Optimization of analytical conditions to identify transformation products by LC-QTOF: application to monitor the wastewater treatment of a vegetation filter.

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Introduction & objetives

A Vegetation Filter (VF), as a type of Land Application System, is a non-conventional water treatment technology where wastewater (WW) is applied for the irrigation of a forestry plantation [1]. In this project we tested the incorporation of two soil amendments to improve natural attenuation of Contaminants of Emerging Concern (CECs) in a VF. However, potential unknown transformation products (TPs) can be produced. The main **objective of this work** is to identify the TPs formed during infiltration through soil and amended soils. A laboratory soil column experiment was performed to test woodchips and biochar potential amendments. Column effluent samples (soil, soil with woodchips and soil with biochar) were analyzed using a non-target metabolomic strategy [2].

Experimental design

Field experiments
WW from the IMDEA Water institute
Irrigation events once a week in each furrow
Humidity variable conditions
Screening for selection of main CECs in water

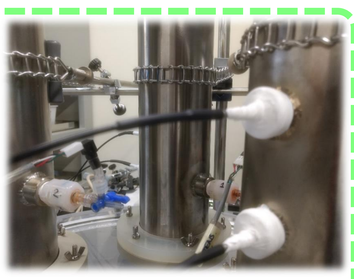
Target CECs and PTs

Anti-inflammatory: Ibuprofen, Ketoprofen, Naproxen
Analgesic: Acetaminophen
Antibiotic: Clarithromycin, Amoxicillin
Hormone: Estrone (E1)
Transformation product: 4-AAA, Atenololic acid
Life-style compounds: Cotinine, Paraxanthine

Woodchips
Promote microbial activity (biodegradation)
Increase sorption sites

Biochar
Increase surface area for microbial community establishment
Greatly increase sorption sites

Laboratory infiltration experiments
3 Stainless-steel columns
Vacuum chamber to simulate unsaturated hydraulic conditions
Synthetic wastewater (SWW) + 1 mg/L of targeted CECs
250 mL (x2 irrigation) each week
12 weeks of experimentation
3 % (w/w) of amendment added and mixed to the soil



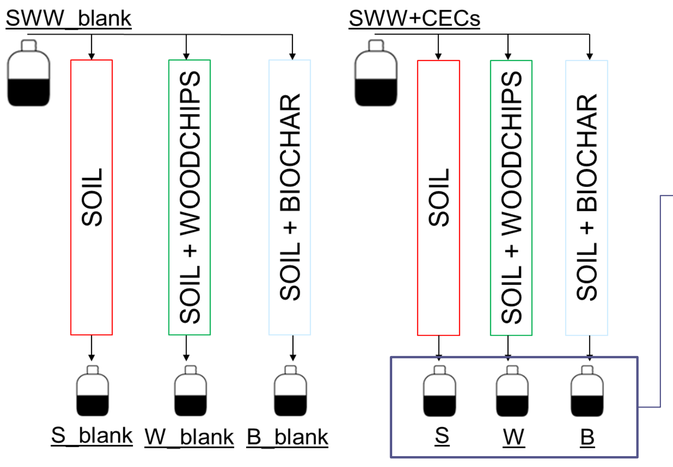


Fig. 1. *Scheme of laboratory infiltration experiments.*

Analytical Methodology

- **LC optimization**:
  - Injection volume (5-20 µL)
  - Chromatographic gradient
  - Different mobile phases at acid and basic pH:
    - Aqueous (A): water with acetate and formate
    - Organic (B): methanol or acetonitrile
  - 4 stationary phases of different polarities
  - Column temperature (30-50°C)

- **MS parameters optimization (Sciex Triple TOF 5600)**:
  - Needle position
  - Ionization mode (ESI positive and negative)

- Ensure analysis precision using control samples.

LC columns

Omega Polar (150 mm x 2.1 mm x 3 µm) (Phenomenex)	**Zic-HILIC** (150 mm x 2.1 mm x 3.5 µm) (Millipore-Merck)
Atlantis dC18 (150 mm x 2.1 mm x 3 µm) (Waters)	**Kinetex F5** (150 mm x 2.1 mm x 2.6 µm) (Phenomenex)

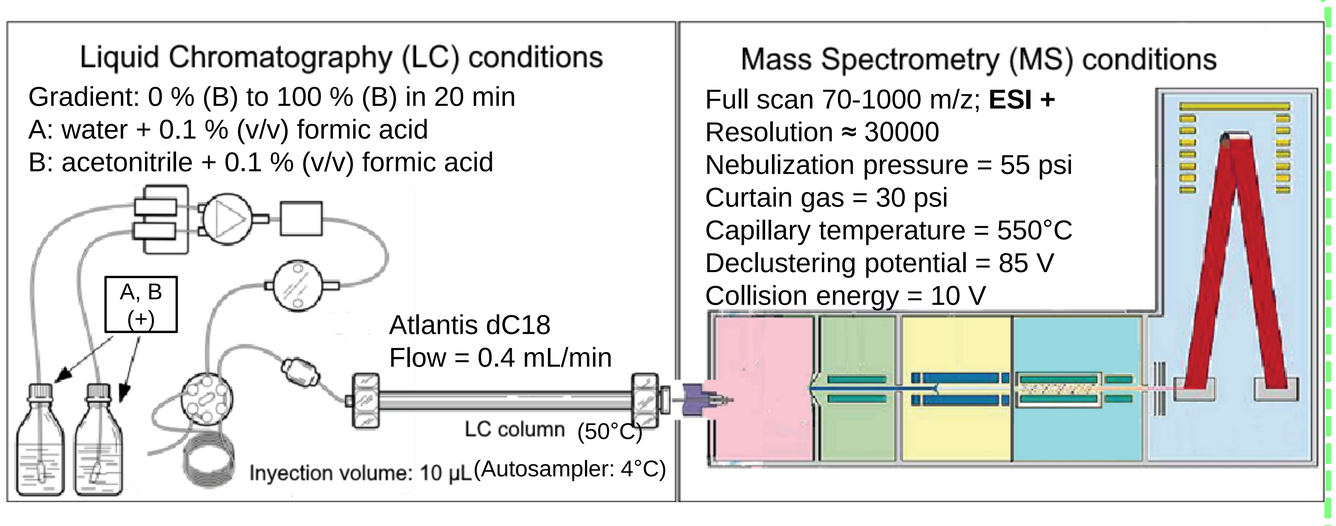


Fig. 2. *A schematic representation and main parameters of LC-QTOF defined method.*

Results

- Once method is defined (as indicated in Fig.2) samples from the 3 laboratory experimental columns (S, W and B in Fig.1) were analyzed by direct injection.

- Data results processed with Sciex OS Software:
  - Minimum peak height / width: 1000 cps / 10 points
  - Non-target screening using formula finder with 5 ppm of mass tolerance
  - Considering only peaks with area ratio threshold of 5 (unknown/control)

- Venn diagrams (Fig.3) show us the comparison of the common compounds as a function of their exact masses in the different analytical conditions used:
  - Positive mode (a.) allows to detect more compounds than in negative mode (b.)
  - Atlantis dC18, in ESI +, allows the detection of the greatest number of compounds: 240

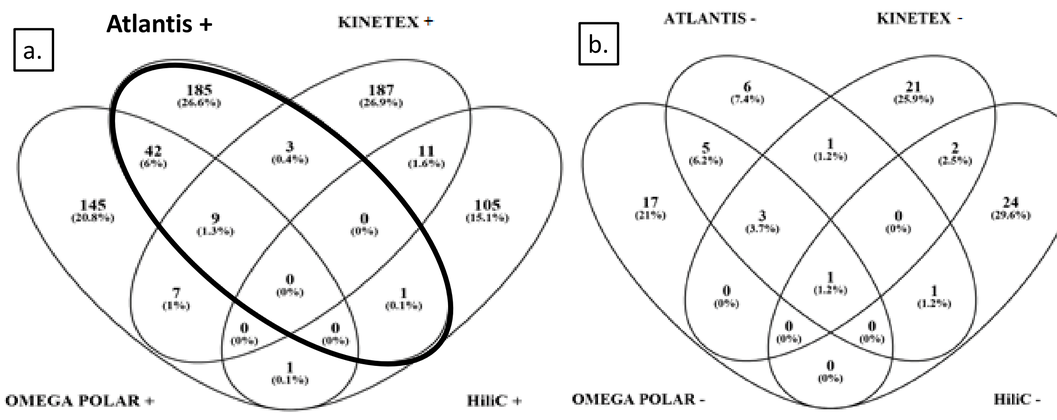


Fig. 3. *Venn diagrams for exact mass in ESI positive (a.) and negative (b.).*

What is next? To identify the unknown compounds more characteristics (intensity or high presence) by statistical approaches. Moreover, differences among tentative TPs detected in the 3 columns will be studied in order to evaluate the effect of the soil on CECs and the formation of TPs.

Conclusions

The analysis strategy used together with the LC-QTOF method allows the detection of a great number of compounds to deepen TPs elucidation.
The results obtained from non-target screening could lead to the identification of new compounds, totally new or recently included in the literature (e.g. [3]).

References

[1] Pérez-Barbón, A., Tortuero, M., Lillo, J., De Bustamante, I. Experiencias piloto en depuración y reutilización mediante filtros verdes intensivos. Congreso hispano-luso. AIH-GE (2016). ISBN: 978-84-938046-5-7.
[2] Boris L. Milman. General principles of identification by mass spectrometry. Trends in Analytical Chemistry 69 (2015) 24–33.
[3] Xuebing Wanga, Nanyang Yua, Jingping Yangb, Ling Jinc, Huiwei Guoa, Wei Shia, Xiaowei Zhanga, Liuyan Yanga, Hongxia Yua, Si Weia. Suspect and non-target screening of pesticides and pharmaceuticals transformation products in wastewater using QTOF-MS. Environment International 137 (2020) 105599.

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