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“On our planet there are simultaneously existing an infinite number of connections and interactions quite difficult to quantify.”



LIFE ACROSS BIOMES

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CONTENT

1

P. 8

**THE WONDERFUL AND MYSTERIOUS COMPLEXITY OF THE
ENVIRONMENT, FROM BACTERIA TO ATMOSPHERE**

PEROSA M.

2

P. 39

**SCIENCE TOLD TODAY: ETHICS, LANGUAGES AND RESPONSIBILITY
IN CONTEMPORARY SCIENCE COMMUNICATION**

IVAN G., COSTANTINI A.

3

P. 49

**PLASTIC POLLUTION AND PLASTIC FIGHTERS:
AWARENESS THROUGH PLAY**

SCHLAPPA, K., MANNA, D., TEMPESTA, M., ZUPPA, F.

The wonderful and mysterious complexity of the Environment, from Bacteria to Atmosphere.

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Abstract: Years of ecological study have taught us that relationships determine the Environment in its complexity, made by the habitat and the organisms that live in it. This research aims to highlight how consequences can be underestimated or recognized only within specific subject areas, and how desirable it is to have greater integration between disciplines and more comprehensive studies to address the lack of available data and better understand these relationships as a whole, in order to prevent the possible development of environmental and ecological problems. The atmosphere is an environment both fragile and resistant, closely linked to the life of the planet as we know it. However, an epochal and fast transformation is taking place in the various atmospheric layers in a way never experienced before in the history of the Earth. The unprecedented electromagnetic saturation combined with chemical and physical contamination of the atmosphere has been considered in the fundamental aspects such as microbial transport, and various forms of pollution. The approach that starts from bacteria is intended to be a stimulus to reconsider the role of unicellular organisms as a foundation of the general environmental structure and therefore it seems interesting to observe how they may respond, due to their proven ability to adapt, react and contribute to Life, when significant changes occur. In every environment they are ecological indicators and already participants in atmospheric processes.

Keywords: environment; microbial ecology; atmospheric pollution; bioaerosol; electromagnetic fields; active satellites; space debris.

1.

Introduction

For a precise approach to the meaning of the term *ENVIRONMENT*, it is interesting to delve into the etymology of the 30 most spoken languages of the world, discovering that the same concept is expressed: "what surrounds, what is around, and circular border". So almost everywhere the term maintains its universal meaning unchanged.

If, therefore, it is so immediate to recognize the meaning of the word precisely, questions immediately crowd in. The environment is around, but what does it surround? How many and what are the different dimensions in which one can or must investigate to define both the environment and its content, all the enormous variety of organisms that inhabit it, and which often contribute to its creation?

By studying the environment, it is easy to observe that many different forms of life are encountered, sharing different quantities and scales of space, sometimes in competition, sometimes in collaboration, often integrating functions and utilities. So, every environment, like for example the vast ocean, is essential for both a Seagull and a Humpback whale, but also for a *Cycliophora* (tiny symbiont of the Lobsters' mouth), or the large variety of algae, plants and groups of minuscule Bacteria. In a similar way a forest, that needs trees, air, water, and soil, hosts ancient and young trees but also mosses, fungi, lichens and a vast variety of vegetable and animal organisms, again connecting the biggest and smallest components, involved in a shared context.

Ecology has long emphasized the importance of relationships and events in shaping environments, which appear fragmented across diverse ecosystems. In an era where human technology increasingly permeates a large quantity of these interactions, we will try to make a journey—undoubtedly synthetic—to cross the different scales of reference, from molecules to the atmosphere, trying to understand how stable or temporary cohabitation of organisms and abiotic phenomena affect the real unstoppable dynamics of each environment.

Leaving aside the infinitesimal particles that make up matter, which at the present time are investigated by the current thought regarding quantum physics, which provides new hypotheses on vacuum, interactions, space-time-energy dimensions and which in any case contracts or decisively expands the concept of environment (Rodríguez et al., 2015), in our journey we move from the molecular level. That is, that space in which fields determined by forces and circumstantial, random elements, create an environment in which chemical-physical reactions can develop through a network of interactions that already require energy passages, chemical potentials, signalling modes, reaction capacities, metabolic networks and competition for active sites. These processes operate on spatial scales ranging from angstroms to nanometres and time scales ranging from femtoseconds to seconds, scales at which electromagnetic fields can directly influence molecular resonances and chemical processes.

From the invisible architecture of molecular fields, we ascend to the unicellular prokaryotic organisms, where the deceptive simplicity of the single cell contains all the fundamental vital functions: gene expression and enzymatic activity that guarantee vital metabolism, the perception of variations in their environment, reactivity and movement, and intercellular communication. So, we intend to focus on the microscopic vastness of bacterial life as representative of the most widespread colonization capacity across any environment.

2. The microbial world

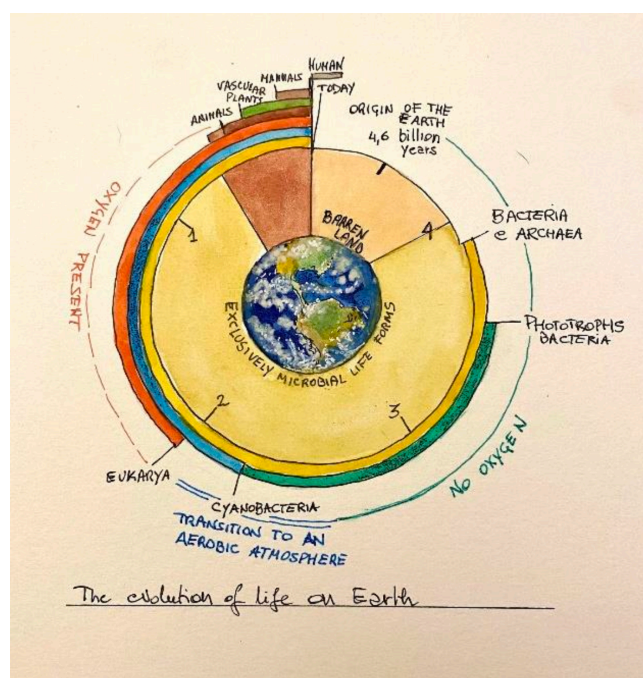


Figure 1. Life evolution on Earth; redrawn from Brock (2022)

The microbial world represents Earth's most ancient and abundant form of life, with microorganisms serving as the fundamental architects of our planet's biogeochemistry. These invisible entities have shaped our world for over 3.8 billion years and continue to drive the essential processes that sustain all higher forms of life (Brock, 2022). Recent advances in microbial ecology research have revolutionized our understanding of these communities, moving beyond traditional culture-based approaches to embrace genomic, proteomic, and systemic-level perspectives.

Bacteria and Archaea have been and still are incredibly spread everywhere, their metabolic versatility allows them to colonize every environment on Earth, from the thinnest biological niches to a complete network of biogeochemical processes. They are sustaining ecosystems across every scale, including the most extreme environments, where both domains thrive in astonishing diversity from the depths of volcanic vents to the frozen landscapes of Antarctica, despite the differences between physical and chemical conditions. Their adaptability allows them to thrive in places where not many other forms of life could persist. They exhibit a lot of physiological and metabolic diversity. Their cellular structures, energy acquisition strategies, and ecological roles vary according to the physicochemical conditions of each habitat. The fundamental division between autotrophic and heterotrophic lifestyles becomes particularly complex in the microbial world.

We will be tracing their differences and distribution across a wide range of environments, with a brief resume of the principal and ending with the Atmosphere.

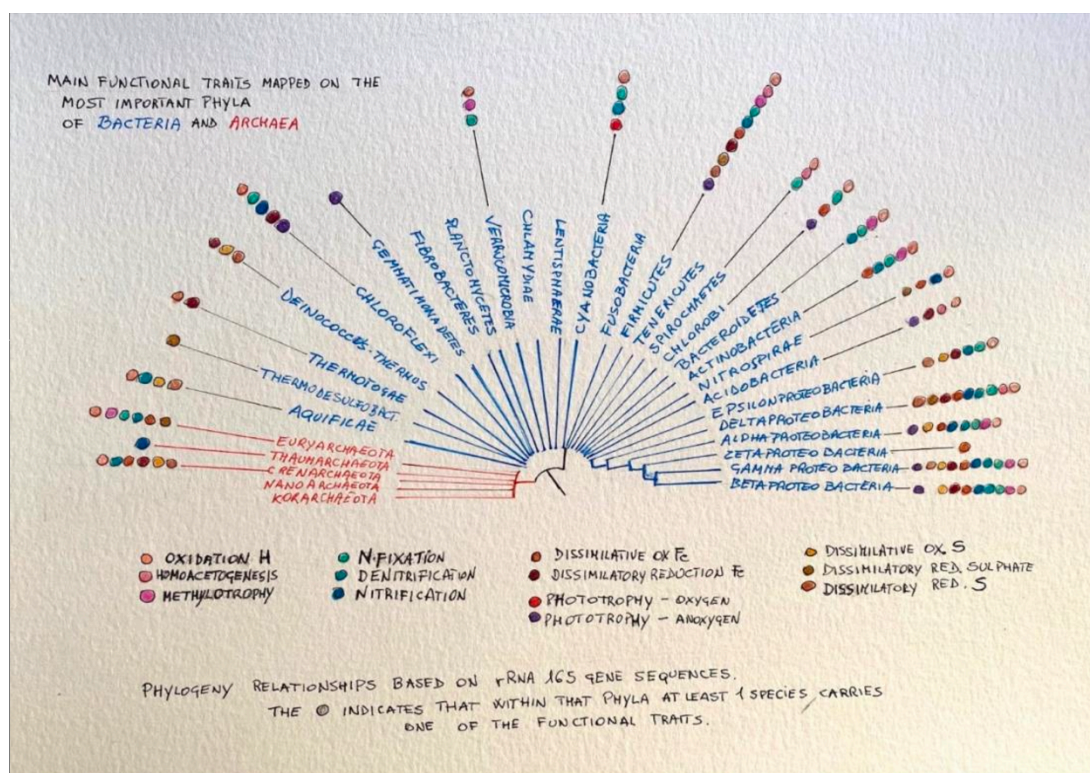


Figure 2. Functional traits; redrawn from Brock (2022)

Aquatic Elements

Freshwater and marine waters host distinct microbial communities with specific physiological, proteomic and metabolic adaptations.

Marine environments host some of Earth's most abundant and ecologically significant microbial communities. Recent large-scale investigations of deep ocean sediments have revealed extraordinarily high proportions of previously unidentified microbial taxa, with studies in the Mariana Trench identifying over 7500 species, 89.4% of which were previously unreported (Xiao et al., 2025).

The ocean's microbial communities display important adaptations to their environment. *Pelagibacter ubique*, likely the most abundant organism in Earth's oceans, exemplifies evolutionary streamlining. These ultra-microbacteria have reduced their genomes to the absolute essentials needed for survival in oligotrophic ocean waters, creating highly efficient cellular machines optimized for life in the vast open ocean.

In contrast, coastal and upwelling areas support much more diverse and metabolically active communities. For example, *Vibrio* species, with their characteristic curved morphology and metabolic versatility, can rapidly respond to changing nutrient conditions, often using sophisticated communication systems (Liu et al., 2025). Other specialized communities of phototrophic sulphur bacteria form deeper layers in the water column, while denitrifiers, and methanotrophic bacteria create complex biogeochemical gradients in sediments.

The Soil Microbiome

Soil represents one of Earth's most complex and biogeochemically active microbial habitats. These terrestrial ecosystems rely on intricate microbial communities that facilitate nutrient cycling, with key genera like *Azotobacter* and *Bradyrhizobium* driving nitrogen fixation, while phosphate-solubilizing bacteria such as *Pseudomonas* and *Bacillus* enhance phosphorus availability for plants.

The relationship between *Rhizobium* bacteria and leguminous plants exemplifies one of nature's most sophisticated symbiotic partnerships. These bacteria invade plant root tissues and establish specialized structures called nodules, where they convert atmospheric nitrogen into ammonia that the plant can utilize for protein synthesis. In return, the plant provides the bacteria with carbohydrates and a protect environment. This ancient partnership has been crucial for maintaining soil fertility and supporting plant communities worldwide.

Actinobacteria, with their distinctive high-GC content genomes and complex life cycles, serve as soil's primary decomposers of complex organic compounds. These organisms produce an extraordinary array of secondary metabolites, including many of the antibiotics that have revolutionized human medicine. Their ability to break down recalcitrant organic compounds like cellulose and lignin makes them essential players in carbon cycling and soil formation.

The Phototrophic Domain

In every environment where light penetrates (water, soil, rocks), complex microbial communities establish themselves in layers where specialized organisms are adapted to their specific combination of physical and chemical conditions, creating a vertical ecosystem of remarkable complexity within just a few millimetres of space. Cyanobacteria, the evolutionary pioneers of oxygenic photosynthesis, typically dominate

the uppermost layers. These remarkable organisms possess specialized cellular structures called thylakoids that house the molecular enzymes necessary for splitting water molecules and releasing oxygen as a byproduct. Many cyanobacterial species also contribute to nitrogen fixation, using specialized strategies to protect their oxygen-sensitive nitrogenase from the oxygen they produce during photosynthesis.

Beneath these oxygen-producing communities, a different world emerges. Members of the Chloroflexi phylum, many forming distinctive filamentous structures, are anoxygenic phototrophs with an alternative photosynthetic strategy. Rather than splitting water, these anoxygenic phototrophs utilize hydrogen sulphide, organic acids, or other reduced compounds as electron sources.

This represents just a simplified example of stratified communities. In nature there exist more examples of niche partitioning, where both vertical and horizontal gradients of light, oxygen concentrations, and chemical conditions create a three-dimensional mosaic of distinct microenvironments.

Chemotrophic Ecosystems

Away from the influence of sunlight, entirely different microbial worlds exist. In these aphotic areas, energy flows through chemical pathways, creating ecosystems that challenge our traditional understanding of life's requirements. Chemolithoautotrophic bacterial and archaeal species drive fundamental biogeochemical processes by oxidizing ammonia and sulphur compounds, transforming these simple inorganic molecules into energy-rich bonds that power cellular metabolism. Among the various processes, there is the nitrogen cycle, essential for all life due to nitrogen's role in DNA, RNA, and protein synthesis. It depends almost entirely on specialized bacteria and archaea that can fix atmospheric nitrogen using the enzyme nitrogenase. Without these microscopic nitrogen-fixers, the vast reservoir of atmospheric nitrogen would remain chemically inert and unavailable to living organisms.

In deeper sediments and anaerobic environments, complex consortia of microorganisms establish syntrophic relationships—mutually beneficial partnerships where the metabolic waste products of one organism become the essential nutrients for another. Fermentative bacteria break down complex organic compounds into simpler molecules, which are then consumed by sulphate-reducing bacteria, methanogens, and other anaerobic specialists. These intricate food webs enable energy flow and nutrient cycling without oxygen and light.

Extremophiles

Perhaps no group of microorganisms better illustrates the remarkable adaptability of life than the extremophiles, thriving in conditions that were once considered completely incompatible with life in consequence of the extreme temperatures, acidic conditions, high radiation levels, or crushing pressures. Often, they belong to Archaea Domain. Thermophilic bacteria like *Thermus aquaticus*, the source of the heat-stable Taq polymerase that revolutionized molecular biology, represent evolutionary masterpieces of protein engineering. These organisms have evolved specialized proteins and cellular mechanisms that remain functional at temperatures exceeding 80 °C, temperatures that would denature the proteins of most other life forms. Their discovery has not only expanded our understanding of life's limits but has also provided invaluable tools for biotechnology and industry.

Halophilic archaea such as *Halobacterium* sp. have evolved entirely different strategies for surviving in hypersaline environments. These organisms have fundamentally altered their cellular architecture, replacing the typical lipid bilayer cell membranes found in most life forms with ether-linked lipids that remain stable in salt concentrations that would rapidly kill conventional organisms.

Extremophiles show relevance for bioremediation applications, with their tolerance to environmental extremes including high concentrations of heavy metals and xenobiotics, they are ideal candidates for cleaning contaminated sites.

Pseudomonas sp., renowned for their metabolic flexibility, can degrade an astonishing variety of organic pollutants, from petroleum hydrocarbons to industrial solvents. *Alcanivorax* bacteria have become specialized marine oil degraders, rapidly proliferating following oil spills and consuming hydrocarbon contaminants. Perhaps most remarkably, *Dehalococcoides* sp. can perform reductive dehalogenation, using chlorinated industrial compounds as electron acceptors in their respiratory metabolism, effectively converting toxic chemicals into harmless byproducts.

Host-Associated Communities: The Microbial Self

The relationship between microorganisms and multicellular hosts represents one of biology's most complex and important frontiers. While pathogenic bacteria like *Salmonella*, *Streptococcus*, and *Mycobacterium tuberculosis* have evolved sophisticated mechanisms to exploit host resources and cause disease, many host-associated microorganisms exist in neutral or beneficial relationships with their hosts.

Bacteroides are beneficial species in the gut of humans and of other animals, they perform essential functions including digestion of complex carbohydrates and synthesize sphingolipids, a particular type of lipid. These communities are so integral to human health that some scientists now consider humans to be "superorganisms" composed of human cells and their essential microbial partners.

The deepest Biosphere

Recent discoveries in subsurface environments have revealed that microbial life extends far deeper into Earth's crust than previously imagined, with organisms adapted to extreme pressure, limited nutrients, and complete darkness. These deep subsurface communities may represent a significant portion of Earth's total biomass. Lithoautotrophic communities in these environments derive energy from mineral oxidation and reduction reactions, essentially "eating rocks" to sustain life. Sulphate-reducing bacteria like *Desulfovibrio* sp. and iron-oxidizing bacteria create complex geochemical systems that influence groundwater chemistry, mineral formation, and even ore deposit formation over geological timescales.

The Atmospheric Microbiome and Electromagnetic Interference

The atmosphere, long considered too harsh and nutrient-poor to support significant microbial communities, has emerged as an important but understudied microbial habitat. Studies have shown that bacteria are one of the many types of biogenic aerosol particles (fungal spores, pollen, etc.) that are ubiquitous in the atmosphere (Burrows et al., 2009). Spore-forming genera such as *Bacillus* and *Clostridium* can survive the extreme

conditions of atmospheric transport, enabling long-distance dispersal between continents (Rodó et al., 2024).

Some atmospheric microorganisms actively influence weather patterns. *Pseudomonas syringae* produces specialized ice-nucleating proteins that serve as condensation nuclei for cloud formation, directly linking microbial metabolism to precipitation patterns and regional climate (Zhen et al., 2017).

3.

The Atmosphere Environment

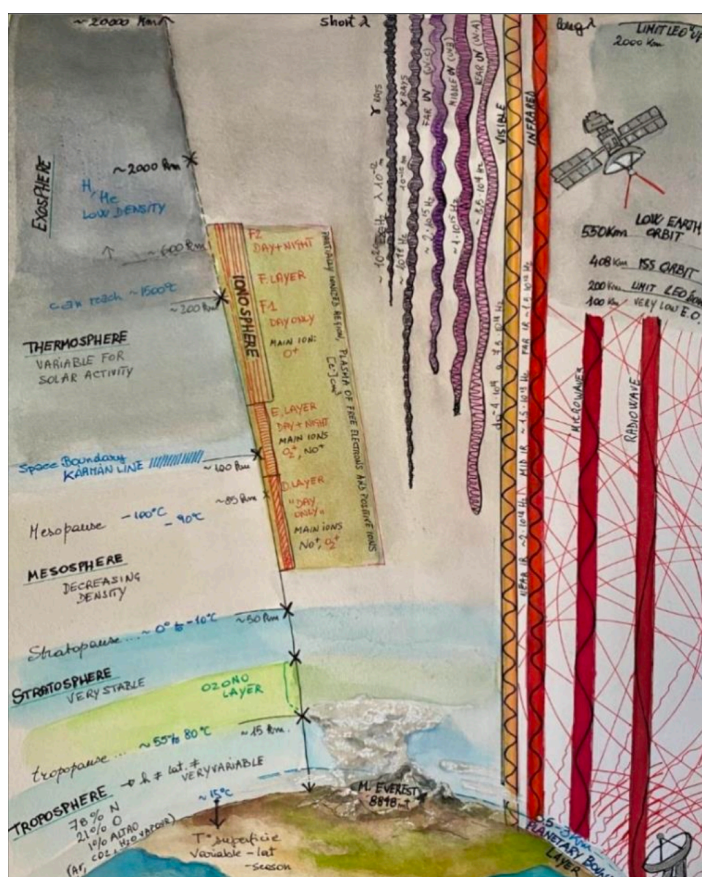


Figure 3. Scheme of the atmospheric layers, temperature, main chemical composition, and incoming radiation

So, we have reached the most external environment, although having had many different states across time, it has successfully surrounded our entire planet for the last 2 billion years. Fluid layers, subject to movements and tides (Covey et al., 2011). The Atmosphere is fundamental to ensure the continuity of the ecosystems that characterize life as we know it. In fact, if it might seem strange to consider the Atmosphere as an ecosystem per se, it is well known that for sure it is the regulatory matrix through which all energy flows, connecting all ecosystems. An interface between space and the Earth's biosphere, practically a fundamental medium that creates ideal conditions for a large part of all

living systems. It should be remembered that this area, in the bottom layer, is populated by birds, both sedentary and migratory, and insects that can fly or be transported to considerable heights, moving within and across continents (Chilson et al., 2017). More than this, the atmosphere also hosts a true "aeroplankton" composed of bacteria, viruses, fungi, protists, algae, spores, and pollen, with concentrations reaching 10^6 microbial cells per cubic meter in the planetary boundary layer (0–3 km altitude), and they might be metabolically active (Tignat-Perrier et al., 2019), decreasing in the free troposphere to approximately $2\text{--}5 \times 10^5$ (200000–500000) cells/m³ and at lower stratosphere to $4 \times 10^1\text{--}8 \times 10^4$ cells/m³ (40–80000) (Bryan et al., 2019; Burrows et al., 2009). Bacteria have, in any case, been detected even in the stratosphere and mesosphere, at the height of 41 km (Burrows et al., 2009). Bacterial genera such as *Bacillus* and *Pseudomonas* dominate these aerial communities, with some organisms like the previously mentioned *Pseudomonas syringae*, actively influencing weather patterns by producing ice-nucleating proteins that serve as condensation nuclei for cloud formation (DeLeon-Rodriguez et al., 2013).

Bacteria enter the atmosphere from all surfaces as aerosol particles and they play a role in the geographical distribution of microbial taxa (Martiny et al., 2006). Many aspects of the phenomenology of bioaerosol formation (Primary Biological Aerosol Particles-PBAPs) (Huffman et al., 2020), and therefore of the dispersion of particles, bacteria, fungi and pollen in the atmosphere, are increasingly studied with advanced systems both from the ground and from space. Terrestrial optical sensors make it possible to identify the bioaerosol, while satellite systems track its movement and/or long-distance transport. The technological advances let researchers have a better ecological understanding of the linking mechanisms between atmosphere, terrestrial surfaces and marine biomes (Šantl-Temkiv et al., 2022). PBAPs may be from a few nanometres to hundreds of micrometres in size. The hygroscopicity of organic aerosols give them efficiency in acting as ice nuclei (IN) and cloud condensation nuclei (CCN). Then PBAPs can be dispersed in the Planetary Boundary Layer (PBL) but, in the presence of specific conditions, they can be displaced above the PBL and enter the free troposphere. Above the PBL, aerosol particles escape diurnal atmospheric constraints and can travel intercontinentally via wind currents or sandstorms (Tang et al., 2022) for long distances and for a long time (several days). Generally, deposition happens with rain, snow or dry deposition (Barberán et al., 2014). To survive the harsh conditions of long-range transport—desiccation, intense UV radiation, and temperature extremes—bioaerosols have evolved protective mechanisms including radiation-shielding pigments, resistant spore formation and attachment to mineral dust particles for physical protection (DasSarma et al., 2020; Griffin, 2004; Smets et al., 2016).

It is also quite important to consider the fact that the exponential increase in launches of satellites can contribute to the bacterial transport to extreme space environments. During a spacewalk and the usual monitoring inside the International Space Station-ISS, viable bacteria have been found both outside and inside of it. *Mycobacterium*, *Delftia*, and *Bacillus* with genetics like that of marine, coastal bacteria, were outside and various hypotheses about their origin have been formulated. It may happen during the phases of ascent or some ultimate space origin (Grebennikova et al., 2018a). A recent paper points out that inside various modules of the ISS a remarkable quantity of different taxonomic bacterial group has been analysed. Despite being a closed and controlled system, it represents an "extreme example of an industrialized environment", and it shows that intensive technological environments fundamentally alter microbial communities in predictable ways (Salido et al., 2025). In this study the microbial sources were human (skin, oral cavity, nose, faeces, and urine), food, and building materials.

Another recent paper, exploring the characteristics of microbes during long-term space missions, describes a new Bacteria, named *Niallia tiangongensis*. It has been isolated from the surface of the hardware on the China Space Station, orbiting between 350 and 425 km heights, a contained environment. Based on phenotypic, physiological and chemotaxonomic characteristics, as well as genome annotation, that differentiate it with terrestrial *Niallia circulans*, is interesting to evaluate whether the space environment can direct microbial evolution or select for autonomous and specific adaptive traits (Yuan et al., 2025).

These discoveries raise questions about whether other anthropogenic changes to space environments may exacerbate current problems related to well-known customary or emerging forms of pollution that pervade the different layers of the atmosphere. From the apparently harmless inflatable party balloons which release microplastics at various altitudes when they burst (and microplastics can likely become substrates for bacteria, as occurs in the ocean), to greenhouse gases, traffic-derived particulate matter, industrial emissions, etc (Luo et al., 2024; Yang et al., 2020). A completely different kind of pollution derives from the considerable presence of orbiting objects, where they have been carried by a rocket. Launches and objects have increased exponentially from the 60s to today, and this reality brings new scenarios that require study and attention, i.e. with regard to the fuels used in relation to the frequency of launches, remembering that the fuel that is not polluting on the ground can be polluting in the upper atmosphere (Caraveo, 2024). Space debris are considered pollutants for many reasons, they have different sizes that means different levels of danger, from millimetres to several meters, (the largest are monitored by SSN-Space Surveillance Network). They are orbiting extremely fast and often with irregular revolutionary trajectories (Perozzi, 2024).

This atmospheric pollution is relevant as the amount of debris raises the issue of collisions and structural damage to other satellites. Also, it causes the release into the atmosphere of particles—difficult to detect—of the various construction materials, fuel residues, etc. The release can happen during the ascent, when a launch failed with rocket explosion, and especially when they burn re-entering the atmosphere. An estimation made with ESA “Master 8 Model Estimate” brings to light a concrete problem that, now, many projects are trying to resolve with solutions that are at the same time creative but also technologically challenging and expensive. After all, the re-entry of the various space objects can also be a problem: the structures are fragmented by dynamic pressure and then melted, vaporized and sublimated by the effect of heating due to high speed, and about 80% of the material persist in the stratosphere (Ott & Bonnal, 2025). The monitoring SABRE mission sampled stratospheric aerosols at < 19 km of altitude, in March 2023. Analysis made it possible to quantify the presence in the stratosphere of about 10% of the metals vapours and aerosols deriving from the burn-up of space objects, include metals, such as Lithium, Aluminium, Silver, Niobium, Hafnium. Consequence of the potential accumulation of this material between 10 and 30 km in altitude is not clarified, nor its radiative impact, but for sure more expanded investigations are required (Maloney et al., 2025). It is not yet known what the effect of an increasing level of metal content may be on the properties of stratospheric aerosol (Murphy et al., 2023a).

Beyond the physical contamination from space debris, the development of satellite operations may originate another possible atmospheric pollution: electromagnetic radiation. After the advent of electrification, more than a century ago, and wireless communications, the level of radiation from devices, land-based infrastructures and then satellites, military and commercial applications, has exponentially grown and the effects of the increasing exposure of non-ionizing radiation, as low-frequency and radiofrequency radiation, have been studied repeatedly, although not sufficiently. A brief

description of what is now orbiting the Earth (ESA's Annual Space Environment Report and Satellite and Space Debris Statistic, 2025).

Table 1. Active Satellites by Orbital Regime (ordered by altitude, updated 08 September 2025). Complete table with all the Orbits, all the type of fragments and other descriptions is available on the website <https://sdup.esoc.esa.int/discosweb/statistics/>

Orbital Regime	Altitude Range	Active Satellites	Total Debris	% of Total Satellites
LEO-Low Earth Orbit	< 2000 km	13263	10435	86.7%
MEO-Medium Earth Orbit	2000-31570 km	76	1115	0.5%
NSO-Navigation Satellite Orbit	~20000 km	293	155	1.9%
GEO-Geostationary Earth Orbit	~35786 km	807	161	5.4%
Other Earth Orbit	Various heights	843	31075	5.5%
TOTAL	-	15282	11866	100%

We therefore face an unprecedented and unknown scientific situation: chronic existence of ground based electromagnetic fields and the major increase of electromagnetic fluxes from the atmosphere, with quantities of emissions that have never existed in terrestrial evolutionary history. Which are the consequences of this vast flux, combined with atmospheric physic-chemical processes to the exposed microorganisms and macro-organisms and the mass of materials orbiting?



Figure 4. Free interpretation of the increasing number of Earth's satellites over time (not to scale)

With laboratory experiments, researchers have started to consider what the effects, the risks and the potential with cells, tissues and organisms can be, of the pervasive and increasing electromagnetic field (EMF) from fast-evolving human technology, that now permeate the whole atmosphere (Romanenko et al., 2017). From the exponential growth of satellites orbiting the planet and therefore to the amazing amount of daily data transmitted, collected, retransmitted, the rapid proliferation of satellite communication systems, including mobile phones, Wi-Fi networks, and 5G infrastructure, there is an objective risk about fundamentally altering the electromagnetic environment in which atmospheric microorganisms, and not only them, are present, survive and function.

In the stratosphere the stressor conditions for microbial life are UV-C and cosmic radiation, freezing temperatures, hypobaric pressure, desiccating condition, starvation and ozone. To resist those elements requires extremophilic characteristics that include tolerance and survival strategies. The stratosphere arguably represents the upper limit for life. However, numerous bacteria and fungi have been found viable. They can survive at these altitudes through sporulation and dormancy, aided by the capacity of aggregation and shielding. The ozone layer, located predominantly within the stratosphere, acts as a protective shield from radiation for our planet, allowing terrestrial life forms to be transported and returned to a viable state from the troposphere and lower stratosphere (DasSarma & DasSarma, 2018). However, the current characterization of these stratospheric stresses remains incomplete with respect to the new environmental scenario determined by the anthropogenic satellite infrastructure. Stratospheric microbial communities, already subjected to multiple extreme stresses, are now chronically exposed to electromagnetic fields of unprecedented evolutionary intensity. The absence of integrated studies linking stratospheric microbiology, bio electromagnetism, and the impact of satellite telecommunications represents a critical gap in our understanding of the contemporary atmospheric environment. It seems relevant to now investigate something that is happening continuously and daily and that—it is worth repeating—is unprecedented in the history of the earth starting from some previous information.

At the lowest level of the Atmosphere, we already have a large amount of electromagnetic fields due to increased communications and increased use of electricity. Also, radar and many other new technologies for the very developed Ground-based monitoring, studying the aerial environment are emitting and receiving back frequencies (An et al., 2024). Natural electromagnetic fields are characterized by relatively weak intensities and a predominantly episodic nature of various phenomenon:

- Schumann resonances, extremely weak electromagnetic waves at 7.8 Hz generated by global atmospheric discharges, reach intensities on the order of pico-teslas—very small magnetic fields—but they are already documented as capable of influencing the permeability of cell membranes and heart rhythms.
- Sporadic impulses generated by lightning are capable of inducing natural electroporation and horizontal gene transfer in microorganisms (Cifra et al., 2021).

The advent of satellite telecommunications has introduced a continuous and pervasive anthropogenic overlay. Modern satellite constellations radiate the entire Earth's atmosphere with signals in the L, S, C, X, Ku and Ka bands. From space, each satellite of the various space agencies or of the growing number of private constellations, whether for meteorology, climate studies, defence, commercial or research purposes, creates a huge flow of emitted/received/transmitted signals that is for sure much higher than the natural reference fields. This chronic electromagnetic exposure represents a completely new condition occurring in the atmospheric layers and an evolutionary selective pressure for aeroplankton, and the other living organisms. The rapidity of this environmental change raises fundamental questions about the possible adaptive responses of microorganisms, including potential mechanisms of electromagnetic resistance that could interfere with conventional microbiological control strategies.

So, laboratory research has revealed that microorganisms are surprisingly sensitive to electromagnetic fields across a wide range of frequencies. Many other studies about bacterial responses to electromagnetic fields, both from the past or from more recent experiments, have shown that exposure can either enhance or reduce cellular activity, with effects varying dramatically depending on field intensity, frequency, exposure duration, and bacterial species characteristics (Hegazy & El-Antrawy, 2025). The mechanisms underlying these effects appear to involve disruption of cellular membrane processes, altered ion channel function, and interference with fundamental metabolic pathways. Generally, these laboratory studies are aimed at finding out which characteristics may contribute a) to the reduction of pathogenicity, b) to the understanding of resistance mechanisms. Sometimes, however, the answers obtained made things more complex, since it emerged, by exposing the bacterial colonies to the influence of electromagnetic fields, that the imposed stress situations can cause different reactions at the EMF exposure, increasing growth in some species (*Stafilococco epidermidis*, *Stafilococco aureus* and *Escherichia coli*) but a decrease in others (*Serratia marcescens*; Tessaro et al., 2015) and an increase in the subsequent viability of the colony itself (Beretta et al., 2019). It should also be considered that bacteria can have variable responses depending on the time scale of environmental fluctuations (Nguyen et al., 2021).

Therefore, at this point, it seems appropriate try to quantify more precisely the extent of the flows we are talking about.

The electromagnetic saturation is multifaceted:

Table 2. Complete electromagnetic spectrum in satellite systems, organised by frequency (Claude Sonnet 4 PRO–Anthropic 2025)

Frequency Range	Wavelength	Satellite, Scope & Applications
380–750 nm (Visible Light)	380–750 nm	Earth observation satellites: Optical imaging, mapping • Military reconnaissance: High-resolution surveillance • Optical ground stations: Some laser communications
0.7–3 µm (Infrared)	700–3000 nm	Earth observation: Vegetation monitoring, land use mapping • Climate satellites: Surface analysis
1064 nm (Near-IR laser)	1064 nm	Inter-satellite laser backup systems
1550 nm (Near-IR laser)	1550 nm	High-speed inter-satellite laser communications (primary wavelength)
3–15 µm (Mid Infrared)	3000–15000 nm	Meteorological satellites: Surface temperature monitoring, climate studies
8–14 µm (Thermal Infrared)	8–14 µm	Military/Intelligence satellites: Thermal surveillance, missile warning systems
15–300 µm (Far Infrared)	15000–300000 nm	Weather satellites: Atmospheric temperature profiling
10 MHz–300 GHz (Radio waves)	30 m–1 mm	Radio astronomy satellites: Space-based radio telescopes
30–300 MHz (VHF)	10–1 m	VHF Communications: Amateur radio, emergency, satellite control
137–138 MHz	2.2–2.1 m	Environmental satellites: Telemetry beacon reception
161.975–162.025 MHz	1.85 m	AIS satellites: Maritime ship tracking from space
300 MHz–3 GHz (UHF)	1 m–10 cm	UHF Communications: Mobile satellite communications, telemetry
400 MHz–40 GHz	75 cm–7.5 mm	SAR satellites: Earth imaging radar, oceanography
406 MHz	73.9 cm	Search & Rescue satellites: COSPAS-SARSAT emergency beacons
1–2 GHz (L-band)	30–15 cm	Navigation satellites: GPS, Galileo, GLONASS, Beidou • Mobile satellite services: Inmarsat, Iridium
└─ 1090 MHz	27.5 cm	ADS-B satellites: Aircraft tracking from space
└─ 1176.45 MHz	25.48 cm	GPS L5: Modern civilian navigation signal
└─ 1191.795 MHz	25.15 cm	Galileo E5: High precision navigation
└─ 1227.60 MHz	24.42 cm	GPS L2: Military/precision navigation
└─ 1575.42 MHz	19.03 cm	GPS L1 + Galileo E1: Primary civilian navigation signals
└─ 1602 MHz	18.7 cm	GLONASS G1: Russian navigation system

Table 2. Continued

Frequency Range	Wavelength	Satellite, Scope & Applications
1–300 GHz	30 cm–1 mm	Weather satellites: Passive microwave sensing (humidity, precipitation)
2–4 GHz (<i>S-band</i>)	15–7.5 cm	Satellite control, space telescope control (Hubble), scientific data downlink
3–30 GHz (<i>SHF</i>)	10–1 cm	Super High Frequency band - Military and commercial microwave communications
└─ 4–8 GHz (<i>C-band</i>)	7.5–3.75 cm	Commercial satellites: uplink/downlink
└─ 8–12 GHz (<i>X-band</i>)	3.75–2.5 cm	Military communications, satellite radar, space telescope data (Chandra)
└─ 12–18 GHz (<i>Ku-band</i>)	2.5–1.67 cm	Satellite TV, broadband internet, space telescope high-speed data (Hubble)
└─ 18–27 GHz (<i>K-band</i>)	1.67–1.11 cm	High-speed satellite communications
└─ 27–40 GHz (<i>Ka-band</i>)	1.11–0.75 cm	High-speed satellite internet, advanced space telescope communications (James Webb)
30–300 GHz (<i>Millimetre waves</i>)	10–1 mm	Military satellites: EHF secure communications
33–75 GHz (<i>Millimetre waves</i>)	9–4 mm	Future satellites: Q/V-band ultra-high-capacity communications
60 GHz (<i>Millimetre wave</i>)	5 mm	Inter-satellite links: Traditional microwave communications

Now, traditional research of scientific publications proves inadequate when seeking integrated data on real-time satellite transmission volumes across competing commercial entities, electromagnetic field measurements at atmospheric scales, and cross-frequency interference patterns from mixed terrestrial/space sources.

The quantitative estimates presented here required **AI-assisted** navigation through:

- Technical specifications buried in engineering reports
- Commercial data released sporadically in investor communications
- Regulatory filings across multiple international agencies
- Scientific papers using incompatible measurement units and methodologies

So, what emerged is that try to quantify the electromagnetic transformation of Earth's atmosphere presents unprecedented methodological challenges. The data required spans multiple domains—satellite engineering, telecommunications, atmospheric physics, and microbiology—each with distinct terminologies, databases, and publication patterns. This methodological complexity itself illuminates a critical research gap: the absence of standardized, accessible databases for monitoring our planet's electromagnetic transformation. It means that “we” are conducting a planetary-scale experiment while lacking adequate tools to document and verify its parameters.

So, estimations were obtained from the research conducted with Claude Sonnet 4 PRO–Anthropic, 2025. The results have been verified more than 20 times with different online

conversations that addressed the same theme, starting from various perspectives, and constantly revisiting the data. The List of the most relevant websites nominated by AI can be found in Web References section.

1. ESA MASTER 2024 data: in the 500–600 km altitude range, the density of active satellites is reaching that of space debris for the first time in history.

2. EARTH OBSERVATIONS SATELLITES:

- Esa/Copernicus (Sentinel 1, 2, 3, 4, 5, 5 P, 6) total ~10–15 TB/day between processed and estimated
- NASA/ Landsat 2–3TB/day (starting from 1972)
- Planet.com > 100 TB/day
- Maxar > 80 TB/day
- Nasa Swot 20 TB/day
- Nisar 80TB/day

3. CONSTELLATIONS

- Mega constellation Space X-Starlink, 7600 active satellites
- One web, 600 active satellites
- Amazon Kuiper 100 satellites (tests, prototypes)
- G60 Qianfan China 50 satellites

4. UNINTENTIONAL EMISSIONS

These emissions represent unintentional electromagnetic pollution that was unforeseen in the original projects; they grow exponentially with mega-constellations; they are not controlled by specific regulations, they can interfere with radio astronomy and terrestrial systems.

“Electromagnetic radiation that is radiated by (or leaking from) electrical devices and systems on board satellites and not necessarily related to the generation and transmission of wanted electromagnetic radiation from antennas used for communication” (Di Vruno et al., 2023). They can derive from: Power systems (converters, batteries) Processors and on-board computers, Electrical cables and interconnects, Thermal control systems, Metal structure of the satellite (acting as an unintentional antenna). It is to be considered that second-generation satellites emit UEMR up to 32 times stronger than first generation. *“The calculated electric field strengths of the detected UEMR exceed typical electromagnetic compatibility standards used for commercial electronic devices as well as recommended emission thresholds from the Radiocommunication Sector of the International Telecommunications Union (ITU-R) aimed at protecting the 150.05–153 MHz frequency range allocated to radio astronomy”* (Bassa et al., 2024).

To understand the actual electromagnetic saturation of the Earth's atmosphere, it is essential to distinguish between two categories of satellites that operate with completely different transmission modes and volumes, considering, however, that the impact derives from the entire satellite population currently active.

Earth observation satellites represent the first systems—like Landsat, Sentinel, Planet.com, and NASA missions—essentially function as sophisticated "space cameras". They continuously acquire images and scientific data of the Earth and then transmit them to terrestrial receiving stations. The flow is unidirectional from the satellite to Earth for passive satellites. These systems collectively generate about 100 petabytes of scientific data per day. However, a significant portion of these satellites are active: they use active sensors-SAR radars, altimeters, scatterometry, space lidar—which actively emit signals

towards Earth and receive its bounce and then transmit the elaborated data to the ground-based data centres, multiplying the effective electromagnetic activity.

This approximation does not consider what is emitted/received/transmitted from many ground radars, for every possible use, even the tracking of dangerous debris (Grebennikova et al., 2018b).

Telecommunications constellations, on the other hand, constitute a completely different universe. Starlink, with its 8100 active satellites, OneWeb, and future mega-constellations do not "observe" the Earth: they connect it. Each satellite functions as a space internet repeater, handling continuous two-way traffic between millions of terrestrial users.

But the satellite ecosystem is much larger. The previous cited ESA 2025 data document updated on 08 September 2025, indicates 15280 satellites still in space, 12500 are still functioning satellites, which means that in addition to the 8100 Starlinks there are thousands operational satellites: GPS/GLONASS/Galileo/BeiDou navigation constellations (about 300 satellites), hundreds of government weather satellites, thousands of military and intelligence satellites, 800 GEO satellites, miniaturized satellites (CubeSats, PicoSats, NanoSats) for traditional telecommunications, as well as a growing population of small satellites for university research, technology testing, and spatial IoT (Internet of Things) services. Each one of these contributes to electromagnetic saturation through telemetry, control, synchronization signals, and often through active emissions for their specific functions. Although they have individually reduced transmission powers, they are very numerous and contribute significantly to the overall electromagnetic saturation.

The quantification of telecommunications traffic presents enormous methodological challenges but considering that the entire satellite population generates a continuous electromagnetic flow, from petabytes of scientific data to massive volumes of internet traffic, from the control signals of all 15280 satellites to the emissions of active sensors—a realistic AI (Anthropic Claude Sonnet 4, plan pro, 2025) estimate is in the order of 5000–10000 petabytes of daily electromagnetic activity through the Earth's atmosphere.

The 5000–10000 petabytes of daily electromagnetic activity translate into a continuous power of 2–3 MW distributed across the entire Earth's atmosphere—the equivalent of turning the entire atmospheric envelope of the planet into a transmitting antenna powered by power plants, with field strengths that can be incredibly higher than the natural electromagnetic signals that have characterized Earth's evolution (Anthropic Claude Sonnet 4, plan pro, 2025).



Figure 5. Free interpretation of artificial Earth satellites (not to scale)

5. Conclusions

This analysis suggests the need for more research. Currently it is not possible to draw conclusions about the short- and long-term effects that this massive occupancy of the atmosphere can have, from the minuscule bacteria or the whole planet. Analysing the comprehensive concept about environment, hides an enormous quantity of unbelievably minuscule environments, included in a vast ensemble, where remarkable or almost unperceivable differentiation can create the conditions for some kind of life, never so simple as the dimensions of organisms can suggest. Accelerating human occupancy and development of aerial habitats requires new regulation of the airspace, and clarification about the habitat concept. Previous experience demonstrates that it is never too much effort to try to protect species when and where they are present (Diehl et al., 2017). On our planet there are simultaneously existing an infinite number of connections and interactions quite difficult to quantify. The principle “everything is everywhere” seems plausible especially for Bacteria dispersal, but also in this case the environment selects and geography per se is not enough to understand the spatial distribution of organisms (Fondi et al., 2016). Yet, emerging research reveals that even the three-dimensional, dynamic environment of the atmosphere hosts distinct microbial biogeographical

patterns. This enhances the fundamental importance of the environmental selection, making it extremely important to better evaluate the massive anthropogenic release of pollutants, the increasing electromagnetic fields and their effects. It appears extremely important to thoroughly explore the effects of non-ionizing electromagnetic fields on bacteria, insects, flora and fauna, in all terrestrial, aquatic and aerial environments (Levitt et al., 2022b) and this increase in exposure to electromagnetic fields is taking place in such a short time that it does not allow the various organisms to adapt (Levitt et al., 2022a). More than this, research on the effects of electromagnetic pollution on atmospheric microorganisms reveals a deep methodological fragmentation that prevents an ecologically relevant understanding of the phenomenon. On the other hand, bio-electromagnetism studies provide precise data on field strengths, frequencies and exposure durations, but they use parameters that are often disconnected from the real environmental conditions that atmospheric organisms encounter daily. Satellite telecommunications research meticulously quantifies transmitted data volumes and emission powers for commercial scope, without however translating these technical parameters into biologically significant exposure units. This methodological disconnect is further compounded by the absence of bridging studies linking space communications engineering with atmospheric ecology. The result is a scientific landscape in which we possess sophisticated technical knowledge in isolated domains but lack an integrated framework that allows us to assess the cumulative impact of satellite infrastructure. There are interesting studies regards the effects on insects and birds' migrations, and also on plants, exposed at radio frequency fields and other type of interferences (Balmori, 2021; Czerwiński et al., 2023; Engels et al., 2014; Wiltschko et al., 2015). Thus, after the perplexities raised by astronomers for light pollution and radio interference (Caraveo, 2020), and those of atmospheric chemists, for the different types of possible pollution, (Murphy et al., 2023b) also from the point of view of the ecology of the environment the need for an interdisciplinary methodological approach is intensified. Therefore, now it becomes not only desirable, but essential to transform fragmented technical data into ecological knowledge that can be used for environmental risk assessment.

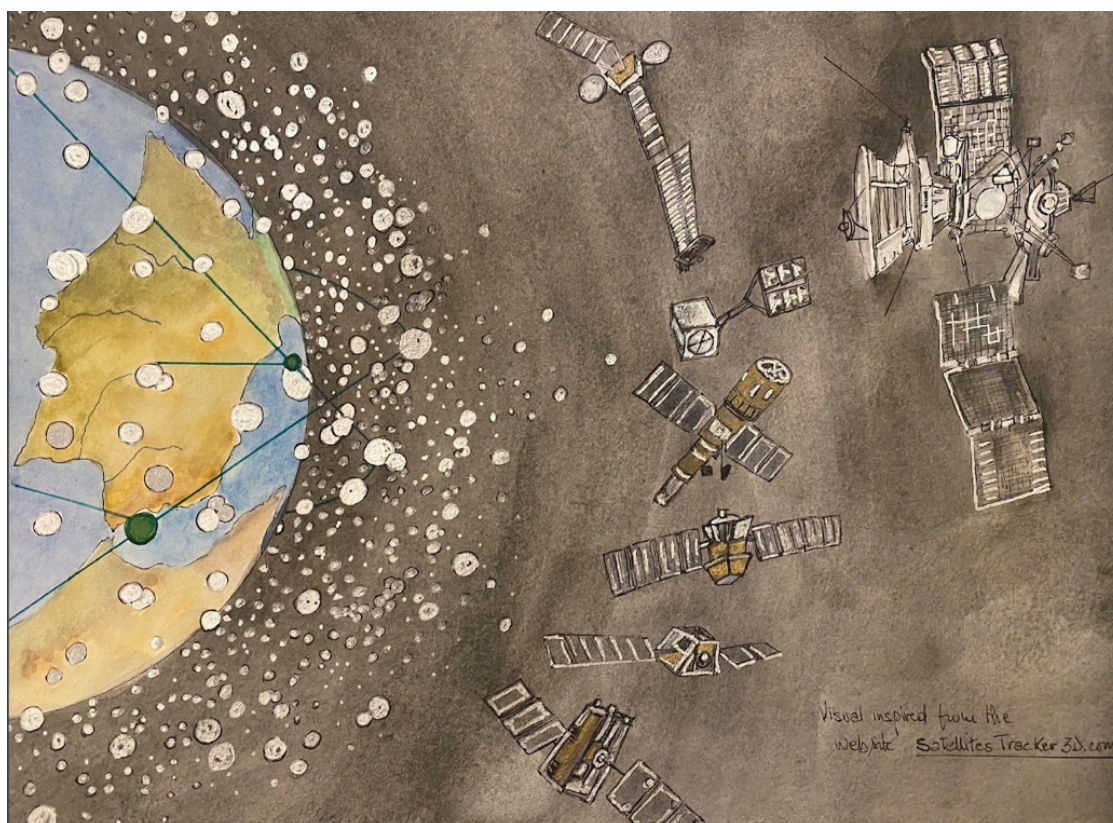


Figure 6. Visual inspired by the Satellite Tracker3D.com website (not to scale)

Author's personal note

During this year, until September 18, there have been approximately 210 space launches and the same day an additional 28 satellites have been deployed on LEO orbit. SpaceX deployed its 2000th Starlink satellite of the year on Saturday September 6, 2025. Without diminishing or denying the importance of technological uses that allow—among other things—a better understanding of the complex relationships and the interaction between radically different forms of life, and the way they fill every environment, it would be desirable that, together with the stratification of data and the useful integrations between research sectors, the backbone of investigations and relationships themselves are shifted further. Historically, it took millennia of trial and error to understand the Solar System, the movements of galaxies in space, the boundaries of the universe and much more, always having to broaden the concept of the centre. We define environment as the context able to surround something intrinsically related with life. Maybe now it is necessary to accept the idea that Human beings will never be the centre around which the environment revolves. Every living organism uses resources coming from its/his own environment and at the same time contributes to creating it, rather than destroying it. If Man were to comprehend this, he could give greater attention to the aspects that, although autonomous, possess great functions of natural integration at very different scales and with an incalculable wealth of life forms. Not all the planet's environments are there only for human consumption. Use doesn't mean exploiting but rather using and giving something back without wasting it or, worse, irreparably ruining or polluting it.

We can finally learn from the Law of Physics that governs life's equilibrium: Nature uses everything with extreme creativity, never looking for profit, but freely giving space and opportunity for new forms and manifestations of her mysterious beauty.

CONFLICTS OF INTEREST: THE AUTHORS DECLARE NO CONFLICTS OF INTEREST.

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Science told today: ethics, languages and responsibility in contemporary science communication

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Abstract: This article investigates the current landscape of science communication through a series of qualitative interviews with young Italian communicators from diverse academic and professional backgrounds. In a media environment increasingly shaped by social platforms and rapidly evolving formats, questions around quality, ethics, and responsibility become central. The voices collected highlight how expertise, intentions, audience awareness, and the choice of language and medium deeply shape the effectiveness of communication. Interviewees emphasize the need to adapt tone and format while preserving accuracy, honesty, and relevance, stressing that science communication does not mean oversimplifying complexity but making it accessible. Overall, this article portrays a dynamic and heterogeneous field held together by shared principles of responsibility, care for the message, and respect for the audience.

Keywords: science communication; ethics and responsibility; digital dissemination; information quality; media and language.

Riassunto: L'articolo esplora il panorama contemporaneo della comunicazione scientifica attraverso una serie di interviste qualitative a giovani comunicatori italiani provenienti da percorsi eterogenei. In un contesto in cui i social media e i nuovi formati digitali hanno ampliato le possibilità espressive e il pubblico raggiungibile, emergono interrogativi cruciali sulla qualità, sull'etica e sulla responsabilità di chi comunica scienza. Le testimonianze raccolte mostrano come competenze, intenzioni, consapevolezza del pubblico e scelta del linguaggio influenzino profondamente l'efficacia del messaggio. Le voci degli intervistati evidenziano l'importanza di adattare *medium* e registro comunicativo, mantenendo accuratezza e onestà, e sottolineano come la comunicazione scientifica non consista nella semplificazione della complessità, ma nella sua accessibilità. Nel complesso, questo contributo rivela un settore dinamico, plurale e in evoluzione, unito da valori condivisi legati alla responsabilità, alla cura del contenuto e al rispetto del pubblico.

La **versione integrale** dell'articolo in lingua italiana è disponibile per il *download* su JECO (Vol. 2), consultabile sul sito www.jecojournal.it

WE COMMUNICATE MORE THAN EVER TODAY, BUT DO WE TRULY UNDERSTAND MORE?

IN STRAMBINO (TO), ITALIAN COMMUNICATORS GATHER TO DISCUSS THE PRESENT AND FUTURE OF COMMUNICATION.

Introduction

In recent years, especially post-COVID-19, science communication has become a constant presence in the communicative landscape: it flows through social networks, university lecture halls, cultural festivals, short videos, independent podcasts, and in the major newspaper. It is fast, accessible, creative, and often capable of reaching an audience that academic or institutional communication no longer catches. This scenario, while opening new horizons, raises urgent questions: what does it mean to communicate science today? Is it possible to distinguish “good” communication from “bad” communication, or does this binary distinction no longer belong to today’s communication landscape?

In a time when anyone can potentially become a communicator via a social media profile, the very definition of quality becomes more complicated: is it a matter of skills? Ethics? Accuracy? Impact? Communication method? Or the type of audience one chooses to address?

These questions guided a small journey of listening and dialogue with young science communicators who today experience science communication from the inside – not as theory, but as daily practice, often self-taught and rooted in highly diverse academic, professional, or creative paths.

This article is structured around various semi-structured qualitative interviews, held at the annual gathering of Italian science communicators, *Folle di Scienza*, with young science communicators, primarily active online but not exclusively, with heterogeneous backgrounds (scientific, artistic, graphic, communicative) and different levels of visibility. The goal is not to quantify a phenomenon or establish normative definitions, but to observe how those who do science communication today interpret their role, their responsibility, and the quality criteria of their work. Involving diverse backgrounds allows us to grasp not only “what” is communicated, but also “how” and “why”. Strategies, priorities, and the very idea of science communication change based on educational background and work context. There is no hierarchy here; the aim is to reveal how, within the heterogeneity of paths, there is something common or something entirely different.

As we said earlier, we communicate every day, continuously. We are immersed in a constant flow of information, but do we truly know how to distinguish who transmits it accurately from who does so in a distorted way? And, above all, is there a single, unequivocally "correct" way of doing science communication? Does it really exist?

Voices from the event

For many, the difference between good and bad science communication exists, but it is never clear-cut: it is a thin line, influenced by context, intentions, and – not least – by the communicator's level of awareness and ethics. Being exposed online means engaging with a heterogeneous audience: people who share our ideas, others who challenge them, and still others who are approaching a topic for the first time. Therefore, talking about science communication necessarily implies talking about the relationship with the audience and the resulting responsibility. Every piece of content carries with it an implicit act of intellectual honesty. And it is precisely in managing this responsibility that what is often called "bad science communication" arises: not so much a technical error, but a matter of intentions. Do we communicate to inform or to polarize? To increase numbers or to build consensus? Or, again, is it the lack of awareness of the effects on the audience that makes communication ineffective – or harmful – even when born with good intentions?

As naturalist and communicator Willy Guasti observes, «it doesn't make much sense to talk about "good" and "bad" science communication: if honesty, responsibility, and source verification are missing, then it's no longer science communication. The only way to do it is to do it well.» This highlights how ethics, intentions, and responsibility are not peripheral but the very structure of those who choose – or think they choose – this profession.

This view finds an echo in those who, like Sara Biancardi, warn against sensationalism. The marine biologist and communicator Sara Biancardi indeed reminds us that when science communication «seeks the sensational or simplifies to the point of distortion.» it risks turning into something other than science communication, even without bad intentions. And here, reflection on the audience becomes paramount: not only “what” we say, but “to whom” we are saying it.

On this very point, psychologist and communicator Graziano Gigante reminds us that «every piece of content has an impact on the receiver and ignoring it can turn a message into something problematic, even without the intention to do so.» And if responsibility is the foundation, then we must also ask “how” we communicate: with what tools, with what language, and with what audience in mind.

Neurology trainee and communicator Paolo Forti indeed emphasizes that the quality of science communication cannot be separated from the competence and relevance of the content: talking about everything, he says, often means talking poorly. «It's easy to venture into topics one doesn't truly know, but quality and accuracy are the minimum basis for being able to talk about science communication.» When this basis is missing, one enters a territory that Forti unhesitatingly defines as “anti-science communication”:

«Interviewing anyone, giving space to even denialist voices, is not informing: it's causing harm.»

From this arises the need to explore an element only briefly mentioned so far, but central to the discussion on science communication: the language and the medium through which we communicate our message.

Our discussion naturally shifts to "how" to communicate: knowing "what" to say is not enough; it is essential to choose the right medium and calibrate the language based on the audience. Social media, books, science festivals, illustrations, or short videos: each channel requires different strategies and a precise awareness of how information is perceived. And on this very aspect, our interviewees offer concrete reflections. It is in this balance between medium and message that the true effectiveness of science communication takes shape.

Indeed, biologist and communicator Andrea Borsa clearly emphasizes the need to be "chameleonic" because «the way of communicating changes, and consequently you must adapt: YouTube is different from a Reel, TikTok is different from a museum, which is different from a TV program. They all have a message to convey, but the target, the tone, and even the words chosen change based on the audience.»

But it's not just about verbal language: visual and graphic language also conveys messages, as explained by Jacopo Sacquegno, biologist and visual thinker: «If I work with universities, researchers, or foundations to give voice to certain complex themes, I have to change style and level of formality depending on the target. Every discipline has its own verbal and visual view: if I address specialists, I can use conventions they understand; if I'm speaking to a festival audience, I'd frame it as re-contextualizing the message and using different kinds of imagery and words that make it accessible, while still respecting the complexity of the topic and the people I spoke to.» Jacopo also emphasizes how different channels influence communication: live events, live scribing, videos, or social media posts require different choices regarding timing, format, and message structure.

If on one side we have social media and scientific or academic conferences, on the other there is more direct and immersive science communication. Marta Depetris, environmental biologist, hiking guide and science communicator, highlights how the role of a field guide allows for modulating language and activities based on direct interaction with the audience: «Every medium has its target... you simply have to adapt it and give it a different form based on the people you want to talk to.» The field experience is not just an alternative channel to social media, but a way to make science communication concrete and multisensory, involving smell and touch as well: touching the change, the place we are in, allows us to surround ourselves with everything that was explained during a journey.

Similarly, the book represents another kind of "touch": slower, more reflective, requiring attention and care from both the author and the reader. Veronica Repetti, linguist and science communicator, highlights how this tool allows addressing an audience that chooses to dedicate time and attention to reading, enabling more articulated and in-depth discussions: «In the case of a book, you can make slightly more articulated discussions,

thus taking things from a bit more of a distance.» However, she adds, one should not take for granted that the reader can absorb every detail: «We, instead, must always find ways to make reading enjoyable, understandable, and engaging», maintaining an accessible style while distinguishing it from academic scientific literature.

Looking ahead

In summary, listening to the voices of those who do science communication today reveals a clear picture: there is no single right way to communicate science, but there are shared principles that determine its quality. Competence, honesty, attention to the audience, ability to adapt to different media and languages, care for the message, and responsibility are the elements that unite even very different approaches. Whether it's an Instagram post, a scientific drawing, a field experience, or a book, the common thread remains the desire to make content understandable, engaging, and meaningful, respecting those who receive it and the challenges that science communication entails. This does not mean trivializing concepts: it is often said that those who communicate science "simplify" content, but as emerges from the interviews, science communication does not mean reducing complexity. It means making it accessible, so that anyone can approach the information, even without specific expertise. It is perhaps one of the greatest challenges a science communicator can face, bringing science out of academic bubbles and transforming it into a shared heritage.

These interviews remind us, therefore, that science communication is never a neutral act: every communicative choice has an impact, and those who communicate must do so with awareness, balance, and respect. Science, like communication, is alive, in constant motion, and those who tell its story must know how to change register, tone, and medium without ever losing sight of the substance of the message and the responsibility towards the audience.

And so, observing the different paths of science communication, one realizes that every communicative choice tells not only the science and the effort behind the scenes, but also the storyteller.

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Plastic pollution and Plastic Fighters: awareness through play

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Abstract: Plastic, due to its properties and versatility, has undeniably contributed to revolutionizing our society within a relatively short period of time. However, with equal rapidity, it has become one of the most concerning environmental issues on a global scale. Awareness-raising represents one of the tools available to help mitigate its effects on both the environment and human health. Games can be considered cognitive and communicative tools capable of facilitating the understanding of complex concepts; the integration of game mechanics with information constitutes “game-based learning”. Based on these principles, “Plastic Fighters” was developed, a game in which participants take on the role of an environmental association dealing with the challenges of plastic pollution. The aim is cooperation to achieve a sustainable balance in the management of the marine-coastal ecosystem. Plastic Fighters is freely available in a “Print & Play” version in both Italian and English.

Keywords: plastic; plastic pollution; marine-coastal ecosystem; game-based learning; cooperative gameplay; awareness-raising.

Riassunto: La plastica, per le sue proprietà e la sua versatilità, ha innegabilmente contribuito a rivoluzionare la nostra società in un arco di tempo relativamente breve. Tuttavia, con altrettanta rapidità, si è trasformata in una delle problematiche ambientali più preoccupanti a livello planetario. La sensibilizzazione rappresenta uno degli strumenti a nostra disposizione per contribuire a mitigarne gli effetti, sia sull'ambiente che sulla salute umana. Il gioco si configura come uno strumento cognitivo e comunicativo capace di favorire la comprensione anche di concetti complessi; l'integrazione tra meccaniche ludiche e informazione costituisce l'apprendimento basato sul gioco (*game-based learning*). Sulla base di questi principi è stato realizzato *Plastic Fighters*, un gioco in cui i partecipanti vestono i panni di un'associazione ambientalista che si confronta con le problematiche dell'inquinamento da plastica. L'obiettivo è la cooperazione per il raggiungimento di un equilibrio per la gestione sostenibile dell'ecosistema marino-costiero. *Plastic Fighters* è disponibile gratuitamente nella versione *Print & Play*, sia in lingua italiana che in lingua inglese.

La **versione integrale** dell'articolo in lingua italiana è disponibile per il *download* su JECO (Vol. 2), consultabile sul sito www.jecojournal.it

1. A “dream” called plastic

Lightweight, flexible, durable, and inexpensive; a material that, thanks to its excellent properties, can be used in agriculture, construction, transportation, thermal insulation, packaging, and electronics – and beyond that – in furniture, toys (Figure 1), leisure products, automobiles, and medicine (Nayanathara Thathsarani Pilapitiya & Ratnayake, 2024).

A dream? No, this is plastic!



Figure 1. Plastic toy abandoned on the beach (photo: Milena Tempesta)

But what is plastic? The term refers to a wide range of polymers (such as polyvinyl chloride – PVC, polypropylene – PP, polystyrene – PS, etc.), mostly synthetic organic materials derived from the petrochemical industry, which can be combined with various chemical substances depending on the production process or the type of plastic being manufactured (Fulke et al., 2025; Nayanathara Thathsarani Pilapitiya & Ratnayake, 2024).

1.1 Plastic pollution is no game!

The development of plastic materials dates back to the second half of the nineteenth century; however, their production increased dramatically starting in the 1940s, and plastic consumption rose by 180 times between 1950 and 2018 (Nayanathara Thathsarani Pilapitiya & Ratnayake, 2024).

Although plastic has been an undeniably revolutionary material, we are now witnessing its “flip side.”

In 2014, the United Nations Environment Assembly identified plastic pollution as one of the ten most urgent environmental issues (Wang et al., 2023). It is estimated that every day the equivalent of 2000 garbage trucks is discharged into rivers, lakes, and oceans worldwide (United Nations Environment Programme, n.d.) with plastic accounting for approximately 80% of marine litter (Fulke et al., 2025).

This form of pollution has become a global problem; today, plastic is present in oceans around the world, on land, in inland waters, and even in the air we all breathe (Stubbins et al., 2021).

Oceans and marine sediments have shown an exponential increase in plastic concentrations (Schwarz et al., 2023). In aquatic environments, plastics can undergo various degradation processes, including photodegradation, thermo-oxidation, hydrolysis, biodegradation, and fragmentation. These processes contribute to the formation of microplastics (< 5 mm) and nanoplastics (< 1 µm). Nevertheless, microplastics may also enter the environment as primary particles, intentionally produced for cosmetic or industrial uses (Brožová et al., 2025). The impacts on species and ecosystems may vary depending on plastic type and particle size (Anastasopoulou & Fortibuoni, 2022).

Within this context, the Mediterranean Sea stands out as one of the most vulnerable marine areas due to the high concentration of anthropogenic activities (such as tourism, maritime transport, fishing, industry, and coastal development, etc.) within its semi-enclosed basin (Sharma et al., 2021).

Scientific research has demonstrated (and continues to provide evidence of) the effects of plastic pollution on aquatic organisms (Benson et al., 2022); however, humans are not excluded from this issue. Microplastics, for example, have been detected in blood, urine, the placenta, and amniotic fluid (Brožová et al., 2025).

Finally, it should be noted that plastic materials represent a source of chemical additives (Henkel et al., 2025), in addition to acting as vectors for pathogens (Magana-Arachchi et al., 2025) and alien species (García-Gómez et al., 2021).

1.2 Games as tools for raising awareness

Although substantial efforts are still required from policymakers, responsible choices made by companies, in their role as producers, as well as by all of us as consumers, can play a decisive role in mitigating the effects of plastic pollution (Plastics, n.d.).

Awareness-raising can be considered an integral part of the responsibility-building process and represents a commitment that must be pursued consistently over time. Raising awareness is the first essential step toward promoting responsible choices, to which we are all called.

For this reason, the social promotion association “DelTa” (*Delfini e Tartarughe nel Golfo di Trieste*), in collaboration with “White Cocal Press” and with financial support from the “Beyond Plastic Med” association (BeMed), developed an awareness-raising tool in the form of a board game, designed to be used both in educational settings and in recreational contexts.

Games, understood as a universal human activity, represent cognitive and communicative tools capable of facilitating the understanding of complex concepts while also encouraging active participation. In recent years, their use in science communication has increased significantly (Giasolli et al., 2006), fostering collaboration among researchers, designers, and communicators (Stachyra & Roughley, 2023). “Game-based learning” is the result of this collaboration and involves the integration of games and game mechanics into learning processes (Caponetto et al., 2014).

Game mechanics can play a key role in educational and communicative processes, as they enable information to be conveyed in an effective and memorable way (Illingworth & Wake, 2021). The challenge lies in balancing learning and enjoyment, while keeping players immersed in the experience.

1.3 Plastic Fighters: the game

The integration of the elements underlying game-based learning led to the development of “Plastic Fighters”, a cooperative board game designed to draw attention to the issue of plastic pollution in the marine-coastal environment. In the game, participants are required to manage the various related challenges, taking into account the complexity of the system in which we live and considering environmental, economic, and social aspects. It is defined as a “cooperative” game because the objective is to achieve a sustainable environmental balance, which can only be reached through collaboration among all players. The game was designed for players aged 8 and older and can be adapted – either by simplifying it or by increasing the level of challenge – depending on age group or educational level.

The following sections present the main components of the game, as well as several practical suggestions that emerged during multiple game simulations carried out during the presentation and distribution of Plastic Fighters.

2. Plastic Fighters: game components

2.1 Game and players

Plastic Fighters is a freely distributed game, also available in a “Print & Play” version in both Italian and English (for download information, please refer to the section following the “Acknowledgments”).

Up to four players can take part in the game. Each player assumes the role of an environmental association characterized by specific skills (Figure 2), which are represented by the “action cards” associated with each role.



Figure 2. The four environmental associations featured in Plastic Fighters. Image from the game materials

These skills can be expanded during gameplay and allow players to increase the range of actions available to mitigate environmental impacts, including those affecting human health.

The game is designed for 2 to 4 players, with an estimated playtime of approximately 40 minutes.

However, since Plastic Fighters was also conceived as a tool for educational activities, and given that a class may reasonably include more than four students (or that the playing group may be larger), it is recommended to divide the class, or group, into additional subgroups of 2 to 4 players, according to the number of associations featured in the game.

In this case, each subgroup represents one association, and each member of the subgroup assumes the role of a member of that association. The Teacher, or Educator, may take on the role of game moderator, while a spokesperson can be appointed for each association.

2.2 The playing area: the game board

The game board constitutes the playing area (Figure 3) and represents a generic mediterranean marine-coastal landscape divided into three interconnected areas – river (R), beach (B), and sea (S) – along which cities are located, and industrial and marine resource exploitation activities take place.



Figure 3. The Plastic Fighters game board depicts a marine-coastal landscape divided into three main sections: sea (S), beach (B), and river (R), each further subdivided into subsections (S1, S2, S3, S4 for the sea; B1, B2 for the beach; R1, R2 for the river). Specific areas allow for the construction of structures designed to limit the dispersion of plastic in the environment (e.g., sea structure, beach structure). Image from the game materials

The arrows shown on the board indicate the dynamic nature of environments, organisms, and waste. For example, as occurs in real life, waste abandoned on the beach may reach the sea (sections S1, S2, S3, or S4); similarly, waste introduced into a river (sections R1 or R2) may be transported by the current to the sea. From there, it may remain in the marine environment or be carried back to the beach (sections B1 or B2).

Organisms, in turn, may exhibit movements of different magnitudes: some are capable of traveling long distances (such as the sea Turtle), others move over more limited ranges (such as the Hermit crab), while others do not move at all.

2.3 Plastic waste

Plastic waste is represented in the game by “waste tokens” (Figure 4) and includes categories such as plastic bags, microplastics, cigarette butts, bottles, and abandoned fishing nets.



Figure 4. Waste categories in Plastic Fighters; from left to right: plastic bags, microplastics, cigarette butts, bottles, and abandoned fishing nets. Image from the game materials

These items represent only a fraction of the plastic-derived waste present in the environment; however, they were selected because they reflect some of the most widespread types of plastic pollution (Accord RAMOGE, 2021).

2.4 Organisms

Organisms, represented in the game by “organism tokens” (Figure 5), include selected animal and plant representatives of the different trophic levels that characterize the marine and coastal system and that may be affected in different ways by plastic materials or by the substances they transport.

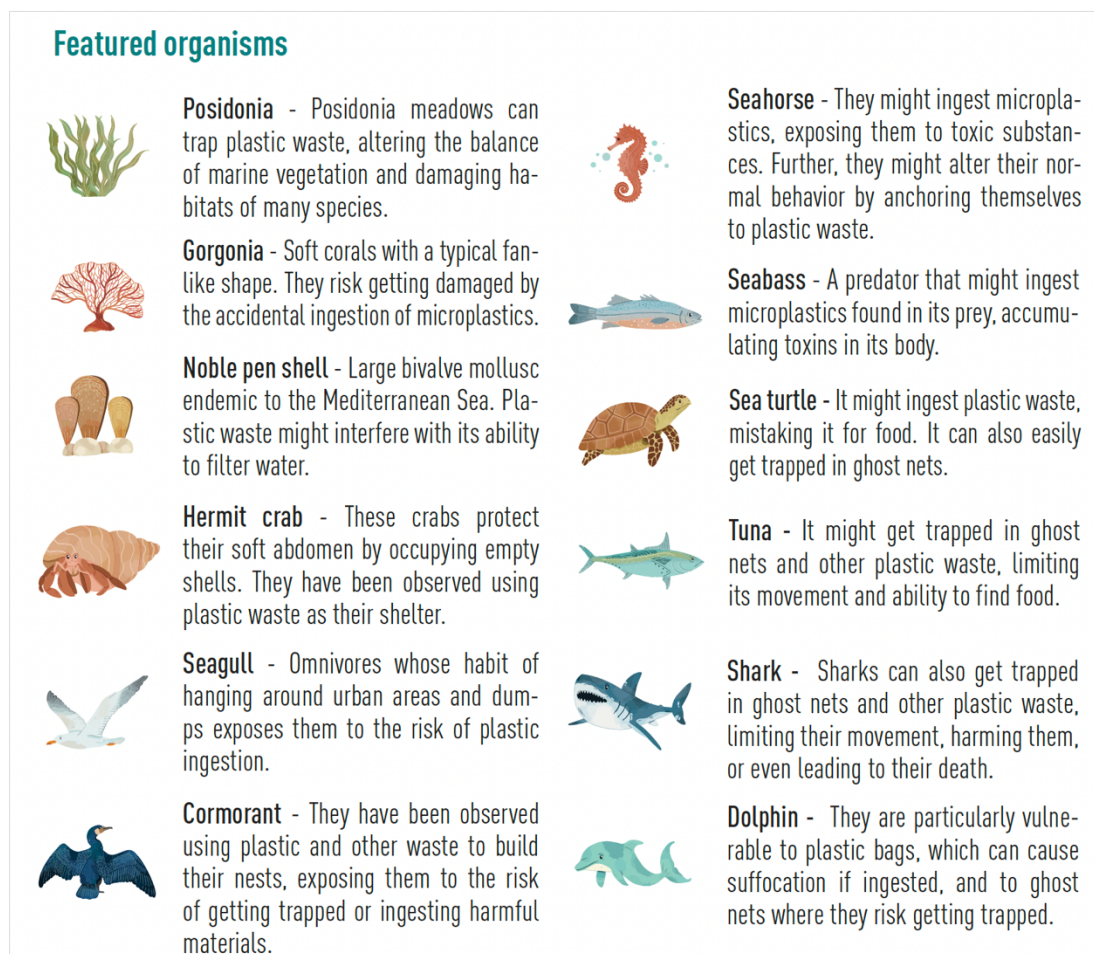


Figure 5. Marine organisms featured in Plastic Fighters. Image from the game materials

Species were selected based on their ecological relevance (e.g. *Posidonia oceanica*, a marine plant fundamental to the stability of coastal ecosystems), their status as Mediterranean endemics (such as *Pinna nobilis*), their importance as food resources (e.g. Bass or Tuna), or their designation as protected and particularly vulnerable species (such as Dolphins and Sharks).

This does not exclude the possibility that other species belonging to different trophic levels, although not directly represented in the game, may also be affected by plastic pollution.

2.5 “Event” cards: “easy” and “difficult” events!

In general, “event” cards (Figure 6) represent the core of the game mechanics: at each turn, a player draws one card, determining the plastic waste release event that must be managed by the environmental association whose turn it is, with the collaboration of the other players.

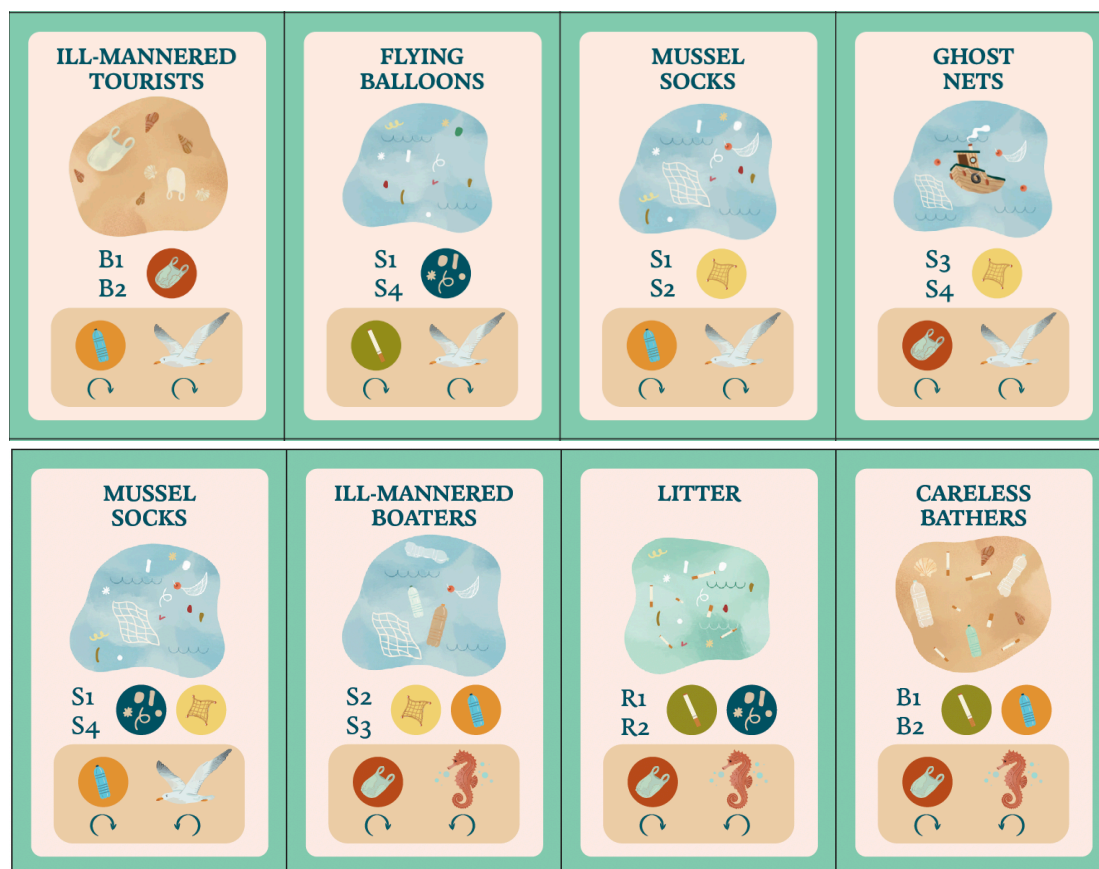


Figure 6. Selection of “event” cards from the Plastic Fighters game deck. The first row shows examples of “easy” event cards, while the second row shows examples of “difficult” event cards. Image from the game materials

Event cards depict situations that may occur in everyday life, such as litter left on the beach by visitors, lost fishing nets at sea, or river floods transporting waste, among others. Each event may lead to different consequences, primarily resulting in the introduction of waste into the environment.

Within the game, an event is generally classified as “easy” when the action described on the card requires the placement of one waste token in two different sections of the same environment. An event is considered “difficult” when the card requires two different types of waste to be placed in two different sections of the same environment.

2.6 The gameplay experience

The resulting gameplay experience consists of addressing the consequences of events, some of which may be potentially lethal, that determine the presence or movement of waste, while also considering the presence or movement of species across areas with different levels of contamination. These consequences can be managed through individual and collective actions, depending on the skills available and on the possibility of investing in the development of structures or services.

Taking part in a game of Plastic Fighters goes far beyond simple gameplay: it is an immersive experience that transforms a complex environmental issue into a challenge lived firsthand.

Plastic waste is no longer just a topic to be studied, but a concrete challenge to be faced together, while endangered marine species become the beating heart of the game board. This dynamic fosters strong player motivation to seek the best solutions as the accumulation of waste increasingly threatens their survival.

In this way, Plastic Fighters activates the core dynamics of game-based learning: learning through experience, emotion, responsibility, and cooperation.

Collaboration among players is not merely a game mechanic, but a deeper message: only by working together can the sea truly be protected – and by playing, players come to understand that the time to act is not tomorrow, but now.

3. Suggestions for use in educational settings

Plastic Fighters was presented and distributed during events such as the European Researchers' Night – “Sharper Night” (Trieste, 2025), at the *Immaginario Scientifico* of Trieste during the *Archeoplastica* exhibition (2025), and at dedicated events aimed at teaching staff from the Friuli-Venezia Giulia Region.

During these occasions, several game sessions were simulated with the aim of explaining the game dynamics and objectives, emphasizing collaboration among players. These events did not merely serve as moments for explanation and simulation but rather became true spaces for discussion and exchange.

Numerous Institutions and Teachers who took part, many of whom did not necessarily come from a scientific background, proposed ideas for reusing the game as a tool for cross-disciplinary learning.

From a scientific perspective, Plastic Fighters can serve as a starting point for addressing and discussing a wide range of topics, spanning from materials chemistry and physics to ecotoxicology, biology, and physiology.

However, disciplines such as civic and environmental education, foreign language teaching, and, more broadly, all areas related to the European key competences for education and training, can also benefit from a playful approach.

In this regard, one of the most interesting suggestions that emerged during meetings with teaching staff concerned the use of the game as an alternative tool for communication in both Italian and English. The possibility of downloading Plastic Fighters in two languages makes its use for foreign language teaching particularly feasible, for example by supporting the acquisition of specific terminology and common species names in an engaging context.

Finally, Plastic Fighters can also serve as a valuable introductory tool for those wishing to approach issues related to plastic pollution, or simply as a game in which cooperation prevails over competition.

4. Final considerations

The experience of Plastic Fighters presented here is intended both as an introduction to the game itself and as a support, or an example, for those who wish to share knowledge and raise awareness through a playful approach. The hope is that the game can be applied creatively and, above all, that it can stimulate moments of reflection on one of the most pressing environmental issues of today and the future.

The game against plastic is still open, and we can win it!

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DOWNLOAD PLASTIC FIGHTERS: THE GAME IS AVAILABLE FREE OF CHARGE IN PRINT & PLAY FORMAT AT THE FOLLOWING LINKS:

- <https://deltaadriatico.wixsite.com/delta/plastic-fighters>
- <https://bora.la/2025/08/06/PLASTIC-FIGHTERS-IL-GIOCO-COOPERATIVO-PER-RIPULIRE-IL-MARE-DALLA-PLASTICA/>

WHO WE ARE: *DELTA – DELFINI E TARTARUGHE NEL GOLFO DI TRIESTE* IS AN ASSOCIATION FOUNDED IN 2016 THROUGH THE INITIATIVE OF A GROUP OF STUDENTS AND MARINE AND ENVIRONMENTAL BIOLOGISTS, WITH THE AIM OF CONTRIBUTING TO THE CONSERVATION OF MARINE-COASTAL ECOSYSTEMS THROUGH KNOWLEDGE. THE ASSOCIATION OPERATES IN THE GULF OF TRIESTE, WHERE IT IS ACTIVELY INVOLVED IN ENVIRONMENTAL MONITORING ACTIVITIES, ALSO CARRIED OUT THROUGH CITIZEN SCIENCE APPROACHES. **WHITE COCAL PRESS** IS A PUBLISHING HOUSE SPECIALIZED IN THE DESIGN OF EDUCATIONAL GAMES FOCUSED ON ENVIRONMENTAL TOPICS, WITH PARTICULAR ATTENTION TO BOTH GAME DESIGN AND THE USE OF LOW-ENVIRONMENTAL-IMPACT COMPONENTS.

AUTHOR CONTRIBUTIONS: KARIN SCHLAPPA: CONCEPTUALIZATION. KARIN SCHLAPPA: METHODOLOGY. DIEGO MANNA, MILENA TEMPESTA, FRANCESCO ZUPPA: VALIDATION. KARIN SCHLAPPA: FORMAL ANALYSIS. KARIN SCHLAPPA, DIEGO MANNA: INVESTIGATION. KARIN SCHLAPPA, DIEGO MANNA: WRITING, ORIGINAL DRAFT PREPARATION. KARIN SCHLAPPA, DIEGO MANNA, MILENA TEMPESTA, FRANCESCO ZUPPA: WRITING, REVIEW AND EDITING. MILENA TEMPESTA, FRANCESCO ZUPPA: SUPERVISION. MILENA TEMPESTA: PROJECT ADMINISTRATION. MILENA TEMPESTA, KARIN SCHLAPPA: FUNDING ACQUISITION. THE AUTHORS HAVE REVIEWED AND APPROVED THE PUBLISHED VERSION OF THE MANUSCRIPT IN ITS ENTIRETY.

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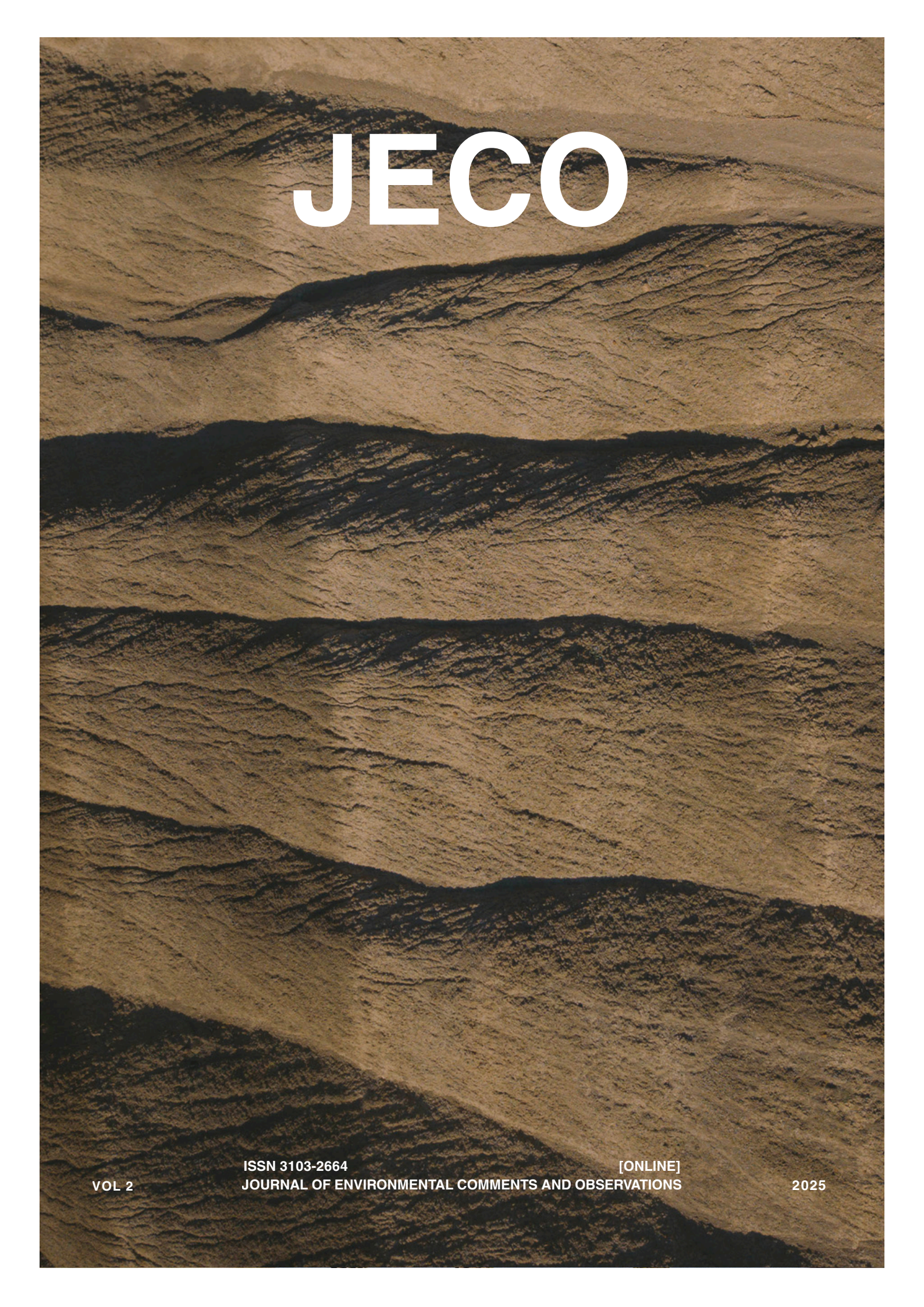
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An aerial photograph of a desert landscape, showing a series of undulating sand dunes. The dunes are illuminated from the side, creating deep shadows that emphasize their ridges and valleys. The overall color palette is a range of browns, from light tan to dark, almost black, in the shadows.

VOL 2

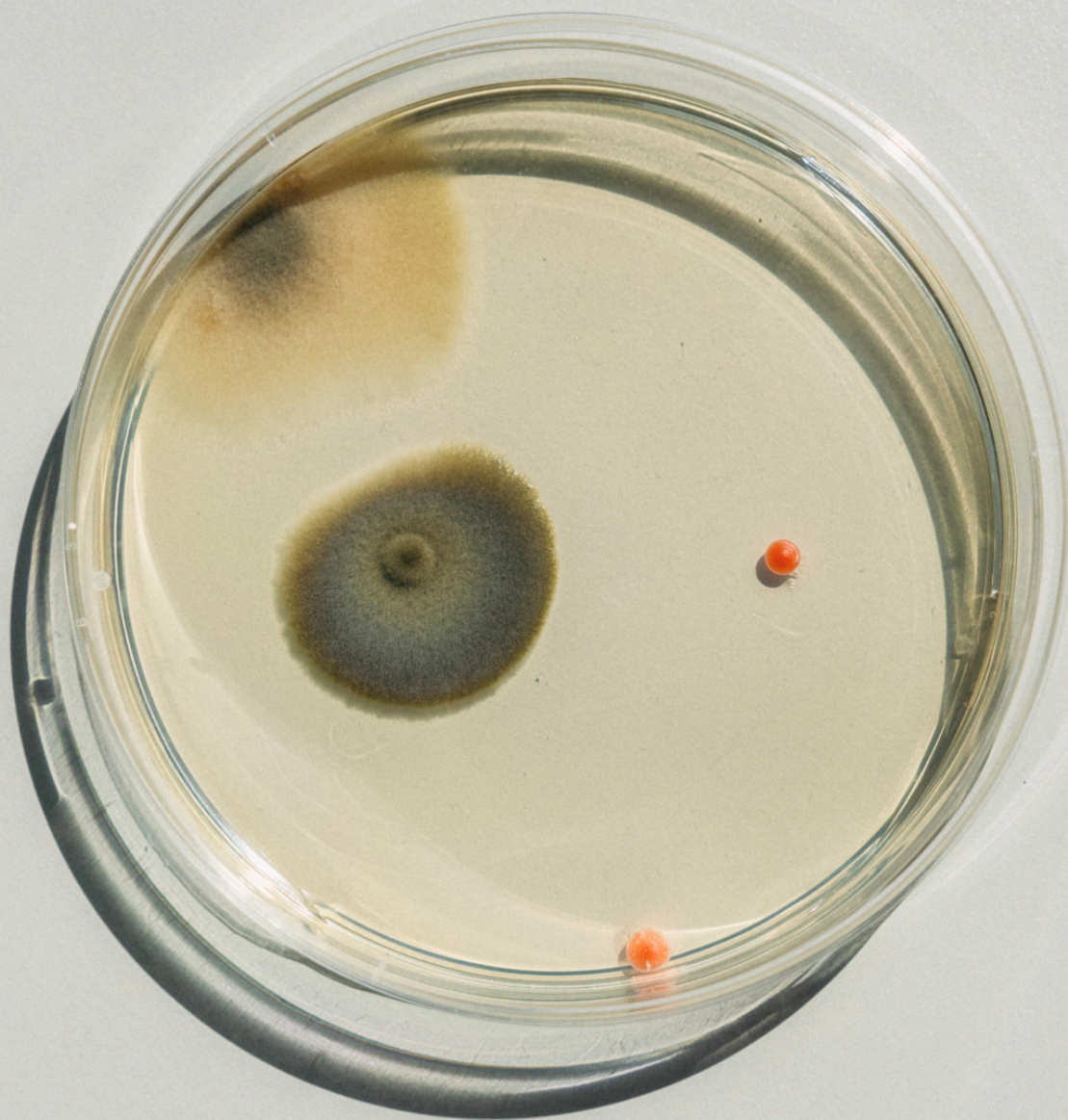
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“large quantities of environmental data are required to carry out
a comprehensive ecosystem economic valuation.”



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EDITORIAL

CREATING A BRIDGE BETWEEN MARINE AND FRESHWATER ECOSYSTEMS

THERE HAS ALWAYS BEEN A DIALOGUE BETWEEN LAND AND THE AQUATIC ECOSYSTEM, WHETHER FRESHWATER OR MARINE, BUT THERE HAS ALSO ALWAYS BEEN A BOUNDARY — A BOUNDARY THAT WAS ONCE CONSIDERED CLEAR AND IMPASSABLE. BUT IS IT REALLY SO? AND, WHAT IF THIS BOUNDARY IS INSTEAD BECOMING INCREASINGLY BLURRED?

There has always been a dialogue between land and the aquatic ecosystem, whether freshwater or marine, but there has also always been a boundary — a boundary that was once considered clear and impassable. But is it really so? And, what if this boundary is instead becoming increasingly blurred? In fact, freshwater and marine systems, which for a long time have been regarded as separate fields both in research and in environmental management, are in truth a single, contiguous, and continuous ecological and hydrological network.

Rivers, for example, which are the first to feed the oceans, carry not only water, but also nutrients, sediments, and, increasingly, traces of human activities. Thus, individual ecosystems are no longer seen as small, separate microcosms, but become connected and linked by infinite bridges.

The theme of this issue of the Journal of Environmental Comments and Observations (JECO) — the third since the beginning of this publication — deals with “*Marine and Freshwater ecosystems*” and invites us to reflect on the connection that exists between lakes and estuaries, streams and river mouths, fish farms and the open sea, between ecosystems in general.

As has become customary, this issue opens with a comic story, created by a young artist, who addresses the topic from an unexpected and direct perspective. By uniting art and science, this artistic form offers a new magnifying lens through which we can enjoy observing the common dynamics of marine and freshwater ecosystems, while also making complex ideas more accessible.

To deepen the understanding of the mechanisms within ecosystems and how anthropogenic pressures interact in them, the scientific contributions included in this issue will guide us on this journey — from one bridge to another.

The manuscript presented in this issue, “*Microplastics in *H. tubulosa*. A case study to support future assessment of the cost of microplastic pollution*”, instead takes us into the marine environment, addressing one of the most widely discussed global problems of recent years: microplastic contamination. Holothurians, commonly known as sea cucumbers, play an essential role in nutrient cycling and sediment ecology. Their detritivorous feeding behavior has allowed researchers to directly observe the contact between microplastics and these organisms, leading to an evaluation of the state of the marine ecosystem.

By quantifying the presence of microplastics and discussing their ecological implications, the authors make it possible to connect the biology of individual organisms with the ecological condition of the ecosystem, thereby expanding knowledge and encouraging conservation actions.

Ecosystems subjected to anthropogenic pressures are not the unique focus of this final JECO issue of 2025. The volume also includes a scientific perspective on plastics — both micro- and nanoplastics — authored by the Editor-in-Chief of the journal.

In her position paper, Prof. Renzi examines microplastics as one of the most pervasive and complex environmental contaminants of the Anthropocene, emphasizing that the primary challenge for research is no longer their detection, but rather the correct interpretation of the data collected. In this context, the scientific community continues to face significant methodological limitations, particularly related to the lack of shared protocols and the use of experimental microplastics that are poorly representative of real environmental conditions.

Scientific journals therefore play a crucial role in promoting methodological rigor and conceptual advancement. Continued publication of microplastics research is essential to reduce current scientific uncertainties and to provide a solid foundation for credible risk assessments and the development of effective environmental policies.

When considered as a whole, JECO Volume 03 reminds us that marine and freshwater systems are not isolated laboratories of study, but intricately interconnected environments. The health of a river influences that of the sea it feeds; likewise, pollutants found in marine environments may originate from inland activities, transported through nutrient flows or plastic particles. The volume also underscores the fundamental importance of standardized methods and in-depth methodological analyses for advancing increasingly precise and robust scientific knowledge.

By combining artistic expression, experimental investigation, and ecological reflection, this issue of JECO embodies the diversity of perspectives necessary to understand and protect aquatic, marine, and freshwater environments.

Connection is what defines the environment and its individual ecosystems — it is up to science to build bridges and links between them.

WORDS BY FRANCESCA PROVENZA
ARTIST CAMILLA BROGI



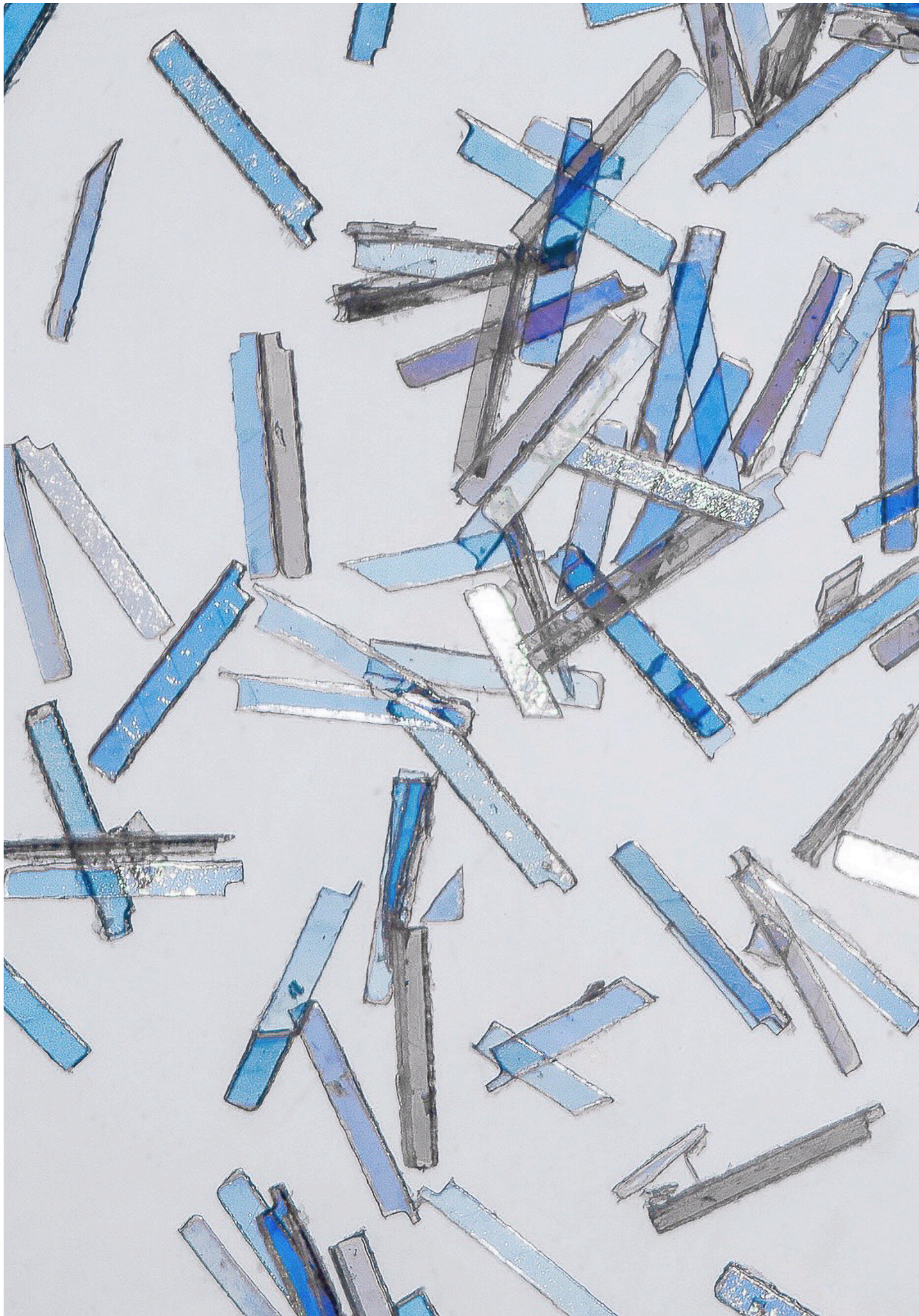


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CONTENT

1

P. 11

**MICROPLASTICS IN *H. TUBULOSA*. A CASE STUDY TO SUPPORT
FUTURE ASSESSMENT OF THE COST OF MICROPLASTIC POLLUTION**

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2

P. 37

**POSITION PAPER – MICROPLASTICS RESEARCH AT A CROSSROADS:
FROM UBIQUITY TO UNDERSTANDING**

RENZI M.

3

P. 39

**SHORT COMMUNICATION – ICT INDEX® V1.0:
AN INTEGRATED FRAMEWORK FOR ASSESSING MARINE TROPHIC
NETWORK INTEGRITY UNDER THE MARINE STRATEGY FRAMEWORK
DIRECTIVE**

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Microplastics in *H. tubulosa*. A case study to support future assessment of the cost of microplastic pollution

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Abstract: Large quantities of environmental data are required to carry out a comprehensive Ecosystem Economic Valuation (EEV) in Marine Protected Areas (MPAs). EEV is used to quantify social and economic costs and benefits that can be associated with nature and ecosystem services. This quantification requires translating ecological changes into welfare impacts. Microplastic (MP) levels are not yet considered in the EEV context due to a substantial lack of consistent and comparable data on levels in sediment and within trophic webs. These data are relevant for translating ecological changes into economic terms. This study reports the levels and chemical composition of microplastics in sediment and benthos species (*H. tubulosa*) collected from the Punta Campanella MPA in Italy following harmonised collection, sampling, extraction, and analysis methods. The reported data is compatible for use in a future EEV in this MPA. Results obtained provided a more thorough understanding of microplastic pollution in the Punta Campanella MPA ecosystem, which could be useful in filling knowledge gaps related to data availability in EEV studies of other similar MPA ecosystems. Results showed that samples contained low levels of MPs, mostly PET and nylon fragments or fibres (in sediment). *H. tubulosa* samples showed lower levels than sediment samples.

Keywords: Sea cucumbers; *H. tubulosa*; sediment; Marine Protected Areas (MPA); microplastics; fibres; Ecosystem Economic Valuation (EEV).

1.

Introduction

Many studies have been performed in recent years to define microplastics (MPs) in marine ecosystems by measuring quantities found in sediment and bottom species. Despite the large quantity of published literature, harmonised methods of extraction and analyses remain poorly acknowledged and referred to, often resulting in an overestimation of microplastic pollution in marine ecosystems and trophic webs. The lack of robust methods in most of the available literature, today, has also been reported by several experts, including Bakir et al. (2024), Koelmans et al. (2019) and Wootton et al. (2024). Principal methodological problems tend to be related to collection, laboratory air-borne pollution of samples, and chemical identification of plastic items among natural substances. Some published papers report identifications performed with visual identification using stereomicroscopy and/or chemical identifications performed on only a percentage of the identified MP items, which is considered problematic (Enders et al., 2020). As microplastic research continues to evolve in terms of sampling, extraction, and analysis of MP particles, the use of existing recommended guidelines and best practices is essential to ensure that resulting data can be compared across studies (Weis, 2019). There were not yet harmonised methods such as the “Guidance on the Monitoring of Marine Litter in European Seas” for MP quantification in complex matrices (MSFD Technical Group on Marine Litter 2023) at the time of the present work’s sampling and analysis. Nonetheless, guidelines such as the GESAMP 2019 Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean and the Harmonised Protocol for Monitoring Microplastics in Biota by JPI-Oceans BASEMAN Project had been published and provided effective strategies which could encourage reproducibility in MP sampling and extraction. These suggestions have been reflected in the updated guidance document by the JRC (i.e. MSFD Technical Group on Marine Litter 2023). Despite advancements in this area, there remains a lack of methodological harmonisation in the study of MPs (Wootton et al. 2024), the resulting non-comparative data could be largely responsible in hindering, for example, ecological risk assessment of this pollutant (Weis, 2019). MP research has not yet comprehensively addressed the current lack of knowledge in quantifying attributes critical to assessing the ecological risks of MPs (Everaert et al., 2018; Li et al. 2025). Although this is likely due to the complexity of the topic, i.e. the many environmental functions that must be considered to accurately demonstrate how MPs interact within an ecosystem, it would benefit the scientific community to implement strategies that have already been established in future studies. It is clear from an environmental modelling point of view that the way in which data is “organized, encoded, and described” allows for or inhibits one’s ability to conduct a meaningful scientific analysis (Horsburgh, 2014). Therefore, to reasonably conduct a

risk assessment of MPs on ecosystems, relevant information should be compiled in a structured way (Waldschläger et al., 2020). In hopes of tackling the lack of organized MP data from a bottom-up approach, this study utilized the most recent guidelines regarding MP analysis, at the time of sampling, (i.e. GESAMP, 2019; Bessa et al., 2019) in marine sediment and biota to create an effective and reproducible method for the extraction of MPs from *Holothuria tubulosa* (*H. tubulosa*) and respective sediment samples.

The purpose of this study is to determine MP levels in both sediment and *H. tubulosa* from the Punta Campanella (Italy), marine protected area (MPA) using an optimised, harmonised, and tested method. The study aims to produce reliable and harmonised data to contribute to the development in assessing the impact of MPs in terms of loss of ecosystem service value (i.e. assessed through Ecosystem Economic Valuation (EEV)) in MPAs. The approach proposed here is not limited to laboratory analysis methods, rather it also considers methods that can allow for more effective data acquisition, organisation, and reporting.

2. Materials and Methods

2.1 Method optimisation

In the development of the methodology presented in this work, all aspects of the study were planned with respect to existing research needs, best practices, and guidelines. These aspects include sampling protocol, sample preparation, extraction, recovery, visual sorting, and chemical analyses of MPs. To start, the Punta Campanella MPA (PCMPA) was chosen as a study site for this investigation because, in theory, the area should contain relatively low levels of MPs due to restrictions on human activity. A lower quantity of in situ MPs can be helpful for protocol validation because it reduces the possibility of interference when accounting for spike particles to establish percent recovery and limit of quantification (LOQ). Furthermore, sediment and biota sampled in MPAs can be of value for ongoing monitoring (European Commission: Joint Research Centre, 2023). Next, *H. tubulosa* was chosen as the study species of interest because benthic invertebrates are inherently effective ecological indicators for the assessment of ecosystem health, particularly in the context of MP exposure (Warnau et al., 2006; Bessa et al., 2019; Cocci et al., 2025). *H. tubulosa* also contributes to sediment health, nutrient cycling, and the transfer of organic matter from detritus to higher trophic levels (Purcell et al., 2016). It is important to note that today the collection and commercial exploitation of *H. tubulosa* are currently prohibited in Italy, meaning appropriate authorisation must be obtained before sampling and using sea cucumbers and bioindicator species for MP monitoring (Pasquini et al., 2022), as well as possible species misidentification, as described by Sertic Kovacevic et al. (2022), however, at the time of sampling for this study (October 2019) this prohibition was not yet in place. However, we did acknowledge the trend in overexploitation of *H. tubulosa*, therefore, we sampled as few of the animal as possible to allow us to test the proposed methods. Future studies which aim to provide data of statistical relevance will likely be required to sample more individuals of the species of interest. Studies show that the concentration of MPs found in aquatic sediment can be orders of magnitude larger than that found in the water column, indicating that benthic species are at a considerably high risk of interacting with these particles (Haegerbaeumer et al., 2019). Despite this, *H. tubulosa* has not yet been heavily studied in MP research, which could be due to prohibitions.

The sample preparation process and extraction methods for the sediment and benthic fauna were developed to ensure that loss of MPs and/or contamination was avoided as much as possible (Bour et al., 2018). Therefore, different analytical methodologies, two for sediment and two for biota, were tested in a trial format at the start of this study to

determine the best practice for the extraction of MPs from the *H. tubulosa* samples and their respective sediment samples based on percent recovery and LOQ results. The trial methodologies focused on the percent recovery in the matrices that were spiked with microplastic particles, the existing environmental pollution and cross-over contamination using negative controls (blanks). Furthermore, the efficiency of the digestion step has previously been applied to complex matrices with successful outcomes.

2.1.1 Sample Preparation

2.1.1.a - Sediment

Each sediment sample had been collected in glass jars with tight closing metal lids directly underneath the location in which the *H. tubulosa* was collected. The sediment was frozen in the same way as the biota. To analyse the sediment, the samples were removed from the -40 °C freezer where they had been stored and were then thawed for approximately 48 hours. The sediment and residual sea water were transferred to a 1.5 L glass jar using a metal spatula. The transfer of the samples was conducted underneath a hood and all equipment used was rinsed with microplastic free distilled water to reduce the possibility of contamination. The jars in which the sediment was collected were opened inside of the transfer jars to catch any sea water that could potentially spill out due to the pressure change. Next, the sample jar was quickly inverted directly above and halfway inside of the transfer jar to pour out any seawater, and the sediment was scooped out using a metal spatula. The original sediment jars were rinsed several times with marine primary filtrate (MPF) sea water until there were no particles visible under the lid or inside the sample jar.

2.1.1.b - Biota

Biota samples were weighed and measured prior to being humanely killed using decapitation, then immediately dissected to avoid any stress which may result in the elimination of contents present in the digestive tract. In detail, the digestive tract and respiratory trees of each *H. tubulosa* had been extracted and weighed at the Punta Campanella sampling site and finally transferred to individual 250 mL glass jars with tight closing lids before being frozen at -40 °C (approximately 1.5 hours post dissection time). At the time of the analysis, each sample was thawed at room temperature for approximately 48 hours, then two samples were prepared for enzymatic digestion with Creon (a pancreatic enzyme) (Trial Method Biota 1: TM1B-S10; TM1B-S5) while the other two samples were digested with Potassium Hydroxide (KOH) (Trial Method Biota 2: TM2B-S10; TM2B-S5).

2.1.2 Spiking of samples

Spike particles were used in this study to quantify the loss of MPs and/or contamination during the application of the protocol. Spike particles were made of low-density polyethylene (LDPE) fragments. They were of varying shapes and sizes and in three colours, i.e. white, pink, and blue. Prior to adding the spike MPs to the sediment and sea cucumber samples, they were measured and photographed using a Nikon SMZ-800 N stereomicroscopic and Nikon ACT-1 imaging analysis software. It was noted which

particles were added to which samples to ensure no double counting or counting of contaminant particles that could have entered the samples during the transfer of the spikes occurred.

2.1.2.a - Sediment

Approximately 50 mL of MPF seawater was used to help transfer spike particles to the four sediment samples. Two subsets of spiked samples were prepared: those spiked with 10 irregular pink, white, and blue MP fragments (S10=2) and those spiked with 5 irregular pink, white, and blue MP fragments (S5=2). Each subset was additionally divided into two different trial methods to evaluate the influence of a drying step on the final results. The residual water from the sampling, transfer and spiking processes of one S10 and one S5-sample was removed via filtration through paper filters of 6 µm threshold (pf-6 µm). These filters were saved to be visually analysed under a microscope after being dried in an oven at 40 °C until the weight reached a constant value. This method was called Trial Method 2 (TM2-S10; TM2-S5). The method that did not include the drying step was called Trial Method 1 (TM1-S10; TM1-S5).

2.1.2.b - Biota

Spike MPs were added to biota samples after the tissue digestion procedure was completed following the same protocol as that described in 2.1.2.a for sediment.

2.1.3 - Microplastic extraction

2.1.3.a - Sediment

The wet samples (TM1-S10 and TM1-S5) were pre-filtered through pf-6 µm. Thus, the MP spike particles were extracted from both wet and dry (TM2-S10; TM2-S5) sediment samples using density separation in 200 mL of saturated NaCl solution. All four samples containing the saturated NaCl solution were agitated at 110 rpm for 20 mins then left to rest for at least 5 minutes. After resting, the supernatant was filtered through pf-6 µm, and the filter was changed and collected whenever it clogged, and no more fluid could pass through. The procedure was carried out three more times for each sample allowing for four NaCl “washes”. The dried samples experienced another step during the filtration process in which the material collected on each filter was rinsed with approximately 20 mL of MPF distilled water after all liquid had gone through the filter, but prior to removing the filter from the vacuum pump system.

2.1.3.b - Biota

2.1.3.b.1 - Enzymatic digestion

This method follows the protocol used in von Friesen et al. (2019). Creon 10000 was added to two of the *H. tubulosa* samples at a weight calculated using Equation 1:

$$\text{g wet weight (w.w.) sample} * 0.2 = \text{weight (in grams) Creon 10000}$$

Next, the sample was added until the pH reached a value of 8. The spike protocol described in section 2.1.2.b was then carried out. Next, the samples were agitated at 110 rpm for two hours before being placed in a 37.5 °C incubator for no less than 14 hours. After the 14 + hour period, the samples were removed from the incubator, agitated again at 110 rpm for 2 hours and placed back in the 37.5 °C incubator until the filtration step (through pf-6 µm). The filter was washed twice with 100 mL of saturated NaCl solution and the supernatant which occurred between washes was filtered again.

2.1.3.b.2 - KOH digestion

The protocol presented by Enders et al. (2020) was followed for the KOH digestion approach used on the remaining two *H. tubulosa* samples. Briefly, a saturated KOH stock solution was prepared by dissolving 560 g KOH salt tablets in 0.5 L MPF distilled water. Next, 150 mL of this stock solution was added to 150 mL of NaClO (14% active chlorine) and 700 mL of MPF distilled water in a tightly closing glass bottle. The quantity of solution added was calculated using Equation 2:

$$\text{g w.w. sample} * 5 \text{ mL} = \text{volume (in mL) KOH digestion solution}$$

The solution was used to help in transferring the spike particles to each sample (15 of each colour in sample TM2B-S15 and 10 of each colour in TM2B-S10), i.e. by rinsing the glass petri dishes in which the spike particles had been kept. Next, the jars containing the samples and the digestion solution were shaken gently and left to incubate at 23 °C for 5 hours. The digested *H. tubulosa* material was filtered through pf-6 µm. Finally, the filters were washed twice with 100 mL of saturated NaCl solution and the supernatant between washes was filtered again.

2.1.4 - Visual identification of spike particles

For both sediment and *H. tubulosa*, the extracted samples were recovered on 6 µm paper filter disks with the help of a filtration glass set, vacuum pump, manifold, and funnel. The funnel and glass filter were rinsed thoroughly with MPF filter water before filtering a new sample. The wastewater from the rinse was collected for each sample and filtered at the end of the process to ensure that no particles were lost during the rinsing phase. All filters collected from the 8 samples were dyed with enough 0.025 concentration Rosa Bengala to cover the entire filter. The filters were kept in covered glass petri dishes inside a 40 °C incubator to allow for more rapid drying of the Rosa Bengala dye before stereomicroscopic analysis.

2.2. Application to experimental sample

In this study, sediment (n=4) and biota (n=4) samples were retrieved by research divers from “Parthenope” University of Napoli. The *H. tubulosa* were identified and obtained by hand, then sediment (2 cm depth) within a surface area of approximately 150 cm² from directly below each animal was collected in 250 mL wide mouth glass jars with aluminium lids. Information regarding the sampling was recorded with the help of “Subtidal Sampling Datasheet” templates (provided in the Harmonized Protocol for Monitoring Microplastics in Biota by JPI-Oceans BASEMAN Project, see Frias et al., 2018 pg. 17). Collected samples were transferred to the laboratory for extraction,

purification and chemical analysis of microparticles recovered using micro-FTIR technology. Efficiency of recovery was evaluated by spiking samples with microplastic particles.

Methodological aspects - By following the guidelines and harmonised protocol proposed by GESAMP and JBI-Oceans, respectively, along with the researchers' simplified methods of obtaining and transferring sediment, the sampling and sample preparation methods used in this study appeared to aid in avoiding the potential loss of MP particles. Both methods are simple and require only one instance in which the sample must be transferred from one vessel to another before the filtration process. The results of the TM1 and TM2 for the extraction of MP particles from sediment favour TM2 as a method to be used in future studies. However, the drying of the sediment required approximately 48 hours, and was seemingly an irrelevant step since the sediment was rehydrated with the saturated NaCl washes. Therefore, it is recommended to simply filter the sediment sample after it is transferred to the larger glass jar for the washes, then add 200 mL saturated NaCl. Rinsing the filters with approximately 20 mL of MPF distilled water appeared to greatly improve visibility. This step is highly recommended for any sample extracted via density separation. *H. tubulosa*, has been shown to eviscerate internal organs due to neurological response to stimuli and soon after death (Byrne, 1986). Given this characteristic, it is important to avoid stressing the specimen at any point during the sampling process to avoid losing the contents of the digestive tract. The sampling protocol used in this study appeared to avoid the encouragement of evisceration given that no material appeared to be expelled until the organs were removed via dissection on site.

Percent recovery values obtained following the digestion and extraction procedures, TM1B and TM2B, indicate that enzymatic digestion results in a higher recovery than KOH digestion and is, therefore, recommended to be used in future studies. The enzymatic digestion not only resulted in more effective removal of the organic material than the KOH but also did not appear to affect the visibility of the white and pink spike particles. Finally, enzymatic digestion has been shown to be gentle on MP particles, whereas KOH can impact polymers as shown in the spectral analysis of this study and described by others (von Friesen et al., 2019).

3. Results

3.1. Method standardisation

Each filter that resulted from the extraction procedures described above was individually analysed using a Nikon SMZ-800 N stereomicroscopic. When a spike particle was found, the Nikon ACT-1 imaging analysis software was used to measure the particle and match it to the measurements made prior to the digestion and extractions. Some images of spike particles before and after each method can be found in Figure 1.

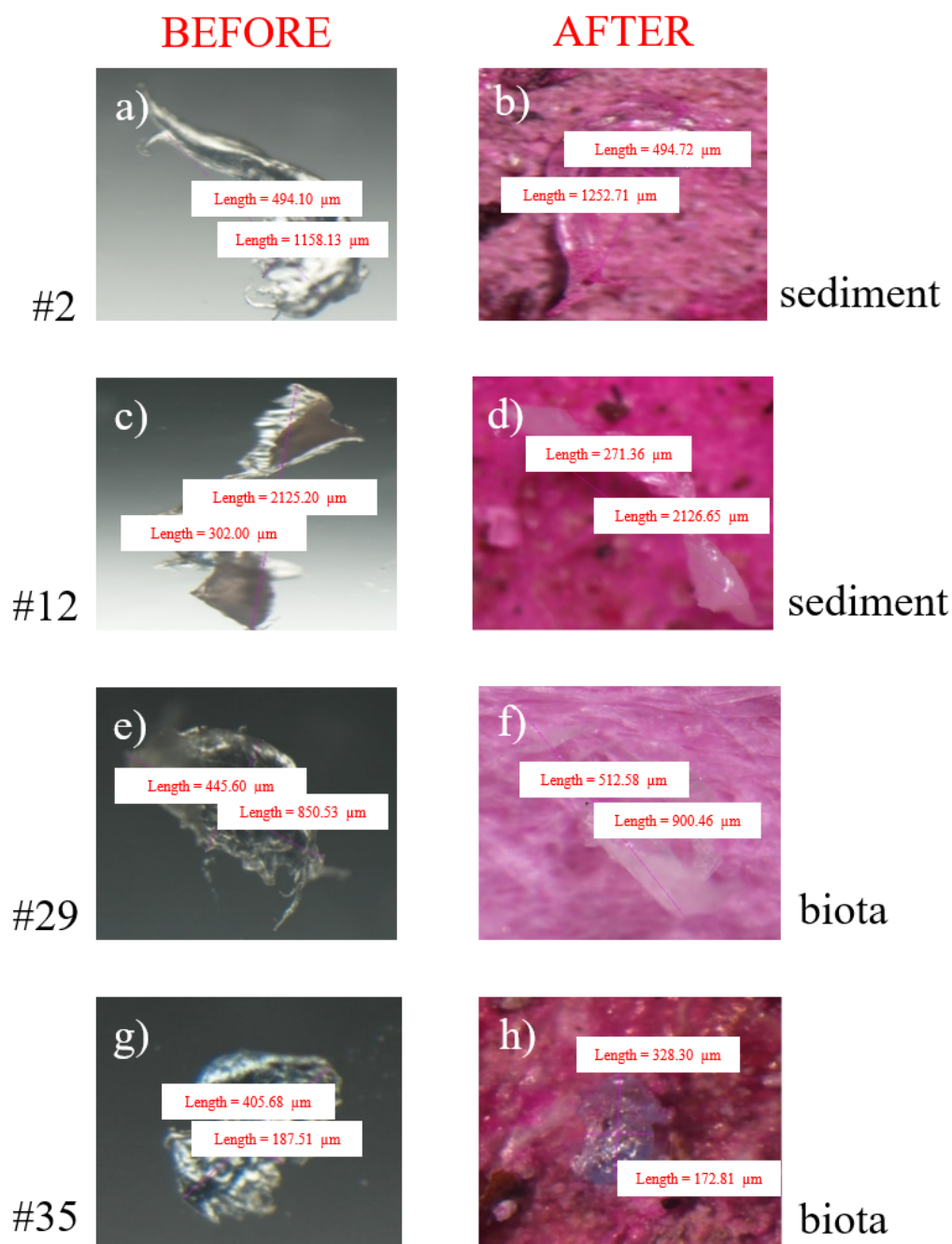


Figure 1. MP spike particles before and after digestion and extraction: a) white particle #2 before being added to TM1-S5/S10; b) white particle #2 from TM1-S5/S10 filter (filtered after first NaCl wash); c) pink particle #12 before being added to TM2-S5/S10; d) pink particle #12 from TM2-S5/S10 filter (filtered before drying) e) white particle #29 before being added to TM2B-S15/S10; f) white particle #29 from TM2B-S15/S10 filter (filtered after KOH digestion); g) blue particle #35 before being added to TM1B-S15/S10; h) blue particle #35 from TM1B-S15/S10 filter (filtered after first NaCl wash)

Regarding sediment samples, TM1 resulted in fibres consisting of low visibility that could be the result of salt crystals forming on the particles from the saturated NaCl solution. The recovery of all particles from all samples is demonstrated in Figure 2.

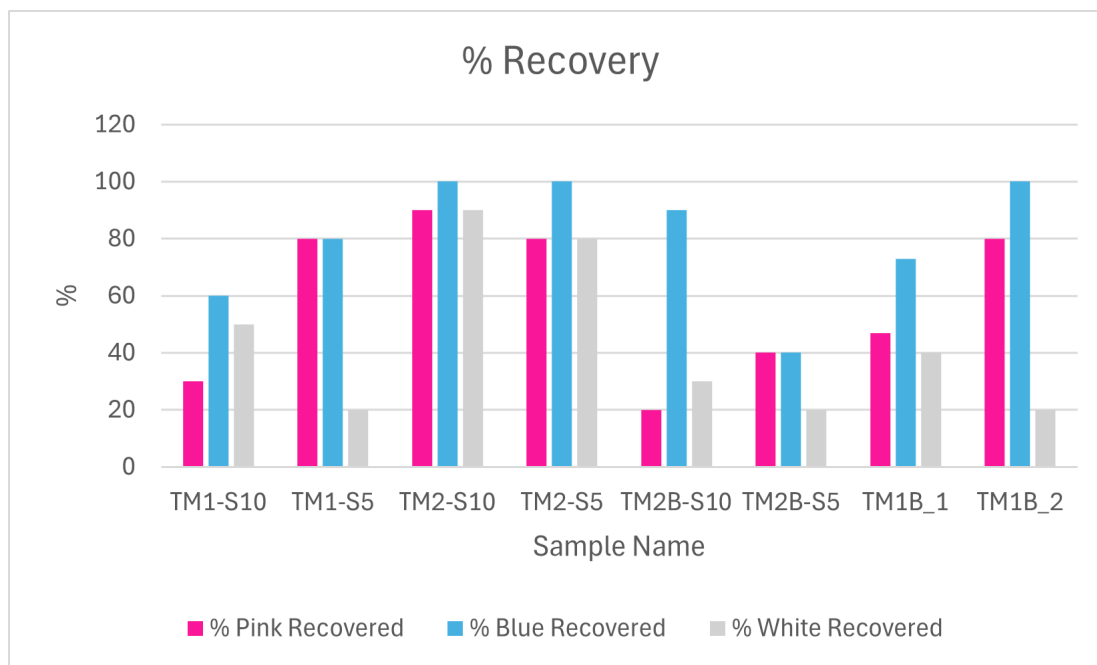


Figure 2. Percent recovery of all samples. Pink bars, blue bars and light grey bars represent the pink particles, blue particles, and white particles, respectively. Exact values can be found in Tables 1-8 along with their respective limit of quantification (LOQ) values

Tables 1 and 2 provide the recovery values of particles in TM1-S10 and TM1-S5, respectively, highlighting the very low recovery rate, particularly for the white and pink particles.

Table 1. Recovery rate of each colour from the MP spikes in sample TM1-S10 (i.e. 10 pink, 10 blue, and 10 white) and respective LOQ values.

	Pink	Blue	White
%	30%	60%	50%
Total Pink Particles Found of 10	3	6	5
LOQ	0.007	0.014	0.012
per kg	6.956	13.911	11.593
Wt. Sample (g w.w.) 431.3			

Table 2. Recovery rate of each color from the MP spikes in TM1-S5 (i.e. 5 pink, 5 blue, and 5 white) and respective LOQ values.

	Pink	Blue	White
%	80%	80%	20%
Total Pink Particles Found of 5	4	4	1
LOQ	0.011	0.011	0.003
per kg	10.724	10.724	2.681
Wt. Sample (g w.w.) 373			

Due to the low recovery rate of TM1, TM2 involved rinsing the filters with approximately 20 mL of distilled MPF water before they were removed from the vacuum pump system. This added step greatly improved the recovery as shown in Tables 3 and 4.

Table 3. Recovery rate of each colour from the MP spikes in TM2-S10 (i.e. 10 pink, 10 blue, and 10 white) and respective LOQ values.

	Pink	Blue	White
%	90%	100%	90%
Total Pink Particles Found of 10	9	10	9
LOQ	0.018	0.019	0.017
per kg	17.442	19.380	17.442
Wt. Sample (g w.w.) 516			

Table 4. Recovery rate of each colour from the MP spikes in TM2-S5 (i.e. 5 pink, 5 blue, and 5 white) and respective LOQ values.

	Pink	Blue	White
%	80%	100%	80%
Total Pink Particles Found of 5	4	5	4
LOQ	0.007	0.009	0.007
per kg	7.472	9.341	7.472
Wt. Sample (g w.w.) 535.3			

Regarding biota samples, the KOH digestion protocol appeared to have decreased the visibility of the spike particles as is shown via the recovery rates listed in Tables 5 and 6.

Table 5. Recovery rate of each colour from the MP spikes in TM2B-S10 (i.e. 10 pink, 10 blue, and 10 white) and respective LOQ values.

	Pink	Blue	White
%	20%	90%	30%
Total Pink Particles Found of 10	2	9	3
LOQ	0.042	0.188	0.063
per kg	41.667	187.5	62.5
Wt. Sample (g w.w.) 48			

Table 6. Recovery rate of each colour from the MP spikes in TM2B-S5 (i.e. 5 pink, 5 blue, and 5 white) and respective LOQ values.

	Pink	Blue	White
%	40%	40%	20%
Total Pink Particles Found of 10	2	2	1
LOQ	0.077	0.0769	0.038
per kg	76.923	76.923	38.462
Wt. Sample (g w.w.) 26			

Furthermore, it is worth noting that the filters often contained a gelatinous layer, covering the particulate matter. Unlike the KOH digestion, the enzymatic digestion resulted in a higher recovery rate also for the white and pink spike particles as shown in Tables 7 and 8.

Table 7. Recovery rate of each colour from the microplastic spikes in TM1B_1 (i.e. 15 pink, 15 blue, and 15 white) and respective LOQ values.

	Pink	Blue	White
%	47%	73%	40%
Total Pink Particles Found of 15	7	11	6
LOQ	0.14	0.22	0.12
per kg	140	220	120
Wt. Sample (g w.w.) 50			

Table 8. Recovery rate of each colour from the microplastic spikes in TM1B_2 (i.e. 5 pink, 5 blue, and 5 white) and respective LOQ values.

	Pink	Blue	White
%	80%	100%	20%
Total Pink Particles Found of 5	4	5	1
LOQ	0.133	0.1667	0.0333
per kg	133.334	166.667	33.333
Wt. Sample (g w.w.) 30			

Sample TM1B_1 was spiked with 15 particles of each colour to allow for a more thorough understanding of the resulting LOQ values.

3.2. Levels in sediment

All tested sediment samples were observed to contain microplastic items (reaching 100% of the total sediment tested), levels recorded were about 0.05 item per gram of sediment extracted. Collected sediment samples showed sizes ranging from 31-17.021 μm with a dominance of black and blue colours and PE (50%), PVC (33%), nylon (15%) plastic types (Figure 3).

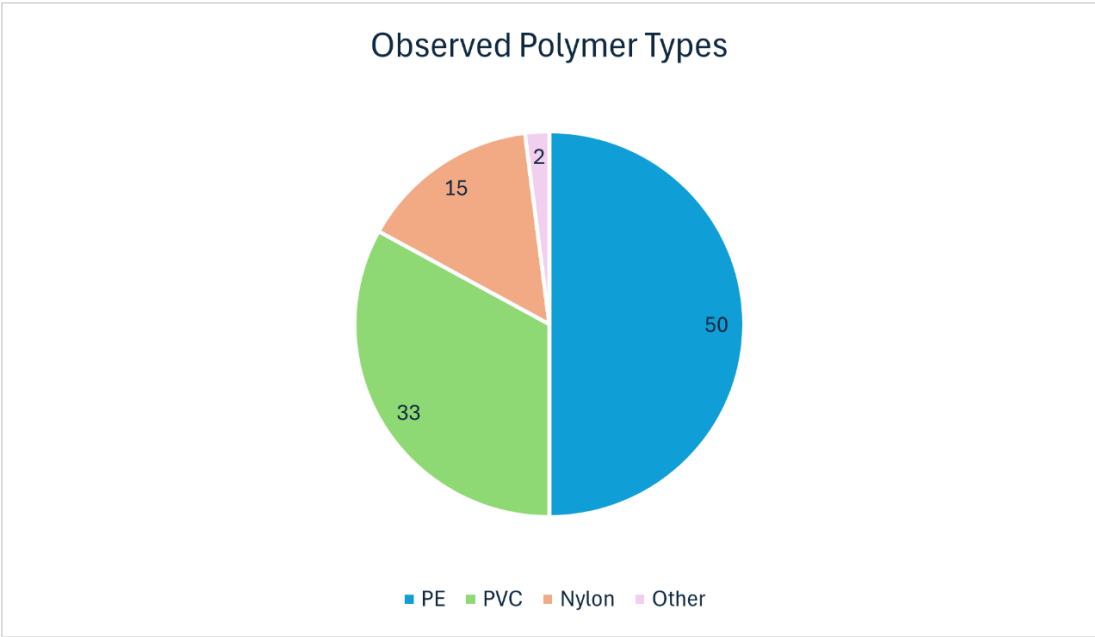


Figure 3. Representation of observed MP polymer types. PE, PVC, Nylon and other shown in blue, green, orange, and pink, respectively

In the sediment samples, about 66% of the recovered microplastic shapes were recorded as fragments while 33% were recorded as fibres. Other shapes were not recorded.

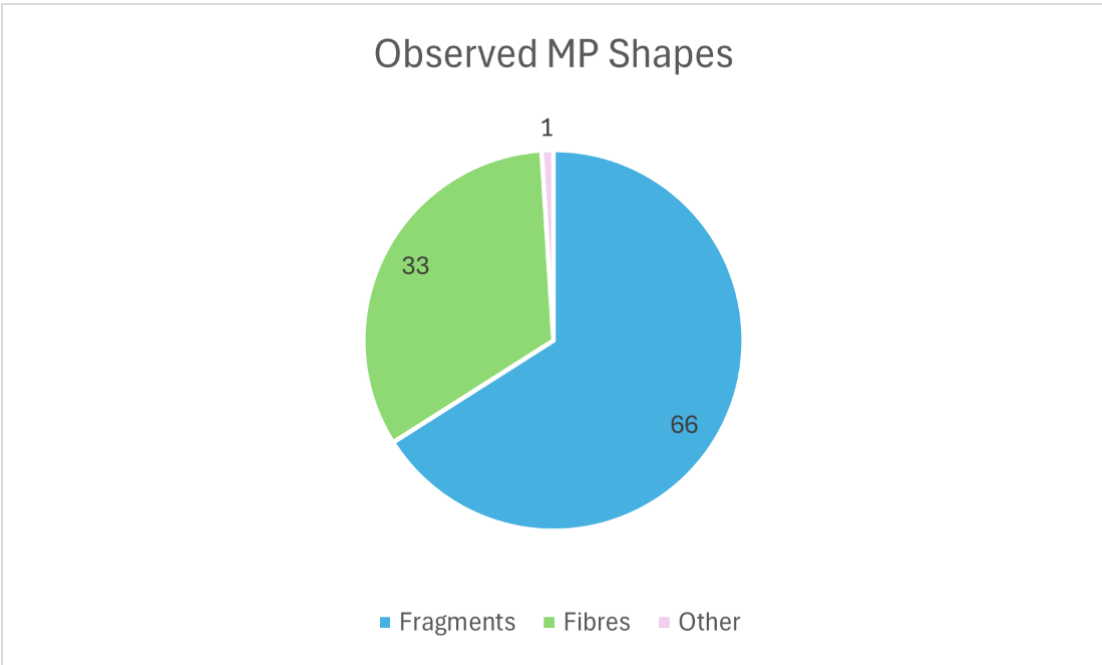


Figure 4. Representation of observed MP shapes. Fragments, fibres, and other shown in blue, green, and pink, respectively

Furthermore, one third of the total amount of recovered fibres were found to be of natural origin (cellulose). It was interesting to observe that the presence of bioclast in sediment seems to actively captures microplastic fibres acting as a sort of filter. Note that this possible scenario was observed on the slides after the complete microplastic extraction protocol and has not been proven with a more thorough investigation (Figure 5).

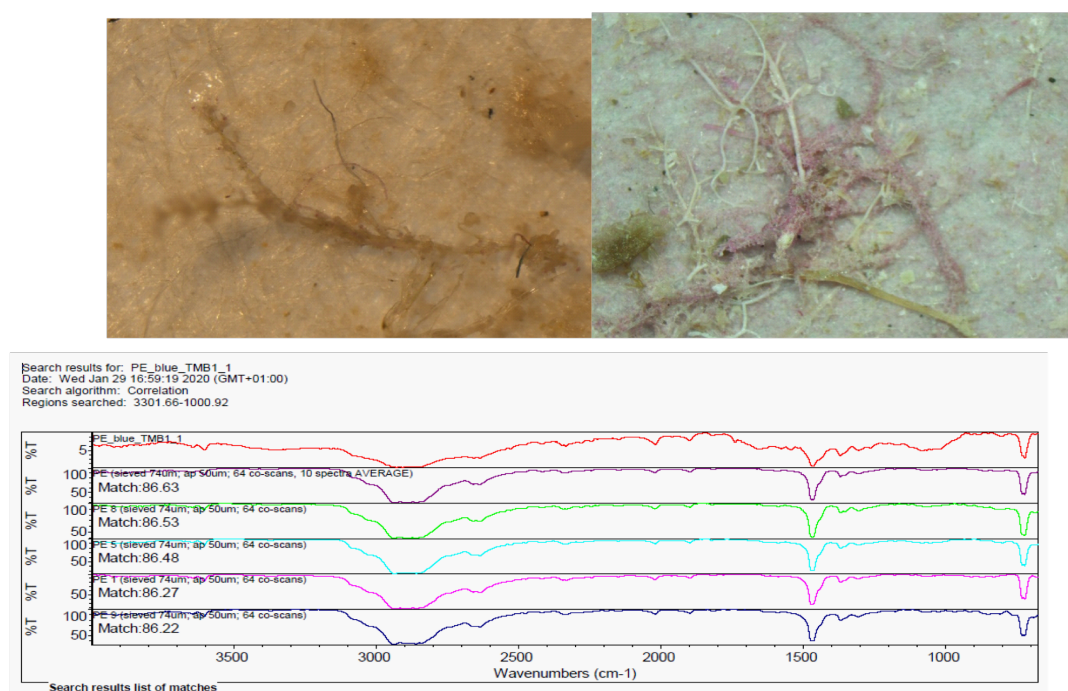


Figure 5. Collecting sediment trapping microfibrils (40X); FT-IR spectra reported as an example

3.3. Levels in sea cucumbers

In the sampled *H. tubulosa*, levels of microplastics were significantly lower than those recorded in the sediment samples. This occurrence could be due to the observed potential trapping activity exerted by bioclast (coralliferous species) as reported in Figure 5. Levels in *H. tubulosa* were on average, 0.5 items/animal. Only PET fibres were recorded in the *H. tubulosa* (100% of types and shapes) showing dimensions lower than 100 µm. In some cases (15% of the total) cellulose/viscose black filament fibres were recorded.

We believe that the results obtained in this study represent reliable data that can contribute to a larger dataset used to determine MPA loss of value caused by MP pollution in both sediment and benthonic species. The tested method of extraction and determination showed high recovery levels of spiking microplastics as shown in Tables 1-8. The efficacy of spiking to control recovery, the efficiency of the extraction, and the fact that MP microchemical analyses are essential in excluding false positive results are helpful analytical tools to generate data needed to more accurately evaluate levels of MPs in the context of EEV. Assessment methods relevant for decision-making, such as EEV, require comprehensive datasets using consistent methodological approaches (Nyadjro et

al. 2023). Proper meta-analysis can only be carried out when there is minimal variability in methodology and reporting of results (Beaumont et al. 2019). Thus, we expect that by following recommended protocols for MP sampling and extraction, our data can be included in future meta-analyses. Furthermore, we demonstrate the feasibility of carrying out sampling, extraction and identification using recommended approaches.

4. Discussion

Coastal areas tend to consist of high biological productivity given their shallow, protected waters and fresh nutritional inputs from rivers (Setälä et al., 2018). In addition, coastal areas are suggested to be at high risk of MP pollution because of their proximity to sources of anthropogenic pollution (Setälä et al., 2018). Furthermore, PCMPA should be relatively clear of MPs contamination given that it is a protected area, therefore, common local sources are expected to exist relatively far from the sampling site. The expected low levels of contamination make the PCMPA optimal when attempting to determine the best practices in sample preparation and analysis because it allows for the researchers to test the applied method without over-saturation of pollution.

MP experts continue to communicate that while there have been several methods used when acquiring biota, sea water, and sediment to analyze MPs, there remains a lack of harmonisation for these methodologies (Barike et al. 2024; Stock et al., 2019; Wootton et al., 2024). However, there do exist guidelines to follow for sampling and analysis (GESAMP, 2019; MSFD Technical Group on Marine Litter, 2023). A combination of referring to published guidelines and personal expert knowledge of the researchers and samplers regarding the PCMPA and *H. tubulosa* was used to carry out this study. In addition, each *H. tubulosa* was sampled along with a respective sediment sample to allow for an investigation of the biota's habitat. We believe this approach is useful in the investigation of the fate of MPs within the marine food web as it ensures a more comprehensive dataset that can be linked to both the species in question and the habitat in question. The method for obtaining samples was based on the GESAMP Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean which describes that the quantity of litter present in an individual biota will represent a combination of the exposure (i.e. quantity of litter in the surrounding environment) and the residence time in the digestive tract or tissues. According to literature, the biota selected for monitoring should be suitable to act as a bio-indicator of plastic contamination (GESAMP, 2019; Stock et al., 2019). For global monitoring schemes, *H. tubulosa* is an acceptable target specific organism, if it is not considered a protected species at the sampling location, given that it plays a key role in the transfer of organic matter from detritus to higher trophic

levels (Purcell et al., 2016) and is an effective ecological indicator for ecosystem health (Cocci et al., 2025; Warnau et al., 2006).

This study aimed to collect important data on the marine benthic invertebrate, *H. tubulosa*, to obtain more comprehensive data that we expect will be useful in quantifying EEV in the PCMPA and to define intake levels from the bottom to the upper levels of the trophic web. Unlike laboratory MP experiments in which the environment of the study animals can be controlled, in situ environments cannot be controlled. This indicates that valuable information could be missing if components of the sampled animals' habitat are not analysed along with the biota. Furthermore, the sediment obtained can be used to better understand the habitat of the species of interest, *H. tubulosa*.

The approach proposed in this study for the analysis of MPs in *H. tubulosa* aims to highlight the importance of comprehensive research from start to finish. Each aspect of the study was planned from a top-down perspective, to pinpoint where in the bottom-up scientific process there might be room for improvement. Perhaps using this approach in the development of future MP studies could be one way to address complex knowledge gaps that continue to inhibit the scientific community's ability to demonstrate the potential ecological impact of MPs on marine ecosystems.

Although not all researchers who investigate the presence of MPs in the marine environment are experts in extraction methodologies, a harmonised approach could allow scientists from other disciplines to contribute their findings as they conduct their individual research. The goal of the work described here is to demonstrate that reasonable quantification of MPs can be achieved with simple methodologies that do not require specialised personnel. The scientific community needs more high-quality data to accurately predict the impacts of MPs (Waldschläger et al., 2020), particularly with respect to socio-economic assessments, however, the availability of data to this extent could require years if limited to MP specialists. Given that there have already been reasonable guidelines and harmonised protocols proposed by experts in the field of MP research, the use of these could catalyse the development of research approaches that provide experts in environmental modelling with adequate data for risk assessment, thus increasing the presence of data relevant for socio-economic assessment and other ecosystem service-based assessments such as EEV.

5. Conclusions

In conclusion, as microplastic research continues to move forward in terms of improved methodologies for sampling, extraction and analysis, there remains a lack of understanding the pollutant's impact at the larger scale. While it has been established that MPs are “everywhere” (Barceló and Picó, 2019; Liu et al., 2019), the scientific community continues to wonder what this means for the big picture in terms of risks (Waldschläger et al., 2020). For this reason, this study aims to address the possibility that lack of necessary information for the risk assessment of MPs on marine ecosystems could be partially due to inconsistent approaches used in the sampling, extraction and analysis of in situ samples as well as the format in which findings are reported.

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POSITION PAPER

MICROPLASTICS RESEARCH AT A CROSSROADS: FROM UBIQUITY TO UNDERSTANDING

MICROPLASTICS CONSTITUTE ONE OF THE MOST PERVASIVE AND SCIENTIFICALLY CHALLENGING ENVIRONMENTAL CONTAMINANTS OF THE ANTHROPOCENE. THEIR UBIQUITOUS PRESENCE ACROSS MARINE, FRESHWATER, TERRESTRIAL, AND ATMOSPHERIC COMPARTMENTS IS THE DIRECT OUTCOME OF EXPONENTIAL PLASTIC PRODUCTION COMBINED WITH PERSISTENT STRUCTURAL FAILURES IN GLOBAL WASTE MANAGEMENT.

With global plastic production exceeding 460 million tonnes per year and less than 10% of plastic waste effectively recycled, environmental leakage has become a chronic and systemic pressure rather than an episodic phenomenon.

Scientific research has unequivocally demonstrated that microplastics occur in sediments, surface and deep waters, biota across trophic levels, and human food items. However, the continued accumulation of occurrence data alone is no longer sufficient. The field has reached a stage where the central challenge is not detection, but interpretation. Microplastics research must now confront its own limitations in order to generate knowledge that is comparable, mechanistically grounded, and relevant for environmental management.

Methodological heterogeneity remains the primary constraint. Differences in sampling strategies, extraction procedures, analytical techniques, and reporting units severely limit cross-study comparability and synthesis. The coexistence of optical, spectroscopic, and thermal methods, often applied without harmonized protocols or interlaboratory validation, continues to fragment the evidence base. These limitations are particularly critical for complex matrices, such as difficult-to-process animal tissues (e.g. holothurians, lipidic tissues) or organic-rich materials (e.g. wastewaters, e.g. agropiles, compost, etc.), for which matrix-specific and standardized extraction and purification protocols are still lacking.

At the same time, experimental research frequently suffers from limited environmental realism. Laboratory studies commonly employ pristine, spherical polymer standards at concentrations exceeding those observed in natural systems, while environmental microplastics are aged, irregular, chemically altered, and associated with additives, sorbed contaminants, and microbial communities. This disconnect undermines the ecological relevance of toxicity data and weakens extrapolation to ecosystem-level risk. The nanoplastics fraction further amplifies these uncertainties, as formation rates, environmental concentrations, and validated detection methods remain poorly constrained.

In this context, Marine Protected Areas (MPAs) should be explicitly recognized as priority research platforms rather than assumed to represent pristine conditions. MPAs offer controlled management settings in which baseline contamination levels can be defined, long-term trends assessed, and local inputs disentangled from regional and long-range transport.

Research conducted in MPAs is therefore essential to move from descriptive monitoring toward mechanistic understanding of sources, fate, and ecological implications of microplastic contamination. Evidence generated in these areas is essential to improve the modelling of microplastic impacts on ecosystem services and to extrapolate potential effects to relatively pristine environments.

We argue that the future of microplastics research must shift decisively from descriptive occurrence studies to integrative and predictive frameworks. This transition requires harmonized methodologies, environmentally realistic reference materials, long-term monitoring strategies, and explicit consideration of multiple stressors under changing environmental conditions.

Scientific journals have a central role in driving this transition by prioritizing methodological transparency, comparability, and conceptual advancement. Continued publication on microplastics is therefore not optional. It is the only means by which the field can resolve persistent uncertainties, consolidate fragmented evidence, and provide a robust scientific foundation for credible risk assessment and effective environmental governance.

EDITOR IN CHIEF
PROF. MONIA RENZI

ICT Index® V1.0: An Integrated Framework for Assessing Marine Trophic Network Integrity under the Marine Strategy Framework Directive

Short communication

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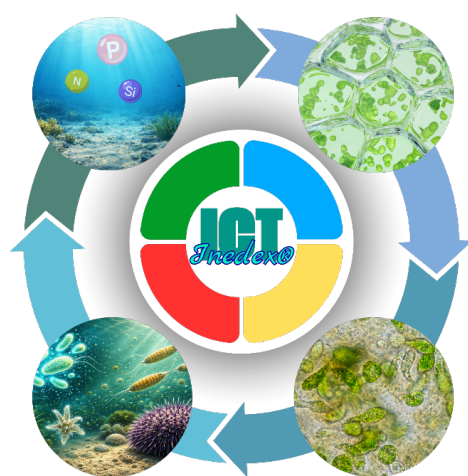
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Abstract: The Directive 2008/56/EC (Marine Strategy Framework Directive – MSFD) establishes the European framework for achieving Good Environmental Status (GES) of marine waters. Descriptor 4 (Food webs) requires that all elements of marine food webs, insofar as they are known, occur at normal abundance and diversity levels capable of ensuring long-term ecosystem functioning. However, despite the clarity of this ecological objective, the Directive does not define a standardized operational methodology for assessing trophic network integrity in an integrated manner. This short communication presents the ICT Index® V1.0 (Trophic Continuity Index) as a methodological proposal designed to address this gap by providing a normalized, transparent and ecologically structured framework for evaluating functional trophic continuity in marine ecosystems.



Keywords: ICT Index® V1.0; Marine Strategy Framework Directive; Descriptor 4; ecosystem-based assessment; primary production; phytoplankton community structure; ecotoxicological response; Weight of Evidence; coastal monitoring.

1.

Rationale and Regulatory Context

Implementation of Descriptor 4 under the MSFD remains methodologically challenging. While numerous indicators are available – such as nutrient concentrations, chlorophyll-a, biodiversity metrics, and ecotoxicological endpoints – these are often assessed independently or only partially integrated. As a result, the interpretation of food web integrity frequently lacks systemic coherence.

The ICT Index® V1.0 was developed to overcome this fragmentation by integrating multiple lines of evidence into a unified functional framework. Rather than reconstructing detailed species-specific trophic interactions, the index evaluates the functional continuity of matter and energy flows across key ecological compartments.

2. Conceptual Structure of the ICT Index® V1.0

The index is based on the fundamental ecological sequence (Figure 1) that characterizes marine coastal systems:



Figure 1. Graphical ecological sequence in marine coastal systems

Accordingly, the ICT Index® V1.0 integrates four independent yet ecologically interconnected functional nodes:

- **Node 1** – Trophic base availability, assessing dissolved nutrient coherence relative to expected natural variability;
- **Node 2** – Primary production, based on chlorophyll-a concentration as a proxy for phytoplankton biomass and energy input;
- **Node 3** – Phytoplankton community structure, expressed through diversity, evenness and dominance indices, reflecting system resilience;
- **Node 4** – Continuity toward higher trophic levels, based on a multi-trophic ecotoxicological battery integrated through a Weight of Evidence approach.

The objective is not to model food web topology but to assess whether energy transfer remains functionally coherent across successive compartments.

3.

Methodological Principles

The ICT Index® V1.0 is defined as a normalized synthetic index ranging from 0 to 1, obtained through the weighted integration of the four nodes. Each node is independently normalized on a 0–1 scale (0 = altered condition; 1 = fully functional condition), ensuring spatial and temporal comparability.

The construction of the index follows four core principles:

- **Prior normalization**, ensuring dimensional consistency and comparability;
- **Functional independence of nodes**, reducing informational redundancy;
- **Ecologically motivated weighting**, assigning greater influence to central energy-flow components (primary production and community structure);
- **Safeguard (anti-compensation) criterion**, preventing severe impairment in one node from being fully offset by high scores in others.

Thus, the ICT Index® V1.0 is not a purely descriptive arithmetic mean but a structurally informed metric grounded in ecological hierarchy and precautionary assessment logic.

4. Ecological Interpretation

The ICT Index® V1.0 represents the degree of systemic trophic continuity.

High values indicate that:

- nutrient availability is coherent with natural variability;
- primary production operates within expected physiological ranges without persistent bloom signals;
- phytoplankton community structure maintains diversity and balance;
- no convergent ecotoxicological stress signals are detected across trophic levels.

Intermediate values reflect physiological variability or localized discontinuities, whereas low values indicate functional impairment due to chemical imbalance, production instability, structural simplification or toxicological interference.

The index therefore integrates bottom-up processes and stress-mediated responses within a unified functional perspective.

5.

Classification of the ICT Index® V1.0

The final value of the ICT Index® V1.0 is interpreted according to ecologically defined functional classes (Table 1).

Table 1. Classification of the ICT Index® V1.0.

ICT Index® V1.0 Range	Functional Class	Ecological Interpretation
≥ 0.85	Fully functional trophic network	Systemic continuity of energy flows; coherence among all nodes; absence of trophic bottlenecks
0.65 – 0.84	Stable trophic network with physiological variability	Natural coastal fluctuations; no convergent critical signals
0.50 – 0.64	Possible local discontinuity	One or more nodes show significant deviations; alteration not necessarily systemic
< 0.50	Functional alteration	Impaired trophic continuity; convergence of imbalance signals across compartments

5.1 Safeguard Criterion

If at least one node scores < 0.50, the ICT Index® V1.0 cannot be classified as “Fully functional trophic network”, even if the weighted average exceeds 0.85. This anti-compensation rule enhances ecological consistency and prevents misleading interpretations due to mathematical compensation among functionally independent compartments.

6.

Relevance for MSFD Implementation

The absence of a harmonized integrated method for assessing food web integrity under Descriptor 4 highlights the need for structured and precautionary frameworks. The ICT Index® V1.0 provides a scientifically grounded and operationally applicable approach aligned with ecosystem-based management principles.

The index does not replace existing indicators; rather, it organizes them within a coherent functional scheme focused on trophic continuity. Its structure is compatible with Weight of Evidence methodologies widely adopted in European environmental assessment systems.

7.

Conclusions

The ICT Index® V1.0 represents a methodological proposal for quantitatively assessing marine trophic network integrity in the context of the MSFD. By integrating chemical, productive, structural and ecotoxicological components into a normalized and precautionary framework, the index translates the qualitative objective of Descriptor 4 into an operational tool.

In a regulatory context where the need to evaluate food web integrity is clearly stated but not operationally defined, the ICT Index® V1.0 may provide a transparent, reproducible and ecologically coherent approach for marine monitoring and ecosystem-based management.

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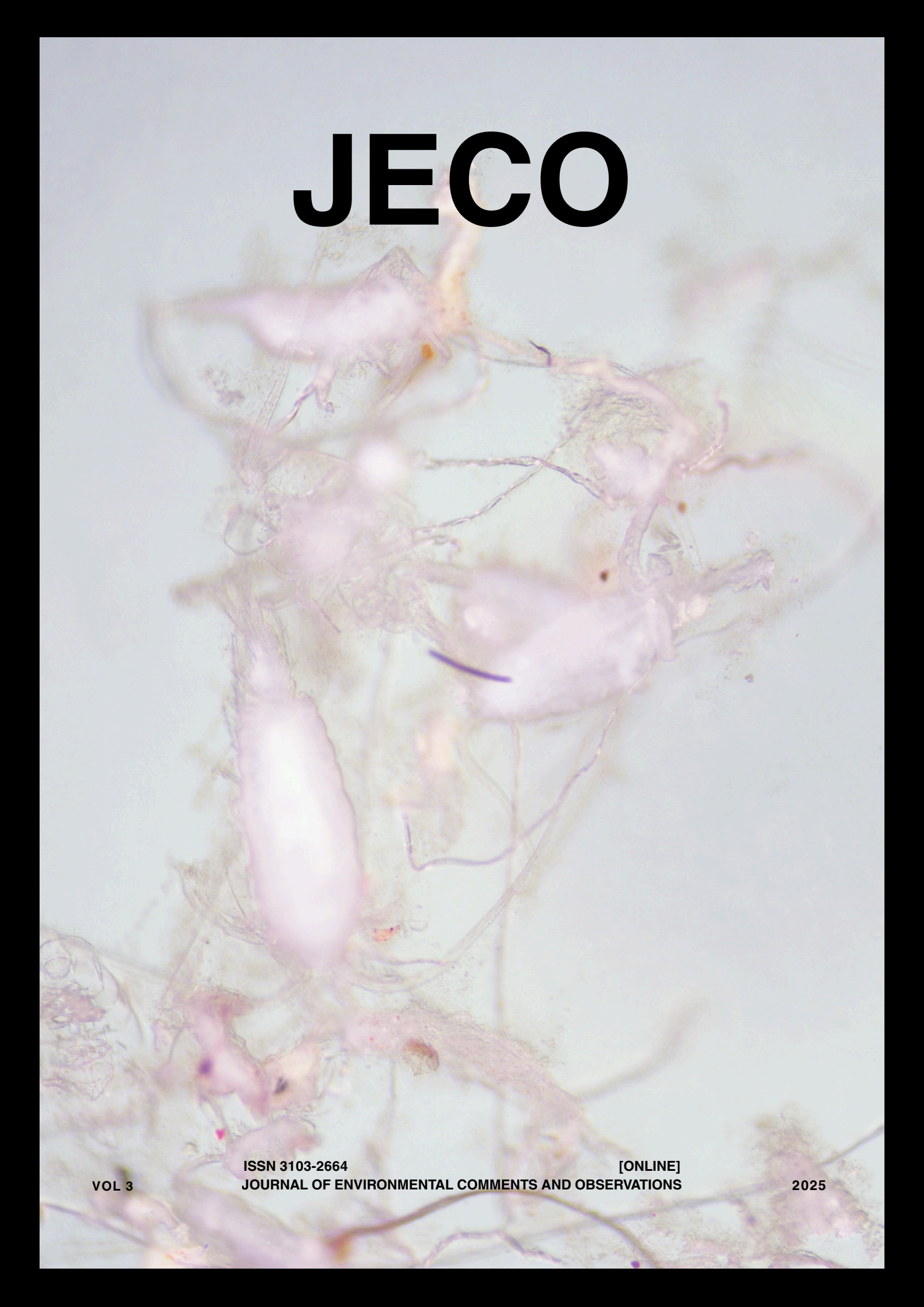
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JECO

A microscopic image of biological tissue, likely a histological section, showing various cellular structures and fibers in shades of pink, purple, and yellow. The tissue appears to be a complex network of cells and fibers, possibly from a developing organism or a specific organ.

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