NON-TARGET SCREENING OF MICROPOLLUTANTS IN GREYWATER USING LIQUID CHROMATOGRAPHY – HIGH RESOLUTION MASS SPECTROMETRY

Melse, L.*, Kryuchkov, F**, Hedström, A*, Kallenborn, R** and Herrmann, I.*

*Luleå University of Technology
**Norwegian University of Life Sciences

Highlights:

- Qualitative data on micropollutant presence in greywater is limited but of importance in the context of water reuse.
- A non-target screening is performed on greywater from a city district (~1000 people) and an apartment complex (~100 people).
- Preliminary results indicate the presence of 66 compounds in the city district greywater, and 58 compounds in the apartment complex greywater.

Keywords: emerging contaminants; non-targeted analysis; trace pollutants;

INTRODUCTION

A major challenge for the reuse of greywater is micropollutants. For identification and characterisation of organic pollutant levels and distribution in environmental matrices a variety of non-target screening strategies and methods (NTS) have been developed (Hollender et al., 2023). For greywater, NTS approaches were taken in only three previous studies. Eriksson et al. (2003) annotated 191 individual compounds using GC-MS in greywater from 38 people living in apartments. Gulyas et al. (2011) annotated 80 organic compounds in raw greywater using GC-MS from 103 inhabitants from an eco-settlement. Gros et al. (2017) identified 31 micropollutants using UHPLC Orbitrap HRMS in greywater from small scale (>5 person) systems.

In this study a qualitative NTS is performed on greywater from Helsingborg, Sweden, and Oslo, Norway. Solid phase extraction (SPE) was used as pretreatment for qualitative analysis using Ultra High Performance Liquid Chromatography with high-resolution Time-of-Flight Mass Spectrometry (UHPLC/MS/MS-QTOF). A first quantitative overview on the identified chemical profiles will be presented and discussed in the context of new sustainable techniques and strategies for greywater treatment and reuse. The results of this study will be used for design of high-sample throughput quantitative analysis of priority pollutants to monitor greywater micropollutant content. The overarching aim of this study and follow up studies is to create a shortlist of micropollutants relevant for long term monitoring in greywater treatment installations targeted for reuse of greywater. While this study is performed on relatively large systems, the resulting shortlist of micropollutants will be used to analyse micropollutant greywater quantity in small wastewater systems as well.

METHODOLOGY

Sampling and sampling locations

An overview of the methodology is given in Figure 1. Greywater was sampled from two locations where the greywater is separately collected. In Oslo (Norway), a sample was taken from Klosterenga, an apartment complex housing around 100 people, where the greywater is treated on-site (Jenssen, 2005). A grab sample was taken from the raw greywater collection tank, prior to any treatment. The second sampling location was in Helsingborg (Sweden), at RecoLab (https://www.recolab.se/). Here, the greywater is collected from the Oceanhamnen city district, currently housing approximately 1000 people and several restaurants, hotels, offices, and beauty parlors. A 24-hour composite sample was taken from the raw greywater collection tank. Both samples were stored in one liter Schott bottles at 4°C until analysis.

Sample preparation

The samples were separated using solid phase extraction (SPE), using Oasis HLB cartridges. The samples were analyzed in triplicate. For each replicate, greywater samples (250 -1000 ml) were passed through SPE cartridges overnight. The target fractions were then eluded from the cartridges and evaporated under a nitrogen stream. Residues were reconstituted with 200 µl of methanol and transferred into µHPLC vials.

Analysis

Normalized samples and laboratory blanks were analyzed on an LC-MS instrument (Agilent 1290 Infinity II coupled to Agilent 6546 QTOF high-resolution mass spectrometer). Two different acquisition modes were applied. Initially, samples were screened using Full Scan mode (*m/z* 50-1700). Subsequently, the LC-MS instrument was configured to perform data-dependent acquisition (DDA or auto MS/MS) in iterative mode (n=4) for one sample of each origin. All MS experiments were conducted separately in positive and negative modes using acidic and basic LC buffers.

Data analysis

The data is currently being analysed using the open-source programs MS-Dial and SIRIUS. Full-scan data files are used for statistical comparison of greywater samples from different origins (Klosterenga and RecoLab) and for the identification of common and unique LC-MS features. DDA data files are used for compound annotation. To achieve this, we search experimental MS/MS spectra against open databases (which are listed on MS-Dial page) and AI-generated data (SIRIUS+CSI:FingerID). This will enable us to create a list of Level II annotations, which will be used in future work to identify and quantify these candidates using authentic standards.

PRELIMINARY RESULTS AND CONCLUSIONS

Currently, only preliminary results of the study are available, as the data analysis is ongoing and expected to be finished in fall. Comparison between the two sites revealed significant differences, the majority of LC-MS features are unique to one origin, with only a few shared features. In figure 2, a volcano plot is shown comparing the signals found in the full scan data files. The blue dots show signals significantly more present in the Helsingborg sample compared to the Oslo sample, the red dots show signals significantly more present in the Oslo sample compared to the Helsingborg sample ($\alpha = 0.05$). The grey dots either are present in both sites or the difference is not significant. As can be seen, even for two Scandinavian cities the difference in compounds present is large, highlighting the importance of having good analysis methods for greywater. By the time of the conference (November 2024), the data analysis will be completed and the full results will be presented.

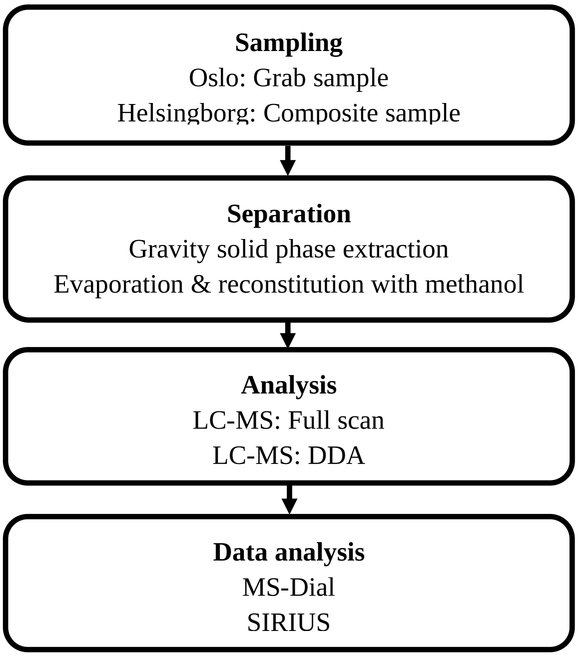

Figure 1: Overview of the methodology used in this study

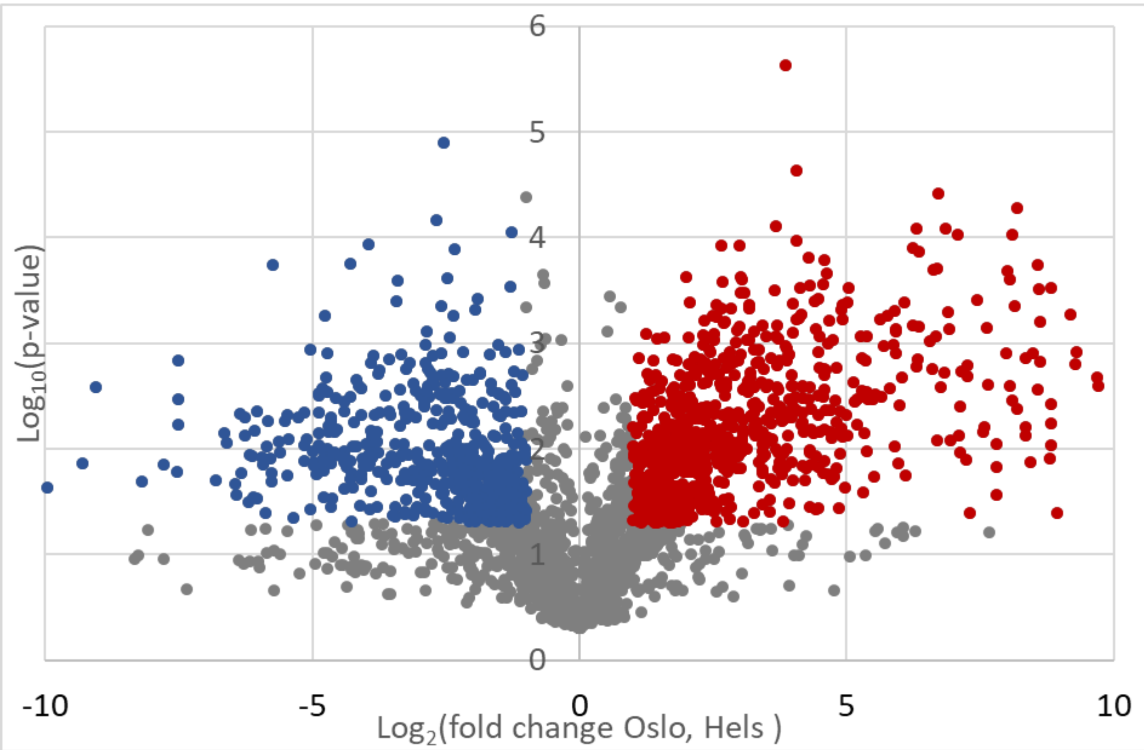

Figure 2: Volcano plot of the signals comparing the Oslo and Helsingborg sites

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