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MARLESS (MARine Litter cross-border awarenESS and innovation actions)

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Report: Use of mussels as suitable tool for water column microplastic purification

AT 6.4

WP 6

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Report: *Activity 6.4 Use of mussels as suitable tool for water column microplastic purification*

1. **INTRODUCTION***:*

Mussels of *Mytilus edulis* complex (*M. edulis*, *M. galloprovincialis* and *M. trossulus*) are widely used as indicators of environment quality and pollution by application of Mussel watch concept. *Mytilus* mussels are a commonly used indicator species for the presence of microplastics (MP) in the marine coastal areas also. Microplastics are small plastic particles that are less than 5 mm in size and can be found in a variety of forms, including fragments, fibers, and pellets. These particles are widely distributed in the environment and can have a significant impact on marine life and ecosystems. *Mytilus* sp. mussels are well-suited for monitoring microplastics in the environment due to their filter-feeding habits. As they feed, they filter large amounts of water and accumulate microplastics along with other suspended particles. This makes them an effective tool for monitoring the presence and distribution of microplastics in the marine environment.

In recent years, numerous studies have been conducted to determine the extent of local and regional Sea microplastics contamination by analyses of water surface, water column and sediment, including indicator species. These studies have provided valuable information about the levels and distribution of microplastics in the world's oceans and coastal areas and have helped to increase our understanding of the impacts of microplastics on the environment, biota and finally on human health.

Brief description of the activity performed

By performed WP 6.4 activities “*Use of mussels as suitable tool for water column microplastic purification*” our intention was assessment of microplastics pollution of Rovinj and Dubrovnik coastal area (5 + 2 locations) by using mussels as a potential suitable innovative tool for monitoring and removing MP from water column, including quality improvement (bioremediation) of the marine environment, e.g. location in vicinity of Rovinj municipal waste water treatment plant Cuvi outlet (Figure 6.4.1).

After proving the concept by laboratory filtration (pseudo/feces) experiments with mussels using cigarette buts cellulose acetate microfibers as model MP, Mussel MicroPlastic Monitor and Purification installations (MMP-M and MMP-P) were designed. Further analyses and monitoring of microplastic at sea surface (manta net), and in water column and sediments, as well as in mussels *M. galloprovincialis* and their collected feces/pseudofeces - suspended matter (MMP-M installations) were performed at 7 investigated sites.

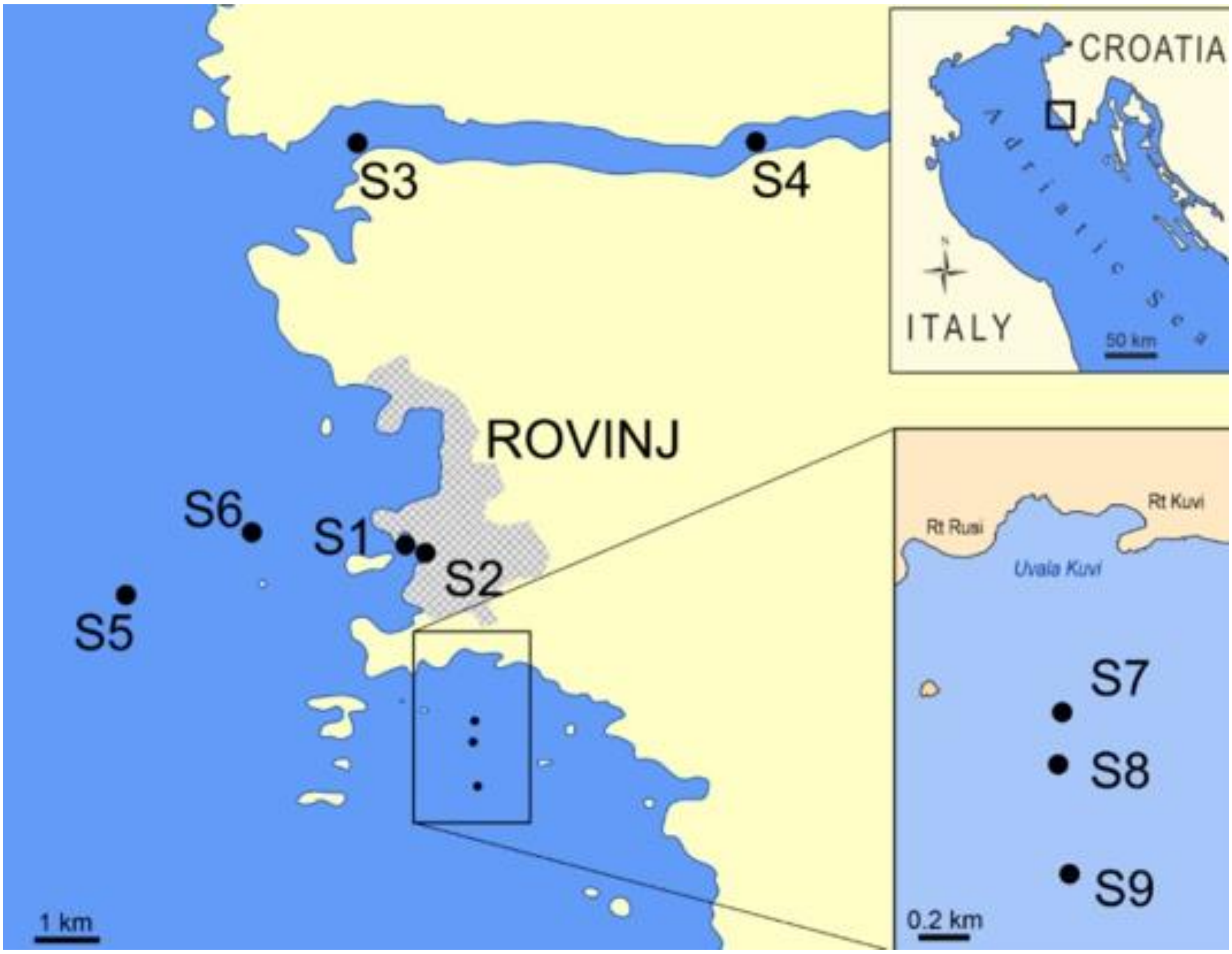


Figure 6.4.1. Investigated area of Rovinj coastal area (S1, S6) and area in vicinity of Rovinj municipal wastewater treatment plant Cuvi outlet (S7, S9, S9).

D6.4.1 Demonstrating Mussel Filtration as an Ecological Approach for Microplastic Removal from the Marine Environment

Microplastic pollution has become an alarming global environmental concern, with pervasive and detrimental effects on marine ecosystems and human health. These tiny plastic particles, measuring less than 5 mm in size, are found throughout the world's oceans, posing a significant threat to marine life and the overall health of our seas. In response to this crisis, scientists and conservationists have been exploring innovative and sustainable solutions to mitigate microplastic contamination. One promising approach is harnessing the natural filtration abilities of mussels, which have the potential to serve as eco-friendly sentinels against this pervasive environmental threat.

Mussels as Filter Feeders
Mussels, a group of bivalve mollusks, are renowned for their remarkable filter-feeding abilities. They reside in intertidal and subtidal zones, where they actively draw in seawater to extract suspended particles, including plankton, detritus, and phytoplankton, as a primary source of nutrition. This feeding behavior has evolved over millions of years, making mussels highly efficient at removing particles from the water column.

Microplastic Capture by Mussels
Recent research has revealed that mussels inadvertently capture microplastics during their filter-feeding process. As they pump and filter water through their gills, tiny microplastic particles, often resembling plankton in size and appearance, become entrapped in the mucus coating their gills. Once trapped, these microplastics are transferred to the mussel's digestive system, where they are encapsulated in mucus and then expelled as pseudofeces. This natural mechanism showcases the potential for mussels to act as biological agents in removing microplastics from the marine environment.

Experimental Demonstrations
To demonstrate the effectiveness of mussel filtration as an ecological approach for microplastic removal, we conducted several experimental studies. In controlled environments, mussels have been exposed to microplastic-laden water to observe their capacity to filter out and retain these particles (Figure 6.4.2).

To estimate and evaluate MP removal/purification efficiency of mussels' filtration, several laboratory experimental exposures with suspended matter (Figure 6.4.2) and concentrations of model microplastic particles (<0.5 mm, cellulose acetate microfibers) formed by cigarette butts decomposition were performed (Figure 6.4.3).

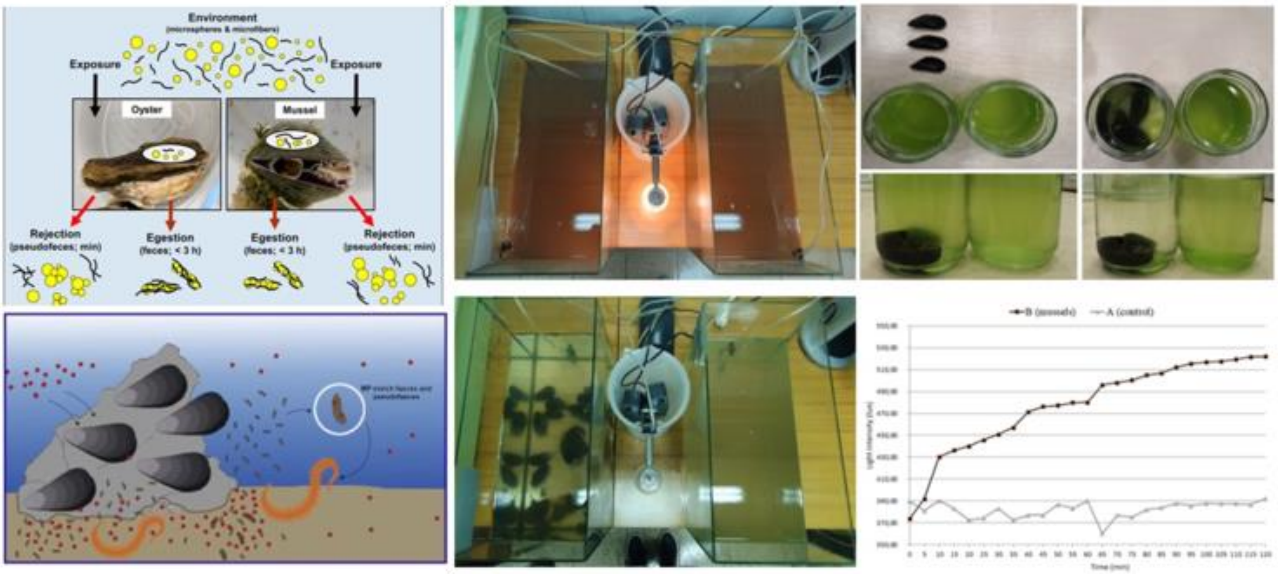


Figure 6.4.2. Demonstration of purification efficiency of mussel's filtration. The decreased turbidity over time up to 2 h measured as light intensity by Hobo data loggers MX2022, as well as optical difference between A) aquaria without and B) aquaria with mussels are direct proof of concept.

The results consistently show that mussels can capture a significant percentage (up to 99%, depending of size and type) of microplastics from the water column, effectively reducing the MP concentration in their surroundings.

Environmental Implications

The implications of using mussels as microplastic filtration agents are profound. Deploying mussel beds in strategic locations within marine environments can potentially mitigate the spread of microplastics. These bivalves can serve as natural filters, reducing the abundance of microplastics in their vicinity, thereby protecting other marine organisms from ingestion and contamination. Moreover, as filter feeders, mussels can potentially aid in the restoration of water quality in areas affected by microplastic pollution.

Challenges and Considerations

While the concept of using mussels for microplastic removal is promising, there are challenges and considerations to address. These include the risk of bioaccumulation within mussel tissues and potential effects on mussel health.

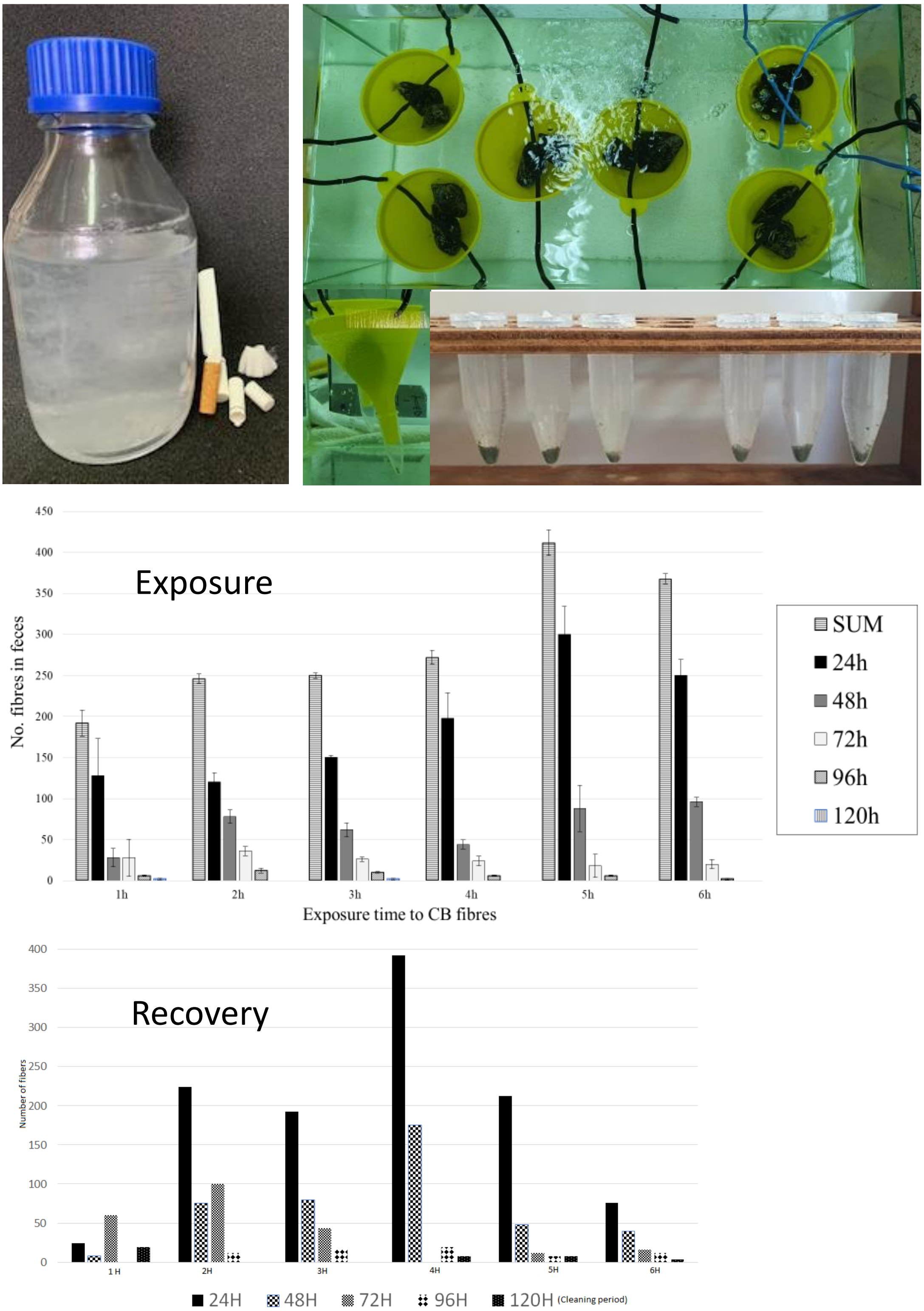


Figure 6.4.3. Demonstration of MP removal/purification efficiency of mussels' filtration by laboratory mussel experimental exposures with different concentration of model cellulose acetate microfibers (<0.5mm) formed by cigarette butts decomposition: A) Exposure (1-6 h) and B) Recovery (24-120 h).

Conclusion

In the face of escalating microplastic pollution in our oceans, harnessing the natural filtration capabilities of mussels offers a promising ecological approach for microplastic removal. Demonstrations of mussel filtration activity have highlighted their ability to inadvertently capture and sequester microplastics, underscoring their potential as eco-friendly sentinels against this environmental menace. As we explore innovative solutions to combat microplastic pollution, the integration of mussels into marine conservation efforts represents a significant step towards a cleaner and healthier marine environment. Nevertheless, further research and careful management practices are essential to maximize the efficacy and sustainability of this approach in addressing the microplastic problem.

D6.4.2. Mussel pseudofeces/feces generation and natural sedimentation as a plastic pump in coastal waters

Coastal waters around the world are increasingly plagued by the insidious presence of microplastics, posing a grave threat to marine ecosystems and human well-being. However, nature often provides innovative solutions to environmental challenges. Here we explores the remarkable role that mussel pseudofeces and feces generation, coupled with natural sedimentation processes, play as a "plastic pump" in coastal waters. Mussels, as filter feeders, inadvertently contribute to the removal of microplastics from the aquatic environment, making them a valuable asset in the fight against plastic pollution.

Mussel Filter Feeding and Microplastic Uptake
Mussels are renowned for their filter-feeding behavior. They actively draw in large volumes of seawater, capturing suspended particles such as plankton, detritus, and even microplastics as they filter-feed. Microplastics, often resembling prey in size and buoyancy, become entrapped in the mucus-coated gills of mussels during this process. Some microplastics are subsequently incorporated into pseudofeces, a mixture of mucus and rejected particles that is expelled by the mussels.

Feces and Pseudofeces as Microplastic Aggregates
The expelled pseudofeces and feces containing microplastics are not immediately lost to the water column. These aggregations of microplastics and organic matter are denser than seawater, causing them to sink. This natural sedimentation process is a crucial aspect of the "plastic pump" phenomenon. As they descend, the pseudofeces and feces act as carriers for microplastics, effectively transporting them from the surface to the seafloor. This mechanism reduces the residence time of microplastics in the water column and facilitates their removal from the ecosystem.

Sedimentation as a Microplastic Sink
The sedimentation of microplastics via mussel pseudofeces and feces plays a vital role in coastal waters. It effectively sequesters microplastics in the benthic environment, preventing them from being ingested by other marine organisms and reducing their potential impact on higher trophic levels. In addition, the burial of microplastics in sediments limits their re-suspension and transport by currents, further confining their distribution and potential harm.

After proving the concept removal of microplastics using cigarette buts cellulose acetate microfibers as model MPs (0.5 mm cellulose acetate fibres) up to 99% by laboratory filtration (pseudo/feces) experiments with mussels (Figure 6.4.3), further experiments in stone basin ($1m^3$) with constructed Mussel MicroPlastic Monitor (MMP-M) installations were performed (Figure 6.4.4 A). Results of suspended matter (POM) sedimentation (Figure 6.4.4 B) by MMP-M installation with and without mussels indicated that mussels filtration and pseudo/feces generation enhanced natural suspended matter sedimentation by 20-60% (December 2021 – December 2022).

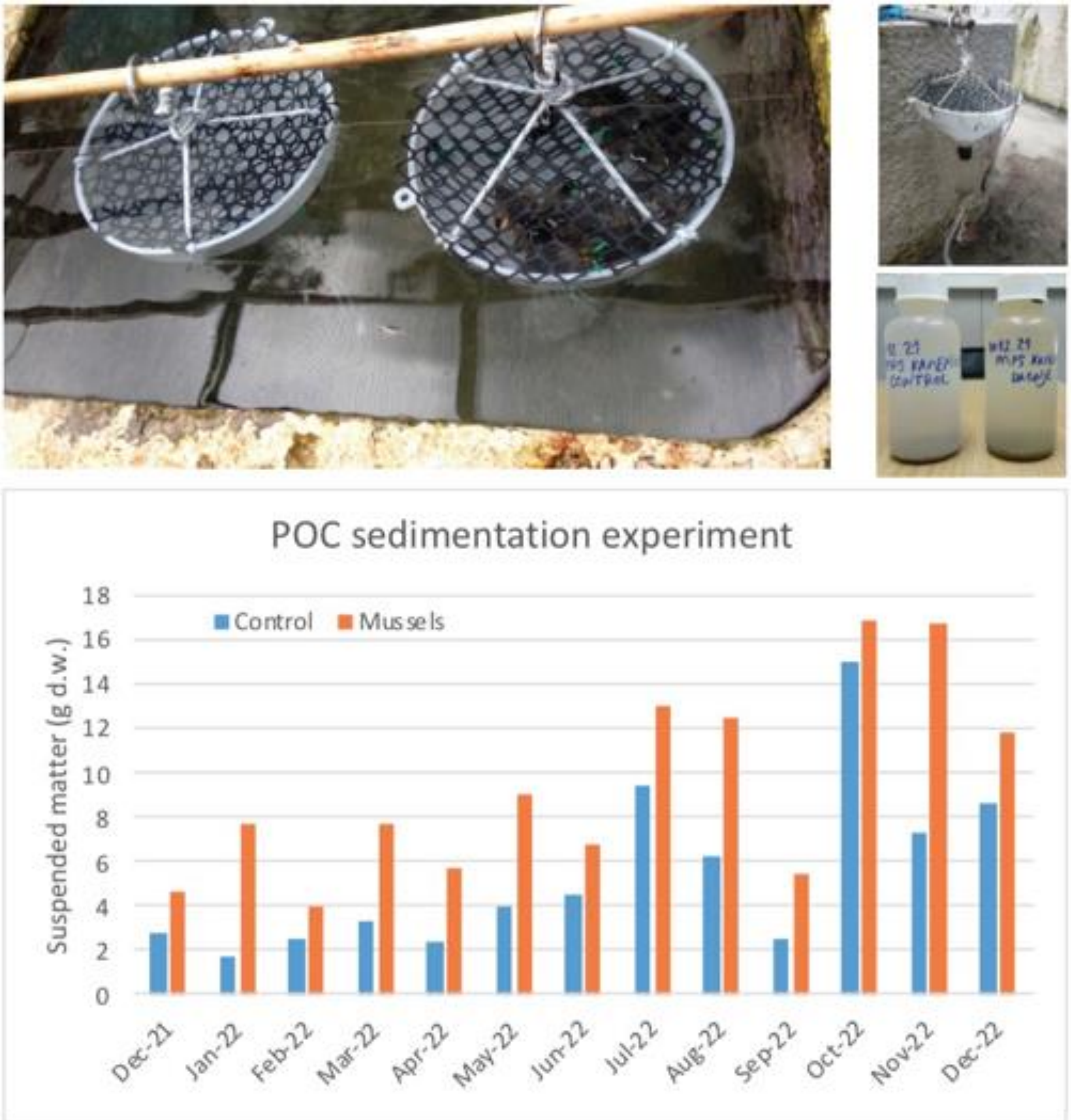


Figure 6.4.4. Demonstration of how mussels' filtration and pseudo/feces generation can enhance natural suspended matter (POC/POM) sedimentation.

While the natural plastic pump created by mussel filtration, pseudofeces, and feces generation is a fascinating ecological phenomenon, there are important considerations. The potential for bioaccumulation of microplastics within mussel tissues and the consequences for mussel health require thorough investigation.

Conclusion

Mussel pseudofeces and feces generation, coupled with natural sedimentation processes, serve as an intriguing ecological mechanism for the removal of microplastics from coastal waters. This "plastic pump" phenomenon showcases the interconnectedness of marine ecosystems and the potential for nature's ingenious solutions to address environmental challenges. While further research and conservation efforts are necessary to fully harness the capabilities of mussels in microplastic mitigation, their role as natural sentinels in the fight against plastic pollution is undeniably promising. Embracing these natural processes can contribute to cleaner and healthier coastal waters, benefitting both marine life and human communities along the coast.

As a single *Mytilus* sp. mussel can filtrate up to 2-5 L of seawater per hour, therewith we demonstrate the concept of bioremediation of the local marine environment (water column) through ecosystem services provided by the mussels *M. galloprovincialis* in the vicinity of the UPOV wastewater outfall Cuvi.

Purification of the water column and prevention of the spread of pollutants were demonstrated in laboratory aquaria (30 L) and backyard pools (1 m^3), where filtration by mussels and pseudo/feces generation enhanced natural suspended matter sedimentation by 20-60% and removal of microplastics by up to 99%.

D6.4.3 Determination of Biological Effects and Mussel Filtration-Feeding Behavioral Changes After In Vitro and Field Microplastic Exposure

Microplastic pollution is a pressing environmental concern with potential adverse impacts on marine ecosystems. Mussels, as filter-feeding organisms, are at the forefront of microplastic interactions in coastal and marine environments. Therefore, we conducted research to understand normal mussel filtration activity (behavior) and possible behavioral changes in mussel filtration-feeding after exposure to microplastics, both in vitro and in field settings.

In Vitro Microplastic Exposure Studies
Laboratory-based in vitro studies have been instrumental in elucidating the biological effects of microplastic exposure on mussels. These experiments involve exposing mussels to controlled concentrations of microplastics under laboratory conditions.

Usually researchers have assessed various parameters, from toxicological effects to accumulation and trophic transfer. We focused our research on Behavioral Changes weil, In vitro studies have also revealed alterations in mussel filtration-feeding behavior in the presence of microplastics. Mussels may reduce their filtration rate, potentially as a protective response to minimize microplastic ingestion. Contrary, by mussel exposure to cigarette butts microfibres (< 0,5 mm; 4000 MP/L) incubated in media with microalgae *Dunaliella salina* (24 h) we observed increase of mussel filtration rate measured by shell opening (valve gape) using Arduino microcontroller and Hall sensors-magnets glued to mussel shells (Figure 6.4.5)

Figure 6.4.5. Valve Gaping Mussel Monitor (VGMM) with 6 sensors/mussels and Arduino microcontroller VG Hall sensors connection schema.

Analysing and comparing VG results before and after mussel exposure to MPs, including controls, it was possible to observe mussels VG normal daily rhythm (> 70% time filtrating with valve open > 50%, with 1-5 resting periods valve open < 20% and occurrence < 30%) and behavior changes comparing exposed vs control mussels (Figure 6.4.6).

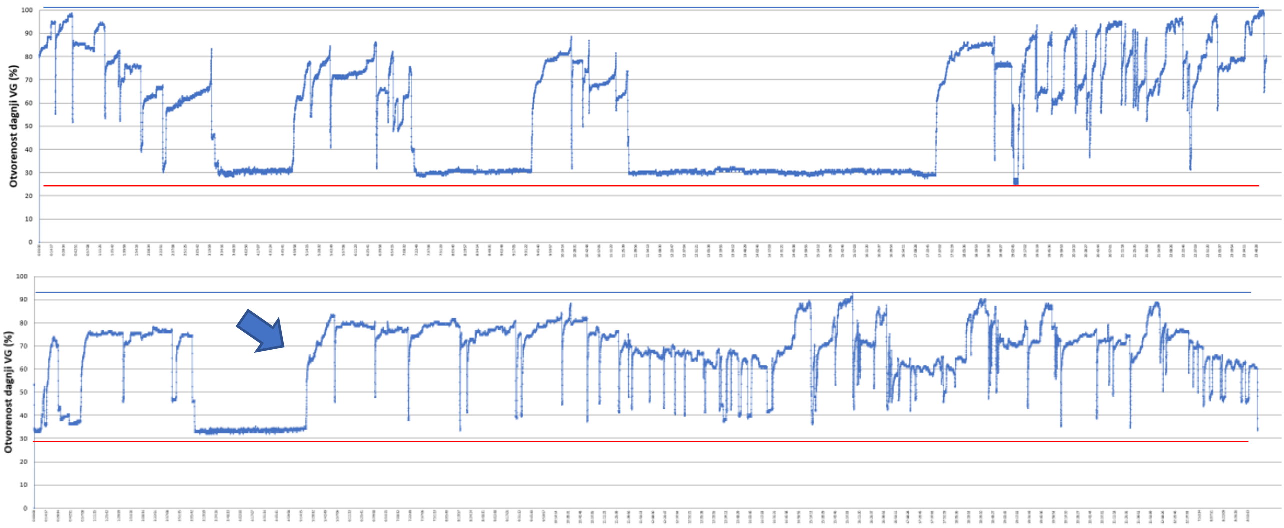


Figure 6.4.6. Real time observation of mussel filtration behavior using valve gaping (VG) mussel monitor: A) normal mussel filtration (24 h, 9:00 – 9:00 h) and B) mussel exposed to model MP (cigarette butts fibres) incubated with microalgae *D. salina*. (blue arow indicated time of addition MP to aquaria, the VG values between red and blue lines represents mussel shell min – max opening)

Field Studies and Real-World Implications

Field studies help identify the types and sources of microplastics that mussels are exposed to. Understanding the sources of contamination is crucial for mitigation and regulatory efforts. Mussels in the field have demonstrated adaptive responses to microplastic exposure over time, such as changes in feeding behavior and the development of protective mechanisms. These adaptations can provide insights into potential resilience strategies for mitigating microplastic impacts.

Conclusion

The determination of biological effects and mussel filtration-feeding behavioral changes after in vitro and field microplastic exposure is essential for understanding the ecological consequences of microplastic pollution in marine ecosystems. While in vitro studies provide controlled environments for uncovering mechanistic insights, field studies offer a real-world perspective on how microplastics affect mussel populations and, by extension, the broader marine ecosystem. Combining the results of both types of research enables scientists and policymakers to make informed decisions regarding the mitigation and management of microplastic pollution, ultimately safeguarding the health of our oceans and marine life. Further research in this area remains critical to fully grasp the scope of microplastic-related challenges and develop effective strategies for addressing them.

D6.4.4 In vitro and in situ mussel MP water column purification efficiency determination

The pervasive issue of microplastic pollution in marine environments has prompted innovative research into potential natural solutions for mitigating this problem. Mussels, with their exceptional filtration capabilities, have emerged as promising agents in the removal of microplastics from the water column. This article explores the methodologies and findings related to the determination of mussel microplastic purification efficiency, both in vitro (laboratory-based) and in situ (field-based) settings.

To achieve the goals: A) Water column microplastics monitoring and B) purification - removing microplastics from the water column and improving local marine environmental quality, two types of MMPP installations were designed and applied for in situ monitoring (MMP-M; Figure 6.4.7) and environment purification (MMP-P).

Figure 6.4.7. Mussel MicroPlastic Monitoring installation (MMP-M).

MMP-M installation is actually device for mussels' pseudo/feces and natural suspended mater, including MPs collection in the water column at chosen depth (20 m). The MMP-M installation is modified funnel construction with mussels (cca 1 kg) on top, hanging on aquaculture rope with buoy on surface and concrete anchor on bottom.

Mussels by filtration concentrate and remove suspended matter and microplastic from sea water column. Using sophisticated gill structures mussels further proceed, separate food (microalgae) from other particles. One part of filtered particles is ingested, digested and excreted as feces usually after > 24 h, and the rest is excreted as mucous pseudofeces (immediately or up to 6 h). Usually, only nanoplastic is passing the intestine barrier and spreading to other tissues (digestive gland, muscle etc.). Anyhow, collected mussels' generated feces and pseudofeces represent concentrated content of microplastics in water column, while one single mussel can filtrate 1-5 L sea water per hour.

Chemical and MicroPlastic Analyses of coastal areas

- Sediment Chemical Analyses

Before the upcoming commissioning of the 3rd stage of municipal wastewater treatment (UPOV Cuvi, Rovinj), marine sediments in the immediate vicinity of the underwater outlet were sampled to determine the initial state of their quality. The surface sediment (< 3 cm) was sampled in May 2022 with a grab at the control location RV001 (S6) 1 NM away from the town of Rovinj, and stations 50 m (S7), 200 m (S8) and 1000 m (S9) distance from the outlet. After analysing the grain size, water content and total organic carbon in the sediment, the analysis of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn), 16 polycyclic aromatic hydrocarbons (PAHs) and total cyclic chlorinated biphenyls (PCBs) was conducted. Additionally, to determine the effect of sediments on biota, a potential toxicity analysis was made by calculating ΣQN1-cumulative risk quotient and QPECm – average risk quotient according French sediment quality guideline (SQGs) regulation (Table 6.4.1).

Table 6.4.1. Results of chemical analyses of Rovinj coastal area marine sediments evaluated according French SQGs legislation (N1 and N2 levels; Q_{PECm}).

Parameters (units)	Sampling sites - 2022									N1	N2
	S1 Harbour	S2 Shipyard	S3 Lim out	S4 Lim middle	S5 Open Sea	**S6 RV001**	**S7 Cuvi 1**	**S8 Cuvi 2**	**S9 Cuvi 3**	**Legal level**	**Legal level**
As / (mg/kg d.w.)	7,90	6,50	5,90	8,20	4,40	4,10	7,10	6,50	5,00	**25,00**	**50,00**
Cd / (mg/kg d.w.)	0,08	0,07	0,08	0,07	0,09	0,05	0,12	0,09	0,07	**1,20**	**2,40**
Cu / (mg/kg d.w.)	25,00	17,00	15,00	16,00	13,00	6,50	15,00	13,00	10,00	**45,00**	**90,00**
Ni / (mg/kg d.w.)	16,00	18,00	30,00	35,00	12,00	8,40	20,00	17,00	17,00	**37,00**	**74,00**
Pb / (mg/kg d.w.)	26,00	15,00	21,00	24,00	11,00	9,00	21,00	13,00	13,00	**100,00**	**200,00**
Zn / (mg/kg d.w.)	47,00	37,00	62,00	68,00	43,00	34,00	66,00	49,00	45,00	**276,00**	**552,00**
Hg / (mg/kg d.w.)	0,30	0,20	0,10	0,10	0,05	0,06	0,20	0,10	0,10	**0,40**	**0,80**
Cr / (mg/kg d.w.)	26,00	16,00	39,00	47,00	19,00	19,00	30,00	29,00	28,00	**90,00**	**180,00**
ΣPAHs / (mg/kg d.w.)	**3,18***	**3,64***	0,11	0,04	0,02	0,07	0,07	0,04	0,04	**1,50**	**15,00**
ΣPCBs / (mg/kg d.w.)	0,33	**0,89***	0,03	0,10	0,10	0,10	0,10	0,10	0,10	**0,50**	**1,00**
ΣQ_{N1}	**5,61**	**6,35**	**2,69**	**3,17**	**1,69**	**1,40**	**2,78**	**2,19**	**2,02**	-	-
Q_{PECm}	**0,56**	**0,64**	**0,27**	**0,32**	**0,17**	**0,14**	**0,28**	**0,22**	**0,20**	-	-

The measured concentrations of heavy metals, individual and total PAHs, and total PCBs in all samples near the Cuvi outlet and control location S6 are in the range of natural content concentrations. Furthermore, the concentrations of all pollutants are below the threshold ERL (Effect of low range 10 percentile) and TEL (Threshold effect level - rarely) values (Official Gazette 28/2021), i.e., their concentrations do not exceed the threshold values (N1 and N2) of the guidelines for the quality of sediments (SQGs) identification of the French regulation.

All examined samples belong to the first category of marine sediments. Ecological risk assessment and probability of toxic effect determined based on QPECm (< 1) average risk quotient indicate that the mentioned sediments, considering the conducted chemical analyses of pollutants, represent a good environmental status and that there is no ecological risk for the environment and organisms.

- Sediment Microplastics Analyses

Results of large and small MP analysis of investigated sediments indicating higher MP abundance in Rovinj sediments (Cuvi 1, Cuvi 2, Cuvi 3 and control RV001) comparing to Dubrovnik coastal area (Pelegrin, control Orašac) sites, as well as MP fragment distribution (Figure 6.4.8).

Figure 6.4.8. Presence of large and small MP with fragments analysis in investigated sediments of Rovinj (Cuvi 1, Cuvi 2, Cuvi 3 and RV001) and Dubrovnik coastal area (Pelegrin, Orašac) sites.

- Sea Surface Microplastics Analyses

Results of surface MP analysis using Manta net methodology indicating higher MP abundance at location in vicinity of wastewater treatment plant outlet (UPOV Cuvi) in comparison to control RV001 site as well as of Dubrovnik coastal area (Pelegrin, control Orašac) sites (Figure 6.4.9, 6.4.10).

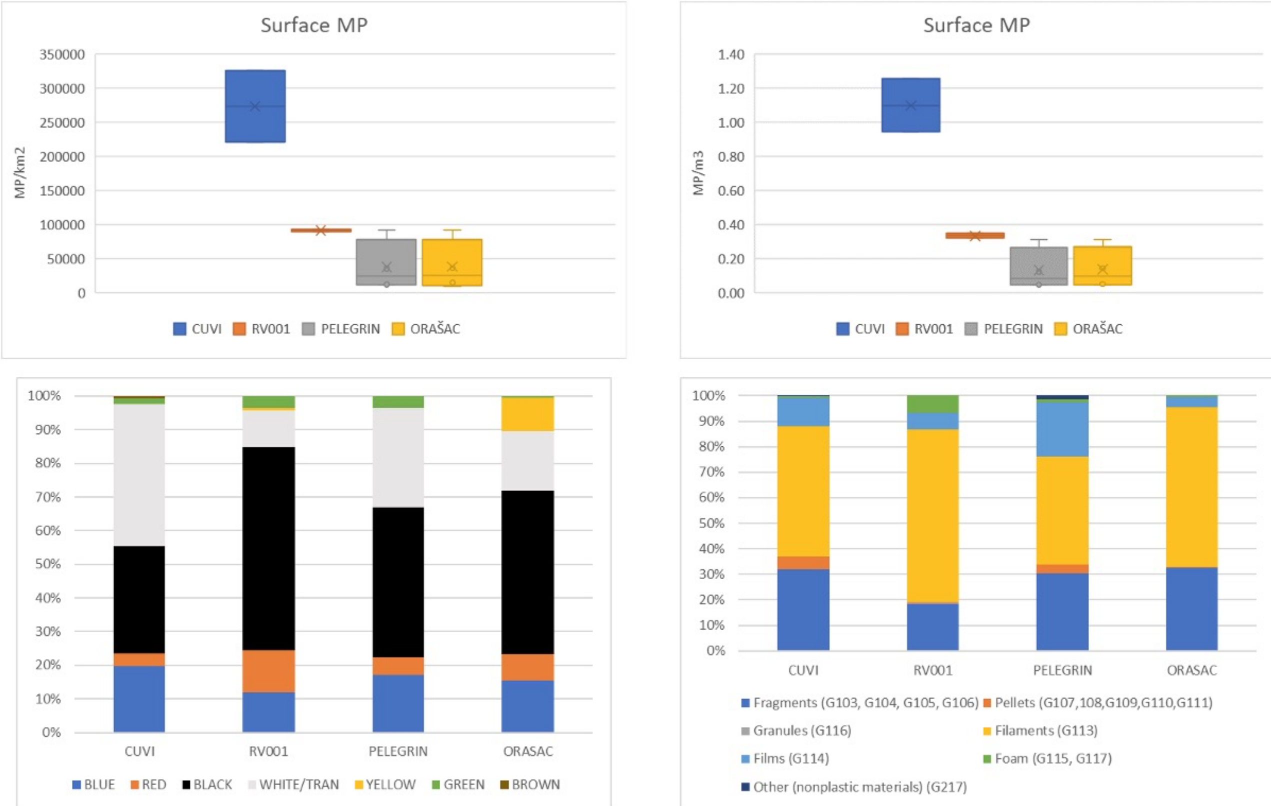


Figure 6.4.9. Results of surface MP analysis at investigated Rovinj (Cuvi, RV001) and Dubrovnik (Pelegrin, Orašac) location using standard Manta net methodology.

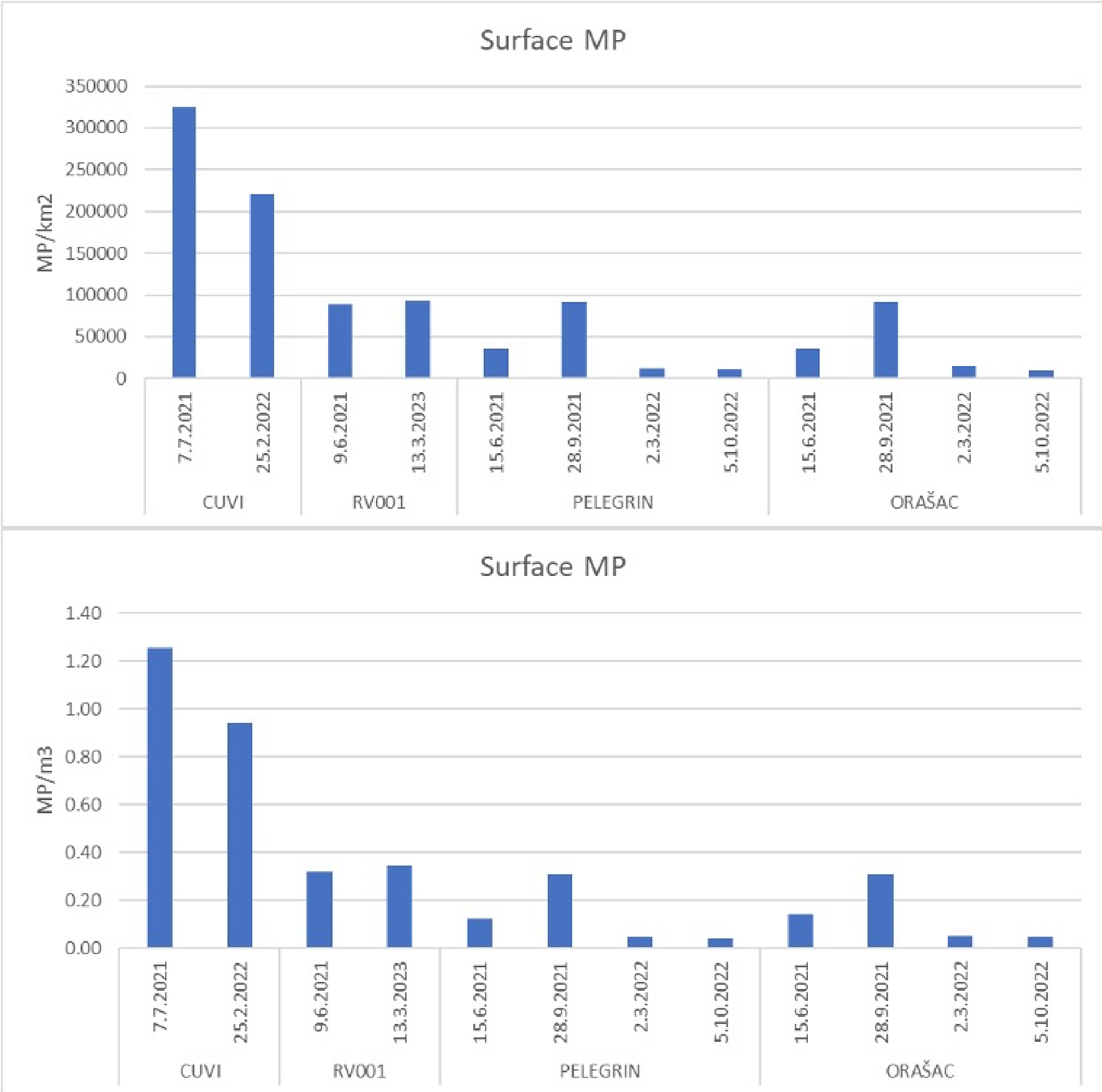


Figure 6.4.10. Results of surface MP analysis at investigated Rovinj (Cuvi, RV001) and Dubrovnik (Pelegrin, Orašac) location using standard Manta net methodology at different seasons.

- Biota Microplastics Analyses

Results of LMP analysis of biota (mussels *Mytilus galloprovincialis* from MMP-P installations) by frequency of MP positive samples methodology indicating higher abundance of MP positive mussels at location in vicinity of wastewater treatment plant outlet (Cuvi 1, Cuvi 2) comparing to control RV001 site as well as of Dubrovnik coastal area (Pelegrin, control Orašac) sites. In contrary, results of SMP analysis of biota (mussels from MMP-P installations) by frequency of MP positive samples methodology indicating less differences between investigated sites of Rovinj (Cuvi 1, Cuvi 2, Cuvi 3) comparing to control RV001 site as well as of Dubrovnik coastal area (Pelegrin, control Orašac) sites (Figure 6.4.11, 6.4.12, 6.4.13).

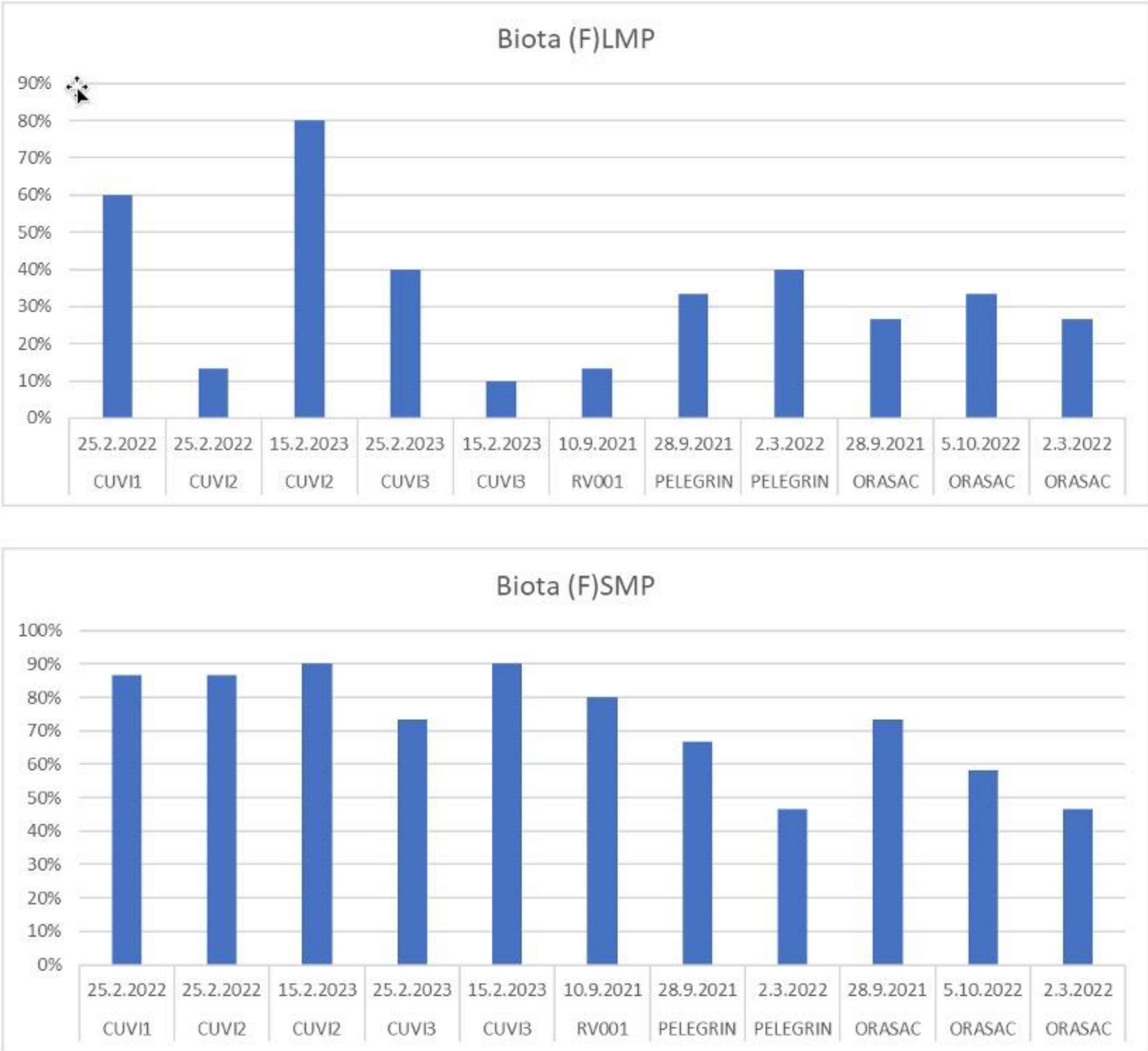
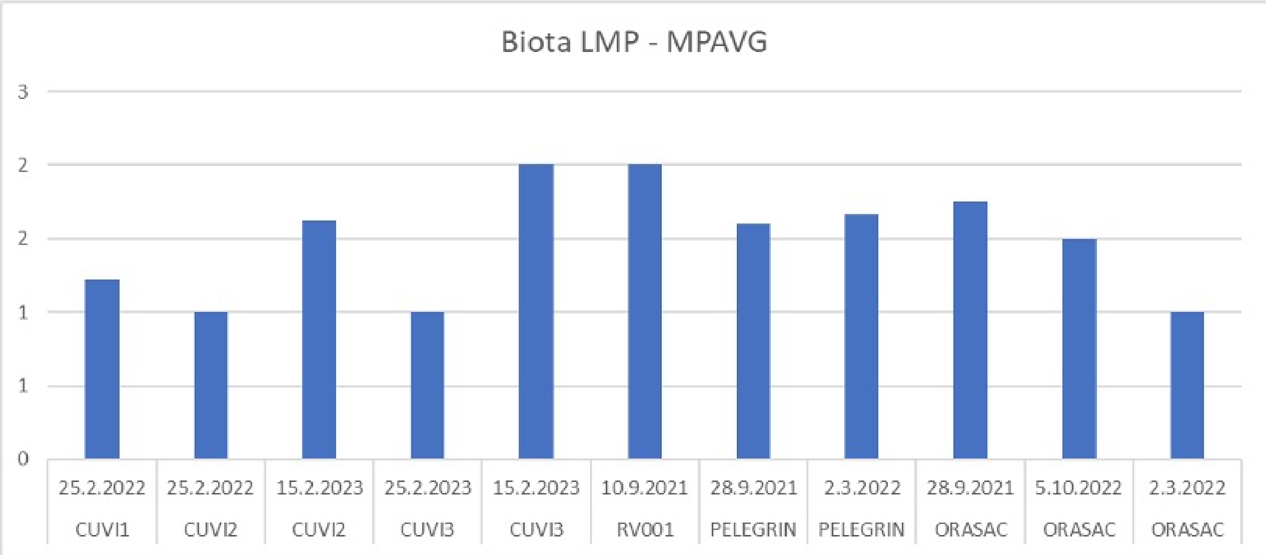


Figure 6.4.11. Results of SMP and LMP analysis of mussels *M. galloprovincialis* from MMP-P installations by frequency of MP positive samples methodology.



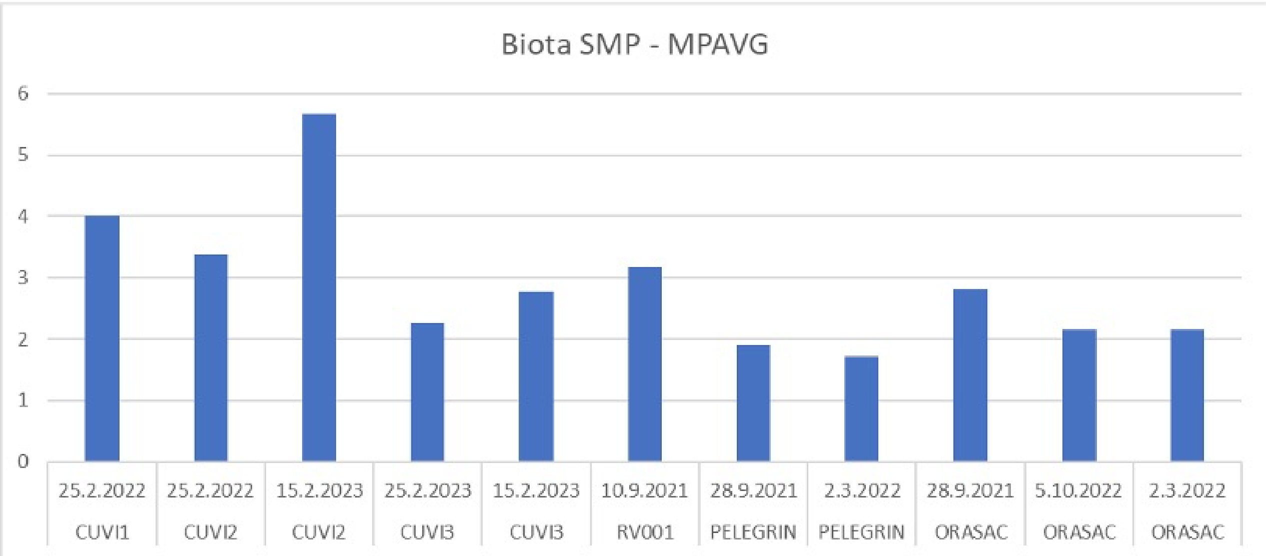


Figure 6.4.12. Results of SMP and LMP analysis of mussels *M. galloprovincialis* from MMP-P installations by frequency of Average MP positive mussel specimens methodology.

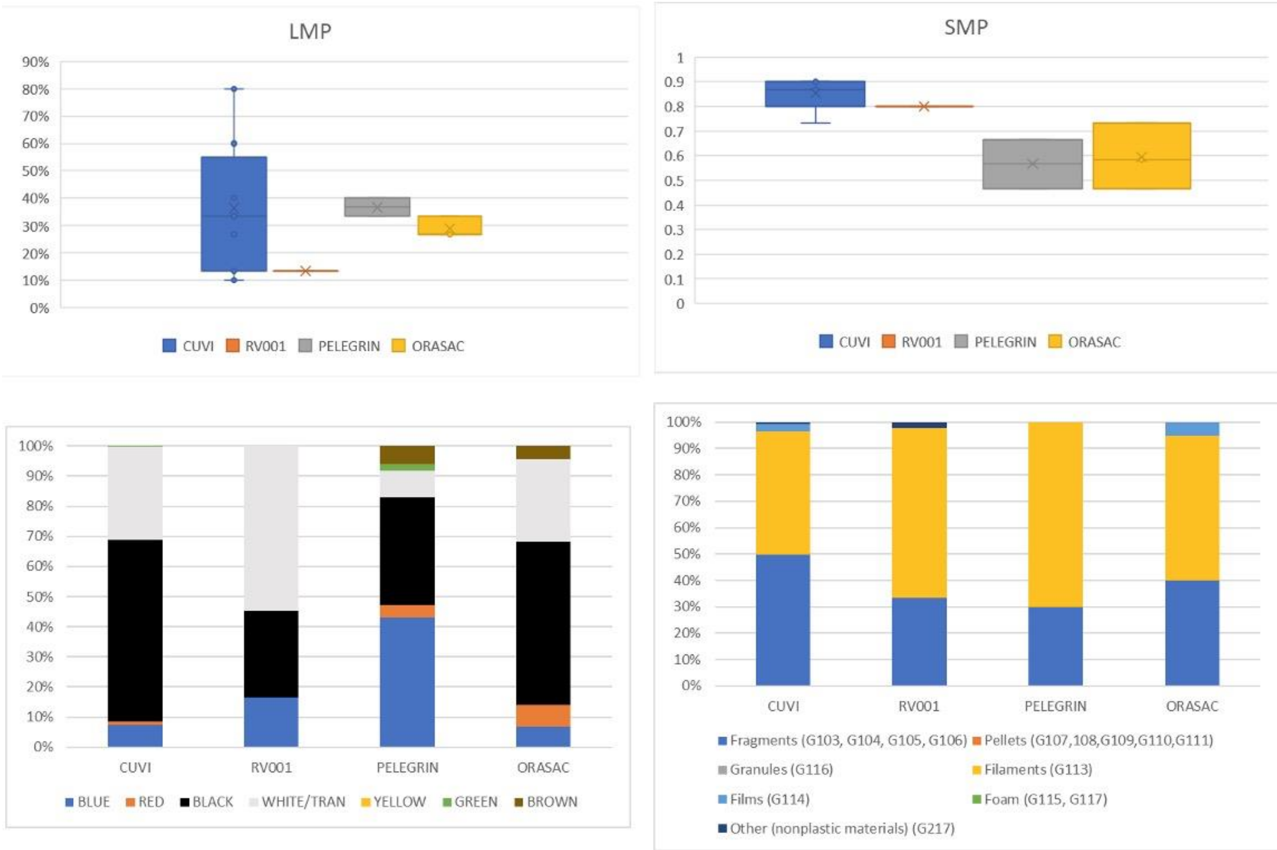


Figure 6.4.13. Results of SMP – LMP, colour and structure-shape analyses of mussels *M. galloprovincialis* from MMP-P installations by abundance methodology.

- MMP-M Mussel Pseudo/Feces/Suspended Matter Microplastics Analyses

Results of MP analysis of biota (mussels *Mytilus galloprovincialis* from MMP-P installations) by frequency of MP positive samples methodology indicating higher abundance of MP positive mussels at location in vicinity of wastewater treatment plant outlet (Cuvi 1, Cuvi 2) comparing to control RV001 site as well as of Dubrovnik coastal area (Pelegrin, control Orašac) sites. In contrary, results of SMP analysis of biota (mussels from MMP-P installations) by frequency of MP positive samples methodology indicating less differences between investigated sites of Rovinj (Cuvi 1, Cuvi 2, Cuvi 3) comparing to control RV001 site as well as of Dubrovnik coastal area (Pelegrin, control Orašac) sites (Figure 6.4.14, 6.4.15, 6.4.16, 6.4.17 and 6.4.18).

Figure 6.4.14. MMP-M installation in “action” collecting MPs and suspended matter by mussel filtration.

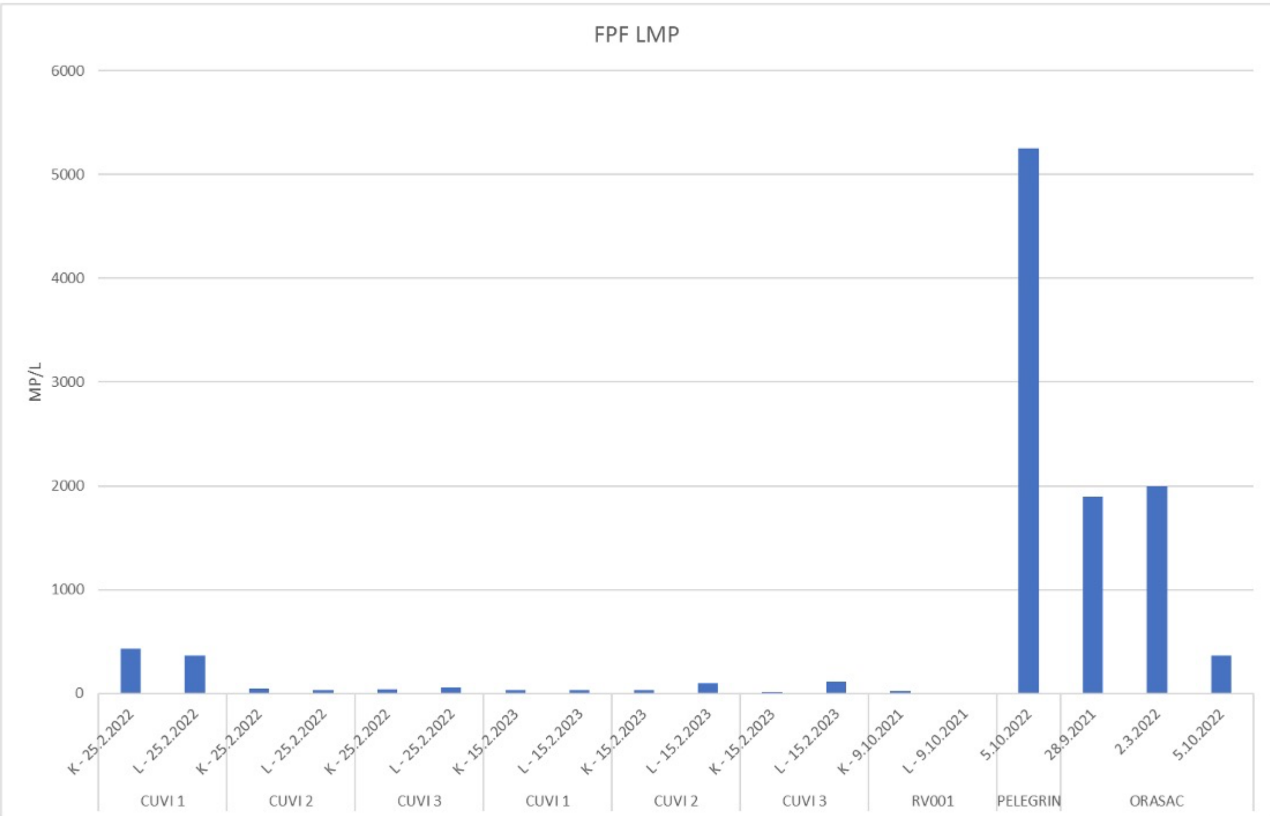


Figure 6.4.15. Results of SMP analyses of mussels suspended matter (mussel pseudo/feces collector) from MMP-P installations by abundance methodology (K – collector, L – funnel).

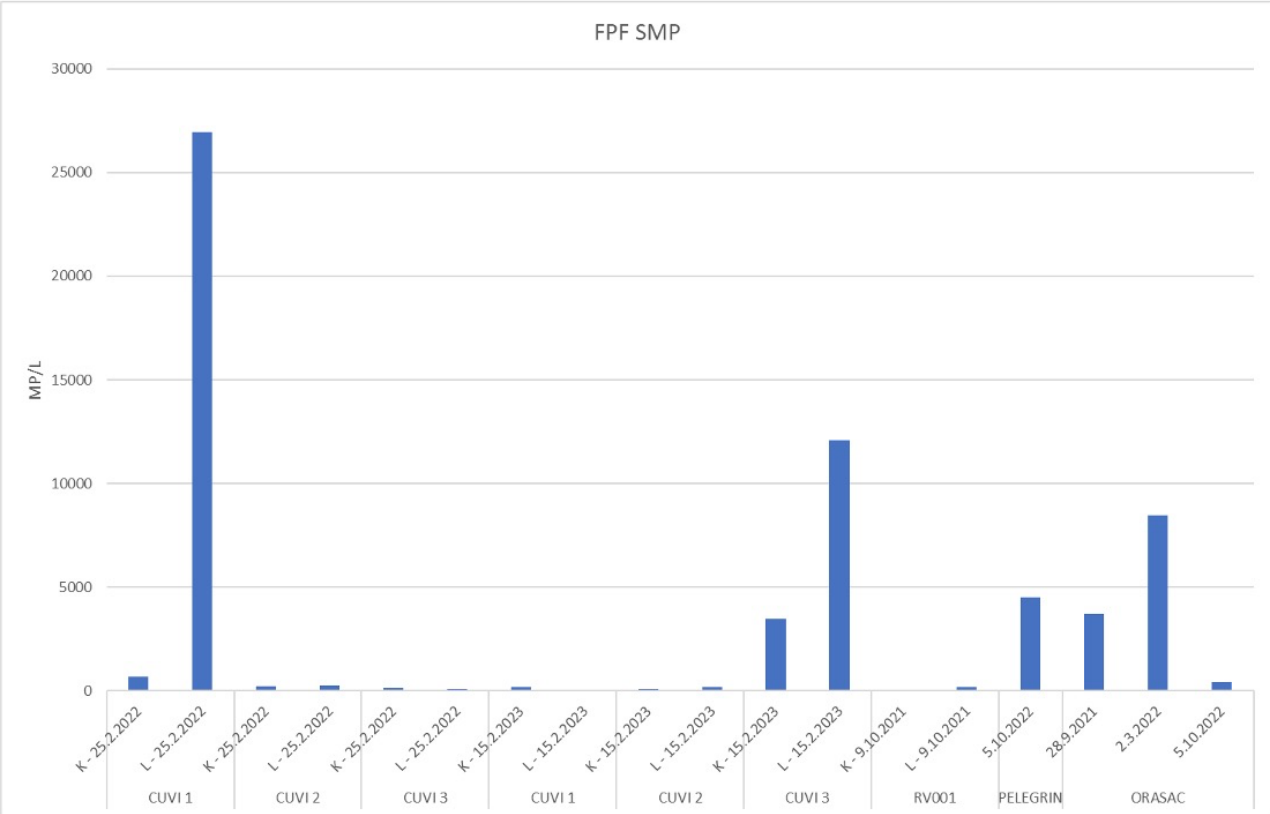


Figure 6.4.16. Results of LMP analyses of mussels suspended matter (mussel pseudo/feces collector) from MMP-P installations by abundance methodology (K – collector, L – funnel).

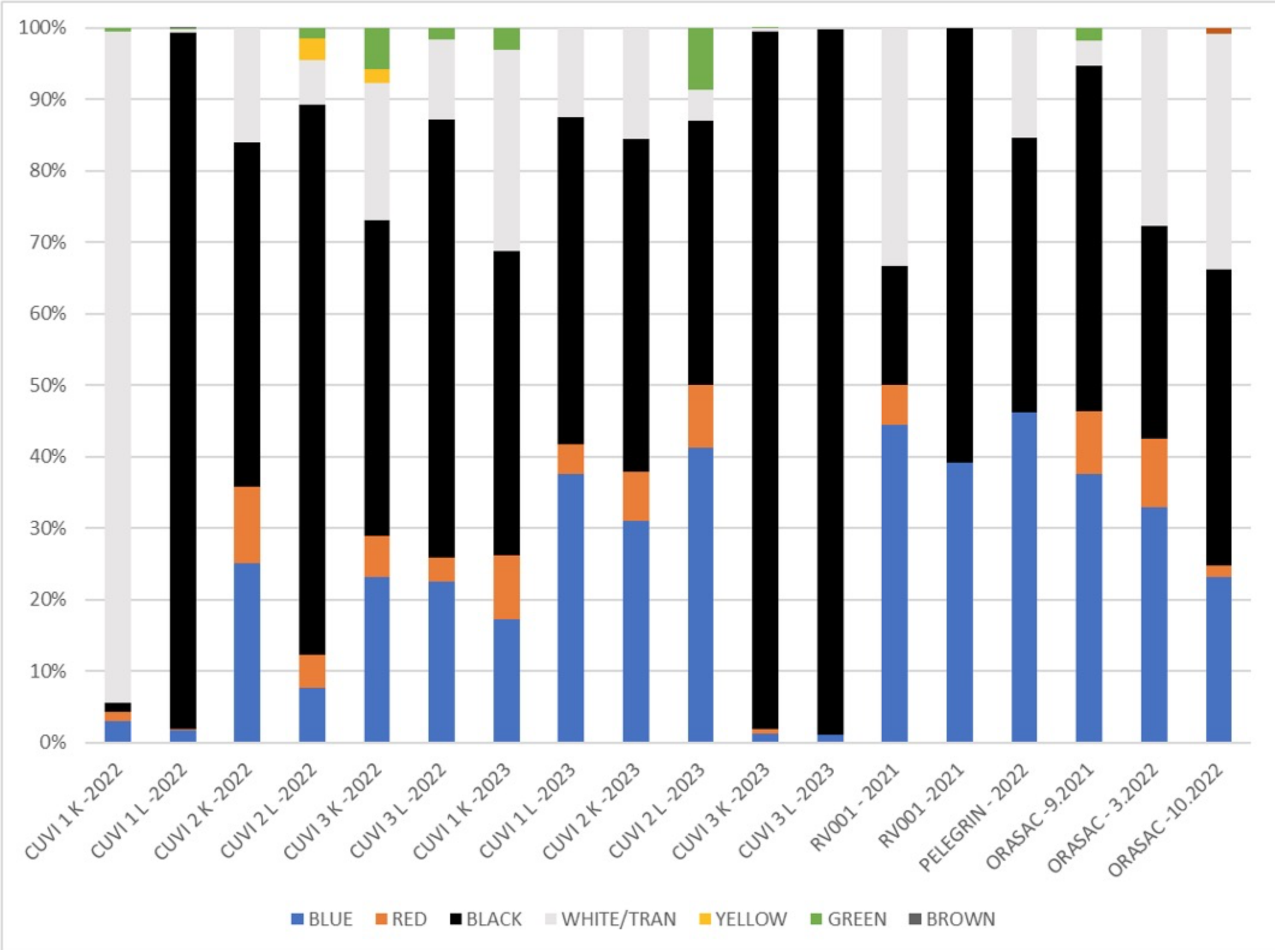


Figure 6.4.17. Results of colour MP analyses of mussels suspended matter (mussel pseudo/feces collector) from MMP-P installations by abundance methodology.

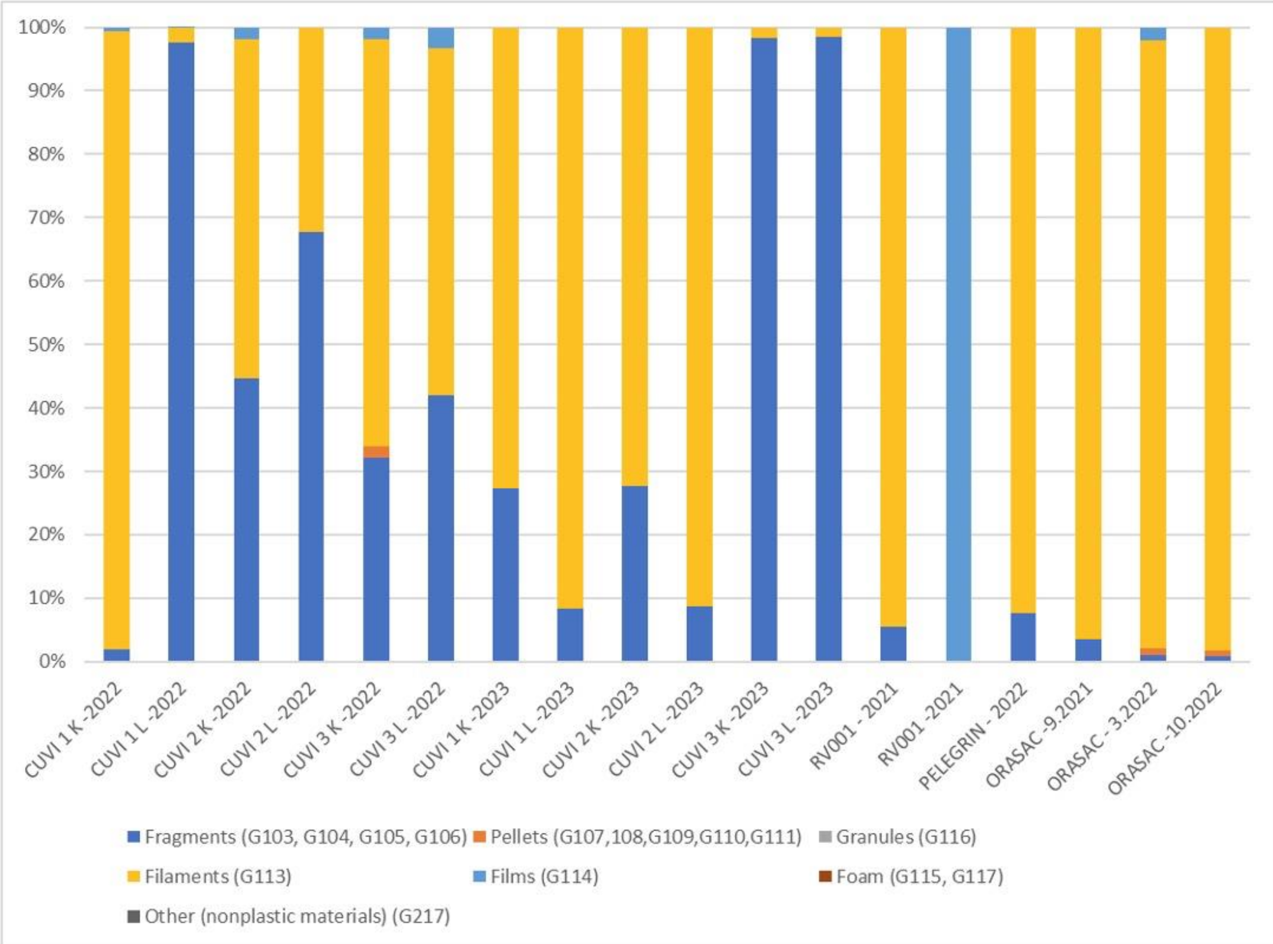


Figure 6.4.18. Results of structure-shape MP analyses of mussels suspended matter (mussel pseudo/feces collector) from MMP-P installations by abundance methodology.

Comparative Analysis and Implications

Comparing in vitro and in situ results is essential for understanding the full extent of mussel microplastic purification efficiency. In vitro studies offer controlled data on filtration rates and microplastic uptake but may not fully capture the complex dynamics of natural environments. In situ studies provide real-world context but may involve confounding factors that make precise measurements challenging with lot of possible problems from weather conditions up to equipment-personnel demand.

The implications of these studies extend beyond mussel biology. They inform strategies for using mussels as natural microplastic removal agents, support the development of conservation efforts, and contribute to a broader understanding of microplastic pollution in aquatic ecosystems. Further research is needed to refine methodologies, investigate long-term effects, and assess the potential ecological consequences of mussel-based microplastic mitigation strategies.

Conclusion

In vitro and in situ studies of mussel microplastic water column purification efficiency play a critical role in addressing the global challenge of microplastic pollution in marine environments. By combining laboratory precision with real-world applicability, these research methodologies offer valuable insights into the potential of mussels as eco-friendly sentinels against microplastic contamination. As the scientific community continues to advance in this field, the findings will guide conservation efforts, inform policy decisions, and contribute to a more sustainable and resilient marine ecosystem.

D6.4.5 Wide application of Mussel MicroPlastic – Purification installations (MMP-P) as a potential suitable tool for removing microplastics from the water column and improving marine environmental quality

By performed WP 6.4 - D6.4.5 activities our intention was to improve (bioremediation) the marine environment, e.g. location in vicinity of Rovinj municipal waste water treatment plant Cuvi outlet and preventing further spreading of MPs.

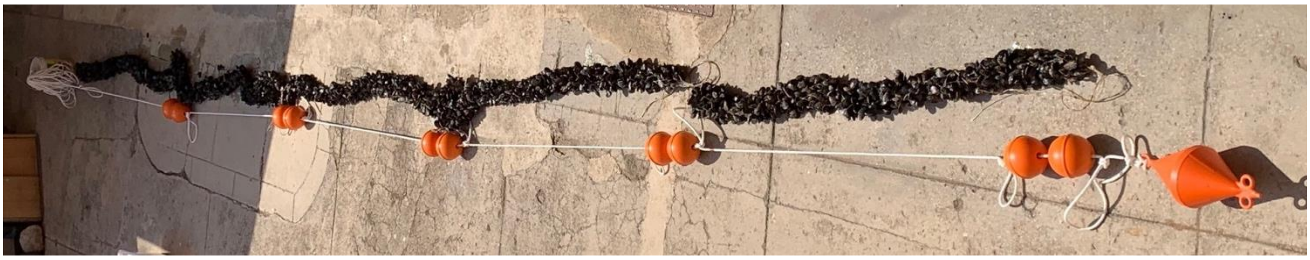


Figure 6.4.19. Mussel MicroPlastic Purification installation (MMP-P).

MMP-P installation is a device for the whole water column purification by mussels' filtration, which is enhancing the natural local sedimentation of suspended matter (POM), as well as MPs and preventing its further spreading. The MMP-P is modified mussel mariculture production installation where mussels in nets (cca 1000 mussels; 5 x 20 kg) are hanging at several depth (5, 10, 15, 20 and 25 m) on aquaculture rope with buoy on surface and concrete anchor on bottom (Figure 6.4.19).

Deliverable location description

Three MMPP installations, with ca 1000 mussels weighting ca 100 kg each, were set up *in situ* in the gradient vicinity of municipal wastewater treatment plant (UPOV Cuvi) at site S7, S8 and S9 (Figure 6.4.6 and 6.4.7) for the final demonstration of the environment purification - bioremediation (August 2022 – May 2023) (Figure 6.4.20 and 6.4.21).

Figure 6.4.20. Occasionally biofouling check and cleaning of MMP-M installations at Cuvi locations. Harvesting of produced mussels at the end of bioremediation period.

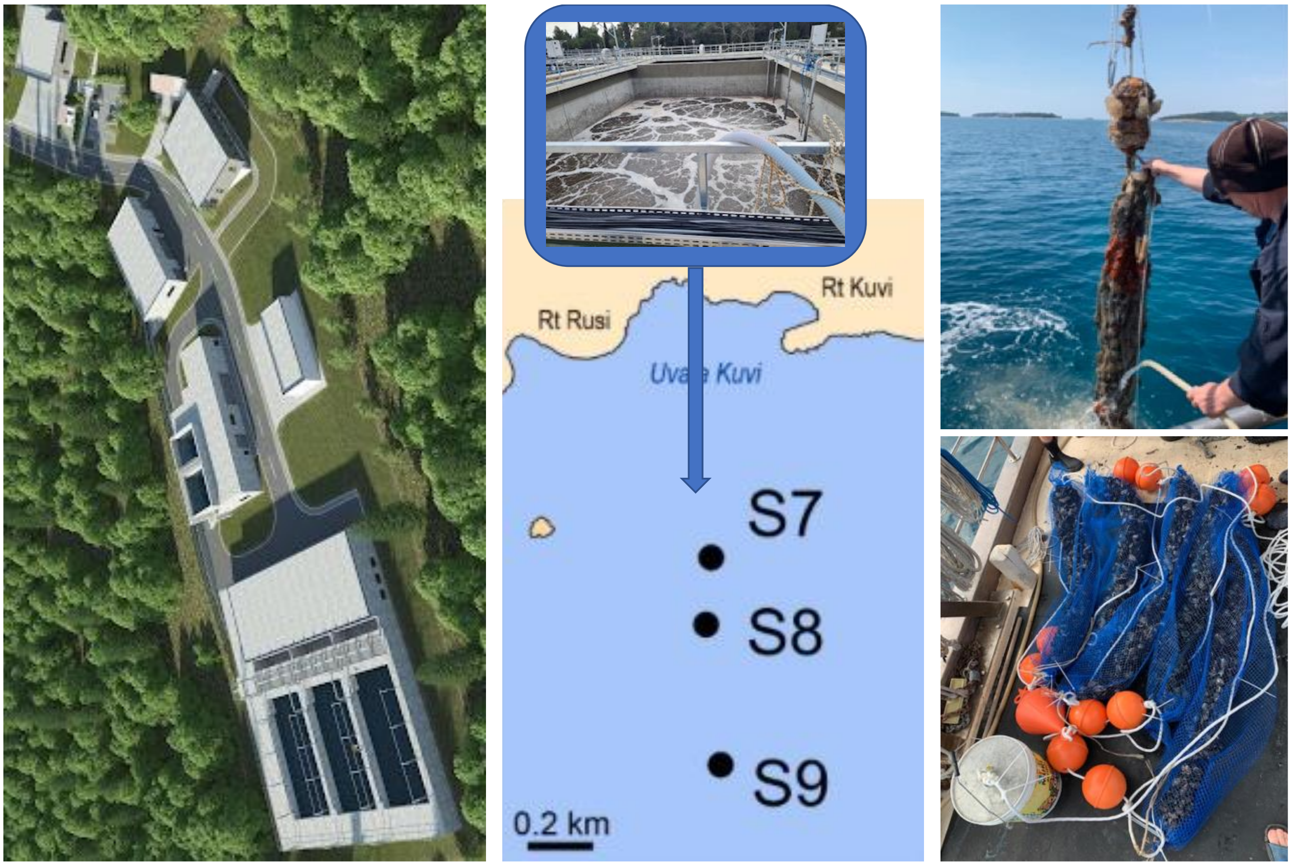


Figure 6.4.21. Map of Rovinj coastal area with MMP-P applied sites (S7, S9, S9) in vicinity of municipal wastewater treatment plant (UPOV Cuvi) outlet (August 2022 - May 2023).

This research was done to investigate the potential of Mediterranean mussels (*Mytilus galloprovincialis*) filtration function as a sustainable tool for water column microplastic purification in field, after demonstrating the concept under the laboratory conditions. The main idea was to take advantage of the effective mussel Microplastic (MP) filtration and sedimentation properties of produced feces and pseudofeces particles, to collect and remove MP from the water column. Therefore, one of the main goals of this research in addition to laboratory testing, was the development of a standardized method/installation that could be used in the natural environment for *in situ* sea water column MPs purification and monitoring purposes, at various locations along the Adriatic coast.

The marine environment purification and contaminants spreading prevention using MMP-P installations can be performed any time. Mussels are filtrating the seawater column all the time - working 24 h per day, seven days per week, etc. Occasionally, each two months it is necessary to check the installation integrity or clean it from biofouling – overgrowth to keep mussel filtration efficacy.

By laboratory experiments we proved the concept of marine environment purification using mussel filtration. Beside environment water column purification and microplastic (MPs) spreading, mussels produced outside shellfish farms can be used further as valuable biomass, including by-products (shells, byssus, fluids) in zero waste circular economy.

Plastic pollution has attracted a lot of attention from scientists and the general public over the last decade. It has been shown that plastic waste has a major economic and environmental impact, especially on the marine biota. Plastic waste opposes mechanical and chemical threat to all living organisms; therefore, regular monitoring is crucial for understanding the abundance of plastic waste and determining the effectiveness of measures to limit or reduce plastic in the environment.

The produced mussel biomass can be further investigated as a valuable source, for e.g. mussel meal production (fish feeding trials e.g. BlueBio project - MuMiFaST) with further potential usage of other by-products (shells, byssus, fluids, etc.).

In general Mussel MicroPlastic – Purification (MMP-P) installations for water column purification in present or modified form can be further exploited as a natural cheap system for bioremediation of marine environment using mussel filtration and ecosystem services they provide.

3. FINAL CONCLUSIONS

The use of mussels as a tool for water column microplastic purification is a promising and innovative approach to combat the escalating issue of microplastic pollution in marine environments. Extensive research, both in vitro and in situ, has provided valuable insights into the potential of these filter-feeding bivalves to act as natural sentinels against microplastic contamination. The following conclusions can be drawn from the current state of knowledge:

Mussels are Efficient Filter Feeders: Mussels exhibit exceptional filtration capacities, processing large volumes of water and capturing microplastics in the process. In vitro experiments have demonstrated their ability to significantly reduce microplastic concentrations in controlled environments.

Natural Purification Mechanisms: Mussels exhibit natural mechanisms for the removal of microplastics from the water column, including filtration, ingestion, and egestion as pseudofeces and feces. These mechanisms help prevent the ingestion of microplastics by other marine organisms and contribute to reducing the abundance of microplastics in marine ecosystems.

In Situ Studies Confirm Efficacy: Field-based studies have provided evidence that mussels can effectively reduce microplastic concentrations in their natural habitat. However, the efficiency of this process may vary depending on factors such as mussel density, environmental conditions, and microplastic sources.

Complex Ecological Considerations: While mussels hold great promise, their use as a microplastic purification tool should be considered within the broader ecological context. Factors like mussel health, population dynamics, and potential bioaccumulation of microplastics within mussel tissues require further investigation.

4. RECOMMENDATIONS

Based on results of performed activities and the current understanding of using mussels for water column microplastic purification, the following recommendations are put forth:

Further Research and Monitoring: Continued research efforts are essential to better understand the long-term effects of mussel-based microplastic removal, including potential impacts on mussel populations and the broader ecosystem. Longitudinal studies are needed to assess the sustainability and ecological consequences of mussel filtration.

Sustainable Mussel Farming Practices: The cultivation of mussel populations for microplastic removal should adhere to sustainable farming practices to prevent overharvesting and habitat disruption. Regulatory frameworks should be developed to guide mussel farming initiatives.

Integrated Approaches: Mussels should be considered as part of integrated strategies to combat microplastic pollution, alongside reduction at the source, improved waste management, and public awareness campaigns.

Ecosystem-Based Management: Microplastic mitigation strategies involving mussels should be tailored to specific ecosystems, taking into account the unique ecological characteristics and challenges of each environment.

Public Engagement and Education: Public awareness and engagement initiatives should be developed to highlight the ecological importance of mussels and promote responsible behaviors that reduce microplastic pollution.

In conclusion, the use of mussels as a tool for water column microplastic purification represents an ecologically sound and innovative approach to addressing a critical environmental challenge. While it is not a panacea, ongoing research, responsible management practices, and a holistic understanding of mussel-mediated microplastic removal can contribute to cleaner and healthier marine environments. These recommendations provide a foundation for further exploration and implementation of mussel-based strategies in the global effort to combat microplastic pollution.

Goals achieved- Activity deliverables:

- D6.4.1 Demonstration of mussels' filtration activity as an ecological approach for microplastic (MP) removal from the marine environment,
- D6.4.2. Mussels' pseudofeces/feces generation and natural sedimentation as a plastic pump in coastal waters - Report,
- D6.4.3 Determination of biological effects and mussel filtration-feeding behavioral changes after in vitro and field MP exposure,
- D6.4.4 *In vitro* and in *situ* mussel MP water column purification efficiency determination,
- D6.4.5 Wide application of Mussel MicroPlastic Purification installations (MMP-P) as a potentially suitable tool for removing microplastics from the water column and improving marine environmental quality.

5. Media coverage:

https://www.glasistre.hr/istra/2023/04/11/dagnje-mogu-ocistiti-more-od-zagadenja-mikro-plastikom-pa-cak-i-od-opusaka-cigareta-856752

https://www.odvodnjarovinj.hr/hr/article/upov-cuvi-posjetili-znanstvenici-sa-instituta-rudjer-boskovic-iz-rovinja-268/

Presentation of MMP-P concept, workshop and lecture for general public - Demonstration of marine environment bioremediation using cultivated mussels *M. galloprovincialis*, and possible fitness monitoring of endangered species e.g. Fan mussel *Pinna nobilis*, Komunalac Fažana d.o.o. 27.03.2023, Fažana
https://www.facebook.com/100063472003918/posts/pfbid02H61gicjEHjoFnCGkuXaDVKR9XCiwB2p7dtNDrjFndsUwrHWc3rz4WiKxdFwaVFNKl/