TOXICOLOGICAL EFFECT OF MICROPLASCTICS IN MARINE ENVIRONMENT - EPHEMARE

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WP1. Adsorption and equilibrium partition of persistent pollutants to microplastics

Objective

To investigate the sorption equilibrium kinetics of model persistent pollutants (PPs) to microplastics (MPs), in order to test the potential role of MPs as vectors of marine pollutants. First tests started with Cadmium (Cd^{2+}) and PFOS

Experiments

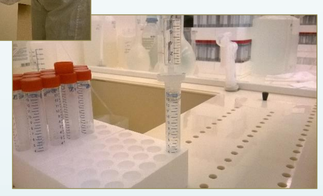
Experiment 1. Surface charge measurement
Different amounts (0, 0.2, 0.4, 2 and 4 g) of polyethylene (PE; 500 - 125 µm) were weigh into 50 mL polypropylene tubes and filled up with 40 mL of 0.03 mol/L KNO_3. Tubes were shaken for 7 days.

Aim: to establish pH of 0 charge (observe plateau on the diagram pH vs weight per L).

g/l	pH
5	5,46
10	5,6
50,25	5,97
100,25	6,34
0	5,35

Fig. 1: pH is increasing with the amount of plastic. The plastic surface becomes positively charged → add buffer to stabilize pH and increase pH to deprotonate plastic surface. pH > 8 $CdOH^+$ precipitates, pH < 8 Cd^{2+} dominates in solution.

Experiment 2: Sorption of Cd^{2+} on PE
Preparation of a CdCl2 stock solution (800 mg/L = 4.362 mol/L) at pH 7. The amount of Cd^{2+} was around 99.8%. Four concentrations of Cd: 80, 8, 0.8 and 0.08 mg/L (n = 3 for each).
Each tube contains 10 g PE per L. Tubes were placed on rotary shaker and samples for analysis were taken after 3 days (no sorption) and after 7 days (test in process).



Experiment 3. Sorption of PFOS on PE
3 replicates for each time point (2nd, 5th, and 7th day) were prepared.
6 mg of PFOS potassium salt and 0.5 g of PE were weigh. 10 mL of deionized water was added and samples were put on the rotary shaker. The samples were filtered by using glass microfiber filters. Debris were transferred into a new tube and extracted with 2 mL MeOH and 30 min ultrasonication. After centrifugation solvent phase was collected and extraction procedure was repeated twice. Before analysis samples were diluted 10 and 100 times in solution mixture: 40% MeOH, 60% ammonium acetate and recovery standard was added.

WP2. Uptake and accumulation of microplastics and associated persistent pollutants

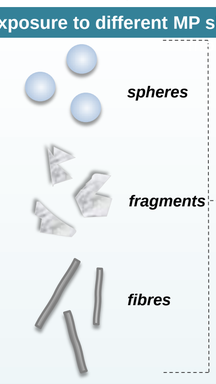
Objectives

- Assess the uptake, accumulation and elimination kinetics of MPs and associated PPs in different model marine organisms
- Investigate MPs interaction with biological surfaces and exchange structures, internal tissue compartmentalisation and localisation
- Develop a dynamic model for MPs and MPs + PPs uptake, accumulation and effects

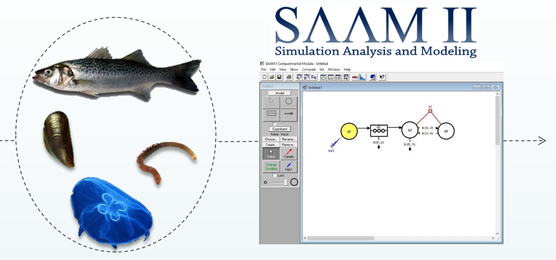
Phase I & II: Modelling MPs

Relevant species from benthic and pelagic ecosystems will be exposed to MPs, including planktonic organisms, pelagic and benthonic fishes, invertebrates (mollusks, echinoderms, polychaeta, and crustaceans).

Exposure to different MP shapes of the chosen animal



Model implementation



Validation in the field

Further steps

- Implement a dynamic uptake model for selected pollutants associated to MPS (benzo-α-pyrene, oxybenzone, PFOS, and cadmium)
- Produce a risk assessment based on model-derived species sensitivity

WP3. Organism Toxicity Assessment

Objectives

- Evaluation of the effect of MPs and adsorbed PPs on a wide range of **biological responses at individual level** (whole organism), using standard **biological models** (from bacteria to fish).
- Endpoints selected are **key physiological functions/processes:** survival, growth, behavior, reproduction (including embryo-larval development).

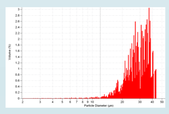
Experiments

For the evaluation of the effects of MPs, sea-urchin larvae were exposed to industrial Polyethylene (PE) and Polyvinyl choride (PVC) at different concentrations.

Raw matherial

Mechanical sievieng using stainless steeel sieves

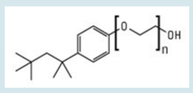
< 40 µm



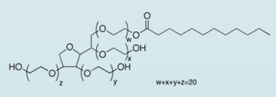
PE	C (mg/L)
2500 p/mL	18,95
1500 p/mL	11,37
150 p/mL	1,14
15 p/mL	0,11

PVC	C (mg/L)
2500 p/mL	18,95
1500 p/mL	9,80
150 p/mL	0,98
15 p/mL	0,10

Two surfactants (Triton®X-100 and Tween®20) were also tested in order to evaluate the toxicity to sea urchin.



Triton® X-100 (Polyethylene glycol tert-octylphenyl ether)



Tween® 20 or Polisorbate 20 (Polyethilene glycol sorbitan monolaurate)

Preliminary results

NOEC (PVC) > 16.34 mg L^{-1}
NOEC (PE) > 18.95 mg L^{-1}
Sea-urchin larvae were not affected by the higher concentration of Microplastics to which they were exposed.
Concerning the surfactants, NOEC was > 4 mg L-1 and > 8 mg L-1 for Triton®X-100 and Tween®20 respectively, both above concentration proposed to dissociate MP agregates in test solutions (0.15 mg L-1).

WP4. Underlying Mechanism of Action

Objectives

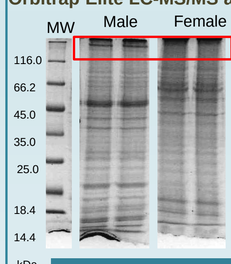
To evaluate the effect of MPs and adsorbed PPs using a variety of **biological responses**, at cellular, biochemical, molecular and physiological levels in a wide range of **biological models** including cell-lines (human and fish), bacteria, bivalves, and fish.

Proteomics and Endocrine Disruption

Proteomic analysis will allow the identification and quantification of all functional proteins that can be modified upon **exposure to MPs.**
Vitellogenin will be used as a biomarker for the detection of the effects of estrogenic EDCs in bivalve mollusks

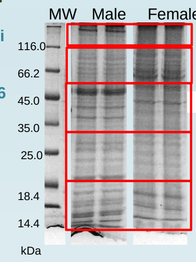
Methodology

For the analysis of Vitellogenin and proteomics in gonad (*Mytilus galloprovincialis*) label-free shotgun proteomics in an Orbitrap Elite LC-MS/MS are being used.

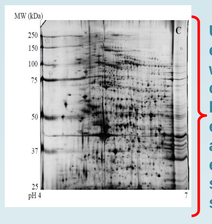


Vitellogenin was detected in the >116 KDa fraction

The indicated fraction will be selected for VTG label-free quantification



The whole proteome will be analysed in selected samples to look for differentially expressed proteins (exploratory proteomics).



Using 2-D electrophoresis the whole proteome can be compared between control and exposed individuals and differential expressed proteins selected for MS/MS sequencing.

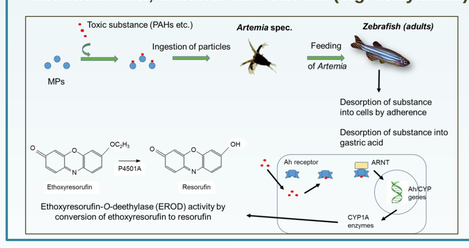
WP5. Trophic Transfer

Objectives

- Identify and quantify transfer of MPs along various simulated food chains
- Clarify tissue distribution and final fate of MPs in organisms representative of pelagic and benthic ecosystems
- Characterize the potential role of MPs as vectors of marine pollutants and their trophic transfer in pelagic and benthic marine food webs

Methodology

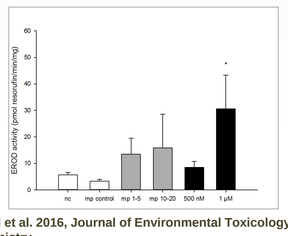
After establishing artificial food chains, MPs will be loaded with POPs (e.g. PAHs) and fed along food chains with focus on accumulation, transfer and effects (e.g. enzymatic) of POPs in model organisms (zebrafish, medaka, sea bass)



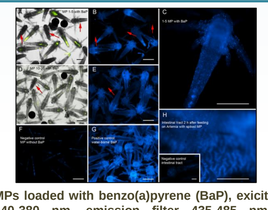
Trophic transfer food chains:
- Artemia spec nauplii → Zebrafish (Danio rerio), Medaka (Oryzias melastigma)
- Paramecium spec → larval zebrafish
- Microalgae (Phaeodactylum tricornutum) → Artemia spec nauplii → Yellyfish (Aurelia aurita)
- Sandworm (Nereis virens) → Sea bass (Dicentrarchus labrax)

Preliminary results

EROD activity after feeding on benzo[a]pyrene (BaP) loaded microplastics:
Negative controls:
a) zebrafish not fed any microparticles (nc)
b) zebrafish fed microplastics without BaP (MP control).
Positive controls:
a) 500 nM (500 nm BaP)
b) 1 µM water-borne BaP (1 µM BaP)



Batel et al. 2016, Journal of Environmental Toxicology and Chemistry



MPs loaded with benzo(a)pyrene (BaP), exicition filter 340-380 nm, emission filter 435-485 nm, visual detection of BaP in Artemia nauplii and zebrafish intestinal tract after feeding

WP6. Field Validation

Objectives

- To analyze quantitative occurrence of microplastics in representative benthic and pelagic species from **4 model geographical areas**
- To define the main characteristic of microplastics detected in biota in terms of size, shape, typology of polymer, chemical load
- To identify differences between geographical areas, benthic vs pelagic species and various trophic guilds, in terms of amount and typology of accumulated polymers
- To integrate field data with ecotoxicological results obtained in WP1-WP4 to identify the most sensitive species and extrapolate the risk of microplastics from the laboratory to the environmental scale.

Investigated species

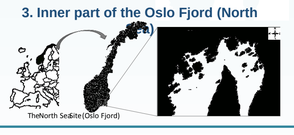
The main species from benthic and pelagic systems will be collected, including planktonic organisms, pelagic and benthonic fishes, invertebrates (molluscs, echinoderms, polychaetes, crustaceans).

Investigated areas

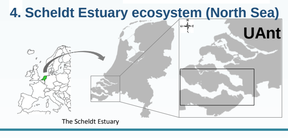
1. North-Central Adriatic Sea (Mediterranean)

2. Open coastal area of Ria Formosa in Southern Portugal (North East Atlantic)

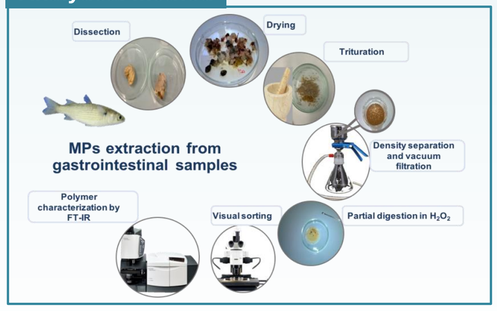
3. Inner part of the Oslo Fjord (North Sea)



4. Scheldt Estuary ecosystem (North Sea)



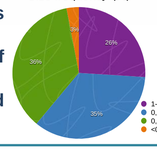
Analytical method



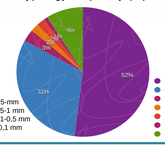
Preliminary results

Preliminary results obtained in Adriatic organisms showed that:
- **45% of analyzed organisms were positive to microplastics ingestion, up to 100% in some benthic species;**
- **size classes between 1 and 0.1 mm accounted for almost 70% of ingested fragments ;**
- **microplastics items were mostly represented by fragments and film, while PE, PS and PA were the dominant polymers.**

Size frequency (%)



Typology frequency (%)



Shape frequency (%)