In situ localization of micropollutants and associated stress response in *Populus nigra* leaves using MALDI-FT-ICR-imaging and LC-MS/MS

L. Maurer[a,b] ,C. Villette[a], J. Delecolle[a], J. Zumsteg[a], M. Erhardt[c], D. Heintz[a]

[a]Plant Imaging and Mass Spectrometry (PIMS), Institut de biologie moléculaire des plantes, CNRS, Université de Strasbourg, 12 rue du Général Zimmer, 67084 Strasbourg, France

[b]Département mécanique, ICube Laboratoire des sciences de l'ingénieur, de l'informatique et de l'imagerie, UNISTRA/CNRS/ENGEES/INSA, 2 rue Boussingault, 67000 Strasbourg, France

[c]Microscopie et imagerie cellulaire, Institut de biologie moléculaire des plantes, CNRS, Université de Strasbourg, 12 rue du Général Zimmer, 67084 Strasbourg, France

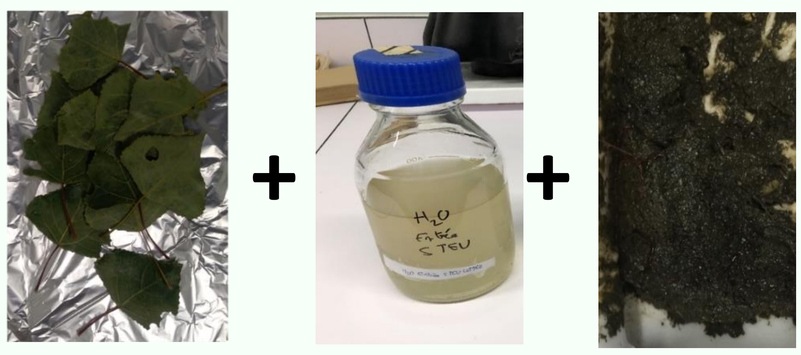
Introduction

Micropollutants and **emerging organic contaminants** are the subject of numerous studies. The use of **mass spectrometry imaging (MSI)** allows the **localization** and also the monitoring of micropollutants in plants leaves. Most of the studies are focused on samples obtained in laboratory conditions. To our knowledge, no studies have been published regarding black poplar (*Populus nigra*) growing in **polluted field conditions**. Non-targeted analysis performed **by MSI coupled with LC-MS/MS** was used to uncover the metabolic profile of a poplar growing in such conditions, but also the localization of micropollutants accumulated in leaf tissues. A **control plant** implemented on the same study site **enforced the conclusions**. This brings the following questions: How do micropollutants diffuse in the environment? What are the mechanisms implemented by poplar to cope with this chronic exposure to diverse micropollutants? To answer these questions, we studied different compartments of the environment: water, sludge and black poplar leaves.

Materials & Methods

Frozen leaves from *P. nigra* were embedded in **M-1 matrix** (Thermo Scientific), **cryosectioned at -20°C and** mounted on an (Indium Tin Oxide) ITO-coated slide. Samples were coated with **HCCA (α-cyano-4-hydroxycinnamic acid) matrix**. The samples were analysed on a **SolariX XR 7T** (Bruker) in **MS positive mode** (50-1,000Da); data were processed using **SCiLS Lab2016b**. Further LC-MS/MS targeted analysis of pigments and hormones levels revealed plant stress responses. Additionally, **sludge** and **wastewater** were collected in the **same square meter** as the poplar. These samples were analysed by **LC-MS/MS to understand the micropollutants distribution in the environment**.

MSI image acquisition from SolariX XR 7T (Bruker)



Poplar leaves + **Wastewater** + **Sludge**

Non-targeted

analysis LC-MS/MS

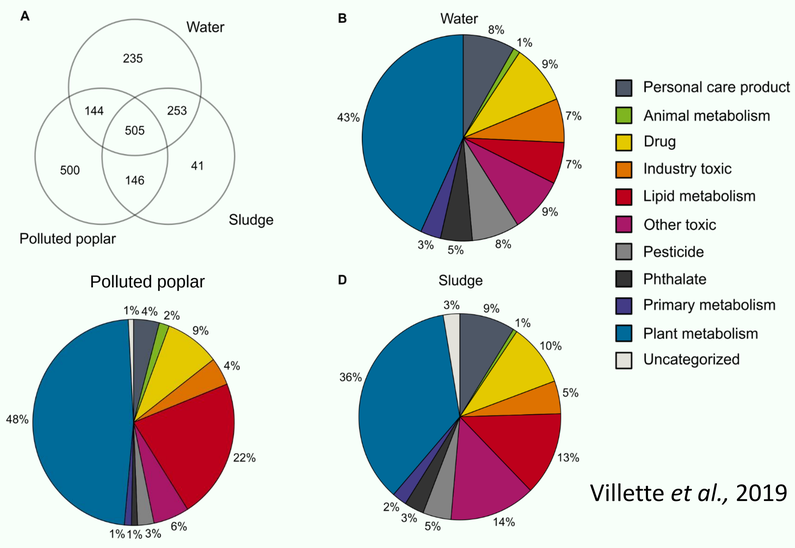
Q-TOF (Impact II, Bruker)
TargetScreener

Annotation with **Metaboscape 3.0** (Bruker)
SmartFormula to generate raw formula
Analyte lists from micropollutants databases

Confirmation of some annotations by commercial standard

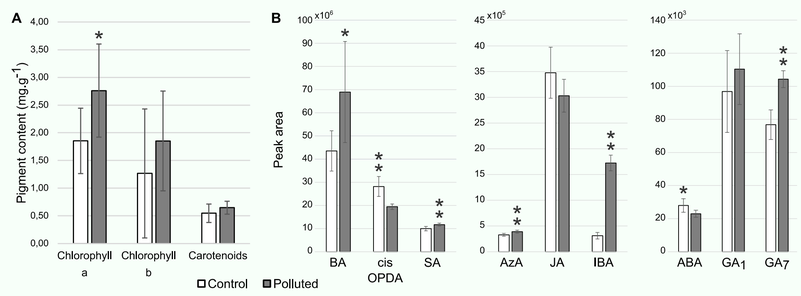
Results & Discussions

Micropollutants distribution in the environment



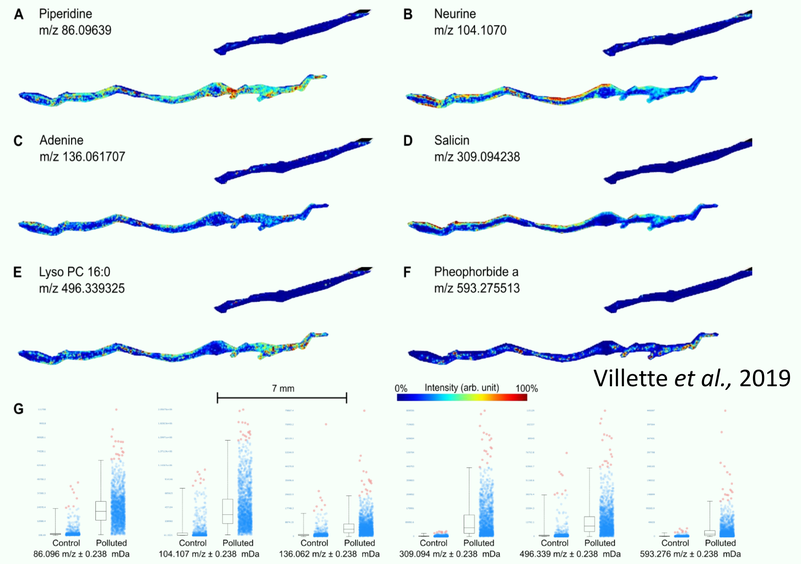
Villette *et al.,* 2019

Responses to stress



Villette *et al.,* 2019

Compounds distribution in poplar leaves



Villette *et al.,* 2019

A **wide variety of micropollutants** as well as their **diffusion** in water, sludge and poplar leaves were described. **Six classes** of micropollutants from **anthropogenic origin** (personal care products, industry toxics, drugs, pesticides, phthalates, other toxics) were detected in all samples.

Chlorophyll a degradation products and IBA were more abundant in polluted leaves. This indicates plant stress and a **higher metabolic turnover**. The plant **produces higher amount of biomass** to **counter** the possible loss due to micropollutants accumulation.

MSI poplar leaves showed an **accumulation of micropollutants in the outer tissues** of the leaves, which is not correlated to the physico-chemical properties of the stored micropollutants. This reveals **active processes** occurring in *P. nigra* leaves to manage the accumulation of exogenous and potentially toxic molecules. These compounds are **probably confined to avoid toxic effects or to promote their degradation.**

Conclusions

The study shows that **micropollutants are diffusing in the different compartments** of the environment. Furthermore, the analysis of *P. nigra* leaves metabolic profile using high resolution MSI highlights the fact that a plant growing in this area **accumulates micropollutants, which show** a **specific distribution**. These pollutants generate a **stress response** from the plant.

References

Villette, C., Maurer, L., Delecolle, J., Zumsteg, J., Erhardt, M., & Heintz, D. (2019). In situ localization of micropollutants and associated stress response in Populus nigra leaves. *Environment international*, *126*, 523-532