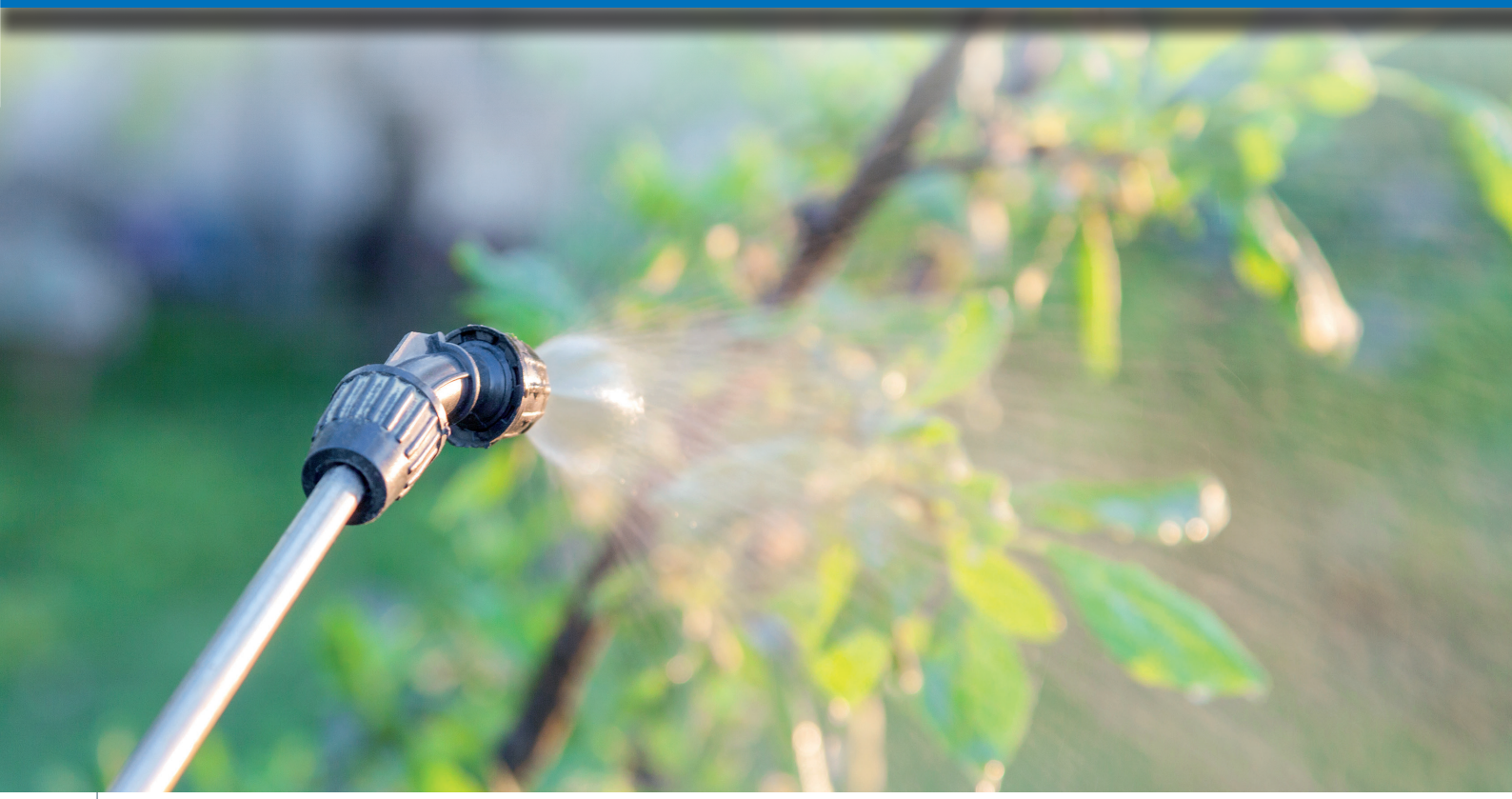

MRMS aXelerate – rapidly detected micropollutants and plant response metabolites in poplar leaves

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

Author: Claire Villette[1], Matthias Witt[2], Aiko Barsch[2], Dimitri Heintz[1]
[1] Institut de Biologie Moléculaire des Plantes (IBMP), CNRS, Université de Strasbourg, France; [2] Bruker Daltonik GmbH, Bremen, Germany

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.

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 $C_cN_{20}H_hO_{40}P_4S_4$ have been considered for molecular formula calculations*

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 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

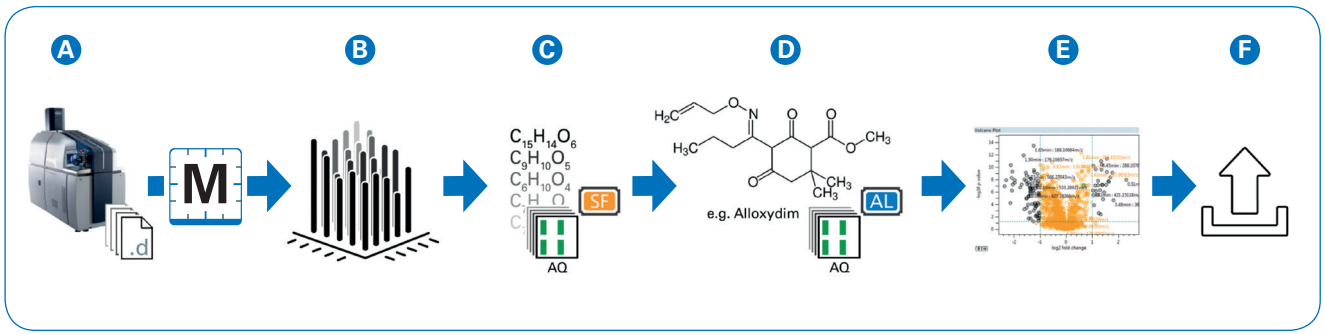

Figure 1: Schematic workflow: A FIA-MRMS acquisition using a scimaX MRMS B Data processing and evaluation using T-ReX 2D in MetaboScape 4.0 C Generate list of molecular formula annotations including annotation qualities D Putative compound annotations using AnalyteList of known and expected compounds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

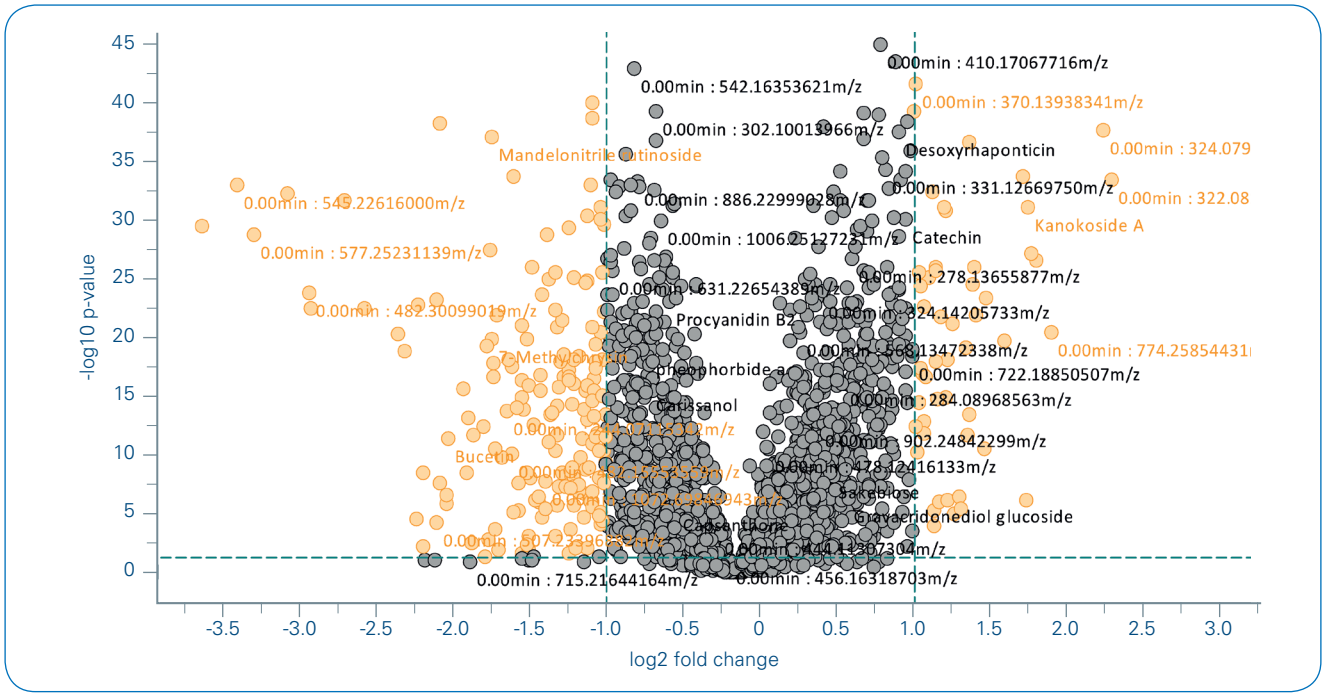

Figure 2: Volcano plot representation of t-test results comparing control vs. polluted sample groups

with sample solution for FIA-MRMS measurements. The sample solution was transported within 6 s to the ion source with a flow of 100 µl/min. The flow was then reduced to 10 µl/min to a have a constant signal for at least 1.5 min. 15 s after sample injection data was acquired for 1.4 min resulting in a total sum of 28 single scans for the final mass spectrum. After 1.8 min the flow was increased to 300 µl/min to wash the line between injector port and ion source as well as the sample loop. Spectra were internally calibrated with Hexakis (1H,1H,2H perfluoroethoxy) phosphazene using a lock mass container. In positive ion mode the potassiated adduct and in negative ion mode the chlorinated adduct were used for internal calibration.

Data Preprocessing

The data processing workflow is shown graphically in Figure 1. The individual mass spectra were loaded into MetaboScape 4.0, where the first step is the creation of a feature matrix (bucket table) using the T-ReX 2D algorithm. Each feature is comprised of the molecular ion and its associated Isotopic Fine Structure (IFS), if available. Additionally, the features may include possible adduct peaks and the associated isotopologues. Features were then annotated with a molecular formula using SmartFormula™ (SF) applying metabolic profiling specific filters to elements and element ratios. Annotation quality (AQ) scores are provided for each result (the first green bar means below 0.2 ppm mass deviation, the second green bar reports a mSigma below 50. This value is an indicator for isotopic pattern or isotopic fine structure matching if available). Features were also matched to Analyte Lists containing known drug and pesticides as well as plant metabolites (names and formulae of plant metabolites were derived from FooDB*** – http://foodb.ca/) for putative annotations of interesting features. Again, matching qualities were provided for each result. Statistical analysis, here a t-test was calculated to identify features of interest. Optionally the data can be exported for advanced statistical analysis.

Results

A total of 3,452 features were recovered from the raw ESI(+) and ESI(-) data (Table 1) using the T-ReX 2D algorithm for mass recalibration, feature extraction, de-isotoping, and pseudo spectra generation in the MetaboScape 4.0 software. Annotation was performed using Smart Formula, and an Analyte List of known compounds, in order to assign drug, pesticide and plant metabolites (Table 1). Annotation was performed with 0.2 ppm (narrow) or 0.5 ppm

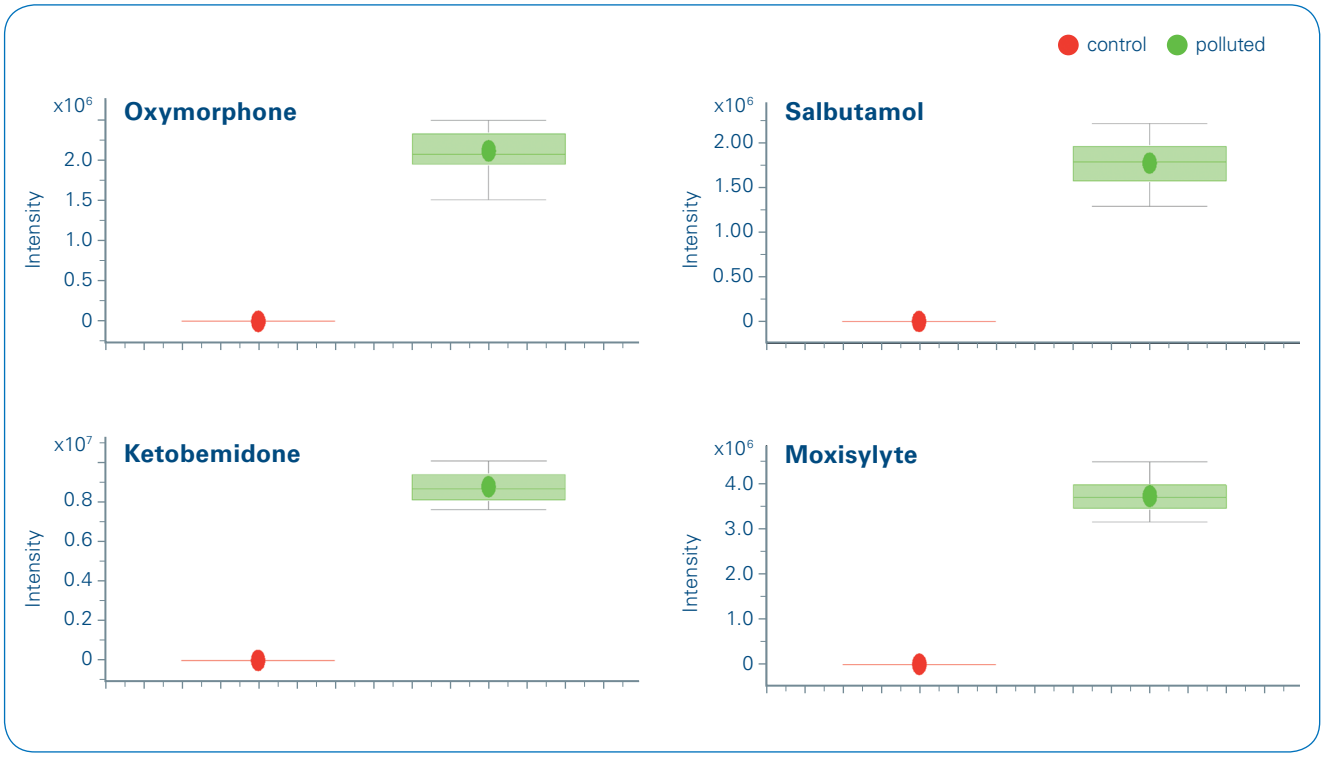

Figure 3a: Bucket statistic (box plots) of detected drugs in polluted poplar samples (green) and control poplar samples (red) detected in positive ion mode

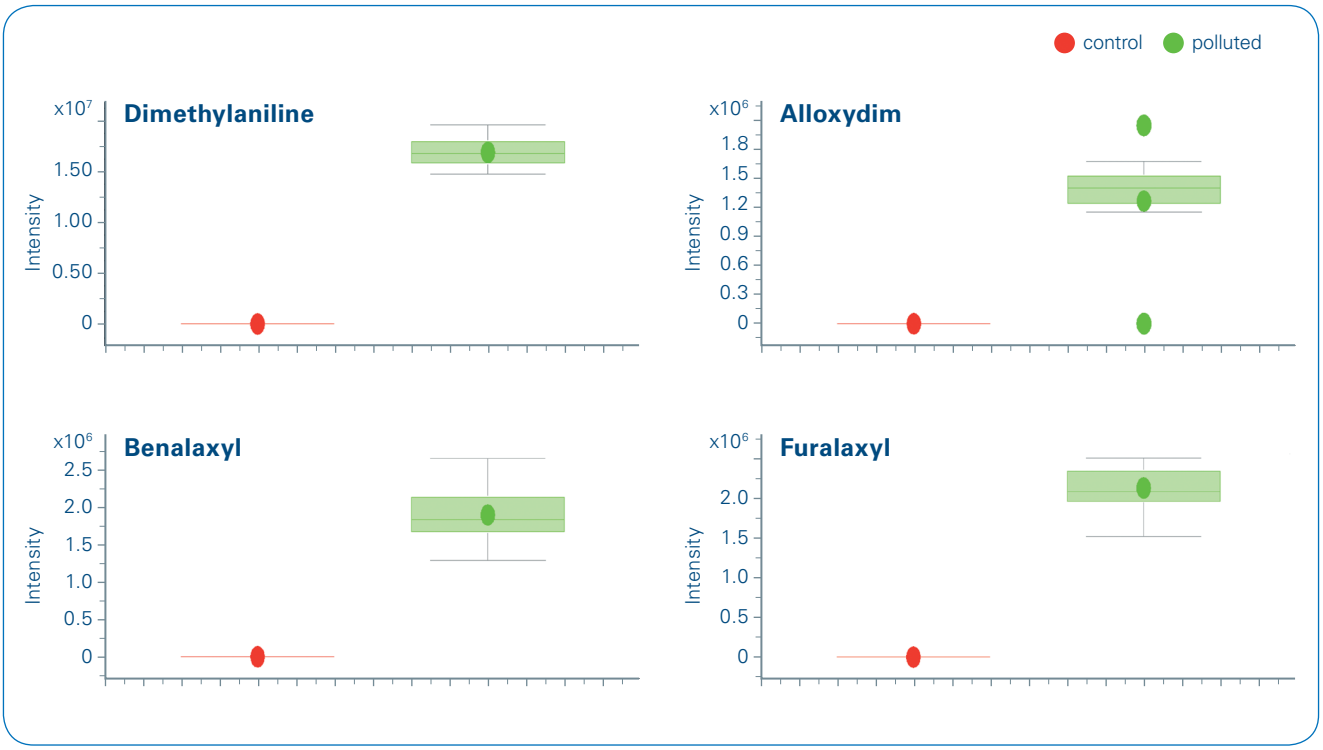

Figure 3b: Bucket statistic (box plots) of detected pesticides in polluted poplar samples (green) and control poplar samples (red) detected in positive ion mode

(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.

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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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Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com