KICK-OFF MEETING, 22.5.2025.

Exposure, biological effects and fate of microplastics in aquatic organisms under different anthropogenic impacts

PlastOrgAnoTox

Methodologies for microplastic assessment

Manca Kovač Viršek National Institute of Biology







Jožef Stefan Institut

Marine Biology Station Piran and Department for Organisms and ecosystem research

Laboratory for microplastic research

WP1 – determination of pure and "aged" MP WP2 – exposure of mussels to MP and study of MP presence in different soft tissues

WP4 – metadata analysis



For the laboratory experiments we have plan to expose mussels to **pure** and **aged MP**. For this purpose we have to discuss and define:

- Caracteristics of pure and aged MPExposure conditions
- •Develop protocols for MP analysis in soft tissue

WP1 and WP2





Determination of pure microplastic

- plastic material:
 - in preliminary tests PP, PE were used;
 - both of this materials can flow in the sea column;
 - are the most frequently produced and also found in nature
 - bluemussels are capable of selecting particles on the base of physico-chemical surface properties
- plastic **shape** (fragments/ fibres/ spheres)
 - Fibres and fragments are more environmentaly relevant shapes
 - Irregularly shaped particles have a higher potential to cause injuries and may attach stronger to internal surfaces.
- plastic size (in preliminary test 40 µm was used)
 - Sizes <60 µm can be efficiently ingested by mussels.
 - In case of larger particles mussels produce more mucus to reject them.



Determination of pure microplastic

- plastic **concentration** (1 mg/l was to much to analyze MP):
 - What is environmentally relevant concentration? Monitoring of MP in the sea water is limited to 300 µm due to nets used for sampling.
 - Suggested that mussels in environment ingest 10 particles/day.
 - The ingestion rate depends of mussel size, fitnes...
 - What units we will use? Mass/volume or count/volume. The best is to have both parameters, due to MP analysis after exposure.
- the type of application of MP (pulsed/ constant concentration)
 - Mussels can ingest all MP in few days.
 - It is difficult to sustain the whole experiment the same concentration.
 - In water environment mussels are not exposed to MP all the time to the same concentration.



Determination of aged MP

How to prepare aged MP?

Several different environmental processes has to be simulated:

- **UV aging** The most frequently used is UV irradiation, which simulates the photoinduced oxidation of plastic polymers due to sunlight exposure:
 - exposure to UV lamps with different wavelengths in air and water
 - not necessarily realistic as the tested MP is usually free of additives that prevent aging
- Chemical oxidation simulation of ozone exposure
- Thermal oxidation exposure of plastic to different temperatures in temperature controlled chambers;
- Mechanical stress due to longitudinal transport and contact with other solids:
 - Orbital shaker or ultrasonic bath with added quartz sand
- **Biotic aging** biofilm formation:
 - Incubation with sinthetic community or in the water from the environment, where MO are already present



Determination of aged MP

We have to determine the protocol for aged MP preparation. We have three options:



To expose the pure MP in the environment

- How long?
- Where in the sea (sea bottom, sea surface)?
- How to evaluate the rate of ageing?
- How much should be aged?



To sample MP in the sea

- The most realistic sample
- Too many variables
- Too many different types of plastic
- Time consuming



To age MP in laboratory conditions

- Easy to control the process of aging and properties of used plastic
- Different properties in comparison with environmentaly aged plastic



Exposure conditions

- Mussels sampling (when, where, how big, how much, in which life cycle)
- The time of exposure (short term exposure 21 days/ 14 days)
- The number of mussels per each aquarium.
- The interval of feading.
- Faeces sampling?
- Water sampling?
- Contamination prevention:
 - To eliminate as possible MP in added sea water.
 - To eliminate as possible air born MP.
 - Evaluation of already present MP.





Preliminary experiment

- It will be performed through this summer.
- To get answer to methodological questions:
- **How much MP** is suitable in the experiment (that is environmentaly relevant and enough high to get response)?
- Which size range?
- What is the optimum time of the exposure?
- How to prepare hystopatological resins?
- How to eliminate possibility of contamination in all experimental steps?





Thank you!

manca.kovac.virsek@nib.si

Zagreb, 22. 5. 2025

What else we have to consider:

- <u>the rate at which particles are rejected from the mantle cavity</u> before ingestion to assess the amount of energy that mussels invest into cleaning themselves from the unwanted particles
- The number of mussels for MP analysis and the protocol of preparation

