KICK-OFF MEETING, 22.5.2025.

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

PlastOrgAnoTox

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general overview of the project

Activities in Slovenia

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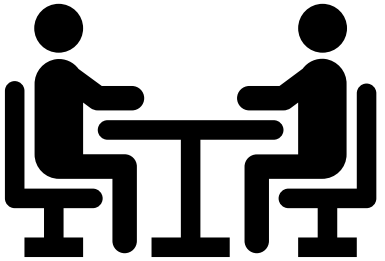
PlastOrgAnoTox, project number N1-0406, financed ARIS, Weave schema
Duration 1. 3. 2025 to 29. 2. 2028

Slovenian partners



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NATIONAL INSTITUTE OF **BIOLOGY**

PlastOrgAnoTox - research objectives

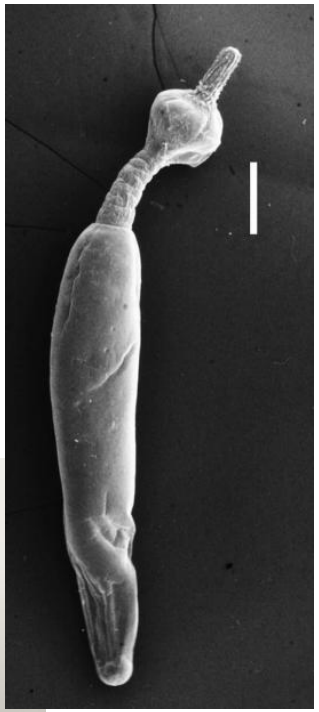
Research objectives of the project

O1 Assessment of the environmental conditions and occurrence of microplastics in habitats with different anthropogenic pressures (freshwater and marine ecosystems, aquaculture)

O2 Investigation of biological changes related to anthropogenic and microplastics exposure in wild populations of aquatic organisms (fish and mussels)

O3 Determination of biological responses and fate of microplastics in selected organisms (mussels, crustacean water flea, fish intestinal parasites) experimentally exposed to microplastics

O4 Publication of guidelines for environmental risk assessment and active communication and dissemination of the obtained data in the scientific and public community



Buchmann& Karami, 2024

Photo: A. Ramšak, NIB

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Hypothesis: PlastOrganoTox

Hypothesis 1. Microplastic (MP) occurrence is detected in marine and freshwater environment, and depends on anthropogenic influences and habitat types → **WP 1**

Hypothesis 2. Environmental exposure to MP results in uptake of MP in freshwater and marine organisms and causes biological changes from cellular to organism level → **WP2**

Hypothesis 3. Laboratory exposure to MP will discriminate biological changes related to MP effects or its combination with different pollutants (metals) and give data on MP fate in soft tissues and hard structures → **WP 3**

Hypothesis 4. Results on MP occurrence in the environment and on effects of MP (hazard data) from our project will be used to assess environmental risk and define novel biomarkers of MP exposure → **WP 4**

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)

Workpackages and tasks/Slovenian partners

WP1. Assessment of the environmental conditions and analyses of MP occurrence in water and sediment	**WP2.** Analyses of MP in aquatic organisms (fish, bivalves)	**WP 3. Assessment of biological effects of MP on aquatic organisms**	**WP4.**/ Environmental risk assessment of MPs
Task 1.1. Analyses of MP in different habitats (freshwater and marine ecosystem, aquaculture) – leader RBI (partners FCET, FS, IWRS, NIB)	Task 2.1. Estimation of anthropogenic impacts and MP exposure in wild fish and mussels – leader RBI (partners FCET, NIB, IWRS)	Task 3.1. Analyses of biological effects due to anthropogenic and MP exposure in the environment – leader RBI (partners NIB, BF)	**Task 4.1. Literature review on risk assessment frameworks for MPs - leader BF (partners RBI, NIB, IWRS, FS)**
Task 1.2. Isolation and preparation of MPs for laboratory experiments – leader RBI (partners FCET, IWRS, NIB)	**Task 2.2. Exposure to MPs and metals in selected organisms under controlled laboratory conditions – leader NIB (partners RBI, UDE, BF, JSI, IWRS)**	**Task 3.2. Biological changes and acute toxicity of MP under controlled laboratory conditions – leader NIB (partners RBI, FCET, BF, IJS)**	Task 4.2. Risk assessment of MPs based on data generated in the WP1, WP2 and WP3 - leader RBI (partners BF, NIB, IWRS, FS)

Workpackages and tasks/Slovenian partners

WP5./ Dissemination and communication – leader RBI (all partners)

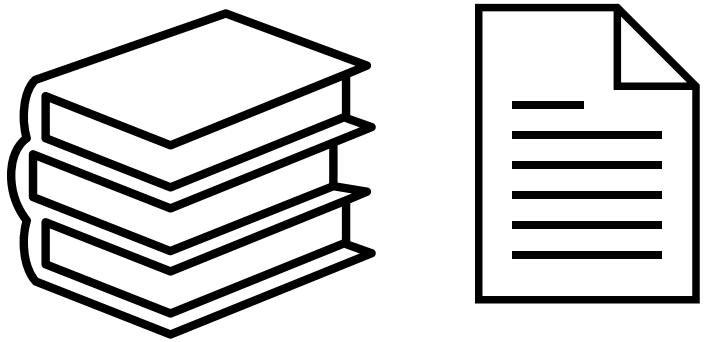
Task 5.1. Dissemination of project results - leader RBI (all partners)

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

WP6./ Project management - leader RBI (all partners)

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

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



Detailed description of work

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)

Task 1.2. Isolation and preparation of MPs for laboratory experiments – leader RBI (partners FCET, IWRS, NIB)
- Preparation of aged MP, or from the environment litter, needed for experiments with aged MP
- Isolation of MP from tissues (mussels)
- Protocols for MP preparation and isolation from mussels

Results needed for set up laboratory experiments in WP2 and analysis WP3

- familiarization with analysis results (especially MP and Cd
- results needed for further experiments under WP3-NIB
- Results needed for guidelines (WP4-BF)

Detailed description of work

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)
- Gut content, gills, intestine and muscle of fish, and mantle, digestive gland and gills of mussels will be appropriately digested and filtered for the subsequent isolation and characterisation (polymer type, size and shape) of MP using FTIR and fluorescence microscopy.

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

A /experiments in mussels and larvae of M. galloprovincialis with MPs and Cd;
- Set up series of experiments based on knowledge and experience from WP1 and WP2 (T 2.1)

B/ trout and acanthocepala -IRB

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)

WP2

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

A /experiments in mussels and larvae of M. galloprovincialis with MPs and Cd; see description in proposal, preparation of detailed working – deadline _____

1/ control – no exposure;
2/ exposure to MPs (one type individually and as combination of different polymer types);
3/ exposure to a coated MP with metal using MP as an intake vector and Cd as a toxic metal;
4/ exposure to “aged” MPs from marine environment to test their capture, ingestion and egestion;
5/ exposure of larvae and adults to fluorescent polystyrene of size about 1 nm to assess possible incorporation in the shells during the stage of faster and slower metabolism.

Experiments

1st experiment / 1st year (September 2025- March 2026), based on data from field sampling

2/ exposure to MPs (one type individually and as combination of different polymer types); 4/ exposure to “aged” MPs from marine environment to test their capture, ingestion and egestion;

2nd experiment /2nd year (March 2026- June 2026)

3/ exposure to a coated MP with metal using MP as an intake vector and Cd as a toxic metal;

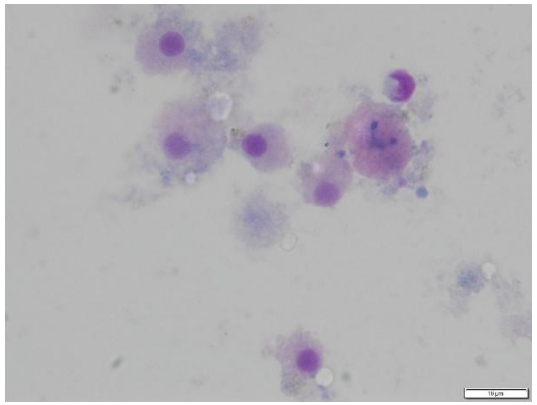
3rd experiment /2nd year (October 2026-March 2027)

5/ exposure of larvae and adults to fluorescent polystyrene of size about 1 nm to assess possible incorporation in the shells during the stage of faster and slower metabolism.

WP3

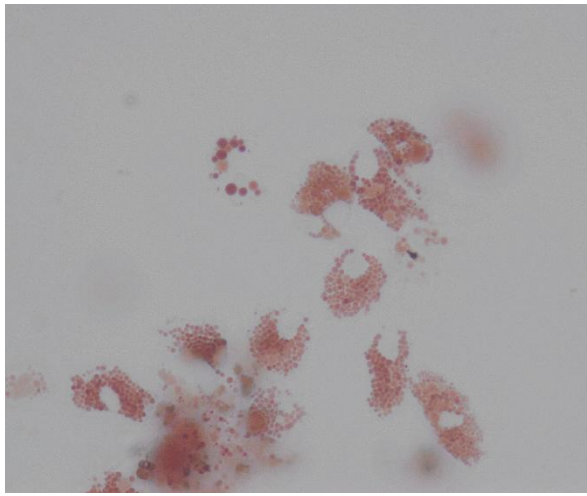
Task 3.2. Biological changes and acute toxicity of MP under controlled laboratory conditions – leader NIB (partners RBI, FCET, BF)

A/ Mussels



Detailed design of experiments with mussels and mussel larvae (see previous slide)
-selection of MP and preparation of MPs (polymer, size, virgin/ageing, adsorbed with Cd)
-experimental groups and number of individuals for exposure experiments
-list of all analyses by tissue (gills and digestive gland) and number of tested individuals for statistics

Endpoints
- immune system (fagocytosis, morphology,…)
- oxidative stress response (oxidative stress - MDA, antioxidant capacity - CAT, GST)
- neurotoxicity (AChE in gills, foot)
- exposure to metals (metallothioneins MT, general stress total protein)
- energetic metabolism (scope for growth, ETS activity, mitochondria respiration, MT intake and excretion in feces, measured intake of food),
- lysosomal destabilisation (neutral red assay, histopatology),
- genotoxic effects measured in haemolymph, digestive gland, gills, and in larvae (Comet assay, MN, cell viability).



- Tissue analysis of metabolites in soft tissues (IJS K. Vogel Mikuš team)
- Deposition into hard structures/shells

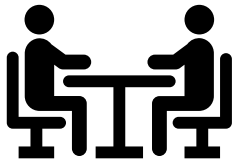
Detailed description of work for NIB

Novelty WP3:

- the **effects of MP** will be **linked to specific biological changes**, from the **cellular to the organismal level**,
- serve for the **selection of novel biomarkers of MP exposure** (related to WP4).
- toxicity testing will provide data on **acute toxicity of specific MP types and their role as a vector** in introducing other contaminants in aquatic organisms, so additionally toxicity will be compared among individual MP types and their combinations with different polymer types and contaminants.

Novelty WP4:

- joint effort of all collaborators will provide **guidance on methodology for isolating and detecting MP** in **different habitats and biological structures**.
- data obtained within the project will serve to assess **environmental risk and biological responses** relevant for MP exposure.



Discussion

Sampling protocols (biota, MPs, adherence of Cd, co-exposure….)

Design of experiments

Timeline for deliverables (reports, analysis, progress of work…)

Data management plan (deliver to ARIS at M6)

Communication plan

Web site

Regular meetings in-person and online