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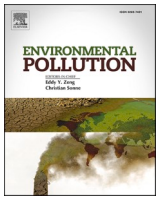

Evaluating macrophyte extracts as eco-friendly antifouling additives for freshwater made-man structures: a field assessment☆

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ABSTRACT

Biofouling on artificial aquatic surfaces yields substantial economic losses and operational challenges. Traditional antifouling strategies often rely on synthetic chemical coatings, which have harmful environmental impacts, thus environmentally sustainable solutions, such as natural antifouling compounds are increasingly being sought. Extracts derived from the aquatic macrophytes *Pontederia crassipes* and *Typha domingensis* have demonstrated potential antifouling properties in preliminary studies; however, their efficacy under natural field conditions remains unverified. This study is the first to evaluate the antifouling potential of these macrophyte extracts when incorporated with epoxy coatings in a natural freshwater environment (Salto Grande Reservoir, Uruguay River). Stainless steel substrates were treated with 2.5, 5, and 10 g L^{-1} of lyophilized macrophyte extracts combined with epoxy and compared to uncoated and epoxy-coated controls. Over a 165-h period, biofouling was assessed via chlorophyll levels, bacterial counts, macro-organism presence and attachment and taxonomic diversity. Analytical techniques, including gas and liquid chromatography, along with Fourier transform infrared spectroscopy, were employed to identify active compounds in extracts. The *P. crassipes* extract at 5 and 10 g L^{-1} exhibited superior antifouling efficacy compared to *T. domingensis*. Coatings with *P. crassipes* significantly reduced bacterial colonization (37 %), algae growth (for different photosynthetic pigments), fungal presence, and macro-organism attachment (not found), while promoting the occurrence of opportunistic taxa less conducive to fouling. The observed antifouling activity may be attributed to specific chemical compounds, including long-chain hydrocarbons and phenolic derivatives, identified in the extracts. The study findings demonstrate the field antifouling efficacy of macrophyte extracts incorporated with epoxy coatings, highlighting *P. crassipes* as a particularly promising, sustainable antifouling candidate. Its high biomass availability and ease of cultivation enhance its potential for industrial-scale development as natural antifouling agents. This work provides critical insights into developing eco-friendly antifouling coatings that minimize environmental impact while maintaining efficacy in biofouling control being the first study to prove in a natural environment the antifouling potential of these macrophytes.

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1. Introduction

Biofouling, defined as the accumulation of biological deposits on artificial aquatic surfaces, poses significant industrial and economic challenges globally (Dobretsov and Rittschof, 2020; Xiao et al., 2020). This phenomenon is particularly problematic in freshwater systems, where invasive species (Satasiya et al., 2025) such as the golden mussel (*Limnoperna fortunei*) colonize critical infrastructure, including hydraulic sensors, turbine cooling systems, and water treatment facilities (Boltovskoy, 2015; Brugnolli et al., 2011). Such colonization obstructs hydraulic systems in hydroelectric power plants, water treatment facilities, and aquaculture systems, leading to structural damage, costly maintenance and operational inefficiencies (Boltovskoy, 2015; Brugnoli et al., 2005; Cámara et al., 2022; Liu et al., 2024). Industries dependent on water resources face an estimated global expenditure exceeding $340 million annually to combat biofouling and repair damaged infrastructure (Cuthbert et al., 2021).

Traditional antifouling solutions often rely on synthetic coatings containing biocidal and/or co-biocide agents. However, these compounds have raised environmental concerns due to their toxicity, persistence in aquatic ecosystems, and potential to harm non-target organisms (Dobretsov and Rittschof, 2020). This has spurred interest in eco-friendly alternatives, with natural antifouling agents emerging as a sustainable solution (Agostini et al., 2021b). Natural antifouling agents have garnered significant attention due to their ecological safety, biodegradability, and lower toxicity compared to synthetic alternatives (Pérez et al., 2021). In this context, aquatic macrophytes like *Pontederia crassipes* Mart. (formerly *Eichhornia crassipes*) and *Typha domingensis* Pers. have emerged as promising sources of bioactive compounds with antifouling potential for freshwater environments (Morales et al., 2024b).

Prior research has demonstrated that extracts from these plants exhibit significant antifouling activity against bacterial biofilms and invasive species without showing toxicity to key aquatic organisms at tested concentrations (Morales et al., 2024b), as well as biotechnological applications such as bioremediation, biogas and biofuel production (Amarilla et al., 2024; Dilshad et al., 2024; Su et al., 2018). Also, *P. crassipes* and *T. domingensis* have an early effect by inhibiting bacterial biofilm to affect intercellular communication - quorum sensing (Morales et al., 2024b). Current antifouling alternatives seek inhibition by this type of action, as they do not affect bacterial planktonic growth and reduce the likelihood of bacterial resistance (Agostini et al., 2019a; Martín-Rodríguez et al., 2015; Neves et al., 2024).

Pontederia crassipes (water hyacinth) species are native to the Amazon basin in South America (Ayanda et al., 2020) and have subsequently been introduced to all continents (Guimarães et al., 2017). *Typha domingensis* (cattail) is native to Brazil, but is currently distributed worldwide (Cruz et al., 2023), in tropical and temperate climate regions (Gomes et al., 2014). Both species are invasive in many regions due to their rapid growth and adaptability, but their high biomass yield, and resilience across diverse ecosystems, making them readily available resources for biotechnological applications (Amarilla et al., 2024; Ayanda et al., 2020; Cruz et al., 2023; Guimarães et al., 2017; Hegazy et al., 2011; Su et al., 2018).

Recent in vitro studies have demonstrated that aqueous extracts from *P. crassipes* and *T. domingensis* inhibit the formation of bacterial biofilms and the attachment of the golden mussel without exhibiting toxicity to non-target organisms, such as *Pseudopediastrum boryanum* and *Daphnia magna*, at concentrations of up to 35 % (Morales et al., 2024b). Despite these promising findings, the efficacy of *P. crassipes* and *T. domingensis* extracts under natural field conditions remains unexplored, in this same sense, studies to control limnic biofouling are scarce. Laboratory-based studies, while essential for initial evaluations, fail to account for the complex interactions and hydrodynamic forces present in natural environments (Romeu and Mergulhão, 2023). Thus, field experiments are essential for validity of their efficacy and the longevity of antifouling compounds present in new solutions (Ghattavi et al., 2024). We also emphasize that conducting *in situ* experiments is critical to bridge this gap, providing a more accurate assessment of the antifouling potential of these natural compounds, where factors such as nutrients, concentrations, temperature, and biotic interactions vary significantly and influence the efficacy of antifouling solutions.

To carry out these tests and their commercial application, natural compounds can be incorporated into paints (Hamidi et al., 2022). Among these, epoxy coatings are widely used for this purpose, due to their excellent mechanical properties, chemical resistance (Vijayan et al., 2022) and release of the antifouling compounds over time through leaching (Pereira et al., 2024). Thus, the present study aimed to evaluate the antifouling efficacy of extracts of *P. crassipes* and *T. domingensis* associated with epoxy coating in a natural environment, the Salto Grande Hydroelectric reservoir, Uruguay river. This location was selected due to its complex hydrological dynamics, diverse ecological conditions, and the presence of significant biofouling challenges, especially due to the golden mussel (*L. fortunei*) invasion (Boltovskoy et al., 2021), making it a representative site for testing antifouling solutions applicable to similar freshwater environments worldwide. Contributing in this way to the global effort to develop environmentally friendly antifouling technologies.

2. Materials and methods

2.1. Painting preparation

Aqueous extracts of *P. crassipes* and *T. domingensis* were prepared according to Morales et al. (2024b). In summary, 6 g of dry plant mass was (mashed/powder) and mixed with 300 mL of sterile natural water (collected from a pond and filtered - 0.2 μm (cellulose acetate filter) and autoclaved). Then the solid phase was separated by (centrifugation/filtration) and then the solution was lyophilized (LIOTOP, L101). The remaining dry mass was then mixed with Hempadur Base 15579 epoxy paint (Hempel - 15570) to obtain the treatments 2.5, 5 and 10 g L^{-1} (g of lyophilizate L^{-1} of paint). These concentrations were chosen because they present antifouling activity and are within the safe concentration for non-target organisms (Morales et al., 2024b).

This paint base is widely used by professionals in the aquatic industry in South America and in addition to epoxy resin, it contains xylene, talc, titanium dioxide, butan-1-ol, n-butyl acetate, 1,3-bis benzene, and toluene (as per product description). Epoxy paint is one of the most recommended polymeric materials because it is very resistant and long-lasting, due to its chemical and mechanical resistance and anticorrosive properties, as well as the potential for antifouling agents to be released slowly, resulting from its entrapment in epoxy paint (Akhter et al., 2025; Pereira et al., 2024; Tonelli et al., 2021; Vijayan et al., 2022). In addition to these treatments, two control treatments were also used: the control with the original substrate, without coating (control A); and the control coated only with epoxy paint (control B). These controls were chosen to establish baseline conditions for biofouling development without intervention (control A) and to evaluate the effects of the paint itself without the active compounds (control B). This approach ensures that any observed differences can be attributed to the antifouling properties of the extracts.

2.2. Experimental procedure

With the aid of a polyester foam roller, these treatments (explained in 2.1) were used to coat stainless steel substrates (12.5 cm^2). The substrates were placed in frames (Fig. 1B) and exposed vertically at a depth of 1m (Agostini et al., 2021a, 2019b) in the aquatic environment (Fig. 1A) near the Salto Grande Reservoir, Lacustrine Zone – Uruguay river (31°16′11.8" S 57°56′44.7" W) for 165 h(Fig. 1C) in April 2023. Samples were taken daily to analyze different variables. This depth and vertically exposure were used because they are already consolidated

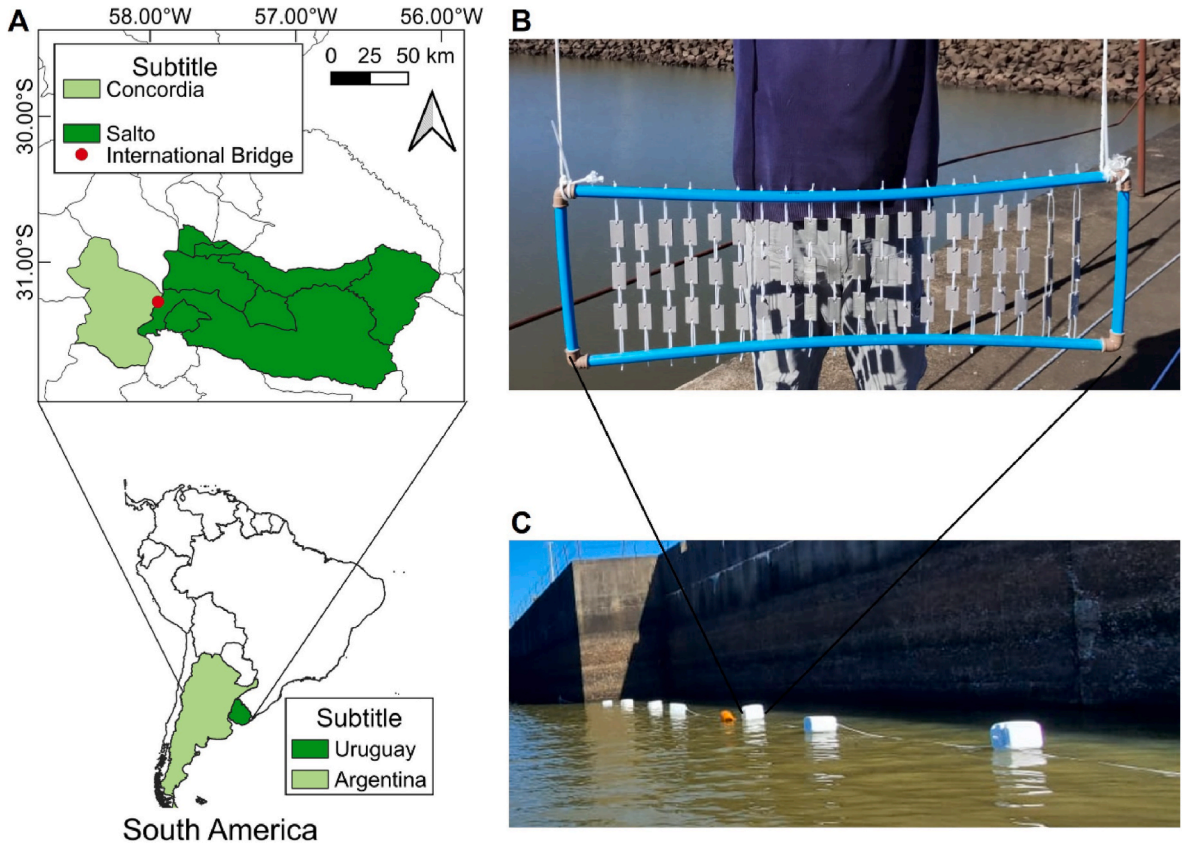

Fig. 1. Experimental site location, surroundings of the Salto Grande International Bridge – Uruguay/Argentina (A) and submerged frames with the substrates painted with antifouling treatments (B–C).

characteristics that guarantee biofouling (Agostini et al., 2019b).

2.3. Study area

Salto Grande is a subtropical fluvial reservoir of approximately 750 km^2 within 100 km of the main channel of the Uruguay River, with an average depth of 6.4 m and a maximum of 35 m, an average annual temperature of 19 °C and annual precipitation of 1,260 mm. It is characterized by a period of ebb and flow from December to March and floods from April to November, with a maximum discharge of 22.000 $m^3\ s^{-1}$ and a minimum of 5.563 $m^3\ s^{-1}$ (O'Farrell et al., 2012). The reservoir is primarily used for power generation, but also for drinking water supply and recreational activities, including sports and fishing. The system is also characterized by the proliferation of cyanobacterial blooms, which varies according to the hydrological conditions of ebb and water level and attains great abundances on the right bank of the reservoir and in the coastal areas closest to the dam (O'Farrell et al., 2012). Just like the Salto Grande reservoir (Boltovskoy et al., 2021), the other main rivers in the Rio de la Plata Basin have a high abundance of the golden mussel *L. fortunei* (Fabián et al., 2021; Silva et al., 2021). This species is one of the most harmful macrofoulers for aquatic industries (Pereira et al., 2022).

2.4. Monitoring of environmental and biological data

For each sampling period (23, 46, 70, 94, 118, 142 and 165 h) in surface (0–1 m), the environmental variables: temperature (°C), dissolved oxygen (mg L^{-1}), electrical conductivity (μS cm^{-1}), pH, and turbidity (NTU) were assessed using a multiparameter probe (YSI DSpro). Water transparency (cm) was also evaluated using the Sechi method (30 cm ⌀). Biological variables: Chlorophyll-*a* (mg m^3), suspended solids (mg L^{-1}), phosphate (mg L^{-1}), total phosphorus (mg L^{-1}), total nitrogen (mg L^{-1}), total ammoniacal nitrogen (TAN) (mg L^{-1}), nitrogen dioxide (mg L^{-1}), nitrate (mg L^{-1}), and abundance of *Microcystis* spp. (cell mL^{-1}), *Dolichospermum* spp. (cel mL^{-1}) and cyanobacteria (cel mL^{-1}), were obtained from data collected by the ecology sector of the Environmental Management area of the Mixta Grande Technical Commission (https://www.saltogrande.org/organizacion.php).

2.5. Biofouling analysis

At exposure times of 23, 46, 70, 94, 118, 142 and 165 h, three replicates (three substrates) per treatment were removed for each microfouling (MIC) and macrofouling (MAC) analysis and stored in falcon tubes with 40 mL of 0.4 % sterile saline solution for MIC and tubes containing 40 mL of 4 % formaldehyde for MAC, respectively. Before storing the substrates in the container, they were washed 3x with sterile saline solution (0.4 %) to remove loose material or planktonic organisms from the samples. The MIC samples were stored in the dark and refrigerated at −18 °C, while the MAC samples were refrigerated at −18 °C and processed later. All methodological procedures are summarized in Fig. 2. The exposure time of up to 165 h was chosen because the bacterial biofilm of the biofouling has already reached its maturation stage II, and this is a critical factor for subsequent ecological succession (attachment of micro and macroorganisms) (Agostini et al., 2021a; Ma et al., 2017; Sauer et al., 2022).

2.5.1. Microfouling analysis

Before being stored, for MIC analysis, immediately after collection, the substrates were scraped with the aid of a sterile metal microbiological loop into a falcon tube and manually stirred. For each analysis, 3 replicates were performed per treatment. An aliquot (1.8 mL) of the samples was taken and stored in a cryotube containing paraformaldehyde/glutaraldehyde (1:0.05 %) for approximately 1–2 weeks

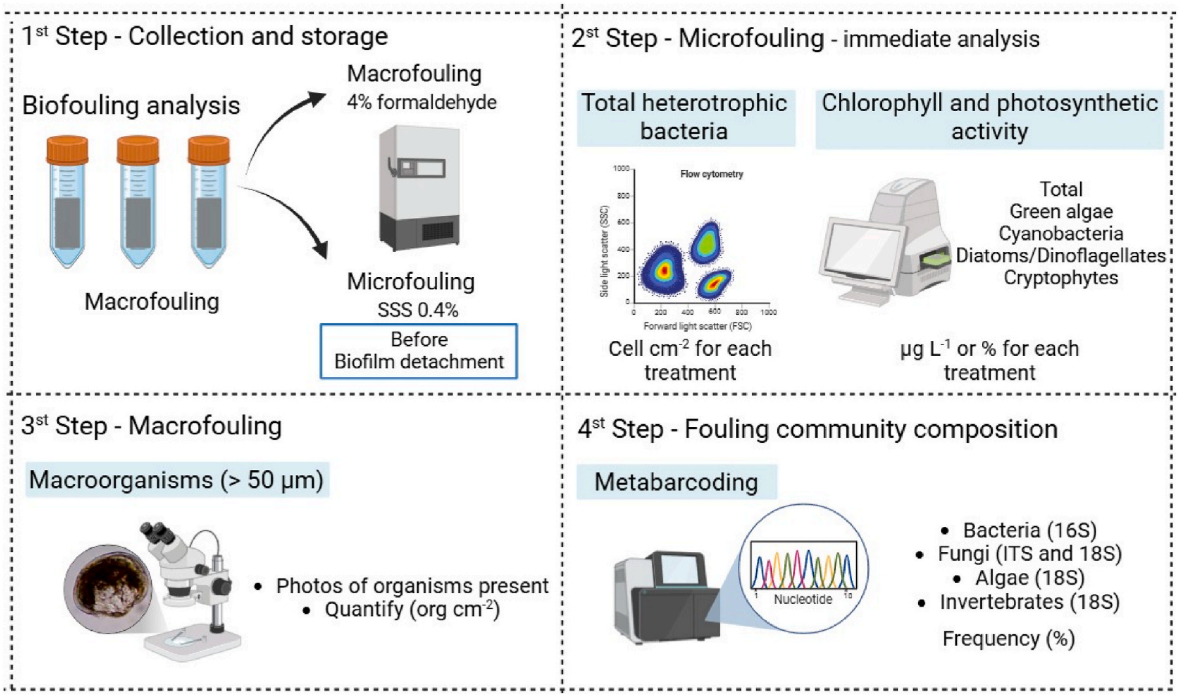

Fig. 2. Scheme of procedures adopted to evaluate the antifouling effect of aqueous extracts of macrophytes in the field.

at −20 °C (Krock et al., 2015), for analysis of total heterotrophic bacteria. Total heterotrophic bacteria were analyzed using flow cytometry, a highly sensitive and rapid method for quantifying bacterial populations (Gasol et al., 1999). Autotrophic organisms were assessed by measuring total chlorophyll and photosynthetic activity, which provide key insights into the productivity and biomass of photosynthetic biofouling communities.

After thawing, samples were sonicated (40 KHz) to improve cell dispersion for 5 min in an SB 3200 DTN ultrasonic cleaner (Krock et al., 2015). Prior to downstream analyses, the cells were stained with SYBR-Green I (SYBR-I, 1:30 dilution of commercial stock; Invitrogen, USA) diluted in dimethyl sulfoxide (DMSO, Merck, Germany) (Marie et al., 2005) in a ratio of 0.001:1 stain:sample for 15 min in the dark at room temperature. The analyses were performed on the Apogee-A40 flow cytometer (Apogee Flow Systems, UK) equipped with argon laser (488 nm). Total bacteria were determined following Krock et al. (2015) by gating and counting cytometric populations events in a scatter plot of 488Green (peak, DNA signal) × 488Red (peak, chlorophyll signal) fluorescence, taking into account the sample volume processed and by using the software FlowJo (v.10.10).The results were expressed as cell cm^{-2} for each treatment.

Subsequently, the remaining samples in the falcon tubes were homogenized for the analysis of autotrophic organisms (in vivo) by measuring total, chlorophyll-*a* total (µg L^{-1}) and photosynthetic activity (%) of green algae, blue-green (cyanobacteria), diatoms/dinoflagellates, cryptophytes with the Algae Online Analyser II (bbe Moldaenke GMBH). Photosynthetic activity was measured by evaluating photosystem II activation.

2.5.2. Macrofouling analysis

To verify the presence of macroorganisms (>50 µm) on the surface of the substrates, three replicates of each treatment were analyzed individually under a stereomicroscope (magnification 40x). To avoid the loss of organisms (principally *L. fortunei* larvae) in the substrate storage solution, the liquid was also analyzed in a Bogorov chamber under the stereoscope. Macroorganisms found were identified, photographed and quantified. The species were identified using specialized literature described by Agostini et al. (2021a, 2019). The presence of macroorganisms in each substrate was used to calculate the quantity (org cm^{-2}) of organisms present in each treatment.

2.6. Fouling community composition

The community composition of fouling organisms was analyzed using metabarcoding techniques for substrates removed at 165 h, which had been stored in sterile 0.4 % saline solution. To investigate the composition of bacteria, fungi, algae and invertebrates, the sequencing of the 16S, ITS and 18S genes was performed by Neoprospecta Microbiome Tecnologia, Brazil. Composite samples (pooled) were made by mixing in equal parts the three replicates per treatment. Amplification were performed with 100 thousand reads with primers 16S rRNA v3/v4 region was performed using 341F (5′-CCTACGGRSGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers (Christoff et al., 2017), ITS1 region amplification was performed using the primers ITS1 (GAACCWGCGGARGGATCA-3′) and ITS 2 (5′-GCTGCGTTCTTCA TCGATGC-3′) (Schmidt et al., 2013), and 18S rRNA v9 region amplification was performed using the 1510r primer (5′-CCTTCYGCAGGT TCACCTAC-3′) (Bradley et al., 2016).

Libraries were sequenced using the NextSeq 1000/2000TM sequencing system (Illumina Inc., EUA) with NextSeq 1000/2000 P1 600-Cycle Kit. The removal of chimeric sequences and grouping of operational taxonomic units (OTU) were performed using UPARSE (Edgar, 2013). Taxonomic identifications were carried out using Blastn v.2.6.0 (Altschul et al., 1990), as well as the reference Silva (v 138.2) (Quast et al., 2012) and Greengenes (De Santis et al., 2006) databases. Rarefaction (normalization) of the metagenomic data (OTUs) was performed using the sample with the lowest number of sequences. Then, based on the relative abundance of sequences in each sample, the frequency of taxa and the richness were calculated for each treatment.

2.7. Chemical characterization of extracts

The chemical composition of the *P. crassipes* and *T. domingensis* extracts was characterized using liquid chromatography (LC-MS) and Fourier transform infrared (FTIR) spectroscopy. LC-MS identified non-volatile and high-molecular-weight compounds, and FTIR provided

functional group analysis. These complementary methods ensured a comprehensive understanding of the extracts' chemical profiles. Results were correlated with antifouling efficacy to identify key bioactive compounds.

For LC-MS, the extracts were resuspended in 30 % acetonitrile (30 % acetonitrile/70 % water). A Shimadzu Shim-Pak GIST HP C18 3 μm 4.5 × 150 mm column equipped with a UV detector was used, using a moving phase gradient of 10 % water and 90 % acetonitrile, both containing 1.1 % formic acid at a flow rate of 1 mL/min. The recorded mass spectra were identified using the standard MASSBANK (http://www.massbank.jp/index-e.html) mass spectra (Tohge and Fernie, 2009). For FTIR, lyophilized samples of the extracts (2 mg) were mixed with 200 mg KBr. Afterwards, the samples were analyzed by Bruker Alpha II infrared spectroscopy (Parameters: ATR Diamond-1 Bounce, 24 background scans, 24 sampling scans, range 4000–400 cm-1 with a resolution of 4 cm-1) and processed with the Opus spectroscopy software. The functional groups of the samples were identified using infrared spectrum tables provided by Sigma-Aldlrich (Naicker, 2024; Sukreem, 2024).

2.8. Statistical analysis

A two-way ANOVA was used for each paint (containing *P. crassipes* or *T. domingensis*), to verify the difference between the treatments (Control A, Control B, 2.5, 5 and 10 g L^{-1}) and the exposure times (23, 46, 70, 94, 118, 142 and 165 h) in the microfouling assays. In all cases, the assumptions of normal distribution of the residues (Shapiro-Wilk test) and homoscedasticity (Levene test) were verified. When alternative hypotheses with a significance level of 95 % were accepted, Tukey's post-hoc test was performed. This method was chosen to simultaneously assess the interaction between treatment types and exposure times, providing a robust framework to address the study's hypotheses regarding the efficacy of antifouling treatments over time and under different conditions. In addition, Hedges'g analysis (method for small samples) was performed in order to show how large the statistical difference is, categorizing the value of g according to Cohen (2013).

Explanatory analyses were performed with the frequency of taxonomic groups (metabarcoding, Bray-Curtis similarity matrix) and total bacteria and autotrophic data (Euclidean distance) through non-metric multidimensional scaling (NMDS). Permutational analysis of multivariate variance (PERMANOVA) was performed to verify differences among groups of treatments for multivariate data. Similarity percentage analysis (SIMPER) was also performed to verify the individual contribution of variables for treatment groups. Statistical analysis was conducted with the use of software Past 4.03.

3. Results

3.1. Monitoring of environmental and biological data

The environmental variables showed minimal variation between the experimental period (Table 1). The water temperature remained at an average of 21.24 °C (±0.56), dissolved oxygen was 8.35 mg L^{-1} (±0.56), chlorophyll-*a* was 9.40 mg m^{-3} (±0.53) and concentrations below the detection level were found for *Microcystis* and *Dolichospermum* species and cyanobacteria (Table 1).

3.2. Microfouling

The microfouling data are summarized in Table 2. The chlorophyll-*a* concentration (μg L^{-1}) of the analyzed groups varied during the exposure time and the treatments (Fig. S1). Total chlorophyll *a* (Fig. S1A–F) and green algae (Fig. S1B–G) increased in concentration from 118 h onwards ($p < 0.05$, Hedge's $g = 1.83$, high effect), diatoms/dinoflagellates (Fig. S1D–I) from 70 h onwards ($p < 0.05$, Hedge's $g = 0.81$, high effect) and the cryptophytes (Fig. S1E–J) from 142 h onwards ($p < 0.05$, Hedge's $g = 1.26$, high effect). Cyanobacteria (Fig. S1C–H) showed a decrease in their concentration between 40 and 70 h ($p < 0.05$, Hedge's $g = 0.65$, moderate effect), but then increased from 94 h onwards ($p < 0.05$, Hedge's $g = 1.43$, high effect). The diatoms/dinoflagellates showed a higher concentration of chlorophyll in relation to the other groups analyzed (Fig. S1D–I).

Table 1
Mean ± SD of environmental and biological variables at the point where the field experiment was carried out. <LQ = below detection limit. TAN = total ammoniacal nitrogen; DL = detection limit. Environmental variables were measured daily throughout the sampling period, while biological variables were evaluated once a week.

Environmental variables	Mean	SD	Biological variables	Mean	SD
Temperature (°C)	21.24	0.56	Chlorophyll-*a* (μg L^{-1})	9.40	0.53
Dissolved oxygen (mg L^{-1})	8.35	0.18	*Microcystis* spp. (cell mL^{-1})	<DL	<DL
Electrical conductivity (μS cm^{-1})	66.14	1.77	Cyanobacteria (cell mL^{-1})	<DL	<DL
pH	7.68	0.09	*Dolichospermum* spp. (cell mL^{-1})	<DL	<DL
Turbidity (NTU)	11.58	1.03			
Water transparency (cm)	87	7			
Suspended solids (mg L^{-1})	5.70	0.50			
Total phosphorus (mg L^{-1})	0.04	0.00			
Total nitrogen (mg L^{-1})	0.63	0.16			
Phosphate (mg L^{-1})	0.03	0.01			
TAN (mg L^{-1})	0.12	0.02			
Nitrogen dioxide (mg. L^{-1})	<DL	<DL			
Nitrate (mg. L^{-1})	0.38	0.32			

Table 2
Summary of the effects on biofouling on substrates painted with aquatic macrophyte *Pontederia crassipes* and *Typha domingensis* extracts at the end of the experiment (165 h). - = decrease, + = increase in comparison to control B.

Endpoints	*P. crassipes* (g L^{-1})			*T. domingensis* (g L^{-1})		
	2.5	5	10	2.5	5	10
Total *chlorophyll a* (*μg. L^{-1}*)	no effect	–	–	no effect	+	no effect
Green algae chlorophyll *a*	–	–	no effect	no effect	–	+
Cyanobacteria chlorophyll *a*	–	–	–	+	no effect	no effect
Diatoms/ Dinoflagellates chlorophyll *a*	+	no effect	no effect	+	+	no effect
Cryptophytes chlorophyll *a*	+	+	+	+	+	no effect
Total heterotrophic bacteria (cells cm^2)	+	–	+	no effect	+	no effect
Presence of L. *fortunei* larvae	Present	present	absent	absent	absent	absent
Frequency (%); richness						
Algae	-;-	-;-	-;-	-;-	-;-	-;-
Bacteria	+;-	+; no effect	+;-	+;-	+; +	+; +
Fungi	+;-	+;-	+;-	+;-	+;-	+;-
Presence of Mollusca (*Sinomytilus harmandi*)	Absent	+	Absent	absent	absent	+

For *P. crassipes* extracts, at the end of the experiment (165 h), the concentration of total chlorophyll *a* (Fig. S1A) was lower in the 10 g L^{-1} treatment ($p < 0.05$, Hedge's $g = 0.89$, high effect), while the chlorophyll *a* of green algae was lower in the 2.5 g L^{-1} treatment compared to control B (Table 2; Fig. S1B) ($p < 0.05$, Hedge's $g = 0.67$, moderate effect). For cyanobacteria chlorophyll (Fig. S1C), there was a decrease with the increase in extract concentrations ($p < 0.05$, Hedge's $g = 1.65$, high effect). For the diatoms/dinoflagellates chlorophyll (Fig. S1D) there was an increase in the 2.5 g L^{-1} treatment compared to the control B treatment ($p < 0.05$, Hedge's $g = 0.42$, small effect) and for cryptophytes chlorophyll *a* (Fig. S1E) there was an increase for all extracts concentration ($p < 0.05$, Hedge's $g = 1.86$, high effect), particularly for 5.0 g L^{-1}.

Following treatments with varying concentrations of *T. domingensis* extracts, and comparison to the control group B at 165 h (Table 2), the total chlorophyll *a* (Fig. S1F) increased in the 5.0 g L^{-1} ($p < 0.05$, Hedge's $g = 1.34$, high effect), the green algae chlorophyll *a* (Fig. S1G) decreased in the 5 g L^{-1} treatment but increased with the 10 g L^{-1} treatment ($p < 0.05$, Hedge's $g = 1.70$, high effect), and the cyanobacteria chlorophyll *a* (Fig. S1H) increased with the 2.5 g L^{-1} treatment ($p < 0.05$, Hedge's $g = 0.98$, high effect). For diatoms/dinoflagellates (Fig. S1I) and cryptophytes (Fig. S1J), both 2.5 and 5.0 g L^{-1} treatments yielded an increase compared to the control B treatment ($p < 0.05$, Hegde's $g = 0.90$ and 1.20 respectively, high effect).

Photosynthetic activity was detected only for the diatoms/dinoflagellates group for treatments containing *P. crassipes* and *T. domingensis* extracts from 118 h onwards and for control B treatment at 165 h only. There was no significant difference in photosynthetic activity between treatments with *P. crassipes* and *T. domingensis* extracts and control B at 165 h ($p > 0.05$) (Table S1).

A general increase in total heterotrophic bacterial counts (cells cm^{-2}) was observed starting at 70 h ($p < 0.05$, Hedge's $g = 0.80$ – high effect). This was followed by a decline at 142 h, before rising again at 165 h ($p < 0.05$, Hedge's $g = 0.77$, moderate effect) (Fig. 3 and Table 2). For the *P. crassipes* treatments (Fig. 3A), during the 165-h period, the 2.5 and 10 g L^{-1} treatments showed a significant increase in total bacteria ($p < 0.05$, Hedge's $g = 0.65$ and 0.70 respectively, moderate effect) compared to the control B treatment, while the 5 g L^{-1} treatment exhibited a significant decrease ($p < 0.05$, Hedge's $g = 0.85$, high effect). For the *T. domingensis* treatments (Fig. 3B), the 2.5 and 10 g L^{-1} treatments showed no significant change compared to the control treatment ($p > 0.05$), whereas the 5 g L^{-1} treatment resulted in a significant increase in total bacteria ($p < 0.05$, Hedge's $g = 1.28$, very high effect).

3.3. Macrofouling

Regarding macro-organisms, a single pediveliger larva of *L. fortunei* was observed at 165 h in the 2.5 g L^{-1} *P. crassipes* treatment (Fig. 4A), while an umbonate larva of *L. fortunei* was found at the end of the experiment in the 5 g L^{-1} *P. crassipes* treatment (Fig. 4B). No representatives of macro-organisms were found in the other treatments or exposure times (Table 2). Additionally, the org cm^{-2} ratio for each treatment is shown in Fig. S2, where at 165 h, the *P. crassipes* 2.5 and 5 g L^{-1} treatments exhibited the highest value of 0.08 org cm^{-2} (Fig. S2).

3.4. Fouling community composition

A total of 12 taxa were recorded, with the bacteria, fungi and algae being the most representative of all samples (Fig. 5A). The control B treatment presented greater richness with 83 taxonomic groups, while the 10 g L^{-1} *P. crassipes* and 2.5 g L^{-1} T. *domingensis* treatments were less rich with only 20 groups each (Fig. 5A). Among bacteria (Fig. 5B), Bacilli were the most frequent for all treatments, followed by Desulfovibrione and Alphaproteobacteria. The 2.5 g L^{-1} T. *domingensis* and 10 g L^{-1} *P. crassipes* treatments exhibited lower richness compared to the control B treatment (Fig. 5B).

For fungi, eight different classes were identified, with Microbotryomycetes, Eurotiomycetes and Tremellomycetes being the most frequent (Fig. 5C). The control B treatment exhibited the highest richness with seven distinct classes, while the 10 g L^{-1} T. *domingensis* treatment demonstrated the lowest richness, containing only three classes, with Microbotryomycetes being highly predominant (Fig. 5C). Regarding algae (Fig. 5D), the Bacillariophyceae class was the most common across all treatments, and was the sole class found in the 10 g L^{-1} *P. crassipes*, and the 2.5 and 10 g L^{-1} T. *domingensis* treatments (Fig. 5D). The control B treatment again displayed the greatest richness, with 38 taxonomic groups, while all the other treatments showed a reduction in richness (Fig. 5D). The abundance of taxa for each treatment is detailed in Table S2.

3.5. Biofouling community analyses

The NMDS analysis of taxa abundance (Fig. S3A) identified eight distinct groups for the tested treatments (STRESS = 0.17), which were supported by the PERMANOVA results ($p < 0.001$). Notably, similarities were observed between the 5 with 10 g L^{-1} T. *domingensis* treatments, as well as between control A and 5 and 10 g L^{-1} *P. crassipes* treatments (Fig. S3A). Bacillariophyceae, Microbotryomycetes and Bacilli contributed 14.02 %, 11.60 % and 11.41 %, respectively, to the formation of

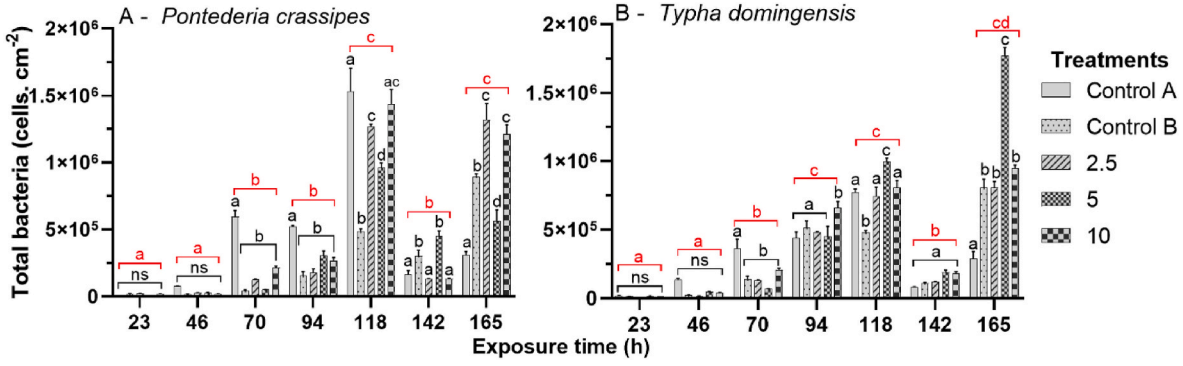

Fig. 3. Mean (±SD) of total bacteria (cell cm^{-2}) of substrates painted with different treatments of aquatic macrophytes *Pontederia crassipes* and *Typha domingensis*. Different letters within each exposure time – statistical difference between concentrations ($p < 0.05$); Different letters for each exposure time (in red) – statistical difference between exposure times ($p < 0.05$); ns – did not show significant difference ($p > 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

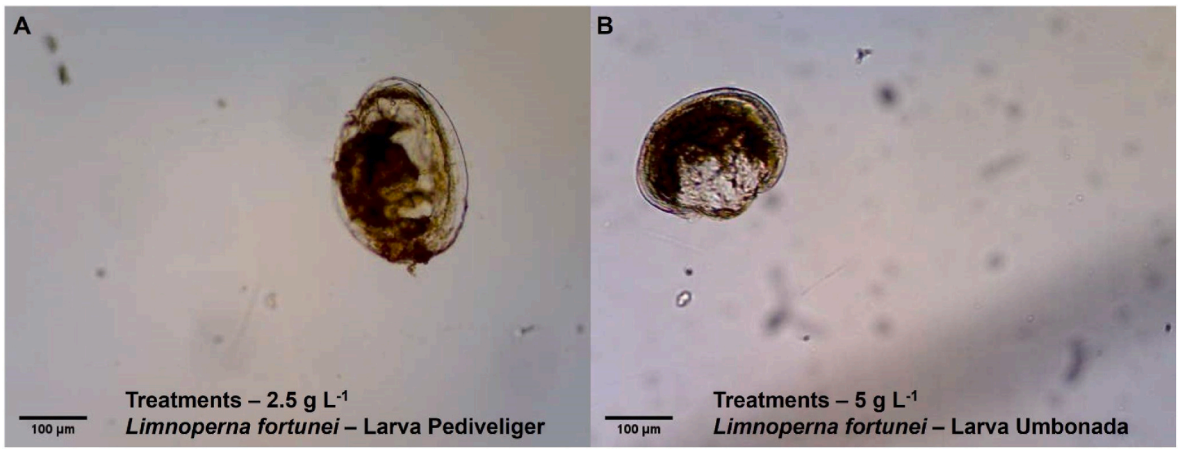

Fig. 4. Macroorganisms found in biofouling samples of different treatments of *Pontederia crassipes* at the exposure time of 165 h by stereoscope microscopy.

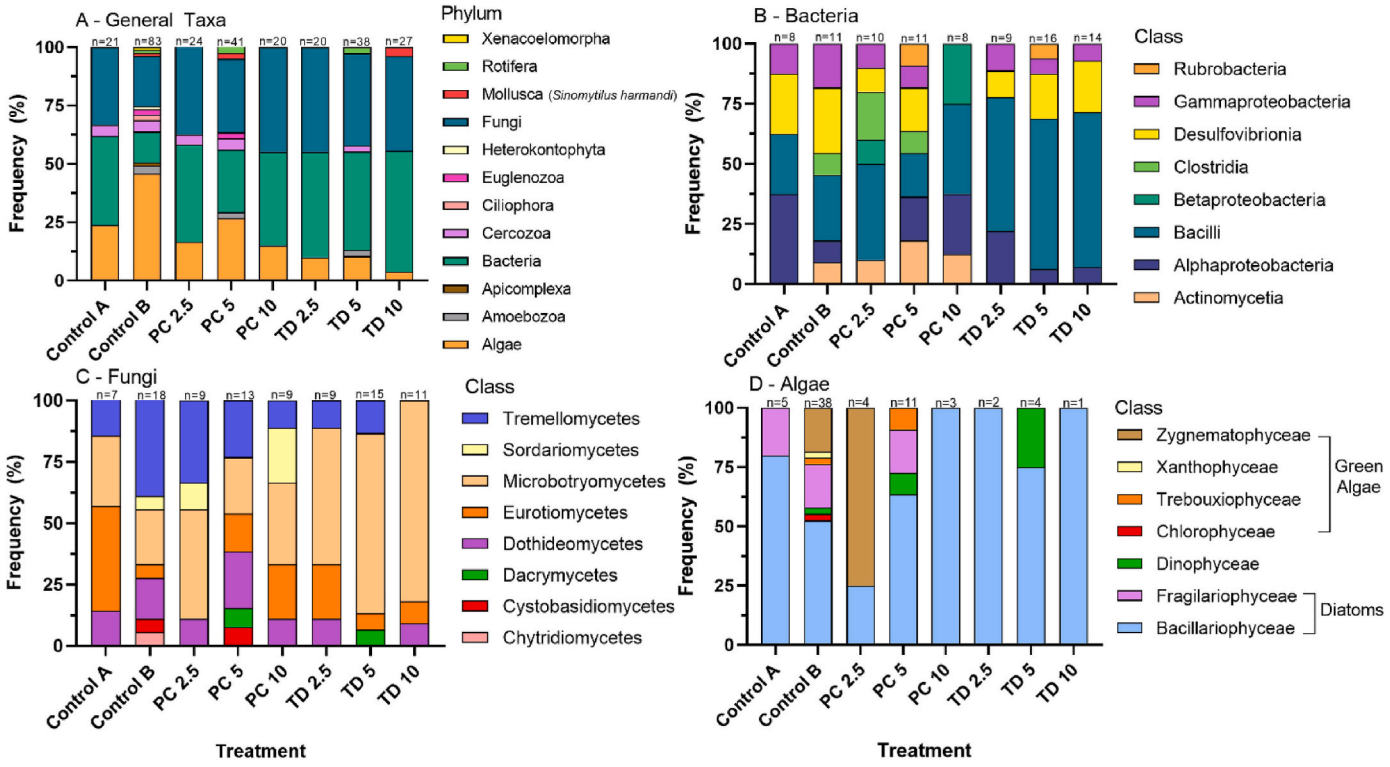

Fig. 5. Frequency of taxa (%) in biofouling samples of different treatments at the exposure time of 164 h. A – 16S, 18S and ITS rRNA, B – 16S rRNA, C – ITS and 18S rRNA, D – 18S rRNA, n – number of taxa in each treatment, PC – *Pontederia crassipes*; TD – *Typha domingensis*.

these groups (Table S3A). In the NMDS analysis of total bacteria and chlorophyll data (Fig. S3B), eight groups were also identified (STRESS = 0.05), with PERMANOVA confirming the result ($p < 0.001$). Similarities were observed between the treatments of 2.5 g. L^{-1} *T. domingensis*, 2.5, 5 and 10 g L^{-1} *P. crassipes*, and control B (Fig. S3B). In this case, total chlorophyll *a* and chlorophyll *a* from diatoms/Dinophyceae contributed 55.25 % and 32.06 %, respectively, to the formation of this grouping (Table S3B).

3.6. Chemical characterization of extracts

For the LC-MS analysis, the three main compounds identified in the *T. domingensis* extract were 4-methylphenethylamine (55.45 %), D-cysteine (15.59 %), and L-lysine hydrochloride (10.73 %) (Table S4). In the *P. crassipes* extract, the primary compounds were 4-methylphenethylamine (63.00 %), betaine-aldehyde (18.21 %), and carbamoyl-DL-aspartic acid (14.81 %) (Table S4). Detailed data, including retention times and chromatographic peaks, are provided in the supplementary material (Table S5 and Fig. S4).

FTIR analysis revealed distinct functional groups for the two extracts (Table S6). The *T. domingensis* extract contained functional groups such as amine, alcohol, aliphatic nitro, aromatic ether, and halo compounds. In contrast, the *P. crassipes* extract primarily exhibited functional groups including alcohol, halo compound, nitro compound, aromatic ether, halide, and alkane.

4. Discussion

Current antifouling alternatives primarily aim to inhibit bacterial biofilm formation, a critical early step that facilitates the adhesion of

other organisms during the successional process of biofouling (Agostini et al., 2021a). In this study, the incorporation of *P. crassipes* and *T. domingensis* extracts into the epoxy coating effectively inhibited both heterotrophic and autotrophic components of microbial biofilms. However, this biofilm inhibitory effect diminished by the end of the experiment (165 h), which might result from a decline in the activity of bioactive compounds. It is important to stress that the half-life of the bioactive present in the extracts is unknown, and loss of efficacy cannot be ruled out. Future studies should incorporate stability testing under various environmental conditions to better understand the degradation rates and persistence of these bioactive compounds. It is also possible that biofilms, once developed and mature, confer protection against chemicals and other abiotic stresses (Chattopadhyay et al., 2022; Davey and O'toole, 2000). This may also have happened since the time of 165h the biofilm is already in its maturation stage II, where its maximum thickness is reached and contains a stable microbial community, in which the extracellular polymer matrix and QS are already well established (Ma et al., 2017; Sauer et al., 2022, 2002). These findings underscore the need to investigate bioactive compound stability and delivery mechanisms to sustain long-term antifouling effects.

Treatments with *P. crassipes* extracts demonstrated greater inhibition of heterotrophic bacterial density, and autotrophic bacteria and algae chlorophyll compared to *T. domingensis* extracts. Laboratory studies attribute this effect to its chemical profile, which likely interferes with bacterial biofilm formation and bacterial quorum sensing pathways (Morales et al., 2024b). Autotrophic bacteria, primarily blue-green cyanobacteria, were the first to exhibit growth; however, their proliferation slowed and then resumed. The same pattern was observed for total bacteria populations, indicating that cyanobacteria likely constituted most of the bacteria via flow cytometry. When compared to control A, treatments with macrophyte extracts delayed the lag phase of microbial growth. Specifically, growth commenced at 94 h on *P. crassipes* coatings and at 70 h for *T. domingensis* coatings. The lag phase represents a period of microbial latency without stable growth, which is then followed by the exponential (log) growth phase (Reischke et al., 2015, 2014). This delay in microbial growth could have practical implications for biofouling management by providing an extended window of time to implement antifouling strategies or treatments before biofilm formation becomes more resistant to interventions. Besides, the rapid lag phase of *T. domingensis* can be explained by the presence of more nutrients in its extract, when compared to *P. crassipes* extract. However, we did not analyze the composition of nutrients present in these extracts, and further studies related to this topic are needed.

Substrates with *P. crassipes* and *T. domingensis* extract-epoxy coatings demonstrated a dose-dependent inhibition for cyanobacteria (blue-green chlorophyll), which was not observed for other photosynthetic organisms. This cyanobacteria inhibition effect is consistent with competition mechanisms reported in prior studies (Neilen et al., 2017; Yu et al., 2019). Chemical compounds in macrophyte extracts can alter cell morphology and enzymatic activity or disrupt photosynthetic machinery by reducing chlorophyll-a and damaging photosystem II (Eigemann et al., 2013; Liu et al., 2021; Tan et al., 2019). However, in our study, due to the low concentration of chlorophyll, it was not possible to detect the photosynthetic activity of cyanobacteria and other taxa. Therefore, to confirm the action of the extracts on the mechanisms of photosynthetic activity, more tests must be performed. However, it is possible to speculate that such effects of the extracts may have inhibited the cyanobacteria by decreasing blue-green chlorophyll, due to possible damage to photosystem I and/or II, effects on decreasing chlorophyll synthesis and/or blocking electron transfer, which may influence adenosine triphosphate (ATP) synthesis (Tan et al., 2019).

Our study documented a distinct succession pattern: autotrophic (cyanobacteria) and heterotrophic bacteria initially proliferated, followed by diatoms (70 h), green algae (118 h), and cryptophytes (142 h). This sequential colonization aligns with reports that diatoms pave the way for adhesion of other photosynthetic organisms (França et al., 2011), with their ability to produce adhesive EPS. Variations in successional biofilm stages can arise from interspecies competition and environmental factors (Zhu et al., 2024), emphasizing the complexity of biofilm dynamics. The initial 48-h phase of biofouling is critical for surface conditioning and nutrient adsorption, and together with temperature strongly influences bacterial adhesion and biofilm development (Rao, 2010). Rao (2010) observed a positive relationship between the concentration of chlorophyll and fouling algae, and between particulate matter and biofouling organisms. Though this was not the primary focus of our study, the environmental variables exhibited minimal variation and remained similar with previous studies in these systems (Boltovskoy et al., 2021). Still in this context, we emphasize that understanding the influence of environmental factors on the dynamics of biofilm formation may be essential for designing antifouling strategies targeting initial succession stages of biofouling.

The extracts of *P. crassipes* and *T. domingensis* reduced the microbial taxonomic richness while altering the taxonomic composition of bacterial, fungal and algal communities. This pattern has previously been observed on surfaces coated with chemical substances, where a decrease in the taxonomic richness of bacterial biofilm communities has been noted (Agostini et al., 2021a; Ammon et al., 2018; Flach et al., 2017). Bacterial communities showed increased frequency of Bacilli, Alphaproteobacteria, and Desulfovibrionia while experiencing decreases of Betaproteobacteria and Gammaproteobacteria. These changes reflect tolerance to environmental stressors such as heavy metals and elevated temperatures (Huang et al., 2024a, 2024b; Keshri et al., 2024).

In the marine environment, Betaproteobacteria and Gammaproteobacteria have a high positive correlation with the settlement of macro-organisms, especially the genus of *Littorinidae* and *Amphibalanus* sp. (Agostini et al., 2021a). Proteobacteria are recognized for their high diversity and abundance in natural and artificial substrates (Agostini et al., 2021a; Bergo et al., 2021; Morales et al., 2024b), being considered the primary colonizers of substrates (Dang and Lovell, 2016; Salta et al., 2013). Thus, inhibiting the bacterial adhesion of this group of bacteria is extremely relevant for the development of new antifouling agents, as noted in Fig. 6, bacterial inhibition of some taxonomic groups can lead to inhibition of the rest of the successional process of biofouling. However, we emphasize that studies to understand the dynamics of the successional process of biofouling in freshwater environments are incipient.

Also in this same context, the anti-biofilm effects of *P. crassipes* and *T. domingensis* extracts on Gammaproteobacteria were previously reported by (Morales et al., 2024b). The response of Gammaproteobacteria was particularly noteworthy, as this group usually thrives on surfaces coated with antifouling agents (Ammon et al., 2018; Flach et al., 2017). Contrary to these trends, our study observed a decline in Gammaproteobacteria, often resilient to antifouling agents, potentially linked to the unique bioactive composition of macrophyte extracts.

Most biofouling research predominantly focuses on bacteria or invertebrates (Sérvulo et al., 2023; Watson et al., 2016), with studies on fungi and algae remaining relatively under-explored, particularly at the class level responses. This study, however, demonstrated significant effects not only on the richness and frequency of bacterial communities but also on fungi and algae. These effects may be attributed to bacterial inhibition, which potentially facilitated the proliferation of opportunistic organisms (Agostini et al., 2021a). For instance, an increase in the frequency of Microbotryomycetes and diatoms (Fragilariophyceae and Bacillariophyceae) alongside a decrease in Dothideomycetes and green algae was observed. The predominance of diatoms, as indicated by metabarcoding data and chlorophyll concentrations, can be attributed to their opportunistic nature, strategic adaptability and specialized traits that facilitate their adhesion, making them the most dominant benthic organisms on substrates (Felisberto and Rodrigues, 2012; França et al., 2011).

The inhibition of bacterial richness observed in the biofilm could also contribute to reduced adhesion of macro-organisms. This suggests that

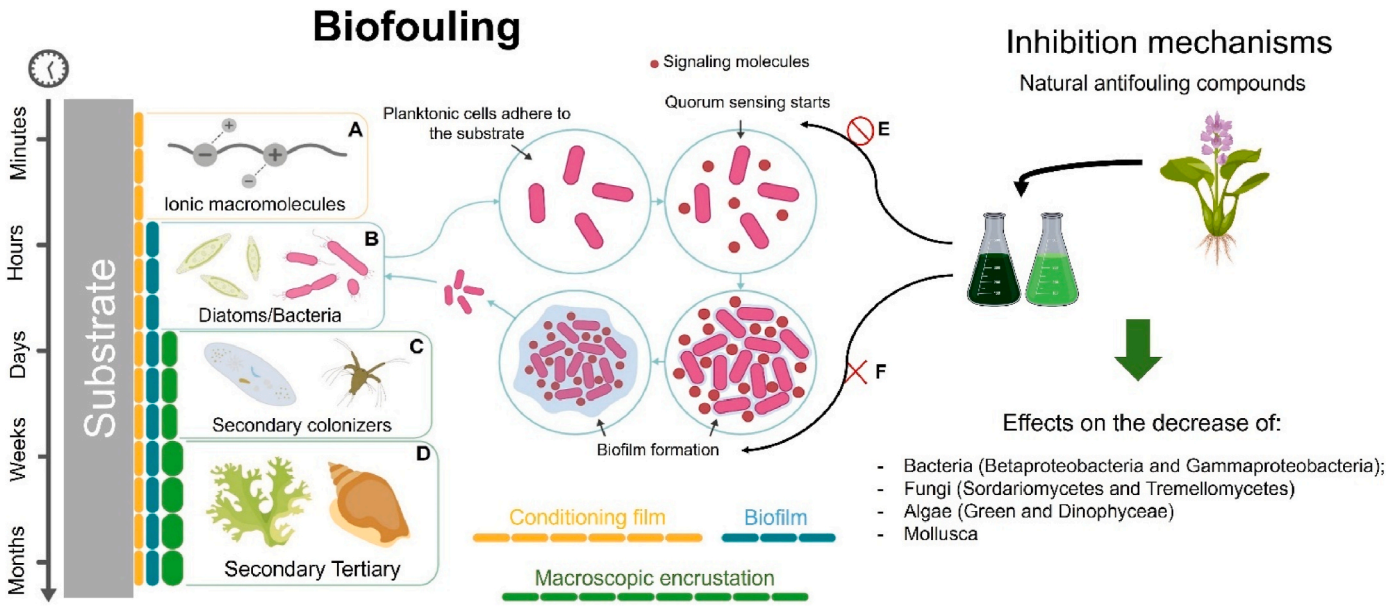

Fig. 6. Conceptual model of the biofouling process and inhibition mechanism of aquatic macrophyte extracts. A – Conditioning film; B – Biofilm formation; C and D – Macroscopic fouling by secondary and tertiary colonizers; E – Blocking the start of Quorum Sensing; F – Inhibition of biofilm formation.

surfaces treated with chemical coatings may foster distinct biofilm communities, which in turn influence the selection of unique macro-organism communities (McNamara et al., 2009). Agostini et al. (2021a) highlighted the positive association between Gammaproteobacteria and macrofouling. In our study, we observed minimal adhesion of macroorganisms (0.08 org. cm^{-2}) on substrates treated with *P. crassipes* at concentrations of 2.5 and 5 $g.L^{-1}$ over 165 h. This could be possibly explained by the effects on reducing Gammaproteobacteria frequency. Another plausible explanation is the low environmental abundance of *L. fortunei* larvae during the study period, as suggested by the absence of macro-organisms even in control treatments. Additionally, it is worth mentioning that during the experimental period, precipitation was moderate, ranging from 0 to 11 mm (Salto, 2025). Thus, further testing is needed to confirm larval abundance in the environment. It is important to note that the experiment coincided with a period typically marked by high larval abundance of *L. fortunei* in the Salto Grande reservoir (Boltovskoy et al., 2021; Cataldo et al., 2022, 2012).

Substrates coated with epoxy paint but without extracts were more prone to biofouling compared to uncoated stainless steel. This finding suggests that the chemical and physical properties of epoxy paint, such as its surface roughness or chemical composition, may inadvertently enhance the colonization of biofouling organisms. Zinc present in low concentrations in stainless steel is essential for bacterial biofilm metabolism, enhancing its attractiveness for organisms (Bongo et al., 2010). However, zinc at high concentrations can be toxic to organisms (Eisler, 1998). Additionally, the surface roughness of stainless steel facilitates the adsorption and absorption of organic and inorganic particles, further facilitating colonization (Patil and Anil, 2005). Therefore, incorporating bioactive compounds, such as macrophyte extracts, into epoxy coatings could mitigate this effect, guiding the design of more effective antifouling paints that balance structural integrity with reduced susceptibility to fouling.

Epoxy bases, a category of polymeric materials containing high molecular weight binders, are often utilized for contact leaching coatings (Pereira et al., 2024). These materials form the basis for antifouling additives (Pereira et al., 2024; Vijayan et al., 2022), due to their mechanical strength, chemical resistance, and anti-corrosive properties, as well as their potential to continuously release antifouling agents (Vijayan et al., 2022). However, the organic solvents used in epoxy paints are currently being discussed in the literature (Akhter et al., 2025; Dey and Shetranjiwalla, 2025; Pereira et al., 2024), such as the toluene and xylene present in the epoxy paint used in the present study. As much as these solvents are being used in biodegradable polymeric coatings (Pereira et al., 2024), they are volatile and contribute to the formation of toxic volatile organic components (Dey and Shetranjiwalla, 2025). However, its cost is justified due to the fast-drying time in its applicability (Dey and Shetranjiwalla, 2025). Thus, as much as this is still debated, we suggest the use of another type of epoxy paint to add the antifouling agent based on *P. crassipes* or *T. domingensis*.

Treatments using epoxy paint with 5 g L^{-1} of *P. crassipes* were more effective in mitigating biofouling compared to those with *T. domingensis*. The lack of a clear dose-dependent response can be attributed to the survival of certain microbial taxa at both higher and lower concentrations, while intermediate concentrations lead to a reduction in their density. The observed dose-dependent non-response can be explained by the fact that some microbial taxa survive at higher and lower concentrations, and at intermediate concentrations end up decreasing their density, also known as the eagle effect (Eagle, 1948; Eagle and Musselman, 1948; Prasetyoputri et al., 2019). This phenomenon highlights the complexity of designing antifouling treatments that balance concentration and efficacy, as suboptimal concentrations might inadvertently select for resistant taxa while reducing the density of others. Addressing this effect requires detailed studies to determine optimal dosing strategies that minimize biofouling in the field, without fostering resilience among microbial communities. For example, field antifouling studies should focus on testing a wide range of concentrations of the treatments tested to verify the effective concentration for biofouling reduction. Besides, in antifouling tests with plant extracts, this effect had already been documented by Agostini et al. (2020, 2019a).

Furthermore, the chemical compounds found in macrophyte extracts may have contributed to the biofouling inhibition observed. Both extracts contained 4-methylphenethylamine, which are recognized for their antimicrobial properties (Rambaran et al., 2024) and are commonly found in high concentrations in aquatic macrophyte extracts like *Cabomba caroliniana* and *Schoenoplectus californicus*, known for their antifouling properties (Morales et al., 2025). In addition, other compounds in lower concentrations in our extracts (*P. crassipes* and *T. domingensis*) are also highlighted in the literature for having antimicrobial, antifungal, antioxidant, and anti-inflammatory activity, as discussed in Table S7. While these compounds likely contributed to the

observed antifouling effects, mainly related to QS inhibition and biofilm formation (Fig. 6), further research is required to clarify their specific roles in biofouling inhibition. It is worth noting that the inhibition of bacterial biofilm, either by direct inhibition of biofilm formation or inhibition of QS, is indicated as an antifouling strategy, due to the biofilm being a prerequisite for the attachment of macrofouling species (Sathicq et al., 2025).

Additionally, the primary chemical groups in the extracts were predominantly alkanes and alcohols, which are integral plant constituents with significant roles in plant physiology. These compounds are often linked to plant-herbivore interactions, such as attracting or repelling pollinators (Lal and Biswas, 2023). Identifying the compounds present in the extracts is a fundamental step in the development of new antifouling agents, as it lays the groundwork for scaling up production and translating these findings into industrial applications. So isolated compounds with high bioactivity can be combined, being possible to increase the antifouling efficacy by combining one or more extracts in different concentrations to cause a synergistic effect with different combinations. To enhance efficiency, isolated compounds could be incorporated directly into paint formulations, advancing the development of a final antifouling product (Hamidi et al., 2022). Antifouling efficacy could be optimized by using organic solvents such as ethanol, ethyl acetate, or methanol, for extraction. These solvents target both polar compounds and lipophilic and hydrophilic molecules, ensuring a broader range of active compounds is extracted (Hamidi et al., 2022).

Traditional antifouling paints based on biocides and co-biocides (e.g., Diuron and DCOIT) have limitations, such as lower efficacy in advanced successional stages of biofouling (Agostini et al., 2019a) and high toxicity to non-target organisms (Soroldoni et al., 2018). On the contrary, natural antifouling alternatives offer hope for replacing traditional biocides, providing efficient biofouling control with reduced environmental impact (Nalini et al., 2025). Such as low and/or non-toxicity to non-target organisms and inhibition of biofouling in its early stage (biofilm formation), as it influences the rest of the biofouling successional process. However though the search for environmentally friendly antifouling alternatives, particularly those derived from natural plant compounds has increased over the years (Agostini et al., 2021b; Hamidi et al., 2022), research on natural compounds from aquatic macrophytes remains limited (Morales et al., 2025, 2024b; 2024a).

Due to the strong competition of resources in the aquatic environment, throughout their evolution, macrophytes began to produce chemical compounds capable of influencing the establishment and growth of other organisms (Gross, 2003). These compounds are mainly related to aspects of defense against herbivory and are reported to contribute to playing key ecological roles in structuring the aquatic environment (Pereira et al., 2021). Despite the knowledge that the chemical defenses of these plants are extensive, they remain unexplored (Gross and Bakker, 2012), mainly related to antifouling activity. In fact, the search for ecologically correct antifouling alternatives derived from macrophytes is explored little (Morales et al., 2025, 2024a; 2024b), and attention has so far focused only on terrestrial plants.

There are still a few studies focused on evaluating the antifouling efficacy of natural plant compounds in real-world field conditions (Pérez et al., 2021). Our study is the first to test the antifouling activity of aquatic macrophyte extracts mixed with epoxy paint in a natural environment. Especially in freshwater environments, where the development of new antifoulants to prevent biofouling is urgent (Sathicq et al., 2025). This field validation strengthens the case for using natural extracts in antifouling technologies by demonstrating their efficacy under real-world environmental conditions, thereby bridging the gap between laboratory research and practical application. The findings from our research not only enhance the understanding of the antifouling potential of aquatic macrophytes but also open possibilities for developing new environmentally friendly and sustainable antifouling solutions.

5. Conclusion

Our study is the first to assess the antifouling efficacy of aquatic macrophyte extracts in combination with epoxy coating under natural environmental conditions. Over a 165-h period, the treatments with 5 and 10 g. L^{-1} of *P. crassipes* extract demonstrated the most significant effectiveness in combating biofouling, with a significant reduction for total bacteria (34 %), cyanobacteria – blue-green chlorophyll (55 %), total chlorophyll (44 %) and macroorganisms. Biofouling inhibition was observed across all groups analyzed, with reductions in bacterial biofilm, chlorophyll content, and the frequency of certain taxa (Mollusca, Clostridia, Betaproteobacteria, Gammaproteobacteria, Tremellomycetes, Sordariomycetes, green algae and Dinophyceae).

The results highlight *P. crassipes* as a promising candidate for the development of new environmentally friendly antifouling paints based on natural products, offering new insights into the potential of such antifouling paints. Thus, as observed in the conceptual model proposed in Fig. 6, our extracts can act in the inhibition of biofilm formation and in the blocking and/or inhibition of quorum sensing, triggering a cascade inhibition for different taxonomic groups. The antifouling effect was promising over the 165-h observation period. However, to better understand the long-term viability, further studies are needed to determine the half-life of the antifouling activity and assess the durability of these paints' effects. We also recommend conducting extended field tests (minimum of one year), to establish a more comprehensive understanding of the long-term effectiveness and durability of these coatings. Thus, as tests to verify the properties/processes of compounds including: Kow, Kd, Koc, H, volatilization, speciation and hydrolysis, as well as the performance of toxicological tests against other non-target organisms, expanding their knowledge about their impact.

To enhance the efficacy of the treatment, we suggest isolating and purifying the antifouling compounds present in the extracts through chromatographic techniques. This would allow for more efficient incorporation of these active compounds into the epoxy base, maximizing the paint's effectiveness. Aquatic macrophytes like *P. crassipes* represent a promising avenue for the development of sustainable antifouling solutions, offering a fresh perspective on the future of antifouling paints. In addition, this species is considered an invasive plant in many locations, and its antifouling benefit can help in its management for biotechnological use, in addition to presenting rapid growth and consequently ease of acquisition of biomass for development on an industrial scale.

CRediT authorship contribution statement

Mikael Luiz Pereira Morales: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Leandro Capurro:** Writing – review & editing, Investigation, Formal analysis. **Facundo Bordert:** Writing – review & editing, Methodology, Investigation. **Hafizah Chenia:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis. **Cecília Alonso:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Fabiana Rey Bentos:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation. **Lucía Boccardi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation. **Ernesto Brugnoli:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation. **Ng Haig They:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation. **Vanessa Ochi Agostini:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation. **Grasiela Lopes Leães Pinho:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Co-author Ng Haig They received research support from the National Council for Scientific and Technological Development – CNPq (Process 404233/2021-0). Co-author Grasiela Lopes Leães Pinho received research support from the Mixed Technical Commission of Salto Grande and Latitud (Uruguayan Technological Laboratory Foundation – LATU).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126952.

Data availability

Data will be made available on request.

References

Agostini, V.O., Macedo, A.J., Muxagata, E., Da Silva, M.V., Pinho, G.L.L., 2020. Non-toxic antifouling potential of caatinga plant extracts: effective inhibition of marine initial biofouling. Hydrobiologia 847, 45–60. https://doi.org/10.1007/s10750-019-04071-6.

Agostini, V.O., Macedo, A.J., Muxagata, E., Da Silva, M.V., Pinho, G.L.L., 2019a. Natural and non-toxic products from Fabaceae Brazilian plants as a replacement for traditional antifouling biocides: an inhibition potential against initial biofouling. Environ. Sci. Pollut. Res. 26, 27112–27127. https://doi.org/10.1007/s11356-019-05744-4.

Agostini, V.O., Macedo, A.J., Muxagata, E., Pinho, G.L.L., 2019b. Surface coatings select their micro and macrofouling communities differently on steel. Environ. Pollut. 254, 113086. https://doi.org/10.1016/j.envpol.2019.113086.

Agostini, V.O., Muxagata, E., Pinho, G.L.L., Pessi, I.S., Macedo, A.J., 2021a. Bacteria-invertebrate interactions as an asset in developing new antifouling coatings for man-made aquatic surfaces. Environ. Pollut. 271, 116284. https://doi.org/10.1016/j.envpol.2020.116284.

Agostini, V.O., Pinho, G.L.L., Muxagata, E., Macedo, A.J., Bentos, F.R., Boccardi, L., Dabézies, M.J., Oliveira, E.B., 2021b. Pinturas Antiincrustantes Derivadas De Plantas Terrestres Una Solución Segura Para El Ambiente En El Control De La Bioincrustación, 22. INNOTEC. https://doi.org/10.26461/22.01.

Akhter, P., Arshad, A., Hussain, M., 2025. A review on environmental impacts of paints and strategies for producing eco-friendly-paints. Int. J. Environ. Sci. Technol. 22, 555–578. https://doi.org/10.1007/s13762-024-05760-z.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Amarilla, S., Samudio-Oggero, A., Nakayama, H.D., Avalos, C., Méndez, C., Ries, A., 2024. Development of a protocol with Typha domingensis Pers. for the treatment of wastewater from paper recycling. CSCEE 9, 100628. https://doi.org/10.1016/j.cscee.2024.100628.

Ammon, U., Wood, S.A., Laroche, O., Zaiko, A., Tait, L., Lavery, S., Inglis, G., Pochon, X., 2018. The impact of artificial surfaces on marine bacterial and eukaryotic biofouling assemblages: a high-throughput sequencing analysis. Mar. Environ. Res. 133, 57–66. https://doi.org/10.1016/j.marenvres.2017.12.003.

Ayanda, O.I., Ajayi, T., Asuwaju, F.P., 2020. *Eichhornia crassipes* (Mart.) solms: uses, challenges, threats, and prospects. Sci. World J. 2020, 1–12. https://doi.org/10.1155/2020/3452172.

Bergo, N.M., Bendia, A.G., Ferreira, J.C.N., Murton, B.J., Brandini, F.P., Pellizari, V.H., 2021. Microbial diversity of deep-sea Ferromanganese crust field in the Rio Grande rise, Southwestern Atlantic Ocean. Microb. Ecol. 82, 344–355. https://doi.org/10.1007/s00248-020-01670-y.

Boltovskoy, D. (Ed.), 2015. *Limnoperna Fortunei*: the Ecology, Distribution and Control of a Swiftly Spreading Invasive Fouling Mussel. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-13494-9.

Boltovskoy, D., Bordet, F., Leites, V., Cataldo, D., 2021. Multiannual trends (2004–2019) in the abundance of larvae of the invasive mussel *Limnoperna fortunei* and crustacean zooplankton in a large South American reservoir. Austral Ecol. 46, 1221–1235. https://doi.org/10.1111/aec.13058.

Bongo, C.W., Malfatti, F., Azam, F., Obayashi, Y., Suzuki, S., 2010. The effect of Zinc exposure on the bacteria abundance and proteolytic activity in seawater. Interdiscip Environ Chem - Biological Responses to contaminants 57–63.

Bradley, I.M., Pinto, A.J., Guest, J.S., 2016. Design and evaluation of illumina MiSeq-Compatible, 18S rRNA gene-specific primers for improved characterization of mixed phototrophic communities. Appl. Environ. Microbiol. 82, 5878–5891. https://doi.org/10.1128/AEM.01630-16.

Brugnoli, E., Clemente, J., Boccardi, L., Borthagaray, A., Scarabino, F., 2005. Golden mussel *Limnoperna fortunei* (Bivalvia: Mytilidae) distribution in the main hydrographical basins of Uruguay: update and predictions. An. Acad. Bras. Ciênc. 77, 235–244. https://doi.org/10.1590/S0001-37652005000200004.

Brugnolli, E., Dabezies, M.J., Clemente, J.M., Muniz, P., 2011. *Limnoperna fortunei* (Dunker 1857) en el Sistema de Embalses del Rio Negro, Uruguay. Oecol Aust 15, 576–592. https://doi.org/10.4257/oeco.2011.1503.10.

Cámara, M., Green, W., MacPhee, C.E., Rakowska, P.D., Raval, R., Richardson, M.C., Slater-Jefferies, J., Steventon, K., Webb, J.S., 2022. Economic significance of biofilms: a multidisciplinary and cross-sectoral challenge. Biof Microb 8, 42. https://doi.org/10.1038/s41522-022-00306-y.

Cataldo, D., Leites, V., Bordet, F., Paolucci, E., 2022. Effects of El Niño-Southern oscillation (ENSO) on the reproduction of migratory fishes in a large South American reservoir. Hydrobiologia 849, 3259–3274. https://doi.org/10.1007/s10750-022-04941-6.

Cataldo, D., Vinocur, A., O'Farrell, I., Paolucci, E., Leites, V., Boltovskoy, D., 2012. The introduced bivalve *Limnoperna fortunei* boosts microcystis growth in Salto grande reservoir (Argentina): evidence from mesocosm experiments. Hydrobiologia 680, 25–38. https://doi.org/10.1007/s10750-011-0897-8.

Chattopadhyay, I., J, R.B., Usman, T.M.M., Varjani, S., 2022. Exploring the role of microbial biofilm for industrial effluents treatment. Bioeng 13, 6420–6440. https://doi.org/10.1080/21655979.2022.2044250.

Christoff, A.P., Sereia, A.F.R., Boberg, D.R., Moraes, R.L.V., Oliveira, L.F.V., 2017. Bacterial identification through accurate library preparation and high-throughput sequencing. White Paper: Bacterial NGS Sequencing.

Cohen, J., 2013. Statistical Power Analysis for the Behavioral Sciences, 0 ed. Routledge. https://doi.org/10.4324/9780203771587.

Cruz, Y. da C., Scarpa, A.L.M., Díaz, A.S., Pereira, M.P., De Castro, E.M., Pereira, F.J., 2023. Influence of seasonal variation to the population growth and ecophysiology of *Typha domingensis* (Typhaceae). J. Plant Res. 136, 665–678. https://doi.org/10.1007/s10265-023-01468-2.

Cuthbert, R.N., Pattison, Z., Taylor, N.G., Verbrugge, L., Diagne, C., Ahmed, D.A., Leroy, B., Angulo, E., Briski, E., Capinha, C., Catford, J.A., Dalu, T., Essl, F., Gozlan, R.E., Haubrock, P.J., Kourantidou, M., Kramer, A.M., Renault, D., Wasserman, R.J., Courchamp, F., 2021. Global economic costs of aquatic invasive alien species. Sci. Total Environ. 775, 145238. https://doi.org/10.1016/j.scitotenv.2021.145238.

Dang, H., Lovell, C.R., 2016. Microbial surface colonization and biofilm development in marine environments. Microbiol. Mol. Biol. Rev. 80, 91–138. https://doi.org/10.1128/MMBR.00037-15.

Davey, M.E., O'toole, G.A., 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev. 64, 847–867. https://doi.org/10.1128/MMBR.64.4.847-867.2000.

De Santis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072. https://doi.org/10.1128/AEM.03006-05.

Dey, S., Shetranjiwalla, S., 2025. Eco-engineered sol-gel primer coatings. J. Sol. Gel Sci. Technol. https://doi.org/10.1007/s10971-025-06752-2.

Dilshad, Rizwana, Khan, K.-R., Dilshad, Rihana, Ahmad, Saeed, Rao, H., Khurshid, U., Ahmad, Sultan, Ahmad, M., Abid, H.M.U., Zaman, M.K., Nisar, R., Khaliq, S., Ghalloo, B.A., 2024. Comprehensive chemical profiling with UHPLC-MS, in-vitro, in-silico, and in-vivo antidiabetic potential of *Typha domingensis* pers; A novel source of bioactive compounds. South Afr. J. Bot. 171, 185–198. https://doi.org/10.1016/j.sajb.2024.06.007.

Dobretsov, S., Rittschof, D., 2020. Love at first taste: induction of larval settlement by marine microbes. IJMS 21, 731. https://doi.org/10.3390/ijms21030731.

Eagle, H., 1948. A paradoxical zone phenomenon in the bactericidal action of Penicillin *in Vitro*. Science 107, 44–45. https://doi.org/10.1126/science.107.2767.44.

Eagle, H., Musselman, A.D., 1948. The rate of bactericidal action of Penicillin in vitro as a function of its concentration and its paradoxically reduced activity at high concentrations against certain organisms. J. Exp. Med. 88, 99–131. https://doi.org/10.1084/jem.88.1.99.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604.

Eigemann, F., Hilt (Nee Körner), S., Schmitt-Jansen, M., 2013. Flow cytometry as a diagnostic tool for the effects of polyphenolic allelochemicals on phytoplankton. Aquat. Bot. 104, 5–14. https://doi.org/10.1016/j.aquabot.2012.10.005.

Eisler, R., 1998. Copper Hazards to Fish, Wildlife, and Invertebrates: a Synoptic Review. US Department of the Interior, US Geological Survey.
Fabián, D., Ferrer, C., Pereira, J., Muniz, P., Capurro, L., Mandiá, M., Failache, G., Brugnoli, E.B., 2021. Variación anual de larvas del mejillón dorado (*Limnoperna fortunei*) en sistemas de refrigeración de centrales hidroeléctricas en embalses del Río Negro. Uruguay. INNOTEC 22. https://doi.org/10.26461/22.03.
Felisberto, S.A., Rodrigues, L., 2012. Dinâmica successional de comunidade de algas perifíticas em um ecossistema lótico subtropical. Rodriguesia 63, 463–473. https://doi.org/10.1590/S2175-78602012000200018.
Flach, C.-F., Pal, C., Svensson, C.J., Kristiansson, E., Östman, M., Bengtsson-Palme, J., Tysklind, M., Larsson, D.G.J., 2017. Does antifouling paint select for antibiotic resistance? Sci. Total Environ. 590–591, 461–468. https://doi.org/10.1016/j.scitotenv.2017.01.213.
França, R.C.S., Lopes, M.R.M., Ferragut, C., 2011. Structural and successional variability of periphytic algal community in a Amazonian lake during the dry and rainy season (Rio Branco, Acre). Acta Amazonica 41, 257–266. https://doi.org/10.1590/S0044-59672011000200010.
Gasol, J.M., Zweifel, U.L., Peters, F., Fuhrman, J.A., Hagström, Å., 1999. Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl. Environ. Microbiol. 65, 4475–4483. https://doi.org/10.1128/AEM.65.10.4475-4483.1999.
Ghattavi, S., Homaei, A., Fernandes, P., 2024. Marine natural products for biofouling elimination in marine environments. Biocatal. Agric. Biotechnol. 61, 103385. https://doi.org/10.1016/j.bcab.2024.103385.
Gomes, M.V.T., De Souza, R.R., Teles, V.S., Araújo Mendes, É., 2014. Phytoremediation of water contaminated with mercury using *Typha domingensis* in constructed wetland. Chemosphere 103, 228–233. https://doi.org/10.1016/j.chemosphere.2013.11.071.
Gross, E.M., 2003. Allelopathy of aquatic autotrophs. Crit. Rev. Plant Sci. 22, 313–339. https://doi.org/10.1080/713610859.
Gross, E.M., Bakker, E.S., 2012. The Role of Plant Secondary Metabolites in Freshwater Macrophyte Herbivore Interaction: Limited or Unexplored Chemical Defences.
Guimarães, M.G.Q., Moreira, A.D.R., Bove, C.P., 2017. Flora do Rio de Janeiro: pontederiaceae. Rodriguesia 68, 103–108. https://doi.org/10.1590/2175-7860201768118.
Hamidi, N., Mohamad Ikhmal Wan Mohamad Kamaruzzaman, W., Amirah Mohd Nasir, N., Syaizwadi Shaifudin, M., Sabri Mohd Ghazali, M., 2022. Potential application of plant-based derivatives as green components in functional coatings: a review. Clean Mat 4, 100097. https://doi.org/10.1016/j.clema.2022.100097.
Hegazy, A.K., Abdel-Ghani, N.T., El-Chaghaby, G.A., 2011. Phytoremediation of industrial wastewater potentiality by *Typha domingensis*. Int. J. Environ. Sci. Technol. 8, 639–648. https://doi.org/10.1007/BF03326249.
Huang, W., Chen, Z., Liu, Y., Li, D., Wei, Z., 2024a. Sulfide-carbonate-mineralized functional bacterial consortium for cadmium removal in flue gas. Chemosphere 363, 142869. https://doi.org/10.1016/j.chemosphere.2024.142869.
Huang, Y., Yang, L., Pan, K., Yang, Z., Yang, H., 2024b. Heavy metal-tolerant bacterium Bacillus cereus BCS1 degrades pyrethroid in a soil–plant system. Journal of Hazardous Materials 461, 142869. https://doi.org/10.1016/j.jhazmat.2023.132594.
Keshri, J., Mankazana, B.B.J., Kachieng'a, L., Momba, M.N.B., 2024. Indigenous metal-tolerant mine water bacterial populations under varying metal stresses. Sci. Total Environ. 948, 174830. https://doi.org/10.1016/j.scitotenv.2024.174830.
Krock, B., Borel, C.M., Barrera, F., Tillmann, U., Fabro, E., Almandoz, G.O., Ferrario, M., Garzón Cardona, J.E., Koch, B.P., Alonso, C., Lara, R., 2015. Analysis of the hydrographic conditions and cyst beds in the San Jorge Gulf, Argentina, that favor dinoflagellate population development including toxigenic species and their toxins. J. Mar. Syst. 148, 86–100. https://doi.org/10.1016/j.jmarsys.2015.01.006.
Lal, N., Biswas, A.K., 2023. Allelopathic interaction and eco-physiological mechanisms in agri-horticultural systems: a review. Erwerbsobstbau. https://doi.org/10.1007/s10341-023-00864-1.
Liu, J., Chang, Y., Sun, L., Du, F., Cui, J., Liu, X., Li, N., Wang, W., Li, J., Yao, D., 2021. Abundant allelochemicals and the inhibitory mechanism of the phenolic acids in water dropwort for the control of *Microcystis aeruginosa* blooms. Plants 10, 2653. https://doi.org/10.3390/plants10122653.
Liu, X., Zou, L., Li, B., Di Martino, P., Rittschof, D., Yang, J.-L., Maki, J., Liu, W., Gu, J.-D., 2024. Chemical signaling in biofilm-mediated biofouling. Nat. Chem. Biol. 20, 1406–1419. https://doi.org/10.1038/s41589-024-01740-z.
Ma, W., Peng, D., Walker, S.L., Cao, B., Gao, C.-H., Huang, Q., Cai, P., 2017. Bacillus subtilis biofilm development in the presence of soil clay minerals and iron oxides. Biof Microb 3, 4. https://doi.org/10.1038/s41522-017-0013-6.
Marie, D., Simon, N., Vaulot, D., 2005. Phytoplankton cell counting by flow cytometry. Algal culturing techniques.
Martín-Rodríguez, A.J., Babarro, J.M.F., Lahoz, F., Sansón, M., Martín, V.S., Norte, M., Fernández, J.J., 2015. From broad-spectrum biocides to quorum sensing disruptors and mussel repellents: antifouling profile of Alkyl Triphenylphosphonium salts. PLoS One 10, e0123652. https://doi.org/10.1371/journal.pone.0123652.
McNamara, C.J., Bearce Lee, K., Russell, M.A., Murphy, L.E., Mitchell, R., 2009. Analysis of bacterial community composition in concretions formed on the USS Arizona, Pearl Harbor, HI. J. Cult. Herit. 10, 232–236. https://doi.org/10.1016/j.culher.2008.07.010.
Morales, M.L.P., Figurelli, G.P., Oleinski, B., Pinho, G.L.L., They, N.H., Agostini, V.O., 2024a. Antifouling activity of aquatic macrophyte extracts on estuarine bacterial biofilms. Chem. Ecol. 1–19. https://doi.org/10.1080/02757540.2024.2321990.
Morales, M.L.P., Guimarães, P.S., De Martinez Gaspar Martins, C., Míguez, D., Bentos, F. R., Boccardi, L., Brugnoli, E., Shaik, A., Chenia, H., Cavalli, R.O., They, N.H., Pinho, G.L.L., Agostini, V.O., 2024b. Aquatic macrophytes as a source of antifouling non-toxic against bacterial biofilms and golden mussel attachment: a possible role of quorum-sensing interference. Environ. Sci. Pollut. Res. 31, 66977–66993. https://doi.org/10.1007/s11356-024-35744-y.
Morales, M.L.P., Neves, L.O. das, Shaik, A., Chenia, H., Maronna, M.M., Soroldoni, S., Nagata, R.M., They, N.H., Agostini, V.O., Pinho, G.L.L., 2025. Aquatic macrophytes as antifouling candidates: anti-attachment and toxicological effects in *Aurelia coerulea* (Cnidaria, Scyphozoa). Environ Toxicol in the Peer Review Process.
Naicker, R., 2024. Investigating the Quorum Sensing and Biofilm Inhibitory Potential of sponge-associated bacteria-derived Crude Biosurfactant Extracts and their Biosynthesised Nanoparticles (Msc Thesis). University of KwaZulu-Natal.
Nalini, S., Venkatnarayanan, S., Inbakandan, D., Clements, C., Riyaz, S.U.M., Dhas, T.S., Murthy, P.S., Dharani, G., 2025. Antifouling activity exhibited by pyrrolo compound isolated from marine sponge associated bacterium *Halobacillus kuroshimensis* SNSAB01 against barnacles. Int. Biodeterior. Biodegrad. 197, 105960. https://doi.org/10.1016/j.ibiod.2024.105960.
Neilen, A.D., Hawker, D.W., O'Brien, K.R., Burford, M.A., 2017. Phytotoxic effects of terrestrial dissolved organic matter on a freshwater Cyanobacteria and green algae species is affected by plant source and DOM chemical composition. Chemosphere 184, 969–980. https://doi.org/10.1016/j.chemosphere.2017.06.063.
Neves, A.R., Godinho, S., Gonçalves, C., Gomes, A.S., Almeida, J.R., Pinto, M., Sousa, E., Correia-da-Silva, M., 2024. A chemical toolbox to unveil synthetic nature-inspired antifouling (NIAF) compounds. Mar. Drugs 22, 416. https://doi.org/10.3390/md22090416.
O'Farrell, I., Bordet, F., Chaparro, G., 2012. Bloom forming cyanobacterial complexes co-occurring in a subtropical large reservoir: validation of dominant eco-strategies. Hydrobiologia 698, 175–190. https://doi.org/10.1007/s10750-012-1102-4.
Patil, J.S., Anil, A.C., 2005. Biofilm diatom community structure: influence of temporal and substratum variability. Biofouling 21, 189–206. https://doi.org/10.1080/08927010500256757.
Pereira, D., Almeida, J.R., Cidade, H., Correia-da-Silva, M., 2024. Proof of concept of natural and synthetic antifouling agents in coatings. Mar. Drugs 22, 291. https://doi.org/10.3390/md22070291.
Pereira, M.L.M., Bastos Vasconcelos, I.M., Macedo, A.J., Muxagata, E., Leães Lopes Pinho, G., Ochi Agostini, V., 2022. Estrategias de control de mejillones invasores: una revisión. INNOTEC 23. https://doi.org/10.26461/23.08.
Pereira, R.C., Nocchi, N., Konno, T.U.P., Soares, A.R., 2021. Diverse traits of aquatic plants cannot individually explain their consumption by the generalist gastropod *Biomphalairia glabrata*. PeerJ 9, e12031. https://doi.org/10.7717/peerj.12031.
Pérez, M., Fernández, L.R., Zambrano, E.E., García, M., Uriburu, M.L., Sánchez, M., Blustein, G., Palermo, J.A., 2021. Use of weed extracts as antifouling additives for marine paints: two case studies. Rev Bras Farmacogn 31, 420–428. https://doi.org/10.1007/s43450-021-00165-2.
Prasetyoputri, A., Jarrad, A.M., Cooper, M.A., Blaskovich, M.A.T., 2019. The eagle effect and antibiotic-induced persistence: two sides of the same coin? Trends Microbiol. 27, 339–354. https://doi.org/10.1016/j.tim.2018.10.007.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.
Rambaran, N., Naidoo, Y., Mohamed, F., Chenia, H.Y., Baijnath, H., 2024. Antibacterial and anti-quorum sensing properties of silver nanoparticles phytosynthesized using *Embelia ruminata*. Plants 13, 168. https://doi.org/10.3390/plants13020168.
Rao, T.S., 2010. Comparative effect of temperature on biofilm formation in natural and modified marine environment. Aquat. Ecol. 44, 463–478. https://doi.org/10.1007/s10452-009-9304-1.
Reischke, S., Kumar, M.G.K., Bååth, E., 2015. Threshold concentration of glucose for bacterial growth in soil. Soil Biol. Biochem. 80, 218–223. https://doi.org/10.1016/j.soilbio.2014.10.012.
Reischke, S., Rousk, J., Bååth, E., 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biol. Biochem. 70, 88–95. https://doi.org/10.1016/j.soilbio.2013.12.011.
Romeu, M.J., Mergulhão, F., 2023. Development of antifouling strategies for marine applications. Microorganisms 11, 1568. https://doi.org/10.3390/microorganisms11061568.
Salta, M., Wharton, J.A., Blache, Y., Stokes, K.R., Briand, J.-F., 2013. Marine biofilms on artificial surfaces: structure and dynamics: marine biofilms: structure and dynamics. Environ Microbiol n/a-n/a. https://doi.org/10.1111/1462-2920.12186.
Salto, G., 2025. Salto Grande - Estácion Metereologica.
Satasiya, G., Kumar, M.A., Ray, S., 2025. Biofouling dynamics and antifouling innovations: transitioning from traditional biocides to nanotechnological interventions. Environ. Res. 269, 120943. https://doi.org/10.1016/j.envres.2025.120943.
Sathicq, Á., Pérez, M., García, M., Romanelli, G., Blustein, G., 2025. Carbonyl-compounds as sustainable antifouling agents against *Limnoperna fortunei*. Biocatal. Agric. Biotechnol. 66, 103591. https://doi.org/10.1016/j.bcab.2025.103591.
Sauer, K., Camper, A.K., Ehrlich, G.D., Costerton, J.W., Davies, D.G., 2002. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. J. Bacteriol. 184, 1140–1154. https://doi.org/10.1128/jb.184.4.1140-1154.2002.
Sauer, K., Stoodley, P., Goeres, D.M., Hall-Stoodley, L., Burmølle, M., Stewart, P.S., Bjarnsholt, T., 2022. The biofilm life cycle: expanding the conceptual model of biofilm formation. Nat. Rev. Microbiol. 20, 608–620. https://doi.org/10.1038/s41579-022-00767-0.
Schmidt, P.-A., Bálint, M., Greshake, B., Bandow, C., Römbke, J., Schmitt, I., 2013. Illumina metabarcoding of a soil fungal community. Soil Biol. Biochem. 65, 128–132. https://doi.org/10.1016/j.soilbio.2013.05.014.

Sérvulo, T., Taylor, J.D., Proietti, M.C., Rodrigues, L.d.S., Puertas, I.P., Barutot, R.A., Lacerda, A.L.d.F., 2023. Plastisphere composition in a subtropical Estuary: influence of season, incubation time and polymer type on plastic biofouling. Environ. Pollut. 332, 121873. https://doi.org/10.1016/j.envpol.2023.121873.

Silva, I., Brugnoli, E., Clavijo, C., D'Anatro, A., Naya, D.E., Mello, F.T., Tesitore, G., Bergonzoni, 2021. Interacciones entre el mejillón dorado y macroinvertebrados bentónicos nativos del Río Uruguay. INNOTEC 22. https://doi.org/10.26461/22.04.

Soroldoni, S., Martins, S.E., Castro, I.B., Pinho, G.L.L., 2018. Potential ecotoxicity of metals leached from antifouling paint particles under different salinities. Ecotoxicol. Environ. Saf. 148, 447–452. https://doi.org/10.1016/j.ecoenv.2017.10.060.

Su, W., Sun, Q., Xia, M., Wen, Z., Yao, Z., 2018. The resource utilization of water hyacinth (*Eichhornia crassipes* [Mart.] solms) and its challenges. Resources 7, 46. https://doi.org/10.3390/resources7030046.

Sukreem, S., 2024. Investigating the Bioactivity of Crude Biosurfactant Extracts and biosurfactant-capped Nanoparticles Synthesized Using Marine sponge-associated Bacteria (Msc Thesis). University of KwaZulu-Natal.

Tan, K., Huang, Z., Ji, R., Qiu, Y., Wang, Z., Liu, J., 2019. A review of allelopathy on microalgae. Microbiology 165, 587–592. https://doi.org/10.1099/mic.0.000776.

Tohge, T., Fernie, A.R., 2009. Web-based resources for mass-spectrometry-based metabolomics: a user's guide. Phytochemistry 70, 450–456. https://doi.org/10.1016/j.phytochem.2009.02.004.

Tonelli, M., Perini, I., Ridi, F., Baglioni, P., 2021. Improving the properties of antifouling hybrid composites: the use of halloysites as nano-containers in epoxy coatings. Colloids Surf. A Physicochem. Eng. Asp. 623, 126779. https://doi.org/10.1016/j.colsurfa.2021.126779.

Vijayan, P.P., Formela, K., Saeb, M.R., Chithra, P.G., Thomas, S., 2022. Integration of antifouling properties into epoxy coatings: a review. J. Coating Technol. Res. 19, 269–284. https://doi.org/10.1007/s11998-021-00555-0.

Watson, M., Scardino, A., Zalizniak, L., Shimeta, J., 2016. Inhibition of invertebrate larval settlement by biofilm ciliates. Mar. Ecol. Prog. Ser. 557, 77–90. https://doi.org/10.3354/meps11825.

Xiao, Y., Seo, Y., Lin, Y., Li, L., Muhammad, T., Ma, C., Li, Y., 2020. Electromagnetic fields for biofouling mitigation in reclaimed water distribution systems. Water Res. 173, 115562. https://doi.org/10.1016/j.watres.2020.115562.

Yu, S., Li, C., Xu, C., Effiong, K., Xiao, X., 2019. Understanding the inhibitory mechanism of antialgal allelochemical flavonoids from genetic variations: photosynthesis, toxin synthesis and nutrient utility. Ecotoxicol. Environ. Saf. 177, 18–24. https://doi.org/10.1016/j.ecoenv.2019.03.097.

Zhu, Y., Tu, X., Bi, Y., Song, G., Mi, W., 2024. Competition in the periphytic algal community during the colonization process: evidence from the world's largest water diversion project. Plants 13, 2067. https://doi.org/10.3390/plants13152067.