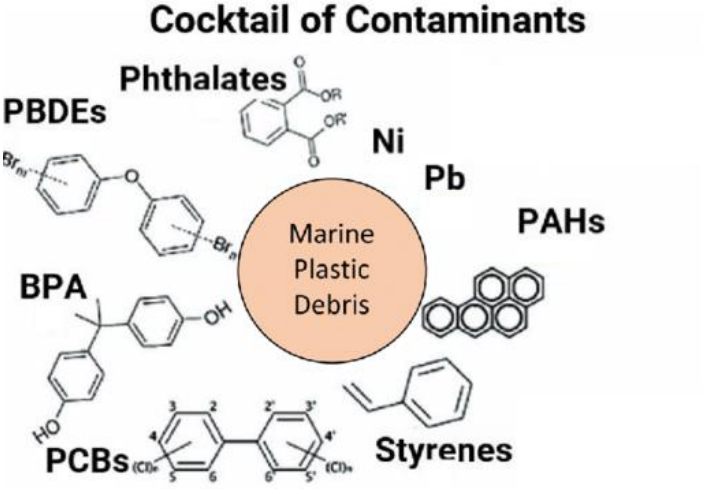
Microplastics as vectors of marine pollutants

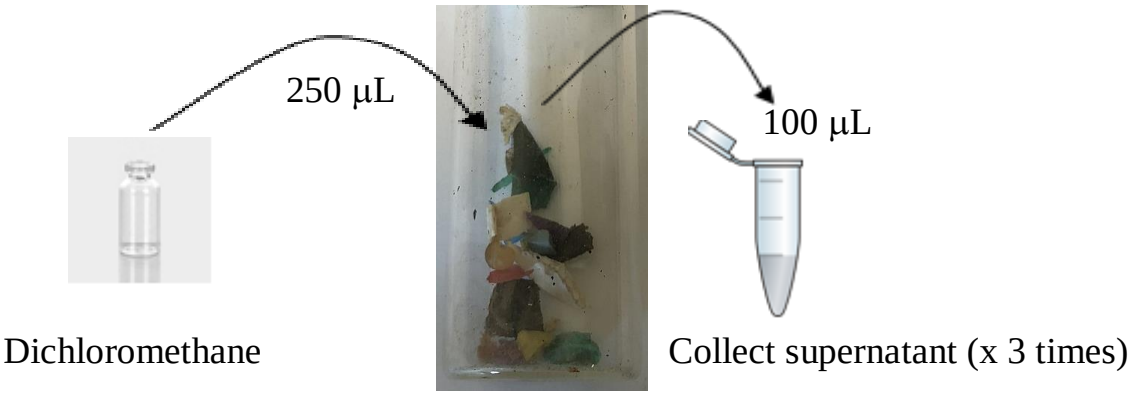
1. *Extraction procedure of chemical contaminants sorbed to microplastics*



The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment – Rochman 2015

Hydrophobic organic contaminants (HOCs) will be isolated from marine debris following the protocol described by Capriotti et al. (2021) with slight modifications.

- Add 250 μL of dichloromethane to each microplastic sample and incubated for 6 min at room temperature, vortexing every 2 min.
- Transfer the supernatant (100 μL) with the extracted HOCs into a new tube.
- Repeat the procedure for 3 times combining the resulting supernatant.



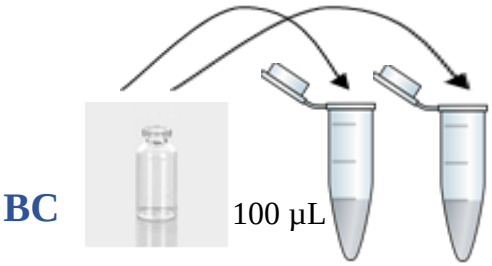
2. *Lactate Dehydrogenase (LDH) Assay Kit (Cytotoxicity)*

This kit is for the rapid, sensitive and accurate measurement of LDH released from damaged cells. The LDH release assay is a cell death / cytotoxicity assay used to assess the level of plasma membrane damage in a cell population. LDH is a stable enzyme, present in all cell types, which is rapidly released into the cell culture medium upon damage of the plasma membrane. LDH is the most widely marker used to run a cytotoxicity assay. The LDH assay protocol is based on an enzymatic coupling reaction: LDH released from the cell oxidizes lactate to generate NADH, which then reacts with the WST substrate to generate a yellow color. The intensity of the generated color correlates directly with the number of damaged cells.

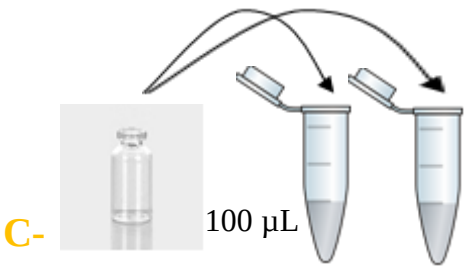
Assay Protocol

Sample Preparation: seed in 0.5 ml microcentrifuge tubes as the following:

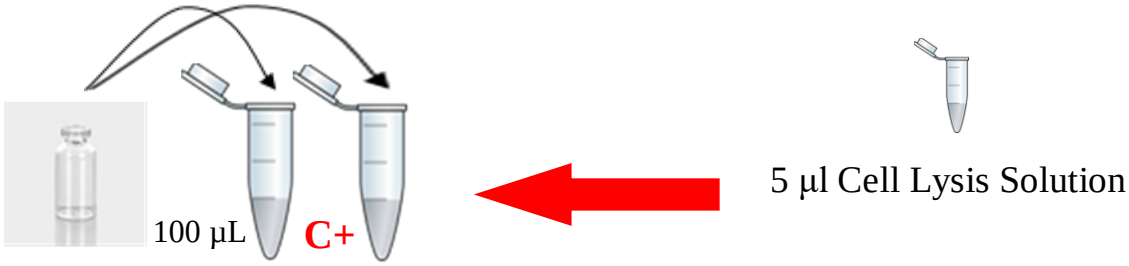
a. **BC** (Background Control): pipette 100 µl culture medium with no cells per tube (in duplicate). The Background Control will measure reagents and LDH background from culture medium serum. The background value has to be subtracted from all other values.



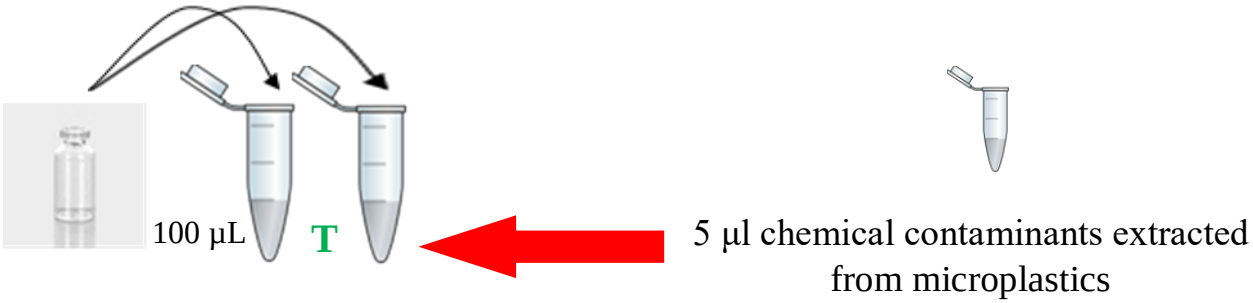
b. **C-** (Negative control): pipette 100 µl cells per tube (in duplicate).



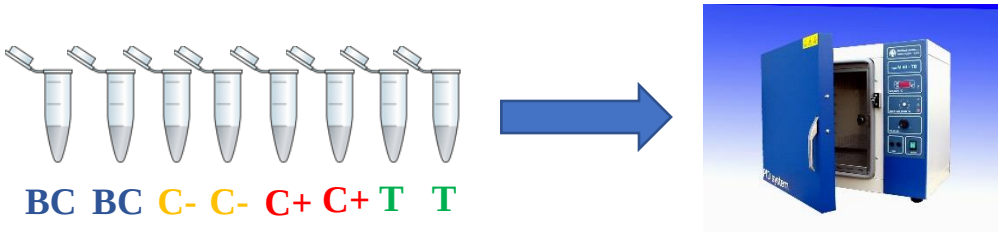
c. **C+** (Cell lysed control): pipette 100 µl cells and add 5 µl Cell Lysis Solution each tube (in duplicate).



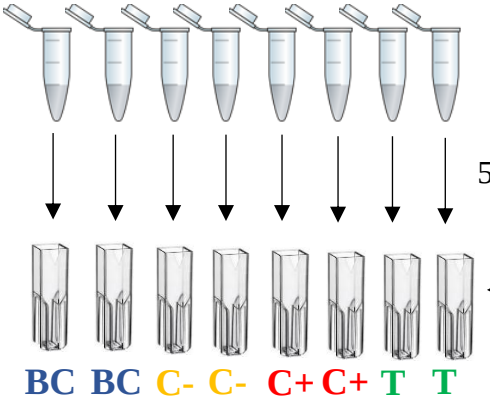
d. **T** (Test Sample): pipette 100 µl cells and add 5 µl chemical contaminants extracted from microplastics each tube (in duplicate).



Sample Incubation: Incubate tubes in an incubator (25°C) for the appropriate time of treatment determined for test substance (20 min).



LDH Reaction: Transfer the clear medium solution (50 µl/tube) into a clear cuvette. Add 50 µl LDH Reaction Mix to each tube, mix and incubate for 15 min at room temperature.



Add 50 µl LDH Reaction Mix to each cuvette

The reaction time can be decreased or increased depending on the color development. Measure the absorbance of all controls and samples with a plate reader equipped with 405/450 nm.

Data analysis

$$\text{Cytotoxicity (\%)} = \frac{\text{Test sample} - \text{BC} - \text{Negative C}}{\text{Positive C} - \text{BC} - \text{Negative C}} \text{ X } 100$$