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Biological Succession and Degradation in Biofouled Plastics

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INTRODUCTION & AIM

Global plastic production has risen sharply over recent decades, now surpassing 400 million tonnes annually [1] . The same durability that makes plastics indispensable in industry also contributes to their persistence as pollutants in marine ecosystems [2]. Only a small proportion of plastic waste is effectively recycled, while a significant share, around 0.5% of total waste each year, ultimately enters the ocean [3]. Once in the marine environment, plastics degrade into macro-, meso-, micro-, and nanoplastic particles , which can negatively affect organisms through ingestion, entanglement, and exposure to leached chemicals [4]. Their distribution is governed by physical factors such as waves and currents, as well as by material characteristics, notably density [5,6]. Yet, despite substantial inputs, the amount of visible floating plastic remains markedly lower than predicted.

Two key mechanisms may account for this “missing plastic” phenomenon: stranding along coastal zones and sinking due to changes in particle density [6] . The attachment and subsequent growth of microorganisms, algae, and invertebrates on plastic surfaces increase surface roughness and mass, which can surpass the density of seawater and lead to sinking [7]. This biofouling process develops through defined stages, starting with the adsorption of organic molecules (forming a conditioning film), followed by microbial adhesion, biofilm maturation, and eventually, colonization by macro-organisms [6,7] .

Beyond altering the physical dynamics of plastics, biofouling also gives rise to a unique ecological niche, the “plastisphere”, that hosts diverse microbial communities [8] . These assemblages may contain both potential pathogens and plastic-degrading organisms, influencing ecosystem health and biogeochemical processes [6,7] . Among common debris polymers, polyvinyl chloride (PVC) is a dense thermoplastic frequently detected in urban and marine environments. However, its biofouling-driven behaviour is still poorly understood. The present study aimed to characterize the temporal succession of microbial communities colonizing PVC surfaces and to quantify the resulting changes in material weight.

METHOD

Experimental Design

An *in situ* experiment was conducted at the Marina of Oeiras (Portugal) (**Figure 1**) to assess the impact of biological colonisation on the weight of PVC. Ten sets of three circular PVC discs (16 cm diameter) were prepared from a homogeneous sheet, roughened to promote microbial adhesion, and suspended vertically at depths of 0.3, 1.2 and 2 m. The lower end of each structure was weighted to ensure stable submersion. The discs were immersed on 27 March 2025 and retrieved at successive intervals after 21 days, 30 days, and 60 days for laboratory analysis.

Figure 1. Location of the Oeiras Marina and schematic representation of submerged PVC structures used in the experiment.

Sampling

Surface material from both the upper and lower faces of each disc was collected with sterile swabs, placed in tubes containing peptone water to enrich microbial biomass without selective bias, and transported at 4 °C. Discs retrieved from the field were processed for visual and microscopic examination, as well as weight measurements.

Species Identification

DNA was extracted using the Qiagen DNA Isolation Kit (Hilden, Germany) and amplified via PCR with NZYTaq II 2× Green Master Mix (NZYTech, Portugal). Bacterial 16S rRNA was targeted with primers 27F/1492R, and fungal ITS with ITS1-F/ITS4.

Sequencing and Analysis

Sanger sequencing was conducted by STAB Vida (Monte da Caparica, Portugal). Sequences were edited in BioEdit and identified via NCBI BLAST against GenBank [web:NCBI].

Microscopy

Disc surfaces were examined at 400× magnification (Olympus CX23, Tokyo, Japan), photographing and classifying dominant taxa (e.g., filamentous fungi, algae, diatoms, bacteria).

Weighing

Discs were weighed pre-immersion (initial), post-retrieval (wet), and after 50 °C drying for 7 days (dry). Increases were calculated relative to initial weight.

RESULTS & DISCUSSION

Microscopic and sequencing analyses revealed a progressive increase in biofilm complexity on PVC discs from 7 to 60 days (**Figure 2**), with the appearance of filamentous algae, diatoms, and diverse microbial matrices. Early stages (7 days) were dominated by pioneer and anthropogenic indicator taxa, intermediate stages (30 days) by mature communities adapted to low-oxygen and nutrient-variable conditions, and late stages (60 days) by stable, specialized microorganisms involved in long-term biofilm maintenance and plastic degradation (**Table 1**).

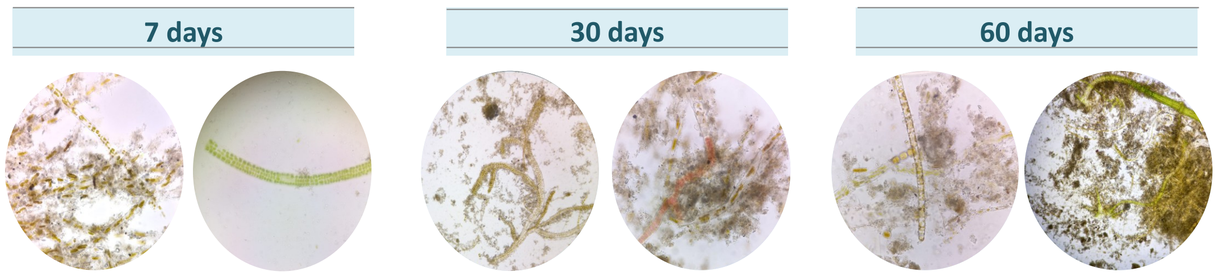

Figure 2. Microscopic observations (400×) of biofilm from PVC discs at 7, 30 and 60 days.

Table 1. Microbial communities (bacteria, microalgae, and fungi) identify by Sanger sequencing in marine biofilms developed over 7, 30, and 60 days.

Period (days)	Dominant bacterial genera/species	Ecological role
7	*Escherichia coli, Enterobacter asburiae, Pseudomonas putida, Raoultella sp.*	*E. coli* and *E. asburiae* indicators of faecal contamination; *Pseudomonas* sp. have been shown to be capable of degrading microplastics [8]
30	*Clostridium botulinum, Vibrio diabolicus, Propionigenium sp., E. cloacae*	*C. botulinum* production of botulinum neurotoxin; *Vibrio* spp. are known as early colonizers to plastic surfaces, *Propionigenium sp.* biofilms and low-oxygen conditions [8].
60	*P. putida ; P. hunanensis*	*Pseudomonas* sp. degrading-microplastics bacteria [8]

Period (days)	Dominant microalgae/diatom genera/species	Ecological role
7	*Nannochloropsis oculata , Melosira tropica*	*Nannochloropsis* genera is microalgae adapted to high light and nutrient conditions. *Melosira* forms chains that help stabilize the surface [9].
30	*Nitzschia nienhuisii , Minidiscus spinulatus*	*Nitzschia* species are stabilizers in benthic ecosystems, showing strong adaptation to fluctuating light, nutrient, and oxygen levels. *Minidiscus spinulatus* contributes to carbon fixation and nutrient cycling [10].
60	*Picocystis salinarum*	*P. salinarum* is known for its extreme salinity tolerance, photosynthetic activity under low light, and ability to dominate hypersaline biofilms [11].

Period (days)	Dominant fungi genera/species	Ecological role
7	*Fusarium acuminatum , F. Tricinctum, F. acuminatum,*	*Fusarium* species are capable of producing enzymes that degrade plastics [12].
30	*Plectosphaerella cucumerina; Cladosporium cladosporioides*	Recognized as some of the most frequent fungal colonizers in marine plastisphere communities [12].
60	*F. tricinctum ; Cladosporium sp.*	Recognized as mature colonizers within the plastisphere, capable of producing extracellular enzymes that degrade complex polymers [12].

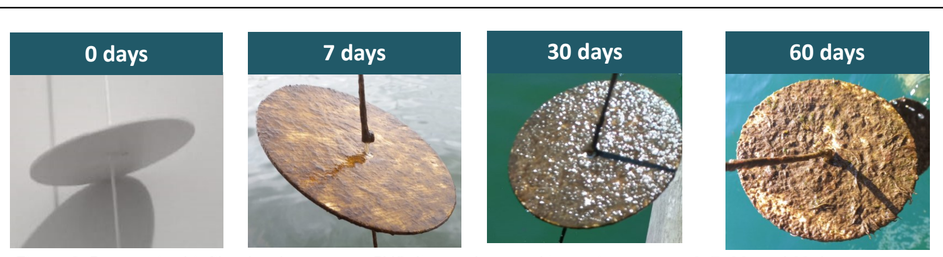

Figure 3. Progressive biofilm development on PVC discs submerged in marine water at 0, 7, 30, and 60 days.

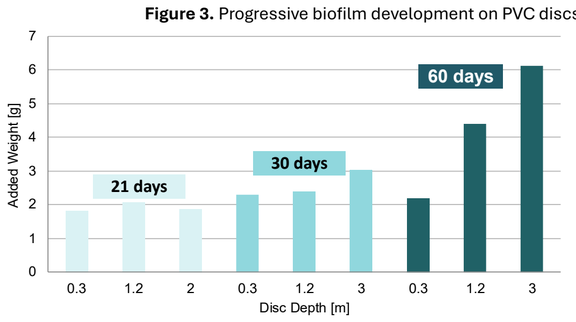

Figure 4. Added biomass (g) on PVC discs at different depths (0.3, 1.2 and 3 m) after 21, 30 and 60 days of marine immersion.

Biofilm growth on PVC discs increased over time, developing from a thin layer at 7 days to a dense, mature biofilm at 60 days (**Figure 3**). Discs dry weight measurements confirmed a progressive rise in biomass and total weight, with deeper PVC discs showing denser biofilms due to lower light and hydrodynamic conditions that promote stable microbial communities (**Figure 4**).

CONCLUSION

PVC surfaces showed progressive microbial colonization over time, with biofilm biomass and weight increasing significantly. Microbial succession from pioneer bacteria and algae to diatoms and fungi revealed ecological maturation within the plastisphere. Biofouling altered plastic properties, increasing density and sinking potential, while plastic-degrading taxa such as *Pseudomonas, Cladosporium*, and *Fusarium* indicated biofilms’ dual role in plastic dispersion and biodegradation.

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