KICK-OFF MEETING, 22.5.2025.

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

PlastOrgAnoTox

PlastOrgAnoTox project general overview

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HRZZ Croatian Science Foundation

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arnis

PMF

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The project objectives are to assess relation of MP occurrence with effects and biological changes in wild and exposed aquatic organisms and point out environmental risks and novel biomarkers

H1 Microplastic (MP) occurrence in the environment

WP 1
T1.1, T1.2
Water, sediment, protocols

leader RBI (CRO)
partners CRO (FCET, FS), SLO (IWRS, NIB)

H2 Presence of MP and pollutants in environment causes biological changes

WP 2
T 1.1, T2.1, T 3.1
MP exposure and fate in biota

leader RBI (CRO)
partners CRO (FCET), SLO (NIB, IWRS, BF)

H3 Laboratory exposure to MP will discriminate biological changes related to MP effects

WP 3
T1.2, T2.2, T3.2
MP effects in biota, biological changes, toxicity

leader NIB (SLO)
partners CRO (RBI, FCET), SLO (BF, JSI, IWRS), DE (UDE)

H4 Assessment of environmental risk and novel biomarkers of MP exposure

WP 4
T1, T2, T3.2, T4.1, T4.2
Risk assessment, novel biomarkers

leaders BF (SLO) and RBI (CRO)
all partners

Project leader RBI (CRO)
all partners

WP 5
Dissemination and communication

WP 6
Project management

WP1 Assessment of the environmental conditions and analyses of MP occurrence in water and sediment

Task 1.1 Analyses of MP in different habitats (freshwater and marine ecosystem, aquaculture) – leader RBI (partners FCET, FS, IWRS, NIB) – 1st year

WATER - physico-chemical parameters, concentrations of 25 metal(loid)s and the quantity, polymer types and shapes of MPs will be determined (7 locations)

SEDIMENT - grain size, mineral composition, particle origin, carbonate content, concentrations of 25 metal(loid)s and the quantity, polymer types and shapes of MPs will be analysed (7 locations)

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Sampling locations:

1 reference location KS1 - Krka River source – wild trout + water + sediment

2 anthropologically impacted location near the town of Knin KK2 – wild trout + water + sediment (wastewater from the municipal runoff and maybe water and sediment from from the pools nearby screw factory)

3 fish farm AQ KF5 – Krka River source – rainbow trout + water + sediment

!!! Industrial wastewater

4 fish farm AQ AF6 - Krka River mouth – sea bream + water + sediment

5 bivalve farm AQ AM7 - Krka River mouth – mussels + water + sediment

6 location near Šibenik AM4 – wild mussels + water + sediment

7 location near Šibenik AF3 – wild sea bream + water + sediment (near shore and off shore)

WP1 Assessment of the environmental conditions and analyses of MP occurrence in water and sediment

Task 1.2 Isolation and preparation of MPs for laboratory experiments – leader RBI (partners FCET, IWRS, NIB) – 1st year

The **"aged" MPs found in investigated environments** (input from Task 1.1) and pristine MPs will be used. **Preparation of "aged" MPs** (by grinding with cryogenic mill and sieving through different filters) will represent **realistic conditions in experimental exposures (irregularly shaped MPs)** and will fulfil a gap in the knowledge. Procurement of **commercial MP, especially fluorescent for mussel shell exposure**

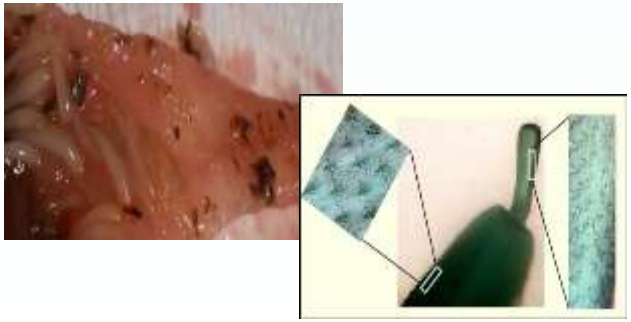
The methods of interest will be also those for **the isolation of MPs from biological components** (tissues as completely biological material, gut content consisting of both biological components and sediment, and shells of mussels containing both organic and calcium carbonate as an inorganic component).

Organic					Inorganic			
base		oxidant		enzyme	acid			
w(KOH)	w(NaOH)	$w(H_2O_2)$	w(NaOCl)		w(HCl)	$w(HNO_3)$	$w(HClO_4)$	$w(H_2SO_4)$
%	%	%	%		%	%	%	%

WP2 Analyses of MP in aquatic organisms (fish, bivalves)

Task 2.1 Estimation of anthropogenic impacts and MP exposure in wild fish and mussels – leader RBI (partners FCET, NIB, IWRS)
Wild and farmed fish (brown trout and gilthead seabream), marine bivalves (wild and farmed mussels), and fish intestinal parasites (acanthocephalans from brown trout).
Gut content, intestine, gills and muscle of fish; mantle, digestive gland and gills of mussels; whole parasites – analyses of MPs and metals – 1st year

Salmo trutta
brown trout



Dentitruncus truttae
Acanthocephala

Mytilus galloprovincialis
mussels

Sparus aurata
gilthead seabream

WP2 Analyses of MP in aquatic organisms (fish, bivalves)

Task 2.2 Exposure to MPs and metals in selected organisms under controlled laboratory conditions – leader NIB (partners RBI, UDE, BF, JSI, IWRS)

A/ Short-term and long-term experiments on adults and larvae of mussels (*Mytilus galloprovincialis*) - determination of the fate of MPs and biological changes caused by the effects of different types of MP relevant for aquatic environment (input from Task 1.2), applied individually and in combinations with other MP types and pollutants (Cd).

B/ Exposure of fish infected with acanthocephalans to MPs - will be carried out on experimentally infected fish with intestinal parasites, which will be exposed to different types, sizes and concentrations of MP (lower and higher exposure levels), following a parasite attachment and growth phase of approximately six weeks after experimental infection of the fish. The exposure will last for 10-20 days, depending on the initial results, and MP levels will be determined before and after exposure in fish gut content, intestine and acanthocephalans.

PS	3rd day	5th day	8th day
CONTROL	6 individ.	6 individ.	6 individ.
0.03 μm, fl	6 individ.	6 individ.	6 individ.
0.1 μm, fl	6 individ.	6 individ.	6 individ.
0.5 μm, fl	6 individ.	6 individ.	6 individ.
1.0 μm, fl	6 individ.	6 individ.	6 individ.

WP3 Assessment of biological effects of MP on aquatic organisms

Task 3.1 Analyses of biological effects due to anthropogenic and MP exposure in the environment – leader RBI (partners NIB, BF)
The outputs from Tasks 1.1 and 2.1 will serve to evaluate biological responses in organisms that will include: **a) organism level** - biometric parameters, condition indices and physiological state; **b) cellular level** – biomarker responses (biomarkers of oxidative stress - MDA, antioxidant capacity - CAT, GST, neurotoxicity - AChE, metal exposure - MT, general stress – TP, genotoxicity - Comet test, cell viability, micronucleus test)

WP3 Assessment of biological effects of MP on aquatic organisms

Task 3.2 Biological changes and acute toxicity of MP under controlled laboratory conditions – leader NIB (partners RBI, FCET, BF)

B/ Toxicity testing on water fleas (*Daphnia magna*) - the acute toxicity of MP individually and in combination with other types of polymers and pollutants (essential and non-essential metals). Planned testing will include:

(1) control – no exposure;
(2) different MP types, separately and in combination, different sizes and concentrations (lower and higher exposure level);
(3) different metals (Cd, Zn, Co, Al, Cr, Fe, Mn, Ni, Ag, Cu, Ca, Mg, lower and higher exposure level);
(4) combinations of different MP types and metals (lower and higher exposure level).

Each test will be performed in triplicate and the effective toxic concentration (EC50) that causes a toxic effect in 50% of the population will be defined.

Water flea (*Daphnia magna* Straus, 1820)

WP4 Environmental risk assessment of MPs

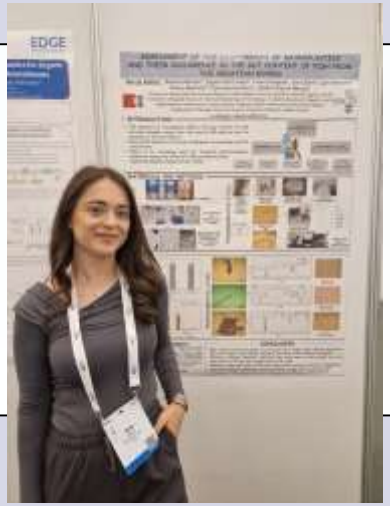
Task 4.1 Literature review on risk assessment frameworks for MPs - leader BF (partners RBI, NIB, IWRS, FS)
We will **review existing risk assessment strategies, with a focus on adapting methodologies tailored to the unique characteristics and pathways of MP pollution**. Additionally, we will explore potential modifications or enhancements necessary to address the specific challenges posed by MPs, such as their persistence, ubiquity, and potential for toxicity and bioaccumulation in organisms throughout the food web

Task 4.2 Risk assessment of MPs based on data generated in the WP1, WP2 and WP3 - leader RBI (partners BF, NIB, IWRS, FS)
Comparison of exposure, effect and toxic concentrations of MPs in both animals taken from the environment and experiments. This approach will enable us to **evaluate the potential risks of MPs across different scenarios, considering real exposure conditions as well as controlled laboratory settings**. We will try to better understand the environmental relevance of laboratory-derived endpoints and **refine our risk assessments** accordingly. Furthermore, incorporating data from both sources will allow us to account for variability in exposure levels and biological responses, **providing a more comprehensive picture of the potential impacts of MPs on ecosystems and organisms**

WP5 Dissemination and communication – leader RBI (all partners)

Task 5.1 Dissemination of project results - leader RBI (all partners)

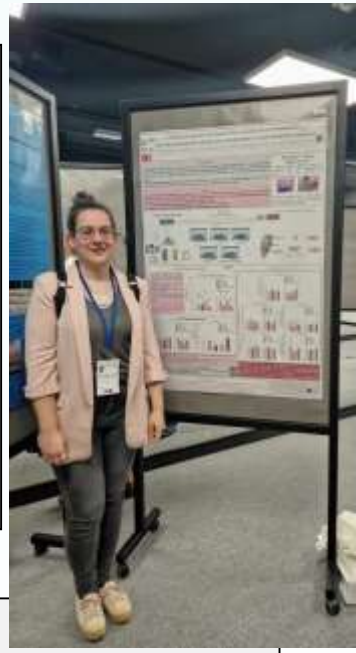
- The **publication of scientific papers** peer-reviewed journals and presentation of results **at international and national congresses and workshops**.
- Dissemination of project outputs to a wider community: **general public and creators of the management and water quality preservation strategies**.
- The results from the project will be incorporated into **teaching activities** of partners at master and doctoral degree studies and scientific workshops for the **popularisation of science** (awareness on MP impact and risks for the environment).



Task 5.2 Communication of project results - leader RBI (all partners)

- The main communication platform will be the **project website**.
- The wider communication will include information sharing by **social networks on domestic/national and international levels and media**.
- Communication within the project collaborators will be assured by **regular project meetings and online communication**.
- Project activities and results will be presented at the Open Days and other **citizen science activities, invited lectures, through the popularisation of science**.

WP6 Project management - leader RBI (all partners)



Task 6.1 Project coordination and progress control - leader RBI (partner NIB)

- Principal investigator will be responsible for the **project management**, assessment of the project objectives, **coordination of reporting, dissemination and communication activities**.
- **Project progress control** will ensure realisation of the planned Working plan and objectives.
- **Regular meetings** of all collaborators will be held in person or online.
- At the kick-off meeting responsibilities between partners will be elaborated into details – 1st year.

Task 6.2 Data and knowledge management - leader BF (all partners)

- Protocol and data sharing will be encouraged, resulting in **publication of the data and methodology in open access journals**.
- If open access allows, all data analysed in a particular paper will be available as supplementary material on the websites of journal publishers. If the publication is not in open access, after the embargo period has passed, the CC BY-NC-ND 4.0 license will be applied.
- **All publications as well as selected metadata will be made available through the national repositories**.

THANK YOU FOR YOUR ATTENTION!