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MArinE Litter SusTainable RemOval and Management

D2.5

Integrated assessment of the cleaning operations effectiveness and their impacts

14/02/2025

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History of changes

Version	Date	Author	Description of changes
V1	30/01/2025	Martina Bocci	Draft version 1
V2	13/02/2025	Martina Bocci, Vanessa Moschino	Draft version 2
V4	14/02/2025	Fantina Madricardo	Final version

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Executive Summary

The MAELSTROM project aims to test and evaluate innovative technologies for the removal of marine litter in different coastal environments, also assessing their impact on the ecosystems in chosen demo sites and evaluating the economic and societal benefits of the MAELSTROM solutions within local economies. The present Deliverable 2.5 assesses the potential environmental effects of the remediation activities performed by the MAELSTROM technologies in the two demo sites considered by the project: the robotic Seabed Cleaning Platform in the Venice coastal area (IT) and the Bubble Barrier in Ave River estuary (PT).

The removal of macro litter represented a first, positive effect on demo sites: the seabed cleaning technology, tested both in the lagoon and in a coastal area, was able to remove 59% of litter items in a heavily anthropized, disturbed and turbid environment inside the Venice Lagoon, and about 75% in the coastal area in front of the Lagoon. In the Ave River estuary, the overall results showed that the Bubble Barrier is already contributing to a significant reduction of floating macro litter, collecting most of these materials before they reach the Atlantic Ocean, contributing to both aquatic ecosystems and human health.

The effect of the technologies on the aquatic environment was assessed by an integrated approach, taking into consideration the physical, chemical and biological parameters as descriptors of the ecosystems.

In the Venice coastal area, the concentrations of microplastics in different environmental matrices (water, sediment and biota), and the variation of macrozoobenthic and fish communities were considered. Integrating all the results from the different matrices and the two areas, it is showed that the removal operations performed efficiently and selectively and that the Seabed Cleaning Platform did not generally affected negatively the environmental status. The post-cleaning conditions were generally similar or improved in comparison the pre-cleaning ones. In particular, microplastic concentrations in sediments and biota decreased over time.

In the Ave River estuary, following the installation of the Bubble Barrier, the ecological assessment results showed no significant changes in any of the evaluated indicators. Any potential significant ecological changes due to the operation of the Bubble Barrier in the estuary are expected to take much longer time than what was available during the project.

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The experience of the MAELSTROM project showed how coastal cities and municipalities, local agencies and local populations can significantly benefit from macro-litter remediation technologies. The successful implementation of this technologies relies on the collaboration of all actors at local level. Endorsement by local authorities is key, as well as the engagement of local communities, schools, and the public. This involvement fostered a sense of shared responsibility and demonstrated the importance of public participation in environmental conservation efforts.

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1 Introduction to the MAELSTROM project

MAELSTROM is a project funded under the Topic CE-FNR-09-2020 Pilot action for the removal of marine plastics and litter. MAELSTROM strives to provide answers and diversified solutions to the complex question of the removal and sustainable treatment of marine litter (ML) legacy. MAELSTROM contemplates the integration of complementary technologies for marine litter removal in different European coastal ecosystems, compounded with a full-fledged circular economy and societal-oriented solutions. In particular, the project (i) sets out a reliable multidisciplinary and scientifically sound approach for the assessment of marine debris distribution and impact on marine life in highly valuable ecosystems and protected areas; (ii) designs and manufactures scalable, replicable and automated technologies, co-powered with renewable energy and second generation fuel, to identify, remove and sort ML; (iii) evaluates over time the performance of ML removal devices along with their impact on local ecosystems; (iv) integrates different technologies to track, sort and recycle all types of collected ML into valuable raw materials for future marketisation; (v) assesses the economic and societal impact of the MAELSTROM solutions providing also a comprehensive life-cycle assessment of the technologies and products; (vi) enhances social awareness about the ML issue and engages citizens and stakeholders in MAELSTROM activities; (vii) interplays with similar projects to maximise innovation uptake for ML removal within and outside the EU.

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2 MAELSTROM Consortium

The MAELSTROM project consortium consists of:

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3 Aim of the deliverable

The present deliverable describes the environmental effects – positive and/or negative impacts – during the life span of the project of the remediation activities undertaken with the use of MAELSTROM technologies in the two demo sites considered by the project: Demo site 1: the Venice coastal area (IT) and Demo site 2: the Ave River estuary (PT). In Demo site 1 a robotic Seabed Cleaning Platform was applied to clean-up the litter deposited on the seafloor of an area located inside the Venice lagoon, and of another sea area located in the Adriatic Sea, in the Venice coastal area. In Demo site 2 the Bubble Barrier technology was applied to prevent the Ave River from transporting litter to the sea, in the coastal area of Vila Do Conde, Portugal.

The deliverable illustrates the results of the integrated ecological assessment performed to evaluate the possible impacts of the remediation activities on the coastal ecosystems of both demo sites. The detailed results of the ecological assessments are presented in D2.3 and D2.4. The integrated assessment presented in this deliverable consists in the comparison of the values of different environmental and biological indicators, assessed before and after the implementation of the remediation technologies. The differences are assessed via statistical analysis.

The description of the impacts of the remediation technologies is complemented with an overview of the results of ML removal in the demo sites. To do so, the report synthetically integrates the results of the effectiveness of the MAELSTROM technologies presented in deliverable D5.2 and D5.4, for the Seabed Cleaning Platform and the Bubble Barrier, respectively.

Finally, the integrated assessment presented in this deliverable makes possible to achieve a comprehensive evaluation considering all the dimensions of sustainability of the technologies: environmental, economic and societal.

4 Methodology

Ecosystem assessments were undertaken in the two demo sites before and after the cleaning operations. The organization of these activities was different in the two sites as it is detailed in the paragraphs below. The ecosystem assessments took into consideration the physical, chemical and biological conditions of the ecosystems where the technologies were applied. The variations in terms of macro litter (ML) and

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micro-plastics (MP) were also considered. The integrated assessment of the results presented in this deliverable takes into consideration the differences in type of ecosystem characterizing the two demo sites.

The overall approach to the environmental impact assessment of the remediation technologies in the two demo sites is illustrated in the following diagram (Figure 1).

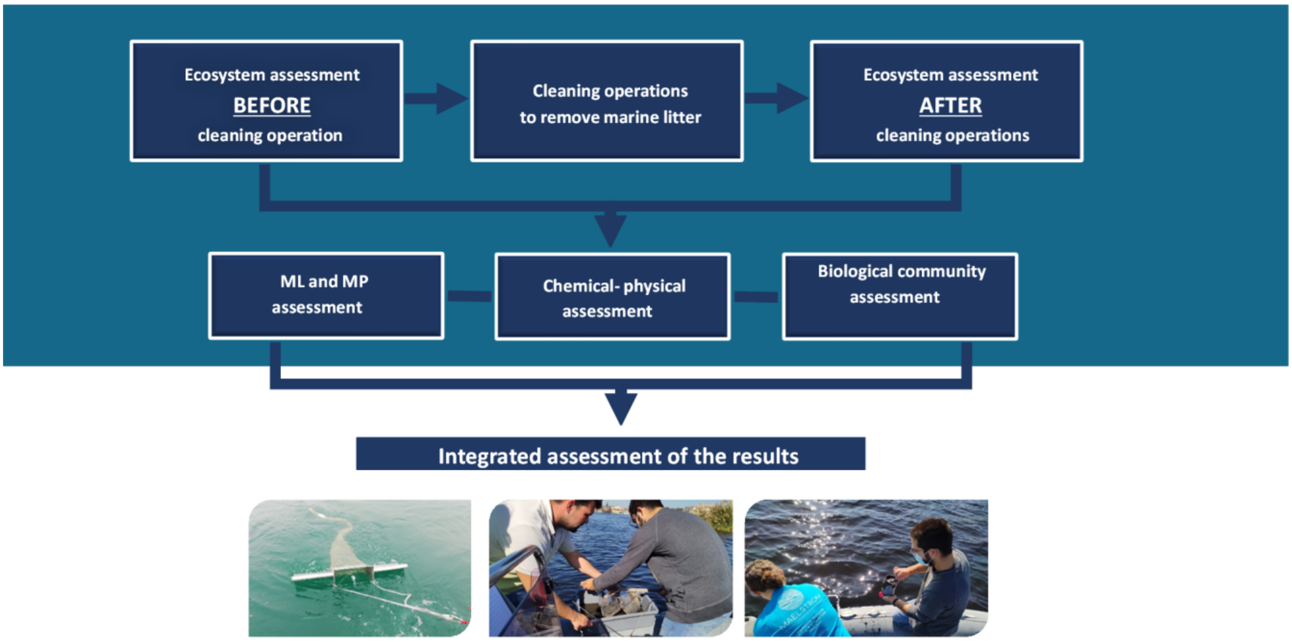

Figure 1 - Design of the assessment of the impact of the remediation technologies in the demo sites.

4.1 Monitoring and cleaning campaigns

The assessment of the effects of the remediation activities on the environment was designed considering the characteristics of each demo site. During the project, the assessment design was adapted to the operational constraints experienced in the implementation of the remediation technologies on site. The timeline of the different actions undertaken in each site, including the monitoring and the remediation campaigns or technology implementation are described in the following paragraphs.

4.1.1 Demo site 1 - Venice coastal area (IT)

In the Venice Demo site, two areas of intervention were considered: a site within the lagoon, close to the city of Venice (Sacca Fisola), and a coastal site (an abandoned mussel farm) located at sea (Figure 2). In the demo sites, the Seabed Cleaning Platform based on cable robotics was used to remove the seabed litter.

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The figure below shows the sites where the cleaning campaigns were performed.

Figure 2 - Cleaned areas in Demo site 1 – Venice lagoon.

The timeline of the activities is illustrated in Figure 3. The lagoon and the coastal sites had slightly different timelines. Pre-cleaning monitoring surveys were performed in both intervention areas between autumn 2021 and spring 2022, consisting in surveys of seafloor litter mapping and samplings related to the monitoring of chemical parameters and biological communities. In the Lagoon of Venice, two cleaning

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campaigns were performed to remove marine litter from the seafloor with the Robotic Seabed Cleaning Platform.

The first cleaning campaign took place from the 12th to the 30th of September 2022, and it was operated in the area of Sacca Fisola, within the lagoon. The cleaning campaign covered an area of approximatively 2000 m^2. The post-cleaning monitoring activities started in that site after the conclusion of the clean-up. Cleaning operations at the abandoned mussel farm were not performed in this period due to adverse weather conditions, and were postponed to spring 2023.

The second cleaning campaign took place from the 22nd of May to the 14th of June 2023. This time both areas were considered.

At Sacca Fisola, four post-cleaning campaigns were conducted (plus an additional one in November 2022 when only microplastics were monitored) whereas three post-cleaning campaigns were conducted in the coastal site.

Figure 3 - Timeline of monitoring and cleaning activities in the Venice demo site.

An integrated framework was defined to compare the quality status of marine ecosystems before and after cleaning operations in the demo site of the lagoon of Venice. The framework is illustrated in Figure 3. To assess the presence of macro-litter on the seabed, to evaluate the removal technology effectiveness and to provide estimates of seafloor cleaning results, mapping activities were performed before and after cleaning operations through underwater acoustic remote sensing. To assess potential impacts on seafloor habitats and biota of cleaning operations, the state of contamination of micro-plastics in various matrices (water, sediments and biota) and the recovery capacity of benthic and fish community after the ML removal were evaluated as well.

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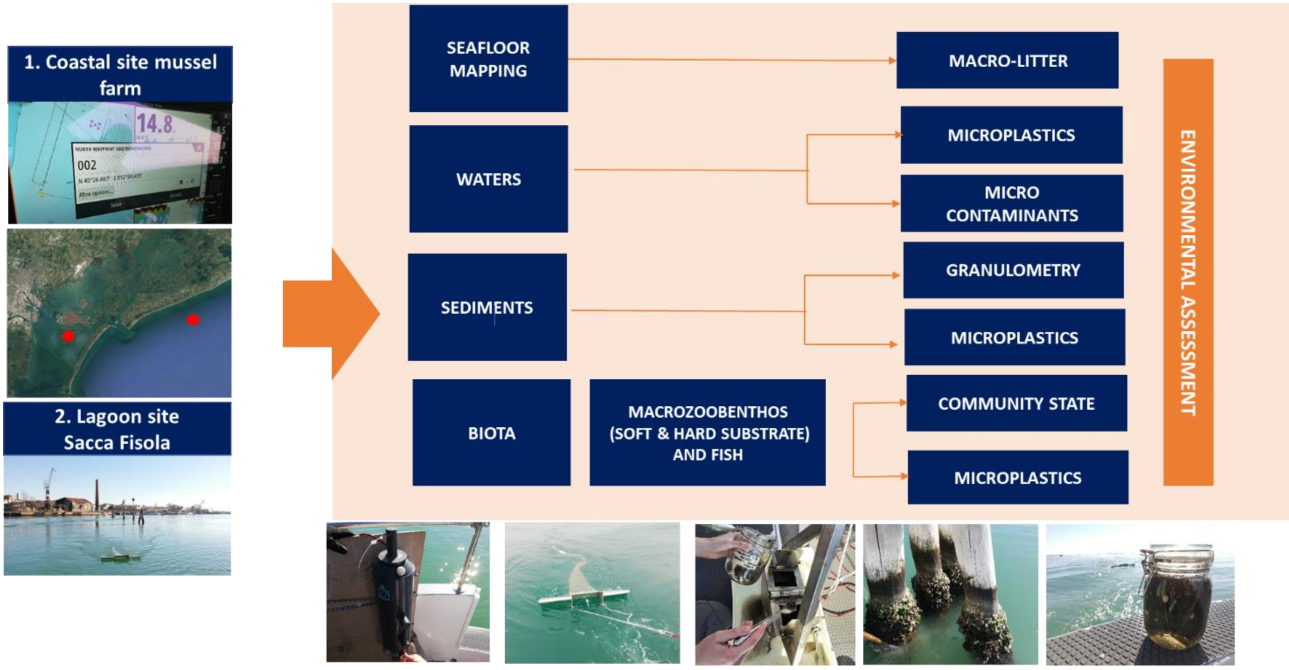

Figure 4 - The integrated assessment framework defined to study the Venice demo site.

4.1.2 Demo site 2 - Ave River estuary (PT)

In the Ave River estuary demo site, the Bubble Barrier, the technology selected for this region, was installed in the lower estuary, as it can be seen in Figure 4.

Figure 5 - Ave River estuary and Bubble Barrier technology, catchment system and compressor seeing from the last Google Earth image (3/2024).

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At the Ave River estuary several campaigns were performed before and after the installation of the technology. Summer and autumn campaigns for physico-chemical, ecological and microplastics characterization were performed between spring 2021 and autumn 2023. After the implementation of the technology (November 2023), the frequency of the samplings was increased to seasonal (winter 24 - two sampling periods-, spring 24, summer 24 and autumn 24) during the last project year. Thus, in total, seven pre-installation and four after-installation campaigns were performed for the Ave River estuary demo site.

5 Impacts of the remediation activities on coastal ecosystems: an integrated ecological assessment

The impacts of the remediation activities in the two Demo sites are presented in this chapter. The results concerning the removal of macro litter are described, as this removal represents *per se* a valuable component of the positive impacts on the ecosystems of the technology application. The results of the ecological assessment and of the microplastics assessment are then detailed. In the presentation of results, emphasis is given to the comparison between the assessments undertaken before and after the implementation of the remediation technologies.

5.1 Demo site 1 – Venice coastal area (IT)

5.1.1 Lagoon site - Sacca Fisola

5.1.1.1 Removal and mapping of seabed litter

To assess the presence of macro-litter on the seafloor before and after the cleaning campaigns performed with the Robotic Seabed Cleaning Platform, high resolution multibeam echosounders were used to map the sites within the lagoon (Sacca Fisola) and at sea in the Venice coastal area (abandoned mussel farm). As well, video inspections with a camera were carried out by divers, mini ROV and by the robotic platform cameras to ground truth the interpretation of the targets identified with the multibeam data. Methodological details are reported in deliverable D2.3 and D2.4.

The seafloor macro-litter distribution of the Sacca Fisola area was assessed through MBES high resolution mapping in four surveys May 2022, November 2022, May 2023 and December 2023. The relative variation in the number items in the Sacca Fisola area between the May 2022 and December 2023 is reported in Table 1 and in Figure 6 is illustrated an example of the distribution and typology of items for the November 2022 dataset. Overall, the number of items per area after the cleaning operations

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decreased from 739 to 472 with a density of 20620.48 items/km² (2.06 items/100m²) and 12226.15 items/km² (1.3 items/100m²), respectively. This improvement is certainly related to the cleaning operations, as we will see below. However, some items may have been buried by sediments or been displaced by currents and therefore not been detected by the MBES.

Table 1 – relative variation in the number items in the Sacca Fisola area between the May 2022 and December 2023.

Year	Month	n° mapped items	Area (Km2)	Items/km²
2022	May	739	0.036	20620.48
	November	472	0.036	13171.97
2023	May	547	0.039	13975.32
	December	418	0.034	12226.15

Out of the full area mapped with the MBES during four surveys in May 2022, November 2022, May 2023 and December 2023, the area of the cleaning operations of about 2000 m² was mapped in detail to show the situation of the seafloor before and after the cleaning (pink rectangle in Figure 6).

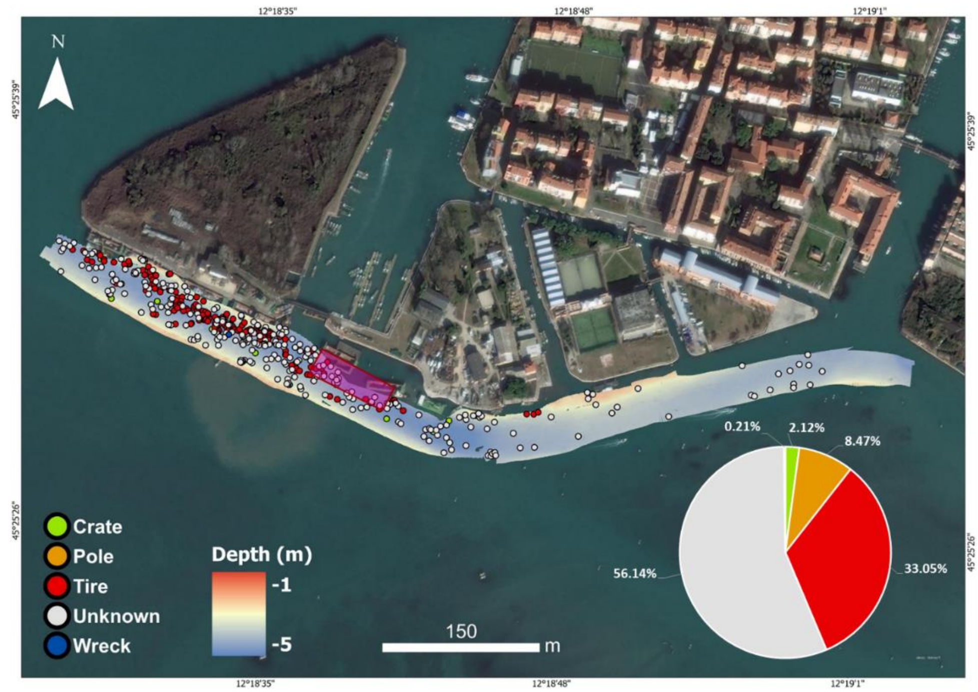

Figure 6 - Map of the Sacca Fisola area with the seafloor items identified with the MBES data in November 2022. The red rectangle identifies the cleaning area described in Figure 7.

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During the first clean-up campaign in Sacca Fisola, conducted between 15 and 27 September 2022, a significant number of marine debris of various types was collected. Among the items recovered were a wooden panel, a rope, a coiled steel cable, several bricks, a plastic net, a steel bar, a trolley, part of a steel wagon, glass bottles, a Venetian cart, twelve truck tyres, a five-metre-long aluminium roof panel, and a steel roll bar. Other materials included plastic sheets, textile, numerous plastic fragments, aluminium cans, an electronic circuit board, a steel and concrete block, wood, electric cables, and steel frames Figure 7).

Figure 7 - Images of marine waste recovery operations during the Sacca Fisola clean-up campaigns: a. Recovery of a piece of steel machinery; b. Removal of a tyre; c. Removal of a 5-metre-long aluminium cover panel; d. Mixed waste collected, including plastic fragments, aluminium cans and an electronic circuit board. These pictures highlight the variety of materials recovered.

During the second cleaning campaign, conducted on 1 and 2 June 2023, the robotic platform employed its grab bucket to collect marine litter in four specific locations within the designated cleaning area at Sacca Fisola. A total of 197 objects were removed, including items such as glass, plastic, metal, and mixed materials. The use of the grab bucket allowed for the efficient collection of larger objects and fragments that were distributed across the cleaning area. Some items, such as metal fragments or small pieces of plastic, were not easily detectable with the MBES but were successfully located and removed thanks to the platform's direct intervention.

In this area, a total of 126 seafloor items were counted in May 2022, while in November 2022 (after the cleaning) only 51 seafloor items were mapped. The comparison between

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the two maps clearly shows the disappearance of the items removed by the Seabed Cleaning Platform (Figure 8 - Map of the area selected for the cleaning operations in May 2022 (up-before cleaning) and November 2022 (down-post cleaning). Since the cleanup covered a total area of about 0.02 km², the density of seafloor items per km² in the rectangle is about 6322 items/km² in May 2022 and 2558 items/km² in 2022, showing a substantial improvement in the cleaned area with **a 59% reduction of the detected items.**

This value reflects the ability of the robotic platform to tackle marine litter effectively, while also highlighting the challenges posed in the heavily anthropized environment of Sacca Fisola. The proximity of a municipal waste collection point contributes significantly to the continuous accumulation of debris in the lagoon and the intense boat traffic contributes to sediment resuspension also due to propellers ploughing the channel seafloor, as shown in Figure 8 - bottom left zoom. In this area, the cable robot could rely almost only to the bathymetric data: the cable robot cameras were under performing due to high water turbidity and low visibility.

A comparative temporal analysis between the data from 2022 and 2023 revealed significant dynamics, including the displacement of debris due to currents or nautical activities and the disappearance of other objects following the cleaning operations (Figure 8). These findings underline both the complexity of the seafloor marine litter problem and the effectiveness of repeated clean-up efforts in mitigating its impact.

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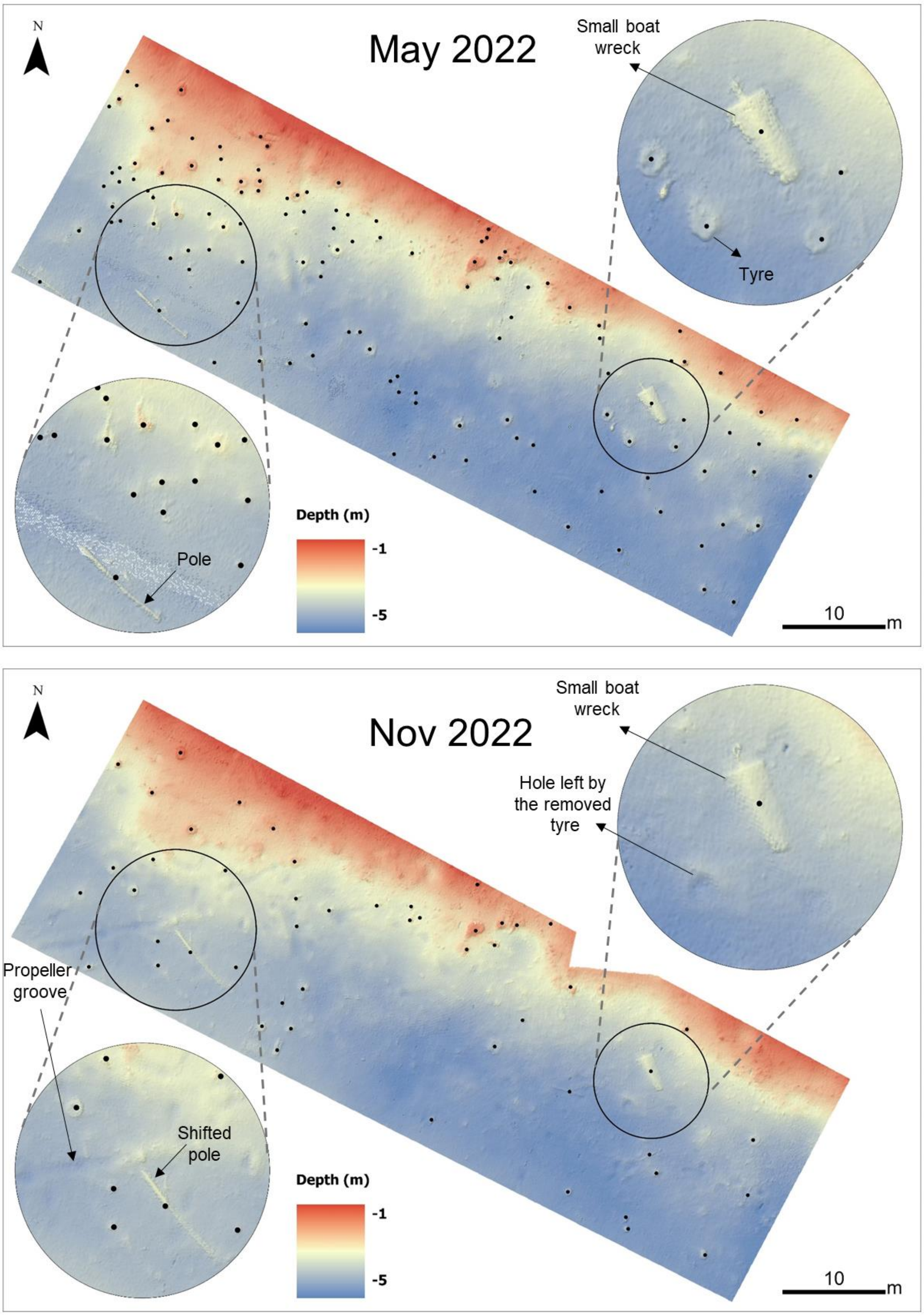

Figure 8 - Map of the area selected for the cleaning operations in May 2022 (up-before cleaning) and November 2022 (down-post cleaning).

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5.1.1.2 Soft bottom macrozoobenthos community

The soft bottom macrozoobenthic community was characterized on stations LV1 (on the cleaning area) and LV2 (control) at the lagoon site (Sacca Fisola) before and after the seabed cleaning operations according to the calendar shown in Table 2.

Table 2 - Soft bottom macrozoobenthos monitoring campaigns in the Lagoon site.

Lagoon site soft bottom macrozoobenthos campaigns		
C1	01/04/2022	Pre cleaning
C2	04/04/2023	1st post cleaning
C3	27/09/2023	2nd post cleaning
C4	12/03/2024	3rd post cleaning
C5	06/09/2024	4th post cleaning

Samples were collected with a 0.1 m^2 Van Veen grab (three replicates for each station) and sieved at 1 mm mesh size. All retained specimens were identified at the level of species or the highest achievable taxonomic resolution. More details on the protocols and results are presented in Deliverables D2.3 and D2.4.

Community structure was described by main univariate ecological descriptors as well as multivariate analyses. Selected univariate descriptors include abundance, richness, diversity, biotic and multimetric indices, as listed hereafter:

- density, Nm^{-2}
- species richness, S
- Shannon index, H'
- AMBI biotic index (Borja et al., 2000)
- M-AMBI multimetric index (Bald et al., 2005; Muxika et al., 2007)

The changes of the univariate descriptors over the five monitoring campaigns have been represented by mean values (+/- standard deviation) in Figure 9.

A two-way ANOVA with stations (two levels) and campaigns (five levels) as fixed crossed factors was performed to assess statistically significant changes for all the descriptors, focusing on C1 (pre-cleaning) and C2 (1st post-cleaning) campaigns (models were checked for homogeneity of variance with Tukey-Anscombe plot and for normality of Residuals with Residual Q-Q plot). A summary of p-values is presented in Table 3 for both factors as well as interactions.

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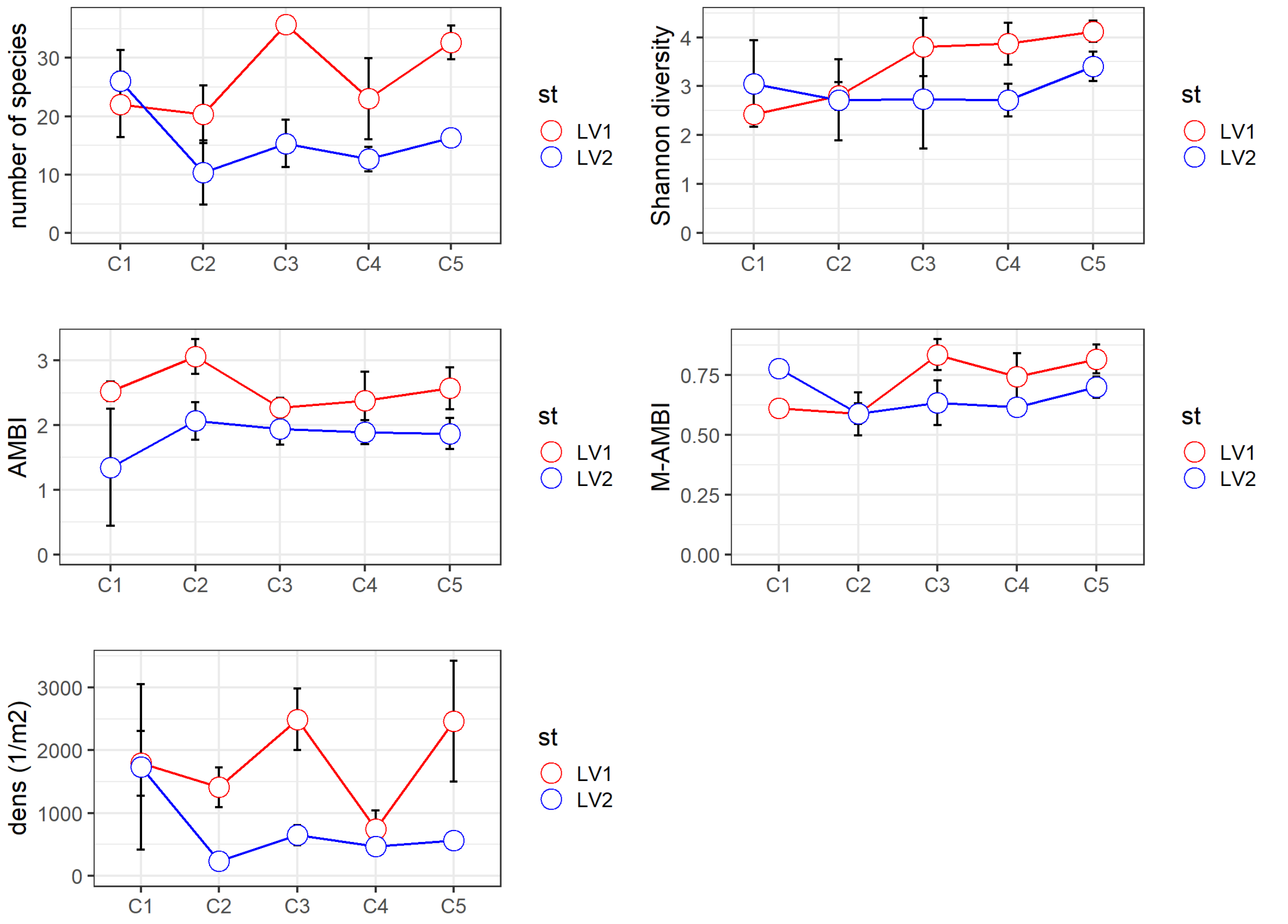

Figure 9 - Lagoon site: changes of the univariate descriptors of macrozoobenthic community over the five monitoring campaigns, where LV1 and LV2 represent the stations on the cleaning area and control, respectively.

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*Table 3 - Lagoon site: statistical significance of changes taking into accounts all the monitoring campaigns (top) and between campaigns (bottom) for each univariate descriptor (see text) calculated by two-ways ANOVA (crossed factors: campaign and station); statistical significance codes: *** = 0-0.001, ** = 0.001-0.01, * = 0.01-0.05, ° = 0.1-1*

ALL CAMPAIGNS	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.00177**	0.03395*	0.10837	0.00180**	0.00872**
station	0.00000***	0.03592*	0.00004***	0.02498*	0.00008***
campaign:station	0.00150**	0.07708°	0.32209	0.00064***	0.03266*
C1-C2	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.02263*	0.95890	0.06125	0.01029*	0.05424°
station	0.35823	0.47088	0.00550°	0.03182*	0.17850
campaign:station	0.05253°	0.35640	0.76350*	0.03080*	0.21717
C1-C3	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.56928	0.24963	0.55329	0.28958	0.66574
station	0.01204*	0.62493	0.02686*	0.62992	0.06006°
campaign:station	0.00133**	0.08367°	0.16541	0.00080***	0.07357°
C1-C4	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.07751°	0.10571	0.52035	0.66637	0.02419*
station	0.32891	0.40540	0.02429*	0.55501	0.70076
campaign:station	0.04647*	0.01841*	0.28289	0.00165**	0.79924
C1-C5	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.84079	0.00643**	0.35579	0.03751*	0.62452
station	0.03368*	0.87444	0.01179*	0.36909	0.08324°
campaign:station	0.00292**	0.04273*	0.43663	0.00053***	0.09933°
C2-C3	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.00325**	0.26215	0.01176*	0.00990**	0.00276**
station	0.00027***	0.21502	0.00151**	0.04869*	0.00003***
campaign:station	0.06847°	0.28046	0.04439*	0.04986*	0.09498°
C3-C4	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.01311*	0.95664	0.85979	0.24718	0.00054***
station	0.00022***	0.01818*	0.03691*	0.00524**	0.00029***
campaign:station	0.07221°	0.90046	0.64016	0.41659	0.00196**
C4-C5	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.01960*	0.03845*	0.66351	0.06229°	0.01458*
station	0.00040***	0.00114**	0.01133*	0.00913**	0.00588**
campaign:station	0.22678	0.28022	0.56721	0.89637	0.02416*

Considering all the campaigns, for **all the parameters there are significant differences between stations and** (with the exception of AMBI-BC) **between campaigns**. The number of species (S) significantly decreases only in the campaign

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C2 (the first post-cleaning) compared to the pre-cleaning situation, in particular for the control station LV2 (without however highlighting significant differences between stations). Thereafter, **the number of species remains always higher in the cleaned station LV1** than in the control station LV2. A similar behaviour is observed for the diversity H', which integrates the specific richness with the distribution of abundances between species (even in the absence of significant variations between C1 and C2). The biotic index AMBI-BC, which expresses the composition of the community in terms of the proportion between sensitive and tolerant species (especially in terms of saprobity, Tagliapietra et al., 2012), shows a significant variation between stations but not between campaigns. The M-AMBI index, as per its nature (Sigovini et al., 2013), averages the three previous metrics, highlighting the significant differences between campaigns and stations (both overall, with an increase in the index starting from C3, and with regards to the transition from C1 to C2, although with a lower p-value, due to a decrease in LV2). Finally, the "vitality" of the community, expressed by density, presents an overall high variability between campaigns, without a clear trend. In any case, it is worth noting that the first post-cleaning campaign was conducted approximately 6 months after cleaning (and one year after the pre-cleaning campaign), and that subsequent campaigns showed a certain degree of change for all parameters.

The multivariate community structure of replicates has been explored through well-established methods. No data transformation before the analyses was deemed necessary. Agglomerative hierarchical clustering was performed, based on Bray-Curtis similarity index. The group average linkage was applied, which evaluates the similarities between the two groups based on the average similarity between all the pairs of objects within the groups. The result has been represented by means of a dendrogram (Figure 10). The results indicate that **in terms of multivariate community structure, stations cluster together by campaign/season only partially, with some replicates/stations showing high average dissimilarity**.

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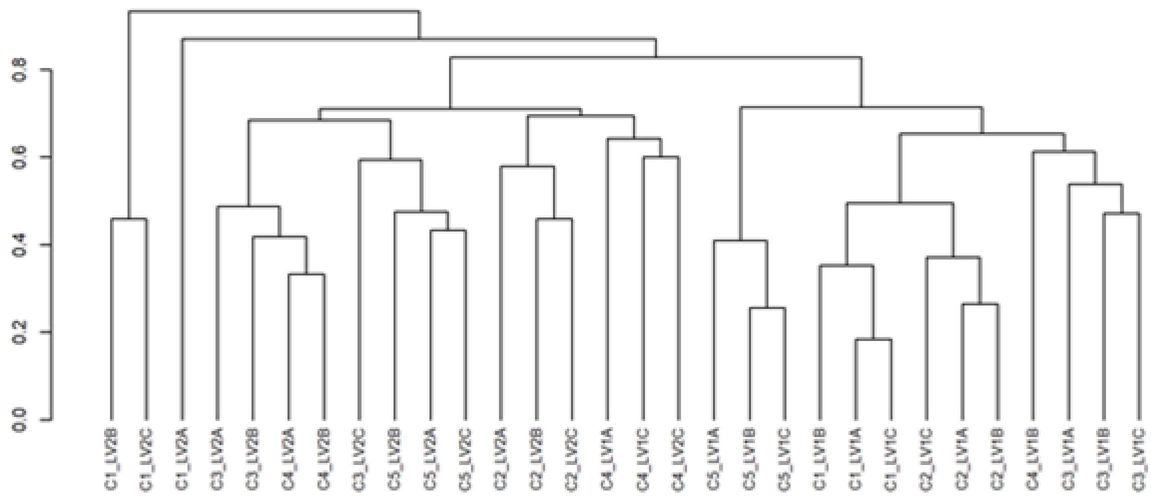

Figure 10 - Lagoon site: dendrogram of agglomerative hierarchical clustering (based on Bray-Curtis similarity index) of macrozoobenthic community data (replicates).

NMDS (non-metric Multidimensional Scaling; Shepard, 1962) ordination was also performed, which considers the rank of (dis-) similarity between samples, and projects the stations into a space with reduced dimensions (usually two-dimensional) through an iterative procedure that minimizes a stress function (considered a measure of the "goodness" of the representation). The ordination is presented in Figure 11. NMDS presents the same overall patterns highlighted by cluster analysis, with **a certain degree of overlapping between campaigns and stations, although in fact a gradient in terms of community composition can be observed between LV1 and LV2, as expected for tidal systems**, however limited the spatial scale.

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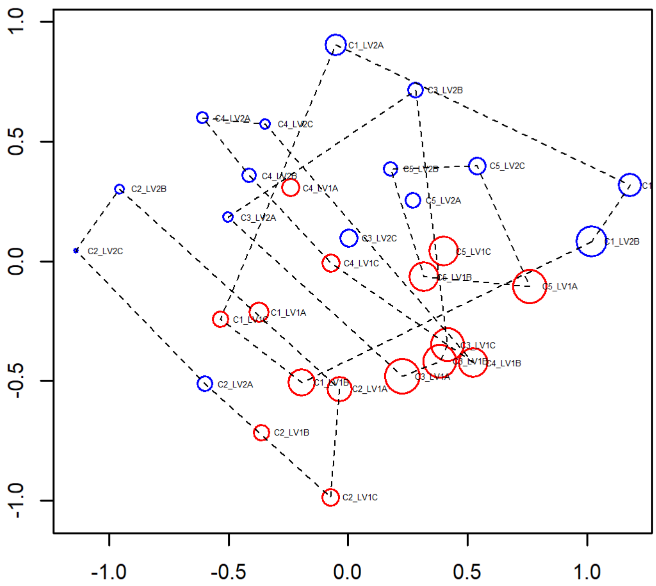

Figure 11 - Lagoon site: NMDS ordination of macrozoobenthic community data (replicates; stress: 0.229); red: cleaning area station LV1; blue: control station LV2; point size is proportional to the number of species; envelopes encompassing each campaign has also been represented.

To test differences in multivariate community composition between stations, two non-parametric multivariate approaches were applied:

- analysis of similarities (ANOSIM, Clarke, 1993), which compare the compositional dissimilarities between the groups to those within the groups and is performed on the rank order of dissimilarity values (a statistic R, comparable to a correlation coefficient, is produced which ranges between -1 and +1, zero indicating completely random grouping); the approach is companion to NMDS; an overall 1-way analysis was performed in both sites, with either sampling stations and campaigns as factors;
- permutational multivariate analysis of variance using distance matrices (ADONIS2, Oksanen et al., 2022), which is based on the principles of PERMANOVA (McArdle & Anderson, 2001) and arguably represents a more robust alternative to ANOSIM; two overall 2-ways analyses were performed in lagoon site, with sampling stations (2 levels) and campaigns (5 levels) or stations and seasons (2 levels) as crossed factors (both considered as fixed).

The results of both non-parametric multivariate tests are summarized in Table 4. A significant difference between stations, as well as campaigns emerges from both

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ANOSIM and Permutational ANOVA, which seems in any case to give no weight to seasonality.

*Table 4 - Lagoon site: results of multivariate non-parametric tests ANOSIM and Permutational multivariate ANOVA on untransformed macrozoobenthos community matrix, with campaigns, stations and seasons as factors; statistical significance codes: *** = 0-0.001, ** = 0.001-0.01, * = 0.01-0.05, ° = 0.1-1.*

	ANOSIM		Permutational multivariate ANOVA	
	R	significance	R2	p-value
station	0.5543	0.001	0.19366	0.001***
campaign	0.3011	0.001	0.29206	0.001***
station:campaign	-	-	0.20111	0.001***
season	0.07629	0.114	0.07782	0.003**
station:season	-	-	0.05090	0.032*

In conclusion, the analyses of univariate and multivariate structure of macrozoobenthos community in the lagoon site shows the absence of any significant reduction in quality in the LV1 sampling station after the cleaning procedure.

At the same time, we analysed the fouling community colonizing the removed marine litter, highlighting its possible role in increasing biodiversity (Deliverable 2.4), which must be considered during preliminary assessments of clean-up actions.

5.1.1.3 Fish community

Fish fauna sampling activities were carried at the lagoon site (Sacca Fisola) before and after the seabed cleaning operations, according to the calendar shown in

Table *5*. Considering that in the lagoon site the cleaning operations were carried out by the Robotic Seabed Cleaning Platform in September 2022, one pre-cleaning monitoring campaign was carried out in March 2022 followed by four post cleaning monitoring campaigns carried out in: April 2023, November 2023, April 2024 and October 2024.

Table 5 - Calendar of Fish fauna campaigns in lagoon area.

Lagoon site fish fauna campaigns	
20-22 March 2022	Pre-cleaning
1-5 April 2023	1° post cleaning
2-6 November 2023	2° post cleaning
8-16 April 2024	3° post cleaning
27-28 November 2024	4° post cleaning

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Fish fauna sampling activities were carried out in collaboration with professional fishermen, using ten fyke nets, which were placed within the sampling site close to the area where removal activities took place according to the procedure described in D2.3 and D2.4.

Abundance and biomass data of fish species were standardised in terms of catch per unit effort (CPUE), i.e. number of individuals captured by fike net in one fishing day, or grams of fish captured by fike net in one fishing day. Mean values (and standard deviations) of CPUE per species were then calculated.

The abundance and biomass data from each campaign were compared by means of boxplots in order to analyse the dataset collected during the sampling campaigns and to identify any changes in the fish community structure because of the cleaning interventions. For each sampling campaign, the stations were treated as replicates.

Abundance and biomass data related to each replicate were used to calculate a set of biodiversity indexes (species richness, Margalef index and Shannon index).

Data were compared in order to evidence possible difference among samples collected before and after cleaning operations using the non-parametric Kruskall-wallis test.

To provide cumulative data on fish community, the diversity indexes were also calculated on the whole data set.

Fish community in the Lagoon site showed a marked difference both in total abundance and biomass, between the spring 2022 campaign (pre-cleaning) and the following ones (post-cleaning). In particular, the standardized number of organisms per catch unit in the 2022 pre-cleaning campaign was 24,4±11,06 ind/fikenet day. The values decreased in the 2023, becoming statistically relevant in 2024 campaigns, when abundance values reached the significative lower values ranging from 0,8±0,63 to 6,4±4,43 ind/fikenet day. Similarly, biomass values resulted higher in 2022 campaign (759,6±279,2 ind/fikenet day) than 2023 and 2024 campaigns (ranging from 36,1±24,2 to 230,3±271, 2 g/fikenet day), showing significant lower values especially in spring samples (Figure 12).

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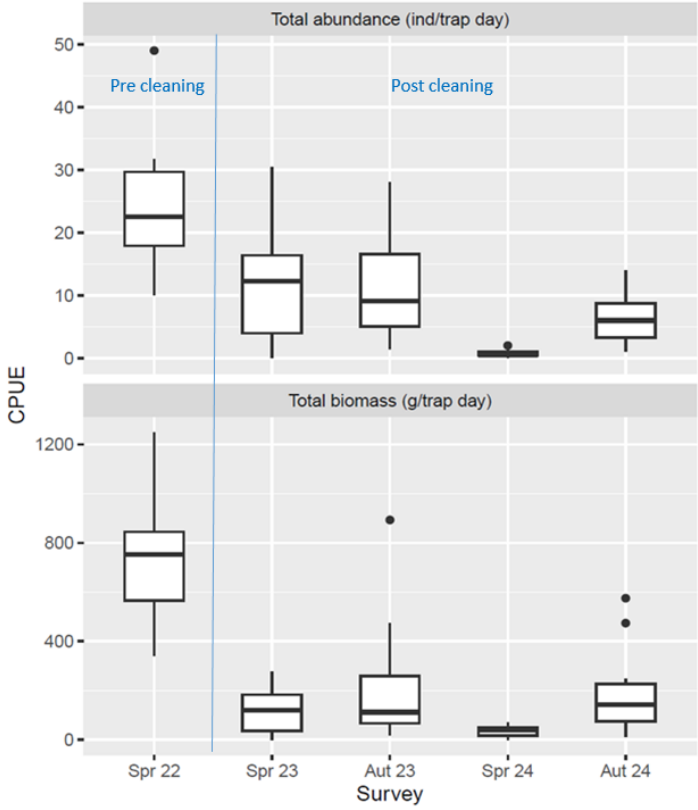

ABUNDANCE	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s.	n.s.	***	***
Spr-23		n.s.	*	n.s.
Aut-23			**	*
Spr-24				n.s.

BIOMASS	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	*	n.s.	*	n.s.
Spr-23		n.s.	n.s.	n.s.
Aut-23			n.s.	n.s.
Spr-24				n.s.

*Figure 12 - Boxplot of abundance and biomass calculated on replicates of fish community collected in the lagoon site and related statistical comparisons (Kruskall Wallis test: n.s= not significant; *=p<0.05; **=p<0.001)*

In general, **abundance and biomass trends highlighted the wide variability between replicates for each sampling campaign due to a large number of species found only occasionally within the lagoon community**. Species such as *Anguilla anguilla*, *Boops boops, Knipowitschia panizzae, Syngnathus abaster, Symphodus roissali* for example were present in only one out of five campaigns. Among the most characteristic species of the lagoon community, *Zosterisessor ophiocephalus* exhibited a marked decrease in abundance before and after the cleaning operation whereas *Atherina boyeri* showed a marked decrease in 2024 compared to previous campaigns.

Data related to biodiversity indexes calculated for each replicate are shown in Figure 13.

The number of species resulted similar in 2022 and 2023 campaigns (range 3,9±2,5 - 5±2.7), whereas a decrease was evident in 2024 campaigns (range 2,3±1,2 - 3,1±2,2), although not statistically relevant.

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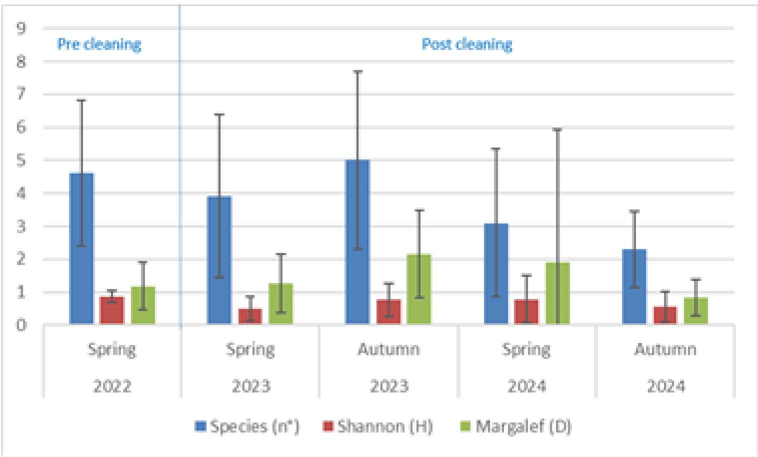

SPECIES	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s.	n.s.	n.s.	n.s.
Spr-23		n.s.	n.s.	n.s.
Aut-23			n.s.	n.s.
Spr-24				n.s.

SHANNON	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s.	n.s.	n.s.	n.s.
Spr-23		n.s.	n.s.	n.s.
Aut-23			n.s.	n.s.
Spr-24				n.s.

MARGALEF	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s.	n.s.	*	n.s.
Spr-23		n.s.	n.s.	n.s.
Aut-23			**	n.s.
Spr-24				n.s.

*Figure 13 - Diversity indexes (mean±St.Dev) calculated on replicates of fish samples collected in the lagoon site and related statistical comparisons (Kruskall Wallis test: n.s= not significant; *=ρ<0.05; **=ρ<0.001;***=ρ<0.0001).*

No specific trends were evident in Shannon and Margalef indices before and after the cleaning campaigns. Shannon index ranged from 0,49±0,36 to 0,86±0,18, and no statistical differences were evidenced among samples. Margalef index ranged from 0,84±0,56 and 2,15±1 and generally no significative differences were present between pre-cleaning and post cleaning campaign, except for Spring 2024 sample which resulted significantly different. For both diversity indices seasonal fluctuations were present, mainly linked to environmental variability.

The values of diversity index calculated on cumulative data obtained by all stations are shown in Table 6.

Table 6 - Cumulative number of species, Shannon and Margalef indexes for fish community collected in lagoon area before and after the cleaning operations.

LAGOON AREA	Spring 2022 pre cleaning	Spring 2023 1° post cleaning	Autumn 2023 2° post cleaning	Spring 2024 3° post cleaning	Autumn 2024 4° post cleaning
Species (n°)	13	12	17	11	13

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Shannon (H')	0.995	0.64	1.17	1.83	2.05
Margalef (D)	1.82	1.73	2.53	2.37	2.89

The number of species did not show marked differences before and after cleaning operations. Nevertheless, lagoon fish community showed an increasing trend in Shannon and Margalef indexes. In particular, the values of Shannon index, ranging from 0.64 to 2.05, resulted within the range of variation determined by Franco and co-authors for the fish community sampled in different shallow water habitats of the Venice lagoon in 2002, using a seine net of 10-m length having a 2 m drop and a knot-to-knot mesh size of 2 mm (Franco et al., 2006).

5.1.1.4 Microplastics

Microplastics were evaluated before and after the cleaning operations in different environmental matrices both at the Lagoon site, i.e. Sacca Fisola. and at sea in the coastal site, i.e. the abandoned mussel farm. Sampling campaigns at Sacca Fisola are shown in the Table 7, below:

Table 7 - Sampling campaigns conducted in Sacca Fisola area. -

Lagoon site - MP campaigns	
February /March 2022	Pre-cleaning
September 2022	Cleaning campaigns
November 2022	1° post cleaning (only for MPs)
March 2023	2° post cleaning
May 2023	2° Cleaning campaign
October 2023	3° post cleaning
March 2024	4° post cleaning
August 2024	5° post cleaning

Microplastics were analysed in different environmental matrices:

- Surface waters: samples collected with the Manta trawl; 3 replicates performed in each sampling campaign.
- Sediments: samples collected with the Van Veen grab; 5 replicates performed in each sampling campaign;
- Bivalves: MPs analysed in filter feeder bivalves, according to their presence at the sampling sites 3 species were used: the mussel *Mytilus galloprovincialis*,

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and the oysters *Magallana gigas* and *Ostreola stentina;* analyses performed in 10 – 20 individuals on the soft tissues.

- Fish: organisms collected by professional fishers using traps. The number of individuals analysed for each sample varied according to the catchment; analyses performed on the gastrointestinal tract of each individual.

The detailed methods used for the collection, extraction and quantification of microplastics in the different environmental matrices are reported in the D2.3 and D2.4.

To compare the MP abundance among sampling campaigns, before and after the cleaning operations, significant differences were assessed by linear mixed models with sampling replay during each campaign (for manta and sediment) or single individual (for bivalve and fish) as the random factor, followed by the Tukey post-hoc correction using the R programme (R Core Team, 2020). For all statistical analyses, the significance level was set at $p < 0.05$.

Results on the presence of microplastics in the environmental matrixes (water, sediments, bivalves and fish) before and after cleaning operations are illustrated in the following figures (from Figure 14 to Figure 17).

With regards to microplastics in water, comparing the results obtained for the floating microplastics before and after the cleaning operations, the statistical analysis does not reveal significant different between samples. Only a slight decrease was observed after macro-litter removal from November 2022 to March 2023. Floating microplastics are strongly influenced not only by the presence of specific sources, but also by the hydrodynamic conditions of the site, consequently their presence could be highly variable, and not directly correlated to the cleaning operations performed at the Sacca Fisola channel (Figure 14).

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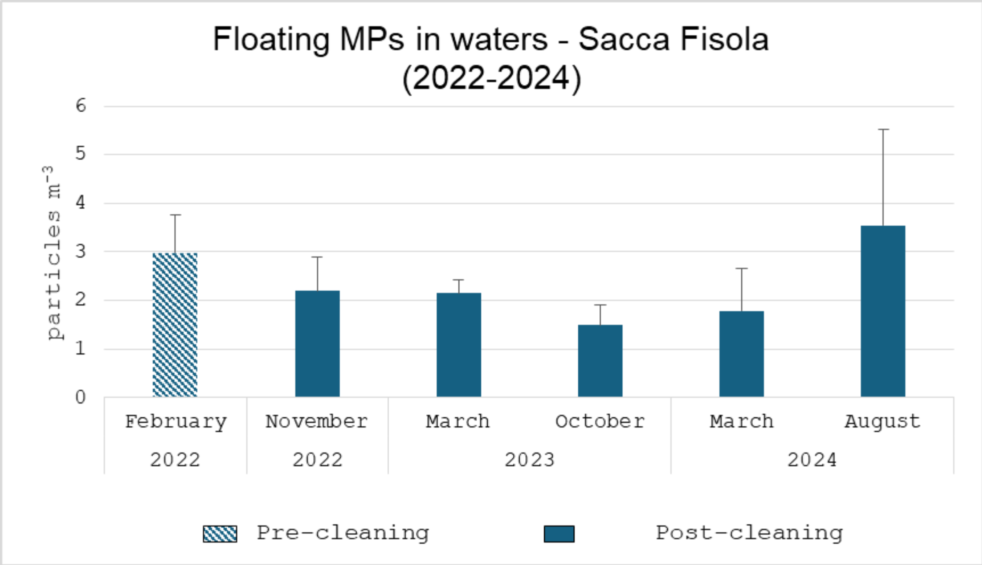

Figure 14 - Floating microlitter items detected in surface waters in Sacca Fisola, before and after cleaning operations. Mean ± s.e., N=3. Tukey post-hoc: $p>0.05$.

Microplastics analysed in sediments showed **a marked decrease comparing the data obtained before and after the cleaning operations** (Figure 15). In particular, the sample performed in February 2022 was significantly different in comparison to all the samples of the post-cleaning campaigns ($p<0.001$). Sediments, compared to waters, are considered a more suitable and conservative matrix to perform long-term monitoring studies useful for potential contamination detection (Ausili et al., 2020). Contaminants tend to accumulate on and into marine sediments through adsorption phenomena and / or due to sedimentations depending on various factors (e.g. sediments texture and particle size, mineralogical composition, hydrodynamic conditions). Microplastics in sediments markedly decreased after the cleaning operations. The observed microplastics were almost exclusively composed of secondary microplastics, thus deriving from the fragmentation of larger items. The decrease of their concentrations in the sediments after the cleaning operations could be related to the fact that their main source, i.e. the macro-litter on the sea-bottom of the area, was removed. However, during cleaning operations sediments were partially resuspended, and consequently microplastics deposited in sediment surface could have been resuspended as well into the water column, leading to the decrease in concentration.

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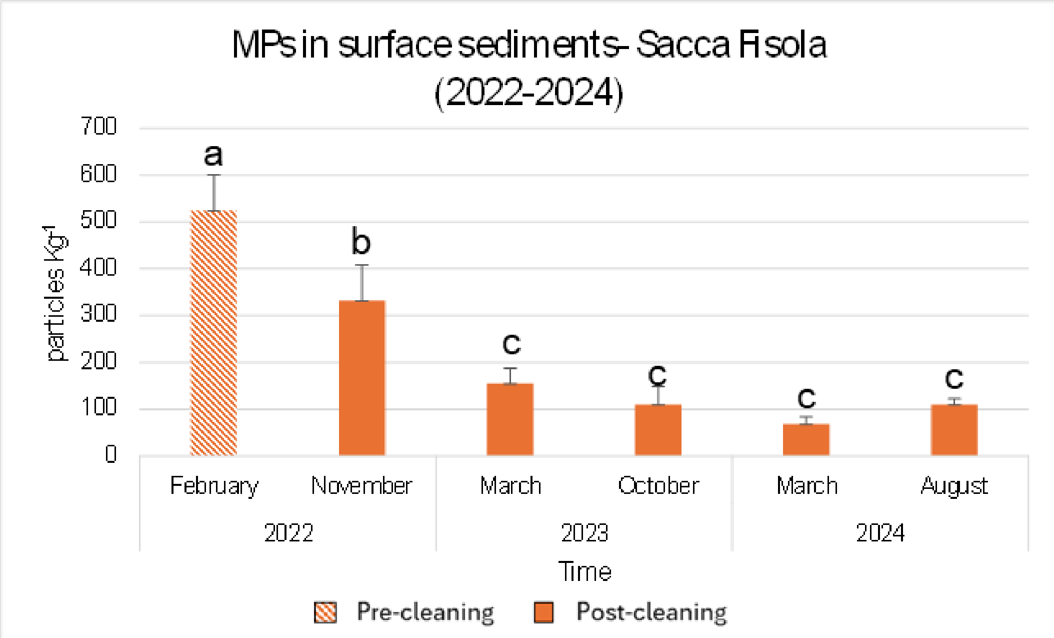

Figure 15 - Microlitter items detected in surface sediments in Sacca Fisola, before and after cleaning operations. Mean ± s.e., N=5. T Different letters indicate statistically significant differences between samples (ρ < 0.05).

Together with sediments, **aquatic biota** is an important matrix to study contamination occurrence in the marine environment, providing specific information on pollutant bioaccumulation processes through different trophic level (Gao et al., 2021). Similarly to what observed in sediments, **microplastics analysed in the soft tissues of filter feeder bivalves showed a marked decrease comparing the data obtained before and after the cleaning operations** (Figure 16). Microplastics detected in bivalve soft tissues in February 2022 (pre-cleaning) was always significantly higher in comparison to those observed in the other sampling campaigns (ρ<0.001).

Microplastics accumulated in the gastrointestinal tract of fish exhibited a significant decrease in concentrations comparing the sample collected in February 2022 to those performed in March 2023, October 2023 and March 2024 (ρ<0.01), but not with the last one performed in August 2024.

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Figure 16 - Microlitter items detected in filter feeder bivalves (M. galloprovincialis, M. gigas, O. stentina) n Sacca Fisola, before and after cleaning operations. Mean ± s.e., N=10 / 20. T Different letters indicate statistically significant differences between samples (p < 0.05).

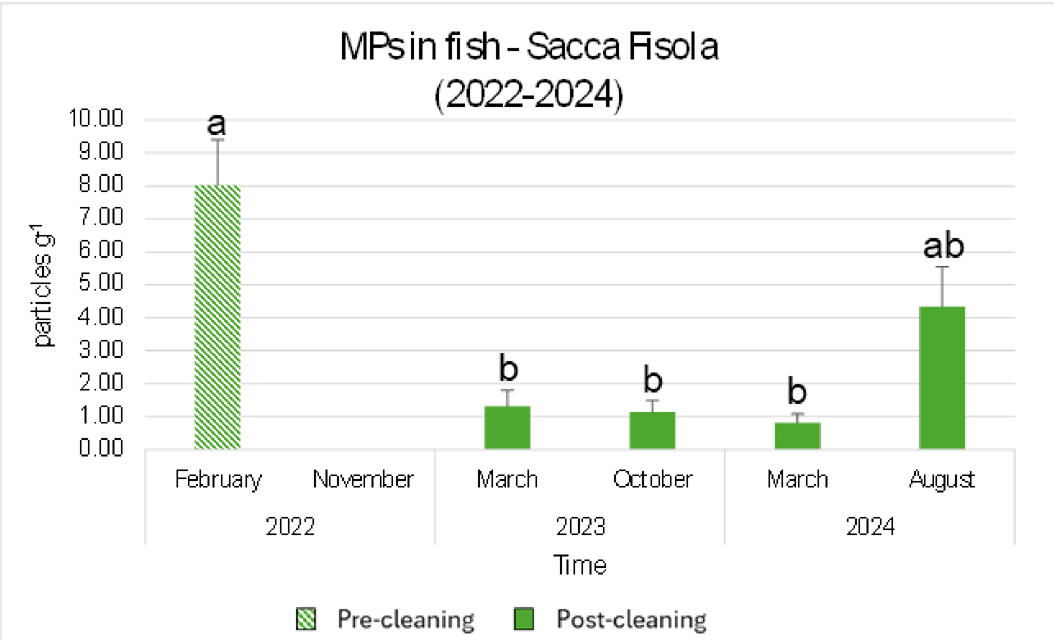

Figure 17 - Microlitter items detected in fish in Sacca Fisola, before and after cleaning operations. Mean ± s.e., Different letters indicate statistically significant differences between samples (p < 0.05).

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5.1.2 Coastal site – abandoned mussel farm

5.1.2.1 Removal of sea bed litter

Bathymetry carried out at the cleaned area in the abandoned mussel farm between 2022 and 2023 shows a significant reduction in the amount of litter on the seabed before and after the cleaning campaign.

The cleaning operations carried out in the abandoned mussel farm yielded significant results, both in terms of the length of ropes removed and the total number of objects successfully eliminated (Figure 18).

The operations on 6 and 7 June 2023 removed waste deriving from mussel harvesting activities, ropes and buoys. On 6 June, the first operation successfully removed an entire rope, while the second operation involved cutting and extracting another rope to ensure its complete removal. On 7 June, the first operation removed a single rope, while the second focused on an assembly of ropes and a 125 x 61 cm buoy. With the help of the Venice State Police Diving Unit, it was also possible to remove two other buoys floating in the water column, identified thanks to the MBES.

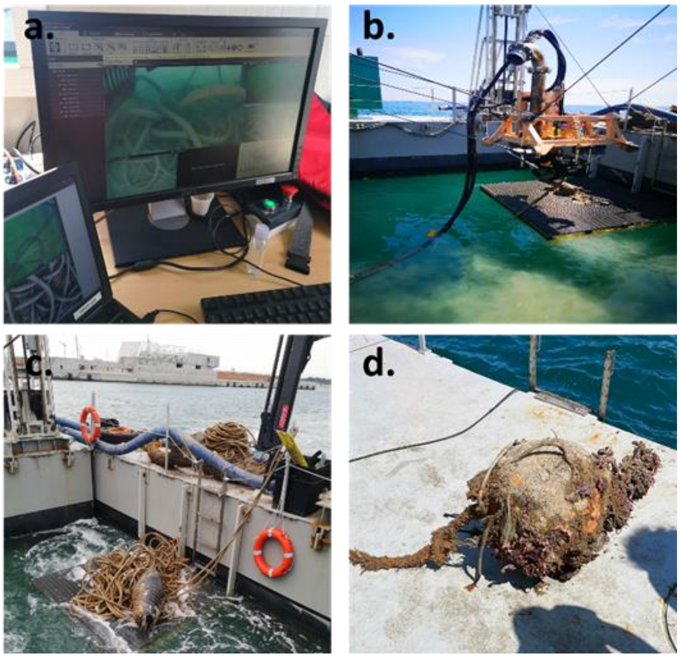

Figure 18 - *Images of the mussel farm clean-up using the robotic seabed cleaning platform: a. Real-time monitoring of the seabed with identification of debris such as ropes and nets; b. Use of the robotic platform to recover debris from the seabed; c. Recovery of ropes and aquaculture equipment, including a float; d. A removed plastic buoy colonized by biological communities (fouling communities) that were sampled for further taxonomic analyses.*

Although four ropes were removed, the post-cleaning MBES mapping showed the presence of three ropes instead of the expected two (Figure 19). This discrepancy can

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be explained by the fact that one of the ropes was partially cut off during removal, leaving a small part on the seafloor. Furthermore, although there are four removal points corresponding to the harvested tops, their positions on the map do not coincide with the original ones. This is due to the fact that the divers bundled the ropes together during the operations to facilitate their removal from the platform.

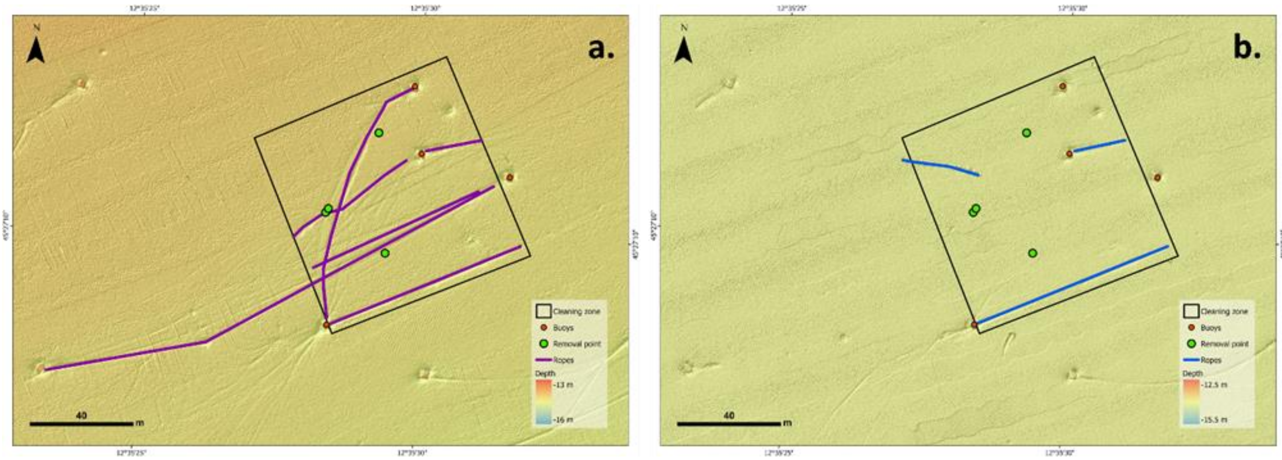

Figure 19 - *The images show the mapped objects on the seafloor (purple and blue lines for ropes and red points of buoys in in the water column): a) before and b) after the cleaning and removal of marine litter in the specific cleaning area (black box) at the abandoned Mussel Farm.*

Within the sub-area where the seabed cleaning platform operated, the **ropes** present on the seafloor were reduced from an initial length of 404 metres in 2022 to 126 metres in 2023, representing a removal rate of 69%. However, an additional rope segment was removed outside the cleaning area. Thus, the total initial rope length interested by the cleaning was of 514 metres, which was reduced to 126 metres, resulting in an **effective removal rate of 75%**. These lengths were calculated by integrating the segments obtained from the bathymetric data with the length calculation tools in ArcGIS Pro.

In terms of **object** removal, the total number of items decreased from 9 (3 buoys and 6 ropes) to 3 ropes, as all buoys were successfully removed. This equates to an **overall removal rate of 67%**, leaving only 33% of the original objects in the area. These results highlight the effectiveness of the clean-up operations and underscore the importance of targeted interventions in addressing marine debris accumulation.

Chemical analysis of the collected ropes identified the material as polyamide 6 (PA6), also known as nylon. This semi-crystalline polymer is robust, has excellent abrasion and chemical resistance, and a density of 1.14 g/cm^3. The total weight of the collected ropes was 260 kg (±10 kg).

Bathymetric data analysis further revealed that the total length of ropes within, and in part outside, the operational area was 514 metres. Assuming a diameter of 2.5 cm,

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the estimated mass of the ropes based on the MBES data corresponded to approximately 287 kg of polyamide 6, aligning closely with the measured weight of the collected ropes. These results demonstrate that multibeam data are not only effective for accurately georeferencing objects on the seabed but also valuable for estimating their physical characteristics, such as weight and size.

5.1.2.2 Soft bottom macrozoobenthos community

The soft bottom macrozoobenthic community was characterized on stations CS1 (on the planned cleaning area, discontinued after the 2nd campaign), CS4 (on the realized cleaning area, since 3rd campaign) and CS2 (control) at the coastal site (abandoned mussel farm) before and after the seabed cleaning operations according to the calendar shown in Table 8.

Table 8 - Soft bottom macrozoobenthos monitoring campaigns in the coastal site.

Coastal site soft bottom macrozoobenthos campaigns		
C1	03/03/2022	1st pre-cleaning
C2	18/04/2023	2nd pre-cleaning
C3	29/09/2023	1st post-cleaning
C4	03/04/2024	2nd post-cleaning
C5	16/09/2024	3rd post-cleaning

Samples were collected with a 0.1 m^2 Van Veen grab (three replicates for each station) and sieved at 1 mm mesh size. All retained specimens were identified at the level of species or the highest achievable taxonomic resolution. More details on the protocols and first results are presented in Deliverables 2.3 and 2.4.

Community structure was described by main univariate ecological descriptors as well as multivariate analyses. Selected univariate descriptors include abundance, richness, diversity, biotic and multimetric indices, as listed hereafter:

- density, Nm^{-2}
- species richness, S
- Shannon index, H'
- AMBI biotic index (Borja et al., 2000)
- M-AMBI multimetric index (Bald et al., 2005; Muxika et al., 2007)

The changes of the univariate descriptors over the five monitoring campaigns have been represented by mean values (+/- standard deviation) in Figure 20.

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Due to the introduction of a new station (CS4) in autumn 2023 to replace the discontinued CS1 station following the relocation of the cleaning area, a two-way ANOVA has been performed only on the last three monitoring campaign, with campaigns (three levels) and stations (two levels) as fixed crossed factors (models were checked for homogeneity of variance with Tukey-Anscombe plot and for normality of Residuals with Residual Q-Q plot). A summary of p-values is presented in Table 9 for both factors as well as interactions.

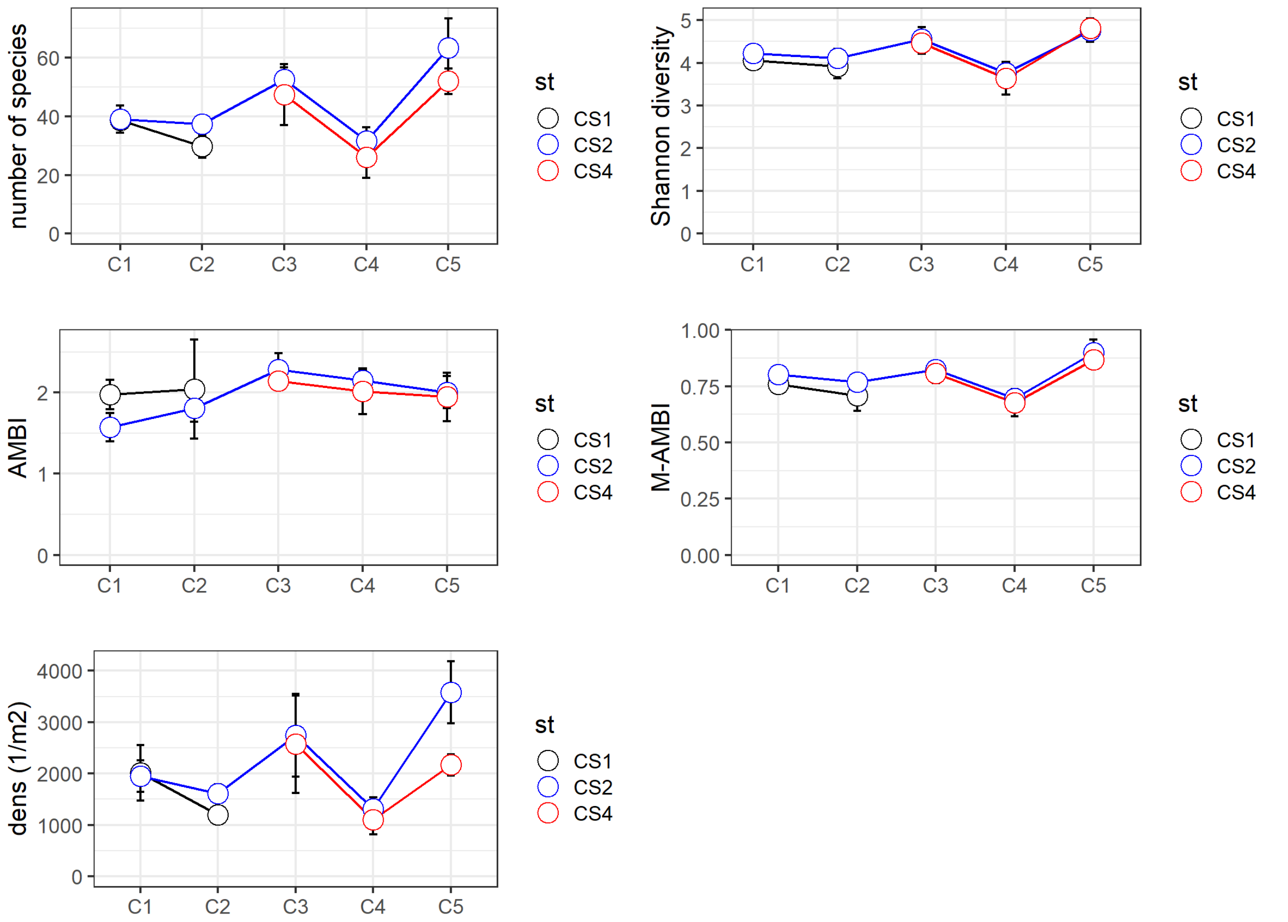

Figure 20 - Coastal site: changes of the univariate descriptors of macrozoobenthic community over the five monitoring campaigns in the cleaning area (CS1-CS4) and in the control area (CS2).

*Table 9 - Coastal site: statistical significance of changes taking into accounts the monitoring campaigns C3-C4-C5 (top) and between campaigns (bottom) for each univariate descriptor (see text) calculated by two-ways ANOVA (crossed factors: campaign and station); statistical significance codes: *** = 0-0.001, ** = 0.001-0.01, * = 0.01-0.05, ° = 0.1-1*

C3-C4-C5	S	H'	BC	M-AMBI	dens. (1/m^2)
campaign	0.00005***	0.00004***	0.20089	0.00003***	0.00070***

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station	0.04942*	0.65769	0.29490	0.29474	0.05202°
campaign:station	0.72759	0.83632	0.93392	0.94418	0.15696
C3-C4	S	H'	BC	M-AMBI	dens. (1/m^2)
campaign	0.00076***	0.00127**	0.28420	0.00089***	0.00473**
station	0.20853	0.52356	0.25172	0.48655	0.62485
campaign:station	0.96795	0.94155	0.96731	0.94415	0.96552
C4-C5	S	H'	BC	M-AMBI	dens. (1/m2)
campaign	0.00009***	0.00012***	0.44435	0.0001***	0.00005***
station	0.06441°	0.81879	0.50579	0.36136	0.00504**
campaign:station	0.49520	0.59619	0.79232	0.81709	0.02140*

Both in case of the overall test C3-C4-C4 and the couples C3-C4 and C4-C5, for all the parameters (with the exception of AMBI-BC, with no significant values at all) **there are significant differences between campaigns, but not between stations** (only *density* shows significant difference between stations).

The performed multivariate analyses of community structure have been described in the previous section. of replicates has been explored through well-established methods. Agglomerative hierarchical clustering, based on Bray-Curtis similarity index and group average linkage, is represented by dendrogram in Figure 21. **Two main clusters distinctly identify the stations sampled in the two seasons, spring and autumn, indicating seasonality as prevailing over spatial variation in structuring the communities at the observed spatial scale in the marine environment. At a lower level of dissimilarity, stations cluster together by campaign.**

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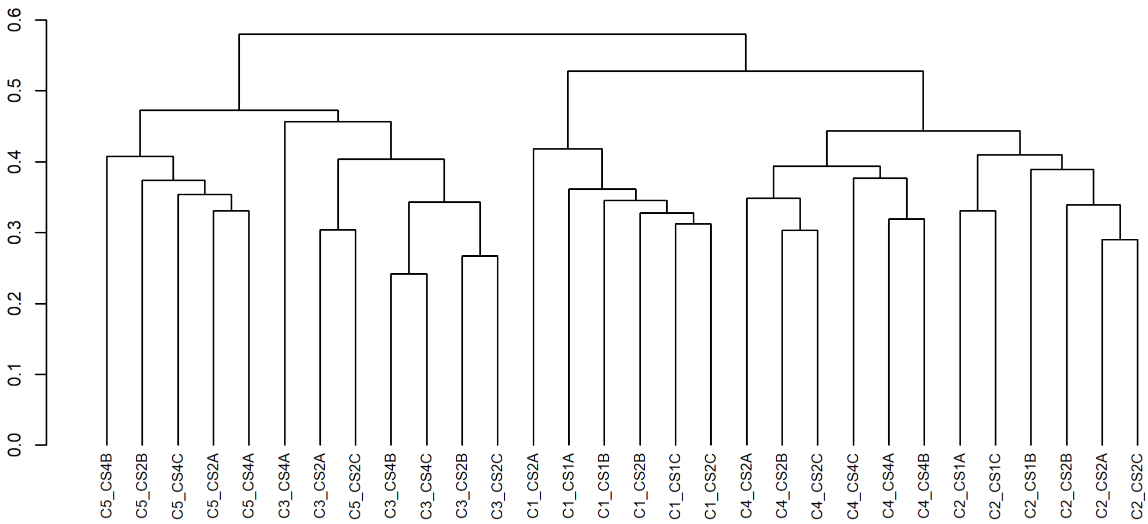

Figure 21 - Lagoon site: dendrogram of agglomerative hierarchical clustering (based on Bray-Curtis similarity index) of macrozoobenthic community data (replicates).

NMDS ordination is presented in Figure 22, which presents the same overall patterns highlighted by cluster analysis, with **clusters corresponding to campaigns, i.e. changes over time, with no clear spatial pattern.**

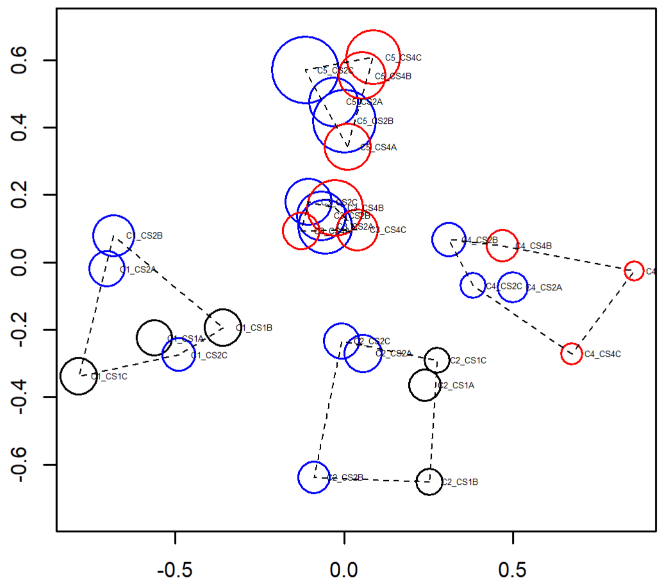

Figure 22 - Lagoon site: NMDS ordination of macrozoobenthic community data (replicates; stress: 0.229); red: cleaning area station LV1; blue: control station LV2; point size is proportional to the number of species; envelopes encompassing each campaign has also been represented.

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To test differences in multivariate community composition between stations, also in this case ANOSIM (as two separate analyses) and permutational multivariate ANOVA (2-ways analysis with sampling stations (2 levels) and campaigns (3 levels: C3-C4-C5) as crossed fixed factors), have been applied. The results of both non-parametric multivariate tests are summarized in Table 10.

The results clearly indicate **a significant difference between campaigns, with no difference between stations**. A further overall one-way multivariate ANOVA on all the monitoring campaigns of CS2 with season as fixed factor indicates a clear seasonality (R^2 = 0.32002, p-value = 0.001).

*Table 10 - Coastal site: results of multivariate non-parametric tests ANOSIM and Permutational multivariate ANOVA on untransformed macrozoobenthos community matrix for monitoring campaigns C3-C4-C5, with campaigns and stations as factors; statistical significance codes: *** = 0-0.001, ** = 0.001-0.01, * = 0.01-0.05, ° = 0.1-1*

	ANOSIM		Permutational multivariate ANOVA	
	R	significance	R^2	p-value
station	-0.0058	0.401	0.06611	0.061°
campaign	0.8529	0.001	0.48497	0.001***
station:campaign	-	-	0.07717	0.241

In conclusion, the **analyses of univariate and multivariate structure of macrozoobenthos community in the coastal site shows the absence of any significant difference between cleaning area station and control station since the first monitoring campaign after the cleaning procedure.**

As done in Sacca Fisola, colonizing the removed marine litter, highlighting its possible role in increasing biodiversity (Deliverable 2.4), which must be considered during preliminary assessments of clean-up actions.

5.1.2.3 Fish community

Fish fauna sampling activities were carried at the coastal site (mussel farm) before and after the seabed cleaning operations according to the calendar shown in

Table *11*. Considering that in the marine coastal area the cleaning operations were carried out by the Robotic Seabed Cleaning Platform in June 2023, the fish fauna assessment was carried out through the organization of two pre-cleaning monitoring campaigns which took place in February 2022 and March 2023, followed by three post cleaning campaigns (in December 2023 and in April and September 2024).

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Table 11 - Calendar of fish fauna campaigns in marine coastal area.

Coastal site fish fauna campaigns	
22-23 February 2022	1°pre cleaning
29-30 March 2023	2° pre cleaning
5-6 December 2023	1° post cleaning
4-5 April 2024	2° post cleaning
22-23 September	3° post cleaning

Fish fauna sampling activities were carried out in collaboration with professional fishermen, according to the procedure described in D2.3 and D2.4.

Briefly, in the coastal site different types of nets were employed in order to sample a wide range of ecological and size fish guilds. In particular, four sets of similar nets, each one consisting of a small trap and three 50 meters long nets (32 mm trammel net; 40 mm gillnet; 50 mm gillnet, for a total of 150 m of net per station) were displaced for 1 day in 4 different stations within the sampling area.

Abundance and biomass data were standardised in terms of catch per unit effort (CPUE), i.e. number of individuals captured by 150 m net in one fishing day or grams of fish captured by 150 m net in one fishing day. Mean values (and standard deviations) of CPUE per species were then calculated.

The abundance and biomass data from each campaign were compared by means of boxplots in order to analyse the dataset collected during the sampling campaigns and to identify any changes in the fish community structure because of the cleaning interventions. For each sampling campaign, the stations were treated as replicates.

Abundance and biomass data related to each replicate were used to calculate a set of biodiversity indexes (species richness, Margalef index and Shannon index).

Data were compared in order to evidence possible difference among samples collected before and after cleaning operations using the non-parametric Kruskall-wallis test.

To provide cumulative data on fish community, the diversity indexes were also calculated on the whole data set.

Fish community observed at the marine-coastal site showed a significant increase in total abundance and biomass during the monitoring period, although seasonal differences were evident (Figure 23). In particular, the highest values of abundance

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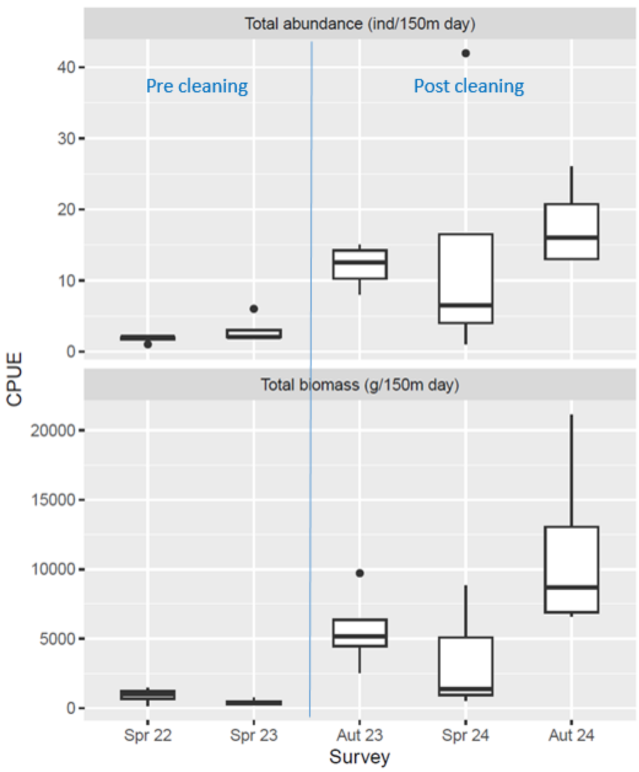
and biomass are observed during the autumn seasons following the project interventions (autumn 2023 and 2024).

Before cleaning operations, the standardized number of organisms per catch unit ranged from 1.75±0.50 (Spring 2022) to 3±2 (Spring 2023). The values were significantly higher after cleaning operations ranging from 12±3.16 (Aut 2023) to 17.75±6.18 (Aut 2024).

Similarly, the **biomass values resulted significantly lower in Spring 2022 and 2023,** ranging from 391±232,7 g to 900,5±544,9 g i**n comparison with the biomass values registered after the cleaning operations**, ranging from 2664,5±4125,6 g (Spr 2024) to 11264,7±6796,7 g (Aut 2024).



ABUNDANCE	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s	*	n.s.	**
Spr-23		n.s	n.s.	*
Aut-23			n.s.	n.s.
Spr-24				n.s.

BIOMASS	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s	n.s.	n.s.	*
Spr-23		*	n.s.	**
Aut-23			n.s.	n.s.
Spr-24				*

*Figure 23 - Boxplot of abundance and biomass calculated on replicates of fish community collected in the marine site and related statistical comparisons (Kruskall Wallis test: n.s= not significant; *=p<0.05; **=p<0.001)*

From autumn 2023 onwards, fish community of the coastal area resulted better structured and characterised by higher abundance and biomass' levels of species such as *Diplodus sargus*, *Mustelus mustelus*, *Solea solea, Sciaena umbra, Trachurus trachurus* and *Umbrina cirrhosa* when compared to the previous period.

Data related to biodiversity indexes calculated for each replicate are shown in Figure 24.

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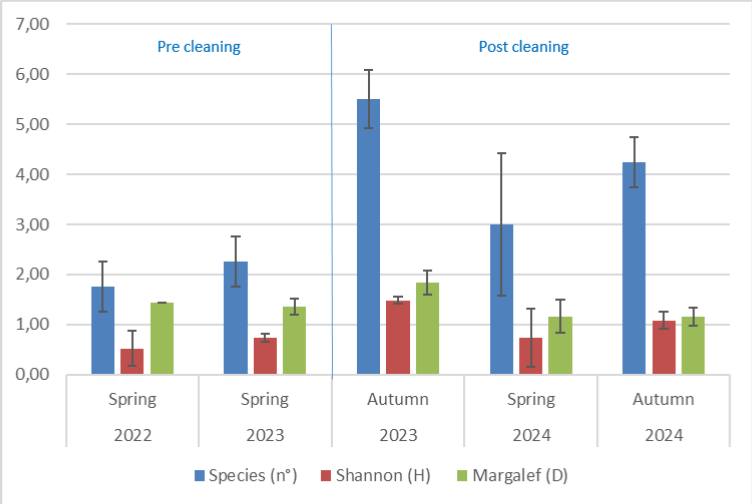

SPECIES	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s	***	n.s.	*
Spr-23		**	n.s.	n.s.
Aut-23			*	n.s.
Spr-24				n.s.

SHANNON	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s.	n.s.	n.s.	n.s.
Spr-23		n.s.	n.s.	n.s.
Aut-23			n.s.	n.s.
Spr-24				n.s.

MARGALEF	Spr-23	Aut-23	Spr-24	Aut-24
Spr-22	n.s.	n.s.	n.s.	n.s.
Spr-23		n.s.	n.s.	n.s.
Aut-23			n.s.	n.s.
Spr-24				n.s.

*Figure 24 - Diversity indexes (mean±St.Dev) calculated on replicates of fish samples collected in the marine site and related statistical comparisons (Kruskall Wallis test: n.s= not significant; *=ρ<0.05; **=ρ<0.001;***=ρ<0.0001).*

The number of species resulted significantly higher in samples collected after the cleaning operations especially in autumn 2023 and 2024. The number of species ranged from 1.75±0.5 to 2.25±0.50 in pre cleaning campaigns and from 4.25±0.50 to 5.50±0.58 during the post cleaning monitoring. **No specific trends were evident in Shannon and Margalef indices before and after the cleaning campaigns.** Shannon index ranged from 0.52±0.35 to 1.48±0.07 whereas Margalef index ranged from 1.15±0.18 and 1.84±0.24. For both indexes no statistical differences were evidenced among samples. Notwithstanding, seasonal fluctuations were present, mainly linked to environmental variability.

The values of diversity index calculated on cumulative data obtained by all stations are shown in Table 12.

Table 12 - Cumulative number of species, Shannon and Margalef indexes for fish community collected in marine coastal area before and after the cleaning operations.

COASTAL AREA	Spring 2022 1° pre cleaning	Spring 2023 2° pre cleaning	Autumn 2023 1° post cleaning	Spring 2024 2° post cleaning	Autumn 2024 3° post cleaning
Species (n°)	4	6	8	7	8
Shannon (H')	1,25	1,62	1,72	1,18	1,34
Margalef (D)	1,54	2,41	1,81	3,41	1,64

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In general, the number of species increased over time, ranging from 4 to 6 in pre-cleaning campaigns and from 7 to 8 during the post cleaning monitoring. No specific trends were evident in Shannon and Margalef indices before and after the cleaning operations, ranging from 1.18 to 1.72 and from 1.54 and 3.41, respectively.

5.1.2.4 Microplastics

At the coastal site, i.e. the abandoned mussel farm, sampling campaigns were performed as shown below in the Table X:

Table 13 - Sampling campaigns conducted in Sacca Fisola area.

Marine site - MP campaigns	
February /March 2022	1° pre cleaning
March 2023	2° pre cleaning
May 2023	Cleaning campai
October 2023	1° post cleaning
March 2024	2° post cleaning
August 2024	3° post cleaning

Microplastics were analysed in different environmental matrices, similarly to Sacca Fisola:

- Surface waters: samples collected with the Manta trawl; 3 replicates performed in each sampling campaign.
- Sediments: samples collected with the Van Veen grab; 5 replicates performed in each sampling campaign;
- Bivalves: MPs analysed in the mussel *M. galloprovincialis*; analyses performed in 10 – 20 individuals on the soft tissues.
- Fish: organisms collected by professional fishers using traps. The number of individuals analysed for each sample varied according to the catchment; analyses performed on the gastrointestinal tract of each individual.

The detailed methods used for the collection, extraction and quantification of microplasticts in the different environmental matrices are reported in the D2.3 and D2.4.

To compare the MP abundance among sampling campaigns, before and after the cleaning operations, significant differences were assessed by linear mixed models

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with sampling replay during each campaign (for manta and sediment) or single individual (for bivalve and fish) as the random factor, followed by the Tukey post-hoc correction using the R programme (R Core Team, 2020). For all statistical analyses, the significance level was set at $p < 0.05$.

The concentrations of microplastics in the different environmental matrices before and after the removal of litter from the seabed are illustrated in the graphs below (from Figure 25 to Figure 28).

Floating microplastics showed a decreasing trend comparing samples before and after cleaning operations, even if no significant differences were observed in the statistical analyses.

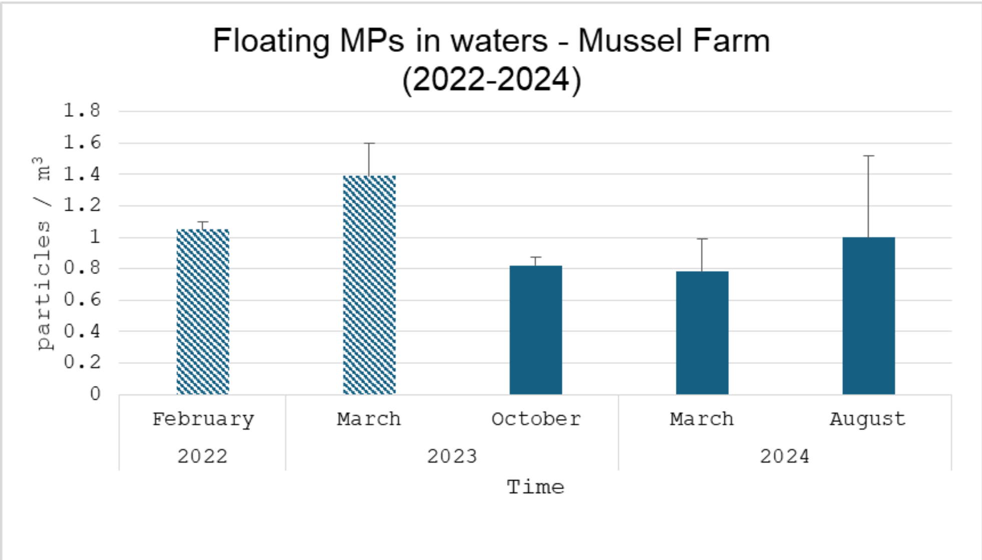

Figure 25 - Floating microlitter items detected in surface waters in the abandoned mussel farm area, before and after cleaning operations. Mean ± s.e., N=3. Tukey post-hoc: n.s. $p>0.05$.

As for sediments, **a significant decrease in microplastics was detected comparing the first pre-cleaning campaigns in comparison to all the other sampling campaigns, including the second pre-cleaning campaign** (Figure 26). This partially confirms the results obtained also at Sacca Fisola, sediments being a more conservative matrix for the assessment of contamination in coastal environments.

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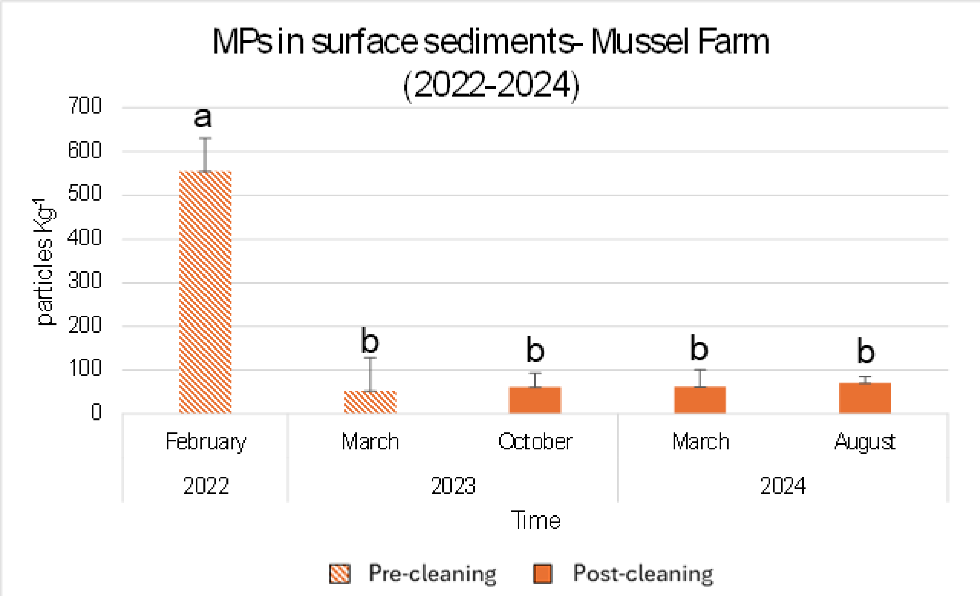

Figure 26 - Microlitter items detected in surface sediments in the abandoned mussel farm area, before and after cleaning operations. Mean ± s.e., N=5. Different letters indicate statistically significant differences between samples ($p < 0.05$).

The evaluation of microplastics in the biota did not show clear trends over time both for mussels and fish (Figure 27 and Figure 28). No differences were observed in the statistical comparisons between samples collected before and after the cleaning operations.

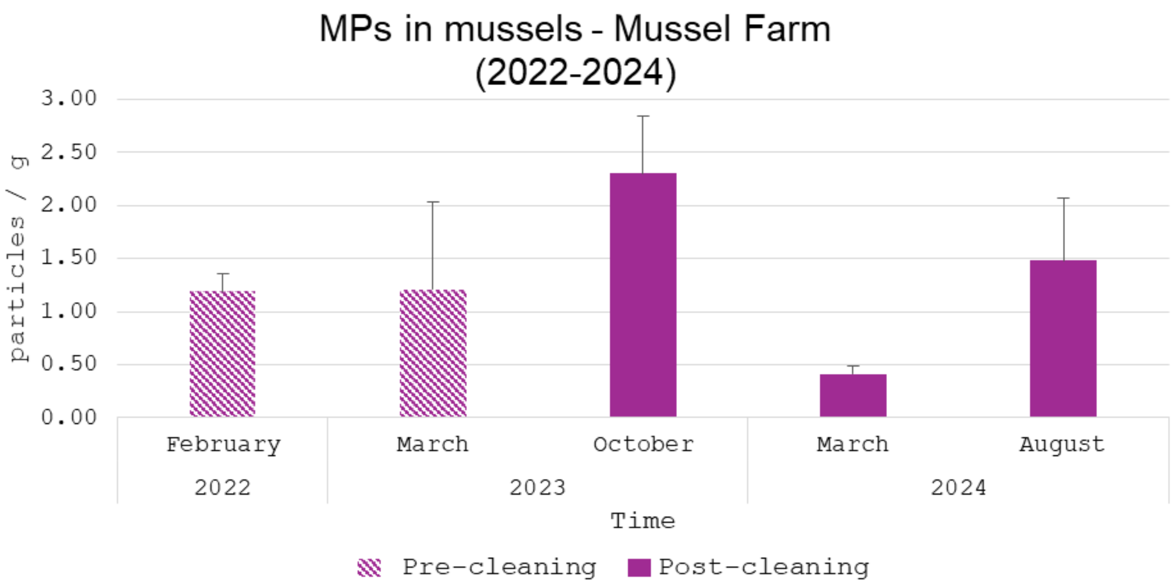

Figure 27 Microlitter items detected in mussels in the abandoned, before and after cleaning operations. Mean ± s.e., N=10 / 20 Tukey post-hoc: n.s. $p>0.05$.

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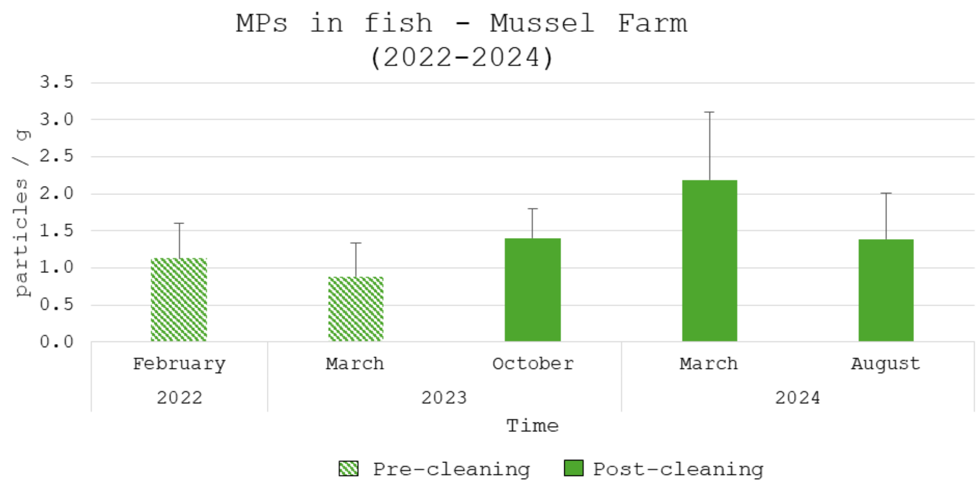

Figure 28 - Microlitter items detected in fish in the abandoned mussel far area, before and after cleaning operations. Mean ± s.e., Tukey post-hoc: $p>0.05$.

5.1.3 Integrated assessment

The Seabed Cleaning Platform based on cable robotics, developed and installed in Venice was tested at the two pilot areas in Venice: Sacca Fisola, close to the city of Venice and an abandoned mussel farm at sea. The scope was to put at hard test and therefore improve the Robotic Seabed Cleaning Platform in the attempt to remove historically accumulated litter.

The Seabed Cleaning Platform was successfully tested in both the lagoon and the open sea coastal environments. The lagoon was a particularly challenging environment given the poor visibility and currents in the lagoon channels. Poor visibility made the removal in the Sacca Fisola area more difficult, and the identification of the ML items was time consuming. The cable robot had to rely mostly on bathymetry data collected before the cleaning during the sea-floor litter monitoring campaigns performed with the multibeam. Once identified the ML item, though, the time to approach the target was very fast and the removal took just few minutes. Even in these extreme conditions, the Seabed Cleaning Platform managed to clean 59% of the items. At the abandoned mussel farm the Seabed Cleaning Platform was able to remove 75%.

The system was demonstrated to be quick and efficient, to be able to operate up to 20 m of depth and to collect items up to 130kg, having a real time control on the deck of all the operations of the cable robot. It was demonstrated that the Seabed

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Cleaning Platform efficiently and selectively operated, collecting more than 2200 kg of seafloor litter.

To measure and evaluate the possible impacts of the Seabed Cleaning Platform on the lagoon and marine ecosystems, various indices were analysed in different environmental components. Generally, aquatic environment quality is monitored trough analysis on physical–chemical and biological parameters as well as contaminant detection, also fulfilling the Water Framework Directive (Directive 2000/60/EC) and Marine Strategy Framework Directive (Directive) 2008/56/EC, which require continuous monitoring of different marine ecosystem descriptors to evaluate their quality and environmental status (Ferraro et al., 2022).

In the framework of the MAELSTROM project, a similar approach was applied to evaluate the possible effects and impacts of the litter removal operations performed with the Robotic Seabed Cleaning Platform in the Venice coastal area. In particular, the concentrations of microplastics in different environmental matrices (water, sediment and biota), and the variation of macrozoobenthic and fish communities were chosen as descriptors.

The information obtained through the main descriptors were then integrated taking into account the statistical significance of results obtained comparing the pre-cleaning with each post-cleaning dataset reported in the previous sections of the Deliverable.

A table was then created where each descriptor is marked with green or red to indicate a statistically significant better or worse condition, respectively, between samples collected before and after the cleaning operations, while orange indicates no significant differences.

Moreover, to elucidate temporal variability among samples, multivariate analyses, Principal Component Analyses (PCA), were performed on a data matrix including the following parameters for both the lagoon site and the coastal site: microplastic concentrations in water, sediments, bivalves and fish; number of species, Shannon and Margalef indices to describe fish community; number of species, Shannon and AMBI indices to describe the macrozoobenthic community. PCAs were performed using STATISTICA 7.0 software package.

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The results of the statistical significance obtained comparing the pre-cleaning and each post-cleaning dataset are reported in Table 14 for Sacca Fisola, and Table 15 for the abandoned mussel farm.

At Sacca Fisola, the results highlighted that the environmental quality generally remain unchanged or improved comparing the pre-cleaning campaign and the post-cleaning campaigns. In particular, better conditions in the indices related to the macro-zoobenthic and fish communities were observed when comparing the pre-cleaning campaign with those performed after one year, i.e. from the 2° post-cleaning on. In the other cases, the condition is unchanged apart for the AMBI index of the macro-zoobenthos that when comparing the pre and 1° post-cleaning campaign highlighted a worst condition. Similar results were also obtained in the coastal site of the abandoned mussel farm, with some differences.

At the abandoned mussel farm, after the relocation of the cleaning area, a new station (CS4) within the cleaned area was introduced to replace the CS1 station, where the pre-cleaning campaigns were conducted. This change was supported by the evidence that in the whole area of the abandoned mussel farm the community is quite homogeneous, and there were no statistically significant differences among sampling stations and trends of the parameters associated with macro-zoobenthos community. This made possible to compare the pre- and post-cleaning data, despite the change of the sampling location. The data presented must be understood in terms of relative trend, generally within the range of variability of the benthic communities, and not of absolute quality values. The coastal site stations in particular show similar trends when comparing the pre- and post-cleaning campaigns trends, and when worst conditions were detected, a recover was observed over time.

These results evidence that the removal operations performed efficiently and selectively with the Seabed Cleaning Platform did not generally affect the biological community. Moreover, if a worst condition is detected, the community is able to recover the initial conditions over time.

Similar results were obtained in a previous study performed in the Northern Adriatic Sea, where the removal of litter (in particular derelict fishing gear) from the seabed lead to a positive impact on the healthy status of the studied rocky outcrop ecosystem. Litter and derelict fishing gear negatively affected the benthic and fish

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community, altering the microhabitat, occupying and covering part of the substrate and organisms (Moschino et al., 2019).

As for the microplastic contents in sediments and biota, at Sacca Fisola the comparison performed between the pre-cleaning and each post-cleaning campaign showed a significant decrease. A similar condition is also observed at the abandoned mussel farm, with sediments and fish being the matrices where higher decreases in MP concentrations were detected. It is known that large plastic items break into smaller particles, as a result of both physical and chemical changes, mainly as a consequence of mechanical breakdown caused by sand abrasion, wave action etc, accelerated by photochemical processes triggered by UV light. During these processes, large plastic debris gradually fragment into smaller pieces, ultimately leading to the production of micro- and nanoplastics (Corcoran et al. 2009; Shi et al., 2023). The removal of macro-plastic items from the seabed of the Demo sites could have led to the local decrease of microplastic concentrations in the main conservative matrices, since macro-plastic items lying on the seafloor for long time are one of the local and main sources of the smaller fragments. Also, for microplastics it was demonstrated that sediments, compared to marine water, might represent a more suitable matrix to perform long-term studies useful for potential contamination detection, as already observed for traditional contaminants.

Table 14 - Changes of the environmental status observed after the cleaning operations at Sacca Fisola, based on the statistical comparison performed between the pre-cleaning and each post-cleaning survey.

	MP water	MP sediment	MP bivalves	MP fish	N. Species Fish	Shannon Fish	Margalef Fish	N. Species Benthos	Shannon Benthos	AMBI Benthos	M-AMBI Benthos
Pre vs 1° post-cleaning	Yellow	Green	Green	Green	Yellow	Yellow	Yellow	Yellow	Yellow	Red	Yellow
Pre – vs 2° post-cleaning	Yellow	Green	Green	Green	Yellow	Yellow	Yellow	Green	Green	Yellow	Green
Pre vs 3° post-cleaning 2024	Yellow	Green	Green	Green	Yellow	Yellow	Green	Yellow	Green	Yellow	Yellow
Pre vs 4° post-cleaning 2024	Yellow	Green	Yellow	Green	Yellow	Yellow	Yellow	Green	Green	Yellow	Green

- Green: Better quality with respect to pre-cleaning
- Yellow: No changes with respect to pre cleaning
- Red: Worste quality with respect to pre cleaning

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Table 15 - Changes of the environmental status observed after the cleaning operations at the abandoned mussel farm, based on the statistical comparison performed between the pre-cleaning and each post-cleaning survey.

	MP water	MP sediment	MP bivalves	MP fish	N. Species Fish	Shannon Fish	Margalef Fish	N. Species Benthos	Shannon Benthos	AMBI Benthos	M-AMBI Benthos
1° Pre vs 2° pre-cleaning	Yellow	Green	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
1° Pre vs 1° post-cleaning	Yellow	Green	Yellow	Yellow	Green	Yellow	Yellow	Green	Yellow	Red	Yellow
1° Pre – vs 2° post-cleaning	Yellow	Green	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Red	Red	Red
1° Pre vs 3° post-cleaning	Yellow	Green	Yellow	Yellow	Green	Yellow	Yellow	Green	Green	Red	Green

Green: Better quality with respect to pre-cleaning
Yellow: No changes with respect to pre cleaning
Red: Worste quality with respect to pre cleaning

The PCAs were performed to highlight the relative distribution of the various samples with respect to pre-and post-cleaning campaigns. At Sacca Fisola (Figure 29), Factor 1 and Factor 2 explain over 70% of total variance in the data matrix. Factor 1 is characterised by positive loadings of the variables microplastics in sediments and biota and negative loading for Shannon Index of macro-zoobenthos. The distribution of the various samples highlights a clear-cut pattern and separation comparing the pre- and post-cleaning campaigns, the highest values of microplastic concentrations in sediments and biota and the lower Shannon index characterizing the samples collected during the pre-cleaning campaign.

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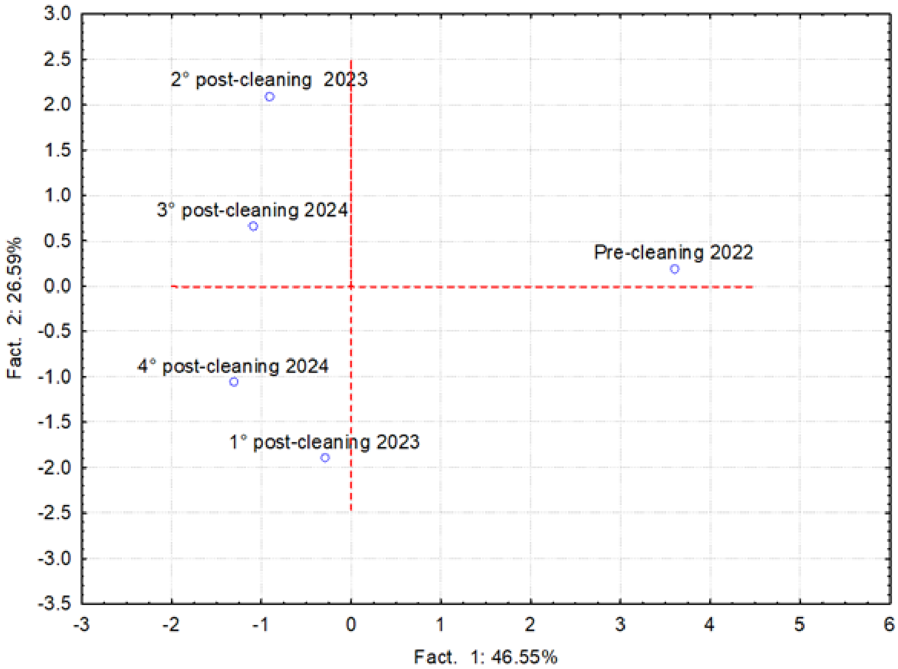

Figure 29 - Principal component analysis (PCA) for the dataset of Sacca Fisola based on average microplastic concentrations in water, sediments, bivalves and fish; number of species, Shannon and Margalef indices to describe fish community; number of species, Shannon and AMBI indices to describe the macrozoobenthic community.

At the abandoned mussel farm (Figure 30), Factor 1 and Factor 2 explain over 75% of total variance in the data matrix. Factor 1 is characterised by positive loadings of the variables microplastics in sediments, number of fish species, and number of species and Shannon index for the macro-zoobenthos. Factor 2 is characterized by the negative loading for microplastic in fish. The distribution of the various samples highlights a clear pattern and separation comparing the pre- and post-cleaning campaigns, the highest values of microplastic concentrations in sediments together with some of the fish and macrozoobenthic indices. This depends on the fact that sometimes the community indices showed higher values in the pre-cleaning campaigns, even if the differences were not statistical different with respect to the post-cleaning, as stress by the results reported in Table 15.

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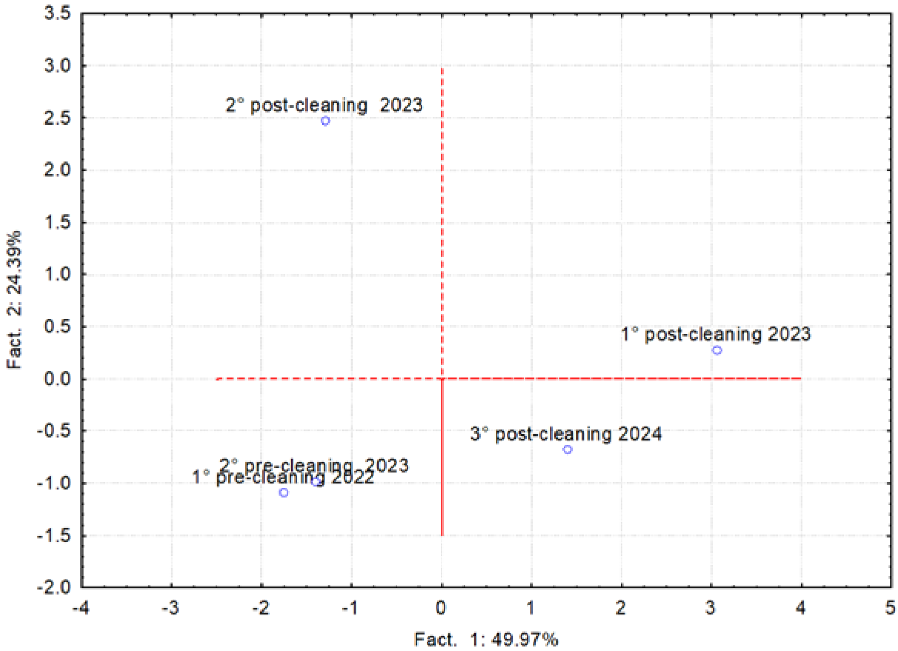

Figure 30 - Principal component analysis (PCA) for the dataset of the abandoned mussel farm based on average microplastic concentrations in water, sediments, bivalves and fish; number of species, Shannon and Margalef indices to describe fish community; number of species, Shannon and AMBI indices to describe the macrozoobenthic community.

Summarizing the results of the integrated environmental assessment, we can conclude that: 1) the Seabed Cleaning Platform was able to remove efficiently and selectively many different items from the sea bottom of the two studied areas, such as tyres, crave, ropes, fishing nets, operating in very different and very challenging environmental conditions; 2) t the platform did not affect and impacted the biological communities; 3) the removal of plastic items from the seabed might have led to the reduction of secondary microplastic concentrations in different environmental matrices, thus improving the environmental status.

5.2 Demo site 2 – Ave River estuary (PT)

5.2.1 Removal of aquatic litter from the Ave River estuary (PT)

In the Ave River estuary, monitoring of Floating Macro-Litter (FML) was undertaken before (June 2021 - December 2023) and after the installation of the Bubble Barrier remediation technology in order to assess its effectiveness.

The results of this chapter are also part and are detailed in the Deliverables 2.4 and 5.4.

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Evaluation of FML followed standard European guidelines (Hanke et al., 2013, 2023) and was conducted through visual monitoring to assess the diversity and relative abundance of debris in the area. Monitoring campaigns were carried out monthly from June 2021 to December 2024.FML observations were performed using binoculars (during ebb tide at three designated locations in the estuary—downstream, midstream, and upstream (S1: 41.340117°N, 8.748913°W; S2: 41.345022°N, 8.745227°W; S3: 41.351156°N, 8.741285°W) Figure 31).

All three observation points were positioned at the same elevation along the estuary margin, approximately 2 meters above the water surface (mean sea level), ensuring a consistent viewing angle for reliable comparisons between locations. Although some FML objects may have been partially obscured by wind-generated ripples or small waves from passing boats, this impact was minimized as observations were conducted on calm days with high visibility. More details on the methodology can be found in D5.4

Figure 31 - Map of the three observation points (S1–S3) for FML monitoring in the Ave River estuary. Reference system: WGS84.

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Significant differences ($p < 0.05$; ANOVA) were observed across seasons, sites, and their interaction (seasons * sites). The data revealed higher FML values in spring and autumn, with significantly lower values recorded in summer (Figure 32). These patterns highlight the **combined influence of seasonal and site-specific factors on FML distribution. Additionally, variations in river discharge, influenced by weather conditions, may also contribute to the observed fluctuations**.

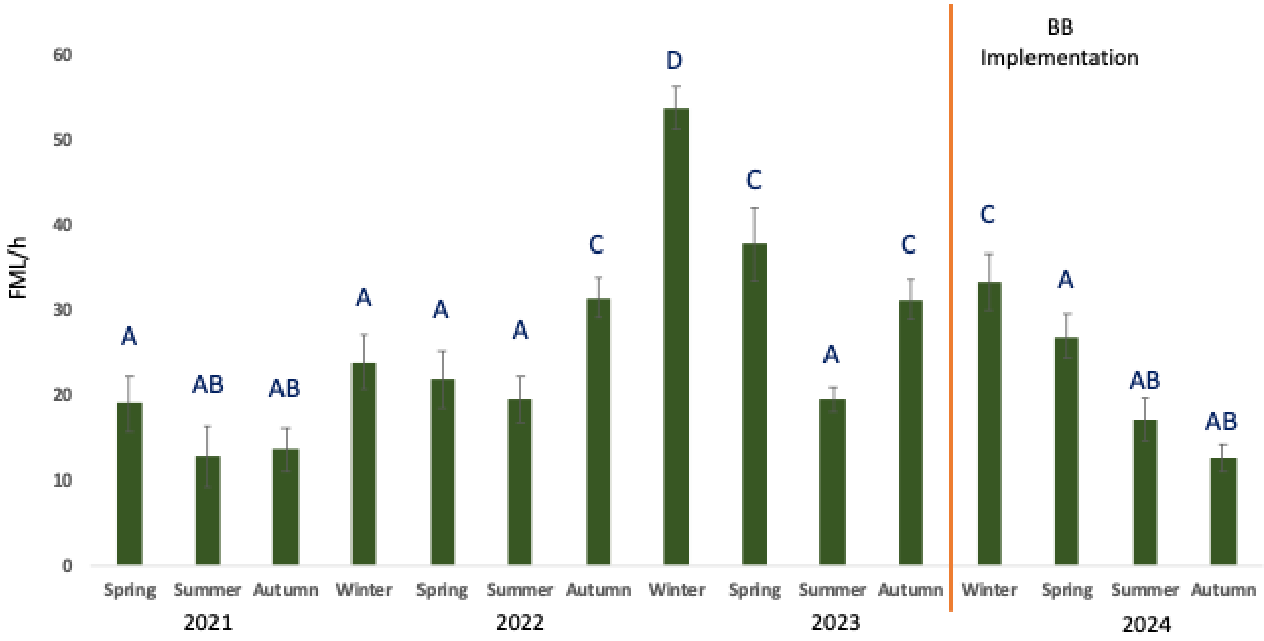

Figure 32 - Seasonal mean (± standard error) of FML items per hour recorded from 2021 to 2024, before and after the installation of the Bubble Barrier (BB). Different letters indicate statistically significant temporal differences ($p < 0.05$).

Before the Bubble Barrier (BB) was implemented, significantly higher floating macro-litter (FML) levels ($p < 0.05$) were recorded near the estuary mouth (S1 - 42.5 ± 6.3 items/h) compared to the other sites (S2 - 22.4 ± 2.1 items/h; S3 - 11.7 ± 2.3 items/h; see Figure 33). These findings emphasize the influence of the urban center on FML accumulation in the estuary, with a rising gradient from S3 to S1.

Following the installation and activation of the BB, a significant decrease in FML ($p < 0.05$) was observed downstream of the installation site (S1 - 25.5 ± 5.9 items/h; see Figure 33). However, no significant changes in FML were detected at S2 (27.6 ± 5.3 items/h) or S3 (12.1 ± 4.7 items/h) after the BB became operational.

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Following the installation of the Bubble Barrier (BB), FML near the mouth of the Ave estuary decreased by approximately 40% (Figure 33), **demonstrating the system's effectiveness in capturing floating debris.**

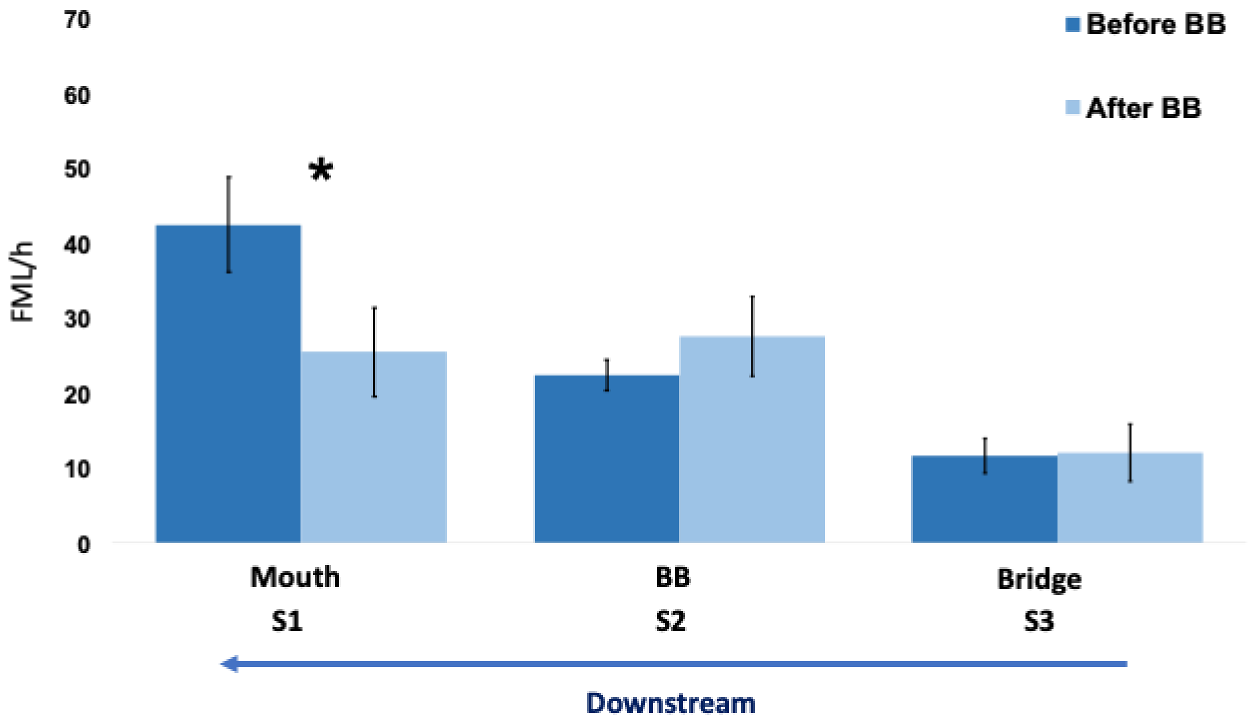

Figure 33 - Comparison of the total FML items (mean ± standard errors) per hour, recorded monthly before and after the Bubble Barrier (BB) implementation, across the three observation sites (S1-S3). Asterisk (*) indicates significant differences between the before and after BB implementation periods ($p < 0.05$.

Despite high variability across months and seasons, artificial polymer materials (plastics) were the most dominant type of floating macro-litter (FML), accounting for over 85.5% of all recorded items. Smoking-related litter (cigarette butts and packaging) followed at 7.3%, with paper and cardboard at 6.4%, while metal objects made up less than 1% of the total observed debris.

At the estuary mouth (S1), downstream of the Bubble Barrier (BB), the top five FML items identified before its implementation were "non-foamed plastic fragments" (39.3%), "foamed plastic fragments" (30.1%), "plastic bags" (12.4%), "cigarette butts" (9.2%), and "paper fragments" (9%). After the installation of the BB, the 5 most commonly identified FML represented, by the same order, 54.7%, 13.1%, 4.1%, 19.8% and 8.3%, respectively.

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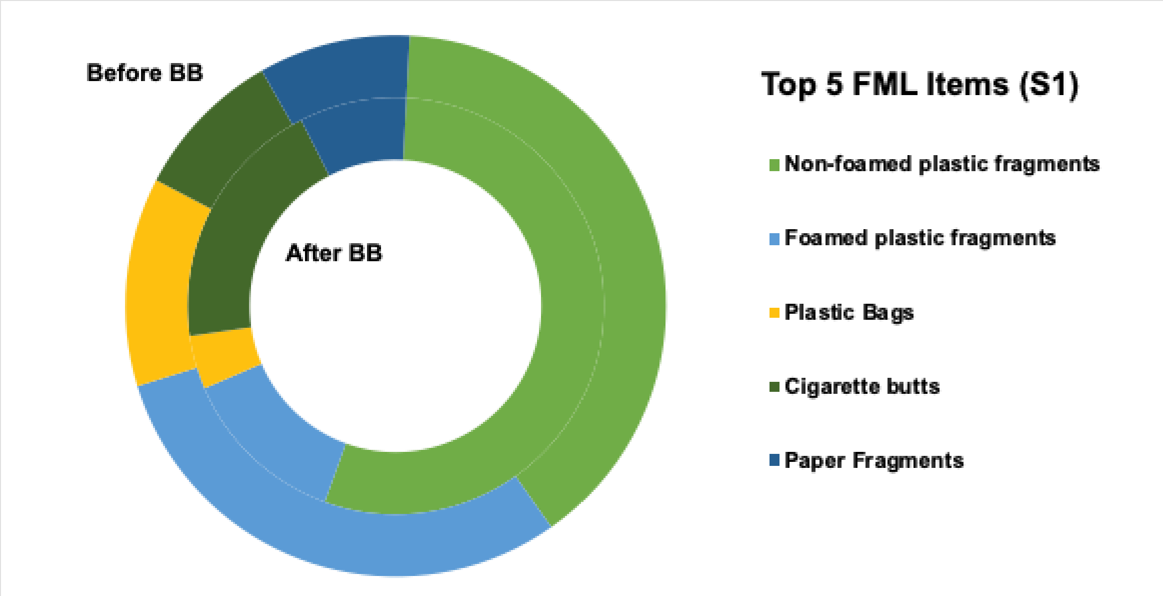

Figure 34 - Classification of the Top 5 materials found in the FML assessment in the estuarine mouth (S1) before and after the BB implementation.

Seasonal patterns and local human activities, such as tourism and recreational pursuits, may also contribute significantly to litter accumulation in this area.

Overall, the results indicate that the **Bubble Barrier is already reducing FML in the Ave River estuary** by **intercepting most of these materials before they reach the Atlantic Ocean**, benefiting both aquatic ecosystems and human health.

Microplastics distribution

Although the Bubble Barrier (BB) is not specifically designed to capture microplastics (MP), an assessment of these emerging pollutants was conducted in the estuary to complement the floating macro-litter (FML) analysis. Since most MP originates from the breakdown of larger plastic items (secondary MP), this evaluation provided additional insight into plastic pollution in the area.

MP particles in the water column were analyzed using standardized protocols (Gago et al., 2018). Samples were collected during low tide conditions with a manta net featuring a 200 µm mesh size. A flowmeter (Hydro-Bios 438115) was attached to the net to measure the filtered water volume. Seasonal planktonic trawls were conducted from 2021 to 2024 along five transects, three located downstream (T1 - T3) and two upstream (T4 and T5) of the BB installation (Figure 35). More details on the methodology can be found in the D2.3 and D5.4.

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Figure 35 - Map of the five selected transects (T1 – T5) for MP in the Ave River estuary. Reference system WGS84. The additional transect, included after the BB implementation is also indicated).

Microplastic concentrations varied significantly across seasons, sites, and their interaction ($p < 0.05$; ANOVA). More than 25 MP/m^3 were recorded in the Ave River estuary, with higher values at upstream transects (Figure 36). Fibres and fragments represented more than 87% of the identified MP (Figure 38). More details on the MP typologies and size distribution can be found in D2.4.

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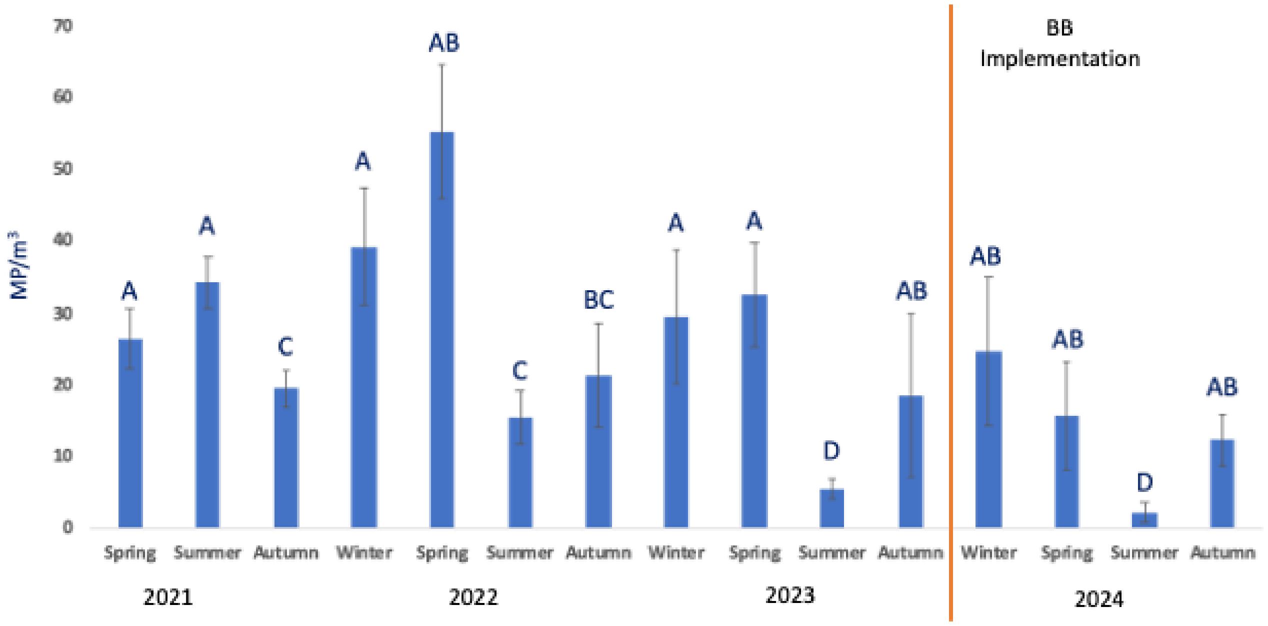

Figure 36 - Mean microplastic (MP) concentration per m³ collected between 2021 and 2024 (mean ± SEM across five transects), before and after the BB installation. Different letters indicate statistically significant seasonal differences ($p < 0.05$).

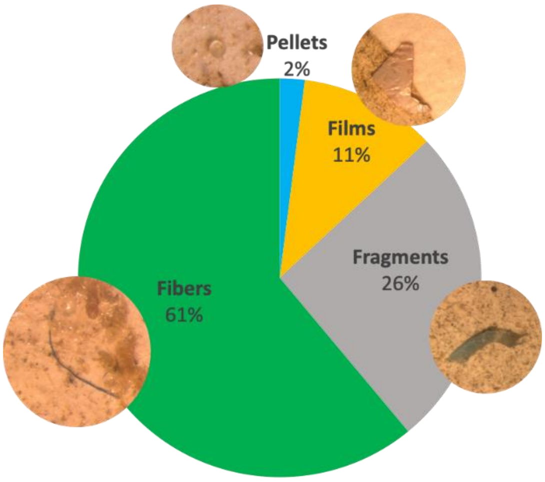

Figure 37 - Percentages of the most common MP collected in the Ave Estuary during the campaigns performed under the scope of MAELSTROM (aggregated observations, seasonal collected between 2021 and 2024).

Following the installation of the Bubble Barrier, an additional microplastic (MP) collection transect was included in the seasonal assessment, over the bubble curtain, using the same methodology and manta net described above. The average MP concentration in this area was estimated at 81 MP/m³, significantly higher, compared with the other 5 transects (.

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These findings highlight the **Bubble Barrier's ability to effectively concentrate MP at the surface of the air bubble curtain** (Figure 38).

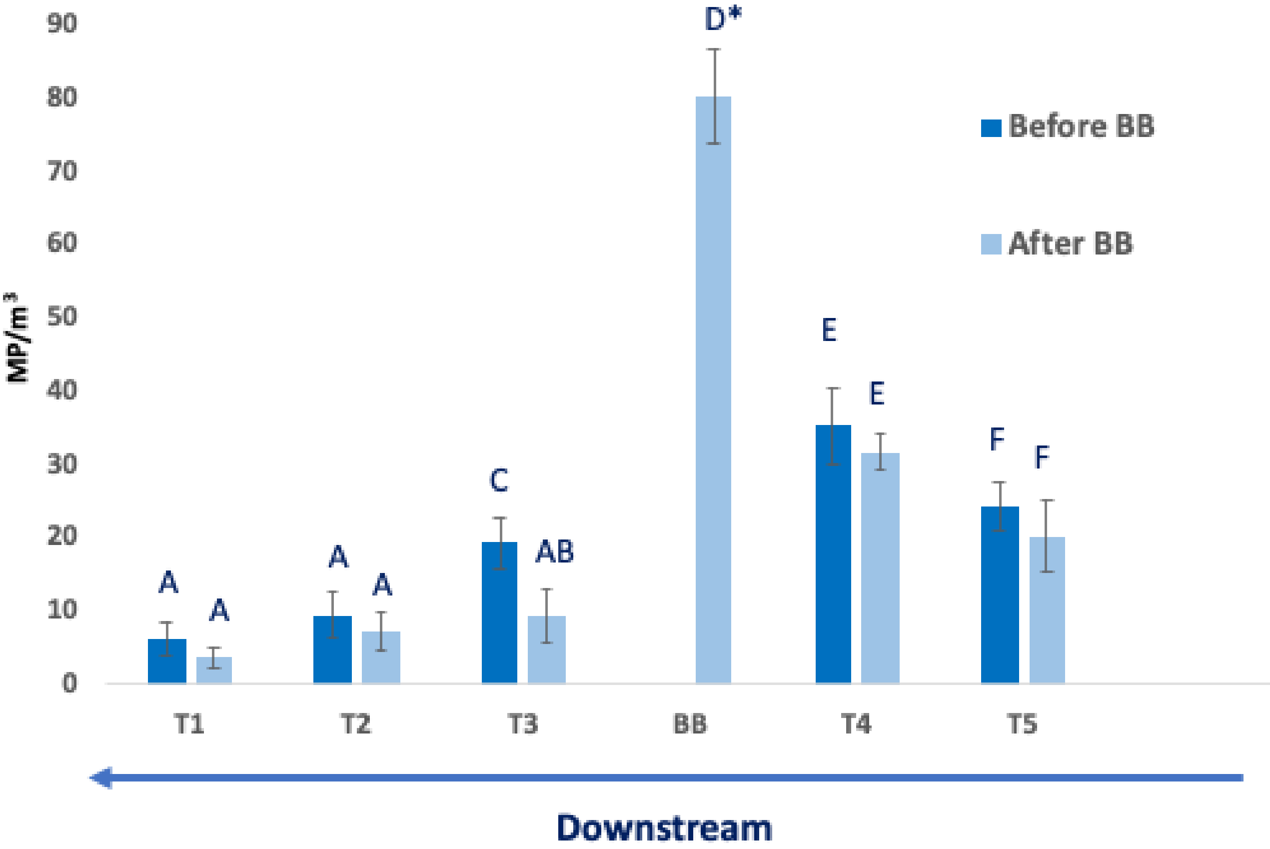

Figure 38 - Comparison of the MP (mean ± standard errors) collected between before and after the Bubble Barrier (BB) implementation. Bars with different letters are significantly different between them ($p < 0.05$) in MP collected before and after the technology operation. BB (*) represents the transect on the bubble curtain.

5.2.2 Integrated ecological assessment

An ecological assessment was carried out prior to the implementation in 2021 and 2023 under the scope of the MAELSTROM project, showing that status of the Ave River estuary can be classified as moderate to poor according to the assessment of general supporting physical and chemical parameters and the biological element (benthic macroinvertebrates and concentration of Chlorophyll a values) (MAELSTROM, Deliverable 2.3; Table 6.1, 6.2 and 6.3). As described in Deliverable 2.3, during ecological assessment campaigns, physical parameters on the water column and MP were also collected. Additionally, observational campaigns of FML were performed.

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After the installation of the marine litter removal technology, the designed campaigns were maintained, and additional analysis were performed, to understand the impact of the technology on the Ave Estuary system.

The ecological assessment of the Ave Estuary has been conducted since the beginning of the MAELSTROM project, covering the Spring and Autumn periods each year (seasons with the highest biological activity for this type of water body) (Table 16). After the installation of the Bubble Barrier, ecological assessments were carried out seasonally to monitor the ecosystem's progression following the implementation of the waste collection technology.

The general supporting physicochemical elements, specific pollutants, and priority substances analyzed indicate that the Ave River estuary has historically been subjected to high nutrient input and diffuse pollution discharges into the ecosystem (Table 17). These results may be linked to agricultural and industrial activities throughout the Ave River Basin.

The biological elements evaluated showed some variation in classification during the study period, particularly within the benthic macroinvertebrate community (Table 18). However, the ecological quality status of the Ave River estuary never achieved a "good" ecological status during the study period. **Following the installation of the Bubble Barrier, the ecological assessment results remained unchanged up to the present (approximately 10 months post-installation), with no significant changes observed in any of the evaluated indicators. Changes in the ecological status of the Ave River estuary will take more time (years) to be measurable.**

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Table 16 - Summary table of the results of evaluated elements used to assess water quality based on WFD metrics throughout the MAELSTROM project, including pre- and post-technology installation phases. Upstream Bubble Barrier (UBB) and Downstream Bubble Barrier (DBB) transects can be found in Figure 35.

Sampling Periods	Ecological Status (1)				Ecological Status	Chemical Status (2)	Overall Status (1) + (2)
	Physical and Chemical Elements	Specific Pollutants (Cu)	Biological elements			Priority Substances (Cd, Pb, Ni, Hg)	
			Chlorophyll *a* P90	BAT			
Spring21	Moderate	*	Excellent	Poor	Poor	Good	Poor
Autumn21	Moderate	*	Excellent	Moderate	Moderate	Good	Moderate
Spring22	Moderate	*	Excellent	Moderate	Moderate	Failing to achive good	Moderate
Summer22	Moderate	*	Excellent	Poor	Poor		Poor
Autumn22	Moderate	*	Excellent	Poor	Poor	Good	Poor
Spring23	Moderate	*	Excellent	Poor	Poor	Failing to achive good	Poor
Autumn23	Moderate	*	Excellent	Good	Moderate	Failing to achive good	Moderate
Bubble Barrier Installation							
UBB_Winter24(1)	Moderate	*	Excellent	Moderate	Moderate	Failing to achive good	Moderate
DBB_Winter24(1)	Moderate	*	Excellent	Good	Moderate	Failing to achive good	Moderate
UBB_Winter24(2)	Moderate	*	Excellent	Bad	Bad	Failing to achive good	Bad
DBB_Winter24(2)	Moderate	*	Excellent	Good	Moderate	Failing to achive good	Moderate
UBB_Spring24	Moderate	*	Excellent	Bad	Bad	Failing to achive good	Bad
DBB_Spring24	Moderate	*	Excellent	Poor	Poor	Failing to achive good	Poor
UBB_Summer24	Moderate	*	Excellent	Ongoing	Ongoing	Failing to achive good	Ongoing
DBB_Summer24	Moderate	*	Excellent	Ongoing	Ongoing	Failing to achive good	Ongoing
UBB_Autumn24	Moderate	*	Excellent	Ongoing	Ongoing	Failing to achive good	Ongoing
DBB_Autumn24	Moderate	*	Excellent	Ongoing	Ongoing	Failing to achive good	Ongoing

* Without reference values

Table 17 - Summary table of the physical and chemical, and hydromorphological results used to assess the water quality based on WFD metrics throughout the MAELSTROM project, including pre- and post-technology installation phases. Upstream Bubble Barrier (UBB) and Downstream Buble Barrier (DBB) transects can be found at Figure 35..

Sampling Periods	Physical and Chemical Elements				Hydromorphological Elements				
	O2 (%)	Nitrate+Nitrites (mg N/L)	Ammonia (mgN/L)	Phosphate (mg P/L)	Organic Matter (%)	Granulometry	Intertidal Zone Structure	Freshwater flow	Exposure to waves
Reference values	109	1.00	0.30	0.11					
Spring21	95.07 ± 1.39	1.41 ± 0.60	0.06 ± 0.006	0.21 ± 0.04	12.03 ± 4.21	Coarse silt/Medium sand	Right margin = artificial - wall Left margin = artificial - wetland	Constant freshwater flow from upstream, with increase in rainy periods	Null
Autumn21	94.37 ± 1.25	1.34 ± 0.22	1.19 ± 0.43	0.42 ± 0.06	9.030 ± 5.66	Coarse silt/Medium sand			
Spring22	92.67 ± 1.25	1.57 ± 0.07	0.92 ± 0.09	0.15 ± 0.02	9.840 ± 6.60	Coarse silt/Medium sand			
Summer22	95.08 ± 1.96				21.04 ± 9.36	Very fine sand			
Autumn22	96.70 ± 0.49	0.47 ± 0.01	0.48 ± 0.06	0.19 ± 0.006	22.85 ± 11.3	Fine sand			
Spring23	87.93 ± 1.28	1.15 ± 0.11	2.75 ± 0.54	0.77 ± 0.19	19.36 ± 6.86	Fine sand			
Autumn23	95.17 ± 0.73	1.12 ± 0.003	0.28 ± 0.02	0.36 ± 0.003	25.15 ± 6.03	Very fine sand			
Bubble Barrier Installation									
UBB_Winter24(1)	98.07 ± 0.39	0.45 ± 0.0006	0.44 ± 0.05	0.25 ± 0.004	10.48 ± 11.9	Very coarse sand			
DBB_Winter24(1)	97.57 ± 0.40	0.45 ± 0.0006	0.46 ± 0.02	0.25 ± 0.00	24.50 ± 3.16	Very fine sand			
UBB_Winter24(2)	99.70 ± 0.42	0.45 ± 0.001	0.38 ± 0.03	0.24 ± 0.002	10.48 ± 10.2	Very coarse sand			
DBB_Winter24(2)	99.17 ± 0.45	0.45 ± 0.001	0.38 ± 0.03	0.24 ± 0.001	20.08 ± 1.83	Fine sand			
UBB_Spring24	95.53 ± 0.48	1.83 ± 0.08	0.39 ± 0.02	0.97 ± 0.26	20.09 ± 8.52	Fine sand			
DBB_Spring24	94.00 ± 016	1.92 ± 0.06	0.41 ± 0.06	1.58 ± 0.12	25.89 ± 6.45	Very fine sand			
UBB_Summer24	83.03 ± 1.90	0.98 ± 0.10	0.39 ± 0.17	0.71 ± 0.28	17.43 ± 8.29	Very fine sand			
DBB_Summer24	82.43 ± 0.17	0.96 ± 0.07	0.39 ± 0.14	0.70 ± 0.27	20.08 ± 5.33	Very fine sand			
UBB_Autumn24	89.57 ± 3.19	1.49 ± 0.03	1.32 ± 0.02	1.40 ± 0.02	26.93 ± 2.97	Very fine sand			
DBB_Autumn24	87.97 ± 0.42	1.54 ± 0.03	1.35 ± 0.03	1.39 ± 0.11	28.89 ± 4.48	Very fine sand			

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Table 18 - Summary table of the biological results used to assess the water quality based on WFD metrics throughout the MAELSTROM project, including pre- and post-technology installation phases. Upstream Bubble Barrier (UBB) and Downstream Bubble Barrier (DBB) transects can be found in Figure 35.

Sampling Periods	Biological Elements						
	Abundance	Richness (S)	Margalef (d)	Shannon-Wiener (H')	AMBI	BAT	Chlorophyl *a* (µg/L)
Reference values			1.9	2.30	2.50		6.67
Spring21	47	2	0.26	0.24	6.00	0.147	4.48 ± 0.83
Autumn21	198	5	0.57	0.79	3.47	0.448	4.64 ± 0.91
Spring22	242	8	1.28	0.98	5.76	0.470	1.47 ± 0.18
Summer22	138	5	0.81	0.29	5.85	0.264	
Autumn22	431	5	0.66	0.23	5.86	0.226	1.28 ± 0.22
Spring23	152	5	0.80	0.16	5.92	0.236	0.79 ± 0.17
Autumn23	141	9	1.62	1.42	4.23	0.698	0.86 ± 0.07
Bubble Barrier Installation							
UBB_Winter24(1)	177	4	0.67	0.63	3.39	0.445	0.09 ± 0.04
DBB_Winter24(1)	2	2	1.44	0.69	1.50	0.707	0.07 ± 0.07
UBB_Winter24(2)	52	4	0.76	0.94	5.02	0.414	0.55 ± 0.26
DBB_Winter24(2)	7	4	1.54	1.28	3.50	0.704	0.39 ± 0.13
UBB_Spring24	37	3	0.55	0.46	5.35	0.277	0.46 ± 0.06
DBB_Spring24	115	1	0.00	0.00	6.00	0.060	0.54 ± 0.08
UBB_Summer24							3.75 ± 0.05
DBB_Summer24							3.45 ± 0.40
UBB_Autumn24							0.51 ± 0.36
DBB_Autumn24							0.34 ± 0.04

5.2.3 Noise pollution

As detailed described in MAELSTROM deliverable 5.4, an acoustic survey was carried out on 11 November 2024 to measure the noise impact of the Bubble Barrier technology in the Ave River estuary. This analysis, initially not foreseen by the project, was introduced to provide some preliminary background information on the possible impact from noise pollution on aquatic species. For a complete assessment of this potential impact component a comprehensive study would be needed, but it was out of scope for the MAELSTROM project.

A recently calibrated ICListen high-frequency hydrophone (https://oceansonics.com/iclisten-hf-hydrophone/) was used to provide absolute measures of biotic, abiotic and anthropogenic sound levels to draw meaningful comparisons of habitats through time and at different locations.

The survey encompasses the sampling at three different locations at the margin of the estuary (piers), the first one at the centre river-side of the Bubble Barrier pier (Pier 1), second on the corner of a small pier near the end of the Bubble Barrier, 285 m from

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the centre of the Bubble Barrier pier (Pier 2), and the last one at the end of a yacht pier, 628 m from the centre of the Bubble Barrier pier (Pier 3). The hydrophone was lowered into the water tied to a line and placed about 50 cm above the estuary bottom for recording. Recordings of at least 5 minutes were taken with the Bubble Barrier switched off and on at all piers and during the start of the Bubble Barrier at Pier 1. For the comparison of conditions with the Bubble Barrier switched on and off, recorded minutes without exceptional noise events were selected to represent base noisescapes. Recordings of the Bubble Barrier switched on and off are represented in Figure 39.

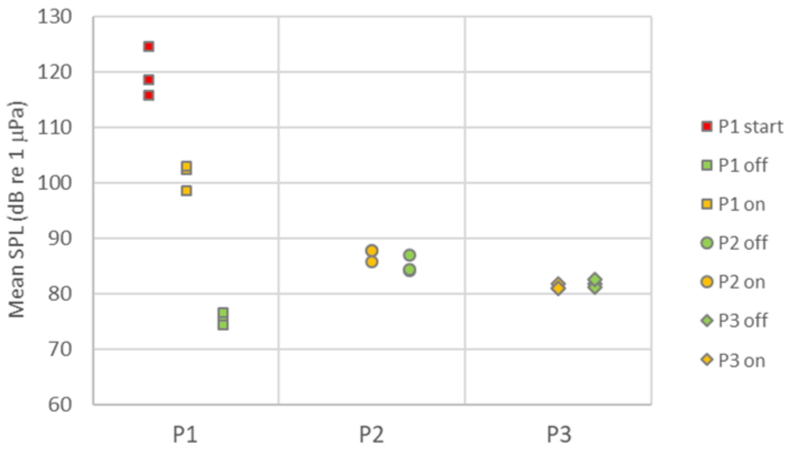

Figure 39 - Mean broadband SPL (20-2000 Hz) of the 3 minutes recorded during the start of the Bubble Barrier, at Pier 1, and of 3 minutes recorded at each site with the Bubble Barrier switched on and off (minutes without exceptional noise events were selected to represent base noisescapes).

The analysis performed revealed that the background noise, with the technology switched off, was lowest at Pier 1. However, with the Bubble Barrier on, noise levels were highest at that site, decreasing with increasing distance from the compressor shed. The effect of the Bubble Barrier technology is clearly visible at Pier 1, but non-existing at Pier 3. At Pier 1, when the Bubble Barrier is switched on, the noise level increases rapidly to values around 120 dB, and decreases, to around 100 dB, one minute after the compressor is working. These noise levels are maintained while the Bubble Barrier is working.

Bearing in mind that noise effects are species and habitat specific (in noisy habitats species adapt to a certain extent) **the measured values suggest that the noise levels produced by the Bubble Barrier technology, particularly when the technology is started, may pose a risk to noise-sensitive species. However, these levels are limited to a small area close to the compressor shed.**

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6 Impacts of remediation activities on local economies and communities

The experience of the MAELSTROM project showed how coastal cities and municipalities, local agencies (e.g. harbour authorities), local economic operators (e.g. touristic beach operators) and local populations can significantly benefit from ML remediation technologies. Municipalities and local environmental authorities responsible for waste collection and disposal management, as well as for environment protection have been involved in the MAELSTROM project. Respectively, the Municipality of Venice and the waste management authority (VERITAS), the Italian Coast Guard and the Italian State Police in Demo site 1 (Venice coastal area, IT) and the Municipality od Vila do Conde, DOCAPESCA - Portos e Lotas, S.A., APA, Vila do Conde Captaincy, the CMIA and the LIPOR, in Demo site 2 (Ave River estuary, PT) have been engaged in remediation activities in their area of competence. The positive environmental effects of the remediation technologies described in chapters 5 and 6 have improved the quality of waters in the territories of the Demo sites. At the same time, implementation of remediation technology made available to responsible authorities, and local communities, a comprehensive knowledge on the characteristics and the dimension of ML problem in their areas, thus paving the way for identification of prevention measures.

An example of added value for local economies and communities is represented by the effective application of ML removal technology in an abandoned mussel farm. In fact, mariculture (mussels and finfish) represents an important source of income for local communities across Europe. The growing trend of the sector requires responsible planning and management authorities to consider the overall life cycle of this activity, including decommissioning phase. Practice of removal of marine aquaculture infrastructures at end-of-life should be more and more standardized and controlled. Nevertheless, the occurrence of sites where some wastes are left at sea is probable, particularly in the areas where the concentration of plants is high. In such areas, the remediation technologies for seabed litter demonstrated in MAELSTROM can provide workable and efficient solution.

Another example of benefit for local territories demonstrated by the project is represented by the capacity of Bubble Barrier to efficiently concentrate microplastics in the area where the barrier is situated. Invasive species have been showed to be

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associated to this plastic litter fraction. Indirect removal of invasive species provides protection to local species and habitats and help preserving the ecosystem services providing benefits to the local communities, like fish species of commercial interest.

Among the technologies provided by the project, the use of seabed mapping techniques provides the possibility to obtain detailed mapping of the presence of litter items deposited on the bottom and help to estimate their size and mass. Such technology can support the identification of cases of illicit disposals and provide useful data to authorities.

The Robotic Seabed Cleaning Platform for seabed litter removal allows for precise identification, quantification and recording of typology and quantity of litter removed, providing reliable data to authorities and local communities and allowing for a transparent assessment of costs and benefits of clean-ups of public areas.

The opportunity to set up potential partnerships between MAELSTROM remediation technologies and local actors within the waste management and remediation market represents another opportunity for local economies that would be involved in the installation and operation of the technologies. As pointed out in the technology exploitation plan (Deliverable D8.6), synergies with other market segments can also provide additional opportunities for local economies, including job creation: highly specialized robotic applications at sea; marine archaeology, for the recovery of underwater archaeological materials; underwater maintenance activities and/ or offshore windmill farm installation for the connection/maintenance of cables.

7 Transferability of the remediation technologies

The Seabed Cleaning Platform was successfully tested in both the lagoon and the open sea coastal environments. The lagoon was a particularly challenging environment given the poor visibility and currents in the lagoon channels. The system was demonstrated to be quick and efficient, to be able to operate up to 20 m of depth and to collect items up to 130kg. Some logistic issues related to the open sea operations could be overcome by upgrading the hull of the platform to improve its navigation properties, speed and manoeuvrability. This technology is particularly suitable to contexts where the conditions do not allow scuba operations, in risky environments e.g. heavily polluted waters, presence of dangerous items, like radioactive waste and munitions, strong currents, etc.

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The Bubble Barrier technology represents a long-term solution to the ML pollution problem, by preventing litter to reach the sea. This technology has the potential to be replicated and adapted for use in riverine and estuarine environments. It was aligned with existing activities in the area and co-designed in collaboration with local stakeholders.

Transferability of remediation solutions was assessed by the technology exploitation plan of the project (Deliverable D8.6): the Total Addressable Market for MAELSTROM solutions (especially for marine litter removal technologies) is represented by c.357 cities with more than 50,000 inhabitants, and 331 of them are in the TEN-T network.

The successful implementation of this technologies relies on collaboration of all actors at local level. Endorsement by local authorities is key, as well as the engagement of local communities, schools, and the public. These actors have generated significant interest and support for the remediation initiatives in both demo sites. This involvement fostered a sense of shared responsibility and demonstrated the importance of public participation in environmental conservation efforts.

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