Combination of targeted and non-targeted workflows for the identification of pollutants in river water using a passive sampling method

Many investigations of environmental or food contaminations have the same basic question, what has changed within place and time. This could be food production at a different location, food processed at different places, or a river polluted at different points on its water course, changing during a time period with different components (pollutants).

Introduction

Typically, the first step would be a targeted approach but most often a comparison in time and/or place will raise many questions. Here a proper non-targeted approach is needed to define the root cause of complex changes.

Many pollutants are ubiquitous in surface waters because of continuous discharges from municipal wastewater treatment plants and we still do not know which pollutants are reaching the environment, the size of the problem for exposed fauna, nor

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Author: Noud van der Borg; Bruker Daltonics GmbH & Co. KG, Bremen, Germany.

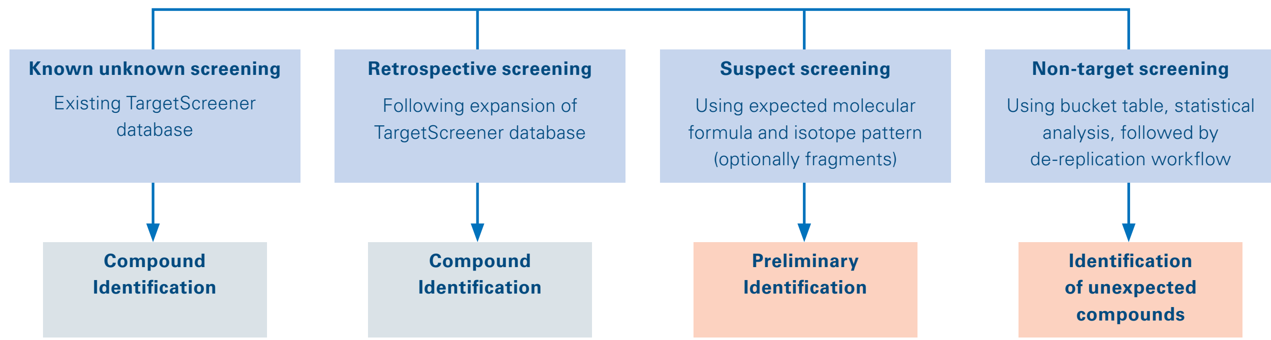


Figure 1: Approach to screen for pollutants in the River Clun using targeted and non-targeted workflows.

what effects, if any, of that exposure may be. Investigative monitoring of water bodies failing ecological standards as set out in the Water Framework Directive is now a requirement of all European Union member states. In this study we test the combination of a passive sampling device in combination with targeted and non-targeted workflows on a QTOF MS for monitoring surface water.

Methods

Chemcatcher® passive samplers were deployed for four weeks in the River Clun, Wales, UK, whose WFD status is classified as poor, to determine which chemicals may be responsible. The extracts obtained were analyzed using an impact II (Bruker) LC-QTOF-MS followed by targeted and non-targeted processing. The extracts were eluted using a 15 min gradient including a flow gradient (0.4-0.6 mL/min) on an UHPLC using a C18 (2.1 x 100 mm, 2.2 μm) column, with acidified water and methanol. For targeted screening and statistical analysis MS data were acquired using bbCID switching between MS and all ion fragmentation MS/MS data. For identification of unknowns some selected samples were analyzed using data dependent AutoMS/MS.

Targeted data analysis for the identification of 'known unknowns' was performed with TASQ (Target Analysis for Screening and Quantification) using a combined database containing pesticides and drugs. Non-targeted data analysis for the identification of 'unknown unknowns' was performed with MetaboScape® (Bruker Daltonics GmbH & Co. KG).

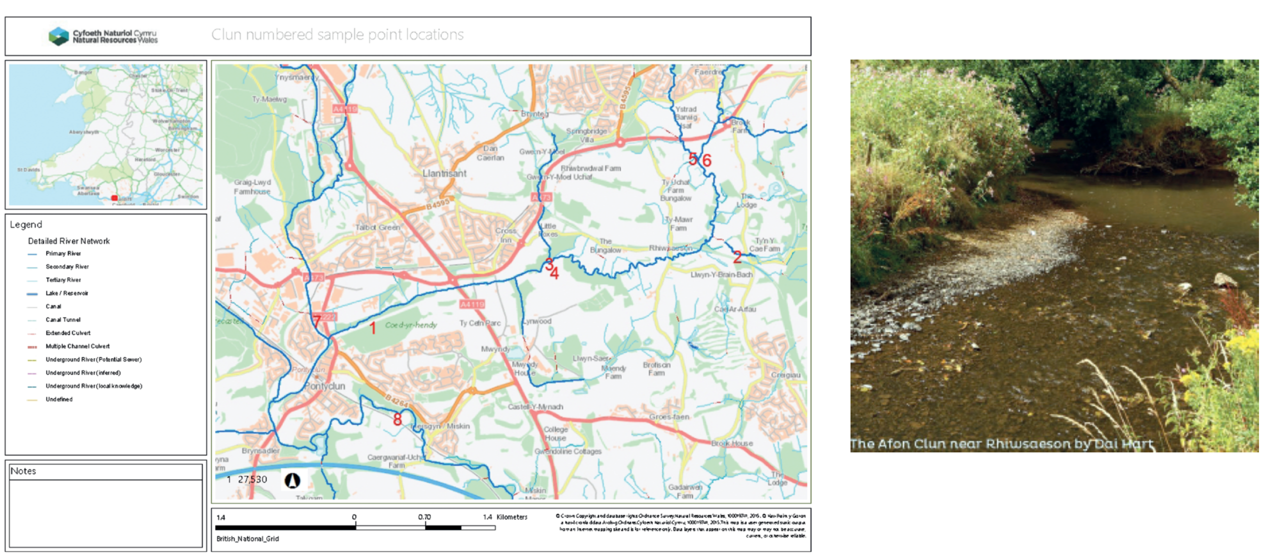


Figure 2: Case study – Clean the Clun: Samples were taken over 10 months from 8 different sampling points using Chemcatcher passive samplers. The samplers stayed in the water for 4 weeks before analyzing the samples.

- Combination of targeted and non-targeted workflows using TargetScreener with TASQ and MetaboScape
- **TargetScreener** contains over 3000 entries and a dedicated database for water analysis as an option containing more than 310 compounds taken from relevant regulations:
    - EU Directive 98/83/EC (EU 2015/1787) drinking water
    - EU Regulation RL 2008/105/ EG surface water
    - Candidates for EU regulation
    - Input from governmental water labs
- **TASQ** allows broad screening of 'known unknowns' and accurate quantitation when calibration curve is available
- **MetaboScape** does statistical analysis to detect unexpected compounds and the identification of 'unknown unknowns' using CompoundCrawler and spectral library search

The River Clun is currently failing to meet its WFD targets on invertebrates and fish, with the main reason for failure sighted as misconnections and sewage pollution.

"Clean the Clun" is a multi-partner landscape-scale initiative. Several partners have key objectives in the area, but all are centered on the flood plain and better ecosystem catchment management to achieve these objectives. Led by the South East Wales Rivers Trust but including other key partners such as RCTCBC, South and West Wales Wildlife Trust and Butterfly Conservation. The partnership is supported internally by NRW's biodiversity, fisheries and Environment Management teams. The project aims to undertake a wide range of activities to restore and protect the natural environment of the River Clun in the Ely Catchment in order to:

- Restore water quality and natural river features to promote ecological recovery
- Remediate and prevent habitat degradation such as landfilling and fly tipping
- Create habitat for species particularly the Marsh Fritillary Butterfly through nature reserves and management agreements
- Create appreciation and steward-ship in local residents through education and activities
- Ensure a lasting legacy through planning policy and volunteer groups

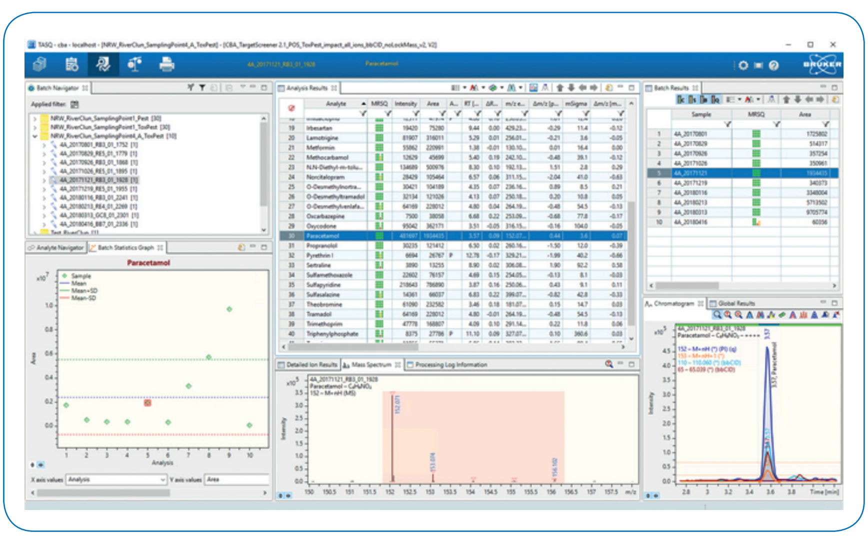
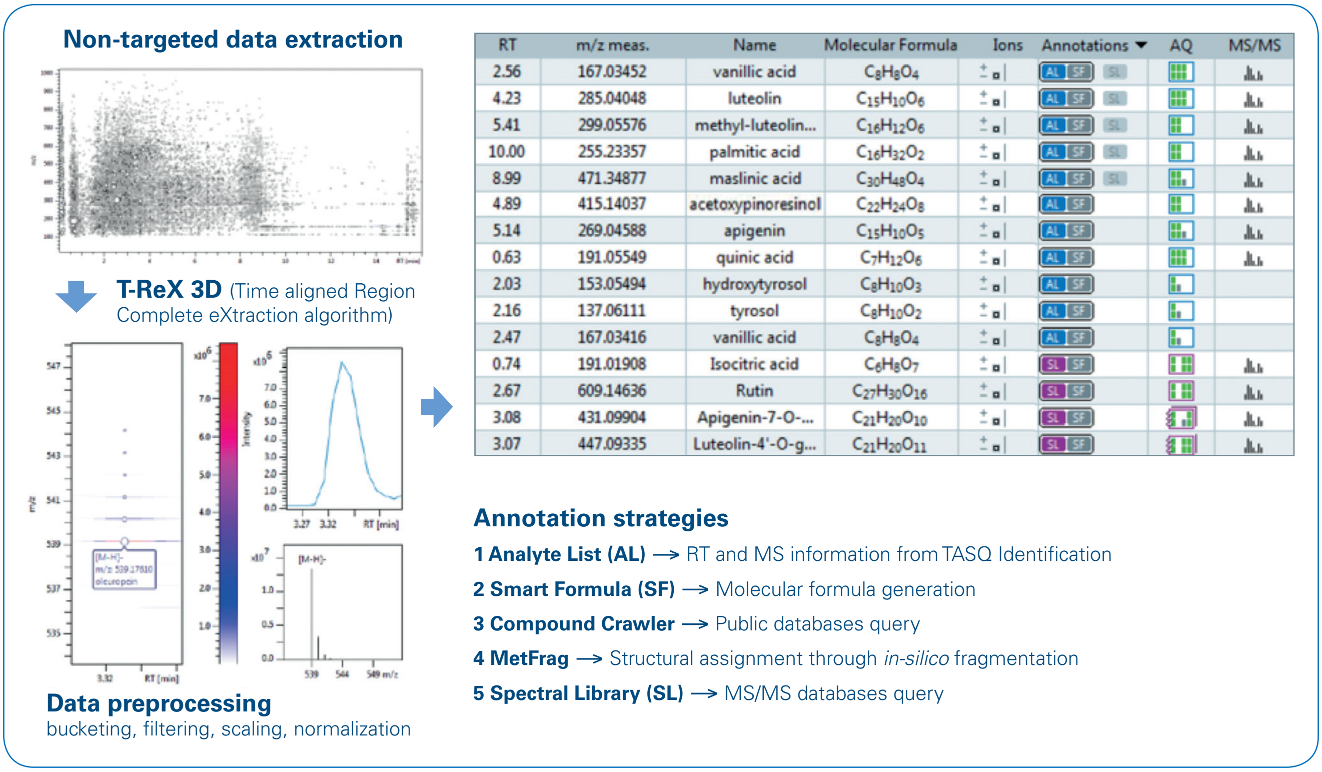


Figure 3: TASQ analysis results from sampling point 4 and the sampling period October 10th until November 21st 2018. 6 pesticides and 36 drugs could be identified with a high MRSQ score and all mandatory qualifier ions found. The batch statistics shows a time course for 10 analyses from a full year. Every data point represents a sampling duration of 4 weeks.

Results

Samples were acquired from July 2017 until April 2018 from 8 different places of the River Clun. For each site there was a time course of 10 data points covering 10 months. With the passive sampling unit every sample is an average of the pollution flowing past the sampling device during the 4 weeks it is submerged in the river. As expected, pollution in the river water due to discharges from municipal wastewater treatment plants varies significantly depending on the location and season. With the targeted approach between 5 and 93 compounds per sample could be identified with high confidence; in average about 50 compounds per sample. Most compounds were pharmaceuticals like Carbamazepine, Codeine, Citalopram, Paracetamol or Tramadol. For statistical analysis (PCA) and the identification of unknowns MetaboScape is used. This is work in progress.

We found that the passive sampling device can be used for monitoring pollutants in water and to obtain time-weighted average (TWA) or equilibrium concentrations of a wide range (2-3 orders) of pollutants in water. Most other sampling



RT	m/z meas.	Name	Molecular Formula
2.56	167.03452	vanillic acid	$C_8H_8O_4$
4.23	285.04048	luteolin	$C_{15}H_{10}O_6$
5.41	299.05576	methyl-luteolin...	$C_{16}H_{12}O_6$
10.00	255.23357	palmitic acid	$C_{16}H_{32}O_2$
8.99	471.34877	maslinic acid	$C_{30}H_{48}O_4$
4.89	415.14037	acetoxypinoresinol	$C_{22}H_{24}O_8$
5.14	269.04588	apigenin	$C_{15}H_{10}O_5$
0.63	191.05549	quinic acid	$C_7H_{12}O_6$
2.03	153.05494	hydroxytyrosol	$C_8H_{10}O_3$
2.16	137.06111	tyrosol	$C_8H_{10}O_2$
2.47	167.03416	vanillic acid	$C_8H_8O_4$
0.74	191.01908	Isocitric acid	$C_6H_8O_7$
2.67	609.14636	Rutin	$C_{27}H_{30}O_{16}$
3.08	431.09904	Apigenin-7-O-...	$C_{21}H_{20}O_{10}$
3.07	447.09335	Luteolin-4'-O-g...	$C_{21}H_{20}O_{11}$

Figure 4: Workflow for the identification of 'unknown unknowns' with MetaboScape.

techniques involve the periodic collection of spot samples of water which is just a snapshot of the conditions at the time of sampling. As pollutant concentrations fluctuate over time and may only be present at trace concentrations, the passive sampling device has many advantages compared to spot sampling techniques.

Summary

High resolution screening with a QTOF system using a combination of TASQ and MetaboScape will not only provide an answer on a known question but will also reveal all changes and contaminations which have occurred during the time period studied.

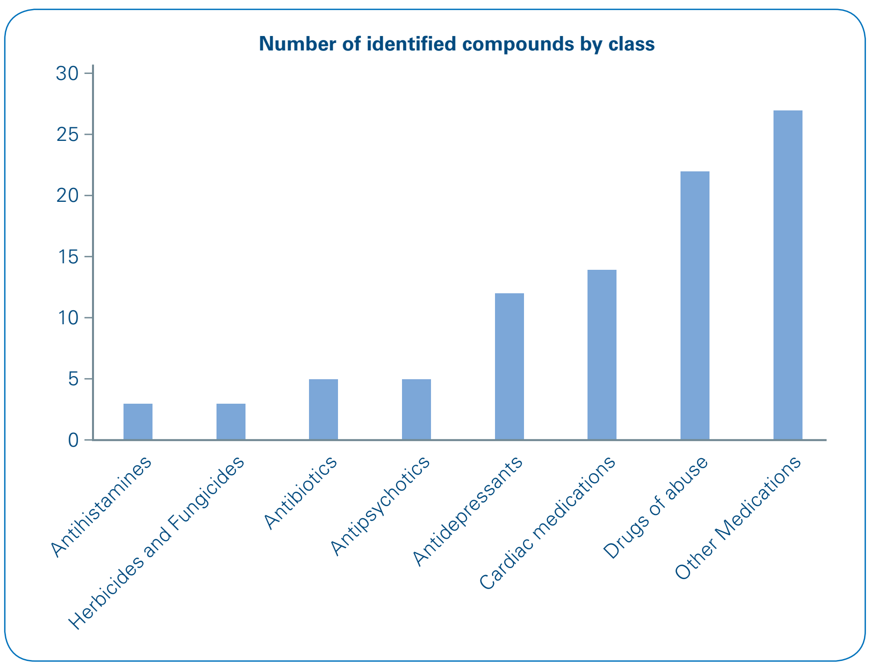
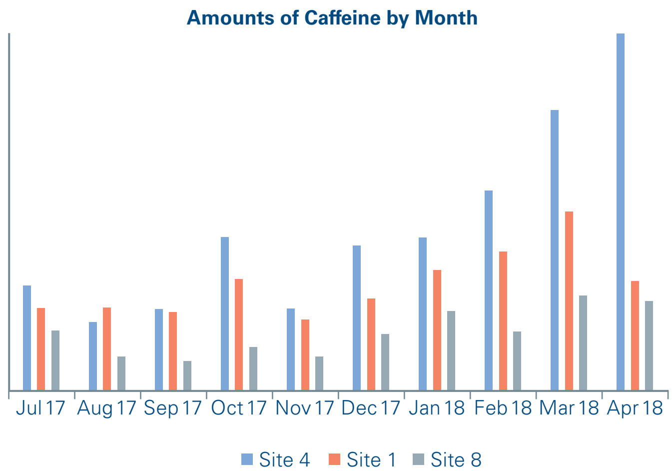
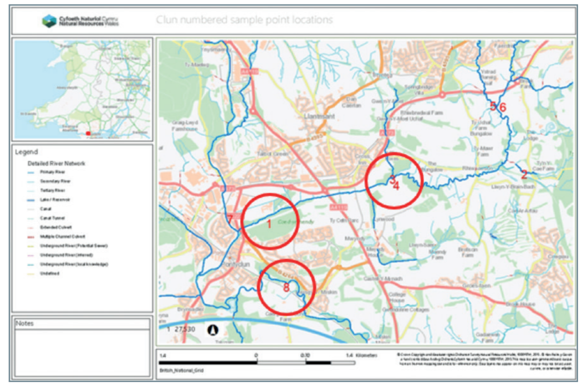


Figure 5: An overview of groups of components which were found in the River Clun.





Work in progress – preliminary data
Dr. Anthony Gravell, National Resources Wales, UK.

Figure 6: The combination of place and time of one of the found components, caffeine, in the river Clun. As the HRMS QTOF collect all data at all times, these graphs could be created for all components found in the river.

Complete analytical solution including UHPLC and impact II

- MS: Fullscan + bbCID MS/MS
- LC: column, mobile phase, accessories
- Databases for environmental, pesticides and other applications.

Software allowing

- Data acquisition via ready to use methods for both targeted and non-targeted modes
- Data processing for
  - Screening
  - Quantitation
  - Monitoring changes
  - Reviewing
  - Reporting

Workflow of TargetScreener

Step 1: Calculating the exact mass of analytes and creating EIC traces

Step 2: Are there any chromatographic peaks available?

Step 3: Verify via diagnostic ions: Scoring based on four criteria

Step 4: Optional quantification: Concentrations of confirmed compounds

Step 5: Create report and optional link to LIMS

Workflow of MetaboScape

Step 1: Acquisition of data

Step 2: Peak picking (T-ReX® 3D) and bucketing

Step 3: Statistics to identify differences

Step 4: Identification/Dereplication

Step 5: Visualization via Pathway Mapping (optional)

Conclusions

- The passive sampling device allows the end user to obtain a more representative picture of pollutants that may be present in the aquatic environment than other sampling techniques.
- TASQ allows rapid screening and quantitation of 'known unknowns' using databases with more than 3000 compounds.
- MetaboScape enables a de-replication workflow for the identification of 'unknown unknowns' using an Analyte List from TASQ, Smart Formula, Compound Crawler, MetFrag and Spectral Library Search.

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Check out the link or scan the QR code for more details.

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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