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Effect based monitoring of emerging organic micropollutant mixtures in conventional wastewater treatment plants effluents in Flanders, Belgium

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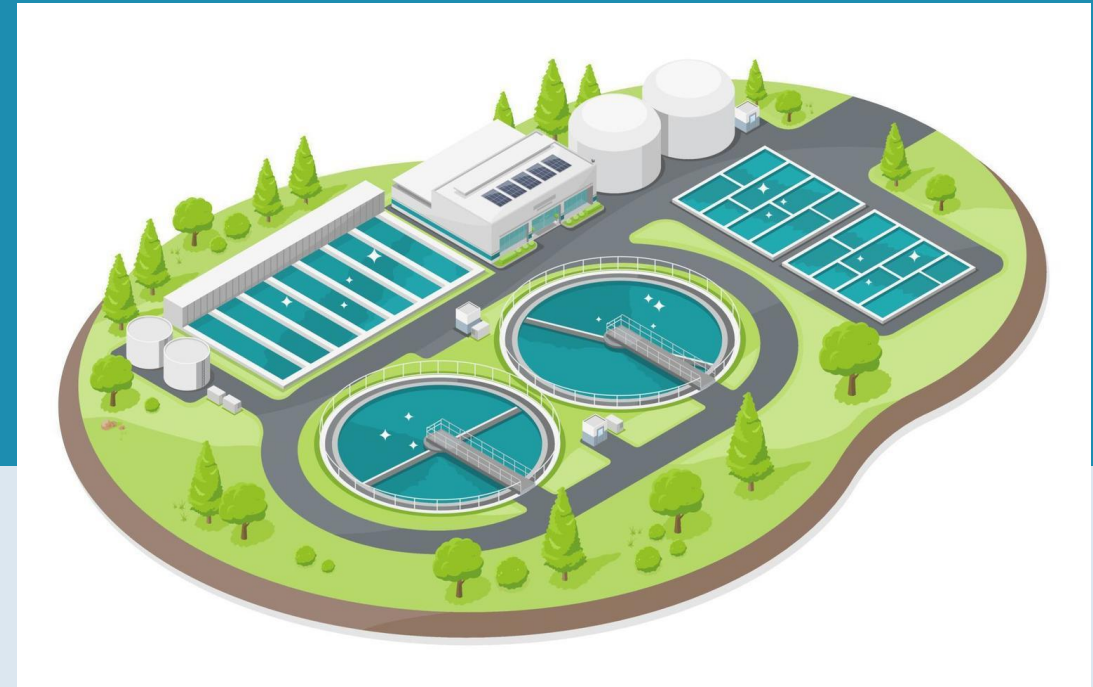
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Dr. Soraya Chapel

Prof. Karel de Schamphelaere

Dr. Annelii Ny

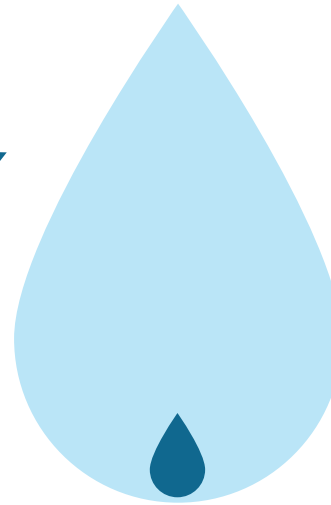


Global freshwater availability

71% of Earth's surface covered in water



2.5% Freshwater (mostly locked up in ice and underground)



1% Readily available freshwater

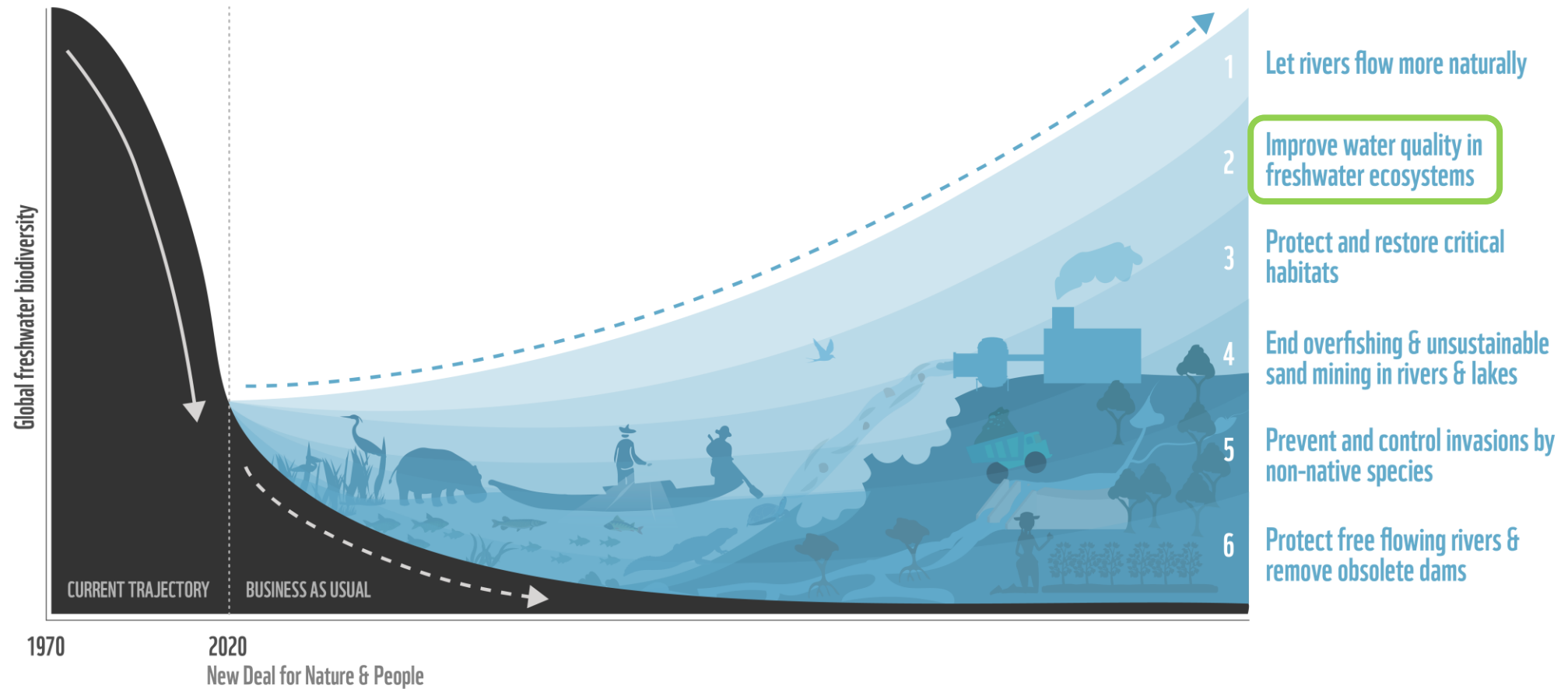


Availability under \uparrow pressure:

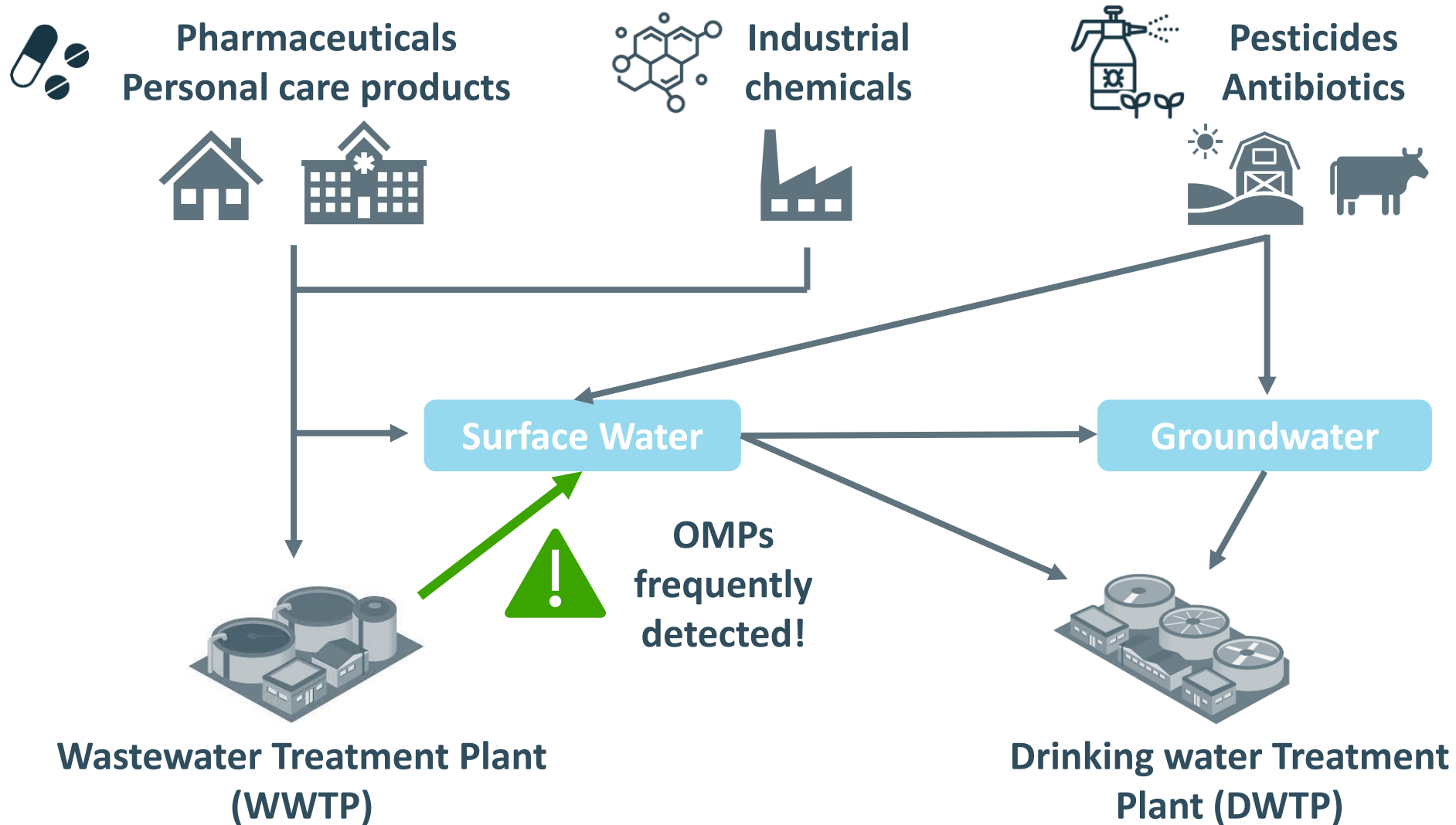
- Climate change
- Increasing demands
- **Pollution**



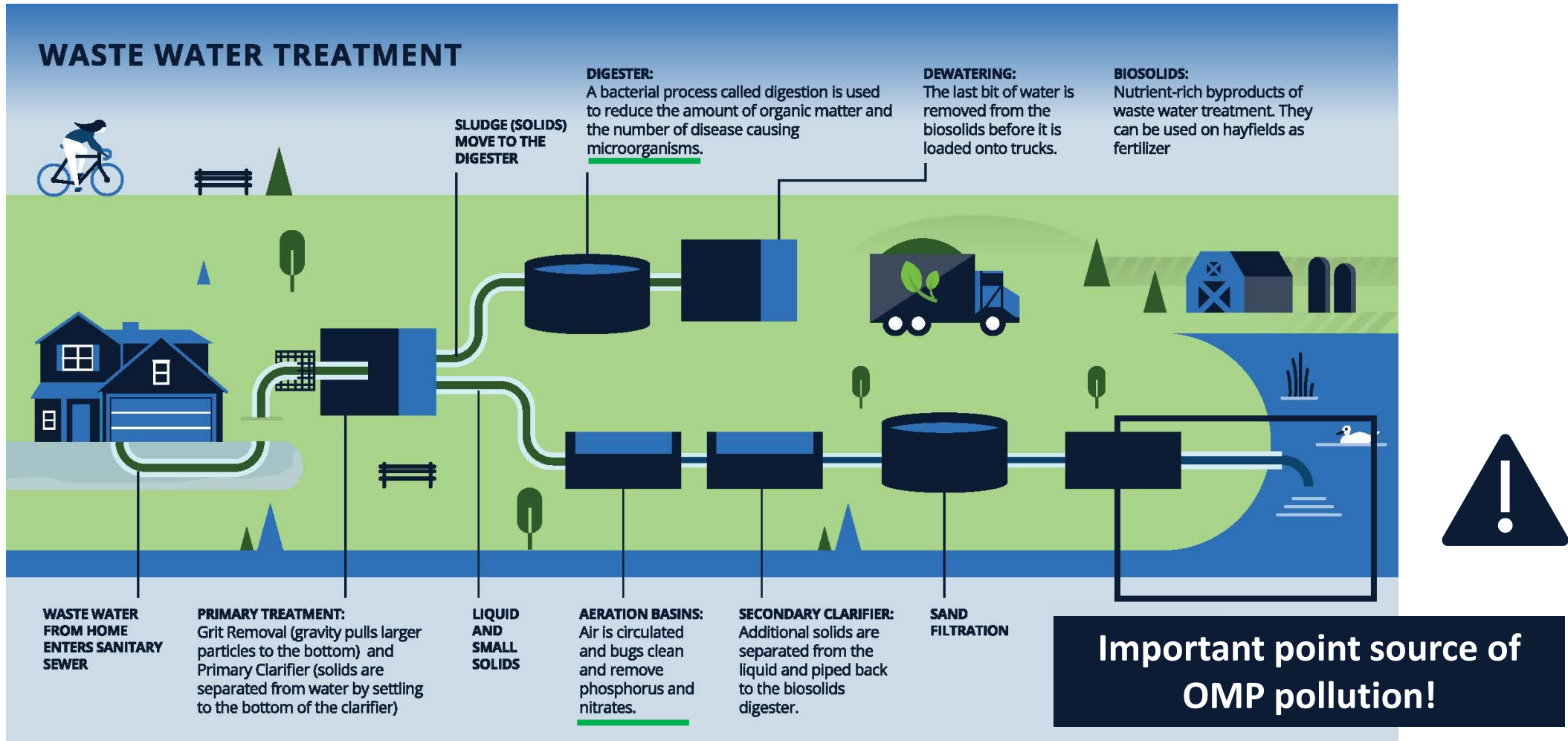
BENDING THE FRESHWATER BIODIVERSITY CURVE - AN EMERGENCY RECOVERY PLAN



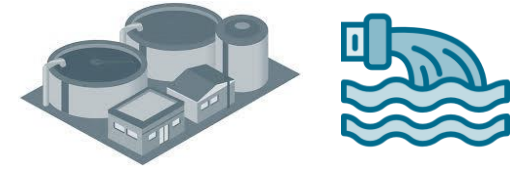
Organic micropollutants (OMPs)



Incomplete removal of OMPs in WWTPs



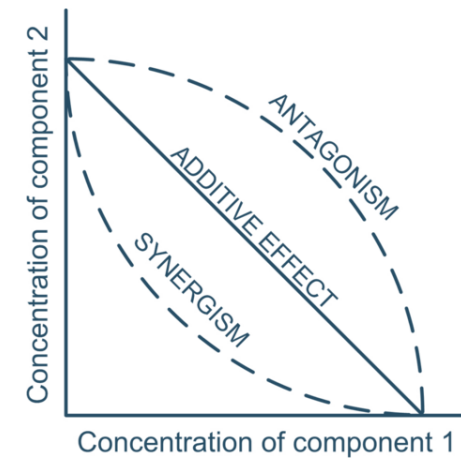
Monitoring of WWTP-effluents: Chemical-based



→ Information on (limited) number of compounds



→ No information on (mixture) toxicity!

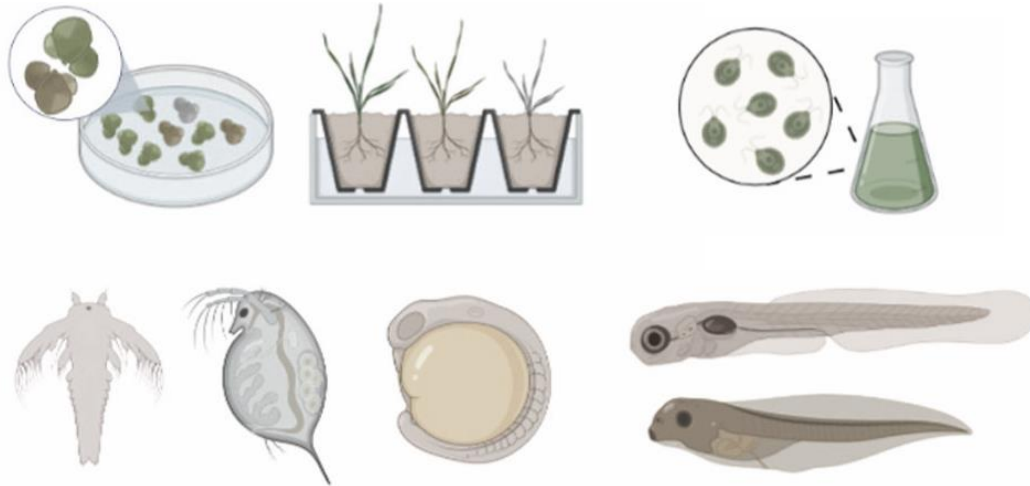


Effect-based monitoring

Fast-screening bioassays using model organisms



In-vivo bioassays (whole organisms)



- Mortality/survival
- Reproduction/developmental effects
- Sublethal effect
- Behavioral effects

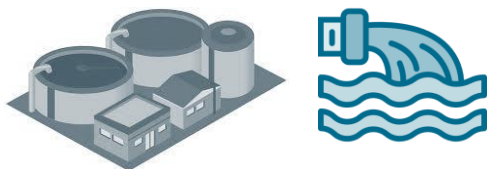
In-vitro bioassays (cell-based bioassays)



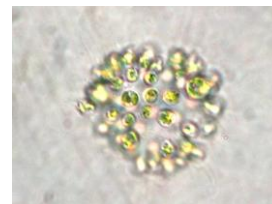
- Reporter gene to amplify a specific response
- Activity of endogenous functions
- Cell viability/growth

Aim of this study

WWTP effluents (Flanders, Belgium)



Toxicity



Cyanobacteria:
Growth inhibition



Zebrafish larvae:
Behavioral effects

Chemical analysis



LC-HRMS



Iceberg
modeling

- Identify main drivers of toxicity in these complex samples
- Better understand impact of these effluents on aquatic environment

WWTP effluents

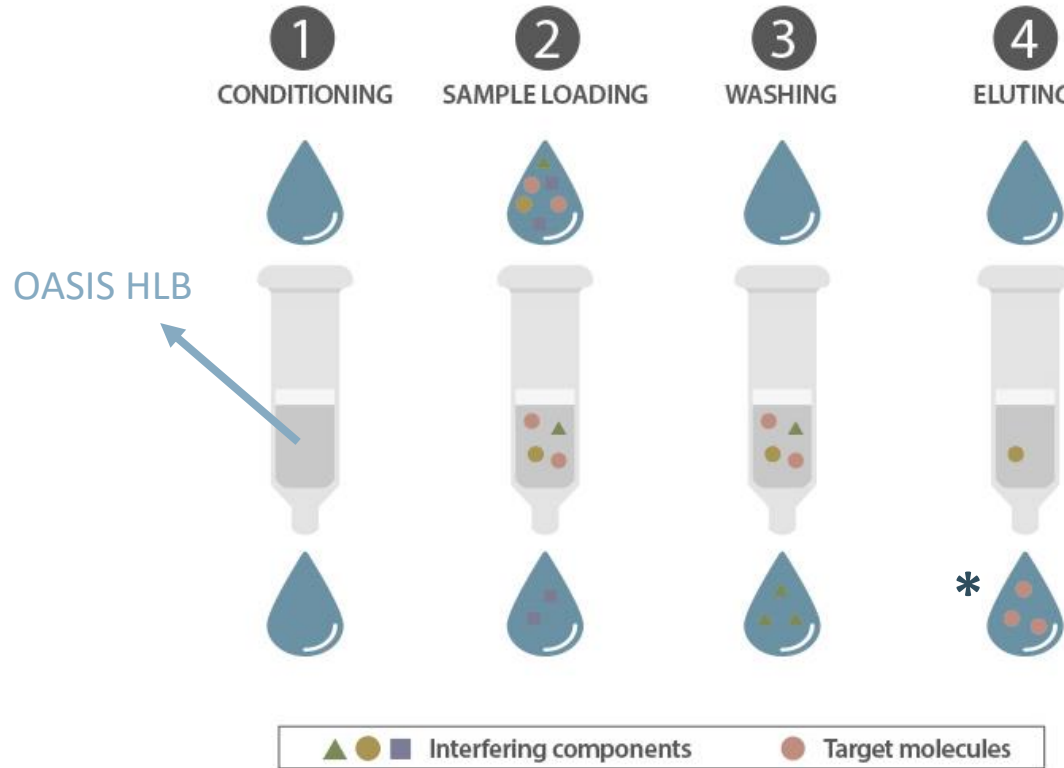


Location	
1	Geel
2	Mechelen-noord
3	Grimbergen
4	Riemst
5	Zichen
6	Schilde
7	Gent
8	Brugge
9	Varenbergbeek
10	Oudenaarde
11	Tessenderlo
12	Bierbeek
13	Tervuren
14	Aartselaar
15	Houthalen-centrum
16	Waregem

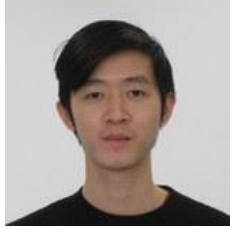
Selection of locations

Based on ecological risk assessment of OMPs from VMM monitoring database (2017-2021)

Sample preparation: solid-phase extraction (SPE)

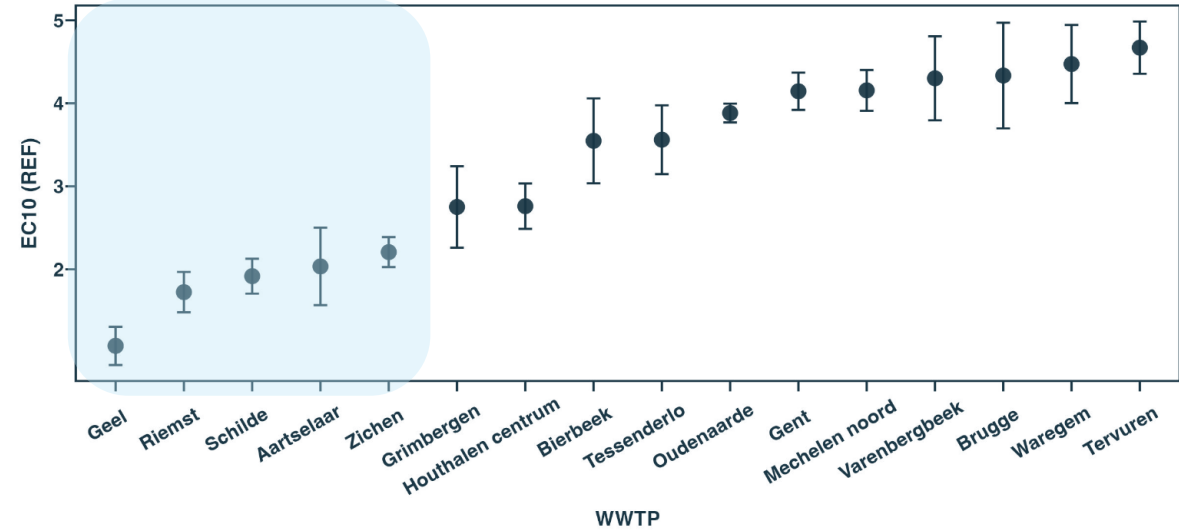
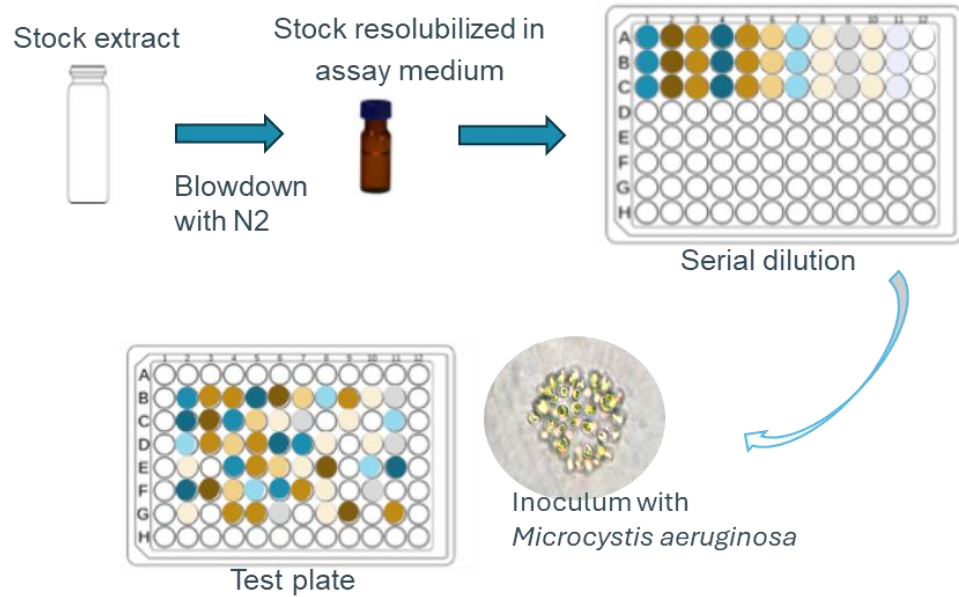


*Evaporated + reconstituted → relative enrichment factor (REF) of 400



Warich Leekitratapanisan

Toxicity: cyanobacteria growth inhibition

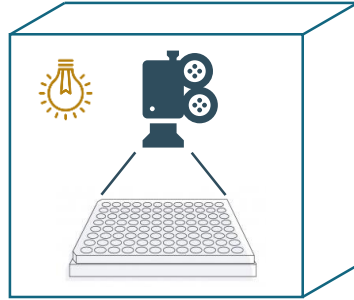


- **Cell density** monitored every 24 hours by measuring phycocyanin fluorescence
- **Growth inhibition** - comparing specific growth rate of control and treatment group calculated from cell density (day 0 to day 4/5)

Overall: EC10 values ranged from 1.7 to 6.6 (average 4.1 ± 1.6)
5 locations: EC10 close to REF 1

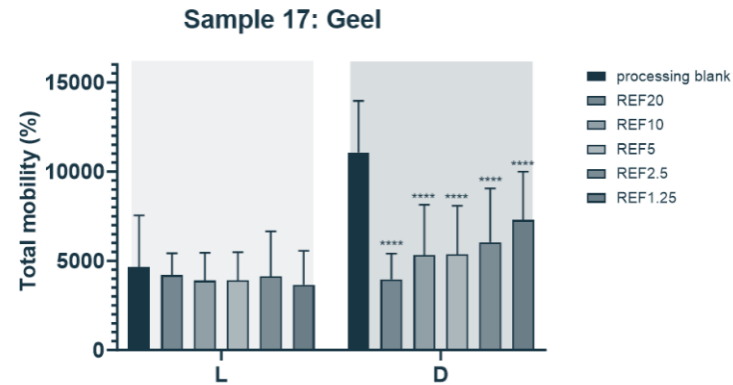
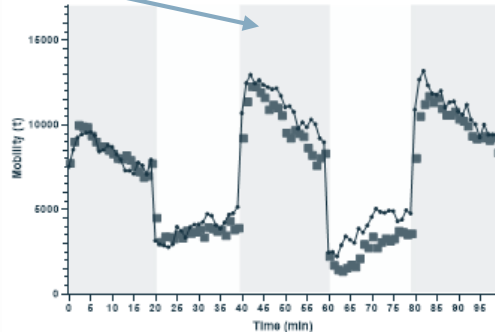
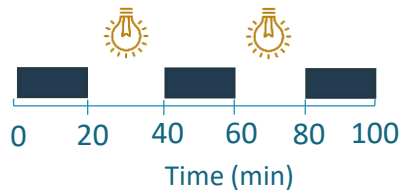
→ High sensitivity of cyanobacteria to WWTP-effluents!

Toxicity: zebrafish behavioral effects



- Wild-type zebrafish (**Danio rerio**)
- Incubated with extracts for 24 hours (start 4 dpf)
- **Behavioral and visual** evaluation at 5 dpf

Dark/Light transitions



Location	
1	Geel
2	Mechelen-noord
3	Grimbergen
4	Riemst
5	Zichen
6	Schilde
7	Gent
8	Brugge
9	Varenbergbeek
10	Oudenaarde
11	Tessenderlo
12	Bierbeek
13	Tervuren
14	Aartselaar
15	Houthalen-centrum
16	Waregem

Overall: 7/17 locations behavioral effect at REF 20
1 location: 60 % lethality at REF 20

- Lower sensitivity of zebafish larvae to WWTP-effluents!
- Differences in toxicity: complementary to cyanobacteria

Chemical analysis: LC-HRMS



- Reversed-phase liquid chromatography (RPLC): **C₁₈ column**
- 20 min gradient
- Different mobile phase for positive/negative mode MS



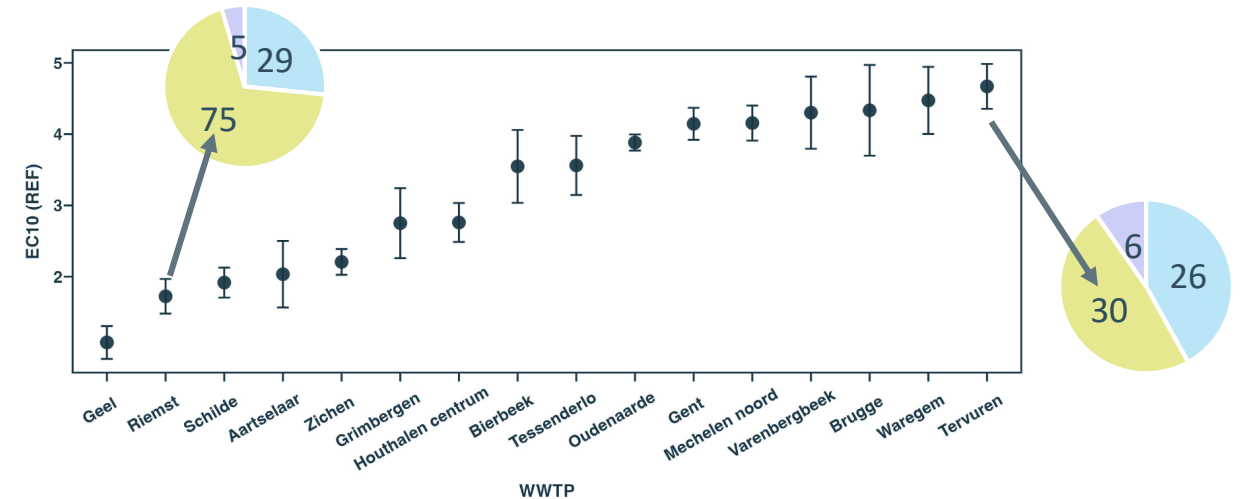
- High-resolution MS: **Agilent QTOF 6530**
- Accurate mass: **compound identification**



Targeted analysis: 335 compounds

Class	#
Pharmaceuticals	60
Pesticides	257
PFAS	18

In total: **136 different OMPs identified** (over 16 locations)



High # of pesticides detected → increased toxicity in cyanobacteria

Link sample composition – toxic effects?

Link toxicity – chemical composition



Iceberg modeling

Quantitative information on 28 selected compounds*

- Cyanobacteria: herbicides, antibiotics
- Zebrafish: neuroactive compounds

*Quantification difficult for all compounds
(matrix effects LC-MS → correction with isotopically labeled standards)

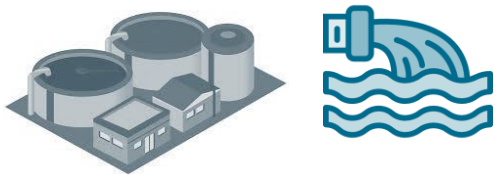
→ LC-MS method validation in progress (LOD/LOQ, SPE recovery, matrix effects)



→ help identifying the main drivers of toxicity in these complex samples

Preliminary conclusions

OMP in WWTP effluents



- Important toxicity of these effluents to different aquatic organisms
- Difference in sensitivity between zebrafish and cyanobacteria
- Toxicological response linked to chemical composition of the sample
 - Variation between samples from different locations

→ Need for **improved wastewater treatment** to remove these pollutants from the water cycle!



Thank you for your attention!

Questions?

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