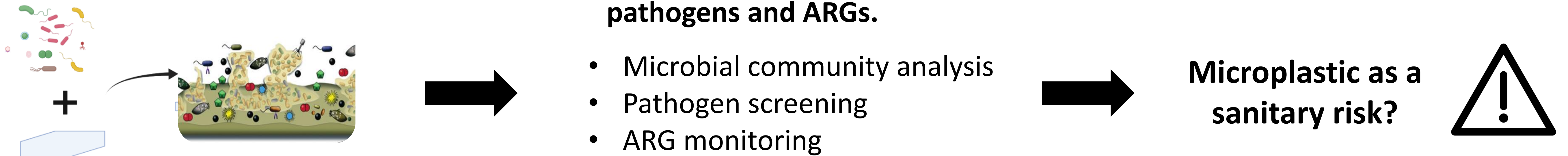


- Microplastics (MP) are detected in all the environments becoming a global concern.
- They are quickly **colonized** by microorganisms forming a biofilm.
- The **health risks** associated to MP are still unknown but debated.
- One of the suggested risks is the potential of **biofilms on MP** to harbor and disseminate pathogens.
- Besides, crowded biofilms are also a hotspot of **horizontal gene transfer** i.e. **antibiotic resistance genes (ARGs)**.
- **Wastewater treatment plants are one of the main sources of MPs, faecal bacteria and ARG in the environment**, where they are in contact.
- Thus, faecal bacteria can get attached to the MP and reach the aquatic ecosystems in a more protected structure than as free bacteria.

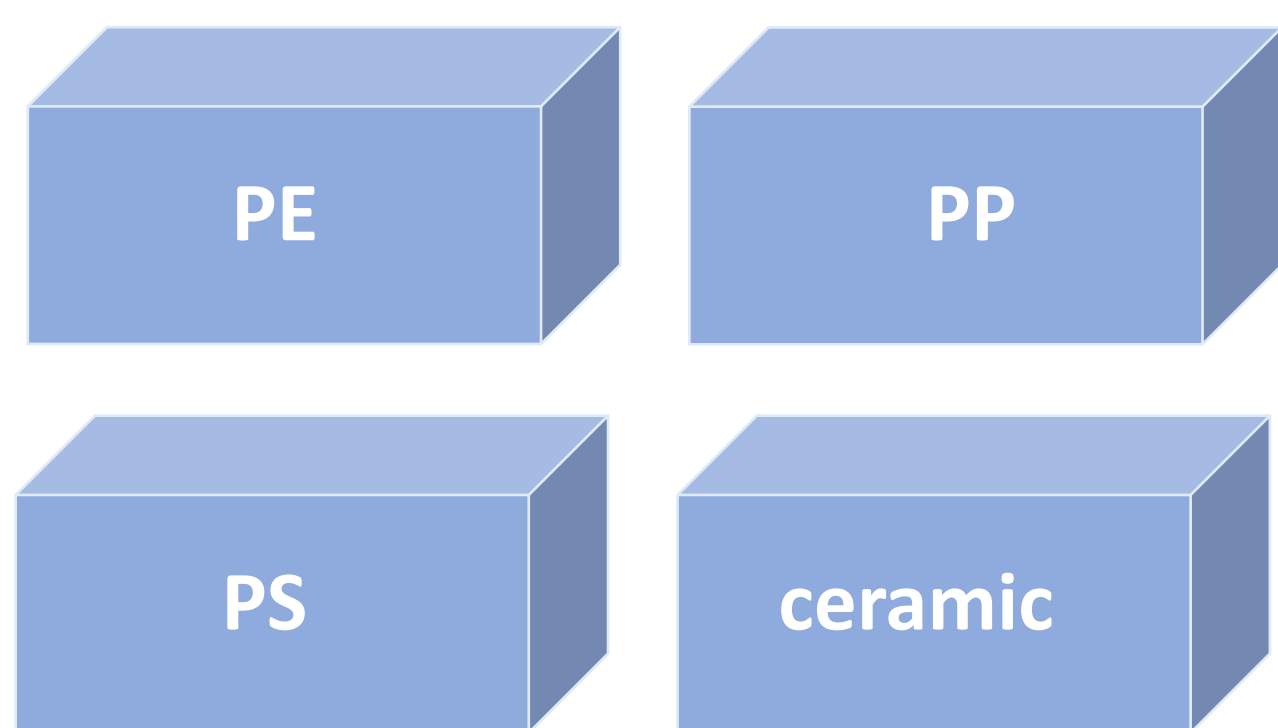
Bact&Plast aims to link MP, sewage and biofilms to evaluate the potential of microplastics to shelter and disseminate waterborne pathogens and ARGs.



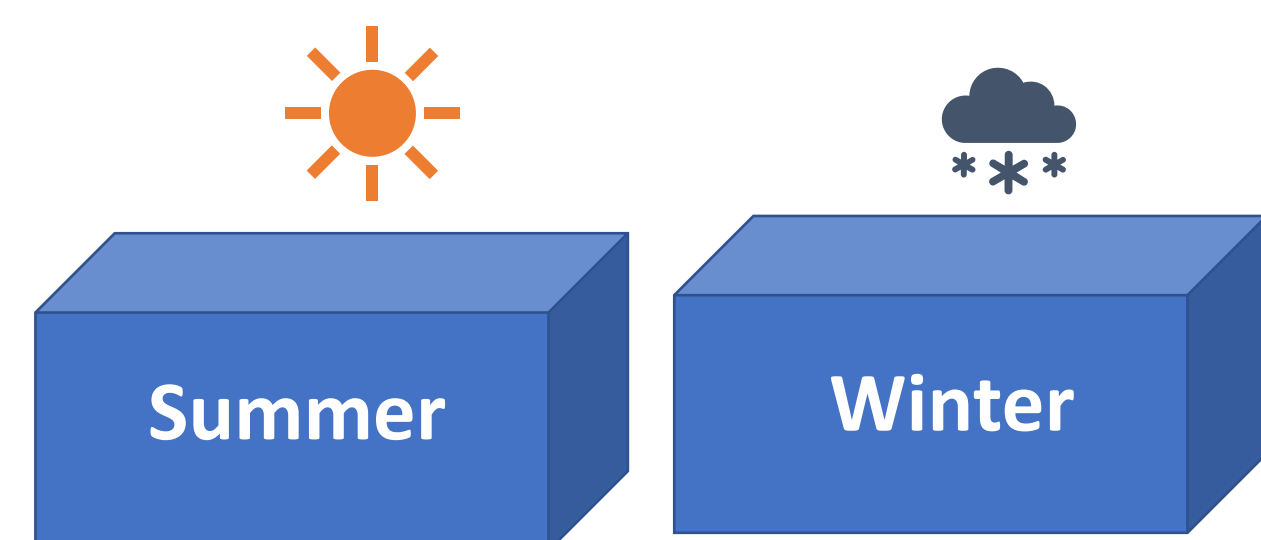
WP1. Evaluation the settlement of bacterial biofilms on microplastics

Task 1. Mesocosmos experiment

Differences between polymers



Differences between seasons



- Mesocosmos will be kept for one month and samples will be collected periodically.

Task 2. Characterization of the bacterial communities of the plastisphere

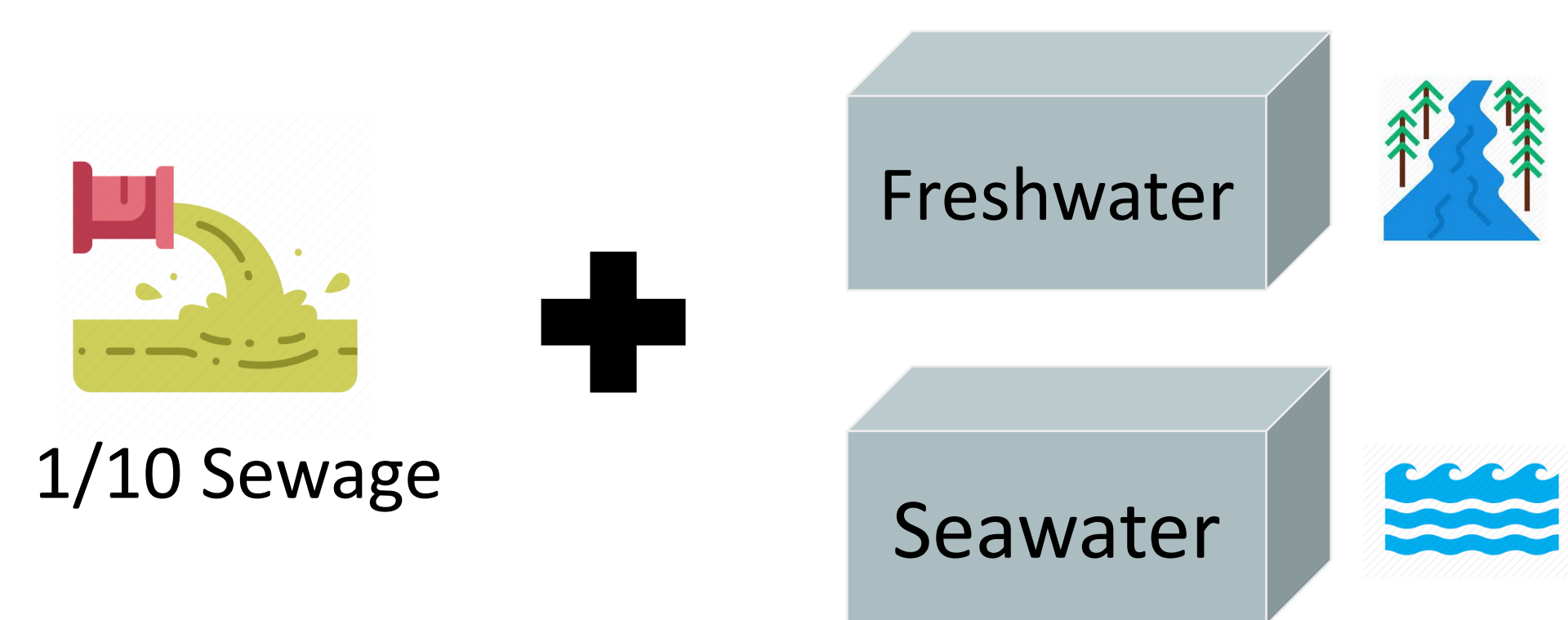
- Epifluorescence microscopy after DAPI staining.
- Scanning electronic microscopy to observe the structure of the biofilm.
- Follow-up of the colonisation process by molecular analysis: 16S rRNA massive sequencing.

Task 3. Characterization of the resistome of the plastisphere

- Shot-gun metagenomics
- Identification of antibiotic resistance genes
- Specific resistant genes will be quantified using qPCR

WP 2. Assess the potential of the plastic biofilm to act as a shelter for pathogens and ARGs

Task 4. Sewage Mesocosmos experiment



Task 5. Monitoring of faecal indicator bacteria, environmental waterborne pathogens and ARGs

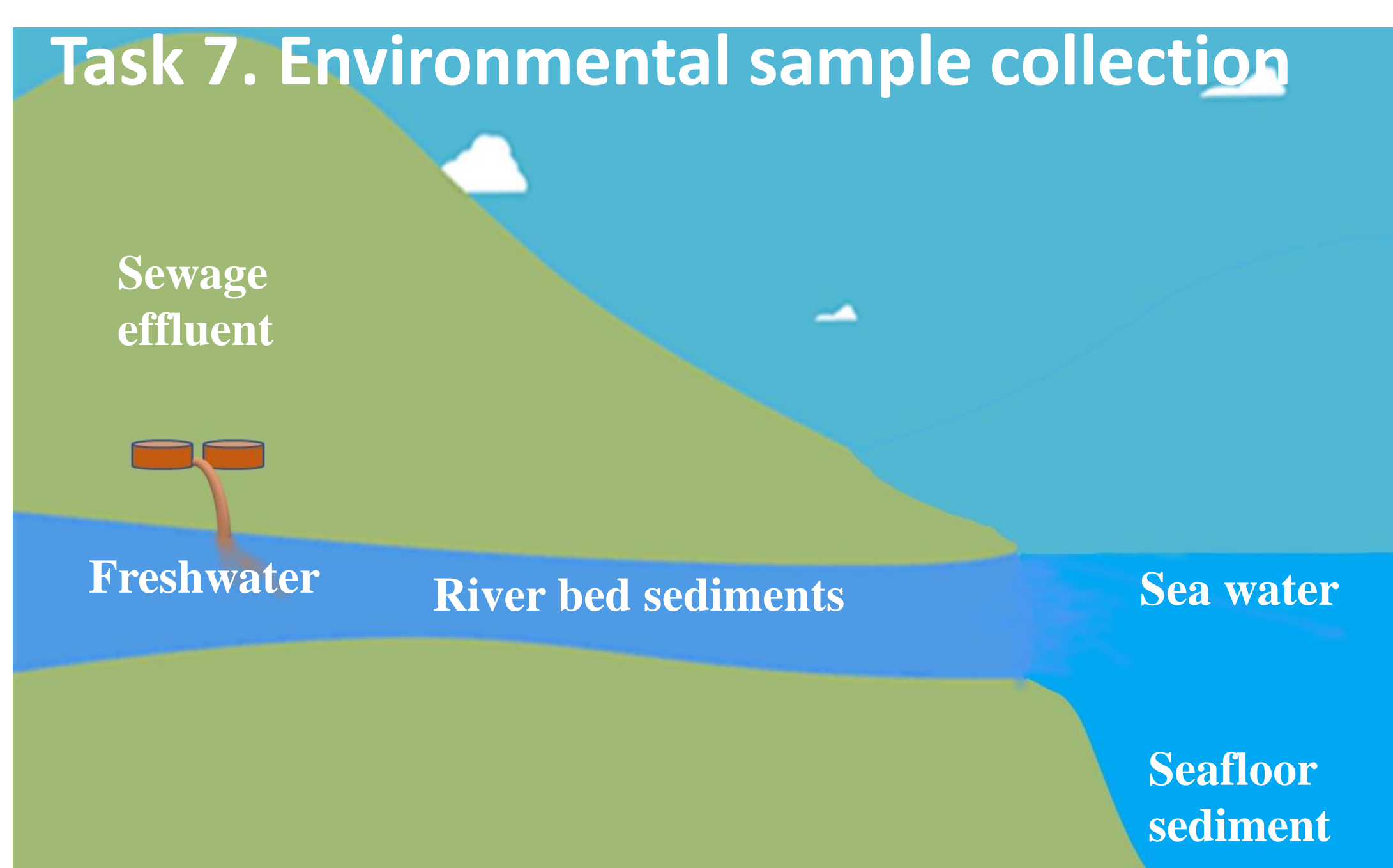
- **Culture methods:** *E. coli* (ISO 9308-1), Enterococci (ISO 7899-2:2000), *Pseudomonas* spp (Pseudomonas agar base), *Vibrio* spp. (TCBS), Total bacteria
- **qPCR:** *E. coli* and Enterococci and ARGs: β -lactamases, aminoglycosides, methicillin, quinolones, sulfonamides and tetracyclines

Task 6. Characterization of the bacterial communities of the plastisphere in a faecally polluted environment

- Massive sequencing of the 16S rRNA gene

WP 3. Analysis of bacterial communities of MP collected from the environment

Task 7. Environmental sample collection



Task 8. Characterization of the microbial community of the plastisphere from environmental samples

- **qPCR:** *E. coli* and Enterococci and ARGs: β -lactamases, aminoglycosides, methicillin, quinolones, sulfonamides and tetracyclines
- Massive sequencing of the 16S rRNA gene
- Shot-gun metagenomics

Task 9. Identification of the plastic polymers

Microplastic particles will be chemically identified using the Fourier transformed infrared spectrometer (FTIR).