



● **MRMS aXelerate – rapidly detected micropollutants and plant response metabolites in poplar leaves**

MRMS aXelerate is demonstrated to be a new and powerful workflow to rapidly profile plant extracts in a context of environmental pollution. This technique enables increased sample throughput by chromatography-free flow injection analysis (FIA) in combination with extreme mass resolution provided by the scimaX MRMS system complementary to deep profiling by LC-MS

MRMS aXelerate incorporates a 3-tier confidence engine provided by MetaboScape 4.0 allowing confident assignments of molecular formulae: a combination of ultra-high mass accuracy, True Isotopic Pattern and Isotopic Fine Structure to ensure confident

assignments at any level. Here it enabled the annotation of plant metabolites from several classes. Additionally, micropollutants (drugs and pesticides) which accumulated in poplar leaves could be detected. This accumulation reflected the growth

conditions of the analysed plants, either near polluted water or using only rain water. This study shows the straightforward workflow from plant crude extracts to detect drugs and pesticides using MRMS aXelerate.

Keywords:
plant metabolites, drugs, pesticides, FIA, MRMS, screening, metabolite profiling, metabolomics

Introduction

Wetlands are used for water depollution purposes in small towns. The waste water from houses is brought to a constructed wetland composed of several ponds planted with reeds (*Phragmites australis*), to be filtered. Before rejection to the natural environment (for example a river), the filtered water goes through a natural pond colonized by endemic species. The study presented here focuses on two poplars (*Populus nigra*) planted either on the natural pond riverbank (called “polluted” poplar), or several meters from the pond (called “control” poplar). FIA-MRMS and subsequent annotation with MetaboScape 4.0 using drug and pesticide databases were successfully used to analyse the plant metabolome and micropollutants.

Experimental

Sample preparation

Poplar leaf samples were collected from a wetland in Alsace (east of France). The differential analysis was permitted thanks to the build-up of the experiment: one poplar was planted on the riverbank of the natural pond of the wetland; a control poplar

Table 1: Number of features and annotations using ESI(+), ESI(-) and combined data

Measurement	Features	Analytes FooDB *** plus drugs and pesticides	Mol. Formula with SF calc. **
ESI(+)	2,093	87/100*	1,801/1,876*
ESI(-)	1,444	277/306*	1,304/1,404*
ESI(+) and ESI(-) combined	3,452	326/383*	2,638/3,116*

Mass tolerance for Analyte List based search: 0.2/0.5 ppm

Mass tolerance for SmartFormula search: 0.2/0.5 ppm

Isotope accuracy for SmartFormula search: 0.2/0.5 ppm

* First value with mass tolerance of 0.2 ppm; second value with mass tolerance of 0.5 ppm

** Elements $C_{20}H_{10}O_4P_4S_4$ have been considered for molecular formula calculations

was planted several meters away from the pond to grow using only rain water. The poplars were planted approximately 2 years before the study occurred. For each condition, eight biological replicates were prepared using 300 mg (FW) of leaves, ground in liquid nitrogen and extracted three times with methanol. The supernatant was collected and dried, then resuspended in 1 ml MeOH. Deuterated abscisic acid was added (1 µg/mL 2H_6 ABA final) prior to extraction as an internal standard. A QC sample was prepared by mixing half part of control and half part of polluted extracts.

MS analysis

8 biological replicates of the “polluted” and the control samples and 2 QC samples were measured in 3 replicates in ESI(+) and ESI(-) modes using a scimaX 7T MRMS system. The stock solutions were diluted 1:1,000 with MeOH for the FIA-MRMS measurements. The detection mass range was set to m/z 107 – 3,000 with a mass resolving power of 1,350,000 at m/z 200 using quadrupolar detection. The measurements were performed with an UHPLC Elute HT system using a 20 µl sample loop. The sample loop was fully filled

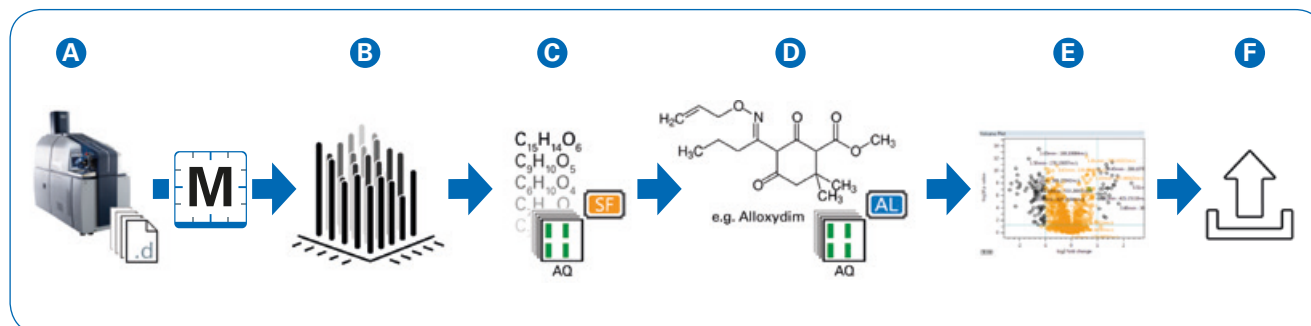


Figure 1: Schematic workflow: **A** FIA-MRMS acquisition using a scimaX MRMS **B** Data processing and evaluation using T-ReX 2D in MetaboScape 4.0 **C** Generate list of molecular formula annotations including annotation qualities **D** Putative compound annotations using AnalyteList of known and expected compounds **E** Statistical analysis to identify features of interest **F** Optional export for advanced statistical analyses

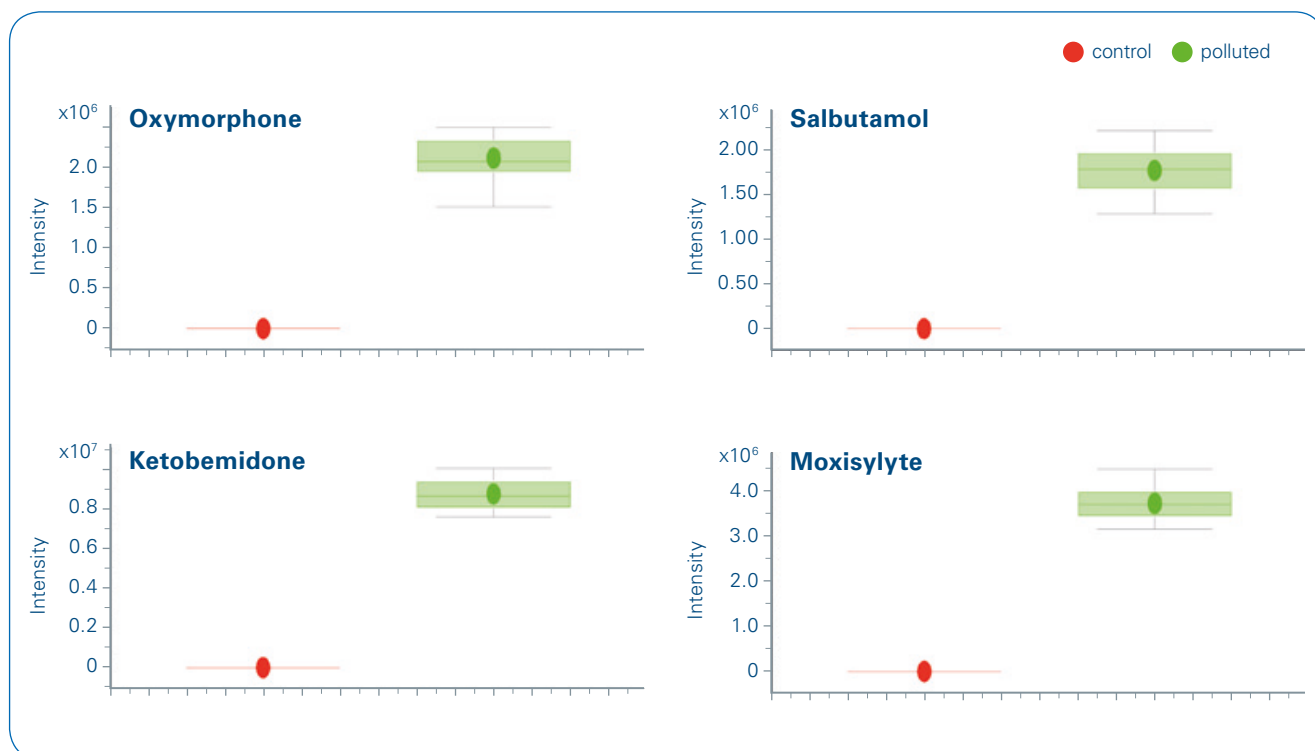


Figure 3a: Bucket statistic (box plots) of detected drugs in polluted poplar samples (green) and control poplar samples (red) detected in positive ion mode

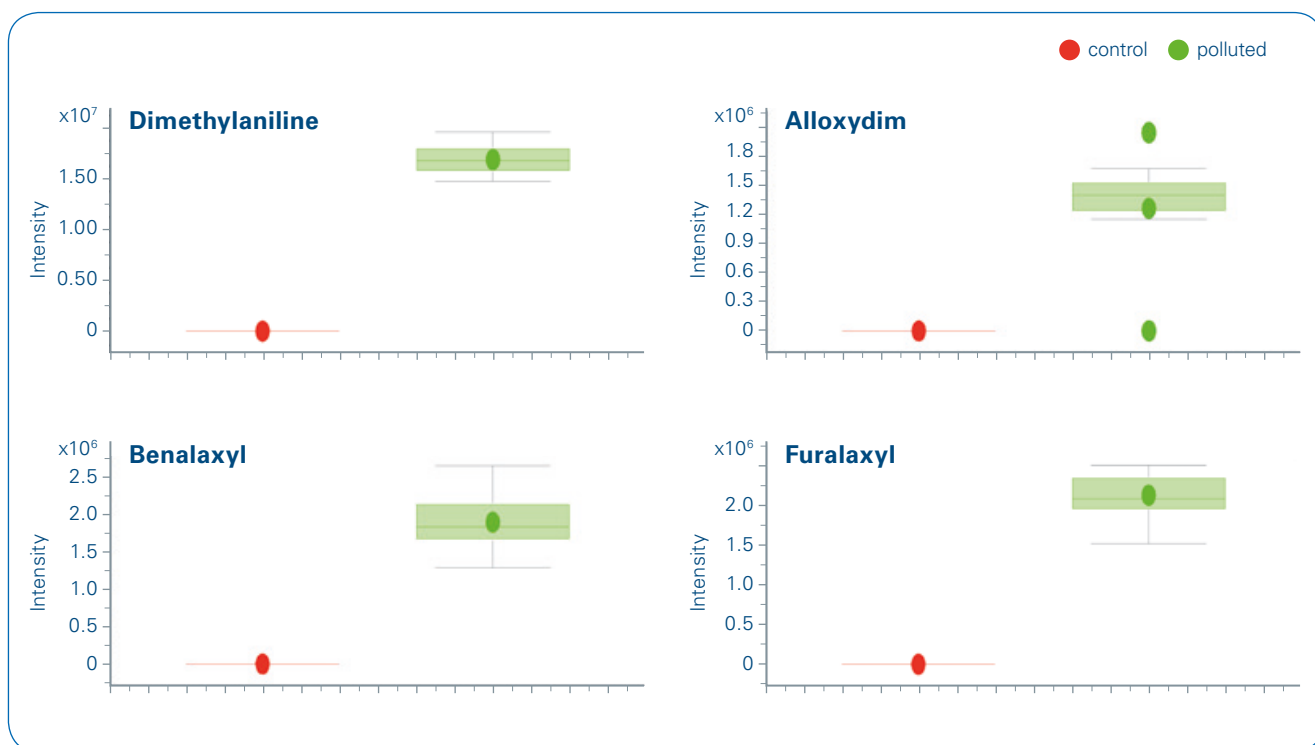


Figure 3b: Bucket statistic (box plots) of detected pesticides in polluted poplar samples (green) and control poplar samples (red) detected in positive ion mode

(wide) mass tolerances and retrieved 326 (0.2 ppm) and 383 (0.5 ppm) compounds with the Analyte List containing plant metabolites, drugs and pesticides. 2,638 (0.2 ppm) and 3,116 (0.5 ppm) molecular formulae were assigned with automatic Smart Formula based annotation. Several classes of plant metabolites could be detected and annotated: chlorophyll and derivatives, lipids, flavonoids, sugars, but also less abundant metabolites as hormones and derivatives. This experiment also showed the presence of micropollutants and drugs in poplar leaves, which were expected due to the growth conditions of the plants. T-test calculation of the T-ReX 2D extracted features in MetaboScape revealed several significant changes between

the two different plants (see Figure 2). Investigating these in more detail by box plot displays in MetaboScape revealed clear differential patterns for several micropollutants (drugs and pesticides) between control and polluted plants (Figure 3a and 3b).

This workflow proves that the assignment of metabolites and micropollutants in chromatography-free non-targeted profiling of crude plant extracts is possible. Additionally, MRMS aXelerate can increase sample throughput using the applied rapid flow injection analysis. Even in these conditions, low abundant metabolites could still be detected and annotated.

*** Note: FooDB is not a Bruker product.

Acknowledgements

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Conclusion

- MRMS aXelerate significantly reduces the sample analysis time by omitting time consuming chromatographic separation.
- Flow Injection Analysis increases sample throughput.
- Low abundant plant metabolites could be detected from crude extracts without dedicated purification.
- Metaboscape 4.0 allows the annotation of plant metabolites and micropollutants based on 3 tier confidence using specific databases.
- This technique enables non-targeted analysis of crude extracts, which could lead to detection of several different micropollutants accumulating in plant leaves.



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