

## (Un)Real Ecologies: Microplastics

### A Workshop with Joana Maclean & Kat Austen

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In context of the interdisciplinary group project *DIY Hack the Panke*

Part of the "Shared Habitat" Project, funded by Nordic Culture Point

#### **Hack the Panke**

Steps and protocols for workshop on microplastics in urban waters

- I. Collect plastic samples from Panke river
- II. Sieve solid particles and examine the plastisphere
- III. Separate plastic from organic matter with wet peroxide oxidation
- IV. Density separation of plastics

The Panke river is a small river that starts about 20 km north of Art Laboratory Berlin close to the small town Bernau. During its way down into the city Berlin, the Panke changes into a straightened urban stream that receives rain- and mixed waters from the surrounding districts. **The 'upper' Panke** around ALB is bordered by **parks, green shrubby watersides** and a swampy floodplain. The more **downstream** we go, the more **influenced** the ecosystem will become **by urban factors**. **The 'lower' Panke** around Gerichtstrasse (Lunch Café Panke e.V on Saturday) shows already **less water plant vegetation** than on the 'upper' part. In order to collect (Micro)plastic from the water stream, we are going to place fine nets inside the river that will collect all particles flowing through that part of the Panke overnight.

To understand the status of human influence in the different parts of the Panke, we want to **compare water from the 'upper' and 'lower' Panke**. **We will search for visible interactions between plastics and biota in and around the Panke**. Asking how organisms and inhabitants of the Panke live-with plastic in their habitat?

*Q: Can we detect plastics in and around the Panke river? Is plastic already a prominent part of the ecosystem? Where does plastic meet the 'natural'?*

Plastics come in different shapes, textures and material properties. Their chemical properties makes them generally very resistant to natural degradation processes by fungi, algae or bacteria. However, the term plastisphere has been used by biologists to describe the living microworld attached to plastic particles in the environment. Surprisingly, plastic seems to be much more than just an human-made waste product: In marine ecosystems it was found that pieces of plastics carry a very specific community of fungi, algae and bacteria. Using a microscope, we can get a close and intimate look into their habitat. In the workshop we want to examine the plastisphere of the Panke and observe the shapes and forms of how organisms interact with plastic. For this step, we will choose some interesting particles from our catch and use the available microscopes to get a close look at plastics and living creatures.

Furthermore, we will use a two step chemical protocol for the analysis of (Micro) plastics in our water samples. In our case, plastics include hard plastics, soft plastics (e.g., foams), films, line, and sheets. Microplastic is defined by its size smaller than 5mm, so with our nets (200 micron) we will be catching all sizes between 5mm and 0.2mm. During the coming two days we want to split our samples into different groups, so that we can compare the different procedures with each other.

The solid particles in soil or waters are a mixture of minerals (abiogenic: not produced by living organisms), organic matter that are carbon-based remains from living organisms or their waste product and recently of synthesized anthropogenic compounds (?) that find their way into both terrestrial and aquatic ecosystems. Each of these compounds have a specific density range and will either float or sink in a given liquid column. In order to divide those three fractions from our water samples, we will separate them according to their density. Using a saturated salt solution will create a liquid in which plastic and minerals clearly separate from each other. But first, we will need to remove all organic compounds, leaving only minerals and plastics! Adding hydrogen peroxide solution (30%) to your sample is a common method for the elimination of organic matter as it strongly reacts with all organic carbon.

## II. Sieve solid particles and examine the *plastisphere*

Plastics come in different shapes, textures and material properties. Their chemical properties makes them generally very resistant to natural degradation processes by fungi, algae or bacteria. However, the term *plastisphere* has been used by biologists to describe the living microworld attached to plastic particles in the environment. Surprisingly, plastic seems to be much more than just an human-made waste product: In marine ecosystems it was found that pieces of plastics carry a very specific community of fungi, algae and bacteria. Using a microscope, we can get a close and intimate look into their habitat. **Today we want to examine the *plastisphere* of the Panke and observe the shapes and forms of how organisms interact with plastic.** For this step, we will choose some interesting particles from our *catch* and use the available microscopes to get a close look at plastics and living creatures. We encourage you to take **notes, draw, make snapshots and videos** and **capture everything** to later compare the status of our different samples with each other.

Furthermore, we will use a two step chemical protocol for the analysis of (Micro) plastics in our water samples. In our case, plastics include hard plastics, soft plastics (e.g., foams), films, line, and sheets. **Microplastic** is defined by its size smaller than 5mm, so with our nets (200 micron) we will be catching all sizes between 5mm and 0.2mm. During the coming two days we want to split our samples into different groups, so that we can compare the different procedures with each other.

*Q: Are plastic particles visible? Do we find organisms in close proximity to plastic particles? Can we see an interaction (attachment, slime, surface growth)? What is the ratio of organic vs. plastic?*

## Experimental steps

### II. Wet sieving of solids in water-column

The following steps will remove the bigger particles and prepare the remaining solids for the wet peroxide oxidation (WPO) reaction by rinsing them with distilled water. We will handle the nets separately in two groups for **A) upper Panke** and **B) the lower Panke**.

1. Start with **rinsing out the nets** into buckets. Use tap water for the first steps
2. Sieving samples through biggest size sieve and **discard** the big organic parts
3. Take **smaller mesh sizes** (cheesecloth) and sieve out the next fraction, liquid flow-through into the bucket
4. **Examine** and **take aside** interesting **organisms** and/or **plastic** pieces for microscope analysis. Use petri-dishes or square trays to keep your specimen
5. **Rinse remaining solids** from smallest mesh size with **distilled water** and transfer into **Erlenmeyer flasks** with a spatula or spoon

#### Possible Output

*Photographs showing interfaces **Biota and Plastic, Drawings** and sketches of all observations and curiosities. Use the digital pinboard to collect your findings and impression.*

### III. Separate plastic from organic matter with wet peroxide oxidation

The solid particles in soil or waters are a mixture of **minerals** (*abiogenic*: not produced by living organisms), **organic matter** that are carbon-based remains from living organisms or their waste product and recently of synthesized **anthropogenic compounds** (?) that find their way into both terrestrial and aquatic ecosystems.

Each of these compounds have a specific density range and will **either float or sink** in a given liquid column. In order to divide those three fractions from our water samples, we will **separate them according to their density**. Using a saturated salt solution will create a liquid in which plastic and minerals clearly separate from each other. But first, we will need to remove all organic compounds, leaving only minerals and plastics! Adding **hydrogen peroxide** solution (30%) to your sample is a common method for the elimination of organic matter as it strongly reacts with all organic carbon. **Caution! The liquid of your wet peroxide oxidation (WPO) will become hot and bubbly.**

*Q: Can we follow the digestion of organic matter? Is it visible under the microscope? Is the plastic fraction floating on top? What is the ratio of plastic vs. mineral? Can we already distinguish between plastic types?*

## Experimental steps

### III. 1. Wet peroxide oxidation (Lab protocol)

**Caution! The mixture is highly reactive. Please use (heat)gloves and goggles**

1. Crush **iron tablets** into fine powder.
2. Place the Erlenmeyer flasks with our solid remains on the table top or magnetic stirrer.
3. **Gloves and goggles!** Pour 20 mL of 30% hydrogen peroxide (caution: this solution can boil violently if heated  $>75^{\circ}\text{C}$ ) into a small beaker and add it into our Erlenmeyer flask. Add 1g of iron tablets and mix gently. Set aside for 5 min
1. Add magnetic stirrer if needed and heat up the mixture up to  $75^{\circ}\text{C}$ . Watch reaction at any time. If reaction is too strong, add distilled water to slow down reaction.
2. Heat up for 30 minutes. If natural organic material is visible, add another 20-mL portion of 30% hydrogen peroxide and repeat heating step until no organic material is visible.
3. Add around 7g of salt (NaCl) per each 20 mL of liquid volume to increase the density of the watery solution
4. Heat mixture to  $75^{\circ}\text{C}$  until all salt dissolves

### III. 2. Wet peroxide oxidation (DIY protocol)

**Caution! The mixture is highly reactive. Please use (heat)gloves and goggles**

1. Crush **iron tablets** into fine powder.
2. Place the Erlenmeyer flasks with our solid remains on the table top or magnetic stirrer.
3. **Gloves and goggles!** Pour 20 mL of 9% hydrogen peroxide hair dye paste (caution: this solution can boil violently if heated  $>75^{\circ}\text{C}$ ) into a small beaker and add it into our Erlenmeyer flask. Fill it up with little water to dilute it further until it becomes more liquid and runny. Add 1g of iron tablets and mix gently. Set aside for 5 min
4. Heat up the mixture up to  $75^{\circ}\text{C}$ . Watch reaction at any time. If reaction is too strong, add distilled water to slow down reaction. Foam will come up if stirred heavily, so better mix it gently.
5. Heat up for 30 minutes. If natural organic material is visible, add another 20-mL portion of 9% hydrogen peroxide hair dye paste and repeat heating step until no organic material (little black dots) is visible.
6. Add around 7g of salt (NaCl) per each 20 mL of liquid volume to increase the density of the watery solution
7. Heat mixture to  $75^{\circ}\text{C}$  until all salt dissolves

#### Possible Output

*Capture WPO under microscope. Bubbles? Pictures of different reactions. Compare DIY and Lab protocols.*

## IV. Density separation of plastics

1. Once cooled down, carefully transfer the WPO mixture into a glass cylinder
2. Rinse the Erlenmeyer flask with distilled water to transfer all remaining solids to the density separator
3. Allow solids to settle for 60 min or longer. Due to the density of most plastics being higher than that of our salt solution, they will float
4. Visually inspect settled solids and floating layer on top for any microplastics
5. Try to catch the floating layer on top and examine plastics under the microscope
6. Explore ways to quantify the amount of plastics in our different samples

### Possible Output

*Counting plastic particles after removal of organics -> Number of particles per mL Water, in a drawn grid on the glass slide or in one minute of observation time? What plastic types are visible?*