



## ● Imaging by MRMS reveals xenobiotics accumulation and plant response in *Populus nigra* leaves

Have you ever wondered how plants growing in a polluted environment cope with chronic exposure to exogenous compounds such as micropollutants? Do you know how plants deal with those compounds, and what they do to the plant? Moreover have you ever imagined that you could get answers to these questions at the tissue level? Here we demonstrate that MALDI Imaging can provide insight into these issues.

### Introduction

The accumulation of exogenous compounds (also known as xenobiotics) in plants has widely been described in the literature, but knowledge about how exactly

the plant deals with these compounds is very scarce. At the exit of a wastewater treatment facility, it has been proven that spontaneous plants in the ecosystem have different capabilities in storing different pollutants,

acting as a team to cope with the mixture of pollutants they are facing [1]. The ability of plants to transform xenobiotics in other by-products (catabolites or conjugates) has also been demonstrated from spontaneous

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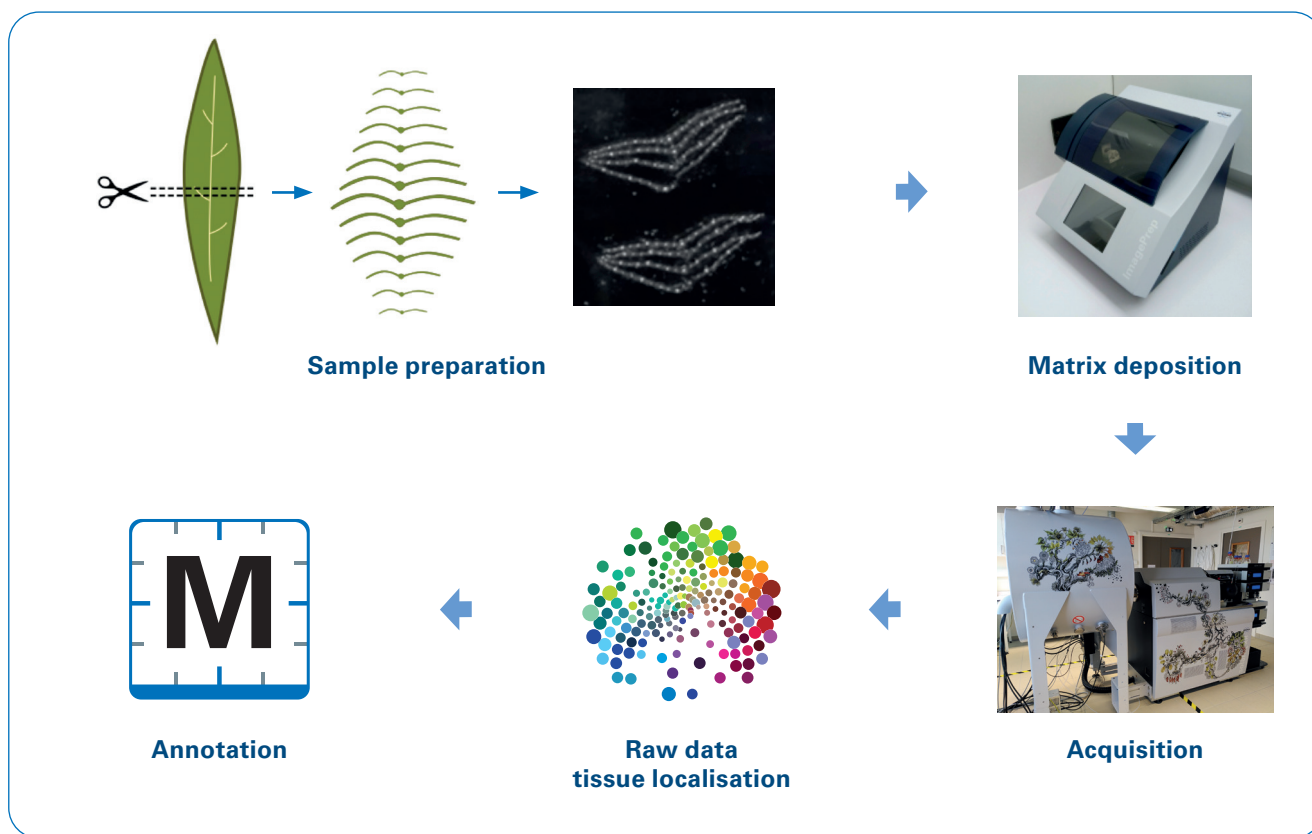


Figure 1: Experimental design from fresh poplar leaf samples to data processing and annotation. Adapted from Villette et al. 2020 [3] with permission.

plants in environmental conditions [2]. To further our understanding of plants' capabilities to manage xenobiotics, mass spectrometry imaging (MSI) is a very valuable tool which allows the *in situ* localization of pollutants and the associated plant response in intact plant tissues. *Populus nigra* (black poplar) leaves were investigated by MSI showing the accumulation of xenobiotics in specific tissues and the accumulation of plant metabolites in response to these exogenous compounds.

## Methods

To check the presence and storage of xenobiotics in poplar leaves, and to assess the plant response to such accumulation, a control is necessary, which is often difficult to obtain in real environmental conditions. We studied a poplar growing at the exit of a wastewater treatment facility and compared it to a control poplar growing several meters away on a drained bank, therefore receiving only rainwater to grow. Plant leaves were

harvested and embedded, frozen and transversally cut with a cryo microtome, then deposited on ITO-coated glass slides. An ImagePrep sprayer (Bruker Daltonics GmbH & Co. KG, Bremen) was used to cover the samples with  $\alpha$ -cyano-4-hydroxycinnamic acid (HCCA) matrix at  $7 \text{ g} \cdot \text{L}^{-1}$  in 50:50 MeOH:H<sub>2</sub>O + 0.2% TFA. The MALDI Imaging acquisition was performed on a solariX XR 7T instrument in positive ion mode, on a mass range  $m/z$  50 - 1000 using a raster width

of 50  $\mu\text{m}$ . The obtained raw data were imported into SCiLS Lab 2016b software (Bruker Daltonics GmbH & Co. KG, Bremen) to visualize spatially resolved images and to perform statistical analysis between the polluted leaves and the control leaves (segmentation and PCA). Then, the discriminative values between control and polluted leaves were searched for using the ROC (receiver operating characteristic) curve. Discriminative  $m/z$  values which were specific to the polluted leaves were exported to be further annotated in MetaboScape 4.0

(Bruker GmbH & Co. KG, Bremen) with analyte lists derived from HMDB (<https://hmdb.ca/>), ChEBI (<https://pubchem.ncbi.nlm.nih.gov/source/ChEBI>) and SwissLipids (<http://www.swisslipids.org/#/>) online databases. The whole workflow from raw samples to data analysis is displayed in Figure 1.

## Results and Discussion

The segmentation performed on the polluted and control leaves showed a distinct metabolic profile between the two samples. This was con-

firmed by the principal component analysis (PCA), which revealed that component 1 was specific to the control leaf, while component 2 was specific to the polluted leaf (Figure 2).

The discriminative  $m/z$  values obtained from the ROC curve were exported to MetaboScape for feature annotation. These annotations revealed an accumulation of xenobiotics in the poplar polluted leaves, while this accumulation was not observed in the control leaves (Figure 3). More precisely, these exogenous compounds were found to be more

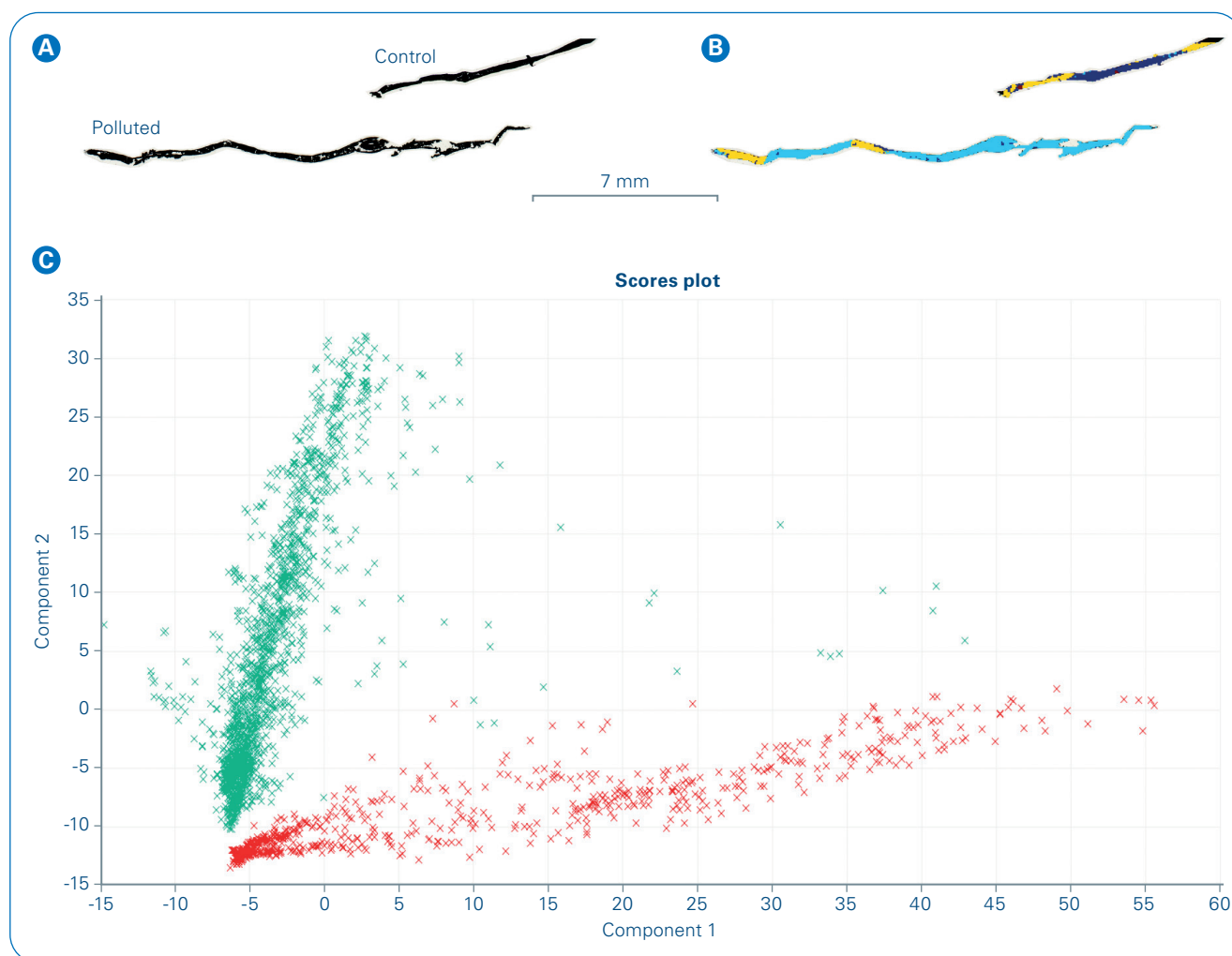


Figure 2: Statistical analysis performed on the control and polluted poplar leaves reveals a specific metabolic profile in the polluted leaves. (A) optical images; (B) segmentation results; (C) principal component analysis (PCA) plot. From Villette et al. 2019 [4] with permission.

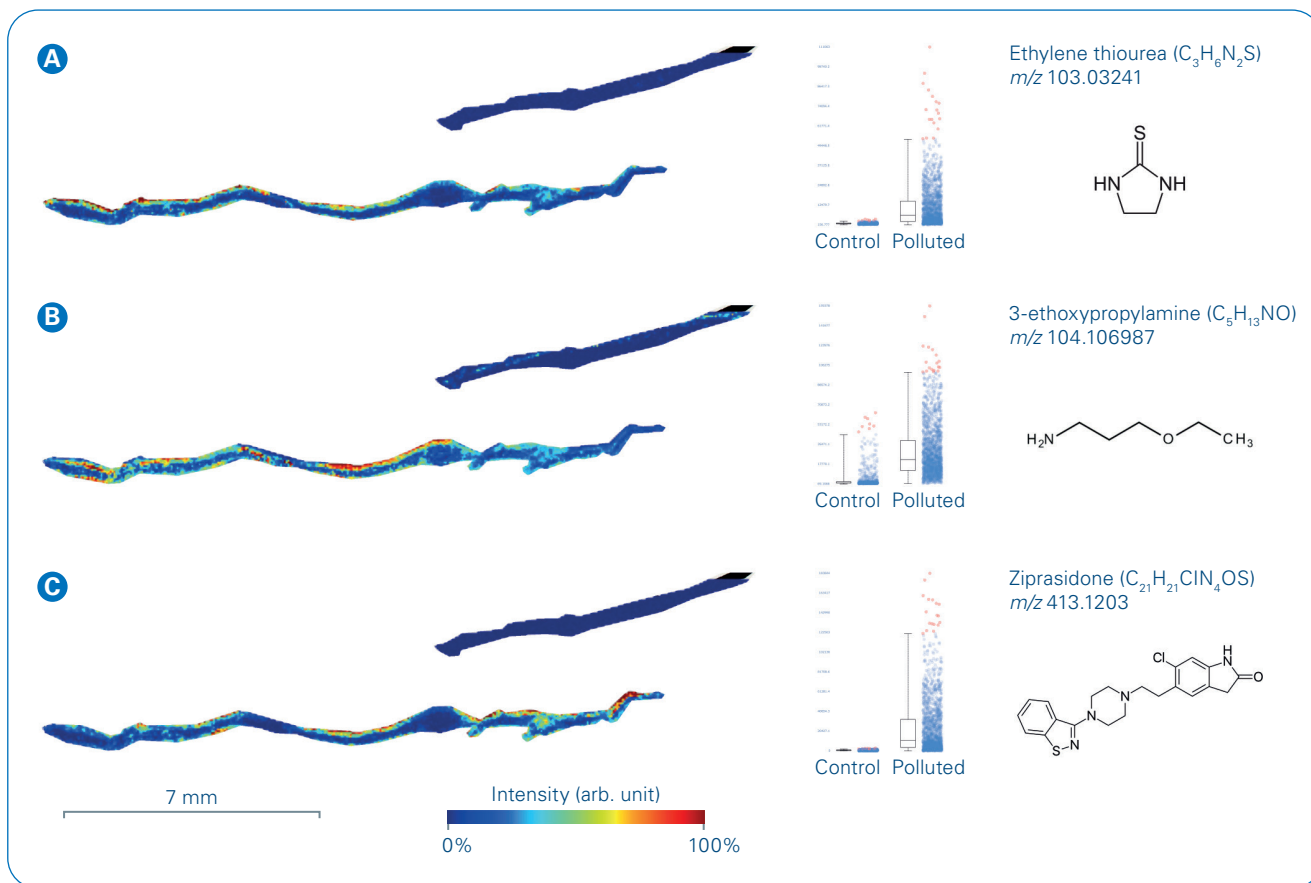


Figure 3: Xenobiotics annotated in poplar leaves. The exogenous compounds were detected in the polluted leaves but not in the control leaves, and were located in the outer tissues on the upper side of the leaves. From Villette et al. 2019 [4] with permission.

abundant in the outer tissues of the leaves, and located at the upper side of the polluted leaves. Among others, the compounds ethylene thiourea, 3-ethoxypropylamine and ziprasidone were found by database search. Ethylene thiourea is a pesticide, 3-ethoxypropylamine is a personal care product derivative and ziprasidone is a drug, which proves that poplar plants are able to manage and store very different types of exogenous compounds.

The second step of this study was to understand how the plant responds

to this accumulation of exogenous compounds. Indeed, complementary LC-MS/MS-QTOF analysis showed that the plant is able to live with one third of its metabolism made of exogenous material, while still looking healthy from a phenotypical point of view [4]. Microscopic observations confirmed that the tissues are not altered in the polluted leaves. However, MALDI Imaging analysis revealed that some plant metabolites can also be found to be differential between the control leaf and the polluted leaf (Figure 4). These metabolites are stress-related com-

pounds, and are more generally distributed in the leaf tissues. Degradation products (neurine is involved in putrefaction, pheophorbide a is a catabolite of chlorophyll) were also discovered. These results show that the poplar plant reacts to the accumulation of xenobiotics, and that the plant is able to move these compounds to specific tissues. However, this reaction has a cost: the metabolic activity in the polluted leaves is higher, and chronic stress could be depicted by the dosage of pigments and hormones [4].

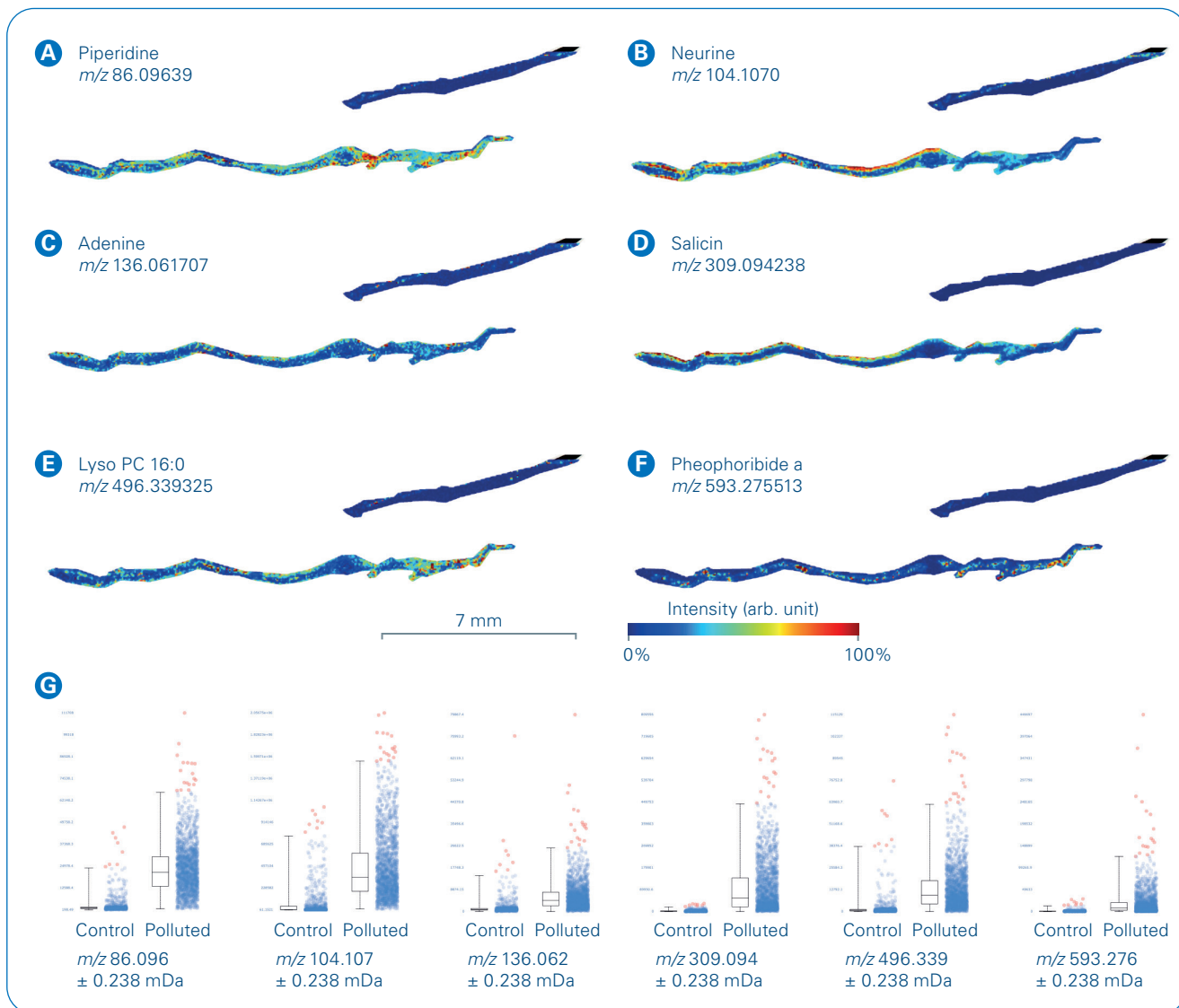


Figure 4: Plant stress-related metabolites annotated in poplar leaves. From Villette et al. 2019 [4] with permission.

## Conclusion

- MRMS MALDI Imaging was applied to the study of polluted *Populus nigra* leaves in real environmental conditions, with implementation of a control plant to make the results robust.
- Exogenous compounds were very accurately detected by MALDI Imaging due to high mass accuracy of MRMS for database search and a specific tissue distribution was observed in the peripheral tissues, on the upper side of the leaf.
- The stress response of the plant to the accumulation of xenobiotics was revealed and confirmed that only xenobiotics are guided to the peripheral tissues by the plant.



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