the biofouling growth rate of 0.054 g/day which was initially in 6th week only worth 0.050 g/day this was influenced by the presence of macro fouling, namely *Pinctada margaritifera* attached to the specimen and the increasing time its size was getting bigger so in 12th week had biofouling growth rate of 0.064 g/day. The increase in growth rate is due to less tannin content. Hence, the anti-inflammatory properties in this treatment are less strong which causes it to be less effective in inhibiting biofouling growth.

3.2. Species Observation

After a month, there are variations in the specimens' state. Figure 5 shows the specimens in the first week, one day after immersion.

The control specimen showed a lot of corrosion on almost the entire surface, while the other specimens showed slight corrosion but no significant change. There is biofilm growth which has slippery textured specimen surface. The growth of biofilm is usually characterized by microbial

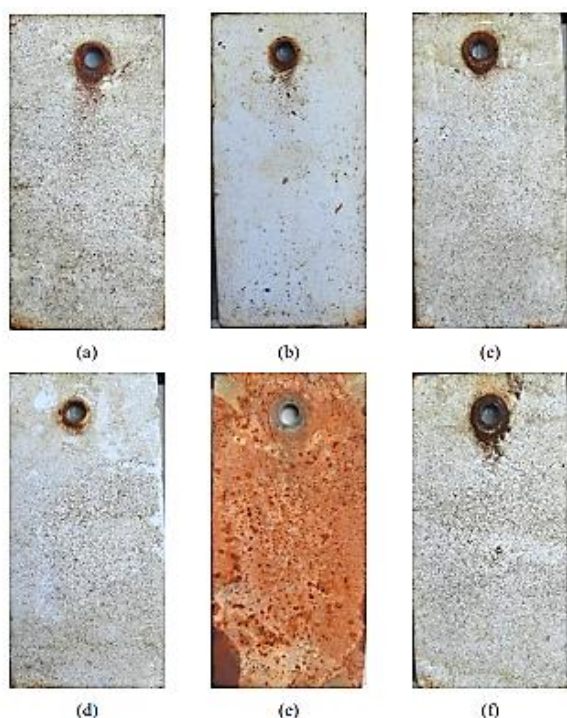


Fig. 5: One week after immersion, (a) 1:1, (b) 3:7, (c) 7:3, (d) antifouling commercial paint, (e) control, (f) oil paint

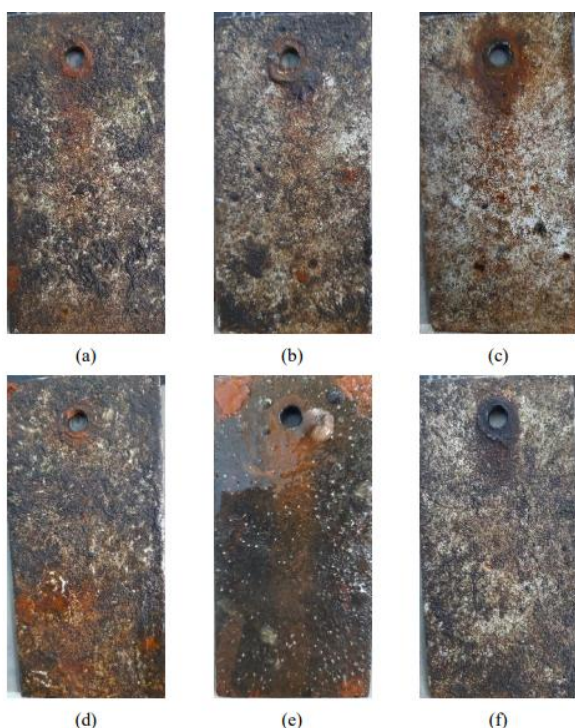


Fig. 6: Seven weeks after immersion, (a) 1:1, (b) 3:7, (c) 7:3, (d) antifouling commercial paint, (e) control, (f) oil paint

colonization of the test specimen with a time of 24 hours to 1 week after the test specimen is immersed ²⁴⁾²⁷⁾.

At week 7 observation (Figure 6), the control specimen had *Planostrea pestigris* and also the growth of *Balanus amphitrite* size, which appeared since week 4, and *Spirorbis* sp., which appeared since week 3 and more and

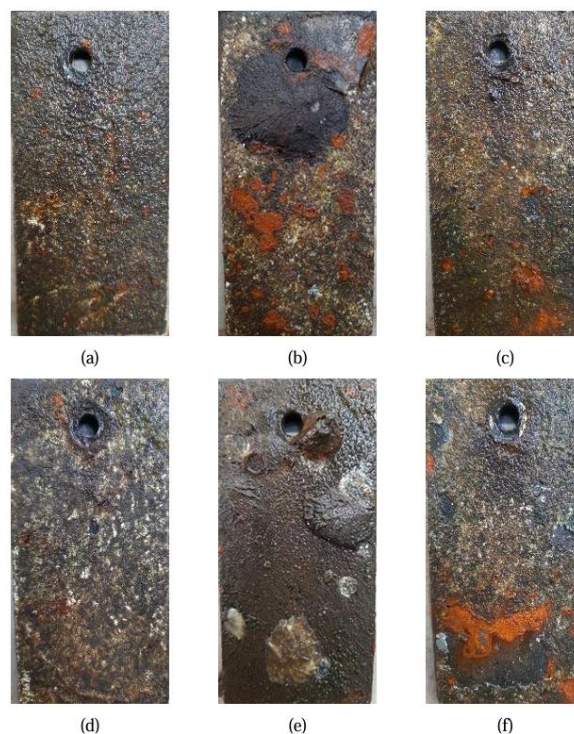


Fig. 7: Twelve weeks after immersion, (a) 1:1, (b) 3:7, (c) 7:3, (d) antifouling commercial paint, (e) control, (f) oil paint

more. In specimen 3:7, there is *Pinctada margaritifera*, which is getting bigger. This species appeared in week 5. Whereas in other test specimens, there is only the addition of biofilm and there is also some microfouling that is degraded. At specimens submerged at Boom Marina, macrofouling typically emerged one month into the immersion period and started to grow in size and quantity at week 7th ²⁸⁾.

In the 12th week of observation (Figure 7), a type of worm was attached to the control specimen, namely polychaeta. Polychaeta is a type of worm included in the phylum of annelids that have setae, a brushy body, and live cosmopolitan in various types of marine ecosystems also this biofouling is a food source for fish ²⁹⁾. *Pinctada margaritifera* and *Balanus amphitrite* are getting bigger. In specimens with oil paint treatment for chipped paint, there is an increase in corrosion. For specimens 1: 1, 7: 3, and commercial paint, only the addition of brown algae occurs, and there is also corrosion caused by fouling degradation. As for the 3:7 specimen on the reverse side, there is a *Pinctada margaritifera* that is getting bigger in size. ABL has potential as an antifouling agent; however, other materials with antimicrobial properties can also be further studied as alternative antifouling agents, such as microalgae *Chlorella vulgaris* ³⁰⁾, silica coating ³¹⁾, Graphene Oxide ³²⁾, and Zirconium dioxide ³³⁾.

3.3. Oceanographic Parameters Influences Analysis

Some of the factors that influence the growth rate of

Table 1: Oceanography parameters data from measurement

Date	Parameter			
	Tide (m)	Current (m/s)	Salinity (‰)	Temperature (°C)
08/18/23	2.20	0.04	34.13	26.89
08/25/23	1.00	0.04	34.19	27.23
09/01/23	2.30	0.04	34.08	27.23
09/08/23	1.10	0.04	34.00	27.44
09/15/23	2.00	0.03	34.11	27.41
09/22/23	1.20	0.04	34.20	27.34
09/29/23	2.00	0.04	34.20	27.77
10/06/23	1.20	0.04	34.19	27.78
10/13/23	1.70	0.04	34.20	28.10
10/20/23	1.40	0.04	34.19	28.26
10/27/23	1.60	0.04	34.18	28.45
11/03/23	1.30	0.03	34.15	28.78

biofouling are the parameters of sea conditions. In this experiment, some of the parameters measured were ocean current velocity, salinity, temperature, and tides. The data can be seen in Table 1.

3.3.1. Control Specimens

Based on observational data, the trend patterns of several oceanographic parameters—including temperature, salinity, current velocity, and tidal fluctuations—relative to the biofouling growth rate on control specimens are shown in Figure 8 and Figure 9. Meanwhile, the correlation results between oceanographic parameters and the biofouling growth rate on control specimens are summarized in Table 2.

The Spearman correlation analysis revealed significant relationships between biofouling growth and environmental parameters. Temperature showed a strong positive correlation ($\rho = 0.804$, $p = 0.0009$), indicating that higher temperatures promote biofouling development, likely due to enhanced metabolic activity and reproduction of fouling organisms. Conversely, current velocity exhibited a strong negative correlation ($\rho = -0.730$, $p = 0.0046$), suggesting that increased water movement may inhibit biofouling by dislodging organisms or reducing settlement efficiency. These findings align with previous studies emphasizing the role of hydrodynamic forces and thermal conditions in shaping fouling dynamics³⁴.

In contrast, salinity ($\rho = 0.171$, $p = 0.5769$) and tidal height ($\rho = -0.257$, $p = 0.3975$) showed weak and statistically insignificant correlations with biofouling growth. These findings imply that, within the observed range, salinity and tidal variation may not be primary drivers of fouling intensity²⁰. Overall, the results highlight temperature and

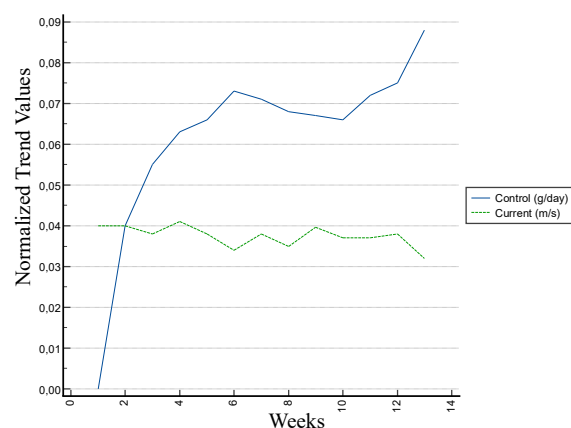


Fig. 8: Temporal trends of biofouling growth rate on control specimens and current velocity over the immersion period. All parameters are presented as normalized values to facilitate trend comparison and Sea Current

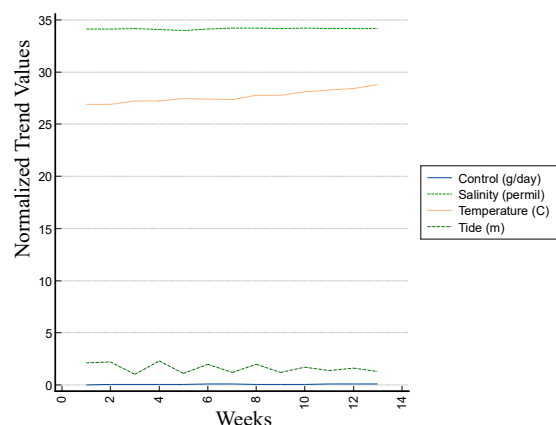


Fig. 9: Temporal trends of biofouling growth rate on control specimens and oceanographic parameters (temperature, salinity, and tidal fluctuations) over the immersion period. All parameters are presented as normalized values to facilitate trend comparison

current as key environmental factors influencing biofouling dynamics, which is crucial for designing antifouling strategies and managing marine infrastructure.

3.3.2. Treatment Specimens

Based on the immersion data discussed earlier, the treatment specimen with a 1:1 formulation demonstrated a Daily Growth Rate (DGR) most comparable to that of the specimen coated with commercial antifouling paint. Therefore, this 1:1 treatment was selected for further analysis to determine whether oceanographic parameters—such as current velocity, temperature, salinity,

Table 2: Spearman Correlation Between Control Specimens and Oceanographic Parameters

		Control Specimens	Current	Salinity	Temp.	Tide
Control Specimens	Correlation Coef.	1.000	-0.730	0.171	0.804	-0.257
	Sig. (2-tailed)		0.0046	0.5769	0.0009	0.3975
	N	13	13	13	13	13

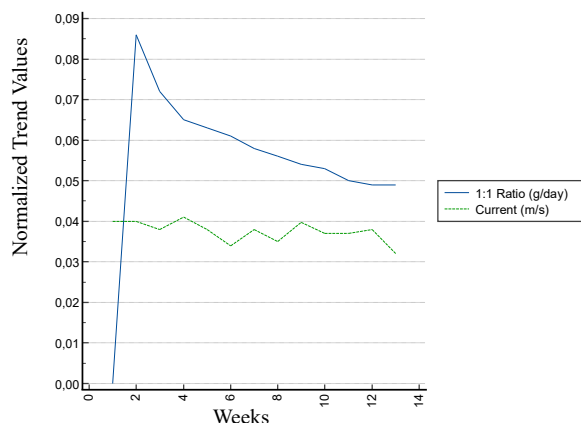


Fig. 10: Temporal trends of biofouling growth rate on treatment specimens and current velocity over the immersion period. All parameters are presented as normalized values

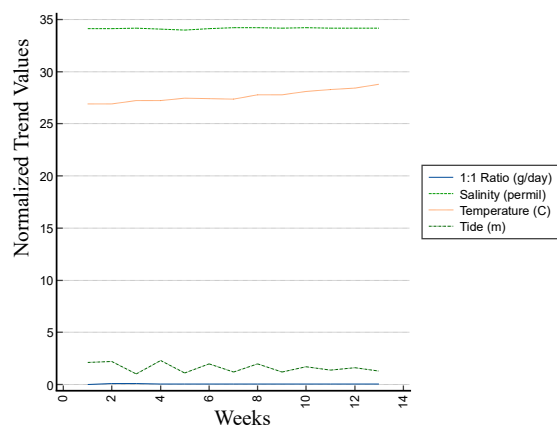


Fig. 11: Temporal trends of biofouling growth rate on treatment specimens and oceanographic parameters (temperature, salinity, and tidal fluctuations) over the immersion period. All parameters are presented as normalized values to facilitate trend comparison

Table 3. Spearman Correlation Between Treatment Specimens and Oceanographic Parameters

		Treatment Specimens	Current	Salinity	Temp.	Tide
Treatment Specimens	Correlation Coef.	1.000	0.295	-0.240	-0.576	0.000
	Sig. (2-tailed)		0.3276	0.4303	0.0395	1.000
	N	13	13	13	13	13

and tidal height—still exert a measurable influence on biofouling development under experimental conditions. This approach aligns with the need to evaluate environmentally sustainable antifouling alternatives while considering dynamic marine factors^{35–38}). The use of Spearman's rank correlation was appropriate for this analysis, given its robustness in handling non-parametric ecological data³⁹), the trend patterns of several oceanographic parameters—including temperature, salinity, current velocity, and tidal fluctuations—relative to the biofouling growth rate on control specimens are shown in Figure 10 and Figure 11. Meanwhile, the correlation results between oceanographic parameters and the biofouling growth rate on treatment specimens are summarized in Table 3.

The Spearman correlation analysis reveals a statistically significant negative relationship between treatment specimens and temperature ($\rho = -0.576$, $p = 0.0395$), suggesting that biofouling growth decreases as temperature rises. Correlations with current ($\rho = 0.295$), salinity ($\rho = -0.240$), and tide ($\rho = 0.000$) are weak and not significant, indicating minimal influence.

Although both treatments were conducted at the same location, the correlation strength between biofouling growth and oceanographic parameters varied considerably between the two specimen types. The difference in correlation patterns between the treatment and control specimens likely stems from the influence of antifouling interventions applied to the treatment group. These treatments—such as coatings or bioactive compounds—

can alter the surface properties and microenvironment, thereby modifying how biofouling organisms respond to oceanographic factors like temperature, salinity, and current. As Carrier et al.³⁵) and Romeu & Mergulhão³⁷) suggest, antifouling strategies can disrupt natural settlement cues and physiological responses of fouling organisms, leading to distinct ecological interactions compared to untreated surfaces.

4. Conclusion

The 1:1 mixture of Averrhoa bilimbi leaf extract and oil-based paint demonstrated the most effective antifouling performance, with the lowest biofouling growth rate among all treatments. Spearman correlation analysis revealed that oceanographic parameters—particularly temperature and current velocity—significantly influenced biofouling in control specimens but had minimal impact on treated specimens. This suggests that antifouling effectiveness is primarily governed by the chemical properties of the coating rather than environmental conditions. Future research should explore longer immersion periods and alternative extract combinations to enhance durability and performance.

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