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MARLESS (MARine Litter cross-border awareN ESS and innovation actions)

Priority Axis: Environment and cultural heritage; Specific objective: 3.3 - Improve the environmental quality conditions of the sea and coastal area by use of sustainable and innovative technologies and approaches

Pilot Actions to prevent, recover, process marine litter, including Stakeholders Toolbox

WP 6

AT 6.4

Use of mussels as suitable tool for water column microplastic purification

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PROJECT MARLESS

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Activity:	AT 6.4 Marine Litter Adriatic Toolbox on Pilot actions
WP Leader:	PP6 Ruđer Bošković Institute
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CONTENT:

ToolBox:

Use of mussels as suitable tool for water column microplastic purification

-Activity 6.4-

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 and 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.

1.1 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).



Figure 6.4.1. Investigated area of Rovinj coastal area (S1, S6) and area in vicinity of Rovinj municipal wastewater treatment plant Cui outlet (S7, S8, S9).

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 microfibrils) formed by cigarette butts decomposition were performed (Figure 6.4.3).

Prove of concept – mussels as purificator of marine environment

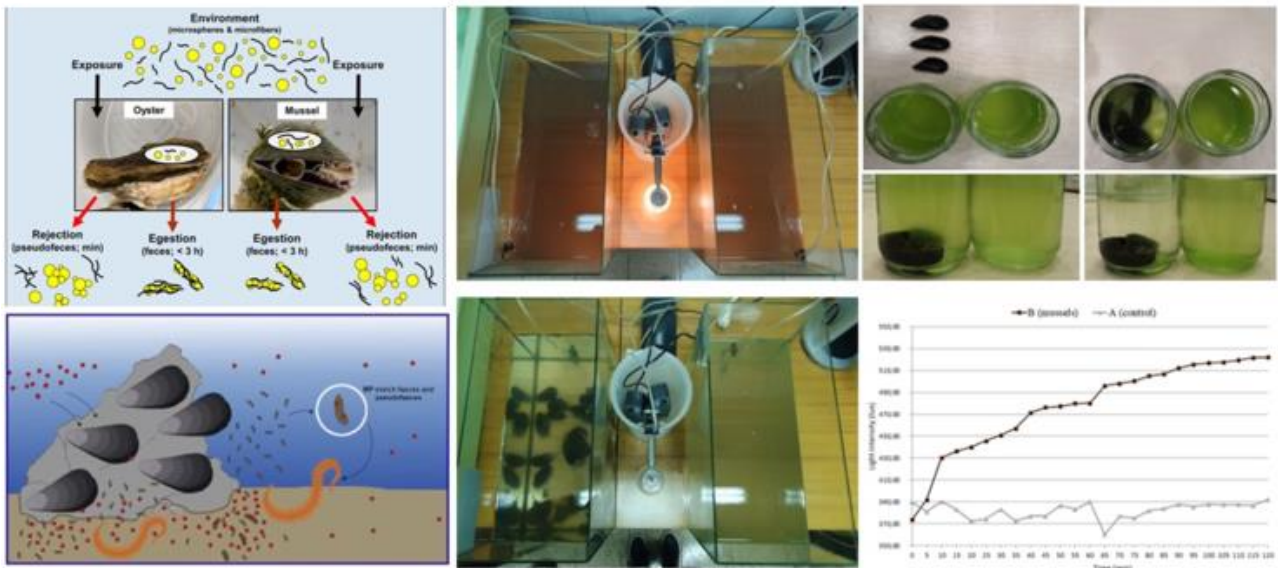


Figure 6.4.2. Demonstration of purification efficiency of mussel's filtration. The decreased turbidity over time up to 2 h measured as light intensity by Hobo data loggers MX2022, as well as optical difference between A) aquaria without and B) aquaria with mussels are direct proof of concept.

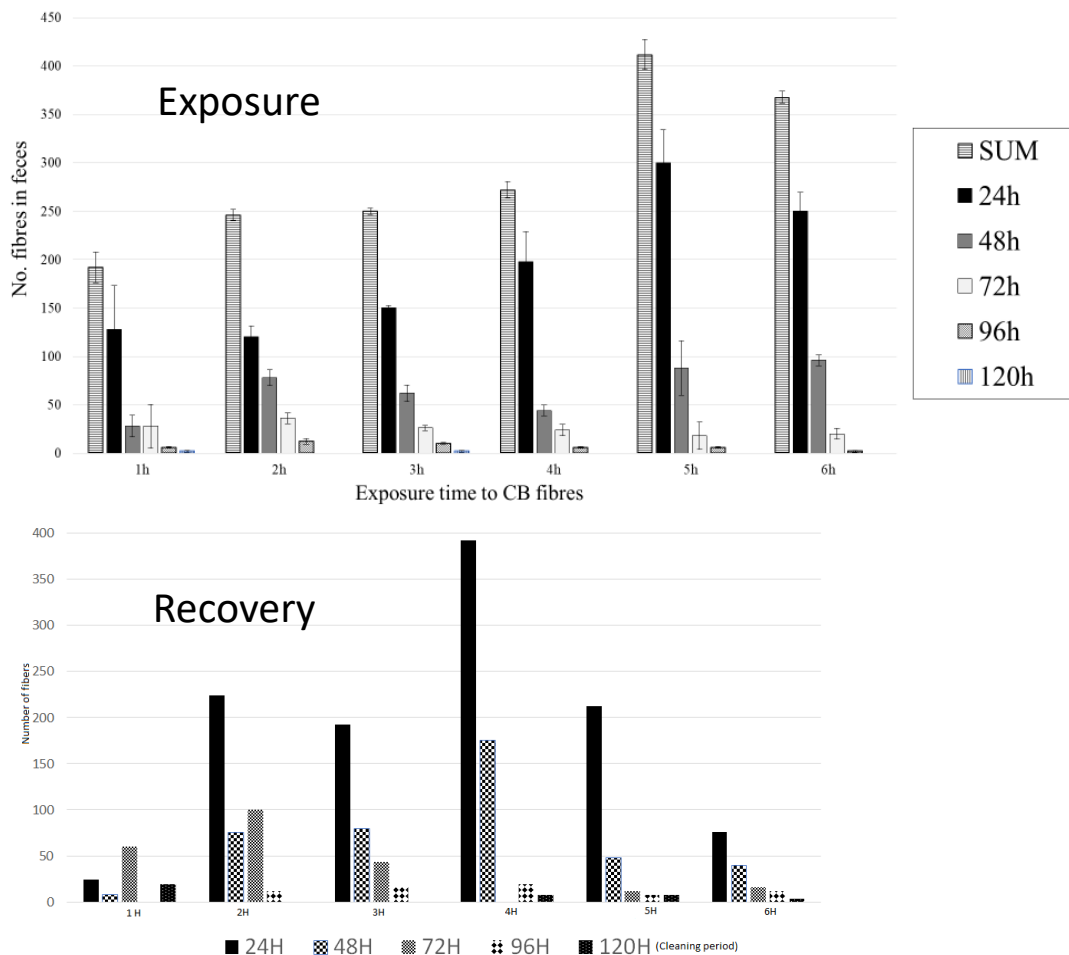


Figure 6.4.3. Demonstration of MP removal/purification efficiency of mussels' filtration by laboratory mussel experimental exposures with different concentration of model cellulose acetate microfibers (<0.5mm) formed by cigarette butts decomposition: A) Exposure (1-6 h) and B) Recovery (24-120 h).

After proving the concept by laboratory filtration (pseudo/feces) experiments with mussels, using cigarette butts cellulose acetate microfibers as model MPs and stone basin (1m³) suspended matter (POM) sedimentation (Figure 6.4.4), Mussel MicroPlastic Monitor (MMP-M) and Purification (MMP-P) installations were designed.

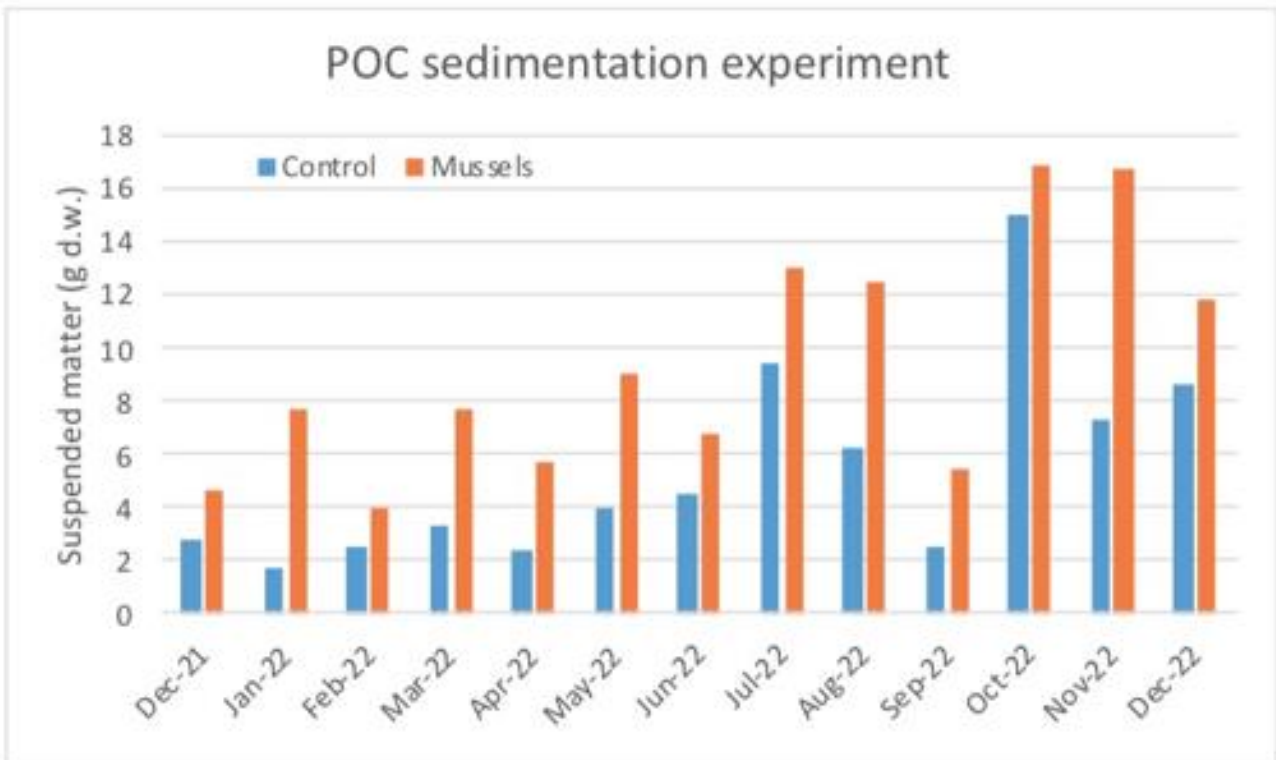


Figure 6.4.4. Demonstration of how mussels' filtration and pseudo/feces generation can enhance suspended matter (POC/POM) sedimentation.

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 m³) (Figure 6.4.2 and 6.4.4), where filtration by mussels and pseudo/feces generation enhanced natural suspended matter sedimentation by 20-60% and removal of microplastics (0.5 mm cellulose acetate fibres) by up to 100% (Figure 6.4.3).

1.2 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.

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 6.4.5) and environment purification (MMP-P; Figure 6.4.6).



Figure 6.4.5. 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.

2. **DELIVERABLE METHODOLOGY:** *(first briefly describe what was done, then some tips for the replication of the action – suggestions are reported below)*

- 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 6.4.6. 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.

The tips for the replication of the action

2.1 *WHERE: Deliverable location description (the location can affect the effectiveness of the action? What about the seasonality?)*

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).

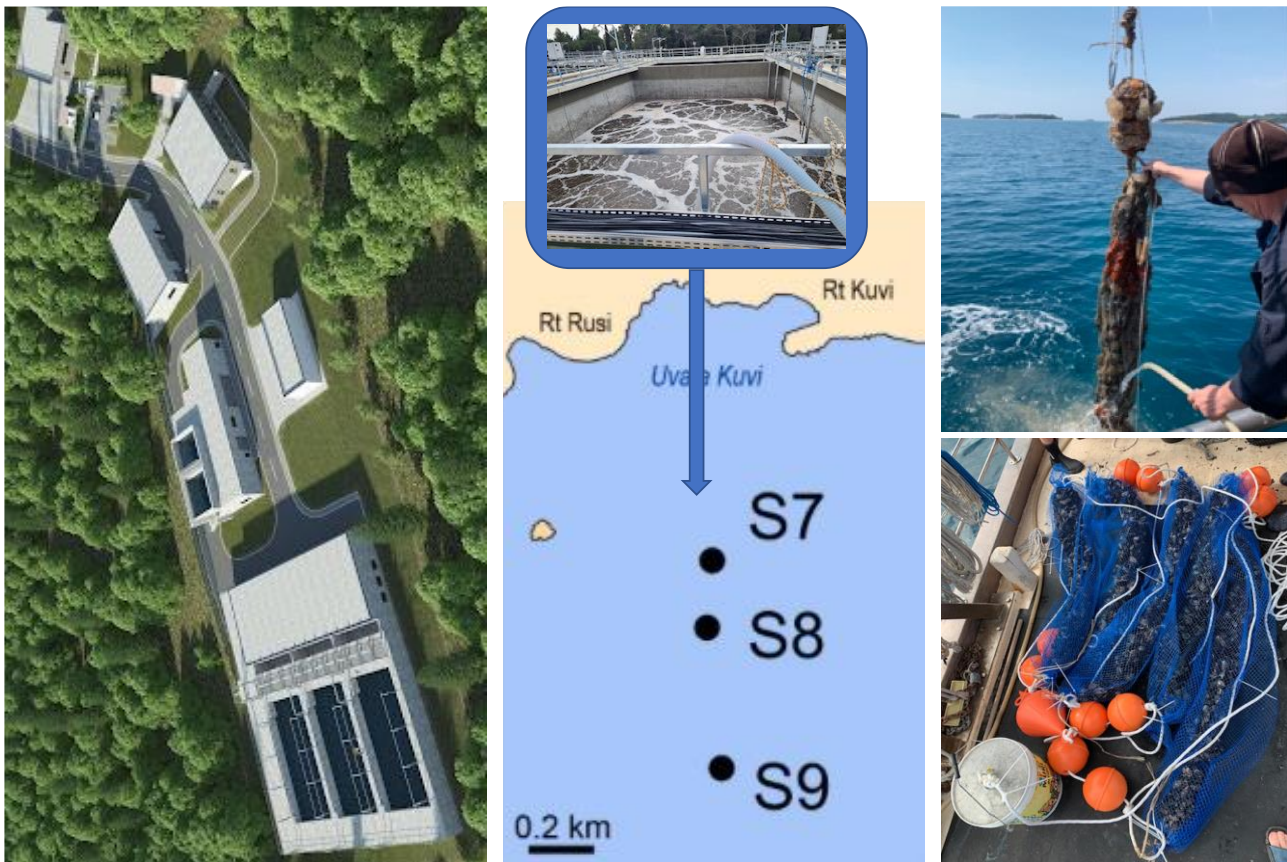


Figure 6.4.7. Map of Rovinj coastal area with MMP-P applied sites (S7, S8, S9) in vicinity of municipal wastewater treatment plant (UPOV Cuvi) outlet.

2.2 **WHO: Experts Involved (Which background do you need to perform well the action? What's the minimum team?)**

The MMP-P installation can be installed at any marine place in the vicinity of a beach, harbor or municipal wastewater outlet. Depending on the chosen site, the minimum team members' number, equipment and infrastructure necessary may vary.

In our case, for setting up a location near the vicinity of UPV Cuvi outlet we needed:

- boat with a winch,
- boat crew (2), 2 scientist and (1) SCUBA diver

2.3 **HOW: Description of the activity realized (specific technology are needed? And the timing needed?)**

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.

2.4 **Outputs achieved vs Outputs expected**

There a lot of benefits using ecosystem services which mussels can provide. 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.

With the performed activity in frame of WP6.4 we demonstrate mussels' efficacy of water column purification and their potential for MPs removal. Further, the characterization of investigated area, according PAHs, PCBs, heavy metals and MPs (sediments), sea surface (Manta net – MPs) and water column (MMP-P mussels – MPs, PAHs, PCBs, heavy metals), including collected mussel pseudo/feces (MMP-M mussels – MPs) were done.

2.5 **SWOT ANALYSIS:** Describe, with the support of the SWOT analysis scheme, how the performed action can be improved

	Helpful	Harmful
Internal Origin	<p>STRENGTH:</p> <ul style="list-style-type: none"> -natural water column purification by mussels' filtration preventing further MPs spreading -high efficacy performed by cheap organisms, working 24h/7 days without batteries -usage of commercial undersized mussels in line with interest of shellfish farmers 	<p>WEAKNESS:</p> <ul style="list-style-type: none"> -occasionally (each 2 months) is necessary to check MMP-P installation integrity and perform cleaning of biofouling
External Origin	<p>OPPORTUNITIES:</p> <ul style="list-style-type: none"> -beside water column MPs bioremediation, the MMP-P installations can increase local biodiversity -easy demonstration of mussel filtration and MPs removal concept using small aquaria with and without mussels and model MPs or suspended matter for different target groups, from kindergarten, schools, general public and stakeholders 	<p>THREATS:</p> <ul style="list-style-type: none"> -any installation in the sea is and object of interest for tourists and the local population -stormy weather (displacing – sinking of installations -predation of mussels by fish Sparus aurata

3. **CONCLUSION / SUMMARY:** *main achievement of the pilot action (after this pilot, can be set a new baseline for new studies? What can be done to continue this pilot actions?)*

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.

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 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.).

4. **TRANSFERABILITY:** *please describe how the experience gained in these pilot actions will allow to define a set of procedures / best-practices for the usage of the selected technologies in the context of the Adriatic sea*

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.

5. **MEDIA:** *If available please add photos and link of videos*

<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/>